

Imperial College London
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Augmented visualisation of tumour boundaries and functioning brain during neurosurgery

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Abstract

Introduction. Surgery for brain tumours represents a major and multidisciplinary challenge. An extensive body of literature suggests a non-linear correlation between the extent of resection and survival. However, resection is often limited by the difficulty of exactly identifying the degree of brain invasion and the presence of functionally active areas.

Hypothesis. The project examines the hypothesis of advancing the current technological set-up in neurosurgery, specifically attempting to visually improve the identification of tumour margins and eloquent brain activation.

Aims. To investigate:

- to evaluate the role and optimization of IntraOperative UltraSound (IOUS) in brain tumour resection;
- to assess how Hyperspectral Imaging (HSI) can enhance the visualization of tumour margins and detect haemodynamic changes in real-time.

Methods. The present thesis expands on the candidate's experience with IOUS, reporting a published analysis of the advantages. The results of performance and technical limitations are also discussed. Regarding the HSI application, the thesis details the results from a pilot study applying these technologies intra-operatively. Hyperspectral data (hypercube images) were processed using various computational techniques, and brain haemodynamics were analyzed through an adapted Beer-Lambert model.

Results. The IOUS series included 391 cases, and the data showed a resection and survival advantage in glioblastomas. Cases operated using IOUS showed an improved resection and a possible survival advantage. Regarding MSI/HSI, 47 patients have been recruited. The following findings were recorded:

- all the algorithms for intra-patient classifications showed excellent performance on all metrics;

- the inter-patient classification methods showed a better performance with meningiomas but more heterogeneous results with gliomas;
- the perfusion maps showed statistically significant changes in brain perfusion, although the clinical significance of these findings is unclear.

Conclusions. The results reported in the present project are promising for the further technological development of both iOUS and HSI.

Acknowledgements

This work is the culmination of four years' effort, but it's really the end point of my formal educational journey in the field of neurosurgery—"formal" is intended as "I have finally finished school," but a neurosurgeon never really stops studying and learning.

Fulfilling a clinical PhD Project is always challenging, and mine hasn't been an exception, considering that it has been a global pandemic.

Through this journey, I have experienced pretty much all the possible feelings of human existence and their opposite: happiness, anger, frustration, relief, anxiety, depression, hope, and the list goes on.

Perseverance has eventually paid off. However, nothing reported here would have been possible without the help of many outstanding professionals whose insight, support, and knowledge made a difference.

0.1 Copyright Statement

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0.2 Statement of Originality

The present thesis is an original work. The candidate wrote each chapter under the suggestion and guidance of the supervisors.

What is not original work but has contributed to the project is therefore detailed in the following paragraph and repeated in the relevant sections of the thesis.

0.3 Contributions

- Dr Zepeng Hu, MS, PhD student: image registration and normalization, coding for CNN in Chapter 4.
- Ms Victoire de Saléon-Terras, MSc student: coding for detecting haemodynamic changes in Chapter 4.
- Dr Iani Jirah Mae Bulatao Gayo, MEng, PhD student: 3D printing of the microscope adapter, Chapter 4.
- Mr Neekhil Patel FRCS, PhD: cross-check of data segmentation for Chapter 4.
- Dr Richard Perryman, PhD: statistical analysis (log rank-test and multivariate) for clinical series on Chapter 2.

0.4 Personal acknowledgments

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- All the wonderful people at Brain Tumour Research (BTR) and Brain Tumour Research Campaign (BTRC) for funding my studies and clinical activity for a long time. This is particularly appreciated considering that funding was not withdrawn nor reduced despite the challenging (to say the least!) financial period of the COVID-19 pandemic.
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- My wife, friend, and life companion Irene, who has always supported every choice I have made and given opinions in an encouraging, loving, and constructive manner.

Dedication



Figure 1: Dr. Girolamo Anichini, circa 1945

This work is dedicated to the enduring memory of Dr. Girolamo Anichini (1914-1987) — a devoted physician, a loving father, and a medical officer with unwavering principles. Compelled to serve in North Africa during World War II, his participation was a reluctant act of fidelity to an Italy he revered, not a regime he loathed.

In the aftermath of the conflict, Dr. Anichini advocated free speech and progressive thought, actively campaigning for international education and collaboration. He inspired his children to seek knowledge across borders, fostering a belief in a world enriched by shared scientific discovery. His daughter (my aunt) emerged as a distinguished visiting professor of chemistry at the University of Pittsburgh; her landmark papers on rare-earth elements are still cited to this day. My father, influenced by his own father's values, instilled in me the importance of language and cultural exchange, enabling me to pursue academic endeavours across Europe and, with his support, in the United States and the United Kingdom.

Dr. Anichini foresaw a future where globalization and cooperation could elevate the human condition through free knowledge exchange. Though his absence has left an indelible void, his foresight's legacy endures. With a heart full of gratitude and pride, I dedicate this work to him, knowing he would be overjoyed to see the fruits of the values he so deeply cherished.

”Being a researcher basically means you’re curious with a deadline.”

Anonymous

Contents

Abstract	i
Contributions and acknowledgments	iii
0.1 Copyright Statement	iii
0.2 Statement of Originality	iv
0.3 Contributions	iv
0.4 Personal acknowledgments	iv
List of Abbreviations	1
List of Abbreviations	1
1 Introduction	5
1.1 The landscape	5
1.2 Histology, treatment, and prognosis	8
1.2.1 Gliomas	8
1.2.2 Meningiomas	10
1.2.3 Metastases	12
1.3 Diffusion patterns of brain tumours	12

1.3.1	Gliomas	13
1.3.2	Meningiomas	15
1.3.3	Metastases	16
1.4	Surgery, tumour infiltration and functional boundaries: challenges related to the extent of resection	16
1.5	Neurosurgical set-up and current limitations	18
1.5.1	Neuro-navigation	19
1.5.2	Fluorescence	20
1.5.3	Awake surgery	21
1.5.4	Neuro-monitoring	22
1.5.5	Visual identification of eloquent brain: state of the art	23
1.6	Experience from our surgical series	25
1.6.1	Materials and methods	26
1.6.2	Results	29
1.6.3	Discussion	32
1.7	Addressing limitations: current research, screening process, and aims of this project	34
1.8	Conclusions	37
2	The role of ultrasound in neurosurgery	38
2.1	Introduction	38
2.2	Ultrasound: physical principles	38
2.3	IOUS in neurosurgery: the available evidence and areas of research	40
2.4	Materials and methods	43

2.4.1	IOUS devices and neuronavigation devices	43
2.4.2	Retrospective data collection	45
2.4.3	Statistical analysis	47
2.4.4	Results	48
2.4.5	Discussion	58
2.4.6	Limitations	60
2.5	Conclusions	61
3	Hyperspectral imaging in neurosurgery: a review	63
3.1	Basic physical principles of light behaviour	63
3.2	Definitions	65
3.3	Hardware overview	66
3.3.1	Sensors	66
3.3.2	Acquisition techniques	68
3.4	MSI/HSI image processing options - a summary	73
3.5	MSI/HSI in neurosurgery: a literature review	82
3.6	Objectives	82
3.7	Materials and methods	83
3.7.1	Inclusion criteria	83
3.7.2	Literature search	83
3.8	Results	84
3.8.1	Clinical data	85
3.8.2	Technical data	87

3.8.3	Algorithms used for image analysis	90
3.9	Discussion	92
3.9.1	The infancy stage of neurosurgical HSI	92
3.9.2	The challenge of reliable ground truth	93
3.9.3	Study heterogeneity	93
3.9.4	Haemodynamic changes	94
3.9.5	Limitations of the present review	94
3.10	Conclusions and rationale for further studies	95
4	Hyperspectral and multispectral imaging in neurosurgery	96
4.1	Materials and methods	96
4.1.1	Set-up: hardware and connections	96
4.1.2	Microscope and light source	97
4.1.3	CCD and CMOS cameras	100
4.1.4	Liquid Crystal Tunable Filter - LCTF	100
4.1.5	Laptop and data storage	103
4.1.6	Ethics application	104
4.1.7	Study design	105
4.1.8	Outcome measures	105
4.1.9	Power calculation	107
4.1.10	Patient recruitment	108
4.1.11	Intra-operative data collection	109
4.1.12	Anonymization process	110

4.1.13	Protocol for images acquisition	110
4.1.14	Image labelling - Ground truth	114
4.2	Data analysis	118
4.2.1	Pre-processing	119
4.2.2	Modules tested for image analysis	122
4.2.3	Analysis of haemodynamic changes	128
4.3	Results	133
4.3.1	Clinical results	133
4.3.2	Adverse reactions and adverse events	137
4.3.3	Intra-operative views	138
4.3.4	HGG-IV	138
4.3.5	Meningiomas	150
4.3.6	LGG and anaplastic gliomas	159
4.3.7	Haemodynamic changes	160
4.4	Discussion	165
4.4.1	Set-up, recruitment and acquisitions: ex-post reflections	165
4.4.2	Tumour identification: Main findings and limitations	166
4.4.3	Detection of haemodynamic changes	170
4.5	Conclusions	173
5	Future developments	174
5.1	Introduction chapter and clinical series	174
5.2	Intra-Operative UltraSound (IOUS) (Chapter 2)	175

5.3	Hyperspectral imaging as a clinical tool (Chapter 4)	178
5.3.1	Ongoing projects and immediate future	180
5.3.2	Long term developments	181
5.3.3	Final summary: the bigger picture	184
A	Publications and supportive work	185
B	Python codes	189
B.1	Code for single hypercube classification	189
B.2	Code written for inter-patients classification	192
	bibliography	205

List of Tables

1.1	Performance of the neuro-monitoring application in our cohort of patients - TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative; PPV = Positive Predictive Value; NPV = Negative Predictive Value.	32
2.1	3D-IOUS histological cohort. GB = Glioblastoma; Oligo = Oligodendroglioma; Astro = Astrocytoma; AO = Anaplastic Oligodendroglioma; AA = Anaplastic Astrocytoma; NOS = Not Otherwise Specified; DNET = Dysembryoplastic NeuroEctodermal Tumour; AVM = Artero-Venous Malformation	49
2.2	Overall number of cases and percentages included for the metrics analysis. Due to incomplete records, not all cases from both cohorts could be included	55
2.3	Performance metrics of the 3D-IOUS compared with the standard neuronavigation.	55
2.4	Summary of glioblastomas cohort characteristics. IOUS = IntraOperative UltraSound; NOS = Not Otherwise Specified; C1, C2 and C3 = Consultant 1, Consultant 2 and Consultant 3.	56
4.1	Components of the HSI/MSI system deployed for the present project	97

4.2	Summary of our dataset. Number HS0043 has been removed from the series as the histological analysis did not confirm neoplastic disease. Notice how the acquisitions at phase 1 were more common, while phases 2 and 3 were attempted less often - see text for more details. ABBREVIATIONS. TOP: ID = Identification; CCI = Charlson Comorbidity Index; EOR = Extent of Resection. HISTOLOGY: Adeno = Adenocarcinoma; Astro = Astrocytoma; GB = Glioblastoma; met = metastasis; Men = Meningioma; NOS = Not Otherwise Specified; Oligo = Oligodendroglioma; PXA = Pleomorphic XantoAstrocytoma. LOCATION: cen = central; cing = cingulate; front = frontal; occ = occipital; par = parietal; sup = Superior; temp = temporal.	135
4.3	Data from the HSI series regarding the main histological subtypes. GB = Glioblastoma; AIV = Astrocytoma Grade IV; LGG = Low Grade Glioma; AII = Astrocytoma grade II; AAIII = Anaplastic Astrocytoma grade III; OligoII = Oligodendroglioma grade II; OligoIII = Anaplastic Oligodendroglioma III; NOS = Not Otherwise Specified; SD = Standard Deviation; EOR = Extent Of Resection; OS = Overall Survival; PFS = Progression Free Survival. All survival metrics are measured in months.	136
4.4	Performance Status (PS) and Clavien-Dindo Score (CDS) for the cohort of HSI patients. N/A = No complications reported; Pts = Patients; Pre-op / Post-op = Pre-operative / Post-operative.	137
4.5	Random Forest classification results	141
4.6	MLPC classification results.	141
4.7	Metrics of the random forest classification approach applied to case HS014. . . .	141
4.8	Decision tree classifier results	143
4.9	Random forest classification results.	143
4.10	SVM classifier results	143
4.11	MLPC results on the HGG-IV series	144

4.12	Performance metrics for each class related to the convolutional neural network (CNN). Abbreviations: ara = arachnoid; cort = cortical gray matter; margin = areas of edema / suspected infiltration; NPV = Negative Predictive Value; PPV = Positive Predictive Value; SD = Standard Deviation; white = white matter. .	145
4.13	Intra-patient random forest Performance Metrics	151
4.14	Random forest classifier results produced when case number HS002 has been used as a validation case - this attempt showed the poorest performance of this model.	153
4.15	Random forest best performance metrics for our cohort of meningiomas, with pixel sampling set at 5000.	154
4.16	Random forest best performance metrics for our cohort of meningiomas, with pixel sampling set at 10000	154
4.17	Performance metrics of SVM applied to our cohort of meningiomas.	155
4.18	Performance metrics for each class related to the convolutional neural network (CNN) in our class of meningiomas. Abbreviations: ara = arachnoid; cort = cortical gray matter; NPV = Negative Predictive Value; PPV = Positive Predictive Value; SD = Standard Deviation	156
4.19	Case HS009 - random forest classifier result.	159

List of Figures

1	Dr. Girolamo Anichini, circa 1945	vii
1.1	A visual representation of the blood brain barrier. Left: 3D visualization showing the nutrients being filtered. Right: section of a brain capillary showing the pericytes, the cytoplasmic branches of the astrocytes, and the epithelial tight junctions. Created in BioRender. Anichini, G. (2024) BioRender.com/s89v610. .	7
1.2	Chart showing Bailey and Cushing classification of brain tumours - From <i>Percival Bailey and the Classification of Brain Tumours</i> [1].	9
1.3	Flow chart showing different treatment pathways according to different histological and molecular sub-types - From <i>EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood</i> [2].	10
1.4	An intra-operative picture from one of the cases reported in Chapter 4. The histology revealed an Oligodendroglioma Grade II - a type of LGG. The dotted blue line highlights the tumour core, and the green line the areas of infiltration. The tumour margins are not visible to the naked eye, and their identification is only possible through the combined use of neuronavigation and, in some cases, intra-operative neuromonitoring. Wernicke's speech area was mapped at the caudal end of the tumour, the top centre of the surgical field in this picture. . .	33
2.1	3D-IOUS system used in the present series	44

2.2	Intra-operative IOUS-neuronavigation coupled view of a left supplementary motor area oligodendroglioma. On the left the image as visualized on the stand-alone neuronavigation, on the right the integration with IOUS. Defined / exophytic LGG are usually optimally visualized using IOUS	50
2.3	A case of a right temporal diffuse LGG. The IOUS signal (right) is challenging to interpret as the lesion appears diffuse, infiltrative in nature, and with no clear boundaries	51
2.4	Intra-operative picture of left frontal GB before (left) and after resection (right). In this case, the necrotic core was well visualized. On the right side, note the presence of a partial shift of the brain orbital gyrus after resection and a cone of hyperechogenic material on the skull base due to the deposit of blood products at the bottom of the cavity.	52
2.5	A case of left frontal recurrent LGG was found to be a transformed Grade IV (GB) after histological analysis. Apart from the size of the lesion requiring multiple acquisitions and therefore causing linear artefacts, the tumour appears challenging to define on IOUS due to the relatively homogeneous hyperechogenic signal.	53
2.6	A case of a parietal metastasis pre (left) and post (right) resection. Even a thin layer of haemostatic material at the bottom of the surgical cavity is causing a significant cone of hyperechogenic artefact.	54
2.7	Pre-operative volumes compared with the EOR. EOR = Extent of Resection; CI = Confidence Intervals	57
2.8	Whilst not independently significant, there was a shift toward longer survival in the IOUS cohort. Perioperative complications had a highly significant impact: 3 patients showed markedly shorter Overall Survival (OS). Radical Chemo-RatioTherapy (CRT) also significantly extends survival times, in keeping with the results of the known literature. P values were determined by the log-rank (Mantel-Cox) test.	58

2.9	Progression-Free Survival (PFS) was poor in all patients, and the differences between groups are insignificant. IOUS does not appear to be significantly associated with significantly improved PFS. Radical CRT promotes longer PFS, as expected. P values are determined by the log-rank (Mantel-Cox) test.	58
3.1	Graphical representation of a random wave function with wavelength and frequency	64
3.2	Different methods for multispectral/hyperspectral data acquisition. Image taken from the work of Shapey and colleagues [3]	69
3.3	Representation of the hypercube acquisition, courtesy of Maria Leiloglou.	71
3.4	Two simplified examples of hypothetical linear (left) and non-linear (right) functions partitioning groups of data	76
3.5	Hypothetical simplified schematic of a neural network for spectral imaging analysis. Adapted from Jiang et al. [4].	81
3.6	Flowchart showing the selection and screening process of the review, as per PRISMA guidelines	86
3.7	Different set-up options found in the current literature - Picture courtesy of Maria Leiloglou.	87
4.1	Schematic of the surgical microscope from the work of Ma and colleagues [5] . . .	98
4.2	Surgical microscope used for the present study, the Zeiss Pentero©, here draped and ready to be used	99
4.3	Draped microscope used during a craniotomy	99
4.4	Varispec© LCTF (two versions, bottom left and top right) with its control box. This is the filter used for the present study	101
4.5	Adapter connecting the Pentero port with the LCTF and the camera	102
4.6	LabVIEW© interface showing the main features	103

4.7	Stages of datacube acquisition during surgery for a brain tumour in a non-eloquent area. These steps were the ones I was interested in acquiring, according to both my experience, our firm's track record, and the reported literature: 1) early cortical / tumour exposure; 2) midway through resection (approximation); 3) end of the resection / final surgical cavity.	112
4.8	Stages of datacube acquisition during surgery for a brain tumour in an eloquent area. The pictured example is a schematic of an awake surgery during a left motor strip tumour resection, and the patient is asked to move the corresponding hand (right side). Again, these steps were the ones I was interested in acquiring, according to both my experience, our firm's track record, and the reported literature: 1a) tumour exposure, no stimulation nor task execution; 1b) tumour exposure, active stimulation or task execution; 2a) 50% of resection performed, no stimulation nor task execution; 2b) 50% of resection performed, active stimulation or task execution; 3a) final surgical cavity, no stimulation nor task execution; 3b) final surgical cavity, active stimulation or task execution. CST = Cortico-Spinal Tracts.	113
4.9	Methodology for image segmentation on one of the cases from our series - HS009. On the left is a sagittal MRI FLAIR reconstruction where the anatomical landmarks are identified and marked. On the right, the intra-operative snapshot is used for image segmentation - note the U-shaped sulcus with the draining veins used as an anatomical landmark.	115
4.10	Case HS009. Segmentation of the intra-operative microscope snapshot showing the different labels. The capillaries were excluded and kept unsegmented outside the labels in this segmentation.	117
4.11	Case HS009. Segmentation of the intra-operative microscope snapshot showing the different labels. In this segmentation, the capillaries are included inside the labels.	118
4.12	Structure of our saved database.	120
4.13	Schematic comparing different image processing models and their data partition and classification features - taken from the scikit-learn website.	124

4.14 Representation of the CNN designed for this study - picture realized by Dr Zepeng Hu.	126
4.15 Flowchart of the protocol to detect haemodynamic changes - from "Use of hyperspectral analysis in neurosurgery", MSc report by Victoire de Saléon Serras. .	132
4.16 Gantt chart showing the different phases of the study.	133
4.17 Case HS017. The result of intra-patient classification algorithms comparison. The decision tree algorithm showed an acceptable result but was outperformed by MLPC and the random forest. Notice that the random forest algorithm was trialled without and with a validation set. "Core" refers to the tumour core, and "margins" refers to the suspected area of tumour infiltration as seen on a pre-op MRI scan.	140
4.18 Case HS017. Overlapping of the mask to the intra-operative microscope snapshot. Random forest has been applied in this case. Top left: intra-operative microscope snapshot showing the tumour exposed; top right: segmentation mask; bottom left: image taken from the hyperspectral stack; bottom right: predicted image overlapped onto the microscope image.	140
4.19 Case HS014. Overlapping of the mask to the intra-operative microscope snapshot. Random forest has been applied in this case. Top left: intra-operative microscope snapshot showing the tumour exposed; top right: segmentation mask; bottom left: image taken from the hyperspectral stack; bottom right: predicted image overlapped onto the microscope image.	142
4.20 Case HS016 - A multifocal, post-central gyrus glioblastoma exposed at the very beginning of the resection. Top left - microscope snapshot: dura has been opened and the tumour is clearly visible. The right side of the picture shows a comparison between predictions from different acquisitions - Exp = Exposure. Bottom left: mask overlapped with the microscope acquisition.	147
4.21 Case HS016, post-debulking - Different exposures don't seem to impact the tumour margins' visualisation significantly.	147

- 4.22 Case HS017, a large temporo-basal glioma. On the top left is the intra-operative microscope snapshot. On the right of the picture are two predictions based on two acquisitions with exposure at 200 and at 50, respectively. Bottom left: the prediction is overlayed on the microscope snapshot to show the corresponding areas. 148
- 4.23 Case HS001 - Comparison between different predictions from acquisitions. This case showed a good exposure of a deep tumour, with more than satisfactory network performance in identifying the tumour core, the margins, and the white matter. 149
- 4.24 Case HS001 - Comparison between different predictions from acquisitions. This case showed a good exposure of a deep tumour, with more than satisfactory network performance in identifying the tumour core, the margins, and the white matter. 150
- 4.25 The result of intra-patient classification algorithm - random forest has been applied in this case. Top left: intra-operative microscope snapshot showing the tumour exposed; top right: segmentation mask; bottom left: image taken from the hyperspectral stack; bottom right: predicted image overlapped onto the microscope image. 152
- 4.26 Intra-operative microscope snapshot for case number HS002. The tumour is deep and poorly visible, with a large number of surgical cottonoids around. These findings might explain the poor performance when this specific case was used for validation in one of the random forest runs. 153
- 4.27 Case HS002 - First meningioma acquired in our series. Top left: microscope snapshot; right side: predicted image; bottom: microscope image with the prediction on top. 157
- 4.28 Bilateral parasagittal meningioma. Top left corner: microscope snapshot. Right side: predictions realized with acquisitions at different exposures. Bottom left: prediction realized with the exposure at 50 used as a mask for the microscope view. 157

4.29	Case HS042 - Convexity meningioma of the supramarginal gyrus. Top left: microscope snapshot showing the exposed tumour with the dura folded on top of the mass. Top right: prediction, exposure at 50. Bottom: prediction compared with the microscope snapshot.	158
4.30	HS009 - Comparison of the different models' performances. Looking clockwise - Top left: mask; top right: random forest classifier output; bottom right: MLPC output; bottom left: mask overlapped with the microscope snapshot.	159
4.31	Case HS009 - intra-operative view. The image is flipped horizontally and seen from the bottom, to compare it with Figure 4.32.	161
4.32	Case HS009 - Tumour exposed. Comparison between HbO and Hb concentrations during task executions.	162
4.33	Case HS009 - Final cavity, comparison between HbO and Hb concentrations during task executions.	163
4.34	Case HS048 - intra-operative view. The image is flipped horizontally to compare it with 4.35.	164
4.35	Case HS048 - THb concentrations and O2 saturation maps.	164
4.36	Case HS048 - Comparison between HbO and Hb concentrations during task executions.	165
5.1	Workflow for an IOUS tissue scanning platform, showing how an IOUS can be linked to a robotic arm for automatic scanning - Picture taken from Dyck et al. [6]	177
5.2	Cubert Ultris S5/SR5 hyperspectral camera module	181

List of Abbreviations

3D-IOUS	3D Intra-Operative UltraSound
5-ALA	5-aminolevulinic acid
ACC	Accuracy
ADC	Apparent Diffusion Coefficient
AI	Artificial Intelligence
AKAZE	Accelerated-KAZE
BBB	Blood Brain Barrier
BOLD	Blood Oxygenation Level Dependent
CBAM	Convolutional Block Attention Module
CBV	Cerebral Blood Volume
CCD	Charge-Coupled Device
CCI	Charlston Comorbidity Index
CDS	Clavien-Dindo Score
CMOS	Complementary Metal-Oxide-Semiconductor
CNN	Convolutional Neural Network
CRT	Chemo-RadioTherapy
CSF	Cerebro-Spinal Fluid
CT	Computer Tomography
CUSA	Cavitron UltraSonic Aspirator
DT	Decision Tree
DTI	Diffuse Tensor Imaging
DWI	Diffusion Weighted Images

EANO	European Association of Neuro-Oncology
EEG	ElectroEncephaloGram
EOR	Extent Of Resection
ES	Effect Size
FLAIR	Fluid-Attenuated Inversion Recovery
fMRI	Functional Magnetic Resonance Imaging
FN	False Negative
FP	False Positive
GB	GlioBlastoma
GFAP	Glial Fibrillary Acidic Protein
GPIB	General Purpose Interface Bus
GTR	Gross Total Resection
HGG	High Grade Glioma(s)
HRA	Human Research Authority
HSI	Hyperspectral Imaging
ICG	IndoCyanine Green
ICP	IntraCranial Pressure
IDH	Isocitrate DeHydrogenase
IFS	Integral Field Spectroscopy
iMRI	intraoperative Magnetic Resonance Imaging
IOUS	IntraOperative UltraSound
IRAS	Integrated Research Application System
IRS	Imaging Replicating Spectrometers
JRO	Joint Research Office
LCTF	Liquid Crystal Tunable Filter
LGG	Low Grade Glioma(s)
MEG	MagnetoEncephaloGraphy
MEP	Motor Evoked Potentials
MGMT	O6-methylguanine-DNA methyltransferase

MLPC	Multi-Layer Perceptron Classifier
MRI	Magnetic Resonance Imaging
MSI	MultiSpectral Imaging
NFP	NeuroFilament Protein
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIR	Near InfraRed
NPV	Negative Predictive Value
nTMS	navigated TransMagnetic Stimulation
NTR	Near Total Resection
OA	Overall Accuracy
ORB	Oriented FAST and Rotated BRIEF
OS	Overall Survival
PFS	Progression Free Survival
PID	Personal Identifiable Data
PIS	Patient Information Sheet
PPV	Positive Predictive Value
PS	Performance Status
RANO	Response Assessment of Neuro-Oncology clinical trials
REIMS	Rapid Evaporative Mass Spectrometry
RF	Random Forest
RGB	Red, Green, and Blue
ROAM	Radiation versus Observation following the surgical resection of the Atypical Meningiomas
ROI	Regions Of Interest
SEN	Sensitivity
SIFT	Scale-Invariant Feature Transform
SMA	Supplementary Motor Area
SPE	Specificity

SRS	Stereotactic RadioSurgery
SSEP	Somato-Sensory Evoked Potentials
SVM	Support Vector Machine
TIVA	Total Intra-Venous Anaesthesia
TMF	Trial Master File
TN	True Negative
TP	True Positive
US	UltraSound
USB	Universal Serial Bus
VEP	Visual Evoked Potentials
ViT	Vision Transformer
WHO	World Health Organization

Chapter 1

Introduction

1.1 The landscape

Brain tumours are uniquely challenging in the landscape of human body cancers.

First, they affect an organ with a unique structure and cytoarchitecture. The brain is not a homogeneous organ like the liver, the lung, and the kidneys; within the brain, areas matter [7,8]. The brain is a profoundly differentiated organ with multiple portions of grey and white matter responsible for different functions and network connections, a concept that has become increasingly clear over time [9–13]. A tumour affecting the right frontal lobe shows a different clinical impact than a tumour involving the thalamus or the brainstem. No portion of the brain is useless, but areas of the brain react differently to disease. The scientific community argues that the brain is arranged in connectomes/clusters of networks, meaning groups of neurons acting in a coordinated fashion to execute specific functions and to elaborate external impulses [7,8,13,14]. Hence, no portion of the brain is without function, but some areas are more plastic and resilient than others. Those areas that do not tolerate a surgical insult used to be defined as "eloquent" [11]. They can now be considered the "hubs" of a network: we can take out some nodes, but taking out a critical hub together with a tumour would be catastrophic [8]. The concept of "eloquent areas" is now considered technically outdated [15], but it still serves a great practical purpose for the neurosurgeon. Specific eloquent areas (focal points in the context

of connectomes) need to be spared by the surgical resection, or the price paid by the patient will be too high, both in terms of life-changing disability and prognosis. A disabled patient might not have adequate Performance Status (PS) to receive adjuvant treatment; therefore, the tumour will recur sooner [2].

Second, the most aggressive primary brain cancers tend to recur locally and not spread metastasis to the rest of the body except in anecdotal cases [16]. While this feature appears advantageous in not causing systemic disease, it also hides another challenge: brain tumours arise from cells and tissues that are profoundly peculiar and show a unique behaviour compared to other cancer cells. Specifically, there is now clear evidence that white matter tracts and leptomeningeal routes are among the preferred pathways for brain cancers to spread, thus confirming a specific diffusion pattern that rarely includes blood or lymph nodes [17–21].

Third, those same cancers rely on a structural advantage: a cytological and biochemical barrier separating the brain from the rest of the body, the Blood Brain Barrier (BBB) [22]. This is not a physical barrier in the ordinary sense of the term (meaning a wall), but rather a microscopic filter made up of astrocytes packed around capillaries and bound together with molecular tight junctions (see Figure 1.1). Said astrocytes actively prevent several molecules from coming into contact with neurons. While this feature makes sense from an evolutionary standpoint [23], as it provides an additional layer of protection for the neurons, it also poses a severe challenge in the context of brain cancer [24]. With very few exceptions, there isn't a single agent or procedure that can successfully cure it. Cancers typically require a variable combination of surgery, chemotherapy, and radiotherapy. Sometimes, part of the chemo-radiotherapy regimen is administered before the surgery; in other cases, it is only administered after. In the specific situation of brain cancers, the presence of BBB does not allow many chemotherapy agents to reach their target. Hence, very few molecules are currently available, and their prognostic impact is limited [2].

Finally, but not less importantly, the infiltration of the tumour beyond macroscopically apparent margins is particularly insidious in the brain. As mentioned, brain tumours infiltrate and spread along white matter tracts, and some tumours tend to affect very eloquent connectomes while

others do not. The more tumour mass that can be resected, the better the prognosis, although the correlation is not linear [2]. The "uncertain" area of tumour infiltration poses a real problem to the surgeon.

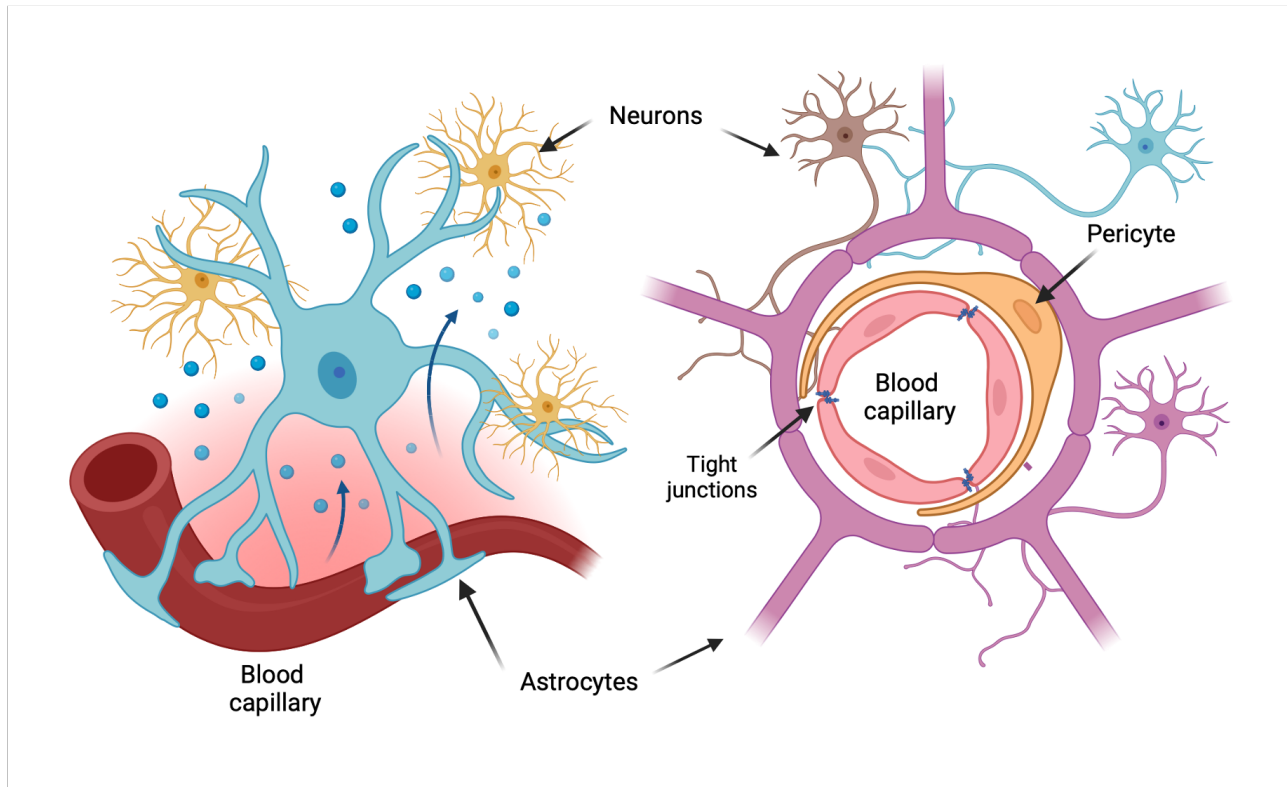


Figure 1.1: A visual representation of the blood brain barrier. Left: 3D visualization showing the nutrients being filtered. Right: section of a brain capillary showing the pericytes, the cytoplasmic branches of the astrocytes, and the epithelial tight junctions. Created in BioRender. Anichini, G. (2024) BioRender.com/s89v610.

The neurosurgical and neuro-oncological communities have been debating this point for a long time [25–29]. Several technological innovations have been introduced in clinical practice. They are currently being tested to refine the Extent Of Resection (EOR), some of which will be discussed and explored in the present research project.

This chapter will discuss the premises of the challenges mentioned above, with specific reference to three of the most common brain tumours: primary brain cancers, also known as "gliomas", primary benign intracranial tumours, also known as "meningiomas", and secondary brain tumours, meaning metastases coming from different organs.

1.2 Histology, treatment, and prognosis

The reason for studying gliomas and meningiomas is intuitive if examining the epidemiological and historical data: they are by far the most common primary intracranial tumours and those that are most commonly seen in neurosurgical and neuro-oncological practice [30,31].

1.2.1 Gliomas

Gliomas are the most common primary brain tumours and arise from glial cells [32]. They are intra-axial, meaning they arise from within the brain parenchyma. They tend to infiltrate the surrounding brain and seed cells distally from their point of origin [18,33,34]. They are highly aggressive, with a variable prognosis depending on the grade (see below).

The first definition of glioma was given by Rudolf Virchow (1821-1902) [35,36]. Several classifications have been proposed since, starting from the first descriptions of brain tumours and gliomas based on their cells of origin [1] (see Figure 4.1), and then evolving, adding more sophisticated and histology-driven descriptions by others [32,37].

The first official World Health Organization (WHO) classification was published in 1979, and all its subsequent editions have served as a reference for pathologists, oncologists and neurosurgeons across the globe [38]. Immunohistochemistry and molecular profiling have been subsequently added, thus offering a more accurate prediction of the behaviour of the specific glioma sub-types and, therefore, a helpful guideline regarding the best therapeutic approach [32,39].

Current treatment options are tailored to the specific grade and histological sub-type, as defined by the European Association of Neuro-Oncology (EANO) [2].

Benign WHO grade I gliomas can be successfully treated with surgical resection alone. Stereotactic radiosurgery or fractionated radiotherapy can be considered as second choice treatment if a residual is left behind or if the tumour is too challenging to be removed through surgery. Intermediate grades, such as grade II, benefit from surgical resection and might not require adjuvant treatment after surgery, depending on the molecular and immunohistochemical features

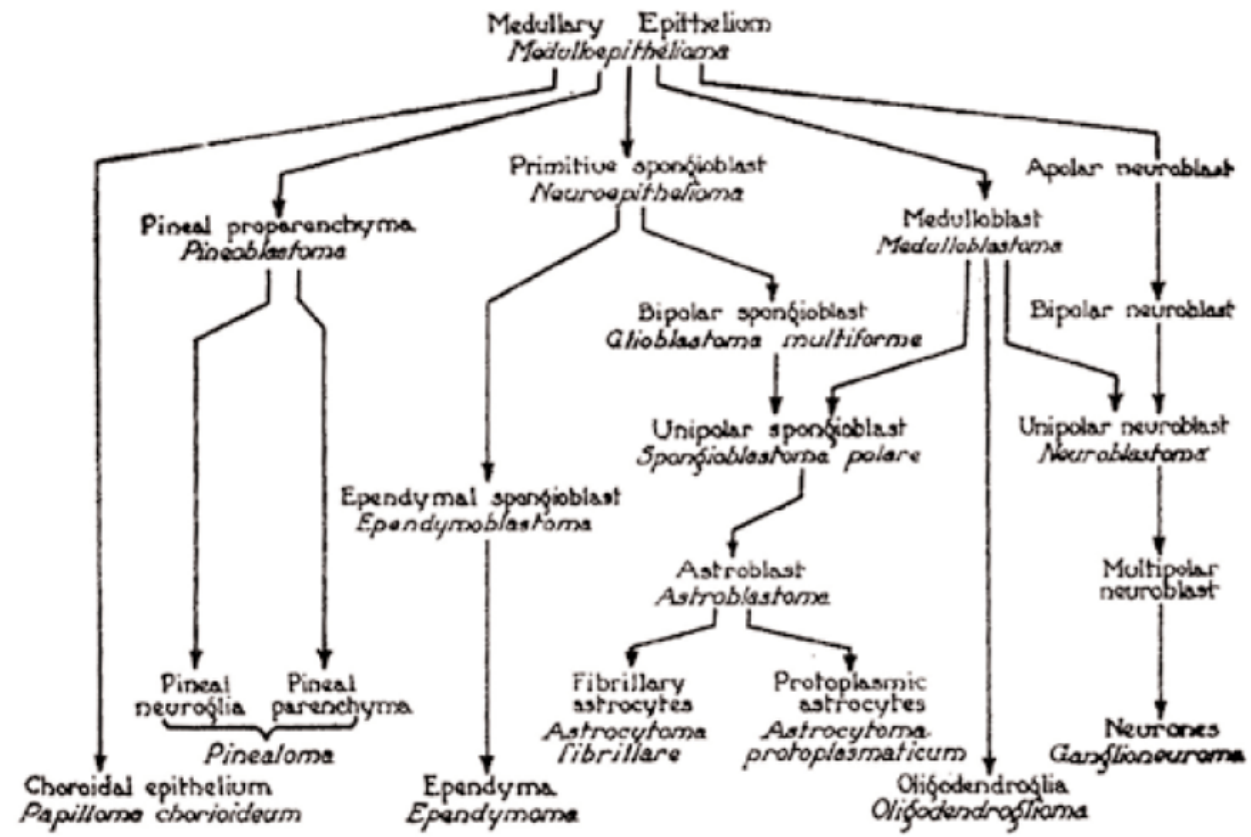


Figure 1.2: Chart showing Bailey and Cushing classification of brain tumours - From *Percival Bailey and the Classification of Brain Tumours* [1].

- See Figure 1.3. For the most aggressive histological subgroups (WHO grades III and IV), treatment consists of a combination of surgery followed by chemotherapy and radiotherapy [2].

The prognosis of brain gliomas varies according to their WHO grade. The details of the molecular specifics are not treated extensively here as they are irrelevant to the scope of the present work. Still, the summary report on 1.3 represents the current state of the art. The prognosis, calculated in terms of Overall Survival (OS) and Progression Free Survival (PFS), is directly correlated to the WHO grade: grade I WHO tumours show an excellent survival if treated radically with surgery and usually are among the few gliomas where the expression "curative treatment" can be used. Grade II WHO - Low Grade Glioma(s) (LGG) - show an OS of more than 5 years if treated radically [40], but variably inferior if radical resection is impossible. Grade III and IV WHO - High Grade Glioma(s) (HGG) - are known to hold the worst prognosis, despite treatment, with an OS of 24 months [40] and 15 months [32] respectively.

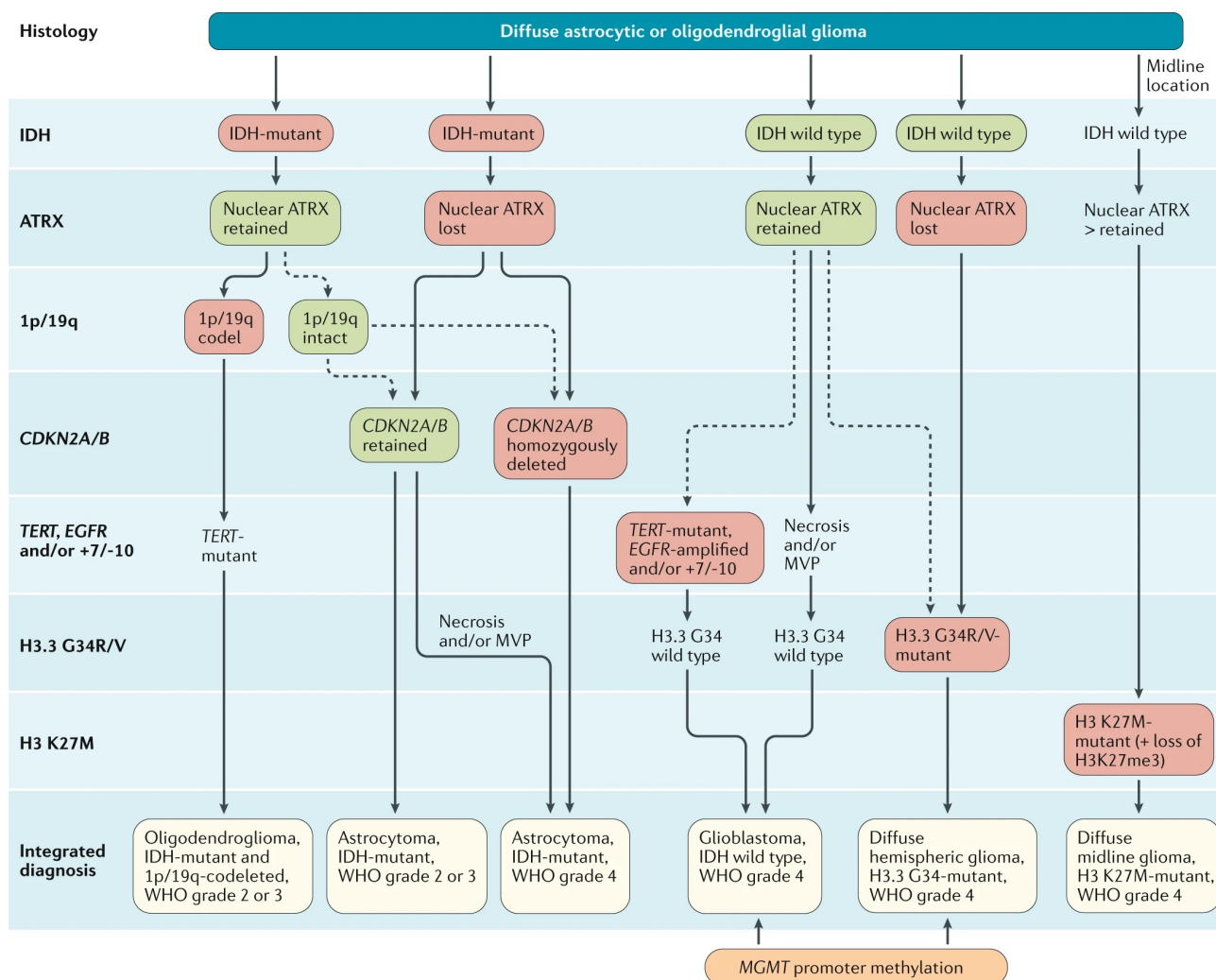


Figure 1.3: Flow chart showing different treatment pathways according to different histological and molecular sub-types - From *EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood* [2].

1.2.2 Meningiomas

Meningiomas are extraaxial tumours arising from the meningotheial tissue. They are also common, although more often asymptomatic, and they tend to be slow-growing and not infiltrative.

Percival Bailey provided one of the first descriptions of meningiomas, both in a dedicated paper [41] and in his book, written in cooperation with Harvey Cushing [42]. Surgery was, and to most extent still is, the treatment of choice as it has shown to be curative in most cases [43]. Some meningiomas are safe to remove altogether, while others can be challenging for a safe resection, which can increase the risk of post-operative neuro-cognitive impairment [44–46]. In 1957, the Simpson classification showed a relatively linear correlation between the amount of

tumour resected and a lower recurrence rate [47]. The neurosurgical community still uses said classification today, although the specifics of what constitutes a total resection are controversial [48]. For post-operative adjuvant treatment, a more binary classification (gross total resection VS sub-total resection) is preferred [49].

The WHO classification divides meningiomas into three histological classes.

Grade I WHO meningiomas are the most common. They are slow-growing, not invasive, and amenable to surgical resection with no need for adjuvant therapy. Surgery was traditionally considered effective in curing the tumour when the mass and the surrounding dura are completely resected [47]. Some authors subsequently pointed out how the non-unusual invasion of bone ("hyperostosis") could also be considered as part of an adequate resection, therefore defining a Simpson Zero grade for those cases where tumour, dura, and overlying hyperostotic bone were resected [50].

Grade II WHO meningiomas represents 15 to 20% of meningiomas. They are known to be more aggressive and require radical surgery to be treated. The ongoing debate on whether delivering radiotherapy post-op in such cases has not reached a consensus. This was the focus of the Radiation versus Observation following the surgical resection of the Atypical Meningiomas (ROAM), which has been closed recruitment in 2021 [51]. The results are still pending. The recurrence rate for grade II WHO meningiomas is considered to be 40-50% [52], but it can be as high as 60% [53].

Finally, grade III meningiomas are rare but highly aggressive lesions with histological features of brain invasion. They show a recurrence rate as high as 90%, and their treatment consists of a combination of aggressive surgery and post-operative radiotherapy [52]. Several possible pharmacological agents, including neo-angiogenetic factors and several oncogenic factors inhibitors, have been tested on grade III meningiomas. However, their use is still under investigation and is only part of clinical trial protocols [54].

1.2.3 Metastases

Brain metastases present different physiopathological profiles and challenges. Their classification does not follow the WHO categories because they are too heterogeneous to classify according to the described histological and molecular parameters. However, they are equally if not even more relevant from an epidemiological point of view, as they represent the most common brain tumour in the elderly population [55]. Brain metastases usually are classified following the traditional TNM (T = Tumour extension, N = lymphNodes involvement, M = distant Metastasis) score, meaning that they are included in a systemic classification rather than a specific one. In descending order, the most common cancers of origins are lung, breast and melanoma, although any of the body cancers can give brain metastases [55, 56].

Treatment of brain metastases varies depending on the patient's PS, the staging of the systemic disease, and the extent of the intracranial involvement [56]. An extensive and detailed discussion regarding indications goes beyond the scope of this chapter. However, to summarize the basic concepts, surgical treatment is usually reserved for isolated metastases with mass effect in patients with controlled systemic disease, reasonably expected prognosis (more than six months) and good performance status. A combination of surgery and stereotactic radiotherapy (also known as Stereotactic RadioSurgery (SRS)) or fractionated radiotherapy on the cavity +/- systemic chemo-radiotherapy is the standard treatment protocol, depending on the histological sub-type [57].

1.3 Diffusion patterns of brain tumours

Brain tumours are heterogeneous in their clinical and biological behaviour and tend to affect the surrounding brain through different physio-pathological mechanisms. Gliomas are intra-axial and infiltrate the brain parenchyma [2, 58], whereas meningiomas are extra-axial and tend to spread through the meninges [59]. The following description details the diffusion routes of the three most common brain tumours and their radiological correlates.

1.3.1 Gliomas

The infiltrative nature of gliomas and the spreading pattern of tumour cells into the typical white and grey matter was initially pointed out by Cushing and Bailey themselves [42], and subsequently re-assessed on the work of Scherer [60]. Early autopsy studies pointed out how glioblastomas tend to invade the contra-lateral hemisphere through the corpus callosum in up to 75% of the examined cases [61], and Walter Dandy reported that even complete hemispherectomies were not aggressive enough to keep the recurrence at bay [18]. The two main diffusion mechanisms are contiguous spreading and white matter tract diffusion [17, 33, 34]. Gliomas rarely spread following blood vessels or breaking through the extracellular matrix, also known as sub-ependymal spread [62]. Still, they can spread through the Cerebro-Spinal Fluid (CSF), although this modality is more frequent in ependymomas [34, 62]. Metastases outside the CNS are anecdotal – 20 cases reported over 200 years [63]. Additional evidence of white matter invasion from surgical reports also helped understand gliomas' invasion pattern and contributed to defining surgical strategies [64, 65].

The introduction of the Magnetic Resonance Imaging (MRI) scan rapidly improved the sensitivity and specificity in the early diagnosis of small tumours and the definition of the extension through the surrounding structures [66–69]. The introduction of Diffuse Tensor Imaging (DTI) for the spatial definition of the white matter tracts confirmed the previously noticed tendency of gliomas to both locally recur or spread through contiguous white matter bundles [19–21]. MRI studies on gliomas diffusion pattern were crucial in defining the anatomy of the sub-cortical white matter, as several bundles were described due to the extensive use of DTI imaging [70, 71].

From a radiological perspective, HGG are avidly enhancing tumours, often showing a hyper-intense enhancing ring with a central hypo-intense (necrotic) core [58]. It is accepted, and part of the international guidelines, that the enhancing component in a newly diagnosed HGG represents tumour tissue [2]. However, the surrounding white matter and grey matter can show a non-enhancing signal change; LGG present a similar challenge, as the exophytic mass can present undefined margins and a degree of signal change fading into regular white and grey matter [58]. This signal change has been a mix of vasogenic oedema due to altered micro-

environment, inflammation, and tumour cell infiltration [72]. The differential diagnosis between reactive peri-lesional oedema and tumour infiltration is a matter of open debate [72, 73].

The suspected cell invasion beyond the radiological margins has been supported by a few histological reports [74–76]. Sahm and colleagues applied immunohistochemical staining to demonstrate glioma diffusion on nine cadaveric specimens from deceased patients with a diagnosis of Glioblastoma (GB), applying Isocitrate DeHydrogenase (IDH)-specific immunohistochemical analysis [74]. Four cases showed evidence of glioma cells spreading through the contiguous white matter to the adjacent lobes and the contralateral hemisphere. Two surgical series of stereotactic biopsies provided similar results and have been used as a benchmark for studies regarding the EOR in brain gliomas [75, 76]. The authors performed a series of MRI-guided biopsies on patients with radiological diagnoses of LGG. The histological specimens were stained with immunostaining markers K-67 - MIB-1, Glial Fibrillary Acidic Protein (GFAP), and NeuroFilament Protein (NFP) [75] or IDH1 [76]. Tumour cells invasion of the surrounding brain parenchyma was confirmed to be up to 2 cm beyond the radiological margins. However, the density of glioma cells was found to decrease exponentially beyond these boundaries. Interestingly, the concentration of tumour cells beyond radiological margins was directly correlated to the tumour growth fraction, with a cut-off of 4% [75, 76].

From a radiological perspective, several MRI scan sequences can identify the degree of peripheral oedema, but differentiating vasogenic signal change from tumour infiltration remains challenging [58, 73]. The available sequences do not show high accuracy and are heavily operator-dependent [73]. For example, vasogenic oedema usually affects the white matter on T2 and Fluid-Attenuated Inversion Recovery (FLAIR) sequences, sparing the grey matter. At the same time, involvement of the cortical layer suggests tumour invasion, a feature that also helps the differential diagnosis between gliomas and metastases [77–80]. Extension of the oedema beyond obvious anatomical barriers is another hint suggesting actual tumour invasion [73]. The hyper-intensity seen on both T2 and FLAIR also seems more pronounced in vasogenic oedema, suggesting an inverse correlation with cell density [81]. Finally, the morphology of the actual non-contrast enhancing tumour more often resembles that of an exophytic mass, especially if multifocal [82, 83]. Diffusion Weighted Images (DWI) and the related Apparent Diffusion

Coefficient (ADC), based on the diffusion of water in tissues, have also been extensively investigated as a tool to define better the extent of tumour infiltration beyond the enhancing margins [84–87]. As a general rule, restricted diffusion is more pronounced in areas of high cellularity, whereas true vasogenic oedema tends to show less restriction [85]. Some authors reported that increased fractional anisotropy, more than diffusion, correlates with increased cellularity, therefore suggesting tumour invasion [86].

Additional MRI sequences have been recently explored to help the differential diagnosis. Elevated Cerebral Blood Volume (CBV) around the enhancing mass usually suggests increased vascular supply, related to the phenomenon of neo-angiogenesis [88]. While this finding does not directly correlate with increased cellularity and is not diagnostic of tumour invasion, it seems to have a predictive value in correctly identifying areas of future enhancement [89]. MRI spectroscopy of the non-enhancing area can highlight regions of elevated choline, although this elevation can also be present in true vasogenic oedema [86].

1.3.2 Meningiomas

Meningiomas are most commonly slow-growing lesions without brain invasion. The perilesional signal change seen in meningiomas is the effect of pure vasogenic oedema [90, 91]. Studies regarding the dura invasion on histological and molecular levels have shown that papillary or fibrillary invasion of the surrounding dura is among the most common mechanisms of spreading [59]. Brain invasion is an exceptionally uncommon finding, and its presence alone is now considered diagnostic of a higher-grade meningioma [92]. As highlighted by several authors and by an extensive literature review, brain invasion is poorly quantified or even identified on peri-operative MRI imaging, and the debate regarding the amount of brain resection to be performed in aggressive meningiomas is still open [93].

1.3.3 Metastases

Metastases are known to recur frequently, with an average of 50% of cases after treatment and a range estimated between 40 and 60%, depending on the histological sub-type and personal risk factors [94]. They are usually well-demarcated lesions and show discrete amounts of peri-lesional oedema [95]. However, on a histological level, their brain invasion patterns resemble that of brain gliomas, with single-cell infiltration being reported as the most common finding and more pronounced in specimens where multiple metastases were present [96]. Despite this point, several authors consider the peri-lesional signal change as a sign of true vasogenic oedema in most cases [72,97]. In this context, DWI sequences are helpful to differentiate metastatic lesions from HGG, as the latter show restricted diffusion possibly linked to increased cellularity [98,99].

1.4 Surgery, tumour infiltration and functional boundaries: challenges related to the extent of resection

All the mentioned histological sub-types of tumours often require surgery as the first step for treatment [2]. However, surgical treatment of brain tumours is technically challenging. The goal of neurosurgeons is to remove the most significant volume of tumour tissue while preserving the surrounding normal brain. This is not always possible. As mentioned, not only the tumour margins are not clearly defined but fade into normal tissue, but a generous resection is often hindered by the presence of the so-called “eloquent areas” of the brain - regions of the brain not amenable to be resected, otherwise the patient will unequivocally develop a permanent disability [8, 100–102]. Surgical removal of a brain tumour, therefore, consists of finding a balance between the extent of pathological tissue removed to prevent recurrence and the physiological boundaries related to the presence of a functionally active brain [2, 7, 8, 58]. Another challenge related to tumour resection is the absence of a visual correlation for the surgeon between the non-enhancing areas of signal change seen on the MRI sequences and the intra-operative finding. While the enhancing tissue appears pathological during surgery in most cases, the non-enhancing component shows the same features of the typical grey or white

matter, indistinguishable from the healthy brain parenchyma (see Figure 1.4).

The body of literature evidence shows a non-linear correlation between improved survival and EOR [25–29]. More recent series, including a “near-randomized” trial [29], highlighted the importance of absolute tumour volume reduction, significantly when both the enhancing and the non-enhancing components are removed [103–105]. However, this evidence is considered Level III or IV, according to most classifications [106]. A detailed analysis of resected tumours vs those that are only biopsied also revealed that a more favourable molecular profile is associated with lesions more amenable of surgical resection, thus suggesting that deep, midline seated gliomas are intrinsically more aggressive than accessible ones [104, 107]. While surgery is indisputably considered part of the treatment and recommended in highly specialized centres, the available consortium guidelines are slightly more cautious about data interpretation due to the lack of randomized controlled trials. However, the same guidelines conclude that “the extent of resection is a prognostic factor and thus, efforts at obtaining complete resections are justified across all glioma entities” [2].

To overcome the problem of brain invasion, a proposed solution is to extend the resection beyond the radiologically defined boundaries and attempt to remove the non-enhancing component of the tumour. This approach is defined as “supra-marginal/supra-total resection”, and has been popularized by some authors especially from the French group [7, 108, 109]. The same concept has been applied to the surgery of glioblastomas by different authors, resulting in a clear survival advantage [110, 111]. However, at the moment, it is still challenging to define precisely what constitutes a beneficial supra-marginal resection, with some surgeons limiting their resection within the area of signal change [108] and others extending it beyond that perimeter [111]. A recent review has also highlighted how the initial enthusiasm related to supra-marginal resection should be at least partially tempered due to the possible increased rate of complications [112].

To balance an aggressive resection with the functional boundaries posed by eloquent areas, the concept of “functional margins” of resection has been popularized by some authors: the tumour resection is extended up to the very boundaries of a functionally active area, and the resection is stopped at that point [7, 108]. The identification of the eloquent regions is performed using

neurophysiological monitoring (see below), with the patient awake or asleep, depending on the location of the specific tumour and its proximity to speech, motor, or sensory areas [7, 8, 102].

The concept of eloquent area in itself is controversial. Initially described by anatomists on a histological level [9], and subsequently by the first epilepsy surgeons [10], it has been re-defined over time [8, 11, 12]. From a more neuroscience-oriented perspective, the current consensus tends to define the brain as a series of "connectomes", meaning macro and micro neuronal networks grouped based on their similar physiological roles, anatomical segregation, and pharmacological profile [113]. While this framework holds a significant clinical advantage, helping the neuro-oncologist to predict the pattern of tumour diffusion more accurately [15], it is less practical from a surgical perspective, where the aim is to avoid damage to critical grey and white matter structures [8, 102]. As an example, an aggressive surgical resection of a glioma arising from the right frontal pole can be carried out without causing significant post-operative disability. This is due to the intrinsic networking properties of the affected brain area. Some areas of the brain, traditionally called "eloquent", serve as a "hub" for specific brain functions [8].

1.5 Neurosurgical set-up and current limitations

Nowadays, a neurosurgical operation to resect a brain tumour is standardized. Once the most superficial area of the tumour is identified through various instruments, a targeted skin incision and surgical approach through a craniotomy are performed. The tumour is typically removed under the microscope or endoscope magnification, using micro-surgical instruments for micro-dissection.

These passages require a heterogeneous toolkit, which includes the following instruments now commonly used:

- a standard cranial surgical set of instruments, including those adapted for microscope magnification, bipolar cautery, suction, drills, and different resection tools, such as Cavitron UltraSonic Aspirator (CUSA) [114]) or laser [115];

- a magnifying device, typically an intra-operative microscope or endoscope with various features (including accommodation, zooming in and out, blue light for fluorescence, and integrated software for video/picture acquisition) [116];

Over the years, some complementary techniques and tools have been developed to increase the EOR and minimize the possibility of post-surgical disability. The following subsections provide a brief description of those used by our group.

1.5.1 Neuro-navigation

Intra-operative neuro-navigation has been widely used in clinical practice since its introduction [117]. It is derived from stereotactic neurosurgery, where spatial coordinates in the three dimensions (x, y, and z) are used to localize a specific target. Commercial neuro-navigation devices typically work using a pre-operative MRI head uploaded into the system, an established number of reference points on the head of the patient, and a camera reference set at a certain distance. While operating, the surgeon can move a probe inside the surgical field. The reference system will detect the probe and match its position with the MRI scan, thus showing the precise position during surgery. The fundamental concept is similar for all the commercially available models [118–120].

Neuro-navigation helps localize the tumour and gives an estimation of the degree of resection [118, 120]. It is now recommended by the National Institute for Health and Care Excellence (NICE) guidelines to use neuro-navigation in any neuro-oncological procedure [121]. However, some structural limitations limit the overall accuracy. Neuro-navigation is not a live imaging technology. It does not account for changes in the surgical field during the resection, as it uses a pre-operative MRI head as a reference [122]. This is particularly problematic in the context of brain surgery, where both brain tissue dehydration and CSF release change the actual position of the tumour and the surrounding brain tissue, with increasing shift and tissue collapse and consequent inaccuracy [120]. This problem has been partially addressed by deploying different solutions, including additional rounds of intra-operative registrations [123], integrating the pre-

operative MRI scan with intra-operative images obtained either through software reconstruction linked with the microscope view [124], or re-imaging the surgical field altogether through intra-operative MRI scan [125]. All of these solutions present problems. The accuracy of repeated registration or additional software for re-calculating the shift is a matter of open debate, and the margin of error remains between 1.5 and 2.5 mm after more than twenty years of refinement [126, 127]. On the other hand, intra-operative MRI and magneto-encephalography are presumably more reliable but expensive and logistically demanding technological adjuncts [128].

1.5.2 Fluorescence

Fluorescence-guided surgery has become an increasingly valuable tool in neuro-oncology, particularly gliomas, to maximize the EOR. There are several compounds used in neurosurgery for different purposes, as the most commonly used agents differ on important properties as pharmacokinetics, fluorescent spectrum, and affinity with the desired target [129].

One of the most commonly used agents in this context is 5-aminolevulinic acid (5-ALA), a prodrug that, when metabolized in the body, leads to the accumulation of protoporphyrin IX. This compound emits light in the red spectral band (610-700 nm) when illuminated with blue light (375-440nm) [129]. Most surgical microscopes used in fluorescence-guided surgery are equipped with blue light filters that allow the PpIX fluorescence to be observed, emitting a pink or red light that the surgeon can see. This allows for the real-time identification of tumour tissue during resection.

The use of 5-ALA has been supported by clinical evidence, including a Phase III trial published in 2006, which reported an increased rate of complete resection of malignant gliomas from 36% to 65% compared to conventional white light surgery and an improved progression-free survival at six months [130]. Due to its high sensitivity and specificity for tumour tissue, 5-ALA has become the standard of care in many countries, including the UK, for surgical resectioning high-grade gliomas [131]. The initial reports on 5-ALA reported limited utility in LGG since it is most effective in tumours with a high metabolic activity, accumulating more protoporphyrin IX [132]. However, a recent review reported that fluorescence in LGG is still helpful in subsets of

tumours that are more metabolically active or show foci of potential malignant transformation, and the coupling with novel tools such as confocal laser endomicroscopy could also increase the accuracy in LGG [133].

While 5-ALA is the most established agent for fluorescence-guided brain tumour surgery, alternatives like IndoCyanine Green (ICG) have also been explored. ICG is a near-infrared fluorescent dye used in various surgical applications, including neurovascular procedures, due to its ability to visualize blood vessels. However, ICG's utility in brain tumour surgery is limited because it lacks the tumour specificity seen with 5-ALA [134].

1.5.3 Awake surgery

The awake surgery technique was introduced in the 1920s and has been used as an additional tool in tumour / epileptic foci resection since the 1950s [135]. The main indication concerns speech mapping, although any neurological function can be tested (and, in fact, often is) through this technique [136–139]. Speech mapping involves the patient being awake and responsive, enabling real-time interaction and feedback during the resection. A small electrical probe stimulates the brain's cortical or sub-cortical regions. At the same time, the patient executes language tasks, such as naming objects, reading, or conversing. Deviations or errors in the patient's speech during stimulation identify essential language areas that should be preserved. This interactive process helps to delineate functional language borders with high precision, minimizing the risk of postoperative language deficits [7, 136, 137].

This technique has limitations. The first is related to patient selection: advanced age and impaired cognitive status are considered contraindications, both in terms of tolerance and performance [140, 141]. The topic is debated, as some series reported acceptable complication rates in elderly patients, although with limited numbers and non-homogeneous settings and metrics [142, 143]. The second challenge concerns identifying non-eloquent areas related to more complex and hierarchically higher brain functions, such as memory, coordination, and meta-cognition. This problem has sparked attention in the neurosurgical and neuropsychological community recently [7, 138, 144]. Finally, the reported performance metrics are variable due to

the structural logistics of the surgical set-up. Arranging an awake surgery session is notoriously demanding and heavily dependent on the experience and the level of training of the surgical, anaesthetic, nursing, and language specialist team [137, 145, 146].

1.5.4 Neuro-monitoring

Intra-operative neuro-monitoring is a complementary tool used for awake or asleep procedures. The surgeon and the neurophysiologist can detect real-time motor and sensory activation changes by positioning electrodes on the patient's distal muscles, nerves, and skin. This is achieved either by active stimulation, like in the Motor Evoked Potentials (MEP), or passive detection, like in the Somato-Sensory Evoked Potentials (SSEP). A partial summary of neuro-monitoring techniques most commonly used in neuro-oncological surgery is provided in this section. The description is not exhaustive, and more detailed information can be found in dedicated references [147].

Motor Evoked Potentials are elicited by transcranial electrical stimulation of the motor cortex, leading to the depolarization of corticospinal motor neurons and subsequent muscle contraction. These evoked responses are recorded via electrodes placed over specific muscle groups. The MEPs indicate the functional status of the motor pathways, and by assessing the amplitude and latency of these responses, surgeons are alerted to potential motor tract injury at a stage when intervention may prevent permanent damage. MEPs are among the most reliable neuro-monitoring techniques when used in the context of spine surgery, with reported sensitivity variable between 70% and 90% [148–150]. However, when used during intracranial surgery, the results are not as reliable due to both deformations induced by the presence of the mass itself, the surgical manipulation of the scalp, and the chosen anaesthesia. Significant variability among different groups is reported [151–153]. This problem has been circumvented by using navigated scalp electrodes [154] or positioning cortical electrodes [155]. Still, it is unclear which solution could provide the optimal performance, and a randomized, double-blinded, multi-centric controlled trial comparing trans-cranial and trans-cortical MEP started in 2021 to investigate this point [156].

SSEPs are conducted by stimulating peripheral nerves and recording the propagated signal at various points along its path to the sensory cortex. Standard stimulation sites include the median nerve at the wrist for upper extremity SSEPs and the posterior tibial nerve at the ankle for lower extremity SSEPs. The primary measured parameters are the amplitude and latency of the cortical and sub-cortical waveforms. SSEPs are highly sensitive to changes in sensory pathway integrity, allowing for compromise detection during surgery [157]. However, the reported sensitivity and specificity during intracranial surgery range between 30% and 65% [158, 159] due to similar anatomical and pathological variations affecting the MEPs.

Visual Evoked Potentials (VEP) are recorded by stimulating the visual pathways, typically using flashing light or a pattern reversal technique [160]. While the patient is under anaesthesia, electrodes placed on the scalp over the occipital cortex measure the brain's electrical responses to these visual stimuli. The evaluation of VEPs during surgery has been advocated in procedures that may affect the visual pathways, such as those involving the pituitary gland, the optic nerve, the optic radiations, or the occipital cortex [161]. Transcortical VEPs seem to be more reliable than transcranial VEPs. However, they are susceptible to significant inter-individual and inter-pathological variations [162].

In conclusion, although feasible, safe, and used as a standard in most neurosurgical centres, this solution is logistically demanding, and its accuracy is debated [147]. Experienced and extensively trained anaesthetic, surgical, and neurophysiological teams are crucial to minimize these challenges. Even so, variability is highly influenced by surgical, anaesthetic, anatomical, and pathological considerations [163].

1.5.5 Visual identification of eloquent brain: state of the art

We lack visual tools to identify the eloquent areas during a neurosurgical procedure. Aside from neuromonitoring, the precise location of an eloquent region can be inferred by integrating some radiological functional imaging technologies into the neurosurgical plan, either manually or through neuronavigation [164, 165].

Functional Magnetic Resonance Imaging (fMRI) is a non-invasive neuroimaging technique that measures brain activity by detecting changes in blood flow, explicitly relying on the Blood Oxygenation Level Dependent (BOLD) signal [166–168]. The physical principle behind fMRI is based on the fact that neuronal activation leads to an increase in local blood flow to the active brain regions, which alters the ratio of oxygenated to deoxygenated haemoglobin. Since deoxygenated haemoglobin is paramagnetic and oxygenated haemoglobin is not, the local magnetic field inhomogeneity changes in active regions, which can be detected using MRI [169]. This method provides a high spatial resolution (1–3 mm), making it sensitive to brain regions activated by cognitive tasks [169]. However, its temporal resolution (1–2 seconds) is relatively low compared to electrical activity measures like ElectroEncephaloGram (EEG). Despite its widespread use, fMRI does not measure neuronal activity directly but infers it from blood flow, which can introduce variability due to the neurovascular coupling process [170]. Studies report that sensitivity can vary between 70% and 90%, while specificity is generally lower due to physiological noise and the indirect nature of the BOLD signal [171]. Accuracy in detecting brain functional regions, like motor or sensory cortices, is typically substantial, but identifying more complex cognitive processes can be challenging. The integration between higher-resolution MRI technologies and Artificial Intelligence (AI) algorithm might improve the diagnostic capability of fMRI in the future [172].

Alternatives to fMRI for mapping brain functions include MagnetoEncephaloGraphy (MEG) [167, 173] and navigated TransMagnetic Stimulation (nTMS) [167].

MEG measures the magnetic fields generated by neural activity in the brain. When neurons fire, electrical currents flow within and between neurons, producing weak magnetic fields on the order of femtoteslas [167]. MEG detects these magnetic fields using susceptible Superconducting Quantum Interference Devices (SQUID), extremely sensitive magnetometers capable of detecting weak magnetic fields [167]. Unlike EEG, which measures the electrical potentials on the scalp, MEG is less affected by the skull and scalp, providing a more direct measurement of brain activity with high temporal resolution [174]. MEG is particularly effective for localizing functional areas related to sensory processing, motor control, and language functions, making it useful in clinical applications such as preoperative brain mapping. While MEG offers superior

temporal resolution (in milliseconds), its spatial resolution is lower [174,175].

On the other hand, nTMS is a non-invasive technique used to map brain functions by delivering brief magnetic pulses to the scalp, which induce electric currents in targeted brain areas [167]. This method temporarily disrupts regular brain activity in specific regions, allowing clinicians to assess their role in motor control or language functions. The "navigated" aspect involves using MRI data to precisely guide the positioning of the magnetic coil over the patient's head, ensuring accurate stimulation of desired brain regions. nTMS is employed in preoperative brain mapping, providing critical information for neurosurgeons to avoid damaging functional areas during tumour resections [176,177].

All these technologies have limitations. Aside from not showing ideal accuracy and sensitivity data, they pose a problem of costs and integration with the neurosurgical equipment [167].

1.6 Experience from our surgical series

Imperial College Healthcare National Health Service (NHS) Trust is a tertiary referring centre specialising in treating complex intracranial tumours.

During this PhD project, I have collected extensive and comprehensive clinical data from the neuro-oncological cases operated at Imperial College Healthcare NHS Trust. The analysis focused on factors that could affect EOR, OS, PFS, and PS, addressing the potential areas of improvement. The aim of this analysis was to:

1. compare the standards of our centre to the results of the relevant literature;
2. identify areas of improvement, specifically in tumour visualization and preservation of neurological function.

1.6.1 Materials and methods

The data collection was entirely anonymized and received approval from the Health and Research Authority—Bloomsbury Research Committee, London. REC reference: 19/LO/1763, IRAS project ID 265404. Five and a half years (01/01/2018 to 01/08/2023) were considered. Only patients operated by a specific firm (Kevin O'Neill, the clinical supervisor of the present project, and the candidate) were included in the analysis.

Data was retrieved using the NHS healthcare search tools and platforms, specifically Cerner© and CareStream PACS©.

Several clinical data were collected, including demographic data (age and gender), tumour features - depth, extension, proximity to eloquent regions [8]), recurrence (Y / N), details of the operation (use of neuro-monitoring, use of IOUS, awake surgery, use of fluorescence -, EOR (expressed in % of tumour resected, calculated based on volumetric measurements on pre- and post-op scans), histology according to the reported WHO classification [2], molecular profile (specifically for gliomas), co-morbidities expressed with the Charlston Comorbidity Index (CCI) [178]; performance status (PS), post-op complications, if present, expressed with the Clavien-Dindo Score (CDS) [179]), postoperative adjuvant treatment, survival metrics - OS and PFS expressed in months.

The statistical analysis of the clinical data was using RStudio© (Version 2023.06.2+561).

Examples of the scripts used for the Cox Proportional analysis are as follows:

```
>library(survival)
>cox_os <- coxph(Surv(time_to_event_OS , status_OS) ~ EOR + factor(
  AdvT) + factor(CDS) + PrePS + PostPS + ATRX + TERT + P53, data =
  HGG, na.action = na.exclude)> summary(cox_os)

> cox_pfs <- coxph(Surv(time_to_event_PFS , status_PFS) ~ EOR +
  factor(AdvT) + factor(CDS) + PrePS + PostPS + ATRX + TERT + P53,
  data = HGG, na.action = na.exclude)
```

```
>summary( cox_pfs )
```

In the cases of some variables, I had to convert the categorical variables into accountable factors - for example, metastases and their organs of origin:

```
>Metastases$Recurrence <- as.factor( Metastases$Recurrence )
>Metastases$Histology <- as.factor( Metastases$Histology )
>Metastases$AdjC <- as.factor( Metastases$AdjC )
```

The analysis focused on five groups of patients with specific histological features: HGG grade IV (HGG-IV), anaplastic gliomas grade III, LGG grade II (LGG), meningiomas, and metastases.

I tested the correlation between surgical and tumour-related factors and EOR and the relevance of more aggressive resection in terms of the complication rate.

A Cox proportional hazard model was used to weigh the role of different patient-related and treatment-related factors on OS and PFS of the HGG-IV subgroup: age, gender, peri-operative performance status, CCI, EOR, histology, O6-methylguanine-DNA methyltransferase (MGMT) and IDH status, post-operative Chemo-RadioTherapy (CRT) - graded as no CRT, palliative CRT, or radical CRT -, and the presence of peri-operative complications. The same model was run for the metastases group but did not include the molecular markers.

Chapter 2 reports a more detailed analysis of the use of IOUS, so this point is not discussed here.

Regarding the deployment of neurophysiological monitoring, I have analyzed the performance metrics as reported in the international literature through Bayesian analysis [180,181], looking at the capability of neuro-monitoring to predict a post-operative deficit.

Specifically:

- True Positive (TP): potential deficit detected during surgery and confirmed to be present post-op;

- True Negative (TN): potential deficit not detected during surgery and confirmed not to be present post-op;
- False Positive (FP): potential deficit detected during surgery but not present post-op;
- False Negative (FN): potential deficit not detected during surgery, but present post-op.

The calculation regarding Sensitivity (SEN), Specificity (SPE), Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Accuracy (ACC) was performed as per standard international literature. Specifically:

$$SEN = \frac{TP}{TP + FN} \quad (1.1)$$

$$SPE = \frac{TN}{TN + FP} \quad (1.2)$$

$$PPV = \frac{TP}{TP + FP} \quad (1.3)$$

$$NPV = \frac{TN}{TN + FN} \quad (1.4)$$

$$ACC = \frac{TP + TN}{TP + FP + TN + FN} \quad (1.5)$$

A Chi-Square test was used to verify whether any specific anatomical location was associated with a higher or lower number of false positives and negatives. The same test was repeated using particular combinations of neurophysiological techniques instead of locations to cross-check whether any methods used (MEPs, SSEPs, VEPs, speech mapping) were associated with a higher or lower number of false findings.

1.6.2 Results

Our firm operated 232 cases in the period considered, averaging 45 cases/year; 163 cases were included for this analysis after different histological subtypes other than gliomas, meningiomas, and metastases were removed.

High-Grade Gliomas - HGG-IV

The linear regression models regarding the correlation between the factors considered and the EOR did not show any statistically significant result. Specifically, an improved EOR was not related to extension, depth, proximity with eloquent areas, use of neuro-monitoring, or IOUS.

The log-rank test and the associated Kaplan-Meier curves directly impact OS, but less on PFS.

The multi-linear regression analysis found no correlation between the possibility of post-op complication, expressed as CDS, and the factors hypothesized to be responsible, including EOR ($p = 0.95$), pre-op PS ($p = 0.14$), age ($p = 0.17$), and CCI ($p = 0.61$). These findings are further discussed in the relevant section.

The best survival was noticed in patients with complete adjuvant treatment, while the worst was in patients without post-op treatment. Patients who had palliative treatment post-op or partial therapy at a total dose did not show a particular difference in survival advantage. Still, their outcomes were worse than those of patients receiving complete treatment. These findings were confirmed in the Cox proportional hazard survival analysis.

The EOR was found to impact OS. For each unit of improved EOR, the hazard decreased by a factor of 0.65 ($p = 0.00832$). Other relevant factors were IDH status, mildly positively correlated with improved OS, hazard ratio 0.79 ($p = 0.045$) and the administration of radical CRT post-op, positively impacting OS, hazard ratio 0.51 ($p = 2.63e-08$).

The factors associated with changes in PFS were the IDH status (weakly correlated with improved PFS, hazard ratio 0.8, $p = 0.05608$) and the administration of radical CRT post-op, positively correlated with improved PFS, hazard ratio 0.35 ($p = 0.00965$).

Anaplastic Gliomas Grade III

In the period considered, 23 patients diagnosed with anaplastic glioma grade III were treated in our department. As expected from the epidemiological data, the size of this sample was also limited, so the statistical analysis did not show any significant correlation between the considered factors and the survival measures.

Low-Grade Gliomas - LGG

In the period considered, 14 patients diagnosed with low-grade glioma were treated in our department. Given this group's small cohort of patients, which reflects the known epidemiological data on these histological types, the statistical analysis did not show any significant correlation between the considered factors and the outcome measures.

Meningiomas

Forty-two patients with a diagnosis of meningiomas were operated on during the period analysed.

In most cases, the EOR was near total or total. The survival data were also compatible with the less aggressive biological behaviour of the disease. Four patients were lost at follow-up. One of the two patients with a diagnosis of Grade III meningioma died due to disease progression despite maximal surgical and radiotherapeutic treatment.

As expected, considering the EOR average, the multi-linear regression analysis did not show any statistically significant factor impacting the EOR.

The Cox survival model was not run for OS in this specific cohort of patients as, except for one outlier perfectly explained by the different histological sub-type (Anaplastic Meningioma Grade III), none of the patients died after surgery or at follow-up. The same model for PFS showed a possible positive impact of the pre-operative PS ($p = 0.00547$) and of the CDS ($p = 0.01951$) on an improved PFS, a result that is challenging to interpret.

Metastases

Thirty-three patients with a diagnosis of intracranial metastasis were operated on during the analyzed period.

In terms of EOR, in this series, all patients had a resection above 80% of the tumour mass, and the vast majority of patients (27) had a near total resection ($> 90\%$). These findings might be due to the intrinsically different nature of the disease (intra-axial but commonly well-defined and with an apparent separation plane) compared to gliomas. Therefore, and partially as expected, the analysis of the factors affecting EOR did not show any significant impact of surgical factors (IOUS, neuro-monitoring, proximity with eloquent areas, histology), except a mild correlation with extension ($p = 0.0315$) and an even weaker correlation with recurrence ($p = 0.08$) and depth ($p = 0.05$).

The survival curves and the relative log-rank tests highlighted an OS improvement in patients undergoing a more generous resection, but the difference was not marked.

The Cox proportional hazards regression analysis highlighted that lung histology (hazard reduction of 90.94%, $p = 0.031$), post-op PS (one unit increase associated with hazard ratio increase of 137.6, $p < 0.001$), and full adjuvant treatment post-op (hazard decrease of 84.81%, $p = 0.010$) were highly significant factors impacting the prognosis.

The only factor mildly impacting PFS was the administration of radical adjuvant treatment post-op, with a hazard ratio of 0.41 ($p = 0.0881$).

Neuro-monitoring

From 01/01/2018 to 01/08/2023, we used intra-operative neuro-monitoring in 124 cases. The impact of neuro-monitoring on the PS and on the EOR was not found to be statistically significant in any of the patient subgroups reported.

The performance metrics are reported in Table 1.1.

TP	TN	FP	FN
26	160	11	22
Sensitivity		0.54	
Specificity		0.93	
PPV		0.7	
NPV		0.87	
Accuracy		0.84	

Table 1.1: Performance of the neuro-monitoring application in our cohort of patients - TP = True Positive; TN = True Negative; FP = False Positive; FN = False Negative; PPV = Positive Predictive Value; NPV = Negative Predictive Value.

The locations giving the highest number of false positives were the superior frontal gyrus (1 FP), the pre-central gyrus (2 FP), the superior parietal lobule (2 FP), and the trigone / temporal stem area (2 FP). The locations showing the highest number of false negatives were the superior frontal gyrus / Supplementary Motor Area (SMA) territory (6 FN), the middle frontal gyrus (4 FN), the insula (2 FN), and the cingulate gyrus (2 FN).

However, the Chi-Square test failed to show any statistically significant correlation between specific locations and the number of false positives ($p = 0.29$) or false negatives ($p = 0.38$), and particular combinations of neuromonitoring and the number of false positives ($p = 0.12$) or false negatives ($p = 0.53$).

1.6.3 Discussion

An overview of the results section and the relative numbers show that, in the period considered, the volumes of brain tumours operated are compatible with national and international standards [2, 182, 183].

A significant majority of patients with a diagnosis of glioma underwent an aggressive surgical resection and received standard post-operative adjuvant treatment when appropriate. The overwhelming majority of the patients with a diagnosis of meningioma received a near-total or gross total resection. A significant majority of patients with a diagnosis of intracranial metastasis received aggressive surgical treatment and post-surgical adjuvant treatment. The overall complication rate varied from 18 to 37%, but complications requiring further surgery

(CDS III) were only observed in 13% of patients ($\pm 2\%$), and 30-day post-surgical mortality was as low as 1%.

In our series, EOR aligns with the prevailing standard average, yet there remains room for improvement in specific histological subtypes. Notably, in the case of HGG-IV, the average EOR is 80%. Therefore, for future research, we speculate that there is a significant opportunity to refine EOR, particularly given the correlation between more comprehensive resections and enhanced survival rates.

In many cases, this lack of complete resection is arguably linked to the intrinsic nature of the pathology, as discussed in the previous sections. Gliomas are infiltrative, and their margins often fade into the normal brain tissue, thus making it challenging to recognize the true extent of the tumour visually - see Figure 4.10.

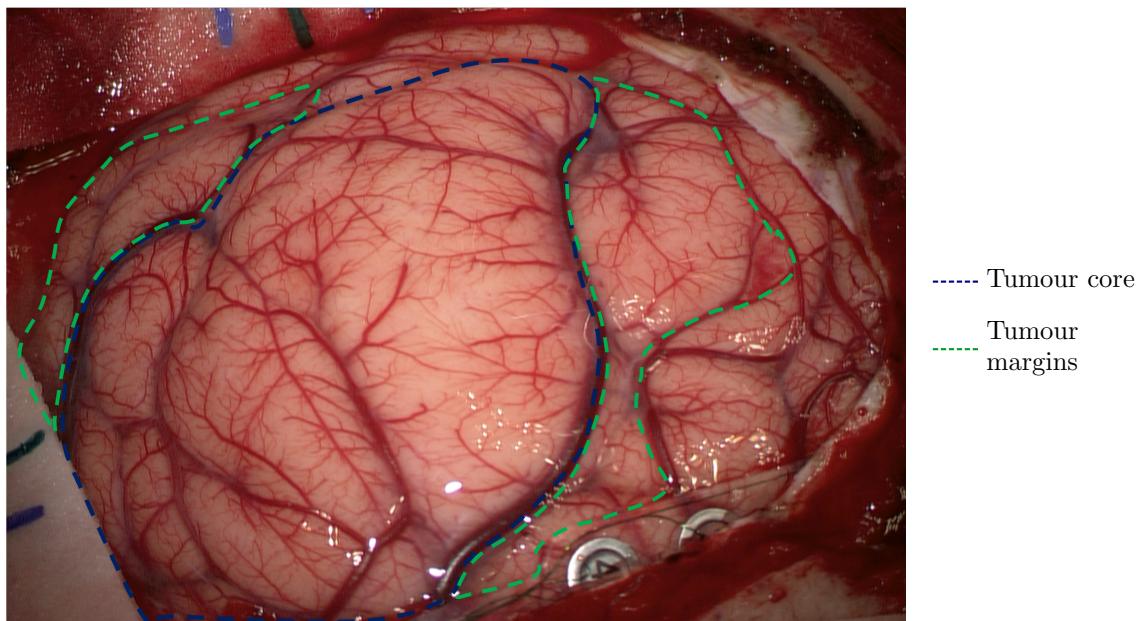


Figure 1.4: An intra-operative picture from one of the cases reported in Chapter 4. The histology revealed an Oligodendroglioma Grade II - a type of LGG. The dotted blue line highlights the tumour core, and the green line the areas of infiltration. The tumour margins are not visible to the naked eye, and their identification is only possible through the combined use of neuronavigation and, in some cases, intra-operative neuromonitoring. Wernicke's speech area was mapped at the caudal end of the tumour, the top centre of the surgical field in this picture.

The data regarding EOR are more encouraging regarding meningiomas and metastases, again most likely due to the more defined dissection plane these tumours tend to present. Yet,

examining the cohort and epidemiological data of intracranial meningiomas, the case for a complete EOR is even stronger: the patients are normally well functioning, and this is a benign disease, amenable to cure with surgical resection alone [92].

Another interesting finding from our series is that a precise subset of patients suffered from neurological complications related to surgical resection. Specifically, there were 6 cases of temporary neurological deterioration in the HGG-IV group, 8 in the anaplastic gliomas cohort, 4 in the LGG group, and 8 in the meningioma cohort. No cases of post-op neurological deterioration were observed among metastases. A more detailed analysis of these cases, crossed with the data taken from neuro-monitoring, revealed that the majority of patients experienced either a post-op SMA syndrome, a variable degree of dysphasia, or seizures. In four cases, such deterioration was directly caused by an ischemic event. In contrast, in the remaining cases, it was thought to be related to postoperative brain oedema and surgical manipulation. Even more interestingly, the data associated with the neuro-monitoring metrics showed that the finding was unexpected in at least 22 data points.

1.7 Addressing limitations: current research, screening process, and aims of this project

The previous sections identified two macro-areas that would benefit further research and development: the extent of tumour resection and the occurrence of postoperative complications, specifically neurological impairment.

The research conducted in this project focuses on those two areas, pursuing both the implementation of known technologies and the testing of new ones with the potential to be introduced in standard clinical practice.

Surgery requires several visual cues, which are instrumental when defining the margins of a glial tumour and its relationship with nearby structures. From a neurosurgical perspective, a visual innovation to push the boundaries of resection by identification of the non-enhancing

tumour area would be preferable to any alternative.

At the beginning of this project, several solutions were considered. The screening process included practical considerations, effectiveness, and novelty of the technology to investigate.

Most regulatory agencies have already approved some of the innovations listed here for clinical use, although the evidence regarding their efficacy is debated.

One example is the use of intraoperative Magnetic Resonance Imaging (iMRI) scan, advocated by several authors [136,184]. As previously mentioned, MRI scans can reliably identify the signal change around the tumour, and the accuracy in distinguishing true vasogenic oedema from tumour infiltration is growing [72,73]. Current literature evidence shows that iMRI might positively impact EOR and complication rates, with clear outcome benefits [185,186]. However, a systematic literature review showed that iMRI is not adopted in most centres due to several limitations, the main ones being that 1) iMRI is not a real-time modality, as surgery must be interrupted to perform the scan; 2) the execution itself is time-consuming and prolongs anaesthetic time; 3) the cost of the machine is linked to the costs of rearranging theatre instruments, building compatible infrastructures, and adequately training the staff to a new setting [136,187]. Moreover, some authors reported equipoise with standard imaging protocols in OS when iMRI is deployed [188]. Other groups have investigated the practicality of ultra-low field MRI, a promising, cheaper, but still not widely spread solution [189]. This option was not considered for this project due to high costs and an inadequate time frame.

A cost-contained and practical alternative to iMRI is IOUS, which has been extensively utilized in the past 20 years [190] and has been adopted by our department since 2010. IOUS was investigated in the context of this project, and the details are expanded upon in Chapter 2.

Other experimental technologies have been considered. These include Rapid Evaporative Mass Spectrometry (REIMS) [191], Raman Spectroscopy [192,193], confocal endomicroscopy [194], and optical imaging, including hyperspectral/multispectral imaging [195] and optical coherence tomography [196]. Most of these technologies are in their infancy and are investigated only in pre-clinical or pilot studies, making them ideal for this project.

An extended discussion concerning all these technologies goes beyond the scope of this introduction. Still, most of these presented issues related to costs, logistics, the project's aims, and appropriate time frames for deployment in the theatre.

For example, our group has investigated REIMS for brain tumour removal [197]. Unfortunately, the lack of funding and a shift in interest towards more commercially profitable medical applications has halted that project in its relatively early stages.

Raman spectroscopy has also shown promising preliminary results and has been investigated by our team in the past [198]. However, its deployment during a craniotomy is logistically demanding and tends to prolong surgery.

Moreover, REIMS, Raman, and confocal endomicroscopy currently all share the common feature of being technologies that work through point analysis, not field analysis. Conversely, this project aimed to investigate a tool that could provide full integration within the neurosurgical toolkit and provide a visual aid to the surgeon covering most, if not all, of the surgical field.

Based on previous work by the same research group, this thesis has pinpointed multispectral/hyperspectral imaging as a valuable technology that merits further exploration. It was chosen due to its excellent cost/benefit profile and the potential to be fully integrated into the surgical microscope or even be used as an adjunct to open surgeries. A more detailed technical analysis is provided in the dedicated chapter.

The multidisciplinary project involved a team of clinicians at Imperial College Healthcare NHS Trust and physicists and engineers at the Hamlyn Centre of Robotics, Imperial College of London.

While the current research addresses a relatively specific problem, its findings are also potentially useful for several other research fields, including neuroscience, hardware developments, deep learning, machine learning, and coding.

1.8 Conclusions

The presented literature analysis and clinical data review highlight that gliomas and meningiomas are the two histological subtypes that would most benefit from further technological advancement.

This project aims to explore novel technological innovations that can enhance tumour resection and reduce the incidence of postoperative neurological decline.

Two technologies, intraoperative ultrasound and multispectral/hyperspectral imaging, have been chosen to be investigated for the mentioned aims.

Chapter 2

The role of ultrasound in neurosurgery

2.1 Introduction

I joined the group responsible for supervising the current project in 2017. Since then, we have been carrying out research regarding the 3D Intra-Operative UltraSound (3D-IOUS) coupled with neuro-navigation since the year 2010, first presenting the results at international meetings [199] and subsequently providing an account of the department's prospective series [200].

The 3D-iOUS has been initially proposed as a field of investigation for this thesis as it is a reliable, real-time tool for visualizing brain tumour tissue, margins, and normal brain.

The results of the clinical and experimental work on iOUS will be presented and critically discussed in this chapter.

2.2 Ultrasound: physical principles

Sound transmits mechanical energy by causing vibrations in adjacent particles, propagating the energy through the medium in a wave-like motion [201]. From a physical perspective, it can be modelled as a wave, taking into account frequency (f) and wavelength (λ), and expressing it as the equation:

$$\lambda = \frac{1}{f}$$

UltraSound (US) technology is based on propagating sounds above audible human frequencies (range 20Hz to 20kHz). The typical US ranges from 2 to 18 MHz.

The US waves interact with tissues similarly to many other waves. Therefore, they are subject to reflection, refraction, transmission, and absorption [201].

The image produced by the interaction between the US waves and the tissues is directly dependent on the velocity of the wave and the density of the tissues themselves. More specifically, this property is expressed by the equation:

$$Z = \rho * c$$

where Z is the acoustic impedance, ρ is the tissue's density, and c is the propagation velocity of the sound wave [202]. Acoustic impedance mismatch is the factor causing the reflection of the ultrasound waves back to the probe acting as a detector.

The US waves are produced by a specific transducer of crystals disposed in a line or a semi-circle. The transducer mechanism is based on the "piezo-electric effect", a property of several materials (including quartz and ceramic) to vibrate when an electrical current is applied and to maintain their original crystallographic structure when the impulse ceases [201]. Conversely, the returning ultrasound echo from the tissues generates an electric impulse proportional to the echo's intensity and can create an image [201,203].

In clinical ultrasound, depth is estimated by measuring the time between pulse emission and echo return, assuming a fixed sound propagation speed of 1540 m/s. Early ultrasound imaging began with A-mode (amplitude mode), where echoes appeared as peaks on a one-dimensional line, showing amplitude but not creating images. B-mode (brightness mode) followed, representing echoes as bright spots with intensity proportional to echo amplitude, providing a two-dimensional image and forming the basis for modern ultrasound imaging [201,204].

Ultrasound image classification relies on echo intensity: hyperechogenic/hyperechoic for higher-intensity echoes, hypoechogenic/hypoechoic for lower-intensity ones, and anechoic for structures like a fluid cyst, which produces typically no echoes [202]. Spatial resolution is critical, comprising axial resolution (along the ultrasound propagation axis) and lateral resolution (perpendicular to it). Axial resolution improves with higher frequencies, but its penetration is lower. Lateral resolution depends on crystal size, with smaller crystals enhancing resolution but compromising penetration depth. Moreover, modern probes can adjust to different frequencies, thus allowing the operator to increase or decrease resolution and depth [201].

In the case of US in neurosurgery, a tissue with high acoustic impedance mismatch (ex., the glioblastoma capsule) is hyperechogenic. In contrast, a tissue with low acoustic impedance (typically the CSF or the white matter) is defined as hypoechogenic.

2.3 IOUS in neurosurgery: the available evidence and areas of research

IOUS is often deployed in neurosurgery for the intraoperative detection and definition of brain tumours [190]. Compared to a standard neuronavigation device, the main advantage is the possibility of real-time imaging through a device that is not as bulky or expensive as the alternatives discussed in Chapter 1.

The first ever reported application of the US in neurosurgery was on a post-mortem case of a 54-year-old woman [205]. Since then, several groups investigated IOUS as a tool to improve visualization during neurosurgical procedures, including neuro-oncological ones [206–210]. Most of these reports were qualitative, describing the deployment of IOUS in small cohorts of cases. In an era without sophisticated neuro-radiological imaging techniques, IOUS represented an innovative and intuitive tool for an intra-operative assessment of the mass, the margins, and the brain shift [208, 210]. Computer Tomography (CT) and MRI scans, introduced in clinical practice during the ‘70s and in the ‘80s, were two significant technological advancements that

allowed neurosurgeons to localize the target lesion more accurately inside the brain [69,211]. Neuro-navigation frameless devices were introduced during the '90s, and they were the first tools allowing intra-operative localisation of the tumour [117]. However, they did rely on pre-operative images rather than real-time acquisition [212]. IOUS appeared to solve the problem of real-time imaging technology and was repeatedly reported to show successful intra-operative tumour identification [213]. Interestingly, in several cases, the signal obtained from IOUS was different when compared to that obtained using traditional imaging technologies (CT or MRI scan), thus some authors speculated that the IOUS could be used not only as an intra-operative aid but also as a complementary tool for those lesions of unclear nature or margins [213]. Moreover, coupling the IOUS with neuro-navigation has dramatically improved the possibility of midline shift adjustment during surgery [214–217] and was proven helpful in enhancing tumour demarcation when close to eloquent areas [164]. In recent years, further technological advancement has led to the development of 3D IOUS [218,219]. The labelling "3D" is slightly misleading: the neuro-navigation software does not provide a 3D reconstruction of the tumour. Instead, it automatically generates the scan projection in different dimensional planes starting from a single IOUS acquisition. When required, the images can be integrated with Doppler angiography so vessel encasement by an intracranial mass or aneurysm can also be detected [219]. More importantly, some systems allow the planning of the surgical resection based on IOUS and recalculating the tumour position after acquiring images, a potentially excellent solution to correct for the brain shift [215]. Recent research has also focused on integrating IOUS with contrast in small cohorts of patients [220–222]. One group used sulfur hexafluoride-filled lipidic microbubbles injected intravenously [221], while another used sodium fluorescein [222]. Despite the exiguous number of patients reported, the reported EOR was improved due to both better visualization and detection of the true residual (as opposite to haemostatic material) inside the surgical cavity [222], a finding that is promising for future developments.

An extensive systematic review and meta-analysis focused on IOUS has shown an average Gross Total Resection (GTR) of 77%, an 82% concordance rate between IOUS and post-op MRI scan, and high sensitivity/specificity at the beginning of surgical resection (more than 90%)

[190]. The analysis included 15 studies, most of which did not report individual patient data. Moreover, while the study's heterogeneity was low, a significant known limitation is the lack of international training standards concerning the IOUS in neurosurgery. The same study failed to find a significant correlation between the use of IOUS and a substantial impact on survival, so we have attempted a quantitative analysis of a homogeneous subgroup of patients. The reported metrics for residual tumour identification (sensitivity, specificity, positive predictive value and negative predictive value) were significantly impaired at the end of the surgery. The authors reported values of 26%, 88%, 62%, and 62%, respectively [190].

Factors influencing the quality of IOUS and the standardization of training have affected its widespread adoption [202]. There remains a steep learning curve due to its limited field of view, unfamiliar topographical representation, artefacts, and the unique visuo-tactile tasks it requires [223]. Additionally, the difficulty in gaining adequate experience outside of the intraoperative environment further complicates the learning process. Concerns have also been raised regarding the imaging resolution and accuracy. These challenges can hinder effectively identifying tumours and their margins, increasing the risk of residual tumour tissue or accidental damage to surrounding healthy brain structures. The advancement resolution and the combined use of contrast agents and Doppler have been proposed as solutions within a larger framework of multi-modality IOUS. Still, the issue of dedicated training to minimize the operator-dependent variability remains [224].

A more recent meta-analysis of 409 diffuse glioma cases compared the accuracy of IOUS with postoperative MRI as the reference standard. The findings indicated that IOUS effectively assesses diffuse glioma resection, showing a pooled sensitivity of 72.2% and a pooled specificity of 93.5% [225]. While these results are promising, the evidence suggests a need to improve IOUS accuracy to be integrated as a standard practice in brain tumour surgery. Trials such as the Functional and Ultrasound-Guided Resection of Glioblastoma (FUTURE-GB) randomized controlled trial, alongside efforts to refine IOUS techniques, are necessary to validate its role in neurosurgical oncology further [226].

The main limitation of IOUS concerns the lack of visualization of fine haemodynamic activity to

identify eloquent brain areas, which is the other goal of the present project. Recent technological developments showed promising results, introducing high-resolution, ultrasensitive ultrasound devices that could potentially detect haemodynamic changes [227, 228]. However, functional UltraSound (fUS) relies on a Doppler signal without decoupling between HbO and Hb. Despite being based on similar physical phenomena, a direct equivalence between the two techniques is not apparent and warrants further studies and data analysis [228]. Moreover, detecting haemodynamic changes remains an indirect measurement of neuronal activity, with limitations linked to timing and physiological constraints [167].

The main focus of the research outlined in the present chapter is to analyze the metrics derived from our clinical series and explore possible solutions to improve diagnostic performance.

The research focuses on a retrospective review and critical analysis of the clinical series of cases operated at Imperial College Healthcare NHS Trust using IOUS. The study focuses on the impact of IOUS on EOR and OS. While Doppler technology was available to detect haemodynamic changes in our department potentially, its use has been inconsistent, and the resolution was too low to attempt any data extrapolation. Hence, it was not considered.

2.4 Materials and methods

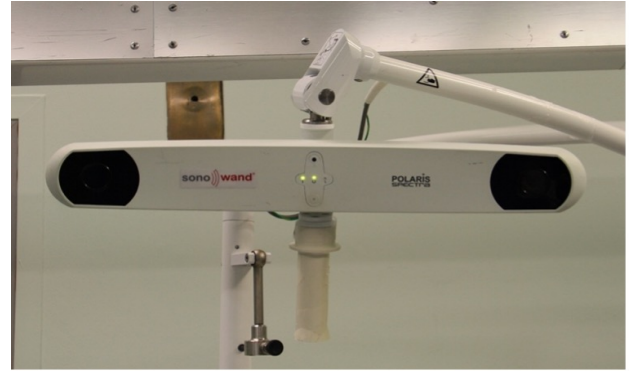
2.4.1 IOUS devices and neuronavigation devices

Our clinical experience was based mainly on two neuronavigation devices. The first one is in an integrated, 3D-IOUS neuro-navigation device – Sonowand©. It consists of an infrared-based neuro-navigation system integrated with two ultrasound probes of different sizes. It can be used stand-alone or combined with the 3D ultrasound, linked to the optical neuro-navigation tracking system through a dedicated tracking device (see Figure 2.1).

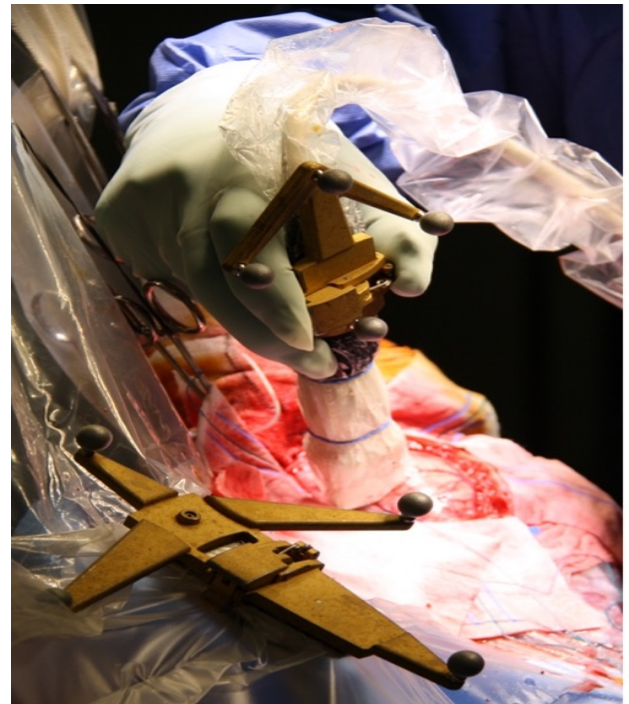
After standard preparation and Mayfield clamp application, the patient’s pre-positioned fiducial landmarks are registered and matched with the pre-operative volumetric MRI scan. The software automatically calculates the registration accuracy and can be amended for specific



(a) Main device: screen, main bulk with probes (bottom centre), pedals (bottom left), infrared camera (top centre)



(b) Detail: infrared tracking camera



(c) Intra-operative use: probe and tracking device linking the movement of the probe with the MRI registration

Figure 2.1: 3D-IOUS system used in the present series

landmarks before finalizing the results. Intraoperative neuro-navigation is then used throughout the procedure. Following the craniotomy flap removal, the ultrasound probe is prepared using a dedicated sterile probe cover and connected to the tracking system. The 3D-IOUS acquisition is therefore performed, linking the IOUS images with the pre-operative volumetric MRI scan. The acquisition is performed through one spatial plane only, with the machine automatically reconstructing the pictures for the remaining two spatial planes. Multiple acquisitions can be performed during the tumour resection so that the operator can check the

extent of the resection in real-time. Sonowand© has been developed by The Sintef Unimed and the Norwegian University of Science and Technology (rights owned by MISON AS), a single-rack CT or MRI-based neuronavigation system with rapid access to high-resolution 3-D IOUS data [229] [230]. As interpreted from the IOUS, glioma margins are histologically accurate [218] [219].

The second device we have used and reviewed for comparison is the Medtronic© Neuro-navigation system. We have selected this device as it shows excellent accuracy and reliability, has been established as a standard intra-operative tool worldwide, and was not integrated with IOUS in our centre, making it ideal for our comparison. This device is also based on an infrared tracking system and can be registered equally using fiducials or anatomical landmarks. Its software is also equipped with a surface tracer option. All cases coming from the present series were operated using either of these two neuro-navigation devices.

The clinical data relevant to the use of 3D-IOUS at Imperial College Healthcare NHS Trust were reviewed. The imaging data were retrieved from the neuro-navigation devices available at the Trust. The following clinical data were also collected: pre-operative images, intra-operative findings, and the post-operative clinical and radiological course to identify whether adding IOUS could achieve a better resection than the standard neuro-navigation device. This retrospective data collection has been entirely anonymized, and it has received approval from the Health and Research Authority – Bloomsbury Research Committee, London. REC reference: 19/LO/1763, IRAS project ID 265404.

2.4.2 Retrospective data collection

All cases retrieved from the two Sonowand machines were collected and reviewed. However, due to the challenges of comparing different histological tumour subtypes, locations, and imaging acquisitions, we limited our review to a descriptive review of most cases (see below). At the same time, a quantitative analysis based on the EOR was performed on a subgroup of cases with a diagnosis of GB. Population samples from the pool of GB patients have been selected from the data stored on three neuro-navigation devices. Collected cases were divided into two

groups: patients operated using the 3D-IOUS-Neuronavigation integrated system have been assigned to one group (exposure group). In contrast, patients operated using the stand-alone neuro-navigation system (Medtronic© Neuronavigation) without IOUS have been assigned to a second group (control group). Two identical and interchangeable IOUS-stealth coupled devices (marked 51 and 55) were used to extract data for the exposure group. Regarding the inclusion criteria, all GB cases where the surgeon was planning to achieve a Gross Total Resection (GTR) (100%) or at least a Near Total Resection (NTR)($>90\%$) of the tumour were collected, as per the operative note record entry. All cases where a partial debulking or a biopsy was intentionally performed were excluded. The candidate of the present project randomly performed the selection of cases, and all the senior surgeons were kept blind to the results. Cases were selected by matching the intra-operative stealth and IOUS scan details and those into the operative notes where a surgeon documented that, in his/her opinion, GTR or NTR was achieved. Patients undergoing surgery without the use of any neuro-navigation devices were also excluded. Cases were selected based on a homogeneous time frame, meaning that all patients included were operated during the same period. Six senior surgeons were equally involved in treating patients from the two groups. Out of this group of consultants, three anonymized surgeons (C1, C2 and C3) were dedicated neuro-oncology surgeons; fifty-two cases of the present series were treated under their care, while different consultants operated eight. All selected patients had a pre-operative and an early post-operative (< 48 hours) volumetric MRI scan T1-weighted (T1w) with Gadolinium (Gd). Patients without volumetric images were not included. Patients undergoing post-op CT scan as a radiological method to check for the extent of resection were also excluded. The following clinical data have been collected from both groups: age at diagnosis, sex, co-morbidities, consultant neurosurgeon responsible for the procedure, adjuvant treatment, OS, PFS, and PS. Pre- and post-operative radiological data in pre- and post-op volumetric MRI scans have been collected for each patient to establish tumour volume and the presence of postoperative residual. A dedicated neuroradiology team has performed pre- and post-operative volumetric calculations on tumour volumes and post-op residuals (when present) on the sequences mentioned above using a dedicated radiological software purchased from the Carestream PACS© package. GTR was defined as the absence

of enhancing residual on an early (< 48 hours) post-operative volumetric T1w with Gd MRI scan [58]. Blind imaging data were provided to the radiology team, so they were unaware of which group the patient was extracted from (exposure vs control) to limit confirmation bias. Two experienced consultant neuroradiologists performed the calculations and two senior clinicians (a neurosurgeon and a neuroradiologist) cross-checked the segmentation. The tumour mass in pre-op and post-op scans was strictly defined according to the Response Assessment of Neuro-Oncology clinical trials (RANO) criteria and was limited to the enhancing component [58]. Location of the tumours in terms of depth and proximity to cortical and subcortical eloquent areas has also been considered in our analysis, as per Spetzler-Martin classification and subsequent updates [8,11]. The primary outcome considered for the present study was the extent of resection (EOR) in the two groups regarding both volume reduction and the presence/absence of the residual. The two groups' secondary outcomes were Overall Survival (OS) and Progression Free Survival (PFS).

2.4.3 Statistical analysis

The decision regarding the 3D-IOUS metrics compared to the standard of care (neuro-navigation) metrics were calculated as follows:

- True Positives (TP): residual tumour detected by the intra-operative device (IOUS or neuronavigation) AND residual detected on post-op MRI scan;
- True Negatives (TN): residual tumour NOT detected by the intra-operative device (IOUS or neuronavigation) AND residual NOT detected on post-op MRI scan;
- False Positives (FP): residual tumour detected by the intra-operative device (IOUS or neuronavigation) BUT residual NOT detected on post-op MRI scan;
- False Negatives (FN): residual tumour NOT detected by the intra-operative device (IOUS or neuronavigation) BUT residual detected on post-op MRI scan.

The calculation of sensitivity (SEN), specificity (SPE), positive predictive value (PPV), negative predictive value (NNV), and accuracy (ACC) was performed as reported in Chapter 1, Section 1.6—Statistical analysis.

Statistical analysis of the clinical data has been performed using RStudio© (ver. 2021.09.2) running R (ver. 4.1.2). A linear regression model has been built to test the effects of each variable on the EOR in R. The following variables were considered: use of IOUS, sex, age at diagnosis, depth of the most superficial and deepest point of the tumour, tumour size, proximity to an eloquent area, surgeon operating, and whether the case was a recurrent GB. Regarding the secondary outcomes (OS and PFS), a Cox proportional hazard model was built to weigh the role of different factors. In addition to the previously mentioned fields, the following ones were considered: peri-operative performance status, percentage of volume resected, MGMT and IDH status, post-operative chemo-radiotherapy (no CRT, palliative CRT, or radical CRT) and the presence of peri-operative complications. Kaplan–Meier curves were built to compare OS and PFS for the following variables: use of IOUS (Y vs N), peri-operative complications (Y vs N) and post-operative treatment (no CRT, palliative CRT, or radical CRT). Plots were generated in GraphPad Prism (ver. 9.3.1). Statistical significance was determined using the Mann-Whitney U test (box-and-whisker plots) or log-rank (Mantel-Cox) test for Kaplan-Meier curves.

2.4.4 Results

Descriptive findings

The present analysis hypothesised that image fusion of IOUS and preoperative MRI would improve the quality of tumour resection and the clinical outcome of surgery. To do this, the pool of cases from the 3D-IIOUS machine was reviewed, and specifics on how different surgeons used this were looked for.

Between the year 2014 and the year 2019, 1243 patients diagnosed with a Central Nervous System (CNS) tumour were operated at Imperial College NHS Trust. Among these, we have

retrieved 391 patients operated using either Sonowand© IOUS-stealth coupled machines (numbered 55 and 51), and 257 operated using Medtronic© stealth Neuronavigation - see Table 2.4. The remaining patients were operated using different neuro-navigation devices or without neuro-navigation.

Histology	N. of cases
GB	127
Oligo grade II	16
Astro grade II	24
AO grade III	6
AA grade III	24
Ependymoma grade I	1
Glioma NOS	2
Pilocytic astrocytoma	1
Metastases	55
Meningioma grade I	41
Meningioma grade II	11
Radionecrosis	4
Lymphoma	4
Abscess	2
DNET	2
Haemangioblasoma	3
Colloid cyst	2
AVM	2
Inflammatory lesion	3
Chondrosarcoma	1
Central neurocytoma	2
Cavernoma	1
Epidermoid cyst	1
Brain haemorrhage	1
Spinal cord / unknown	55
TOTAL	391

Table 2.1: 3D-IOUS histological cohort. GB = Glioblastoma; Oligo = Oligodendroglioma; Astro = Astrocytoma; AO = Anaplastic Oligodendroglioma; AA = Anaplastic Astrocytoma; NOS = Not Otherwise Specified; DNET = Dysembryoplastic NeuroEctodermal Tumour; AVM = Artero-Venous Malformation

As expected, reliance on 3D-IOUS was variable among different surgeons and very operator-dependent. While post-operative 3D-IOUS checks might have been performed in many cases, 3D acquisitions were not always recorded and stored in the Trust devices. Another finding concerns the 3D acquisition. The US probe is connected to the probe localizer to acquire

images for the software to reconstruct. The pins need to be oriented towards the infrared tracking system. From the imaging data retrieved, this acquisition can occasionally generate some degree of artefacts (see Figure 2.5) and needs to be carefully performed. Multiple attempts are required to obtain the best view.

Regarding the types of tumours treated, we have found that most surgeons heavily relied on the IOUS when dealing with primary or secondary intra-axial, mostly subcortical lesions. Extra-axial lesions, such as meningiomas, were rarely operated on using IOUS, and the machine was mainly used as a standard neuro-navigation device. This was specifically true for convexity / parasagittal meningiomas.

Regarding IOUS during glioma surgery, we have observed different imaging features in our pool of cases. Low-grade gliomas (LGG) appeared well-defined and had clear margins on the MRI scan, which were definitively more accurately seen and defined with IOUS. However, diffuse LGG were more challenging to explain, and the boundaries are difficult to visualize (see Figure 2.2 and Figure 2.3).

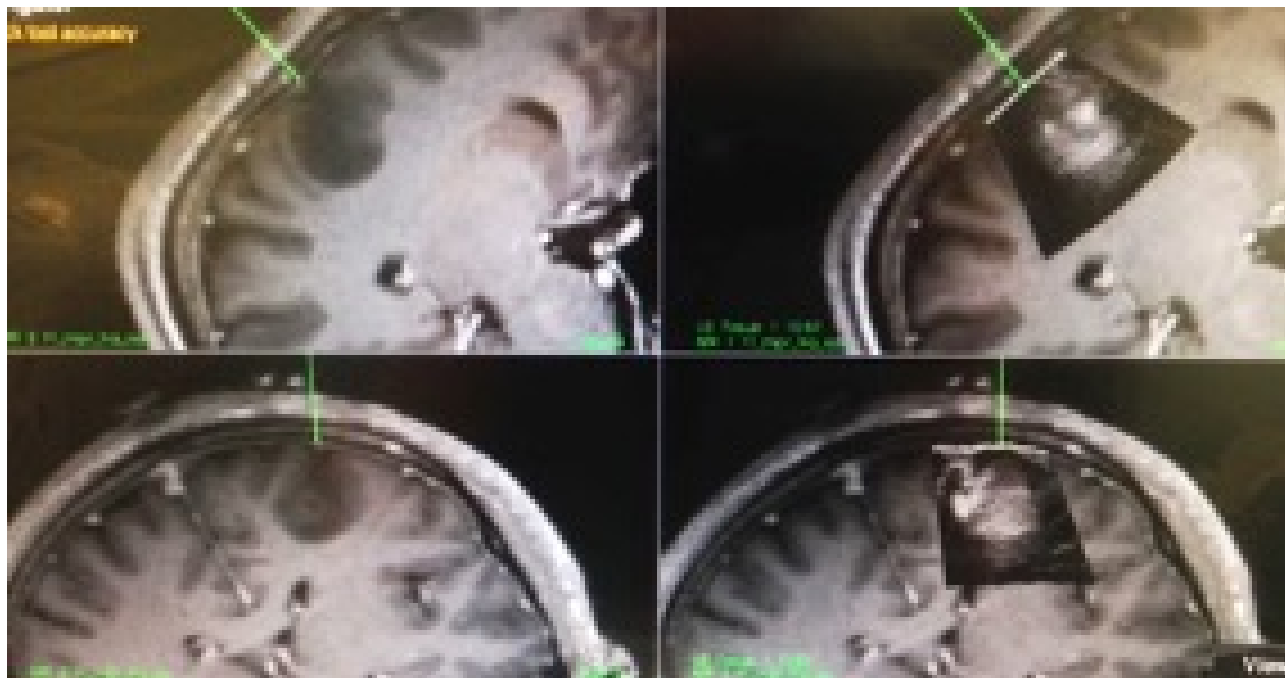


Figure 2.2: Intra-operative IOUS-neuronavigation coupled view of a left supplementary motor area oligodendroglioma. On the left the image as visualized on the stand-alone neuronavigation, on the right the integration with IOUS. Defined / exophytic LGG are usually optimally visualized using IOUS

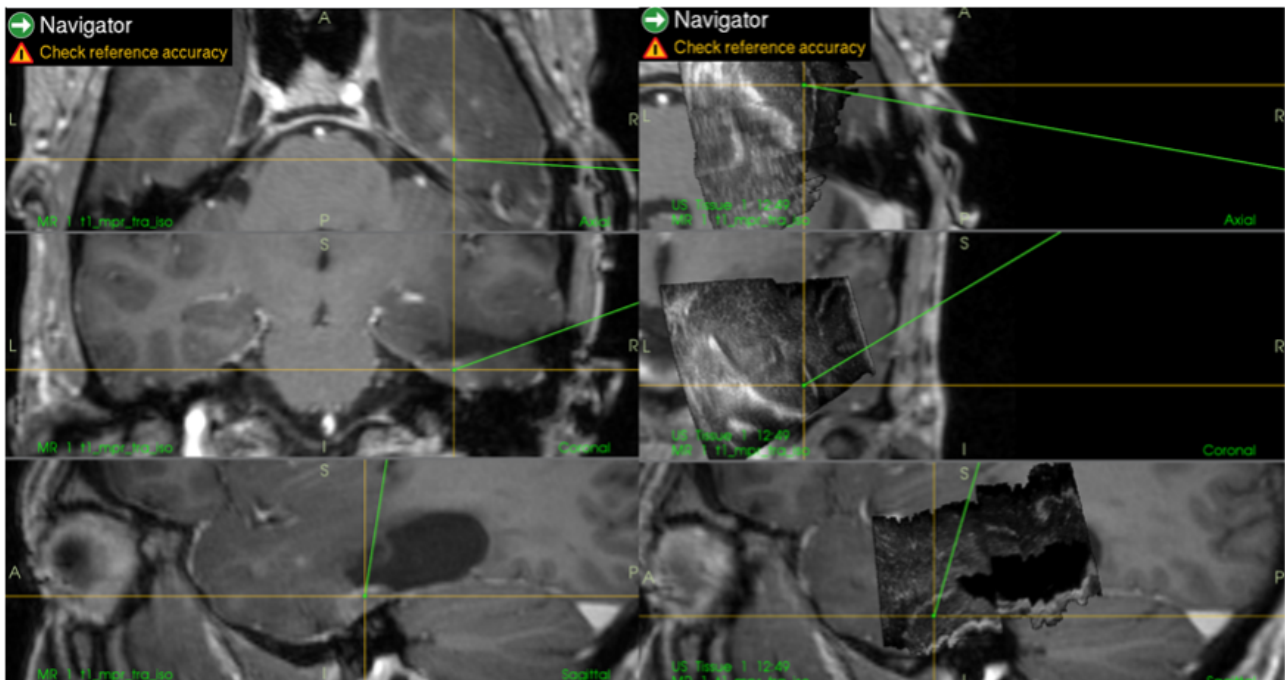


Figure 2.3: A case of a right temporal diffuse LGG. The IOUS signal (right) is challenging to interpret as the lesion appears diffuse, infiltrative in nature, and with no clear boundaries

More heterogeneous results were also noticed with high-grade gliomas (HGG) and GB. Anaplastic astrocytomas and oligodendrogliomas were often tricky to define, as the boundaries were frequently mixed with peri-lesional oedema. However, in most cases, it was not particularly challenging to visualize the areas of necrosis and cystic degeneration. The same findings were observed on primary GBs, which appear hyperechoic, inhomogeneous masses with a necrotic core and poorly defined boundaries (see Figure 2.4). Recurrent gliomas were probably the most challenging tumours to visualize on IOUS (see Figure 2.5).

As highlighted by other authors [190], intra-operative imaging quality varies based on the stage of surgical resection. Superficial, small-sized lesions were typically very well visualized by the acquisition and during resection. However, even a modest to moderate amount of bleeding causes a visible artefact that can hamper surgical view beyond the limits of resection (see Figure 2.6).

Accurate haemostasis and removing all haemostatic material from the surgical cavity are crucial to detecting residual tumours. This aspect was seen as the most variable and can explain the significant inter-operator variability seen on both intra-operative imaging and post-op scan.

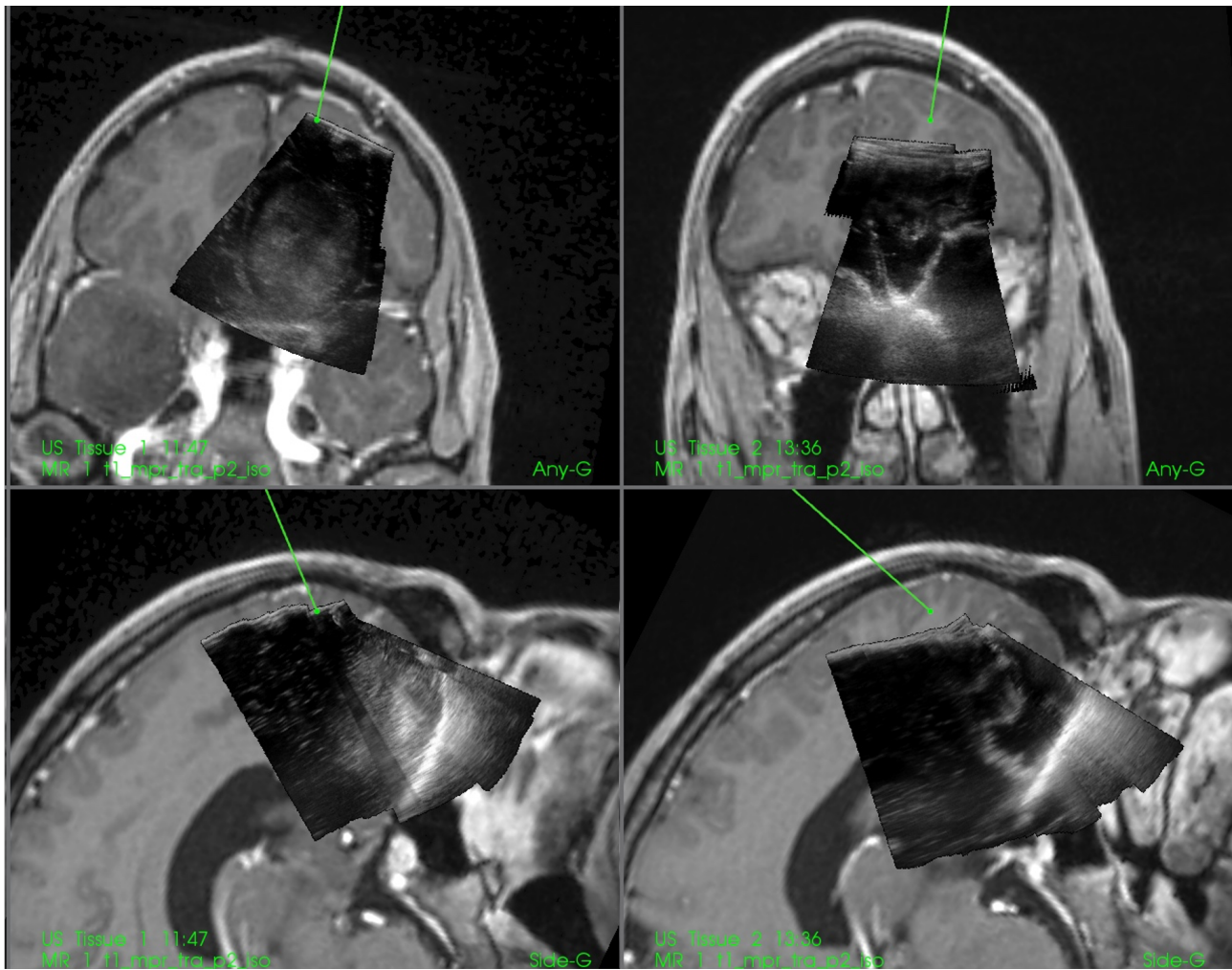


Figure 2.4: Intra-operative picture of left frontal GB before (left) and after resection (right). In this case, the necrotic core was well visualized. On the right side, note the presence of a partial shift of the brain orbital gyrus after resection and a cone of hyperechogenic material on the skull base due to the deposit of blood products at the bottom of the cavity.

3D-IOUS metrics

Based on the inclusion criteria, thirty GB cases operated using Sonowand© IOUS-stealth coupled devices and thirty operated using Medtronic were selected.

The statistical analysis was based solely on these cases, as the two samples were considered acceptable size-wise, and they all had complete clinical and radiological data.

Regarding the metrics of the 3D-IOUS performance, the details regarding the number of cases included are reported in Table 2.2. Although the IOUS counted 30 patients included, 4 cases had to be removed from the metrics analysis due to incomplete data, thus the total number of

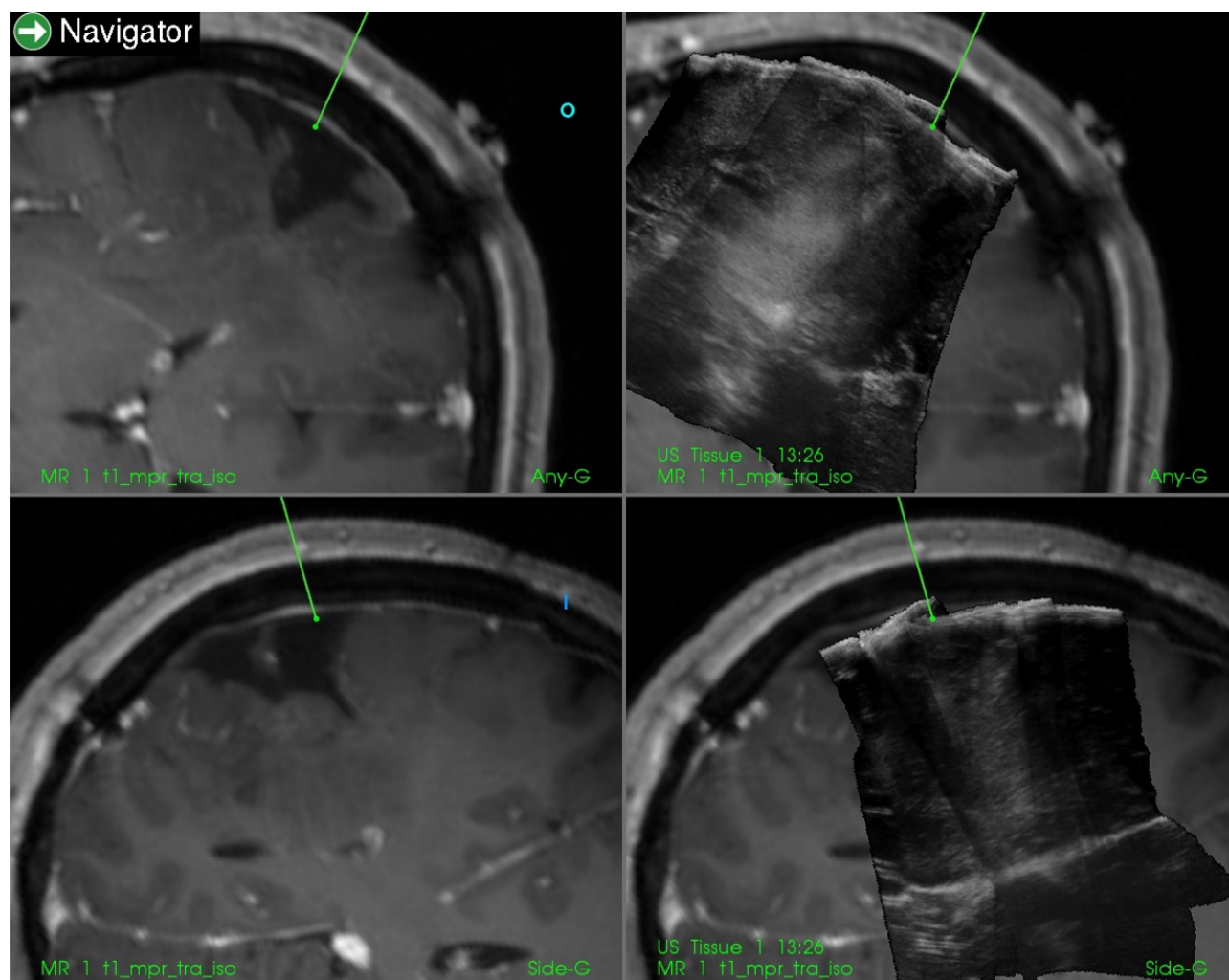


Figure 2.5: A case of left frontal recurrent LGG was found to be a transformed Grade IV (GB) after histological analysis. Apart from the size of the lesion requiring multiple acquisitions and therefore causing linear artefacts, the tumour appears challenging to define on IOUS due to the relatively homogeneous hyperechogenic signal.

cases considered was 26. The same issue was related to the neuronavigation cohort, where 5 cases had to be excluded.

The metrics calculation are summarized on Table 2.3. The 3D-IOUS and neuro-navigation performances are similar, although results must be interpreted cautiously (see the Discussion section).

Clinical results

All patients included had surgery between the 1st of January 2014 and the 31st of December 2017. Clinical and radiological features of those cases are summarized in Table 2.4.

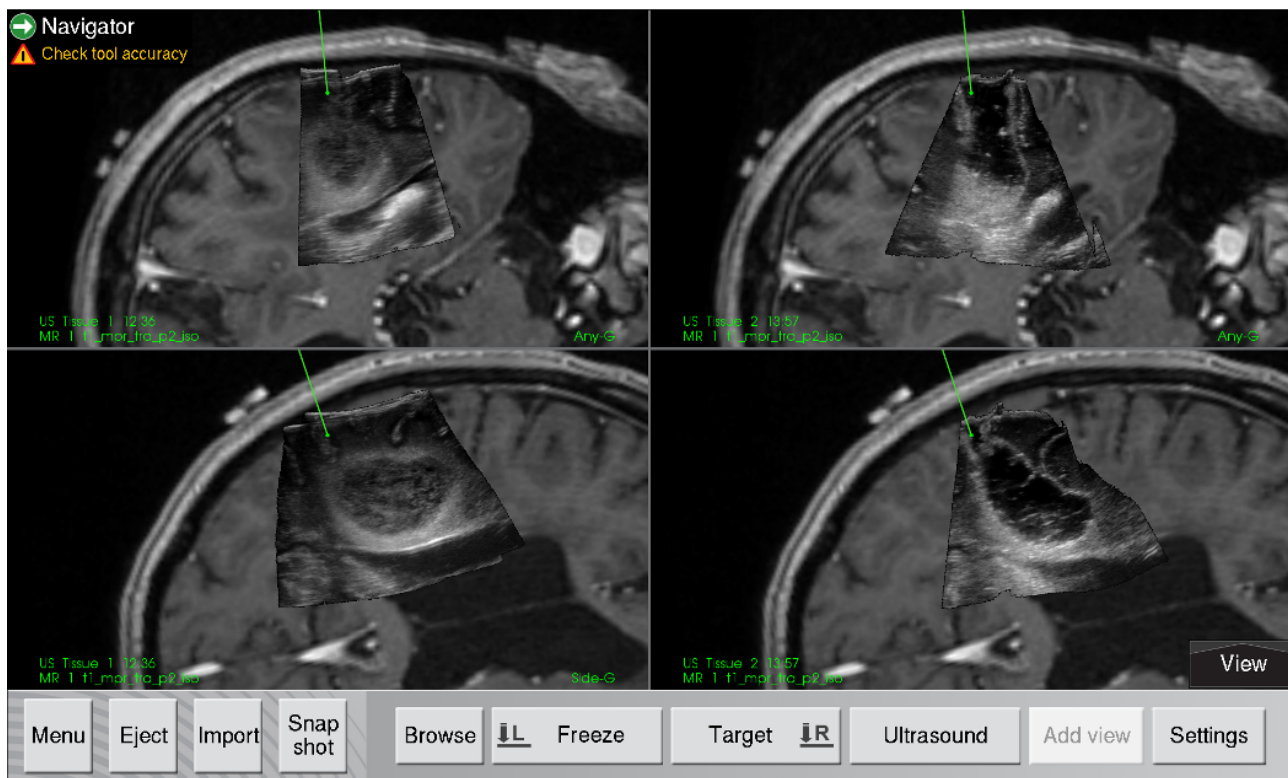


Figure 2.6: A case of a parietal metastasis pre (left) and post (right) resection. Even a thin layer of haemostatic material at the bottom of the surgical cavity is causing a significant cone of hyperechogenic artefact.

The mean age of the patients at diagnosis was 55 years (range 26-84), 21 of whom were females and 39 males (M:F ratio = 1.8). No secondary GBs were included in the present series. The average tumour volume for the whole pool of cases was 91.16 cm^3 (min 4.94 cm^3 , max 384 cm^3 , SD ± 72.69). Tumour volume was higher in the IOUS group (mean 118 cm^3 , min 18 cm^3 , max 384 cm^3 , SD $\pm 86 \text{ cm}^3$) compared to the control group (mean 64.14 cm^3 , min 5 cm^3 , max 170 cm^3 , SD $\pm 42 \text{ cm}^3$, Figure 2.7, $p = 0.007$). Depth points of the tumours were comparable between the two groups: the average most superficial point was 1 cm in both groups (min 0 cm, max 3, SD ± 0.8), while the average deepest point was 5 cm deep (min 3, max 8, SD ± 1.29 and 1.44 in the IOUS group and in the control group, respectively). Six and seven cases were near eloquent areas in the IOUS and the control groups, respectively. All cases located near an eloquent area were operated on using neurophysiological monitoring, awake surgery, or a combination of the two, depending on the indications. Five patients were operating using 5-AminoLevulinic Acid (5-ALA), 2 in the IOUS group and 3 in the control group. The routine use of 5-ALA in GB surgery was introduced as standard in clinical practice in the UK only

	3D-IOUS		Neuronavigation	
	N. of cases	%	N. of cases	%
TP	12	46%	11	44%
TN	4	15%	5	20%
FP	0	0	1	4%
FN	10	38%	7	28%
TOTAL	26	100%	25	100%

Table 2.2: Overall number of cases and percentages included for the metrics analysis. Due to incomplete records, not all cases from both cohorts could be included

	3D-IOUS	Neuronavigation
SEN	54.76%	61.11%
SPE	100%	83.33%
PPV	100%	91.67%
NPV	28.30%	41.67%
ACC	61.62%	66.67%

Table 2.3: Performance metrics of the 3D-IOUS compared with the standard neuronavigation.

in 2018. This was not part of the department's routine practice before that date, and given the exiguous number of patients treated using 5-ALA, this factor was not included in the final analysis. As expected, EOR was found not to be normally distributed, with two clear peaks seen in the density distribution, separating a large population with optimal resection ($n = 51$, mean $92\% \pm 8\%$) and a small population with sub-optimal resection ($n = 9$, mean $55\% \pm 6\%$). The average EOR in the IOUS group was 93% (range 49%-100%, median 96%, SD $\pm 10\%$), while in the control group, it was 80% (range 44%-100%, median 81%, SD $\pm 17\%$).

There was a significant increase in EOR in the IOUS group compared to the control group, independent of other variables (see Figure 2.7, $p = 0.0004$) known to affect resection, such as tumour depth and location near an eloquent area. Superficial access to the tumour was also linked to an improved EOR ($p < 0.03$). Interestingly, a weak association between consultants not usually performing neuro-oncological surgery and an enhanced EOR has also been found ($p = 0.05$). The IDH1 gene has been reported as wildtype in 44 cases, mutant in 9, and was not analysed in the remaining 7 cases. The MGMT gene was unmethylated in 33 cases, methylated

		IOUS	Stealth	Total
Age (years)		58 (± 14.8)	53 (± 14.4)	55 (± 14.6)
Sex		23M, 7F	16M, 14F	49M, 21F
Tumour volumes (cm ³)		118 (± 86)	64 (± 42)	91.16 (± 72.69)
Tumour location	<i>Frontal</i>	9	8	17
	<i>Temporal</i>	11	13	24
	<i>Parietal</i>	8	6	15
	<i>Occipital</i>	0	2	2
Proximity with eloquent areas		6	7	13
PS pre-op		0 in 29 pts; 1 in 1 pt	0 in 14 pts; 1 in 15 pts; 2 in 1 pt	0 in 42 pts, 1 in 17 pts, 2 in 1 pt
PS post-op		0 in 27 pts; 1 in 1 pt; 4 in 2 pts	0 in 13 pts; 1 in 10 pts; 2 in 2 pts; 3 in 5 pts	0 in 40 pts; 1 in 11 pts; 2 in 2 pts, 3 in 5 pts and 4 in 2 pts
IDH Status	<i>Wildtype</i>	21	23	44
	<i>Mutant</i>	6	3	9
	<i>NOS</i>	3	4	7
MGMT Status	<i>Unmethylated</i>	16	14	30
	<i>Methylated</i>	7	1	8
	<i>NOS</i>	5	9	14
Consultant operating (years of experience)	<i>C1 (23)</i>	17	5	22
	<i>C2 (23)</i>	4	19	23
	<i>C3 (26)</i>	2	4	6
	<i>Other (NOS)</i>	5	2	7

Table 2.4: Summary of glioblastomas cohort characteristics. IOUS = IntraOperative Ultra-Sound; NOS = Not Otherwise Specified; C1, C2 and C3 = Consultant 1, Consultant 2 and Consultant 3.

in 12 cases, and was not analysed in the remaining 15 cases. The pre-operative performance status (PS) was 0 in 42 patients, 1 in 17 patients, and 2 in one patient. Immediate post-operative performance status was 0 in 40 patients, 1 in 11 patients, 2 in 2 patients, 3 in 5 patients and 4 in 2 patients. Four patients experienced significant post-operative complications: two in the form of severe post-operative cognitive and neurological impairment (PS 4), two developed acute hydrocephalus post-op, treated with ventriculoperitoneal shunting. One of the patients with hydrocephalus also developed aspiration pneumonia, which was successfully treated with antibiotics. We did not observe post-operative ischemic complications in either of the groups. Where observed, the worsening post-op status was in all cases related to postoperative oedema. The average OS was 13.4 months (range 0-32 months), and the average PFS was 7.4 months

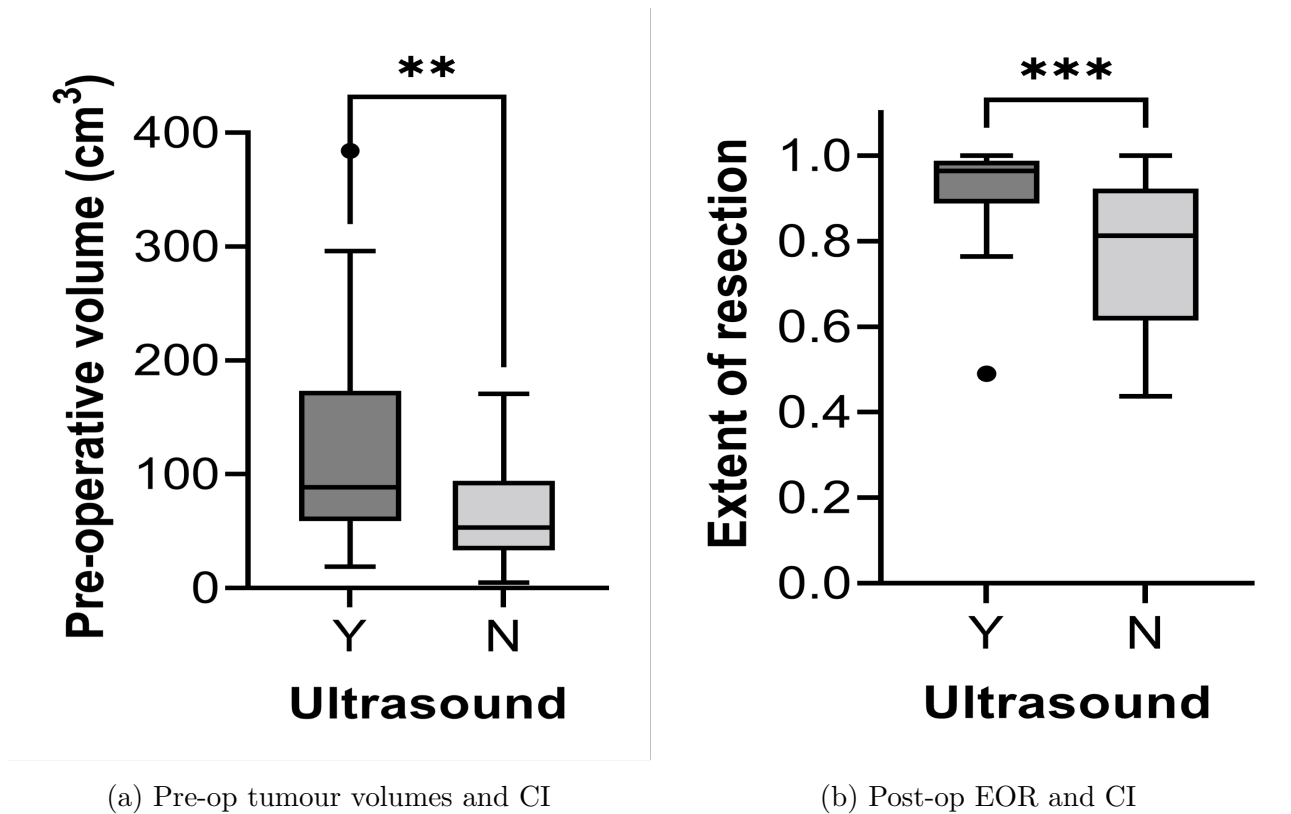


Figure 2.7: Pre-operative volumes compared with the EOR. EOR = Extent of Resection; CI = Confidence Intervals

(range 0-28). The Cox Proportional hazard model showed a significant advantage in OS on patients operated using the IOUS, with a Hazard Ratio (HR) = 0.196 and $p = 0.017$. As expected, administration of radical CRT was also associated with improved OS (HR = 0.199, $p = 0.006$). The post-operative complication negatively affected survival (HR = 15.612, $p = 0.002$). We also observed differences in OS based on the presence of the tumour near an eloquent area (HR = 0.349, $p = 0.013$), the tumour volume (HR = 1.007, $p = 0.047$), and the surgeons performing the operations, both the “other” group (HR = 11.729, $p = 0.004$) and surgeon C3 (HR = 7.703, $p = 0.013$). However, only the peri-operative complications and application of CRT had independent effects on survival times (see Figure 2.8). Only the administration of radical CRT was significantly associated with increased PFS (HR = 0.242, $p = 0.013$), and this association was seen as independent of other factors (see Figure 2.9).

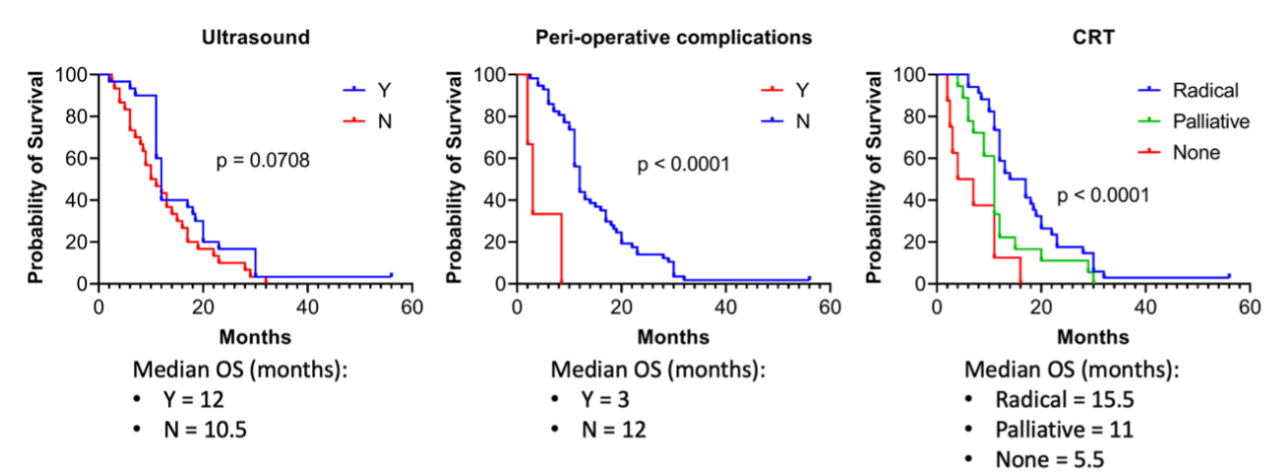


Figure 2.8: Whilst not independently significant, there was a shift toward longer survival in the IOUS cohort. Perioperative complications had a highly significant impact: 3 patients showed markedly shorter Overall Survival (OS). Radical Chemo-RatioTherapy (CRT) also significantly extends survival times, in keeping with the results of the known literature. P values were determined by the log-rank (Mantel-Cox) test.

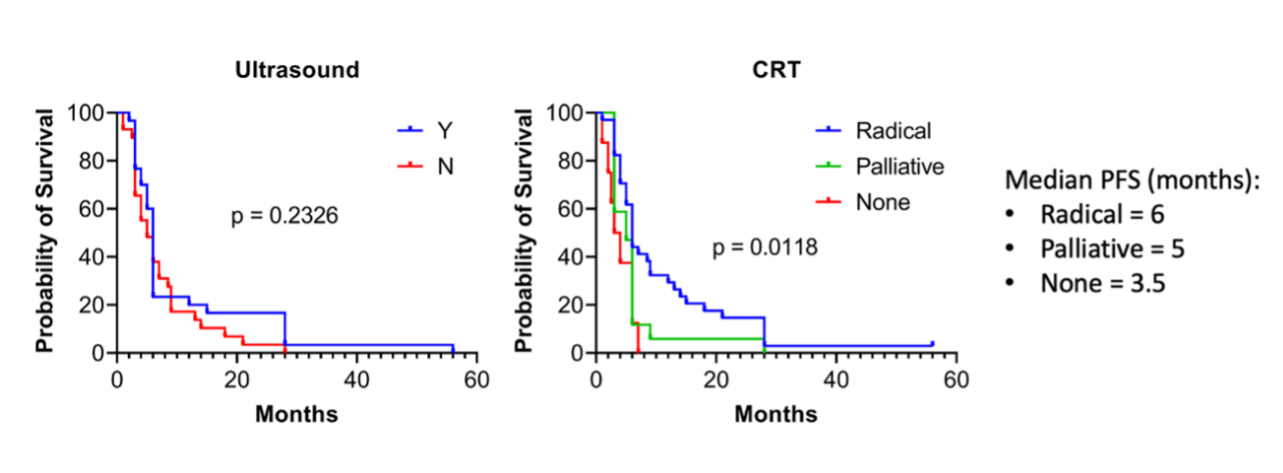


Figure 2.9: Progression-Free Survival (PFS) was poor in all patients, and the differences between groups are insignificant. IOUS does not appear to be significantly associated with significantly improved PFS. Radical CRT promotes longer PFS, as expected. P values are determined by the log-rank (Mantel-Cox) test.

2.4.5 Discussion

The 3D-IOUS metrics analysis on this series has shown that 3D-IOUS is not significantly superior to neuronavigation regarding residual detection. There are significant limitations in this finding. The first and obvious one is that the cohort of patients is limited (26 vs 25), and retrospective data collection is, by definition, subject to bias. However, the second and most important is the practical absence of FP for both technologies. This is a known issue, already

reported in the relevant literature [219]. The reason is intuitive: while 3D-IOUS might have a lower threshold to detect residual tumours, it's unlikely for both 3D-IOUS and neuronavigation to detect a residual when the residual is not there (FP). Because of this technical problem, sensitivity and negative predictive values are significantly skewed towards the low side. However, the large number of FN in the IOUS cohort most likely directly reflects the technical problems reported, specifically regarding artefacts hiding a possible residual. A comprehensive literature review showed a pooled sensitivity of 49% and a specificity of 98% [225]. Our data show slightly improved performance (61% sensitivity and 100% specificity), which might be explained by the fact that IOUS has been extensively used in our department, and most of the surgeons were highly experienced with its use.

Regarding the clinical data, we have included two comparable groups of cases operated by a heterogeneous pool of surgeons. The purpose of this selection was to choose a sample that would resemble as much as possible a cohort seen in an "average" neurosurgical service, with heterogeneous tumours' locations, surgeon's styles and preferences, and tumour sizes. In our series, the tumours included had different locations, sizes, and proximity to the surface. However, the number of tumours located near eloquent areas was similar between the exposure and the control group (6 vs 7, respectively). Moreover, the average depths of the tumours were comparable between the two groups in terms of the most superficial and the deepest portions. On the other hand, pre-operative tumour volumes were higher in the IOUS group than in the control group, which was presumably a stochastic effect. Despite the average larger tumour volume suggesting that surgical resection might result in more challenges, the EOR was still more generous in the IOUS group, thus indicating that IOUS makes a difference in the quality of EOR regardless of tumour size. Our results show that, when all possible factors are considered, the EOR is increased when IOUS is used. Interestingly, using IOUS also resulted in a statistically significant improvement of OS in this series, although the advantage was not massive. The small number of patients included and the numerous confounding variables suggest that this outcome should be taken cautiously and that further investigation would be warranted. A potential prospective study enrolling a more conspicuous pool of patients, with stratification based on tumour location, size, and depth, might be helpful to verify this finding. It is also worth stressing that the

same finding did not apply to PFS in the present series. Another interesting finding concerns the stratification of patients according to the surgeon operating on them. We have purposely decided to include this variable in the analysis to consider the inevitable inter-operator variability: different surgeons have different approaches and techniques when resecting a tumour. As expected, most patients were operated by dedicated neuro-oncology consultants, with a few being operated by general neurosurgeons (grouped and marked as “others”). EOR did not show significant differences among the three neuro-oncology surgeons operating, although the Confidence Interval (CI) was broader in C2 compared to C1 and C3. Differences in resection technique, patient PS, comorbidities, and proximity to eloquent areas could account for the discrepancy. However, even more interesting, the differences in EOR did not translate directly into differences in OS and PFS, with C1 being the consultant with the highest associated survival, but EOR was comparable with that of C3. Also, C1 has operated on a lower number of cases included, which might have skewed the analysis. Tumour depth has not significantly affected EOR, OS or PFS. Still, this effect is likely linked to pre-operative case selection: deeply located lesions are unlikely to be selected for GTR. This is also true for the performance status, MGMT and IDH1 status and their impact on OS and PFS. However, some limitations related to the model are reported in the next section.

2.4.6 Limitations

The present data collection and analysis has several limitations. First, this is a retrospective review based on previously collected data. Many cases have been excluded due to incomplete or missing data pools—e.g., patients not scanned within 48 hours post-op, lack of a volumetric T1 with Gd scan, patients lost at follow-up, etc. Secondly, the dataset is quite heterogeneous, and some surgeons preferred using the IOUS to start with precisely because of the integration between stealth and ultrasound. This is why the operating surgeon was included as a possible confounding variable in the statistical analysis. Still, given the small pools of cases analysed, this factor may have had more impact than shown. Thirdly, the small sample included in

the analysis did not allow for a more precise stratification of the data, meaning that tumours similar in locations and size could not be precisely compared. In our study, we have partially tried to solve this problem by accounting for tumour location in terms of eloquence proximity, pre-operative size, and depth. However, ideally, it would have been more adequate to compare outcomes from pools of tumours in similar locations. It is also worth noting that eloquent areas proximity is an outdated concept (see Chapter 1, Introduction) and therefore a more precise stratification is desirable for future studies. Finally, we have relied on the clinical notes to include patients in the statistical analysis. We assumed the surgeon's aim to achieve a GTR or NTR of the enhancing component was not biased. A relatively wide CI on EOR suggests that a degree of error was present in some cases. This is also the case when analysing the impact of the tumour molecular profiles in relationship with OS and PFS: MGMT and IDH statuses were missing in a significant minority of cases because the present series dates to 2014, and molecular profiling was not included in the WHO classification yet, so their actual impact was difficult to determine with the data available.

2.5 Conclusions

The 3D-IOUS is a promising technology that is easy to integrate into the neurosurgical toolkit for tumour resection.

The hardware is cheap, reliable, not bulky and suitable for integration into neuro-navigation, which is already part of the neurosurgical set-up.

Our clinical results highlight the improvement of both clinical outcomes (in terms of EOR and PFS) and performance metrics.

In the short to medium term, the team's research goals would be to:

- identify the best software to refine IOUS accuracy;
- increase the dataset of available images for training and testing purposes.

The long-term goal is to integrate algorithm-based software onto the IOUS device so that the provided image can be enriched by probability maps for the brain/tumour interface, automatic artefact detection, and eventually integration into robotics platforms.

Several clinical limitations emerged from this work. Most of them are already highlighted in the relevant sections, but summarizing here:

- the difficulty in exactly quantifying the advantage of using IOUS in a heterogeneous group of cases with different clinical, histological, and imaging features;
- the operator-dependent nature of the technology, coupled with the lack of standardized national or international training;
- the relatively low sensitivity in detecting tumour margins due to both the infiltrative nature of some tumours and the presence of artefacts;
- lacks diagnostic capabilities to detect eloquent areas.

While some of these limitations have been addressed here, multispectral and hyperspectral imaging have been selected as an additional field of research. The following chapters are dedicated to this technology.

Chapter 3

Hyperspectral imaging in neurosurgery: a review

3.1 Basic physical principles of light behaviour

To understand how hyperspectral imaging works, it is necessary to understand how light interacts with matter. Light is a form of electromagnetic radiation made up of oscillating electric and magnetic fields. These fields travel together through space at the speed of light, approximately 299,792 kilometres per second in a vacuum. Light can exhibit properties of both a wave and a particle, a duality that is one of the fundamental principles of quantum physics [231].

- **Wave Nature of Light - Electromagnetic Waves.** Light as an electromagnetic wave consists of oscillating electric and magnetic fields propagating through space. These fields are perpendicular to each other and the direction of the light's travel. Light waves have specific wavelengths and frequencies. The wavelength is the distance between successive crests of the wave, while the frequency is the number of waves that pass a point in space per second (see Figure 3.1). The colour of visible light is determined by its wavelength.
- **Particle Nature of Light - Photons.** Light can also be described as a stream of particles called photons. Each photon carries a discrete amount of energy that depends

on the light's frequency.

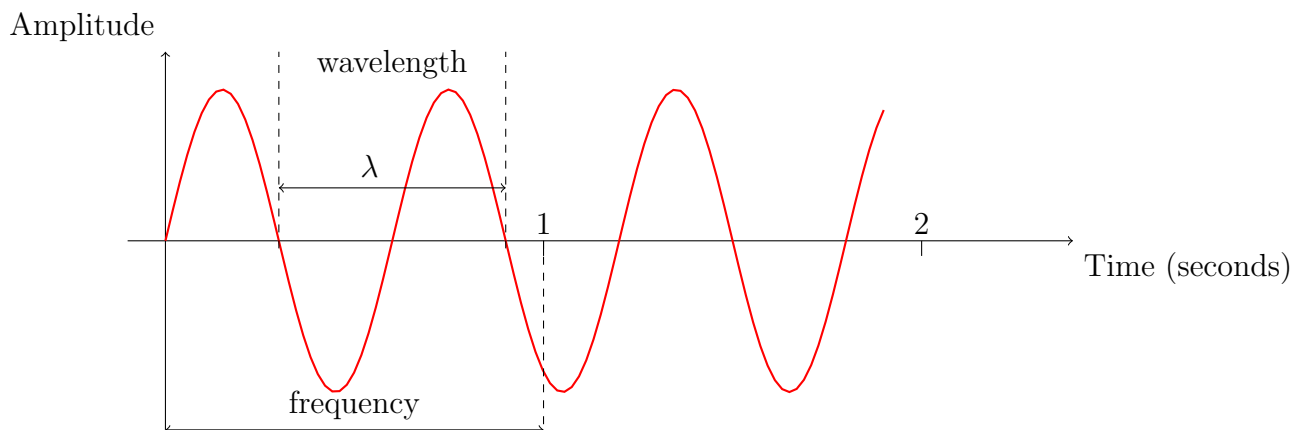


Figure 3.1: Graphical representation of a random wave function with wavelength and frequency

A *chromophore* is a molecular entity within a compound that is responsible for the compound's colouration. Chromophores contain electrons in specific energy levels. When light hits a chromophore, certain wavelengths (colours) of light are absorbed by the molecule, causing its electrons to cross from a lower energy level (ground state) to a higher energy level (excited state) [232]. The energy of the absorbed light corresponds to the difference between these two energy levels. The wavelengths that are not absorbed determine the colour we see because they are reflected or transmitted to the observer.

When light interacts with biological tissue, several fundamental processes occur, primarily involving absorption, scattering, and transmission [233]. The interaction is strongly influenced by the molecular composition and structure of the tissue and the specific wavelength of the light. Absorption is the process of chromophores, such as haemoglobin and melanin, absorbing particular wavelengths of light, converting the energy into heat or initiating photochemical reactions [234].

Scattering is a property related to light propagation through media. When light encounters microscopic structures within the tissue, such as cells and organelles, its path is altered, and the light is redirected in different directions. This process can be described by Mie scattering for larger structures and Rayleigh scattering for smaller particles [235]. Scattering causes the light to diffuse, leading to a loss of directional information and influencing the appearance

of tissue, which often results in a soft, diffused light characteristic of biological matter. The combination of absorption and scattering dictates the penetration depth of light in tissues, with longer wavelengths (e.g., near-infrared) generally experiencing less absorption and scattering, allowing deeper tissue imaging [236].

In addition to these primary processes, other phenomena linked to the deviation of the light path, such as reflection and refraction, occur at tissue boundaries due to differences in refractive indices between tissues and surrounding media. However, different from scattering, reflection and refraction are coherent processes where phase relationships are preserved across the wavefront when interacting with smooth surfaces. This coherence allows a surface to act as a mirror or prism, giving us well-defined reflections or refraction effects [234].

In a biological tissue such as the brain, haemoglobin (oxy and deoxy) is the primary chromophore within the visible spectrum. In contrast, lipids such as myelin show a small peak in the visible spectrum, more pronounced in the Near InfraRed (NIR) range [233]. These chromophores exhibit an absorption and a reflectance spectrum. When correlated with the concentration of single chromophores, this information holds a clinical value regarding the tissues' nature [3, 237, 238].

Hyperspectral imaging, a technology used to obtain the combined reflectance spectrum for each pixel in an image of a scene, focuses on several of the light-tissue interaction phenomena mentioned. By capturing and analyzing the spectrum at each point in the image, hyperspectral imaging allows for detailed chemical and structural analysis of biological tissues.

3.2 Definitions

MultiSpectral Imaging (MSI) and HSI involve acquiring image data across the electromagnetic spectrum at multiple wavelengths. While traditional imaging captures data in the three primary bands of the visible spectrum Red, Green, and Blue (RGB), MSI/HSI extends this capability by further dividing the visible spectrum into narrower wavelength bands. There is no universal agreement regarding the distinction between multispectral and hyperspectral [3]. As a rule of

thumb, the number of spectral signatures acquired for MSI is 10 or 20, while in HSI, hundreds of contiguous spectral bands are recorded for every pixel in the image.

In this section, a brief description of the central scanning systems used in the neurosurgical literature is reported.

3.3 Hardware overview

Hyperspectral imaging can be acquired through different cameras and setups, each tailored to the specific scanning requirements of the intended application [233].

From a clinical perspective, the primary considerations when choosing the appropriate cameras concern the acquisition times, the resolution, and the logistics. These elements must be harmonized to ensure adequate data collection that provides high quality within the constraints related to the surgical and anaesthetic times.

This section discusses some basic features of the most commonly discussed sensors and acquisition techniques, specifically focusing on why we have chosen our specific set-up.

3.3.1 Sensors

Several camera types are commonly employed for acquiring hyperspectral imaging, each with its unique mechanism for capturing spectral information instantaneously. The following are the most frequently encountered in the relevant neurosurgical literature [3]. Many groups have reported several comparisons between the different types of cameras [233, 239–242].

Charge-Coupled Device (CCD) and Complementary Metal-Oxide-Semiconductor (CMOS) cameras are among the most widely used cameras for a variety of commercial and research purposes [243]. They differ in structure and mechanism of light capture but are adaptable to snapshot acquisitions.

The fundamental feature of CCD cameras consists of an array of pixels, each functioning as a receptacle for photons [244]. Each pixel accumulates photons and converts them into an electrical charge, which must then be transferred across the entire array of pixels to a common output node, where it is amplified and digitized. This charge transfer involves sequentially moving the accumulated charge across the chip from one pixel to the next. This process is known as *serial charge transfer* and has some limitations:

- each pixel's charge must be passed through adjacent pixels, one by one, until it reaches the output node - the further a pixel is from the output, the longer the process takes;
- it requires more power to move the electrical charge across the array;
- the sequential transfer process can be relatively slow, limiting the speed at which images can be captured and processed.

Each charge eventually reaches an output node, also known as a *charge-to-voltage converter*, where the charge is quantified and transformed into a digital image. The magnitude of the voltage corresponds to the amount of light captured by that particular pixel. A brighter spot in the image will produce a larger voltage, while a darker spot will produce a smaller voltage. Once the charge has been converted to a voltage, the signal is amplified, which is boosted to ensure the differences in voltage (corresponding to light intensity) are strong enough to be accurately digitized. After amplification, the analogue voltage signal for each pixel is fed into an *analogue-to-digital converter*. This component converts the continuous voltage levels into discrete digital numbers. These digital numbers represent the light intensity values of the pixels, effectively encoding the brightness and, if using colour filters, the colour information of the scene. [244].

In contrast, CMOS sensors have an active pixel structure. Instead of relying on a central output node, each pixel in a CMOS sensor has its circuitry for amplifying and converting the light into a digital signal directly within the pixel itself [245]. This localized processing within each pixel eliminates the need for serial charge transfer across the chip. As a result:

- each pixel handles its conversion and readout, allowing for faster processing since the conversion happens in parallel across all pixels rather than sequentially;
- CMOS sensors use less power because the charge doesn't need to be physically transferred across the chip. The digital conversion happens right where the light is detected;
- CMOS sensors can capture and process images much faster than CCDs due to this localized, parallelized approach, which makes them better suited for applications like high-speed photography or real-time video capture.

Initially showing lower image quality and sensitivity, advancements in CMOS technology have significantly enhanced performance, which is now considered superior to CCD [245].

Each camera type holds specific advantages and trade-offs regarding spectral and spatial resolution, sensitivity, and complexity. The choice of camera depends on the particular requirements of the hyperspectral imaging application, including factors like the need for speed, resolution, and the environment in which the system will be used. Some features of the most commonly used cameras deployed for hyperspectral acquisitions are summarized in the works of Clancy [233] and Lu [240]. For the choice of the ideal camera to adapt to this study, our perfect device would be cost-constrained, medium to high resolution, but with a quick acquisition timing that could minimize movements and guarantee snapshot acquisitions, compatible with the surgical timing. Despite the high costs, the availability of CCD and CMOS cameras in our centre made them ideal candidates.

3.3.2 Acquisition techniques

The acquisition of the spectral bands can be performed through several methods, namely:

1. **spatial scan**, further divided into

- point-scan;
- line-scan;

2. spectral scan;

3. snapshot.

All the scanning options have the goal to simultaneously acquire spatial data and spectral data or spectral signature, thus allowing the creation of a "datacube" or "hypercube" - See Figure 3.2.

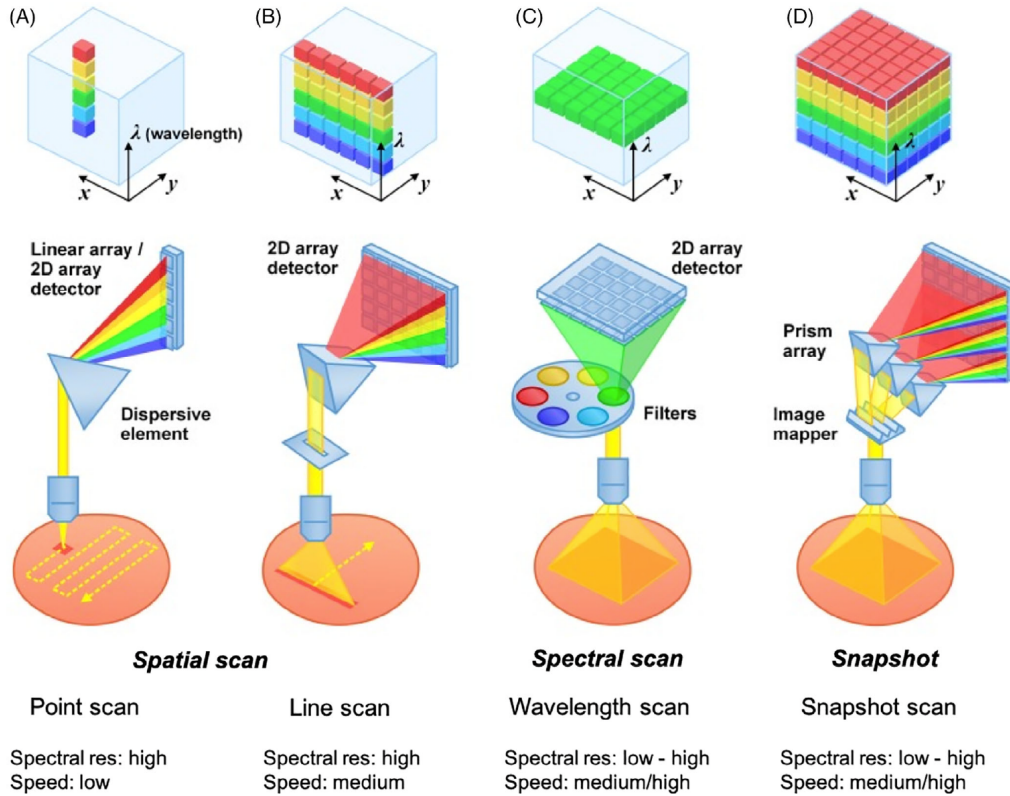


Figure 3.2: Different methods for multispectral/hyperspectral data acquisition. Image taken from the work of Shapey and colleagues [3]

Spatial Scanning

Spatial scanning acquires the complete wavelength spectrum per pixel or line of pixels, continuously scanning through the image [246]. Pixel scanning relies on whisk-broom [246] cameras, while linear scanning is performed using push-broom cameras [247–249], respectively.

The central component of a whisk-broom camera is a single detector or a small array of detectors juxtaposed with a rotating mirror or a set of mirrors. The specific orientation of these mirrors

allows them to sequentially redirect incoming light from different portions of the scene toward the detectors. Dispersive elements, such as prisms or diffraction gratings, are also integrated into the system and employed to dissect the incoming light into its constituent spectral components, enabling the detector(s) to capture spectral information at each point. The "whisking" action, a side-to-side sweeping motion facilitated by the oscillating mirrors, is pivotal in this design, allowing the system to progressively scan the entire field of view and build the corresponding hypercube [240].

A typical push-broom camera incorporates a dispersive optical element and a 2D sensor array, allowing simultaneous spectral data acquisition from a singular spatial line of the target scene. Dispersive optical elements are located in the light path, allowing the decomposition of the incident light into a spectrum across the array. This configuration allows each pixel in the linear array to capture a specific wavelength, encoding comprehensive spectral information for each point in the observed line. The camera progressively scans the image, synthesizing consecutive lines of spectral data like the movement of a broom sweeping dust (hence the name). This systematic motion facilitates the construction of the hypercube [240].

Spatial scanning provides high spectral resolution (a few nm) across hundreds of wavelengths [233]. However, spatial resolution is constrained by the number of scan lines and tissue motion. The need for multiple dispersive optical elements, especially in push-broom systems, makes miniaturization challenging [233]. Motion artefacts and the difficulty of aligning spatial slices post-acquisition remain limitations for surgical applications. In vivo, imaging of internal anatomy using flexible endoscopes has been demonstrated [237,248], with scan times minimized to reduce motion effects [250]. Alternatively, simultaneous wide-field imaging with a second camera can correct motion [251].

Spectral scanning

Spectral scanning techniques sequentially acquire images at different wavelengths [233].

Wavelengths are switched mechanically with a filter wheel [252] or electronically with a Liquid

Crystal Tunable Filter (LCTF) [233, 240]. These systems are compatible with high-brightness surgical light sources and can be integrated into clinical instruments with minimal modifications [248, 253, 254]. However, tissue motion may cause artefacts in the datacube, which can be corrected post-acquisition [255].

The process repeats for each wavelength, one after the other, to create the hypercube (see Figure 3.3).

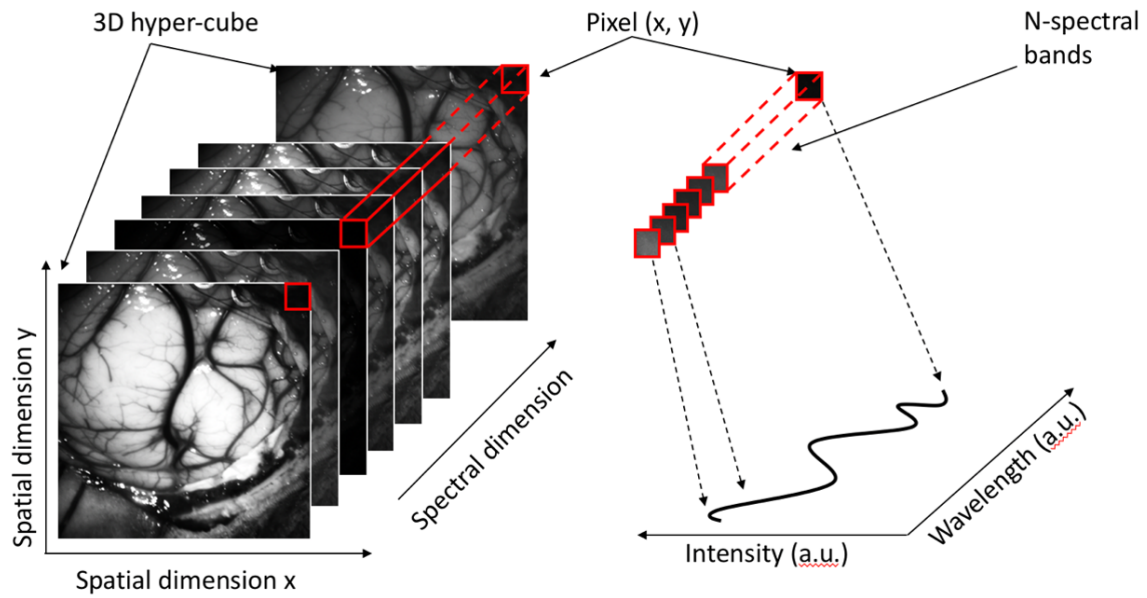


Figure 3.3: Representation of the hypercube acquisition, courtesy of Maria Leiloglou.

An LCFT selectively parses light into its constituent wavelength bands with high precision. Liquid crystals are substances with phases that exhibit order like crystals but can flow like liquids. When subjected to varying electrical stimuli, these liquid crystals reorient, altering the filter's optical path length. The LCTF acts as a gatekeeper, sequentially admitting specific wavelengths of light to reach the detector, thus enabling the acquisition of images across a broad spectral range [233, 240].

Snapshot scanning

Snapshot scanning in hyperspectral imaging refers to the capability to capture the entire hypercube in a single shot, as opposed to scanning mechanisms that require more time to record different wavelengths sequentially [233]. This approach is crucial for applications requiring real-time data, dynamic scene analysis, or where the subjects are in motion. While they allow fast acquisition, they compromise spatial resolution, limiting the number of wavebands to the MSI domain [233]. For example, a 9-band snapshot imager based on a 2048×1088 sensor has a final spatial resolution of 0.25 MP, which drops to 0.09 MP for 25 bands [256]. These images are useful in applications like fibrescopes [257], and performance can be enhanced using fibre bundles for spatial mapping [258]. However, due to multiple optical elements, their weight and complex design have limited their accessibility until recent advances in spectral imaging sensors. Companies now offer tiled and mosaic designs with spectral filters directly on the sensor [259], making these systems more accessible for clinical applications like rigid endoscopes, fibrescopes, and operating microscopes [248].

Mosaic filter cameras and Imaging Replicating Spectrometers (IRS) can be used for snapshot scanning. Mosaic filter cameras utilize band-pass filters, each permitting a specific spectral band to pass through and arranged in a mosaic pattern directly on the camera sensor. This design enables each pixel to capture light from one particular part of the spectrum, depending on the filter it lies behind. Although mosaic filter cameras can generate hyperspectral images in real-time, they compromise spatial resolution as different filters share sensor space [260,261]. In contrast, IRS employ mirrors or prisms to replicate the scene onto different sensor areas, with each replica passing through its spectral filter. This allows for the simultaneous capture of multiple spectrally distinct images of the same scene [262]. Another approach, Integral Field Spectroscopy (IFS) cameras, also known as hyperspectral light-field cameras, use a microlens array positioned before the sensor to capture both spatial and spectral data in a single shot. Each microlens generates a small scene image, each representing a different view or spectral range [263].

3.4 MSI/HSI image processing options - a summary

The hypothetical aims of MSI/HSI analysis in neuro-oncological surgery are to 1) allow the differential diagnosis of healthy vs pathological tissue and 2) identify brain areas based on their functional activation. Spatial data and spectral data are analysed for these purposes. A clear partition of data focusing on both spectral and spatial data is required to obtain a diagnostic advantage. While the defining feature of MSI/HSI consists in detecting the spectral signatures, most groups have integrated both spatial and spectral information in their analysis, as spatial features are relevant to developing an application capable of visually identifying features like margins, texture, shapes [195, 264, 265].

Machine learning (ML) algorithms are commonly deployed for MSI/HSI analysis. These can be classified into supervised and unsupervised [266–268].

A supervised ML algorithm uses a set of labelled examples, and each data point is defined as a *feature vector* [266]. In the specific example of HSI applied to tumour surgery, a possible feature vector is the spectral signature found within a pixel of the image acquired. For example, making the case that the pixel falls into the labelled category of "tumour", the ML model is trained to classify tumour vs healthy brain based the set of labelled training data.

In supervised learning, the dataset is usually split into [266, 268]:

- *a training set*, used by the model to learn patterns from the labelled set and adjust its internal parameters to minimize the error;
- *a validation set*, typically a smaller set used to tune the parameters of the model;
- *testing set*, a set used to evaluate the model's performance on completely unseen data.

An unsupervised ML algorithm uses unlabelled data, so it attempts to identify hidden patterns within the data [266, 268]. Back to the example of tumours vs normal tissue, an unsupervised ML algorithm would attempt a classification by grouping pixels with similar spectral signatures without the label's input on the pixels themselves.

The ideal model for any form of data analysis, including imaging analysis, is evaluated in terms of its performance in classifying data based on what it has learned during training [266–269]. This evaluation uses metrics such as (among many options) accuracy, sensitivity, specificity, or other measures of deviation from the reference values [270]. A well-performing model achieves high scores across these metrics on training and unseen test data, indicating that it has generalized well [266, 267]. Overfitting occurs when a model learns the training data too closely, capturing noise and details that do not generalize to new data. This leads to high performance on training data but poor performance on test data [269]. In contrast, underfitting happens when a model fails to capture underlying patterns in the data, often due to overly simplistic assumptions, resulting in poor performance on training and test data [269].

Several modifications and evaluations can be attempted to refine the model’s performance, including calibration of the hyperparameters, fine-tuning, and regular evaluation [266].

Hyperparameters are the settings that dictate how the learning process unfolds and control the behaviour of the model, some examples being the kernel function, the layers of a neural network, or the depth of a decision tree (see below) [266]. *Fine-tuning* refers to making minor adjustments to a model’s hyperparameters to improve performance.

Several ML algorithms are used for MSI/HSI analysis, each offering unique strategies to decode the spectral and spatial data. A detailed description and explanation of these algorithms go beyond the purpose of this thesis, and therefore, only a summary will be reported here. Moreover, it is important to stress that image analysis is a vast and ever-developing field; thereby, the attention will be focused on the main algorithms mentioned in the literature review reported in this chapter.

Principal Component Analysis (PCA). PCA is a dimensionality reduction technique that helps identify patterns within high-dimensional data by transforming it into a set of orthogonal (uncorrelated) components. PCA assumes independence between variables, seeking to capture the directions of maximum variance within the data [271]. In hyperspectral imaging, the goal of applying PCA is to reduce the large number of spectral bands into a smaller set of principal components, each representing significant patterns in the data [272].

Hyperspectral images contain hundreds of spectral bands with unique intensity values for each pixel. After normalization and noise reduction, where the spectral bands are scaled and artefacts are minimized, PCA can be used as a pre-processing step to reduce the dataset's dimensionality. This reduction is achieved by identifying eigenvectors and eigenvalues, defining the principal components, and capturing the most relevant spectral features without retaining every spectral band. *Eigenvectors* indicate the directions of maximum variance in the data (principal components); *eigenvalues* measure the importance or weight of each eigenvector in explaining the data's variance. Applying PCA allows for a more manageable and computationally efficient dataset, potentially simplifying subsequent analysis [273].

In the context of brain tumour imaging, PCA has been used to identify significant spectral components that may correlate with tissue properties, such as oxygenated and deoxygenated haemoglobin (Hb), which can aid in distinguishing between pathological and healthy tissue [274,275]. However, PCA is unsupervised and does not classify data into predefined categories. It simply reduces dimensionality, which may result in the loss of subtle features that do not correspond to the largest variances in the data [276,277]. This limitation is particularly relevant in surgical intra-operative imaging, where the subtle spectral differences between tissue types are crucial for reliable prediction [277].

Support Vector Machine (SVM). SVM is a supervised learning model employing algorithms that categorize data into two or more distinct classes. The main goal of an SVM is to find a hyperplane that best separates data points from different classes. This separation is achieved by maximizing the margin between the hyperplane and the closest data points of each class, known as *support vectors*. In cases where data are not linearly separable or data that need to be mapped into a higher dimensional space, such as some hyperspectral dataset, the SVM formula can be slightly adjusted, introducing an element of "tolerance", also known as *soft margin*, and modifying the weight vector with a function called *kernel function*. This mathematical function calculates the similarity between data points in a higher dimensional space [267,278].

There are different types of kernel functions, depending on the complexity of the data [278] - see Figure 3.4.

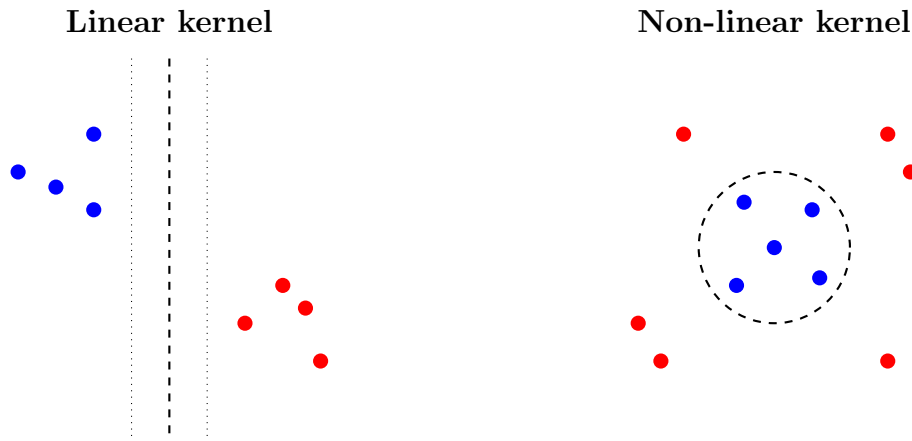


Figure 3.4: Two simplified examples of hypothetical linear (left) and non-linear (right) functions partitioning groups of data

In the context of HSI for surgical imaging, one group has initially used SVM with labelled datasets using a linear kernel [279–283]. However, the same groups subsequently applied a more complex kernel, based on the Radial Basis Function (RBF), as it seemed to improve the performance [284]. In most of these reports, the authors used SVM as a control ML option to test its performance against some more complex ML algorithms, such as CNN [280–283].

The main challenges related to SVM are that it requires careful parameter tuning when deploying non-linear kernel functions, the computation time can be significant with large datasets, and it may struggle with noisy data [285].

Decision Tree. This algorithm constructs a tree-like model where each node represents a decision point based on spectral features, and each branch leads to outcomes corresponding to different material or object classifications [286]. It begins at the root with the entire hyperspectral dataset and splits it into branches based on specific spectral characteristics, such as wavelength intensities. These splits are determined by criteria designed to maximize the homogeneity of the resultant subsets [287]. Each leaf node in the tree represents a classification category, effectively segmenting the hyperspectral image into distinct areas corresponding to different tissues. The number of layers of the decision tree defines the *depth*.

In the case of HSI intra-operative acquisition, a decision tree model can be trained to “learn” the spectral signatures of each tissue type from a labelled dataset and subsequently test the performance on an unseen batch. In a hyperspectral cube containing several classes of tissues

from a neurosurgical procedure, the decision tree starts from the *root node* and proceeds at each layer by setting thresholds that best separate broad groups of spectra from the others.

Decision trees are easy to interpret and understand, making them a good choice for complex clinical settings, including HSI in the context of brain tumour surgery [283]. They can handle both categorical and continuous data. They can be prone to over-fitting, especially in high-dimensional data like hyperspectral images. This could lead to overly complex models that generalize poorly to new data [288].

Random Forest. Random Forest is an *ensemble learning method*, a technique in machine learning where multiple models, often called *weak learners*, are combined to solve a problem more effectively than any of the individual models could alone [289].

A random forest model builds numerous decision trees (the *weak learners*) on different subsets of the training data. Each tree is trained on a randomly selected subset of the data (both samples and features), a technique known as *bagging* or *bootstrap aggregating*. When constructing each tree, the algorithm doesn't consider all the features at each split. Instead, it randomly selects a smaller subset of features to decide the best split at each node. This randomness further reduces the likelihood that the trees will learn the same patterns, increasing diversity within the forest. Once the random forest is trained, it makes predictions by having each tree in the forest "vote" on the outcome. The class with the most votes across the trees becomes the final prediction for classification tasks.

Back to the example of hypercube analysis, each decision tree within the forest attempts to classify the hyperspectral pixels by weighting the spectral signatures with the labelled classes. However, because each tree only uses a subset of data and features, each pixel's path might differ from tree to tree, creating varied classifications across the forest. Once all the trees have classified a pixel, the random forest compares these predictions to make a final decision using majority voting (a process called *aggregation*), where each tree "votes" for a class based on its individual decision. The class with the most votes becomes the final classification for that pixel. For example, if most trees classify a pixel as "tumour", the final decision for that pixel will be "tumour".

The main advantage of random forest algorithms lies in their capacity to reduce overfitting, thereby enhancing the reliability and accuracy of clinical predictions, making them efficient for large, noisy datasets, typical in medical hyperspectral imaging [283, 290]. However, Random Forest models can become computationally expensive with high-dimensional data like hyperspectral images [291].

Optimization Algorithms. Optimization algorithms (OA) are deployed to systematically search for the best solution within a defined problem by maximizing or minimizing an *objective function*, a mathematical function designed to measure the performance of a solution. OAs aim to find the “optimal” set of parameters or variables that give the best possible result.

Several OAs are used to fine-tune model parameters or select relevant features from hyperspectral data.

- *Ant Colony Optimization (ACO)*. ACO mimics the behaviour of ants in finding optimal paths between a source of food and their nest by spreading pheromones. [292]. More specifically, in “the double bridge experiment”, scientists noted that ants were exploiting positive feedback to choose the shortest bridge (from a selection of two options) to a food source. This dynamic inspired the ACO model, where an ant is a virtual agent that moves through a series of nodes (options) to find the optimal paths [292]. The ant’s movement is guided by probabilities based on pheromone levels. Once the ant has completed its path, the pheromone level on each path is updated. Based on spectral similarity, this update reflects how good that path was, helping guide future ants. In the case of hyperspectral images, each ant starts from a random pixel and then moves to neighboring pixels based on the similarity of their spectral signatures. The more similar the spectral data between pixels, the more likely an ant is to move in that direction, thus changing the probabilistic value (the pheromone) of that particular group of pixels. In the relevant literature, ACO has been used for feature selection for hyperspectral images [280]. It has been reported to be particularly useful in selecting relevant spectral bands, improving the accuracy of disease detection. Still, it can be computationally intensive and slow to converge on the best solution [280, 293].

- *Particle Swarm Optimization (PSO)*. PSO has also been inspired by a biological analogy, specifically how groups of animals move together to avoid dangers or towards food sources. In a typical natural environment, a valley might represent the best food source, while the mountain peak could be an area to avoid. In PSO, a swarm of particles move around the data environment, starting from a random spot and attempting to find the best solution to the problem [294]. Each particle updates its speed and direction in each step based on specific rules. Over multiple passages, particles get closer and closer to the best solutions by adjusting their movement according to their own experiences and the group's experiences. In a dataset of spectral signatures linked to different tissues, the goal would be to optimize the spectral bands and imaging parameters to enhance the contrast between different classes (for example, tumour tissue vs healthy brain parenchyma) and identify the best spectral bands that can be representative of the respective tissues [280]. PSO has the advantage of optimising feature selection and model parameters for HSI, which can significantly improve classification performance but can converge to solutions that are satisfactory for the surrounding pixels (*local optima*) but not adequate for the whole dataset (the *global best solution*), requiring fine-tuning hyperparameters [280, 295].
- *Genetic Algorithm (GA)*. GA is inspired by natural selection, using processes like mutation and crossover to evolve a population to adapt to the environment at its best. In GA, the population is the group of possible solutions, and each member of the population has a set of genes that describe its features [296]. Several solutions are tested, the best are selected, and subsequently combined (crossover) or changed slightly (mutation) to find another improved solution. This process is repeated until the ideal solution is found. In the context of hyperspectral data, the task consists of classifying different tissue types based on their spectral signatures. The GA starts with a set of random or simple classification methods (each being a candidate solution), with each solution having different criteria for recognizing tissue types, such as certain combinations of wavelengths or intensities. The fitness of the possible solution is calculated by comparing the results of the GA's classification to the labelled data. The best solutions are chosen, and crossover/-mutations are applied to explore other possibilities. The process is repeated, and after

several rounds, the GA will arrive at an optimized set of classification parameters that can reliably identify tissue types, providing a model that can be applied to future hyperspectral images. As with other optimization algorithms, GA is highly flexible and can find the best possible performance among many, making it useful for feature selection in hyperspectral data. However, GA requires many iterations to converge on a solution, making it computationally expensive for high-dimensional data [280, 297].

Deep Learning. Deep Learning is a subset of ML that uses *neural networks* with many layers (hence, "deep") to learn complex patterns in large datasets.

A neural network is a computational model inspired by the human brain, consisting of interconnected layers of nodes, similar to neurons, to process data, recognize patterns, and make decisions [4, 266]. Each neuron receives inputs, processes them by applying a mathematical operation, and passes on the result to the next layer.

The *input layer* receives the data. Each neuron in this layer corresponds to a feature of the input data (example: blood supply in the example given in Figure 3.5). The hidden layer, sitting between the input and output layers, performs the core of the processing. In *deep neural networks*, there are multiple hidden layers. Each hidden layer extracts increasingly complex features from the data. The output layer provides the final prediction based on the processing done by the previous layers. The parameters that determine the strength and influence of the connections between neurons are called *weights* and *biases*. Weights are adjusted during training to minimize errors in the predictions.

After a neuron computes a weighted sum of its inputs, an activation function determines whether it should activate and pass on information to the next layer.

A neural network typically works using *forward propagation* and *backward propagation*. The data is passed from the input layer through each hidden layer to the output layer (forward propagation). The output layer produces a prediction, which is then compared to the actual answer. This difference is calculated using a *loss function*, which measures how far the prediction is from the correct answer. The network updates the weights and biases based on the

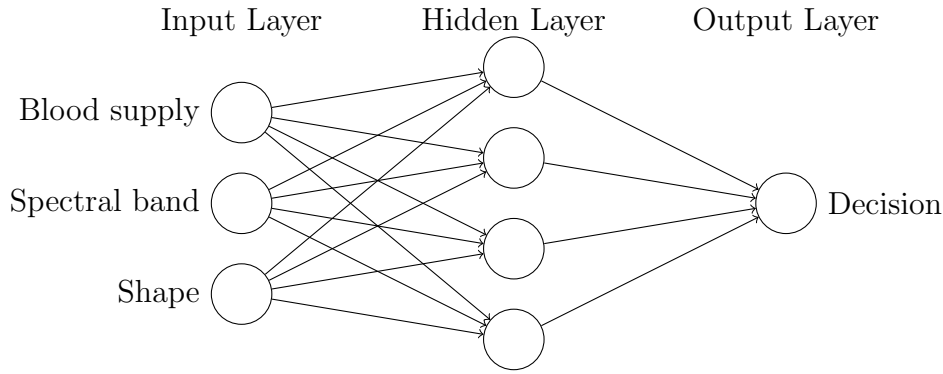


Figure 3.5: Hypothetical simplified schematic of a neural network for spectral imaging analysis. Adapted from Jiang et al. [4].

loss to minimize errors (backward propagation). Using an optimization algorithm, the network adjusts the weights to reduce the loss over many iterations. Through this iterative process, the network learns to recognize patterns.

An example of a neural network is the *MultiLayer Perceptron Classifier (MLPC)*, a network with all the structural characteristics just discussed: an input layer, a hidden layer, and an output layer. The MLPC's hidden layers can perform an operation called *features extraction*, where increasingly complex data patterns are extracted, and it is typically trained through back-propagation [266].

Neural networks can vary in complexity and architecture [298]. A Convolutional Neural Network can process visual data using a mathematical operation known as *convolution*. A convolution is a mathematical operation that combines two functions to produce a third function, highlighting specific features of the input data. In the context of neural networks for hyperspectral images, a convolution operation involves overlapping a small matrix, called a *filter*, over the input data - in this case, the HSI datacube with the relevant spectral/spatial data. The filter detects patterns in the data by looking at small, localized regions at a time [298].

CNNs can be built with several modules, optimization algorithms, and functions to analyse the complexity of the data, which is particularly relevant for hyperspectral analysis [273, 280, 283]. Several authors reported the best predictive power using CNNs when compared to more traditional machine learning algorithms such as PCA, SVM and random forest [195, 281, 283, 299]. However, CNNs require large amounts of labelled data for training and demand significant

computational resources.

3.5 MSI/HSI in neurosurgery: a literature review

Clinical applications of MSI/HSI include colorectal, breast, plastic, and obstetric surgeries [233, 237, 300–302].

In neurosurgery, the clinical utility of MSI/HSI concerns two main applications:

1. improvement of tumour detection;
2. study of the haemodynamic changes after vascular manipulation and injury (either iatrogenic or secondary to the disease) or intra-operative stimulation.

The hypothesis behind the clinical use of HSI/MSI is that the spectral signatures of different tissues can be acquired and labelled to serve diagnostic purposes. Specifically, it can help discriminate between pathological and normal tissue or reflect the degree of oxygen perfusion by indirectly detecting the concentration of haemoglobin and deoxyhaemoglobin.

The present work focuses specifically on hyperspectral/multispectral imaging as it has the potential to become a real-time, cheap, and reliable technology, which can also be integrated into the neurosurgical toolkit without adding additional encumbrance in theatre.

3.6 Objectives

The purpose of the present chapter is to review the existing literature concerning the application of HSI/MSI in neurosurgery. The aim is to report the state of the art of this technology and its potential application as a standard neurosurgical tool for surgical oncology.

3.7 Materials and methods

3.7.1 Inclusion criteria

The rationale of the present search was to focus the literature review on intra-operative HSI/MSI technologies that could be integrated with the neuro-surgical toolkit during procedures on human patients. For this reason, studies regarding the application of HSI/MSI imaging in neurosurgery have been selected on the bases of being in-vivo, intra-operative, and on human subjects. Studies on confocal microscopy, Raman laser, optoacoustic/photoacoustic spectral analysis, or agents-related fluorescence have been excluded unless HSI/MSI were integral part of the acquisition hardware. Studies on neuronavigation and/or 5-ALA fluorescence during neurosurgery have also been excluded, due to the established presence of clear clinical benefit, unless a specific combined application of 5-ALA fluorescence and HSI/MSI was reported. Radiological studies, such as those concerning the use of optic tomography or intra-operative MRI, have also been excluded.

3.7.2 Literature search

The relevant literature search has been performed according to the PRISMA guidelines. The following databases have been used for the search: PubMed, SCOPUS, and Web of Science. On the initial research, simple word association with one Boolean operator was used, such as "hyperspectral imaging OR multispectral imaging AND neurosurgery". One hundred and one studies were retrieved from PubMed using this method, most of them irrelevant to the purpose of the review. Specifically, many papers regarding neuro-navigation, fluorescence, 5-ALA, photoacoustic, and radiological technologies were retrieved and had to be consequently excluded. Similar results were achieved with SCOPUS (53 papers). Using the above-mentioned string, Web of Science gave back 45,112 studies. Therefore, three research strings have been developed using specific MESH terms and Boolean operators to retrieve studies more strictly compatible with the inclusion criteria.

The following strings were used for the final search:

- PubMed - ((((((“multispectral”[Title/Abstract] OR “multispectral imaging”[Title/Abstract] OR “multispectral analysis”[Title/Abstract] OR “hyperspectral”[Title/Abstract] OR “hyperspectral imaging”[Title/Abstract] OR “hyperspectral analysis”[Title/Abstract] OR “optical imaging”[Title/Abstract] OR “diffuse optical imaging”[Title/Abstract]) AND “neurosurgery”[Title/Abstract]) NOT “magnetic resonance imaging”[Title/Abstract]) NOT “neuronavigation”[Title/Abstract]) NOT “MRI”[Title/Abstract]) NOT “5ALA”[Title/Abstract])).
- SCOPUS - multispectral AND imaging OR hyperspectral AND imaging OR optical AND imaging OR multispectral OR hyperspectral OR diffuse AND optical AND imaging AND neurosurgery AND NOT mri AND imaging AND NOT 5-ala AND NOT neuronavigation AND NOT MRI AND NOT optic AND tomography.
- Web of Science - multispectral imaging (All Fields) and neurosurgery (All Fields) or hyperspectral imaging (All Fields) and neurosurgery (All Fields) or optical imaging (All Fields) and neurosurgery (All Fields) or multispectral analysis (All Fields) and neurosurgery (All Fields) or hyperspectral analysis (All Fields) and neurosurgery (All Fields) not MRI (All Fields) not 5-ALA (All Fields) not photoacoustic (All Fields) not magnetic resonance imaging (All Fields).

The following clinical trials registries were also scrutinized: Clinicaltrials.gov (US), European Clinical Trials (EU), German Clinical Trial register, and the ISRCTN registry. The resulting list of papers was cross-checked by the candidate and Dr Maria Leiloglou for the final selection.

3.8 Results

The PRISMA flowchart for the present review is illustrated in Figure 3.6. No automation tools were used for the present review. The PubMed search retrieved 39 papers; further screening

selected 30 articles. The Scopus research retrieved 54 papers. Finally, the Web of Science research retrieved 51 papers. After duplicates removal, two more papers were retrieved. A total number of 34 papers were selected for the final screening. The final selection included only papers where HSI/MSI were used for diagnostic purposes in the context of brain tumour and detection of haemodynamic changes. This was due to the incomplete data of some of the papers retrieved and features of some of the technologies applied, where the labels HSI/MSI were used in the context of near-infrared or standard RGB/white light optical signal applications. The final proposed review presented here includes 20 articles: 15 papers concerning the application of HSI/MSI during tumour resection, and 5 papers concerning the application of HSI/MSI to detect significant haemodynamic changes during neurosurgical procedures overall.

3.8.1 Clinical data

All the studies included in the present series were pilot/proof-of-concept studies. No randomized clinical trial was found, and reported clinical data are often incomplete.

We found seven studies reporting number of patients and images used and the diagnostic performance metrics: sensitivity, specificity, and accuracy, two of which have reported the additional metric of AUC, all of which were used for the meta-analysis. The count of the total number of patients considered for this review needed to account for six papers that used different methods for data analysis on the same dataset, namely the HELICoiD database [284] studies, which included 22 [284], 6 [282], 5 [195], 16 [273], 6 [303], 9 [281], and 16 [280] patients respectively. By analysing these papers as a single dataset, thus considering only the maximum number of patients included (22) and adding the number of patients from the remaining papers, 88 patients overall were included in the present review. The hyperspectral images were either taken from the intra-operative view of a primary brain tumour, specifically gliomas (45 patients) or normal brain cortex (54 patients), while in the remaining cases images from different histological subtypes were acquired. In all cases, a standard craniotomy was performed, and the acquisitions were collected once the dura was opened, with no significant deviations from international protocols regarding cranial neurosurgical procedures. No significant side effect

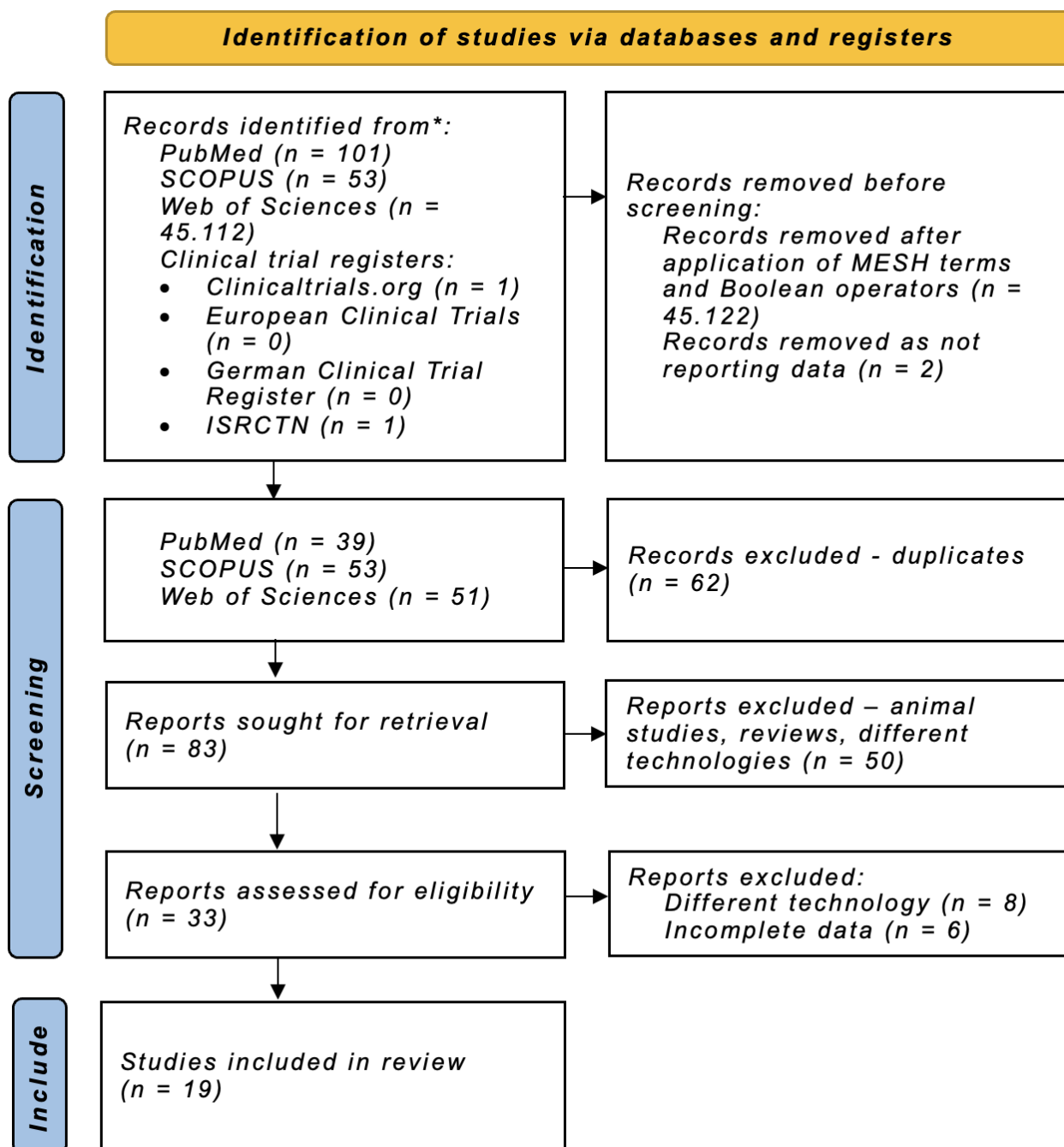


Figure 3.6: Flowchart showing the selection and screening process of the review, as per PRISMA guidelines

was reported, which is partially expected with this being a non-invasive imaging technology. The studies retrieved focused on two main neurosurgical applications for which HSI/MSI was used. The first one is neuro-oncological surgery, specifically for the purpose of better identifying the tumour, its margins, and the normal brain cortex. The second application concerns the detection of haemodynamic changes and perfusion variations in the context of either vascular

neurosurgery or epilepsy surgery. The presented results are discussed referring to this specific categorization.

3.8.2 Technical data

The intra-operative set-up for images acquisition was relatively homogeneous among groups, but the hardware and software used were variable.

The intra-operative set-up for image acquisition was relatively homogeneous among groups, but the hardware and software used were variable, as reported on Figure 3.7.

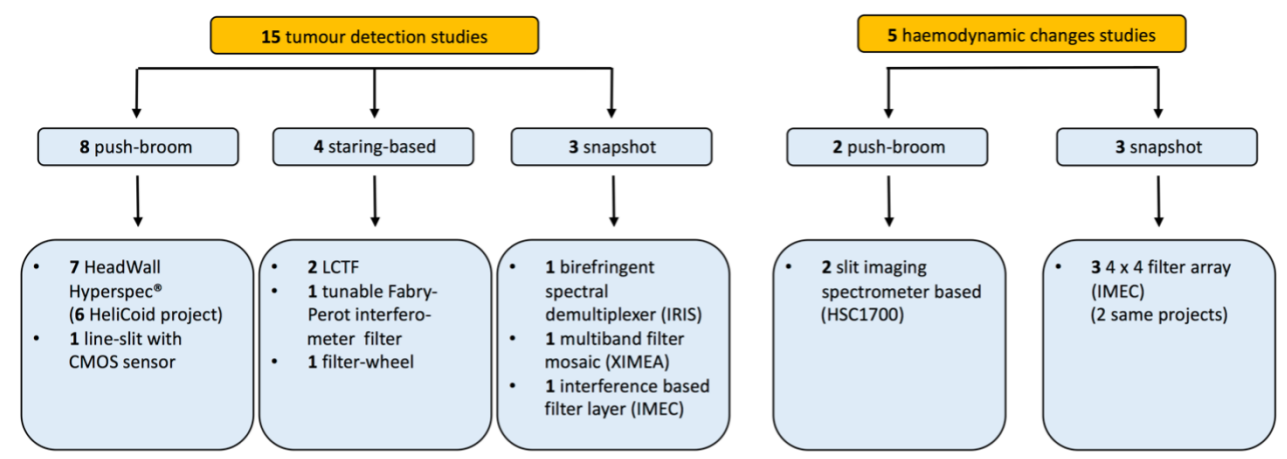


Figure 3.7: Different set-up options found in the current literature - Picture courtesy of Maria Leiloglou.

In most studies, the camera has been integrated onto one of the microscope's ports so that the acquisition would be as similar as possible to the standard intra-operative white light microscope view [238, 264, 290, 304–308]. In the remaining reports, including those from the HELICoiD project, the acquisition was performed through a custom-made scanning platform in an open setting [195, 273, 280–282, 284, 303].

The choice concerning the type of camera used was heterogeneous, with Charged Couple Device (CCD), pushbroom, and Complementary Metal Oxide Semi-conductor (C-MOS) cameras being the commonly used. The spectral range reported also varied, from a minimal of 655 to 975 [283] to a maximal range of 400 nm to 1000 nm in the HELICoiD studies [195, 273, 280–282, 284, 303]. Similarly, the spectral sampling step or interval ranged from a minimal of 0.73nm in the

HELICoiD database [195, 273, 280–282, 284, 303] to a maximal of 45nm [309]. Therefore, most groups acquired bands in the order of hundreds, thus more compatible with the definition of hyperspectral imaging.

Eight [195, 273, 280–282, 284, 290, 303] out of 15 studies for tumour detection have used a push-broom based approach. Six [195, 273, 280–282, 303] of these studies deployed the same system developed for the Helicoid project encompassing the HeadWall photonics Hyperspec© VNIR model. Four out of 15 studies used a staring-based (spectral scanning) approach, out of which, two [238, 307] used an LCTF (liquid crystal tuneable filter), one [264] with a tuneable Fabry-Perot interferometer, and one [309] with a filter wheel for switching spectral bands. Three [283, 306, 307] out of 15 studies used a snapshot-based approach, capturing both spatial and spectral information simultaneously. In these studies, a birefringent spectral demultiplexer (Image Replicating Imaging Spectrometry - IRIS instrument) [306], a mosaic of multiband filters (from XIMEA©) [283], and a layer of interference-based filters (from IMEC©) [307] were deployed to acquire different spectral bands at different sensor locations.

Three [259, 304, 305] out of the five studies for haemodynamic change detection have used a snapshot-based approach with the 4x4 filter array in front of the sensor as implemented by IMEC. Two studies [310, 311], used a push-broom approach with a slit imaging spectrometer camera (HSC 1700).

As mentioned in the “Technical overview” section, the acquired hypercubes require comparison with a “ground truth” image for validation. This is the case for both tumour detection and haemodynamic changes detection, which will be here discussed separately.

Tumour detection studies

In most studies the ground truth consists of a white light picture labelled with the categories of interest. This step is challenging, as it is important to achieve an accurate segmentation so that the model is trained on a reliable ground truth and the adherence with the pre-defined labels is as strict as possible. Segmentation is frequently performed by the neurosurgeons based on

the white light microscope image itself. The HELICoiD project relies on a more sophisticated method based on a semi-automatic segmentation tool. First, after opening the dura, intra-operative fiducial markers were placed on the tumour surface according to pre-operative MRI neuronavigation images [273,280,282,284,303]. The tumour was then biopsied on the position of the marker as an additional confirmation. post-operatively, the reconstructed RGB image was loaded onto a semi-automatic segmentation software. The surgeon selected four image pixels, each point representing one of the specific labelling classes (tumour, brain, vessels, background). The software deployed a Spectral Angle Mapper (SAM) method to segment the rest of the image. SAM is an algorithm where the spectral information is represented as a vector, and pixels with similar spectral values are compared, measuring the differences between their vector angles [312]. A labelling tool for a supervised selection is applied to a reference pixel in the HS image where the neurosurgeon is confident that it belongs to a certain class. Subsequently, the SAM is computed in the entire image, linking the values to the reference pixel, and a threshold is manually established to identify the pixels with the most similar spectral properties to the selected one [280]. Puustinen and colleagues segmented the ground truth images using similar labels to the HELICoiD project, but with a cross-check by two experienced neurosurgeons and removing the background areas from the final analysis [264]. In other cases, the intra-operative view was compared with a pre-operative MRI scan. Specifically, an elegant solution was proposed by Bravo et al. [238], who overlayed the intra-operative view with the corresponding reconstructed MRI image. Other authors adopted the solution of comparing the intra-operative picture with the MRI and segmenting according to the surgeon's experience, but without detailing what criteria were followed to precisely mark the regions of interest [290,307]. Finally, in the two reports of HSI/MSI applied together with fluorescence, the fluorescence measurement and the corresponding comparison with the intra-operative images was performed by overlapping the RGB image with the fluorescence image [308].

Detection of haemodynamic changes

Iwaki et al. used a dedicated pre- and post-bypass SPECT image to evaluate the oxygenation in the regions of interest [310]. Mori and colleagues relied on a visual qualitative assessment

of the surgical field pre- and post-anastomosis [311]. without using a labelling method, and compared that to the oxygenation maps. In the remaining papers, although a manual segmentation of blood vessels and cortex was performed, the ground truth confirmation regarding the haemoglobin changes was achieved indirectly by comparing the timing of the signal with that of the epileptic spikes registered through intra-operative strip or grid electrode registration [259, 304, 305].

3.8.3 Algorithms used for image analysis

Based on the image-processing section of the Technical Table, the image processing pipelines could be broadly divided into three steps:

1. pre-processing;
2. feature extraction or dimensionality reduction;
3. classification.

Tumour detection studies.

The pre-processing routines included correction for dark noise with the use of the dark reference images captured with the sensor of the camera covered. Also, the correction for variable illumination light conditions with the use of the white reference image captured upon illumination of the spectralon reflectance standard target. This step could also include correction for the spectral response of the camera, normalization of pixel values to correct for imaged scene texture/ orientation etc. and removal of extreme bands because of noisy data. The feature extraction routines were mainly dimensionality reduction approaches (for example, Principal Component Analysis (PCA), Ant Colony Optimization (ACO)) to extract a composite set of spectral bands, minimizing redundancy and noise in the data. For cases where the data represented fluorescence, spectral fitting was performed to extract the corresponding fluorophore

(for example PPIX) concentration or fluorescence intensity channels. The classification step has been implemented with:

- Traditional machine learning approaches: a Support Vector Machine approach in six [195, 273, 280–282, 284, 303] studies, Random Forests in two studies [283, 290] and K-Nearest Neighbour (KNN) in three studies [195, 284, 303] for spatial homogenisation of the classification results;
- Fluorescence concentration map or fluorescence signal in four studies [238, 306, 308, 309];
- Deep learning models: convolutional neural network [CNN]-based approaches in four studies [195, 281, 283, 307] and deep neural network (DNN) in two studies [195, 281];
- Spectral angle threshold in one study [290];
- Minimum distance approach where the distance from each of the ‘end-member’ class in one study [282].

Haemodynamic change detection studies

The pre-processing step in these studies was found to be similar with those of the tumour detection studies. Four of the studies [259, 304, 305, 311] used the diffuse approximation fit to assign a per pixel probability to be blood or cortex tissue. This is a process where the tissue’s optical properties are estimated from the hyperspectral data, and a model of how light interacts with tissue, proportional to the tissue composition itself, is applied. In these studies, fitting of the Beer-Lambert law extracted the relative concentration of oxy- and deoxy-haemoglobin. The haemodynamic activity was visualised by extracting the frequency [259, 304] or temporal [305]. Domain variations of the oxy- and deoxy-haemoglobin signals from image regions of interest (e.g. blood vessels). Iwaki et al. extracted the relative change of oxygen saturation SO₂ and used ROC analysis to define a threshold for predicting cerebral hyperperfusion syndrome (CHS) [310]. Mori and colleagues used the retrieved oxy and deoxyhaemoglobin concentrations to calculate oxygen saturation and visualized the latter as a two-dimensional colour map [311].

3.9 Discussion

The present review analysed a total of 20 studies, 15 of which focused on testing HSI/MSI as a tool to refine the intra-operative identification of brain tumours, 5 focused on the detection of haemodynamic changes in the brain during different procedures. Although the technology looked very promising and the reported results were significant in most cases, several challenges and limitations emerged from the updated literature analysis.

3.9.1 The infancy stage of neurosurgical HSI

A small number of studies have been retrieved, and an even smaller number was used for the purpose of the review. There are no multicentric prospective studies or randomized trials available to this day to further evaluate the possibility of applying HSI/MSI in neurosurgery. This is presumably due to a few factors, partially already mentioned: the technical set-up and the technology still being investigated using several different options for analysis. Regarding the set-up, most groups used either a dedicated camera attached to one of the microscope ports, or a dedicated scanning platform, often operated by a separate team. It is safe to assume that both solutions are required to become more integrated into the (already bulky) neurosurgical theatre setting and become more user-friendly to be adequately tested. The software and algorithms used for image analysis were also time-consuming and were used after the surgery. This is also an important limitation: it is clearly necessary to convert the hypercubes into a readable image that can be used as a mask or as a map of probability in real time, so that they can be used for decision making during surgery. Further software engineering and algorithm implementation are required to overcome this obstacle. The most important limitation of the technology (hardware and software) includes all the above: the lack of a real-time or quasi-real time system. In an ideal neurosurgical setting, the hypercube acquisition should take a fraction of a second, and so should the algorithm analysis. This way, hypothetically, a probability mask highlighting the tumour areas, the tumour margins, and the normal brain could be produced rapidly and overlap the surgical view. As discussed, several implementations are still to be

addressed to both standardize the technology and to provide results more quickly.

3.9.2 The challenge of reliable ground truth

The challenging step of obtaining a ground truth for brain imaging analysis is that the tissue cannot be confirmed histologically by random sampling like in other districts of the human body. This resulted in heterogeneous segmentations in most cases, and it could have easily impacted on the good performance of the models. In three of the retrieved studies, the pre-operative MRI head has been taken as ground truth to process the signal [290, 307, 310], but only one group pursued a direct comparison by using a dedicated software [310]. In other cases, a fitting model was built so that the categories could adequately segregate when compared with the MRI signal, and the authors have indirectly stressed the challenge of training the model by choosing to mark as “healthy cortex” pixels that were adequately distant from the tumour core [290]. The need for a homogeneous approach regarding the ground truth source is crucial for the reliability of the whole technology and needs to be addressed with further studies. There is lack of consensus or standard on how many labels to use and how to exactly segment infiltrating primary brain tumours, which can be problematic without an objective definition of tumour core, margins, and unaffected cortex.

3.9.3 Study heterogeneity

The reported studies proved to be quite heterogeneous both in terms of set-up, data collection, and image analysis algorithms used. Different groups have proposed different algorithms for images analysis, especially for tumour detection. Specifically, SVM, CNN, decision tree, random forest or even least-square regression were used and, in the case of the HELICoiD project, even compared in terms of specificity, sensitivity, and accuracy. Moreover, five out of these seven studies worked on the same dataset, which is an important limitation.

3.9.4 Haemodynamic changes

Different optical imaging technologies have been used to study the possibility of haemodynamic changes, including simple RGB imaging and fluorescence. HSI/MSI has also been considered by multiple authors, but most of the reports are still in the preliminary phases and lack data for a more in-depth analysis. Results regarding different degrees of perfusion and haemodynamic changes in the brain during neurosurgery are extremely promising, but image processing is even more challenging. The first and obvious problem is that only four studies were included in the present review, as per the above-mentioned problem of limited data available in many of the excluded reports. Three of the included studies [259, 304, 305] based their analysis on the Beer-Lambert law principle, which establishes a proportional correlation between the amount of reflected light and the concentration of chromophores in the illuminated tissue. In the other study the authors simply established regions of interest (ROI) comparing them with MRI and SPECT data and classified the spectral signature accordingly [310]. Different approaches for data analysis have been followed, with the main limitations being that the pool of patients included was small, and the accuracy metrics were not reported.

3.9.5 Limitations of the present review

The main limitation of the present review is that no automatic screening tools were used. This might have affected the precision of our search, and consequently it is possible that some of the relevant papers have not been found. The nomenclature regarding this technology is also relatively heterogeneous, with the distinction between MSI and HSI still loosely defined and several papers using equivocal terms (such as “optical imaging”). On the other hand, considering that the application of hyperspectral imaging in neurosurgery is still in its infancy and that automation tools are often problematic in terms of flexibility, we believe that the risks of excluding relevant papers has been relatively contained

3.10 Conclusions and rationale for further studies

Multispectral and hyperspectral imaging are promising tools that have shown the potential to be integrated into the neurosurgical toolkit for both tumour resection and detection of haemodynamic changes. However, the present review shows that the technology is still not real time, and that the image analysis process needs to be more homogeneous. The hardware requires more standardized implementation, with a faster set-up, and to become more user friendly, whereas reliable software with built-in image analysis algorithms need to be produced. Further studies on larger cohorts of patients, producing more extensive databases of hypercubes for training/testing purposes are therefore desirable for hyperspectral imaging to become a clinical standard in neurosurgery.

Chapter 4

Hyperspectral and multispectral imaging in neurosurgery

4.1 Materials and methods

4.1.1 Set-up: hardware and connections

The first stage of this study was dedicated to adapting the HSI system to the neurosurgical toolkit available at Imperial College Healthcare NHS Trust. This phase took place from December 2018 to October 2019.

As mentioned in Chapter 1, the theatre set-up includes one out of three available neurosurgical microscopes (two Zeiss Pentero© and one Leica©) and an endoscope.

The available multispectral camera systems were previously adapted to the endoscope due to previous laparoscopic studies [233]. Therefore, it needed to be adapted to the microscope ports. The choice to adapt it to the surgical microscope has been driven by crucial logistical factors:

1. the acquisition through the available camera allows for a more precise resolution when adapted to a port;

2. the microscope is by far the most commonly used visual tool to resect intracranial brain tumours.

The complete list of the system components used in the present study is explained in Table 4.1.

Components		
Name	Position	Function
<i>Xenon light</i>	Built into the microscope	Illumination
<i>Coupling device</i>	Connected to the surgical microscope's port	Aligns light beams
<i>CCD Camera</i>	Fixed on the LCTF device	Acquisition component
<i>CMOS Camera</i>	Fixed on the LCTF device	Acquisition component
<i>LCTF</i>	Connected to the coupling device and the laptop	Filters the white light source dividing it into single spectral bands
<i>Laptop</i>	Connected to the CCD/CMOS camera and the LCTF	Main hardware for data storage

Table 4.1: Components of the HSI/MSI system deployed for the present project

4.1.2 Microscope and light source

Two surgical microscopes have been used for the present studies, both Zeiss Pentero©, although different versions. These microscopes are currently used worldwide as the standard of practice. A detailed description of the microscope components goes beyond the scope of the present thesis. However, an excellent overview has been provided by Ling Ma and colleagues [5].

In summary, current surgical microscopes are equipped with a light source and a system of lenses arranged to convey binocular vision that reproduces the 3D view of the surgical field to the observer.

The Zeiss Pentero© model, specifically, is equipped with four ports. The first surgeon typically uses the main port with two handles on both sides. The handles allow the head of the microscope to be moved in any direction, including the switches for zoom, focus and blue light. The main objective needs to be calibrated (weight balanced) once switched on as the first task before the whole system is used. The remaining three ports are for the assistant, but only two ports

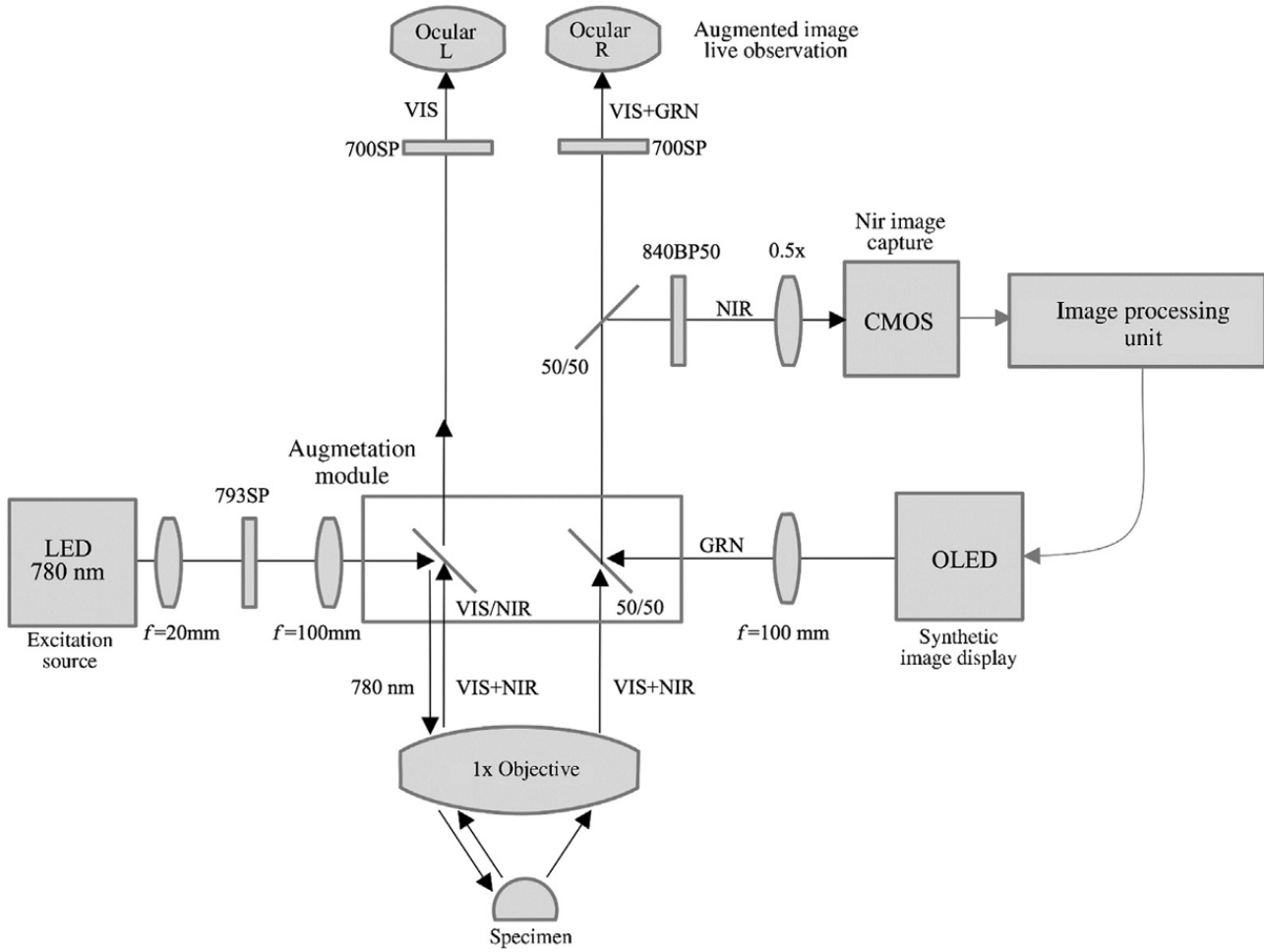


Figure 4.1: Schematic of the surgical microscope from the work of Ma and colleagues [5]

(including the main one) can be used simultaneously. Ports can be switched to accommodate the assistant surgeon to the proper position. The light source is incorporated into the main body of the microscope and is far from the surgical field, so no heating is delivered. The main objective module is connected to the body through a flexible arm. Such an arm not only conveys the light to the operator and the visual information to the main body but also connects the optical system to a built-in computer for data storage, specifically videos and image registration. Finally, the draping system of the microscope reaches the entire length of the microscope arm (see 4.3) and allows for surgical manoeuvres in a sterile environment.



Figure 4.2: Surgical microscope used for the present study, the Zeiss Pentero©, here draped and ready to be used



Figure 4.3: Draped microscope used during a craniotomy

4.1.3 CCD and CMOS cameras

We used a CCD and a CMOS camera. Chapter 3, section Spectral scanning, reports an overview of the main features.

Our CCD camera was a ThorLab© DCC22 model with a resolution of 1280 x 1024 and a maximum frame rate of 30 per second.

The CMOS camera was a ThorLab© DCC326 model, with a resolution of 1936 x 1216, with a maximum frame rate of 60 per second.

4.1.4 Liquid Crystal Tunable Filter - LCTF

The LCTF used for this project was a Varispec© filter built by Cambridge Research & Instrumentation Inc. It was connected to the microscope adapter through a General Purpose Interface Bus (GPIB) cable and to the laptop through a Universal Serial Bus (USB) 1/2 cable, as shown in Figure 4.4.

The microscope port and the camera port have different circumferences. Moreover, the camera must perfectly align with the port exit to avoid visual field restrictions. For this purpose, a Thorlabs© adapter coupling the microscope with the camera and the LCTF cable was used and adapted to the microscope port. The adapter was 3D printed at the Hamlyn Centre of Robotics and was part of the MEng project that the candidate of the present work has supervised (see Acknowledgments).

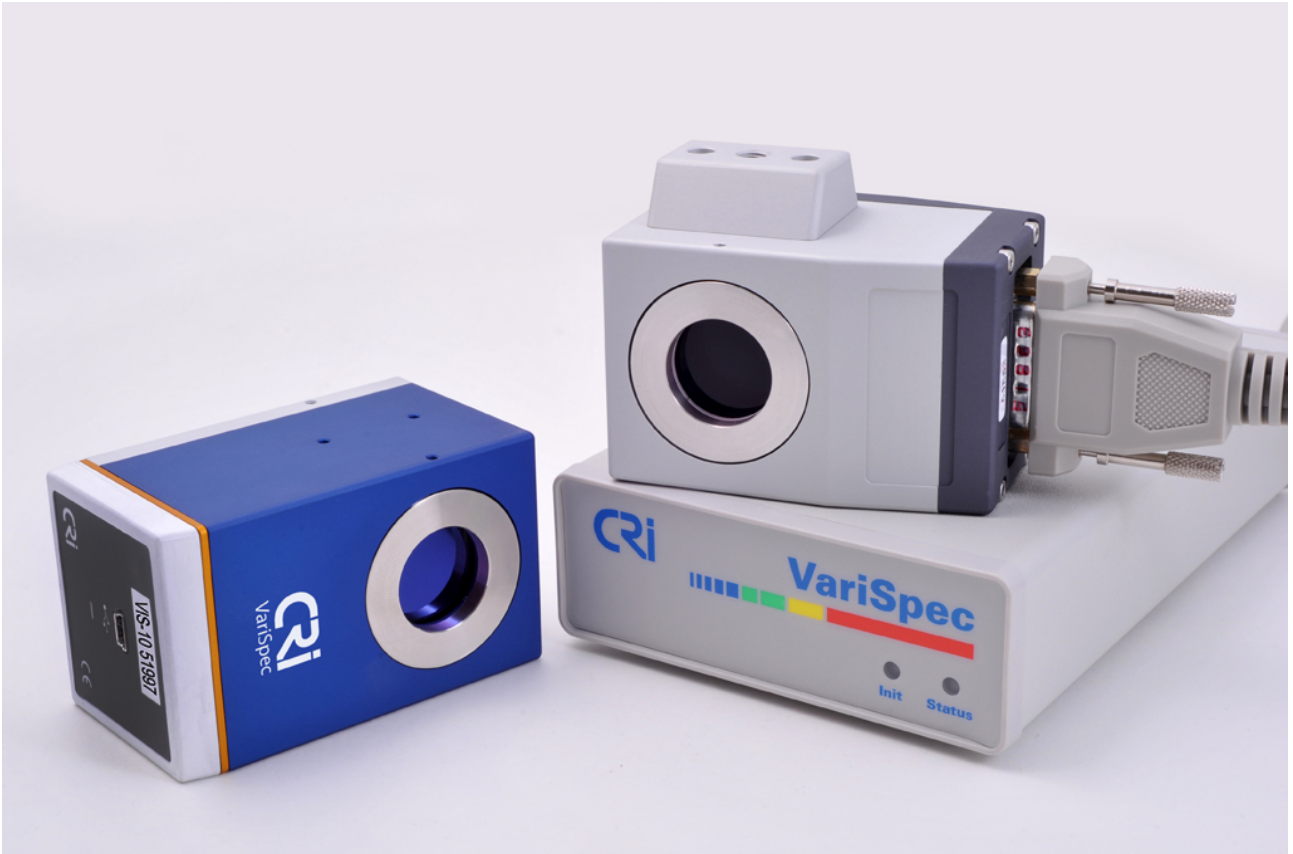


Figure 4.4: VariSpec© LCTF (two versions, bottom left and top right) with its control box. This is the filter used for the present study



Figure 4.5: Adapter connecting the Pentero port with the LCTF and the camera

4.1.5 Laptop and data storage

As mentioned, the CCD camera and the LCTF were connected to a Laptop through a USB-3 / USB cable and a GPIB / USB cable, respectively. The laptop used for the present study was a Hewlett Packard (HP) 6.2 Pavilion G6©, and the program used for the image acquisition was LabVIEW©, a C++-based software from National Instruments Corp. This program was suggested by the supervisor, Professor Dan Elson, as it was previously used in similar studies [237], is reliable, and user-friendly with a steep learning curve.

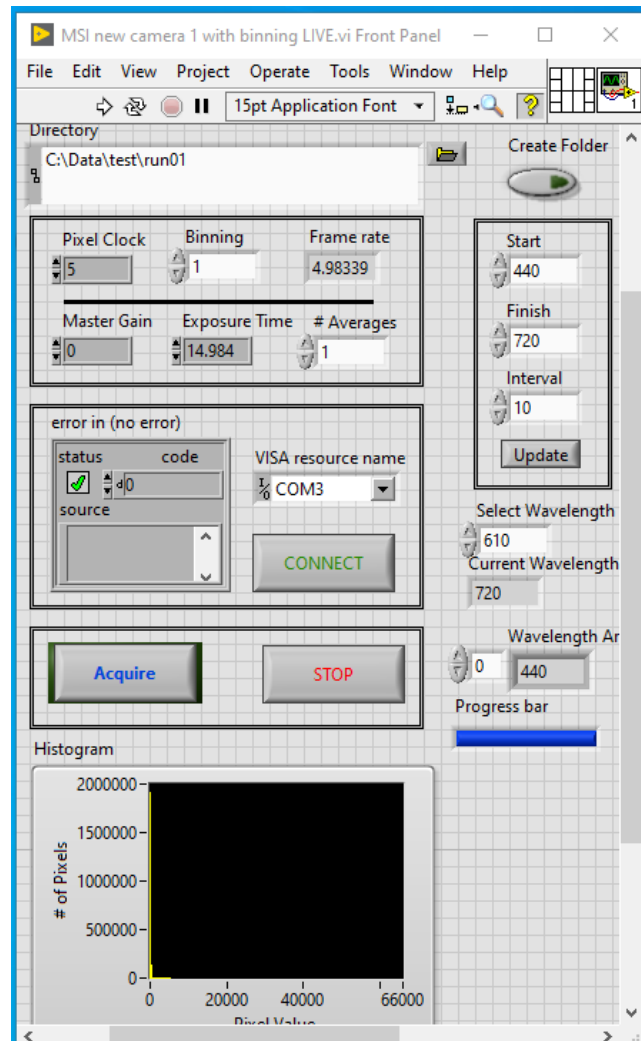


Figure 4.6: LabVIEW© interface showing the main features

The main features seen in Figure 4.6 are:

- Directory - on the top, the specific destination folder to save the hypercubes can be set up in advance using the "Create Folder" button and establishing the pathway into the

relevant box;

- Averages - number of single images acquired per band (ex: at a band of 450 nm, two averages correspond to 2 images at 450 nm);
- VISA resource name - the port to be selected to make sure the images are acquired;
- lateral box - "start", "finish", and "interval" define the wavelength spectral range (from 440 nm to 720 nm in this case) and the interval (10 nm between one image of the hypercube and the other).

During image acquisition, the program allows the camera's image stack to be stored in dedicated folders that can be pre-created or created directly from the main LabVIEW interface.

I have, therefore, created our database and stored our anonymized data on this College laptop for future analysis. The ethics application describes the anonymisation process in detail in the following section.

4.1.6 Ethics application

The ethics application was submitted through the dedicated Integrated Research Application System (IRAS), the platform for applying for clinical research studies in the United Kingdom and submitting the relevant documentation to the Human Research Authority (HRA).

Before submission to the ethics committee, the Imperial College Joint Research Office (JRO) supported the clinical team to draft the relevant study protocol (see below) and insurance documents.

The application received approval from the South Central—Berkshire B Research Ethics Committee, on behalf of the HRA, on 04/02/2020, REC reference 19/SC/0583, IRAS ID 258210.

The original plan was to recruit patients for three years, ending the study in February 2023. This proved impractical due to the 2020 global COVID-19 pandemic, which effectively stopped

any non-COVID-19-related research study in the Trust for 12 months (see Results section). Therefore, an extension was requested to October 2023.

4.1.7 Study design

The present study aimed to investigate the potential application of hyperspectral imaging during neurosurgical procedures, specifically during brain tumour resection. The research procedure consisted of real-time image acquisition and a second, separate data processing stage.

The study aims were as follows:

1. to detect structural differences between brain tumour tissue and the surrounding brain;
2. to detect structural differences between eloquent/functional and non-eloquent/non-functional brain areas

The study was designed as a pilot/proof of concept study. The data collection was prospective, and the study was foreseen to last three years.

The planned study sites were Imperial College Healthcare NHS Trust—Charing Cross Hospital for data collection and Imperial College London—South Kensington for data analysis. As per the power calculation (see below), our target for patient recruitment was 50.

4.1.8 Outcome measures

The primary outcome measure considered was the differentiation between tumour, tumour margins, and normal brain tissue during surgery for brain tumours in terms of sensitivity, specificity, and overall accuracy.

The secondary outcome measure identified haemodynamic changes during brain tumour surgery, either in task execution in awake patients or neurophysiological stimulation in anaesthetized patients.

For this purpose, I planned to compare the images acquired with pre-determined ground truth (see the relative section below), labelling the different Regions Of Interest (ROI) according to the current standard, and calculate the metrics as follows:

- True Positives (TP): positive ROI identification as established by the conventional diagnostic capabilities and positively predicted by the specific algorithm chosen for image analysis;
- True Negatives (TN): negative ROI identification as established by the conventional diagnostic capabilities and negatively predicted by the specific algorithm chosen for image analysis;
- False Positives (FP): negative ROI identification as established by the conventional diagnostic capabilities but positively predicted by the specific algorithm chosen for image analysis;
- False Negatives (FN): positive ROI identification as established by the conventional diagnostic capabilities but negatively predicted by the specific algorithm chosen for image analysis.

The calculation regarding sensitivity (SEN), specificity (SPE), positive predictive value (PPV), negative predictive value (NNV), and accuracy (ACC) was performed as defined in Chapter 1.

The algorithms used for image analysis built a confusion matrix by comparing the pixel values in the mask obtained from the labelling (the ground truth) with those from the hypercube.

The original ethics application also included the acquisition of images through MSI/HSI during fluorescence imaging, specifically the blue light provided by the Zeiss Pentero microscope after administration of 5-AminoLevulinic Acid, a contrast agent commonly used for detection of brain tumour margins (see Chapter 1). However, this outcome measure was removed as the MSI/HSI acquisition was not sensitive enough to detect significant visual changes when applied to a blue light field.

4.1.9 Power calculation

Given the assumptions and estimations made for this complex study, the goal was to determine the number of patients needed to achieve statistical significance for different diagnoses and detect haemodynamic changes in the brain. The hypotheses were:

- **Null Hypothesis (H0):** Hyperspectral imaging does not significantly differentiate between tumour and normal brain tissue or between active and inactive brain areas.
- **Alternative Hypothesis (H1):** Hyperspectral imaging significantly differentiates between tumor and normal brain tissue or between active and inactive brain areas.

Type I and Type II errors were defined according to international literature:

- **Type I Error (False Positive):** The null hypothesis is true but is incorrectly rejected.
- **Type II Error (False Negative):** The null hypothesis is false but is incorrectly accepted.

The Effect Size (ES), defined as a measure of the magnitude of the expected difference, was cautiously estimated to be between 0.05 and 0.15. The significance level (α) was set at 0.05, and the power ($1 - \beta$) was targeted between 80% and 90%. Variability or standard deviation (SD, σ) was estimated at around $\pm 5\%$. Cohen's coefficient (δ), expressed as the ratio between the ES and the SD (σ), was used for sample size calculations:

$$n \approx \left(\frac{Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}}{\delta} \right)^2 \times 2 \quad (4.1)$$

The formula 4.1 was taken from the work of Zhang and colleagues [313]. In our case, it was particularly challenging to estimate the correct ES, given the minimal data available in the body of literature in 2019, when the study started [195,273]. The sample size calculations, with the corrected Cohen's δ , for the given examples, were:

- For $ES = 0.1$ and $\sigma = 0.05$, the calculated δ was 2.0, with an estimated sample size of approximately four patients per group - I have considered it inadequate compared to the existing data ([195,273] and discarded it.
- For $ES = 0.05$ and $\sigma = 0.1$, the calculated δ was 0.5, with an estimated sample size of approximately 63 patients per group - this result was considered plausible and in keeping with the limited literature evidence [195,273]
- For $ES = 0.1$ and $\sigma = 0.1$, the calculated δ was 1.0, with an estimated sample size of approximately 16 patients per group.

Aside from the power's calculation, some practical limitations related to the NHS challenges and the epidemiological data (brain tumours are uncommon in the general population, and not every brain tumour is treated through surgery [2]) had to be considered. A total pool of 50 patients was established after discussion with the ethics committee, expecting to recruit 30 ± 5 gliomas, 10 ± 5 meningiomas, and 10 ± 5 patients with other histological subtypes.

4.1.10 Patient recruitment

Patients were approached about this study at their first neuro-oncology clinic consultation. A research team member who was also involved in direct care was present at the consultation and explained in detail the study's purposes and methods. At this point, the patient was given a detailed Patient Information Sheet (PIS).

I have obtained all the patients' consents at preoperative assessment or in a private room in the admissions lounge, clinic, or hospital ward at Charing Cross Hospital. The consultation length was expected to vary depending on the complexity of the clinical procedure planned. Surgery was usually scheduled at least a few days after the clinical consultation, in most cases a few weeks after, and in a minority of cases, some months after. The consent form for research was signed within the day before the surgery, thus allowing sufficient time for questions and personal research on the topic.

4.1.11 Intra-operative data collection

The system was assembled before every operation described in Section 1 - Set-up. The system (microscope + connected camera) was draped using sterile drapes per standard neurosurgery procedure.

The patients who agreed to participate in the study were admitted to Charing Cross Hospital, Neurosurgery Department, the day before the surgery, per standard NHS practice. Except for further discussion of the study details and signing the consent form for research, no additional tests were required for the patients other than those for standard NHS clinical care.

The procedure was carried out as expected per the clinical indication on the day of the surgery, which was performed in Charing Cross Hospital. I was responsible for data acquisition in every operation of the present series and was the primary surgeon in most cases.

The patient was anaesthetized and placed on the surgical table. The WHO checklist and the operating procedure were performed per standard NHS practice. The operating surgeon used the standard NHS neurosurgical equipment described in Chapter 2 during surgery. During each surgical intervention, a variable number of spectral data were collected at specific stages detailed in the research protocol (see below, subsection Data Acquisition)

All imaging data were completely anonymized before being provided to the researchers at the Imperial College London. From a clinical perspective, I have also collected:

- clinical data such as diagnosis, age, sex, clinical status, and medical background;
- radiological data, specifically MRI head pre- and post-op
- histological data, specifically diagnosis and molecular profiling

Surgery was conducted following standard NHS procedures.

4.1.12 Anonymization process

To ensure anonymization, each patient was given a specific protocol number—ID log. I performed anonymous image acquisition to sync spectral data collected with the surgical procedure and link it with the ID log. These data were stored in secure Imperial College Computers and College Servers, accessible only by research team members and under password protection.

Regarding the clinical data, these were stored on NHS Trust Encrypted computers in a semi-anonymized form and transferred onto College Computers in a pseudo-anonymized form for study purposes. NHS computers follow the NHS Trust confidentiality policy and comply with the Data Protection Act. Semi-anonymization means these data include NHS, hospital number, age, and protocol numbers. When needed, pseudo-anonymization was performed before transferring clinical data to College Computers. Pseudo-anonymization means that these data did not include any identifiable information, including NHS number and hospital number, but only the protocol number – ID log.

According to the College retention policy, Personal Identifiable Data (PID) will be stored for ten years.

The signed consent forms were archived inside the patient’s medical records and will be available for audit and research quality control activities. All patients have been given a copy of the signed consent form. A Trial Master File (TMF) containing hard copies of the consent forms has been kept in the Neurosurgery Department in Charing Cross Hospital 3 South Corridor (offices). Entry into these facilities is restricted and controlled in and out of hours; only staff members have access. No other copies were made or retained.

4.1.13 Protocol for images acquisition

Intraoperative acquisition aimed to capture several phases of the tumour resection and a relatively wide range of possible cases so that the hyperspectral system could be applied to a diverse cohort of images.

For this purpose, I have established the acquisition protocols to capture images of tumours, brain tumour interface, and haemodynamic changes in the context of either neuro-stimulation or task execution during awake surgeries.

In a standard tumour resection case, without neuromonitoring, I aimed to perform acquisitions at three stages (see Figure 4.8):

1. at the level of tumour exposure;
2. halfway through the resection;
3. of the surgical cavity;

The number of acquisitions to perform per stage was deliberately not specified, as I predicted that it would vary depending on the degree of exposure, light intensity, and specifics of the single case. However, I aimed at setting the microscope light intensity between 70% and 100%, as the Zeiss microscope has an automatic adjustment feature linked to the zoom function. I have attempted to maintain luminosity at 80% with an acquisition distance of 35 to 45 cm in all cases.

In a tumour resection case with neuromonitoring, the acquisition protocol was slightly modified, adding acquisitions pre- and post-stimulation or pre- and post-task execution to detect potential haemodynamic changes. Specifically:

- 1a. tumour exposure, no stimulation nor task execution;
- 1b. tumour exposure, active stimulation or task execution;
- 2a. $\approx 50\%$ of resection performed, no stimulation nor task execution;
- 2b. $\approx 50\%$ of resection performed, active stimulation or task execution;
- 3a. final surgical cavity, no stimulation nor task execution;
- 3b. final surgical cavity, active stimulation or task execution.

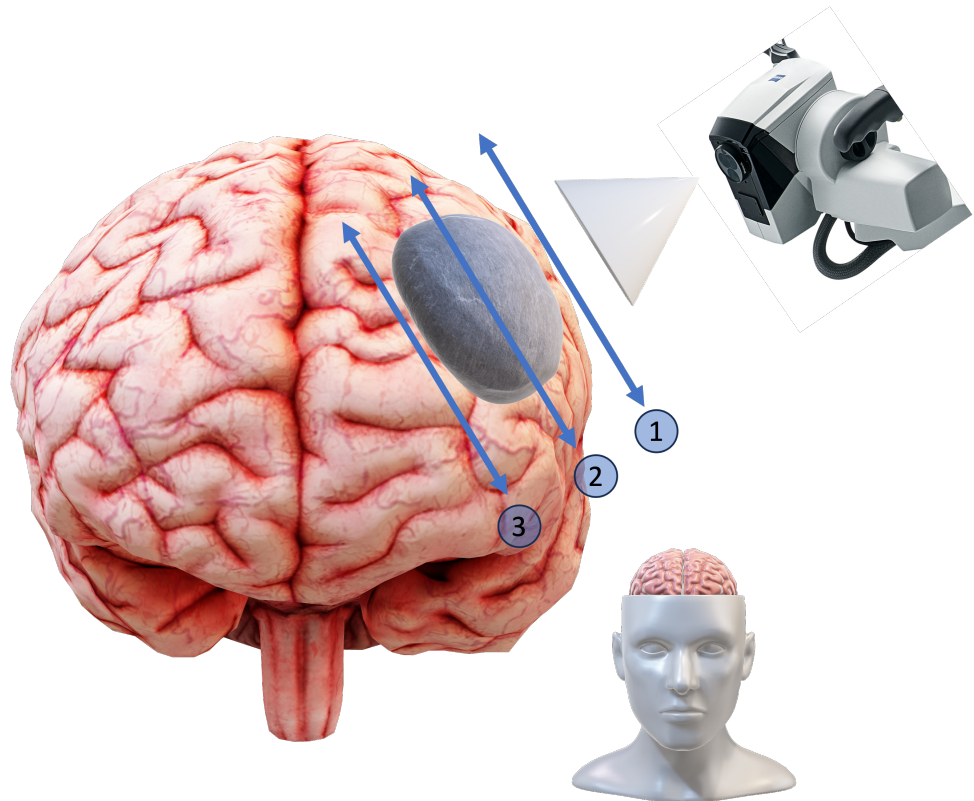


Figure 4.7: Stages of datacube acquisition during surgery for a brain tumour in a non-eloquent area. These steps were the ones I was interested in acquiring, according to both my experience, our firm’s track record, and the reported literature: 1) early cortical / tumour exposure; 2) midway through resection (approximation); 3) end of the resection / final surgical cavity.

Practically, and as explained in detail in the results and limitation sections, strict adherence to this specific protocol was rarely possible due to a number of factors, case and/or time-specific.

For each acquisition, an RGB microscope snapshot was taken to be used as a ground truth for image segmentation and to train the models. This process was not always followed due to logistical and technical issues with the microscopes available (see results and limitations sections).

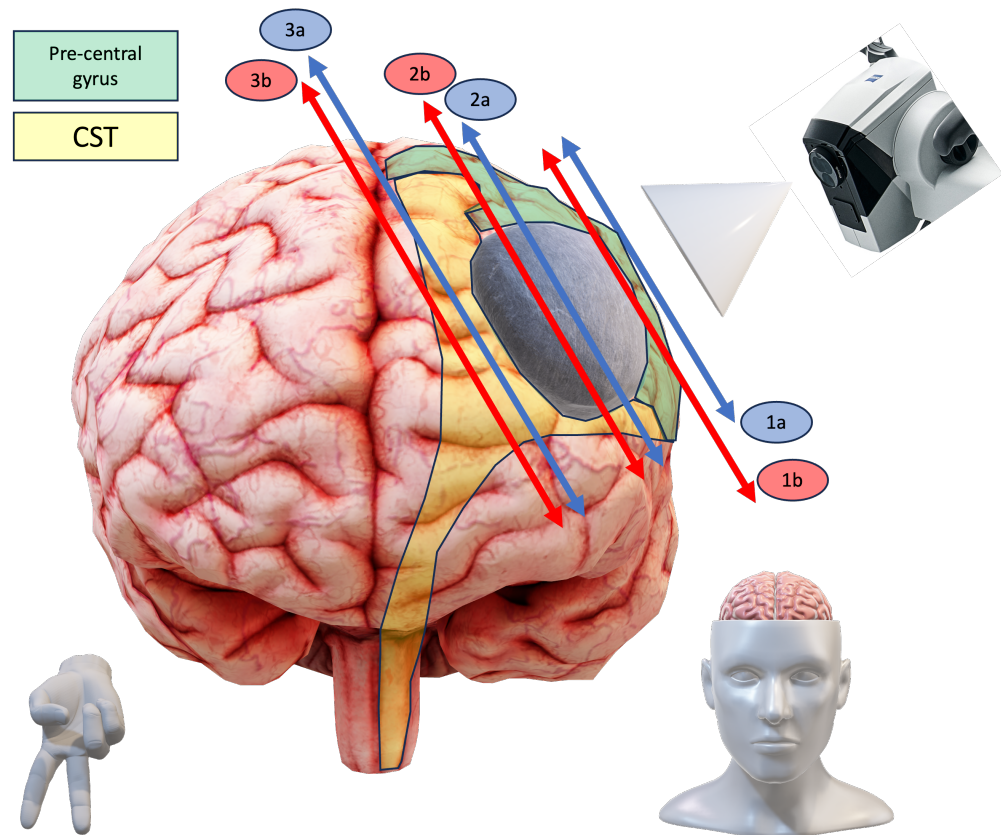


Figure 4.8: Stages of datacube acquisition during surgery for a brain tumour in an eloquent area. The pictured example is a schematic of an awake surgery during a left motor strip tumour resection, and the patient is asked to move the corresponding hand (right side). Again, these steps were the ones I was interested in acquiring, according to both my experience, our firm's track record, and the reported literature: 1a) tumour exposure, no stimulation nor task execution; 1b) tumour exposure, active stimulation or task execution; 2a) 50% of resection performed, no stimulation nor task execution; 2b) 50% of resection performed, active stimulation or task execution; 3a) final surgical cavity, no stimulation nor task execution; 3b) final surgical cavity, active stimulation or task execution. CST = Cortico-Spinal Tracts.

4.1.14 Image labelling - Ground truth

One of the main obstacles to assessing the diagnostic reliability of HSI / MSI is to have a ground truth to test machine learning and deep learning algorithms. This is due to the intrinsic limitation of the tissue analyzed: in a human, in vivo, intra-operative study, the confirmation of the visualized tissue cannot be extrapolated from multiple histological biopsies from the different tissues visualized. This could be possible for the tumour core, which needs to be resected in the first place. Still, it is rarely possible for tumour margins, and it is ethically unacceptable for a normal brain, even if not eloquent.

To overcome this problem, some authors have compared the intra-operative acquisition with the pre and post-operative MRI scans using a sophisticated merging software [238]. This has also been our choice for most cases. However, I have manually performed the comparison, which was cross-checked by three neurosurgeons (the candidate, Mr Neekhil Patel, and Mr Kevin O'Neill). Specifically, the internal Imperial College Healthcare NHS Trust PACS Carestream© system was used to obtain a realistic 3D reconstruction of the case analyzed based on the MRI images. The MRI scan sequences were compared with the intra-operative microscope snapshot when available or with the registered image when the microscope snapshot was unavailable. I acquired and reconstructed the MRI images from the pre-operative MRI scan for the initial acquisitions (1 acq in Figure 4.7). In comparison, the post-op MRI head was used for the acquisitions taken at the end (2 and 3 acq in Figure 4.7) as it better corrected for midline shift and surgical cavity collapse.

After selecting the appropriate MRI images for comparison, a dedicated labelling software was deployed to segment the images for the specific acquisitions. The aim was to label tissues recognized by the surgeon and identified through the MRI signal and produce an image to be used by the model for subsequent training and testing.

Two main segmentation protocols were established, with different labels for specific tissues depending on the type of case examined. Labelling was established to maximize the reliability of the models used for the data analysis, specifically aiming to avoid overfitting and improve

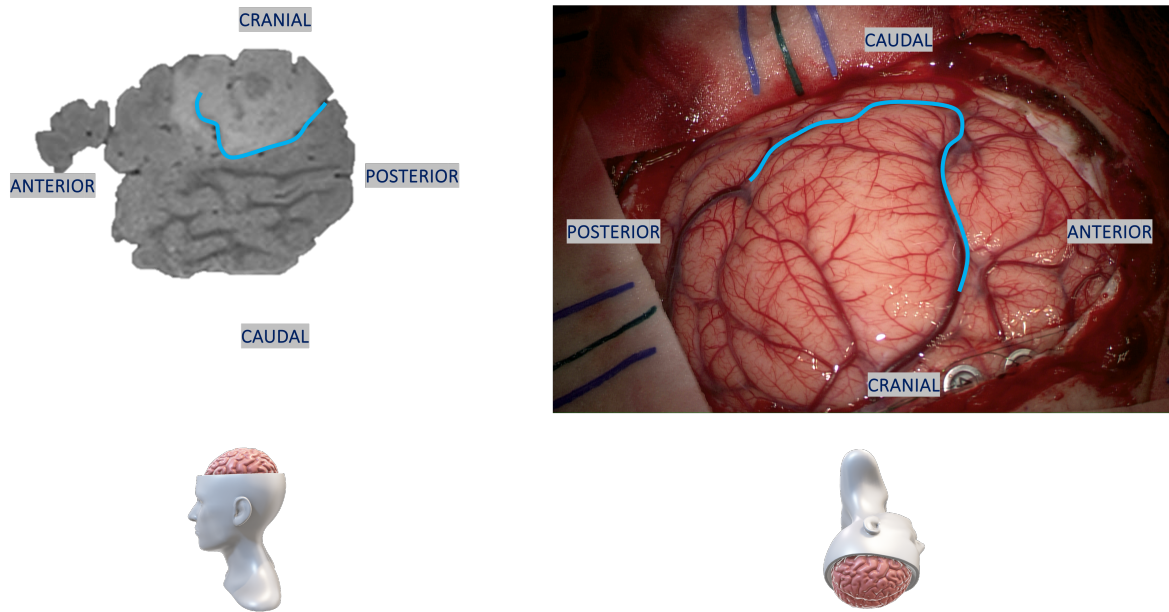


Figure 4.9: Methodology for image segmentation on one of the cases from our series - HS009. On the left is a sagittal MRI FLAIR reconstruction where the anatomical landmarks are identified and marked. On the right, the intra-operative snapshot is used for image segmentation - note the U-shaped sulcus with the draining veins used as an anatomical landmark.

Overall Accuracy (OA).

Therefore, for brain gliomas and metastases, the following labels were used:

- **tumour core** - the main bulk of the tumour confirmed both by radiological investigations and intra-operative surgeon's impressions;
- **tumour margins** - areas around the tumour that show some degree of signal change on MRI imaging (see below for further details);
- **normal grey** - brain surface around the tumour not affected by any signal change or anomaly detected either during surgery or on the pre-op MRI;
- **normal white** - deep white matter around the tumour not affected by any signal change or anomaly detected either during surgery or on the pre-op MRI;
- **blood vessels** - arteries and/or veins;

- **blood** - bleeding coming from either the margins of the surgical field or from the tumour resection itself;
- **arachnoid** - layer of soft meningeal tissue (leptomeninges) often present at the level of clefts/gyri of the cortical surface.

The label "tumour margins" was not used in the clinical sense of the term. The margins of glioblastoma are traditionally referred to as its enhancing capsule. In contrast, in exophytic low-grade gliomas, this definition refers to the identifiable peripheral area that separates the tumour from the normal grey/white matter. In the present series, "tumour margins" refer to the signal change around the tumour mass, suggesting infiltration or inflammation affecting the healthy brain. The potential role of HSI/MSI in intra-operative identification of this signal change, seen on the MRI scan but not detectable with the naked eye, is one of the primary outcome measures evaluated by the present study.

For meningiomas, a slightly different set of labels was used:

- **dura** - all the dura mater around and on top of the tumour, where the tumour usually arises;
- **tumour core** - main bulk of the meningioma confirmed both by radiological investigations and intra-operative surgeon's impressions;
- **normal grey** - brain surface around the tumour not affected by any signal change or anomaly detected either during surgery or on the pre-op MRI;
- **normal white** - deep white matter around the tumour not affected by any signal change or anomaly detected either during surgery or on the pre-op MRI
- **blood vessels** - arteries and/or veins;
- **blood** - bleeding coming from either the margins of the surgical field or from the tumour resection itself;

- **arachnoid** - layer of soft meningeal tissue (leptomeninges) often present at the level of clefts / gyri of the cortical surface.

The label "tumour margins" was removed for meningiomas and replaced with "dura" because, clinically, meningiomas do not usually invade the brain but the dura, except in the rare Grade III.

A critical decision was also needed regarding the segmentation of the blood vessels, as the main arteries and veins tend to branch at the level of the cortical surface into progressively smaller capillaries. The decision-making regarding what to label as capillary and what to label as brain cortex was challenging, and there is no literature reference to rely upon. After discussions with the supervisors, I have opted for two equally valid segmentation techniques. In one (see Figure 4.10), the capillaries were excluded from the segmentation as much as possible, while in the other (see Figure 4.11) they were included. I have made both segmentations available for further data analysis.



Figure 4.10: Case HS009. Segmentation of the intra-operative microscope snapshot showing the different labels. The capillaries were excluded and kept unsegmented outside the labels in this segmentation.

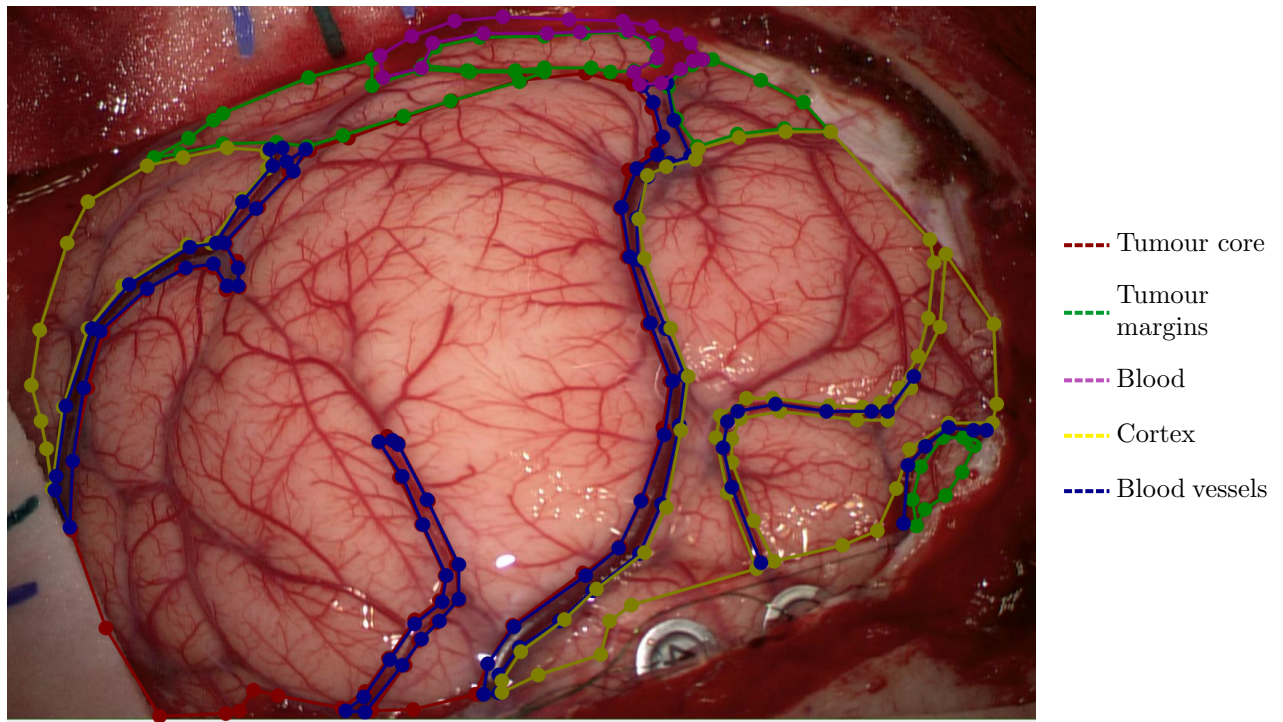


Figure 4.11: Case HS009. Segmentation of the intra-operative microscope snapshot showing the different labels. In this segmentation, the capillaries are included inside the labels.

4.2 Data analysis

When several hypercubes are acquired, data analysis is a complex process in which a ground truth must be identified and compared with the data obtained through the acquisition.

As detailed above, our ground truth consisted of an equivalent, labelled RGB image taken from the microscope simultaneously with the single hypercube acquisition.

An algorithm must be trained and tested to ascertain whether HSI is accurate and reliable in identifying the different structures and tumours from the surrounding brain. Training implies feeding the algorithm with the ground truth and the hypercube so the module can compare the two. In contrast, testing means the algorithm will analyse a new hypercube without ground truth after the training to verify the performance.

However, the hypercube first needs to be pre-processed through registration and normalization.

Registration is the process of aligning all the images of the hypercube with each other and using a microscope snapshot. It is a mandatory step to eliminate all the noise and the movement

artefacts due to the gaps between the acquisition of a single hypercube slice and the next, a process during which heart pulsation, breathing patterns, and other confounding factors impact the position of some of the picture components, such as the brain itself.

Normalization involves adjusting pixels' intensity range to a standard scale without information loss. This process ensures that results are not skewed by areas that give little information but present extreme values.

The pipeline for image analysis followed three steps:

1. pre-processing, consisting in registration and normalization;
2. hyper-cube analysis, consisting in algorithm training and testing;
3. results analysis, interpretation, and audit of **point 2**.

As detailed in Chapter 3, the acquired hyper-cubes were analysed by applying specific algorithms selected from those tested in the international literature.

Analysing hyperspectral images represents a complex and evolving area of study. The methodology applied in this instance is somewhat provisional and introductory. Experiences from various research groups corroborate this perspective, which also indicates that numerous aspects remain to be tested and perfected in this field.

A summary of the registration, normalization, and analysis algorithms used is provided here.

4.2.1 Pre-processing

Database structure

All cases were loaded, in pseudo-anonymized form, onto the College drive. The folders were organized as shown in Figure 4.12.

Creating a centralized online database was crucial to granting all project professionals access. The database was also intended to serve future projects and further dataset analyses.

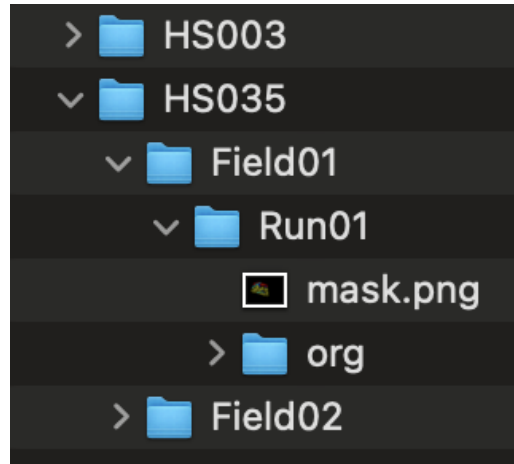


Figure 4.12: Structure of our saved database.

Image registration

Dr Zepeng Hu of the Hamlyn Centre of Robotics, under the supervision of Professor Daniel Elson, formalized and applied the image registration and normalization process.

Each acquired hypercube was formed by a stack of grayscale images captured at wavelengths ranging from 440 nm to 720 nm alongside their corresponding standard white light microscope images. The operative microscope images served as the basis for ground truth annotations. Compared to the standard microscope images, the multi-spectral images exhibited various distortions, including rotation and inversion. Most importantly, we observed expected minimal changes in the pixel's position between one image to another due to physiological movements (heart pulsation, respiration, etc.) of the human tissues during surgery.

Image registration was mandatory to align the multi-spectral images with the ground truth. This process involved two primary steps: one for aligning repeated pictures and another for aligning image stacks. The critical algorithms considered for registration were Scale-Invariant Feature Transform (SIFT), Oriented FAST and Rotated BRIEF (ORB), and Accelerated-KAZE (AKAZE).

- **Scale-Invariant Feature Transform (SIFT)** [314]: SIFT is an algorithm used for detecting and describing local features in images. It is particularly effective when the images may be subject to scale, rotation, and illumination changes. By identifying distinct

key points and generating a unique descriptor for each one, SIFT allows the matching of features across different images.

- **Oriented FAST and Rotated BRIEF (ORB)** [315]: ORB is a fast and efficient feature detection and descriptor extraction algorithm. It is designed as a more computationally inexpensive alternative to SIFT.
- **Accelerated-KAZE (AKAZE)** [316]: AKAZE is an algorithm for feature detection and description that is known for its effectiveness in handling images with non-linear scale space. It employs a method of constructing scale space using non-linear diffusion filtering, which enhances its ability to detect and describe features in images with complex textures and patterns.

All these algorithms were tested, and I, Dr Zepeng Hu, and the supervisors discussed the results during weekly meetings. The SIFT algorithm was chosen as it has given the most robust results regarding scale, rotation, and fidelity with the microscope image.

Normalization

Normalization is a data preprocessing technique that adjusts an image's pixel intensity range. The primary goal is to transform the pixel values to a standard scale without distorting the ranges of values or losing information. This process is essential for comparing and combining data from different sources or feeding it into machine learning algorithms, which helps speed up the learning process and achieve better performance. Normalization ensures that each pixel contributes equally to the analysis, preventing features with more extensive ranges from dominating those with smaller ranges.

In this study, two distinct data pre-processing methodologies were employed and compared. The techniques are detailed as follows:

1. **Min-Max Normalization:** This approach involves scaling each pixel value to a stan-

standardized range between 0 and 1. The formula for Min-Max Normalization is given by:

$$x = \frac{\text{value} - \min}{\max - \min} \quad (4.2)$$

where *min* and *max* represent the minimum and maximum pixel values within the image dataset, respectively.

2. **Mean-Variance Standardization:** This method focuses on normalizing the data based on the mean and variance. The standardization is achieved using the formula:

$$x = \frac{\text{value} - \mu}{\sigma} \quad (4.3)$$

Here, μ denotes the mean, and σ represents the standard deviation of the pixel values across the image dataset.

Both approaches were repeatedly compared for different hypercubes, and I, Dr Hu, and the supervisors visually analyzed the results. The approach detailed in Equation 4.2 showed the best prediction with maximum noise reduction, so we chose to normalize our dataset.

4.2.2 Modules tested for image analysis

As the candidate for this project, I have tested several algorithms for the present project using Python coding on Pycharm platforms. As this was not my area of expertise and I had to consider a learning curve, I first developed a code to analyze a single intra-operative acquisition by comparing the labelled microscope image with the respective hypercube (See Appendix B, first code). For this purpose, I have applied some of the standard algorithmic methods discussed in Chapter 3 to test their performance and learn the basic principles of coding in Python. I have subsequently written a new code to apply the same algorithms on groups of hypercubes taken from the pool of patients with a specific histological diagnosis (See Appendix B, second code). This code was more complex, and I used Dr Zepeng's feedback to refine it. I then performed several runs adjusting the hyperparameter tuning, the pixel sampling methods, and

the dataset distribution through training and testing to evaluate the model's performance with different settings.

The theoretical future development of these codes is to be modified and potentially adapted to microscope software so that an analysis of hyperspectral stacks acquired during surgical procedures could be performed efficiently, almost in real time. The concept would be to create an overlay mask to make the different classes visible to the surgeon as a filter to be activated during the procedure to check the extent of the tumour and the infiltration boundaries within the healthy brain (or dural) tissue.

The codes were run using a workstation with a AMD ryzen 9-7900x 12 core processor with 64gb of RAM and a Nvidia RTX 4090 Graphical Processing Unit (GPU) to run our experiments.

We have tested the following algorithms for image analysis:

- Decision Tree (DT) classifier;
- Random Forest (RF) classifier;
- Support Vector Machine (SVM) classifier;
- Multi-Layer Perceptron Classifier (MLPC);
- 2D and 3D convolutional neural networks.

A visual representation of the different classification methods used for image analysis is offered in Figure 4.13. We have considered several models, basing our choice on the need to test supervised algorithms for image analysis and on the experience of other groups discussed in Chapter 4, where a brief explanation of the most common models considered is reported.

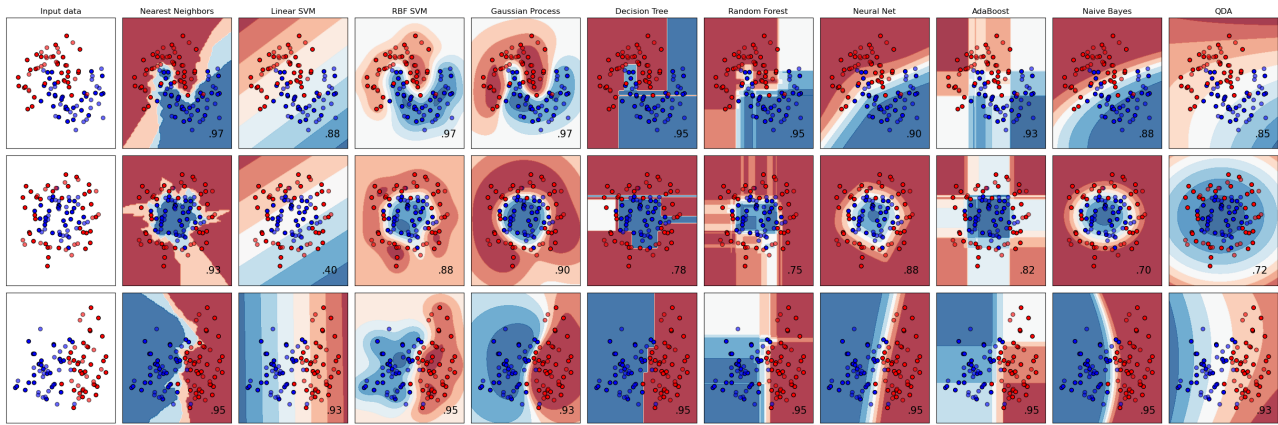


Figure 4.13: Schematic comparing different image processing models and their data partition and classification features - taken from the scikit-learn website.

Single hypercube classification models

I created and perfected the first example of hypercube classification code, which I used as a first attempt to develop code that could process multiple hypercubes.

The code, written in Python using PyCharm, facilitates comparing the hyperspectral cube with a mask derived from segmentation - see Appendix B, first code. The system includes interchangeable modules for machine learning algorithms to optimise performance, such as DT, RF, SVM, and MLPC. These modules were methodically tested to determine the most effective algorithm for this application.

I have structured the code into three main blocks:

1. image loading and reading;
2. comparison between the mask and the hyperspectral cube using the different modules;
3. calculation and printing of the metrics.

For each case, 60% of the pixels were used for training, and 40% were used for testing, of which 20% for Validation and 20% for testing.

Inter-patient classification models

I applied SVM, decision tree, random forest, and MLPC algorithms for the inter-patient classification model.

I have written the script starting from a structure similar to the code in the previous subsection. I have then progressively modified several passages to adapt the code to a larger dataset, with support from Dr Zepeng, primarily for error correction and identifying crash sources - See Appendix B, second code. I have executed the code personally and worked on fine-tuning, modification of hyperparameters, and auditing process on the results, modifying the data selected for training, validation, and testing through a trial and error process until several factors were corrected and the best possible data were obtained.

The Python code's core architecture remained consistent across all the options mentioned above, with modifications limited to the lines about the selected algorithm.

The code included the following steps:

1. dataset loading and reading;
2. normalization;
3. sampling of the hyperspectral cubes;
4. reshaping of the sampled data into a 2D format where each row (array) represents a patch of pixel and columns represent spectral features;
5. feed the prepared data to the image analysis algorithm;
6. calculate and print the performance metrics.

Convolutional Neural Networks - CNN

The most refined algorithm for image analysis we have used is a Convolutional Neural Network (CNN) mixing spatial and spectral analysis features. Dr. Zepeng Hu developed and updated

this algorithm entirely.

The architecture of the model employed in this study is depicted in Figure 4.14.

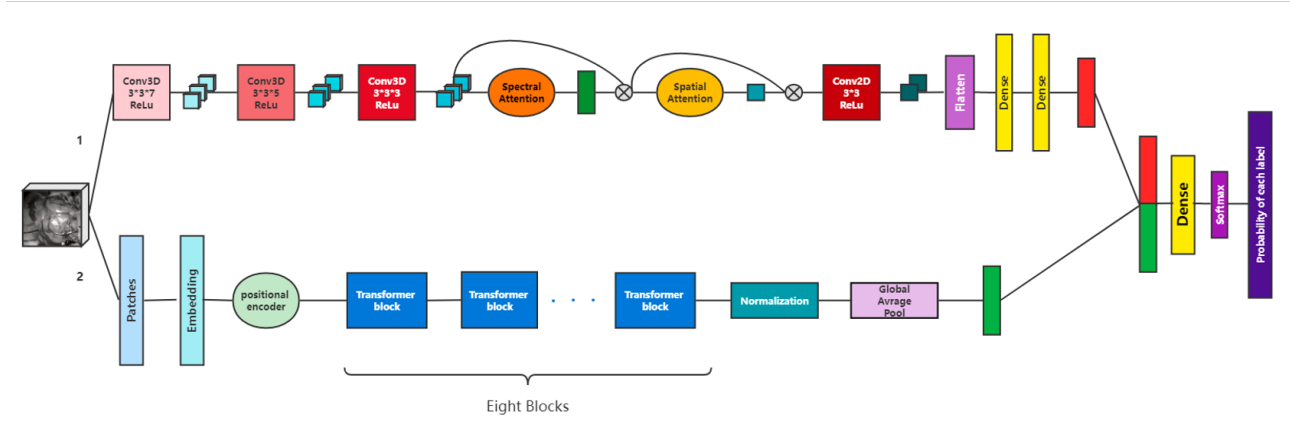


Figure 4.14: Representation of the CNN designed for this study - picture realized by Dr Zepeng Hu.

It represents a combination of 3D convolution, 2D convolution, and transformer networks, inspired by the Hybirdsnn framework and the Convolutional Block Attention Module (CBAM) [317], integrating both spatial and spectral attention mechanisms to enhance multi-spectral image classification.

The first component of the model combines 3D and 2D convolutions, incorporating spectral and spatial attention blocks. This combination is important for processing multi-spectral images, where both spatial and spectral information are pivotal. While 2D CNNs excel in spatial information processing, they cannot discern spectral features comprehensively. Conversely, 3D CNNs can extract both types of features, albeit with increased computational demands. The model adopts the Hybrid approach of utilizing three blocks of 3D convolution followed by a 2D convolution block, effectively preserving spectral information while efficiently capturing spatial details.

The spectral attention module incorporates pooling operations, convolutions, and a sigmoid activation. An attention module is a mechanism that allows the network to focus on specific parts of the input more than others, assigning higher weights to regions more relevant to the task. In this particular case, the spectral attention module explores inter-channel relationships among different wavelengths to prioritize relevant spectral features. Pooling operations are

processes deployed to reduce the spatial dimensions, making the network more efficient by focusing on the most prominent spectral features and reducing sensitivity to small variations. The sigmoid activation function is a mathematical function that introduces non-linearity and assigns values between 0 and 1 to produce a feature map with enriched spectral attention: values closer to 1 imply higher relevance, while those near 0 are less significant.

Similarly, spatial attention adjusts parameters across spatial dimensions of feature maps, directing focus towards areas of significance. This is achieved through pooling operations, concatenation (a process where multiple spatial features are combined), convolution, and sigmoid activation, resulting in a feature map with enhanced spatial attention.

These attention blocks are strategically placed after the third 3D convolution layer and before the 2D convolution to maximize the retention of spectral information, which is crucial for accurate classification in multi-spectral imagery.

The second line of the model draws inspiration from the Vision Transformer (ViT) architecture. ViT, as proposed by Dosovitskiy et al. [318], divides an image into fixed-size patches, embedding these patches and processing them through a transformer encoder as suggested by Vaswani et al. [319]. Encoders are components of the CNN designed to reduce the input data. An encoder typically compresses the spatial information but retains relevant features (for example, edges, angles, curves, textures), also called *the feature map*.

In our context, where the input consists of stacked greyscale images, the model adapts the ViT approach to handle a higher volume of images. Direct application of ViT's embedding technique would result in fragmented spectral and spatial relationships. To overcome this, the model employs Tubelet Embedding. This method treats the input as a cohesive cube, extracting volumes rather than individual patches, thereby preserving the spectral coherence. Consequently, each $25 \times 25 \times 21$ image cube is processed as $5 \times 5 \times 21$ volumes, ensuring integrated analysis of both spectral and spatial information.

4.2.3 Analysis of haemodynamic changes

A brief introduction regarding the properties of light is presented in Chapter 3.

Victoire de Saléon Terras developed this section's code and procedure, summarising her MSc. project.

I have run the code together with Dr Zepeng Hu for several cases. However, the cases reported here are the ones I ran, as they showed the most promising results.

The code consisted of several modules that:

1. launch a Monte Carlo simulation (see below);
2. load the hyperspectral cubes;
3. computes the spectral intensities for the specific ROI;
4. fitting process by combining the result of the Monte Carlo Simulation and the spectral intensities;
5. calculation of the haemoglobin, deoxyhaemoglobin, and saturation values.

We are interested in two primary phenomena for haemodynamic change detection: absorption and scattering. Absorption plays a significant role due to chromophores, such as haemoglobin. Haemoglobin's absorption characteristics vary depending on its oxygenation state, with oxyhemoglobin (HbO_2) and deoxyhemoglobin (Hb) being the primary absorbers in the visible range of 400-800 nm in brain tissue. Deoxyhaemoglobin absorbs more light at 660nm, while oxyhaemoglobin absorbs more light at 940 nm. The comparison between these two specific wavelengths would have been ideal for our experiment. However, our CCD and CMOS cameras were not equipped with IR-bandpass filters to detect NIR, nor did we have comparable equipment for NIR acquisition. Hence, bands outside the visible spectrum were not acquired. We have focused our analysis on the more subtle differences in reflectance spectra between the two within the visible range.

The relative concentration changes of these haemoglobin states affect the absorption of brain tissue. The absorption coefficient, μ_a , is defined as the probability of photon absorption per unit path length in a medium, typically averaging 0.1 cm^{-1} in biological tissues.

The equation governs the attenuation of light in an absorbing medium:

$$\frac{dI}{I} = -\mu_a dx \quad (4.4)$$

where I represents light intensity, and x is the distance of light propagation. Integrating this equation yields Beer's Law, which establishes that as light travels through a medium, its intensity decreases exponentially and as a proportion of the absorption coefficient:

$$I(x) = \exp(-\mu_a x) \quad (4.5)$$

In lay terms, the absorption of a sample is calculated as the sum of the concentrations of each chromophore multiplied by its molar extinction coefficient, $\epsilon(\lambda)$. Knowing the concentrations C_{HbO_2} and C_{Hb} , we can compute the oxygen saturation SO_2 and the total hemoglobin concentration Ct as follows:

$$SO_2 = \frac{C_{HbO_2}}{C_{HbO_2} + C_{Hb}} \quad (4.6)$$

$$Ct = C_{HbO_2} + C_{Hb} \quad (4.7)$$

Biological tissues, consisting of various structures such as cells and extracellular components, significantly impact light scattering. This scattering phenomenon results in a change of direction for the propagating light, leading it to follow multiple paths within the tissue. The extent of scattering is influenced by factors such as the tissue's composition and structure and the wavelength of the incident light. The scattering coefficient, denoted as μ_s , quantifies the

likelihood of photon scattering within the tissue.

The reduced scattering coefficient, μ'_s , adjusts for the scattering angle distribution, effectively reducing the scattering coefficient to account for forward-directed scattering, making it more useful in diffusion approximation. μ'_s is characterized by its value at a specific wavelength, typically 500 nm, and a dimensionless factor known as the "scattering power."

The concept of absorptivity in biological tissues is governed by the Beer-Lambert law. This law establishes a relationship between the absorption of light and the concentration of various components within the tissue, in this case, Hb and HbO. Absorptivity, or the absorbance spectrum, is dependent on the concentration of these components, their molar extinction coefficients, and the distance between the light source and the detector, as expressed in the equation:

$$A(\lambda) = \mu_a(\lambda) \times x + \mu_s(\lambda) \times x = \sum_X C_X \varepsilon_X(\lambda) \times x + \mu_s(\lambda) \times x \quad (4.8)$$

where $A(\lambda)$ is the absorptivity, C_X is the concentration of the component X , ε_X is the molar extinction coefficient of the component X , and x is the distance, through the sample, between the light and the source-detector.

The intensity spectrum $I(\lambda)$, as recorded by the imaging system, is intricately linked to this absorptivity and can be expressed as follows:

$$I(\lambda) = I_0 10^{-A(\lambda)} \quad (4.9)$$

OR

$$A(\lambda) = \log_{10}(I_0) - \log_{10}(I(\lambda)) \quad (4.10)$$

It's important to highlight that equation 4.9 assumes that the light intensity is constant at every wavelength, but this is not the case for most commercially available microscopes and we

have not tested our equipment before our study (which might be a significant limitation of this model for our results). Due to the optical properties of biological tissues and the wavelength of the light source, photons often experience multiple scattering events. Consequently, the actual optical path length, denoted as $l(\lambda)$, is considerably greater than the direct distance, represented as x , between the source and the detector. The Differential Path-length Factor (DPF) is incorporated to account for the elongated path length caused by scattering in the absorptivity calculations:

$$A(\lambda) = \mu_a(\lambda) \times x \times DPF(\lambda) = \sum_X C_X \epsilon_X(\lambda) \times x \times DPF(\lambda) \quad (4.11)$$

and the optical path length is expressed as:

$$I(\lambda) = x \times DPF(\lambda) \quad (4.12)$$

To summarize, the final equation accounting for molar extinction coefficients, absorption coefficient, and DPF is as follows:

$$A_{\text{corrected}}(\lambda) = \epsilon_{\text{Hb}}(\lambda) \cdot C_{\text{Hb}} \cdot x \cdot DPF(\lambda) + \epsilon_{\text{HbO}_2}(\lambda) \cdot C_{\text{HbO}_2} \cdot x \cdot DPF(\lambda) \quad (4.13)$$

From this, we can build the fitting model based on the following equation:

$$I_{C_{\text{HbO}_2}, C_{\text{Hb}}}(\lambda) = I_0 10^{-(C_{\text{HbO}_2} \epsilon_{\text{HbO}_2}(\lambda) + C_{\text{Hb}} \epsilon_{\text{Hb}}(\lambda)) \cdot l(\lambda)} \quad (4.14)$$

In the scope of this section, we intended to determine the unknown concentration changes represented by C_X . This endeavour utilizes the absorptivity model delineated prior (wherein the absorptivity is deduced from the light intensities recorded by the imaging device as formulated in equation 4.9).

The implementation of a wavelength-specific fitting algorithm that incorporates C_X as its pa-

rameters necessitates the precise determination of the optical path length, denoted as $l(\lambda)$. The behaviour of photons in biological tissues was simulated using Monte Carlo sampling.

The *Monte Carlo simulation* applies random sampling to model light behaviour in biological tissue, simulating many photon paths influenced by scattering and absorption. This stochastic process occurs in a 3D tissue model, with the z-axis perpendicular to the tissue surface and the xy-plane aligned. The simulation calculates the *DPF* by tracking the journey of each photon until re-emission or absorption and averaging these distances. Its accuracy depends on the tissue's specifics, optical properties, and photon count, noting that a higher photon count increases accuracy and computational load.

Once the Monte Carlo simulation was performed, and the reflectance spectra were extrapolated from the data, a fitting model was built using least square algorithm to calculate the spectra relative to Hb, HbO₂, and SO₂ concentrations, and their changes over different hyperspectral stacks.

The overall procedure is exemplified in Figure 4.15.

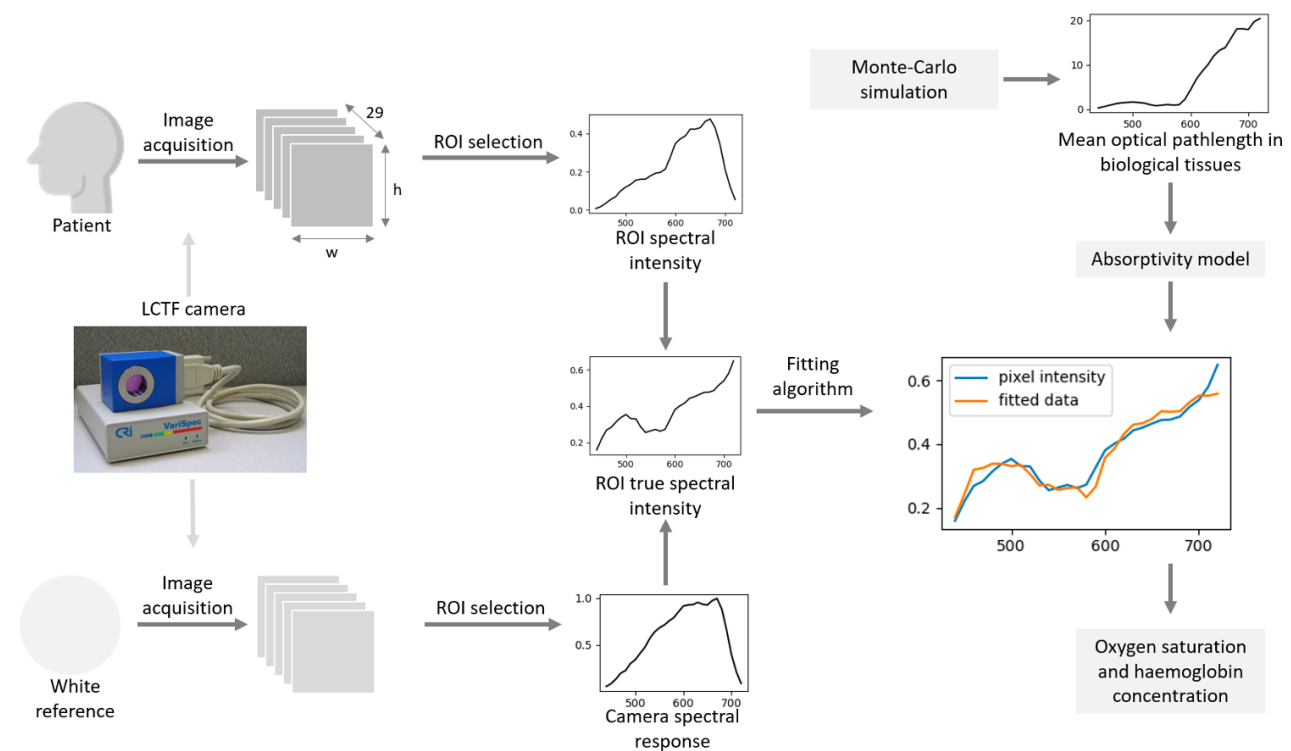


Figure 4.15: Flowchart of the protocol to detect haemodynamic changes - from "Use of hyperspectral analysis in neurosurgery", MSc report by Victoire de Sal  on Serras.

4.3 Results

The study timeline is summarized in Figure 4.16. As mentioned in Section 5.1.6, the final ethics approval was granted on 04/02/2020. After the initial NHS set-up, the plan was to start patient recruitment in March 2020. However, the COVID-19 pandemic had its outbreak in the UK from that month and carried on for a significant portion of 2020 and part of 2021.

The present PhD project was suspended for one year for two reasons:

- the Imperial College Healthcare NHS Trust froze every non-Covid19 related study for an indeterminate period;
- the candidate returned to full clinical service to support the Department of Neurosurgery during this critical phase.

While the pandemic did not stop in 2021, the overall NHS logistics had improved to a stage where most of the research studies could restart. The Trust gave the green light to start this project in February 2021, and the first patient was consented to and recruited in March 2021.

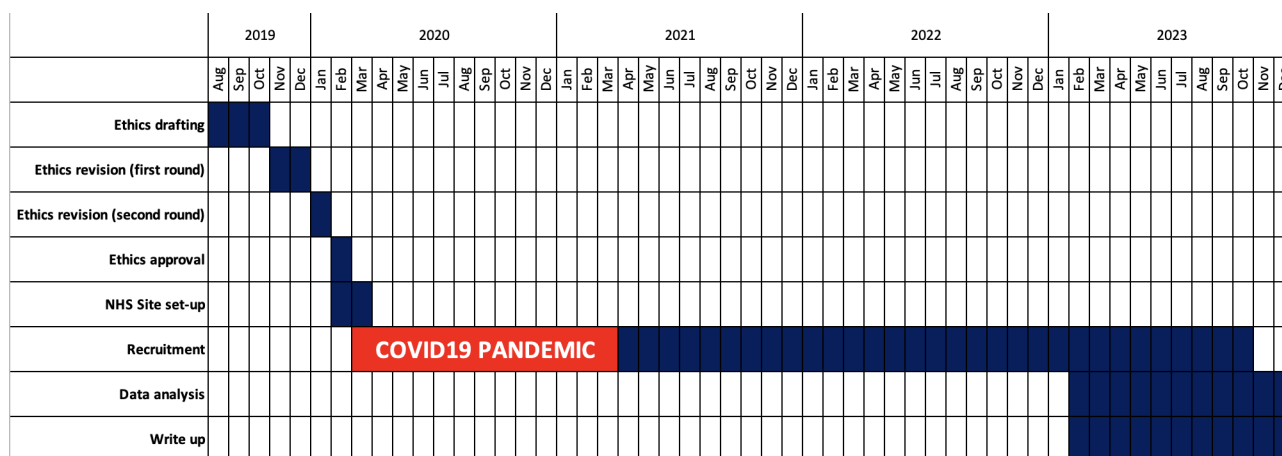


Figure 4.16: Gantt chart showing the different phases of the study.

4.3.1 Clinical results

Forty-seven patients have been recruited in the present study. A total of 62 patients consented, but in 15 cases, the consent was either withdrawn or the logistics in the theatre did not allow

sufficient time for image acquisition. In one case, a patient was recruited with an initial suspicion of lymphoma, but the subsequent histological analysis revealed a granulomatous disease (not neoplastic), so the images were discarded.

Each patient has been pseudo-anonymized using the log ID HS (HyperSpectral) + case number: the first patient recruited was patient HS001, the fifteenth patient was patient HS015, and so on.

A summary of the dataset is reported in Table 4.2. Further details for some of the specific histological types, the survival metrics, and the complication rates are summarized in Tables 4.3 and 4.4, respectively.

Overall, the histological diagnoses, survival metrics, and pre- and post-operative performance metrics were compatible with the results of similar histological subtypes exposed in Chapter 2.

ID	Gender	Age (yrs)	CCI	Histology	Location	Acquisitions	EOR
HS001	M	72	5	GB	Sup front gyrus	1 + 2 + 3	90%
HS002	F	61	5	Men grade I	Inferior falx	2 + 2	100%
HS003	M	71	9	Melanoma (met)	Precuneus	1a + 1b + 2	100%
HS004	M	65	2	GB	Paracentral lobule	1a + 1b + 2	30%
HS005	F	31	0	Astro grade III	Sup front gyrus	1 + 2 + 3	98%
HS006	M	66	2	GB	Inf temp gyrus	1 + 2 + 3	30%
HS007	F	73	3	Men grade I	Sup par lobule	1a + 1b + 3	100%
HS008	M	57	4	Men grade II	Pre-cuneus	2 + 3	95%
HS009	M	30	0	Oligo grade II	Supramarg gyrus	1a + 1b + 3	95%
HS010	M	54	1	GB	Post cent gyrus	1 + 2	99%
HS011	M	72	4	GB	Middle front gyrus	1 + 3	90%
HS012	M	59	1	GB	Sup par lobule	1a + 1b + 2	90%
HS013	M	71	10	Adeno (lung)	Occ pole	1a + 1b + 3	100%
HS014	M	74	3	GB	Temp-occ gyrus	2	98%
HS015	F	42	0	Men grade I	Sup front gyrus	1	100%
HS016	M	62	2	GB	Post-cen gyrus	1 + 3	50%
HS017	F	73	5	GB	Middle temp gyrus	1 + 3	98%
HS018	M	36	2	Astro grade II	Insula	1a + 1b	60%
HS019	F	39	0	Astro grade IV	Middle front gyrus	1 + 2 + 3	50%
HS020	F	53	1	GB	Middle temp gyrus	1 + 2 + 3	95%
HS021	F	28	0	Epidermoid cyst	Atrium	2 + 3	95%
HS022	M	69	2	GB	Pre-cuneus	2	90%
HS023	M	85	4	Men grade II	Paracentral lobule	1a + 1b + 3	80%
HS024	F	62	2	Oligo grade III	Middle front gyrus	1 + 3	50%
HS025	M	73	3	Not diagn (likely GB)	Inf front gyr	1	0%
HS026	M	69	2	GB	Supramarg gyrus	1 + 3	20%
HS027	M	50	2	GB	Middle temp gyrus	1 + 2 + 3	95%
HS028	F	62	3	Men grade I	Supramarg gyrus	1	100%
HS029	M	70	5	GB	Middle front gyrus	1a + 1b + 3	94%
HS030	F	49	0	GB	Sup par lobule	2	20%
HS031	F	38	0	Men grade I	Sup front gyrus	1 + 2	95%
HS032	M	30	0	PXA grade III	Sup front gyrus	1a + 1b + 3	80%
HS033	F	73	3	Men grade I	Temp pole	1 + 2	100%
HS034	M	68	3	Lymphoma	Sup par lobule	1 + 3	100%
HS035	F	60	2	GB	Cing gyrus	1 + 3	100%
HS036	M	44	0	GB	Angular gyrus	1 + 3	100%
HS037	F	70	3	GB	Temp pole	1 + 2 + 3	98%
HS038	M	84	4	GB	Orbital gyrus	1 + 2 + 3	60%
HS039	F	32	0	Oligo grade III	Sup front gyrus	1a + 1b + 3a + 3b	80%
HS040	F	53	2	GB	Temp-occ gyrus	1	60%
HS041	M	58	1	Astro grade IV	Insula	1	60%
HS042	F	55	1	Men grade I	Angular gyrus	1	100%
HS044	M	62	3	GB	Middle temp gyrus	1 + 2	50%
HS045	F	81	7	GB	Inf par lobule	1 + 3	95%
HS046	F	46	1	Men grade I	Pre-cuneus	1	98%
HS047	M	51	5	Glioma NOS	Insula	1a + 1b	0%
HS048	M	32	0	Astro grade II	Sub-central gyrus	1a + 1b	60%

Table 4.2: Summary of our dataset. Number HS0043 has been removed from the series as the histological analysis did not confirm neoplastic disease. Notice how the acquisitions at phase 1 were more common, while phases 2 and 3 were attempted less often - see text for more details. ABBREVIATIONS. TOP: ID = Identification; CCI = Charlson Comorbidity Index; EOR = Extent of Resection. HISTOLOGY: Adeno = Adenocarcinoma; Astro = Astrocytoma; GB = Glioblastoma; met = metastasis; Men = Meningioma; NOS = Not Otherwise Specified; Oligo = Oligodendroglioma; PXA = Pleomorphic XantoAstrocytoma. LOCATION: cen = central; cing = cingulate; front = frontal; occ = occipital; par = parietal; sup = Superior; temp = temporal.

HGG-IV				
TOTAL	24			
Gender (M:F)	16M, 8F (2:1)			
Histology	22GB, 2AIV			
	Average	Median	Mode	SD
Age	63	64	72	11.35
EOR	73%	90%	90%	28%
OS	13	9	9	14.62
PFS	6	4	4	6.6
MENINGIOMAS				
TOTAL	10			
Gender (M:F)	8F, 2M (4:1)			
Histology	8 grade I, 2 grade II			
	Average	Median	Mode	SD
Age	59	59	73	14.9
EOR	97%	100%	100%	6%
OS	18	16	16	9.33
PFS	16	13	9	9.08
LGG + ANAPLASTICS				
TOTAL	8			
Gender (M:F)	6F, 2M (3:1)			
Histology	2 AII, 3 AAIII, 1 OligoII, 1 OligoIII, 1 glioma NOS			
	Average	Median	Mode	SD
Age	39	32	32	15.2
EOR	59%	70%	60%	39%
OS	24	16	N/A	21
PFS	12	8	N/A	8.7
OTHERS				
TOTAL	4			
Gender (M:F)	3M, 1F(epid)			
Histology	2 Mets (lung + melanoma), 1 lymphoma, 1 epidermoid			
	Met (mel)	Met (adeno)	Lymphoma	Epidermoid
Age	71	71	68	28
EOR	100%	100%	100%	95%
OS	34	19	5	104
PFS	30	18	1	18

Table 4.3: Data from the HSI series regarding the main histological subtypes. GB = Glioblastoma; AIV = Astrocytoma Grade IV; LGG = Low Grade Glioma; AII = Astrocytoma grade II; AAIII = Anaplastic Astrocytoma grade III; OligoII = Oligodendroglioma grade II; OligoIII = Anaplastic Oligodendroglioma III; NOS = Not Otherwise Specified; SD = Standard Deviation; EOR = Extent Of Resection; OS = Overall Survival; PFS = Progression Free Survival. All survival metrics are measured in months.

Metric	N. of Patients	
PS - Grade	Pre-op	Post-op
0	39	36
1	5	6
2	3	5
3	0	0
CDS - Score	N. of Pts	
N/A	40	
I	2	
II	1	
III	4	
IV	0	
V	0	

Table 4.4: Performance Status (PS) and Clavien-Dindo Score (CDS) for the cohort of HSI patients. N/A = No complications reported; Pts = Patients; Pre-op / Post-op = Pre-operative / Post-operative.

4.3.2 Adverse reactions and adverse events

We have observed no evidence of adverse reaction to the technology or the protocol deployed in any of the cases recruited.

We recorded the following adverse events:

- *CSF leak*: 2 cases (4%), one treated conservatively (CDS 0), the other resulting in an infection requiring a washout (CDS II);
- *post-operative infection*: 3 cases (6%), treated successfully with washout and antibiotic therapy (CDS III);
- *post-op stroke*: 2 cases (4%), both of minor entity, treated conservatively (CDS I and II);
- *intra-operative haemorrhage*: 1 case (2%), successfully identified and treated during surgery, and not requiring further intervention;
- *post-op neurological deterioration*: 3 cases (6%), 2 self-resolved (CDS 0), one requiring ITU admission (CDS III) but recovered.

We have recorded no deaths directly or indirectly related to this protocol. On the meningioma subset, one case is significantly affecting the average OS and PFS - an 84-year-old gentleman

with severe comorbidities and a diagnosis of atypical meningioma grade II, who passed away one year after the surgery due to tumour progression.

4.3.3 Intra-operative views

Specifics of the intra-operative views are discussed within the single histological sub-types. However, an acceptable variety of cases with different locations were recruited in the present series, as shown in Table 4.2.

Using the current setting, it was clear after the first few cases recruited that deep-seated tumours were more challenging to visualize, and the exposure was significantly worse; therefore, cases showing more superficial lesions were privileged. For similar reasons, acquisitions 1 and 2 were more commonly acquired than 3. During surgery, we noticed that depending on the surgeon's technique and preference, a variable amount of blood, haemostatic materials, and cottonoids / surgical electrodes were hindering the view.

We have managed to maintain luminosity at 80% with an acquisition distance of 35 to 45 cm in most cases, although some variations were observed due to the specificity of the cases (example: the surgeon occasionally adjusting the luminosity or changing the focus as clinically required).

In most cases, the same acquisition phase was taken at an exposure of 200 ms (frame rate 0.05 Hz) and one of 50 ms (frame rate 4.3), with few exceptions.

The specific technical details and the complete anonymised database will be publicly available approximately one year after submitting this thesis.

4.3.4 HGG-IV

When reviewing the glioma dataset in the present series, it emerged that it was heterogeneous regarding locations and surgical approaches.

While some of the surgeons preferred a wide craniotomy with a precise exposure of the tumour (as seen in case HS017), others preferred more key-hole-based approaches.

The consequence of the second choice was the inevitable reduced exposure to the surgical field, more noise due to intra-operative elements (such as blood and surgical instruments), and consequently reduced visibility even when the microscope was applied.

This difference in approaches resulted in a highly heterogeneous dataset, which caused the performances of some of the algorithms examined to fluctuate.

Single hypercube classification model

The execution timing of this algorithm was 1 minute and 9 seconds $\pm$ 10 seconds, depending on the different modules applied, with the sole exception of the SVM classifier, which is much more computationally demanding.

The algorithm was applied to several cases, but in this section, I focused on HS017 and HS014 as trials as examples of two radically different approaches—wide craniotomy versus key-hole surgery.

In case HS017 (a left temporal tumour), a wide craniotomy was performed, and a clear distinction between the tumour mass and the cortex was visible.

In this case, the decision tree classifier's performance was satisfactory but inferior to that of the MLPC classifier and the random forest, which showed the best performance.

The results of the single classes identified through the intra-patient classification using the Random Forest classifier and the MLPC algorithm are reported on Tables 4.5 and 4.6.

A visual comparison between the predicted images produced by the different algorithms is shown in Figure 4.17, and the application of the mask onto the intra-operative snapshot is shown in Figure 4.18.

In case HS014 (a temporo-occipital tumour), a key-hole craniotomy was performed, limiting

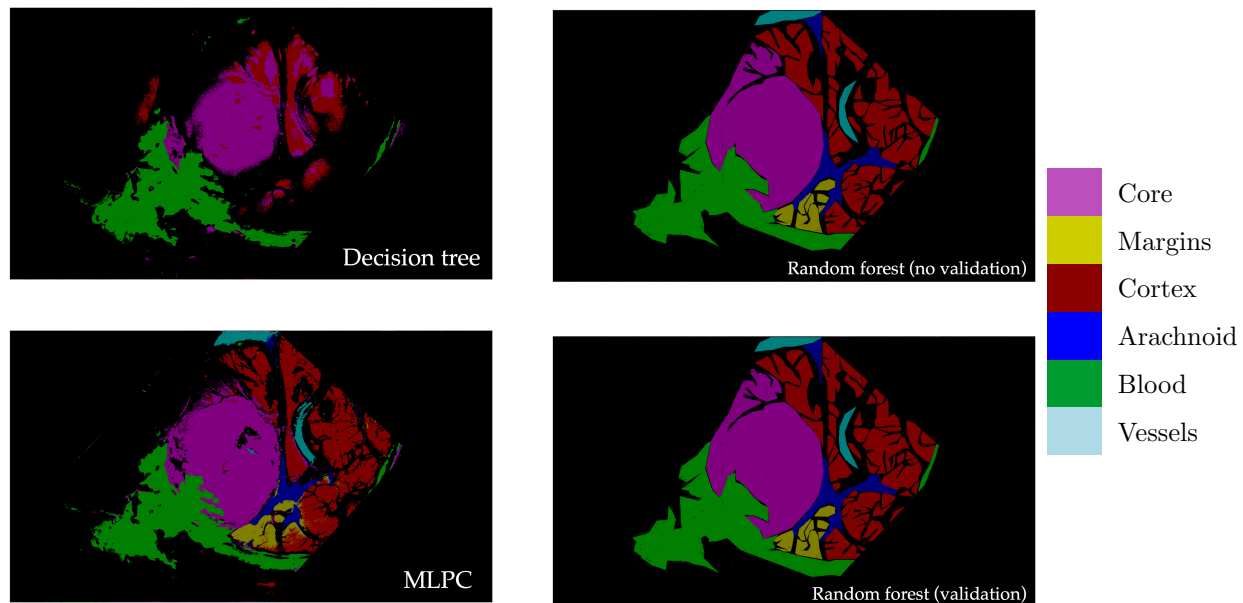


Figure 4.17: Case HS017. The result of intra-patient classification algorithms comparison. The decision tree algorithm showed an acceptable result but was outperformed by MLPC and the random forest. Notice that the random forest algorithm was trialled without and with a validation set. "Core" refers to the tumour core, and "margins" refers to the suspected area of tumour infiltration as seen on a pre-op MRI scan.

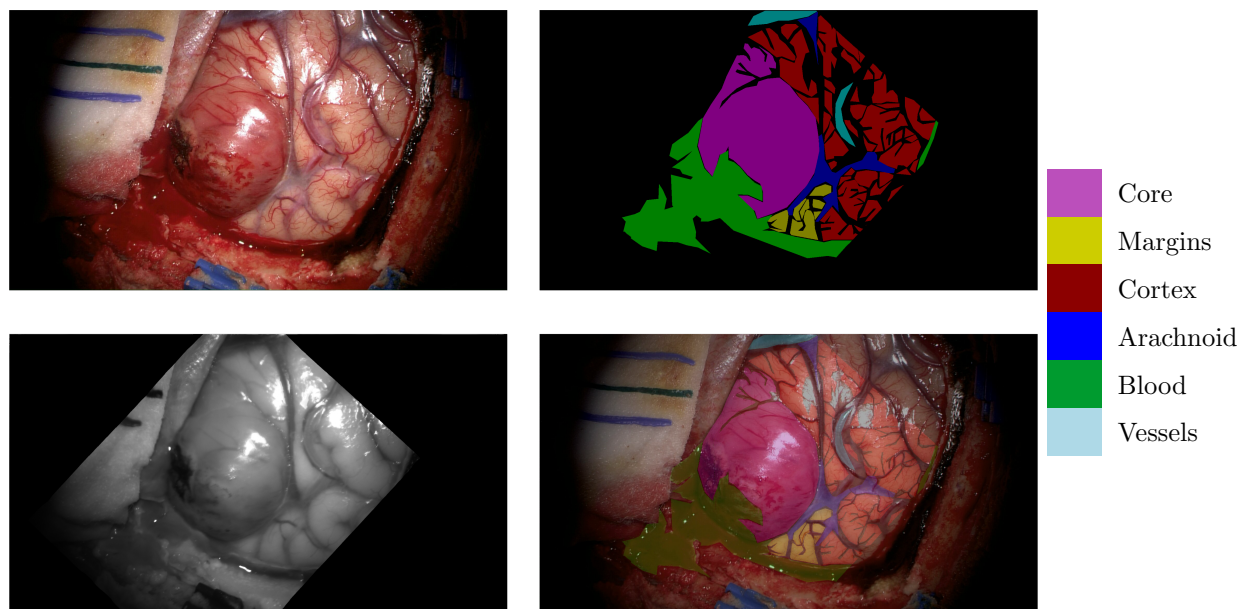


Figure 4.18: Case HS017. Overlapping of the mask to the intra-operative microscope snapshot. Random forest has been applied in this case. Top left: intra-operative microscope snapshot showing the tumour exposed; top right: segmentation mask; bottom left: image taken from the hyperspectral stack; bottom right: predicted image overlapped onto the microscope image.

Class	Sensitivity	Specificity	Overall Accuracy
bkg	0.99	0.96	0.98
core	0.98	1.00	1.00
cortex	0.96	1.00	0.99
arachnoid	0.93	0.99	0.99
vessels	0.90	1.00	1.00
margins	0.90	1.00	1.00
blood	0.97	1.00	1.00

Table 4.5: Random Forest classification results

Class	Sensitivity	Specificity	Overall Accuracy
bkg	0.95	1.00	1.00
core	0.99	0.98	0.99
cortex	0.99	1.00	1.00
arachnoid	0.97	1.00	0.99
vessels	0.98	1.00	1.00
margins	0.95	1.00	1.00
blood	0.98	1.00	1.00

Table 4.6: MLPC classification results.

the exposure of the more deeply seated tumour. A certain amount of subarachnoid blood was also seen on the cortical surface.

In this case, all the algorithms tested showed a poorer performance when compared with the testing in case HS017, as shown in Table 4.7.

Class	Sensitivity	Specificity	Overall Accuracy
bkg	0.99	0.94	0.98
core	0.95	0.99	0.99
cortex	0.88	1.00	0.99
arachnoid	0.87	1.00	1.00
vessels	0.75	1.00	1.00
margins	0.89	1.00	1.00
blood	0.17	1.00	1.00

Table 4.7: Metrics of the random forest classification approach applied to case HS014.

Figure 4.19 shows the visual results. The model's performance is still excellent, but there is increased noise.

In all cases, and partially as expected, the "tumour margins" and the "arachnoid" classes showed the worst classification metrics. These results have substantial implications for the

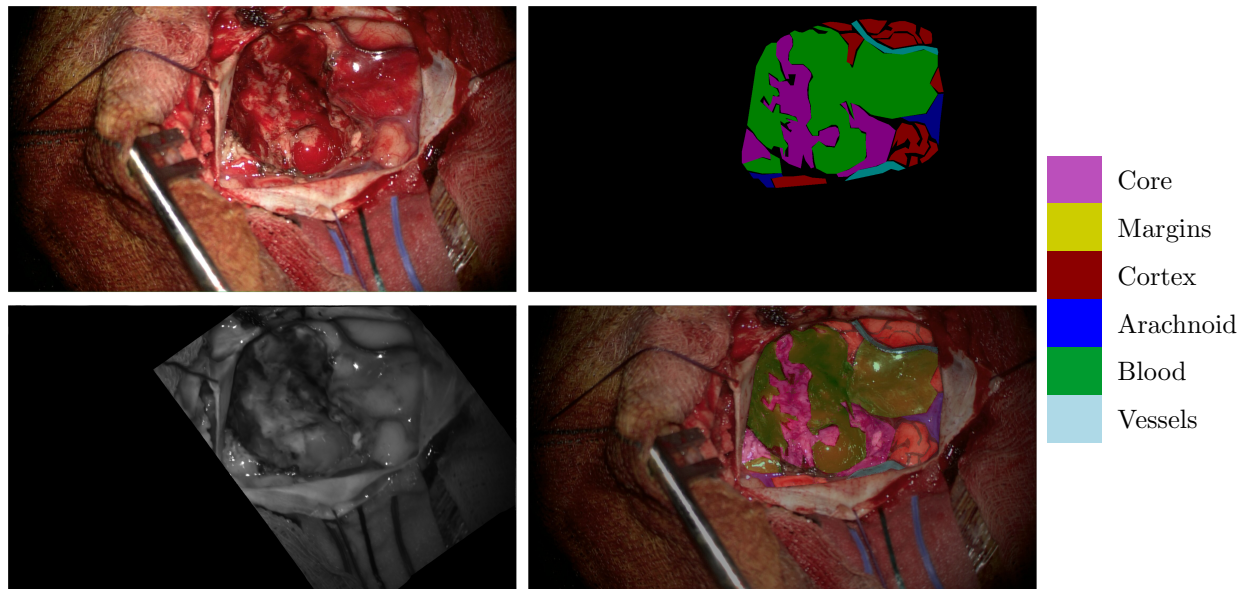


Figure 4.19: Case HS014. Overlapping of the mask to the intra-operative microscope snapshot. Random forest has been applied in this case. Top left: intra-operative microscope snapshot showing the tumour exposed; top right: segmentation mask; bottom left: image taken from the hyperspectral stack; bottom right: predicted image overlapped onto the microscope image.

next section.

Inter-patient classification

The initial inter-patient classification of the HGG-IV dataset performed poorly regardless of the algorithm used, especially regarding the tumour core.

As a matter of example, see Tables 4.8, 4.9, 4.10, and 4.11.

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	0.6383	0.7209	0.7076	0.9122	0.3049
blood	0.5048	0.9052	0.8784	0.9622	0.2767
cort	0.3304	0.7411	0.6749	0.8522	0.1967
core	0.0364	0.9561	0.8173	0.8481	0.1285
margins	0.0348	0.8207	0.7943	0.9608	0.0067
vessel	0.2150	0.9767	0.8663	0.8801	0.6094
white	0.0040	0.9988	0.7185	0.7188	0.56

Table 4.8: Decision tree classifier results

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	0.6866	0.7595	0.7444	0.9027	0.4272
blood	0.7748	0.9215	0.9114	0.9822	0.4225
cort	0.6783	0.8023	0.7908	0.9609	0.2581
core	0.0953	0.9730	0.7962	0.8100	0.4709
margins	0.0077	0.9963	0.8257	0.8280	0.3037
vessel	0.8293	0.8869	0.8773	0.9632	0.5929
white	0.0243	0.9787	0.8908	0.9082	0.1033

Table 4.9: Random forest classification results.

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	-	0.8414	0.8414	1.0000	0.0000
blood	0.7430	0.7353	0.7366	0.9364	0.3529
cort	0.3142	0.8070	0.6832	0.7781	0.3532
core	0.0920	0.9538	0.7734	0.7987	0.3450
margins	-	0.9403	0.9403	1.0000	0.0000
vessel	0.3404	0.9680	0.8629	0.8795	0.6814
white	0.0707	0.9223	0.7441	0.7894	0.1941

Table 4.10: SVM classifier results

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	0.5403	0.8464	0.7830	0.8757	0.4789
blood	0.8555	0.8589	0.8587	0.9877	0.3100
cort	0.7018	0.8133	0.8030	0.9641	0.2760
core	0.0982	0.9730	0.7968	0.8105	0.4780
margins	0.0001	0.9988	0.8265	0.8273	0.0233
vessel	0.8401	0.8459	0.8450	0.9638	0.5199
white	0.0460	0.9698	0.8848	0.9093	0.1339

Table 4.11: MLPC results on the HGG-IV series

These results did not significantly change when changing the combination of cases. Specifically, I have attempted these different solutions:

- 60% training set, 20% testing set, 20% validation set;
- leave one out for testing and validating;
- exclude all cases acquired using the second camera (from case HS030 to HS048).

CNN classifier

Due to the poor performance of the other algorithms, we have applied CNN to a selected group of cases. In most of them, the tumour was relatively well exposed, and the normal brain tissue was adequately visible on the microscope snapshot.

The cases selected for this analysis were:

```
['HS001', 'HS012', 'HS016', 'HS017', 'HS019', 'HS020', 'HS026',
'HS029', 'HS030', 'HS037']
```

This algorithm was far more computationally demanding, requiring 8 hours to analyse the dataset.

The main results for the CNN classifier on HGG-IV are shown in Table 4.12.

Class	Metric	Mean $\pm$ SD	Best (Value)	Worst (Value)
ara	Sensitivity	0.45 ± 0.30	0.92 (HS001, Run15)	0.00 (HS037, Run8)
	Specificity	0.95 ± 0.06	1.00 (HS037, Run8)	0.75 (HS030, Run2)
	Accuracy	0.90 ± 0.10	1.00 (HS030, Run3)	0.50 (HS030, Run1)
	NPV	0.91 ± 0.13	1.00 (HS030, Run3)	0.28 (HS019, Run6)
	PPV	0.55 ± 0.29	1.00 (HS001, Run22)	0.00 (HS030, Run3)
blood	Sensitivity	0.56 ± 0.31	0.97 (HS001, Run18)	0.00 (HS001, Run13)
	Specificity	0.94 ± 0.07	1.00 (HS001, Run15)	0.76 (HS037, Run6)
	Accuracy	0.89 ± 0.10	1.00 (HS019, Run4)	0.54 (HS030, Run3)
	NPV	0.93 ± 0.11	1.00 (HS012, Run4)	0.38 (HS019, Run8)
	PPV	0.46 ± 0.34	0.95 (HS020, Run6)	0.00 (HS012, Run4)
vessel	Sensitivity	0.53 ± 0.25	1.00 (HS019, Run9)	0.00 (HS001, Run14)
	Specificity	0.96 ± 0.06	1.00 (HS001, Run14)	0.57 (HS037, Run3)
	Accuracy	0.92 ± 0.07	1.00 (HS030, Run3)	0.57 (HS037, Run3)
	NPV	0.95 ± 0.07	1.00 (HS037, Run3)	0.54 (HS019, Run5)
	PPV	0.55 ± 0.29	0.97 (HS001, Run13)	0.00 (HS037, Run3)
cort	Sensitivity	0.54 ± 0.34	0.97 (HS012, Run4)	0.00 (HS037, Run3)
	Specificity	0.79 ± 0.18	1.00 (HS037, Run3)	0.29 (HS030, Run1)
	Accuracy	0.78 ± 0.12	0.99 (HS019, Run5)	0.45 (HS030, Run1)
	NPV	0.84 ± 0.15	1.00 (HS037, Run8)	0.52 (HS001, Run12)
	PPV	0.45 ± 0.35	1.00 (HS020, Run7)	0.00 (HS037, Run8)
tumor	Sensitivity	0.48 ± 0.27	0.91 (HS029, Run7)	0.00 (HS037, Run8)
	Specificity	0.88 ± 0.13	1.00 (HS037, Run4)	0.34 (HS020, Run5)
	Accuracy	0.82 ± 0.11	1.00 (HS001, Run6)	0.52 (HS020, Run5)
	NPV	0.89 ± 0.12	1.00 (HS030, Run1)	0.59 (HS020, Run5)
	PPV	0.38 ± 0.36	0.91 (HS017, Run3)	0.00 (HS037, Run8)
margin	Sensitivity	0.38 ± 0.27	0.97 (HS020, Run4)	0.00 (HS016, Run4)
	Specificity	0.86 ± 0.14	1.00 (HS016, Run5)	0.36 (HS020, Run4)
	Accuracy	0.79 ± 0.13	1.00 (HS001, Run12)	0.45 (HS037, Run8)
	NPV	0.88 ± 0.13	1.00 (HS030, Run3)	0.45 (HS037, Run8)
	PPV	0.36 ± 0.35	1.00 (HS016, Run5)	0.00 (HS030, Run3)
white	Sensitivity	0.14 ± 0.12	0.37 (HS030, Run3)	0.00 (HS037, Run5)
	Specificity	0.97 ± 0.07	1.00 (HS037, Run5)	0.64 (HS037, Run8)
	Accuracy	0.91 ± 0.09	1.00 (HS012, Run4)	0.64 (HS037, Run8)
	NPV	0.94 ± 0.08	1.00 (HS012, Run4)	0.78 (HS001, Run22)
	PPV	0.31 ± 0.37	0.97 (HS026, Run5)	0.00 (HS012, Run4)

Table 4.12: Performance metrics for each class related to the convolutional neural network (CNN). Abbreviations: ara = arachnoid; cort = cortical gray matter; margin = areas of edema / suspected infiltration; NPV = Negative Predictive Value; PPV = Positive Predictive Value; SD = Standard Deviation; white = white matter.

The following cases with the relative runs and categories showed the best performance (1.00/100%):

- HS030, Run3 (Four occurrences): Accuracy (ara), NPV (blood), Accuracy (vessel), NPV (margin).

The following cases with the relative runs and categories showed the worst performance (0.00 / 0%):

- HS030, Run3 (Four occurrences): Accuracy (ara), NPV (blood), Accuracy (vessel), NPV (margin);
- HS037, Run8 (Five occurrences): Sensitivity (ara), Specificity (blood), Accuracy (cort), NPV (margin), PPV (tumor);
- HS012, Run4 (Four occurrences): Sensitivity (blood), Specificity (white), Accuracy (white), PPV (white);
- HS037, Run3 (Three occurrences): Specificity (vessel), Accuracy (vessel), PPV (cort).

These results show that the CNN performance on gliomas is heterogeneous, with some promising performances in the categories of interest. For example, the average accuracy in detecting the tumour core was 0.82 ± 0.11 , and the NPV was 0.89 ± 0.12 , both in keeping with the outcomes reported in the international literature (see Chapter 4). However, the sensitivity was not satisfactory (0.48 ± 0.27), and so the PPV (0.38 ± 0.36). As seen in Table 4.12, white matter and margins also did not offer good performance in terms of sensitivity.

The Discussion section expands on these results further. Below is a selection of cases for visual analysis of the predictions.

The algorithm automatically assigns a random colour to everything outside the pre-labelled mask, but the values seen on the background are excluded from the metrics calculations.

Figures 4.20 and 4.21 offer a first example of the results. This was a case of a post-central gyrus multifocal glioblastoma. The clinical and radiological features are specifically interesting

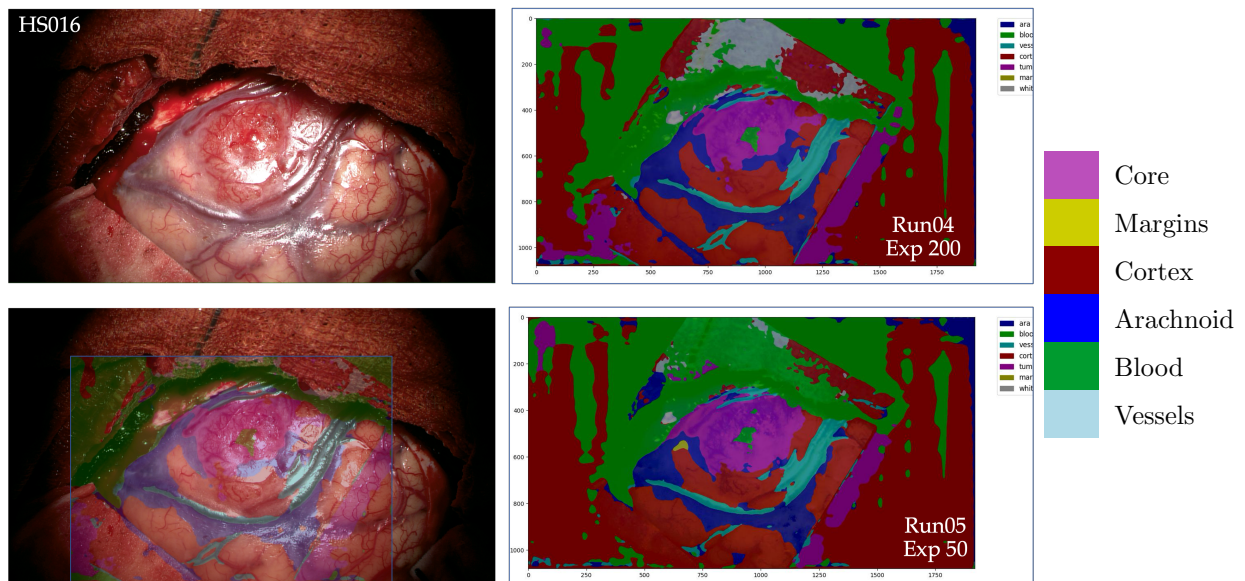


Figure 4.20: Case HS016 - A multifocal, post-central gyrus glioblastoma exposed at the very beginning of the resection. Top left - microscope snapshot: dura has been opened and the tumour is clearly visible. The right side of the picture shows a comparison between predictions from different acquisitions - Exp = Exposure. Bottom left: mask overlapped with the microscope acquisition.

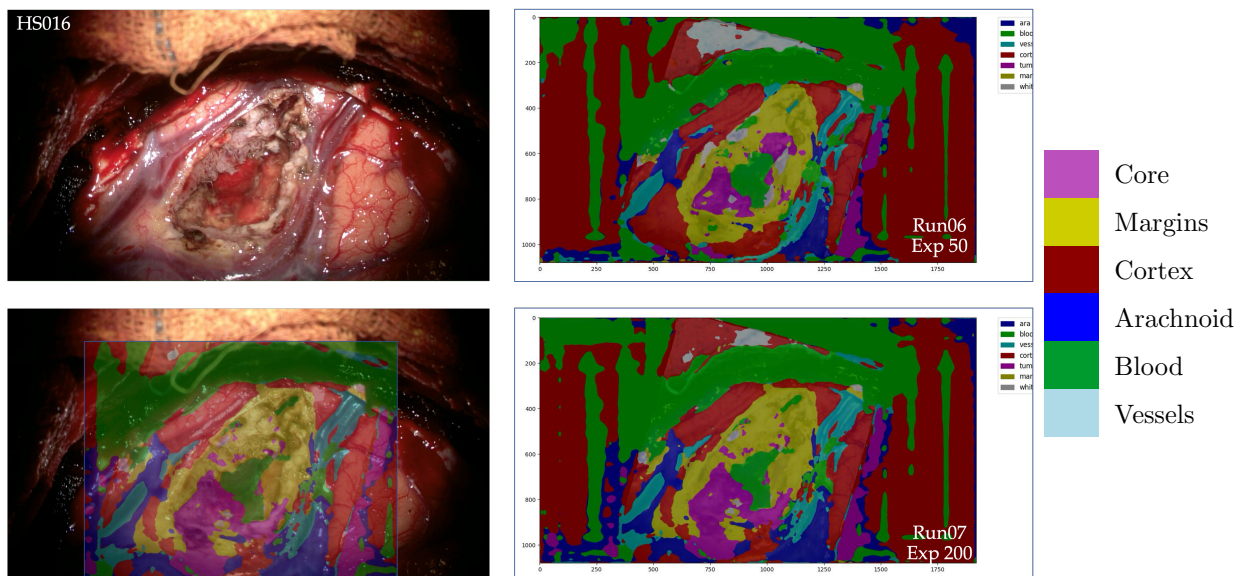


Figure 4.21: Case HS016, post-debulking - Different exposures don't seem to impact the tumour margins' visualisation significantly.

since, per the pre-operative MRI scan, the tumour bulk seen in the picture was surrounded by oedema / altered signal, meaning that the visible cortex was marked as "margins" during the labelling process. Contrary to the expectation, the CNN produced an image with relatively clear identification of the tumour core, the arachnoid, the blood vessels, and the blood extravasation, but not the margins. All cortical surfaces have been interpreted as cortical surface/brain grey matter. In Figure 4.21, the post-resection cavity shows a degree of residual tumour and cauterized tissue that the CNN has interpreted as tumour margins. In both cases, the different exposures don't significantly affect the network's performance.

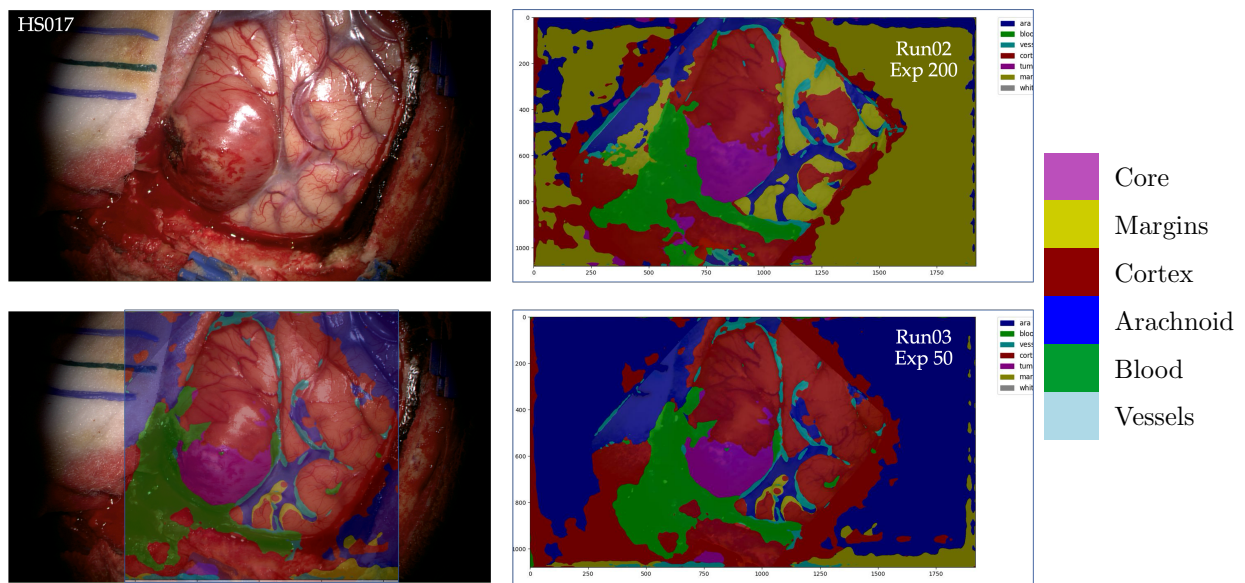


Figure 4.22: Case HS017, a large temporo-basal glioma. On the top left is the intra-operative microscope snapshot. On the right of the picture are two predictions based on two acquisitions with exposure at 200 and at 50, respectively. Bottom left: the prediction is overlaid on the microscope snapshot to show the corresponding areas.

Figure 4.22 shows another case with obvious tumour visualization. This was an example of a temporal-basal glioblastoma, where a reverse-horseshoe skin flap was harvested and a relatively large craniotomy performed to allow brain expansion and reduce the trauma from manipulation. The algorithm only partially identifies the tumour core (purple patch) on CNN processing. In this case, the tumour is fading and partially displacing the superior temporal gyrus, which appears similar to the tumour border in several points. This element alone may be responsible for the partial mislabelling of the predicted image, a point to be expanded on in the discussion section. The CNN is acceptable for identifying cortical surfaces, arachnoids, and blood vessels. However, more interestingly, identifying the areas labelled as tumour margins (the yellow)

low patches) is variable according to the exposure selected, even if the other categories remain stable. This finding is difficult to explain.

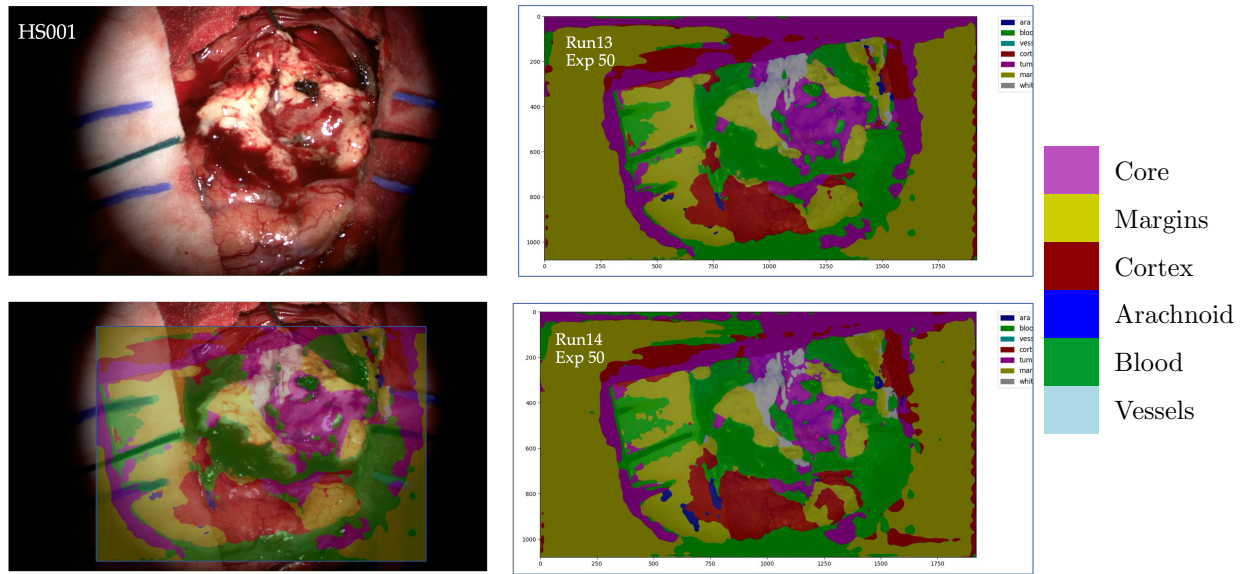


Figure 4.23: Case HS001 - Comparison between different predictions from acquisitions. This case showed a good exposure of a deep tumour, with more than satisfactory network performance in identifying the tumour core, the margins, and the white matter.

Case HS001 was a parasagittal glioblastoma. The first acquisitions were showing an intact cortical surface with no tumour seen. This case offered an excellent example of the network performance when the tumour was not surfacing, thus requiring adequate exposure with prolonged haemostatic phase to show a decent interface between the tumour and brain tissue. The CNN predictions are shown in Figure 4.23. In this case the algorithm showed an excellent visual identification of the tumour core, the margins, and white matter, as pre-labelled in the mask.

Figure 4.24, finally, shows a partially resected glioblastoma of the superior parietal lobule. This was an exhaustive example of a case showing some of the technical challenges related to the present study (see Discussion section). First, the Labview software froze during the first acquisition (cortical surface) and had to be restarted, therefore the first acquisition was performed during the number 2 phase. Secondly, the microscope camera did not acquire the snapshot, so the cases were segmented using one of the hyperspectral cube images. Third, a key-hole approach was chosen for this case, thus limiting the exposure of the "normal" brain. As a consequence of these factors, the intra-operative picture selected shows a mixture of elements,

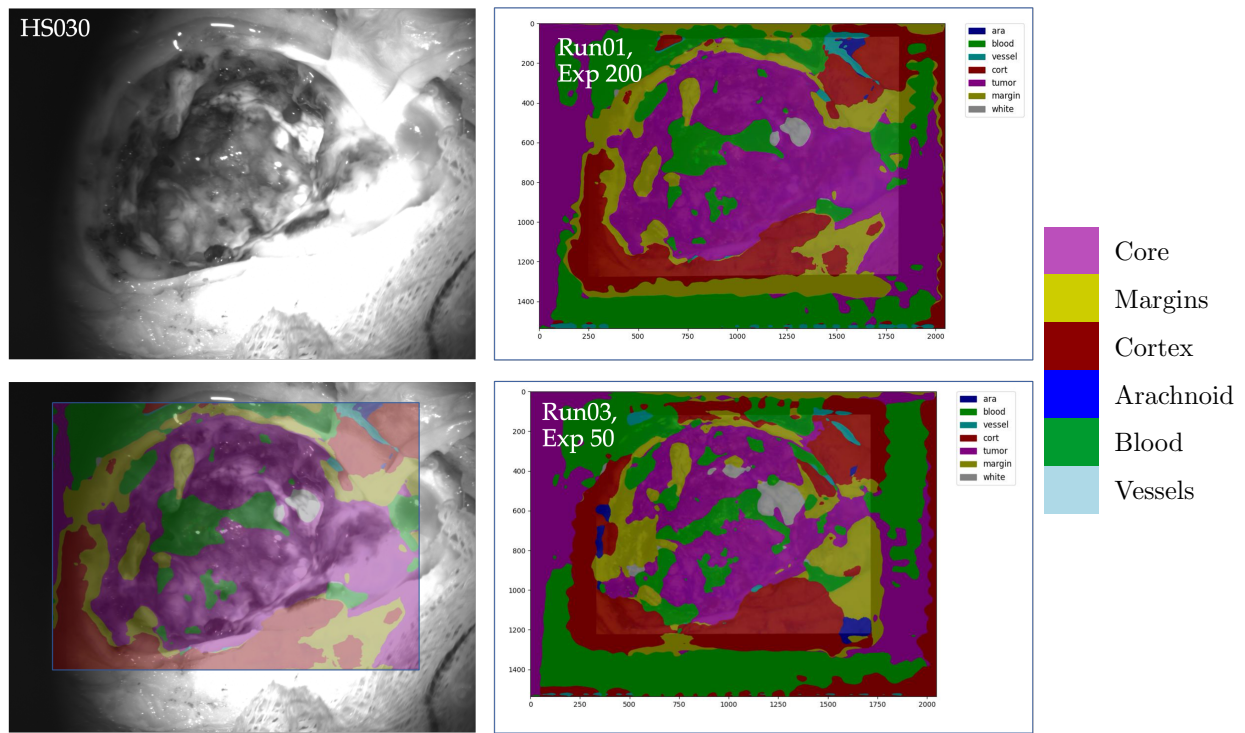


Figure 4.24: Case HS001 - Comparison between different predictions from acquisitions. This case showed a good exposure of a deep tumour, with more than satisfactory network performance in identifying the tumour core, the margins, and the white matter.

including blood, CSF, white matter, and tumour residual, resulting in a poorer performance of the CNN. Specifically, the tumour core is more extended than expected when considering the pre-op MRI scan, and the minimal amount of surrounding cortex is also overly interpreted as tumour margin.

4.3.5 Meningiomas

The intra-operative pictures showed an interesting variety of cases for anatomical location, tumour visualization, and access to visualization.

Eight meningiomas were located in the sagittal or parasagittal region, with one (HS002) being a meningioma of the inferior falx. Two meningiomas were situated on the convexity.

The single hypercube classification model showed excellent results with all algorithm variants used.

The inter-patient classification showed more heterogeneous results.

- The random forest reached a sensitivity of 0.7 and specificity of 0.6 for the tumour core class, with an accuracy of 0.6 - however, there was marked variation depending on which case was chosen for validation. Changing the estimators and the random states and adding class re-balancing did not significantly change the performance metrics.
- The SVM reached a sensitivity and a specificity of 0.8 for the tumour core class, with an accuracy of 0.8.
- The decision tree and random forest classifiers reached a sensitivity of 0.7 for the tumour core class, with an accuracy of 0.8.

Intra-patient classification

I used the model for case HS015 as a trial. The results of the intra-patient classification using the Random Forest classifier are reported in Table 4.13.

Class	Sensitivity	Specificity	Overall Accuracy
bkg	0.98	0.98	0.98
core	0.99	0.99	0.99
cortex	0.96	1.00	1.00
arachnoid	0.94	1.00	1.00
vessels	0.92	1.00	1.00
dura	0.93	1.00	1.00
blood	0.93	1.00	1.00

Table 4.13: Intra-patient random forest Performance Metrics

As expected, the performance metrics showed excellent sensitivity, specificity, and accuracy for all labels. This procedure seems to apply perfectly to the specific features of the single case. However, this is also its limitation, as it needs to be generalized to an inter-patient classification (see discussion).

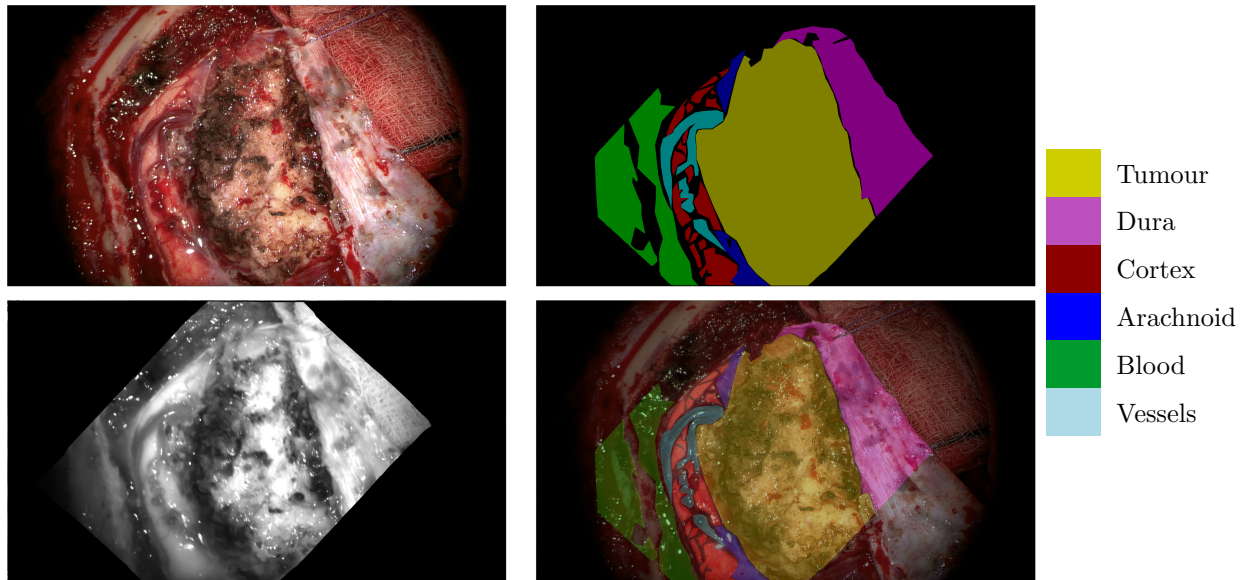


Figure 4.25: The result of intra-patient classification algorithm - random forest has been applied in this case. Top left: intra-operative microscope snapshot showing the tumour exposed; top right: segmentation mask; bottom left: image taken from the hyperspectral stack; bottom right: predicted image overlapped onto the microscope image.

Inter-patient classification

The **random forest classifier** showed heterogeneous results.

I have attempted different combinations of cases, sampling, and estimators. As specific categories (especially arachnoid and blood vessels) were scarcely represented in the segmentation mask, pixel re-sampling was allowed in some cases.

When looking at tumour vs dura differentiation, the worst performance was obtained with the following combination of cases:

```
data_path = '/Users/Giulio/Desktop/Meningiomas/'
tra_pathlist = ['HS015', 'HS008', 'HS033', 'HS042', 'HS046', 'HS023', 'HS031', 'HS028']
val_pathlist = ['HS002']
test_pathlist = ['HS007']
```

The results of this combination are reported in Table 4.14.

The reason for such poor performance is unclear. One possible explanation is that both the training and the validation cases showed a minimal amount of visible tumour. Specifically, case

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	0.4238	0.9035	0.8266	0.8915	0.4561
blood	0.8144	0.6979	0.7103	0.9692	0.2437
cort	0.4546	0.8804	0.8040	0.8806	0.4541
core	0.0839	0.9766	0.7763	0.7865	0.5095
dura	0.1223	0.8029	0.7011	0.8388	0.0983
vessel	0.1250	0.9157	0.7738	0.8271	0.2450

Table 4.14: Random forest classifier results produced when case number HS002 has been used as a validation case - this attempt showed the poorest performance of this model.

HS002 presented a deeply seated para-falcine tumour that had to be manipulated, cauterized, and dissected from the surrounding brain with multiple cottonoids. The poor exposure and numerous artificial elements might have been at the root of this issue.

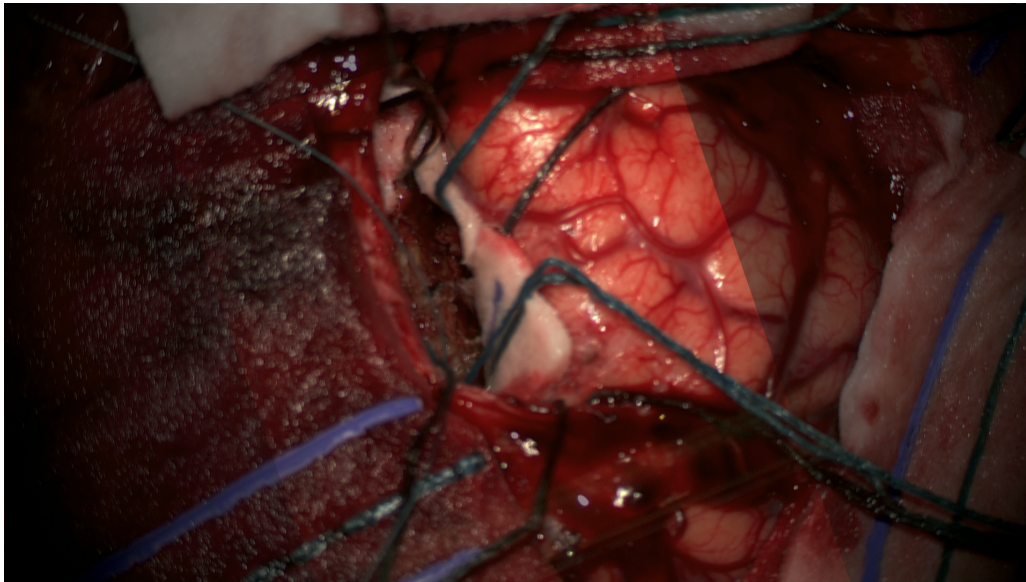


Figure 4.26: Intra-operative microscope snapshot for case number HS002. The tumour is deep and poorly visible, with a large number of surgical cottonoids around. These findings might explain the poor performance when this specific case was used for validation in one of the random forest runs.

The best metrics were otherwise obtained with the following combination of cases:

```
data_path = '/Users/Giulio/Desktop/Meningiomas/'
tra_pathlist = ['HS015', 'HS008', 'HS042', 'HS046', 'HS023', 'HS031', 'HS002', 'HS028']
val_pathlist = ['HS033']
test_pathlist = ['HS007']
```

Table 4.15 reports the results obtained by sampling 5000 pixels per class, while Table 4.16

shows the results obtained with 10000 pixels sampling per class. This was the best performance achieved by applying a Random Forest algorithm to our cohort of meningiomas. The sensitivity was at 0.74 for the tumour core, although with a poor PPV (0.2) and a borderline accuracy (0.6).

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	0.0865	0.9882	0.8309	0.8366	0.6070
blood	0.5548	0.8385	0.8056	0.9348	0.3112
cort	0.2631	0.9186	0.8271	0.8849	0.3440
core	0.7340	0.6506	0.6651	0.9205	0.3074
dura	0.5233	0.8863	0.8441	0.9340	0.3770
vessel	0.1069	0.9257	0.6971	0.7280	0.3577

Table 4.15: Random forest best performance metrics for our cohort of meningiomas, with pixel sampling set at 5000.

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	0.1175	0.9894	0.8280	0.8315	0.7165
blood	0.5553	0.8655	0.8245	0.9274	0.3861
cort	0.2354	0.9199	0.8224	0.8787	0.3279
core	0.7447	0.5973	0.6154	0.9433	0.2064
dura	0.5392	0.8979	0.8505	0.9275	0.4459
vessel	0.0946	0.9363	0.6966	0.7220	0.3716

Table 4.16: Random forest best performance metrics for our cohort of meningiomas, with pixel sampling set at 10000

The best performance for the **SVM classifier** was achieved with the following combination of cases:

```
data_path = '/Users/Giulio/Desktop/Meningiomas/'
tra_pathlist = ['HS008', 'HS042', 'HS046', 'HS023', 'HS031', 'HS002', 'HS028', 'HS015']
val_pathlist = ['HS033']
test_pathlist = ['HS007']
```

Results metrics are reported on Table 4.17.

Class	Sensitivity	Specificity	Accuracy	NPV	PPV
ara	0.5415	0.9414	0.8716	0.9067	0.6612
blood	0.5683	0.8285	0.7982	0.9359	0.3034
cort	0.4838	0.8347	0.7857	0.9088	0.3218
core	0.8435	0.8367	0.8379	0.9620	0.5220
dura	0.2275	0.9640	0.8784	0.9047	0.4536
vessel	0.2022	0.9541	0.7442	0.7554	0.6306

Table 4.17: Performance metrics of SVM applied to our cohort of meningiomas.

CNN classifier

The CNN algorithm's performance on meningiomas was also computationally demanding, requiring 8 hours to be completed.

The cases selected for this analysis were:

'HS015', 'HS008', 'HS042', 'HS046', 'HS023', 'HS031', 'HS002',
'HS028', 'HS033', 'HS007'

The main results for the CNN classifier on our cohort of meningiomas are shown on Table 4.18.

The algorithm showed its best performance (1.00/100%) on several more cases, runs, and categories when compared to the HGG results. The tumour class showed an average sensitivity of 0.71 ± 0.24 , and an accuracy of 0.86 ± 0.15 .

The Discussion section discusses these results further. Below is a selection of cases for visual analysis of the predictions.

The algorithm automatically assigns a random colour to everything outside the pre-labelled mask, but the values seen on the background are excluded from the metrics calculations.

The first meningioma recruited in our series was case HS002, which is exposed on Figure 4.27. This was a case of a left-sided parasagittal meningioma located 1 centimetre deep on the falx. Some degree of dissection needed to be performed to reach it, meaning that the tumour was cauterized on several occasions and at least partially lost its natural texture. The predictions showed an excellent result for the tumour core (sensitivity 0.8, specificity and accuracy 0.9).

Class	Metric	Mean $\pm$ SD	Best Value (Runs)	Worst Value (Runs)
ara	Sensitivity	0.45 ± 0.26	0.99 (HS028, Run7)	0.00 (HS033, Run5)
	Specificity	0.96 ± 0.08	1.00 (HS008, Run8)	0.65 (HS028, Run4)
	Accuracy	0.91 ± 0.11	1.00 (HS007, Run6)	0.57 (HS031, Run3)
	NPV	0.94 ± 0.11	1.00 (HS007, Run5)	0.51 (HS031, Run3)
	PPV	0.44 ± 0.36	1.00 (HS008, Run5)	0.00 (HS007, Run5)
blood	Sensitivity	0.73 ± 0.21	0.99 (HS031, Run3)	0.00 (HS033, Run3)
	Specificity	0.88 ± 0.15	1.00 (HS033, Run3)	0.19 (HS031, Run3)
	Accuracy	0.86 ± 0.12	1.00 (HS046, Run4)	0.47 (HS031, Run3)
	NPV	0.91 ± 0.20	1.00 (HS046, Run4)	0.12 (HS028, Run4)
	PPV	0.50 ± 0.40	0.99 (HS031, Run5)	0.00 (HS046, Run4)
vessel	Sensitivity	0.45 ± 0.33	0.99 (HS033, Run3)	0.00 (HS031, Run3)
	Specificity	0.96 ± 0.11	1.00 (HS031, Run3)	0.46 (HS033, Run3)
	Accuracy	0.93 ± 0.10	1.00 (HS028, Run4)	0.54 (HS007, Run5)
	NPV	0.94 ± 0.17	1.00 (HS023, Run5)	0.09 (HS007, Run5)
	PPV	0.52 ± 0.37	0.99 (HS007, Run5)	0.00 (HS023, Run5)
cort	Sensitivity	0.82 ± 0.15	0.99 (HS033, Run5)	0.30 (HS008, Run5)
	Specificity	0.90 ± 0.07	0.99 (HS023, Run8)	0.69 (HS007, Run5)
	Accuracy	0.86 ± 0.08	0.99 (HS023, Run8)	0.67 (HS007, Run6)
	NPV	0.88 ± 0.18	1.00 (HS033, Run3)	0.15 (HS007, Run6)
	PPV	0.71 ± 0.27	0.99 (HS007, Run6)	0.00 (HS033, Run3)
dura	Sensitivity	0.59 ± 0.27	1.00 (HS002, Run2)	0.07 (HS008, Run5)
	Specificity	0.93 ± 0.06	1.00 (HS028, Run7)	0.78 (HS015, Run4)
	Accuracy	0.86 ± 0.09	0.99 (HS033, Run5)	0.64 (HS008, Run5)
	NPV	0.89 ± 0.11	1.00 (HS023, Run7)	0.66 (HS008, Run5)
	PPV	0.63 ± 0.34	1.00 (HS028, Run7)	0.00 (HS023, Run7)
tumor	Sensitivity	0.71 ± 0.24	1.00 (HS023, Run10)	0.16 (HS046, Run7)
	Specificity	0.91 ± 0.15	1.00 (HS002, Run2)	0.33 (HS008, Run5)
	Accuracy	0.86 ± 0.15	0.98 (HS007, Run9)	0.33 (HS008, Run5)
	NPV	0.94 ± 0.10	1.00 (HS007, Run6)	0.60 (HS015, Run4)
	PPV	0.58 ± 0.39	1.00 (HS002, Run2)	0.00 (HS007, Run6)

Table 4.18: Performance metrics for each class related to the convolutional neural network (CNN) in our class of meningiomas. Abbreviations: ara = arachnoid; cort = cortical gray matter; NPV = Negative Predictive Value; PPV = Positive Predictive Value; SD = Standard Deviation

However, on visual inspection, there was a clear over-representation of the class tumour, most likely due to the overuse of cauterization during surgery, which made the tumour surface appear more as coagulated blood / dry tissue - note the blood-soaked cottonoid on the left, with a similar appearance, interpreted as tumour by the algorithm.

Figure 4.28 shows the intra-operative view on case HS008 and the comparison between the two

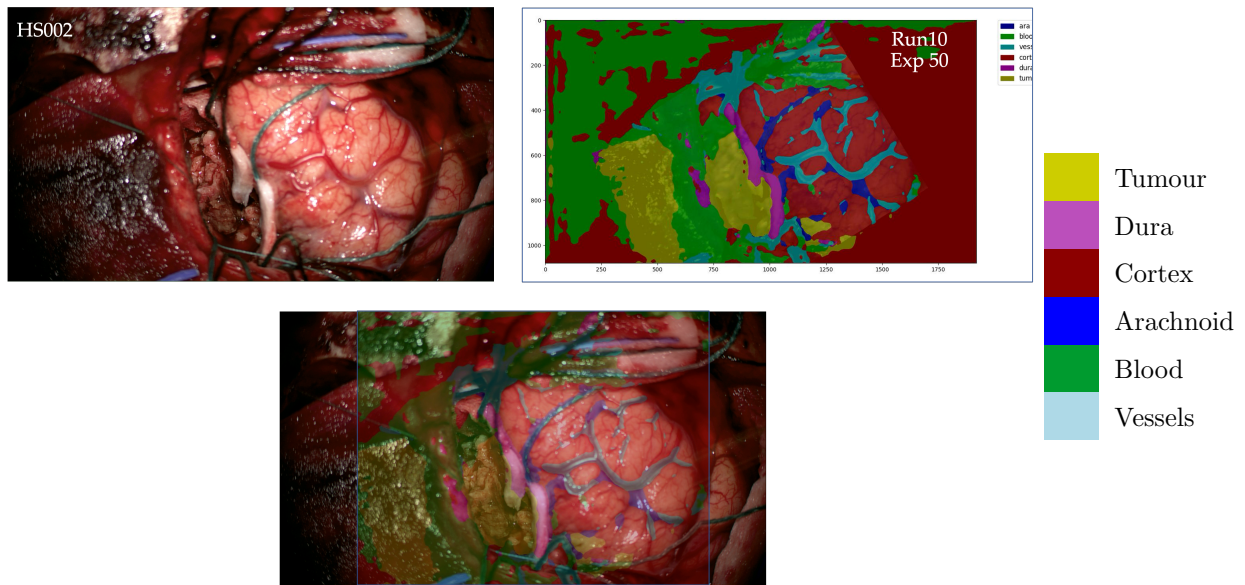


Figure 4.27: Case HS002 - First meningioma acquired in our series. Top left: microscope snapshot; right side: predicted image; bottom: microscope image with the prediction on top.

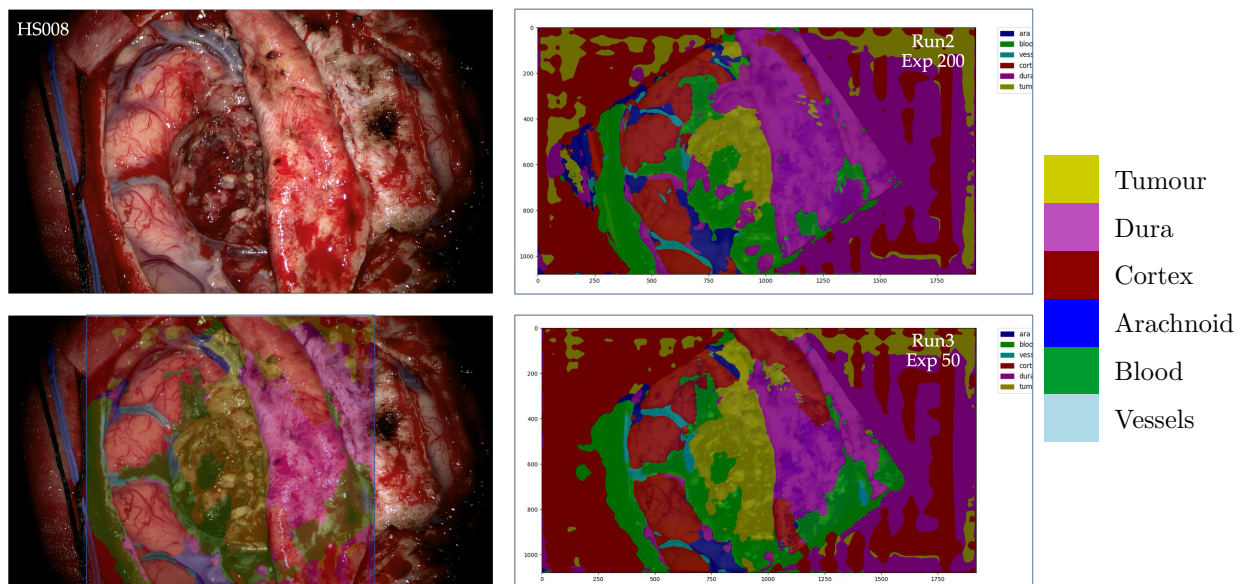


Figure 4.28: Bilateral parasagittal meningioma. Top left corner: microscope snapshot. Right side: predictions realized with acquisitions at different exposures. Bottom left: prediction realized with the exposure at 50 used as a mask for the microscope view.

exposures (200 and 50, respectively). In comparison, the performances do not immediately appear different, the exposure at 50 seems to produce slightly improved metrics (sensitivity 0.8, PPV 0.7, accuracy 0.94) compared to the one at 200 (sensitivity 0.6, PPV 0.8, accuracy 0.92), which is a similar result observed in the HGG series, Figure 4.22. This result is confirmed visually when looking at the tumour (dark yellow), showing a more precise representation at 50.

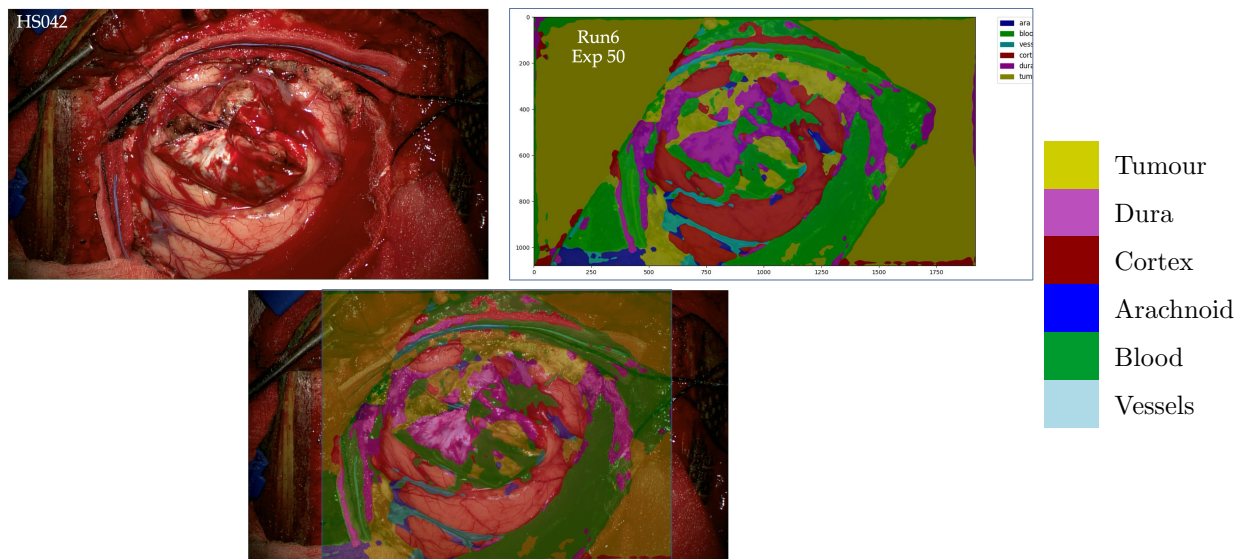


Figure 4.29: Case HS042 - Convexity meningioma of the supramarginal gyrus. Top left: microscope snapshot showing the exposed tumour with the dura folded on top of the mass. Top right: prediction, exposure at 50. Bottom: prediction compared with the microscope snapshot.

Figure 4.29 shows a convexity meningioma case. The tumour was exposed through a wide craniotomy, and the intra-operative snapshot shows excellent visualization of the brain-tumour interface and the dura overlying the tumour. The prediction was obtained from an acquisition performed with an exposure of 50. Upon visual inspection, the algorithm correctly predicted most of the areas and the relative classes, although some patches of the dura were interpreted as tumours. This might be due to a very similar spectral signature of the dura compared to the tumour core. Still, the possibility of actual tumour infiltration on the apparently normal dura cannot be excluded, although more data will be needed to confirm.

4.3.6 LGG and anaplastic gliomas

The dataset of LGG and anaplastic gliomas was too limited to create two different classification models to process them separately or together.

I have only used the intra-patient classification methods to analyze some of the cases collected. The performance metrics obtained by running the random forest classifier are shown in Table 4.31

Category	Sensitivity	Specificity	Overall Accuracy
BKG	0.98	0.80	0.95
Core	0.82	0.98	0.97
Cortex	0.74	0.99	0.98
Arachnoid	0.46	1.00	1.00
Vessels	0.42	1.00	0.99
Margins	0.60	1.00	1.00
Blood	0.12	1.00	1.00

Table 4.19: Case HS009 - random forest classifier result.

The random forest classifier outperformed both the decision tree and the MLPC in identifying the tumour and the cortex. A visual comparison is shown in Figure 4.30.

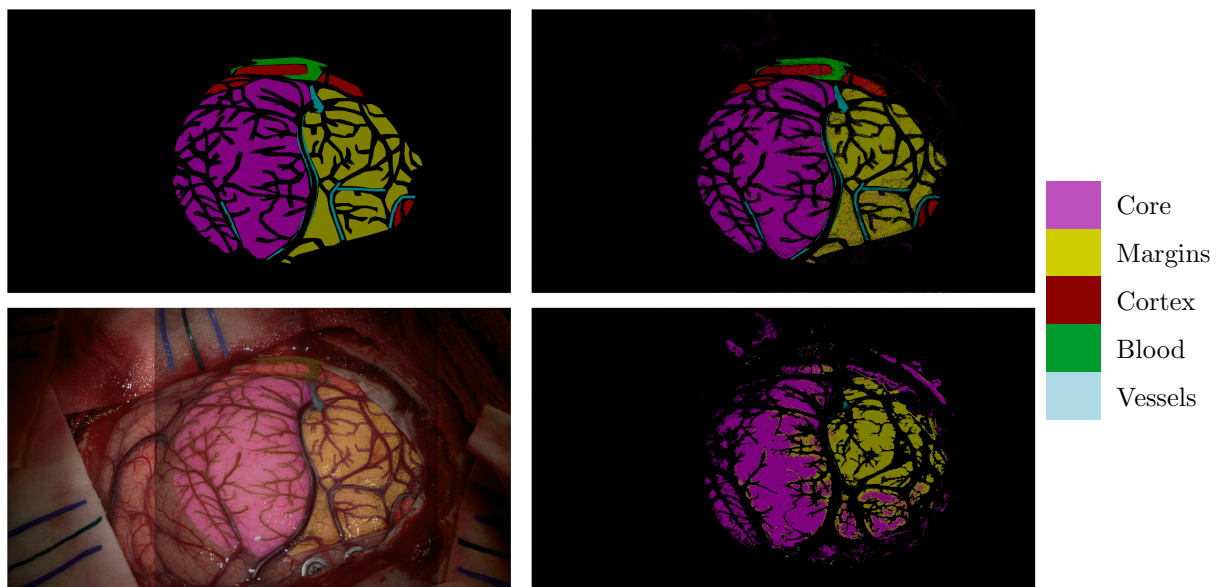


Figure 4.30: HS009 - Comparison of the different models' performances. Looking clockwise - Top left: mask; top right: random forest classifier output; bottom right: MLPC output; bottom left: mask overlapped with the microscope snapshot.

4.3.7 Haemodynamic changes

All the patients operated on and included in this analysis followed the same clinical and anaesthetic protocol, the asleep-awake-awake technique. They were first anaesthetized and intubated with a laryngeal mask. Peripheral electrodes, arterial lines, venous accesses, and urinary catheters were positioned to guarantee adequate monitoring and drug infusion. The Total Intra-Venous Anaesthesia (TIVA) was kept constant using a combination of propofol infusion and opioids (fentanyl or remifentanyl).

The local anaesthesia provided was a combination of bupivacaine 0.5% and adrenaline 1:200.000, with a dose ranging from 40 to 80 mls, depending on the weight of the patient. After skin incision, muscle dissection, and craniotomy, local anesthetic using lidocaine 2.5% (no adrenaline) was repeated at smaller dosage (5 to 10 mls) on both the muscle and the dura.

The TIVA was stopped and the patient was typically woken up and kept awake for the rest of the procedure.

The image analysis protocol detailed in Figure 4.15 was applied to the four awake cases of this series.

The first finding was that some apparent changes in perfusion between different phases and tasks were observed in all cases analyzed. The second observation was that the changes were not localized to specific areas but were more generalised. The final, more challenging observation was a discrepancy between some expected and some observed changes. For example, comparing the acquisitions between an anesthetized patient and the patient executing a task, we noticed that in some cases the expected differences were subtle, while comparing a patient awake but not executing tasks, and the same patient executing a task, marked differences were observed.

The following subsections show part of the results, where two cases are exposed with their relative Figures and commented on.

For each case, there are three groups of figures:

1. intra-operative picture;

2. total haemoglobin (THb) compared to oxygenation: on each figure, the total haemoglobin concentration is shown on the top row and the oxygen saturation in the bottom row - the spectrum varies from **low concentration**, expressed in dark blue, to **high concentration** expressed in red;
3. oxyhaemoglobin (HbO) / deoxyhaemoglobin (Hb) changes - on each figure, the oxyhaemoglobin concentration is shown on the top row and the deoxyhaemoglobin concentration is shown on the bottom row - the spectrum varies from **low concentration**, expressed in dark blue, to **high concentration** expressed in red.

Case HS009

This was a case of a left-sided grade II oligodendroglioma located at the level of the supra-marginal gyrus, displacing the suspected Wernicke's area inferiorly and the arcuate fasciculus more deeply and anteriorly.

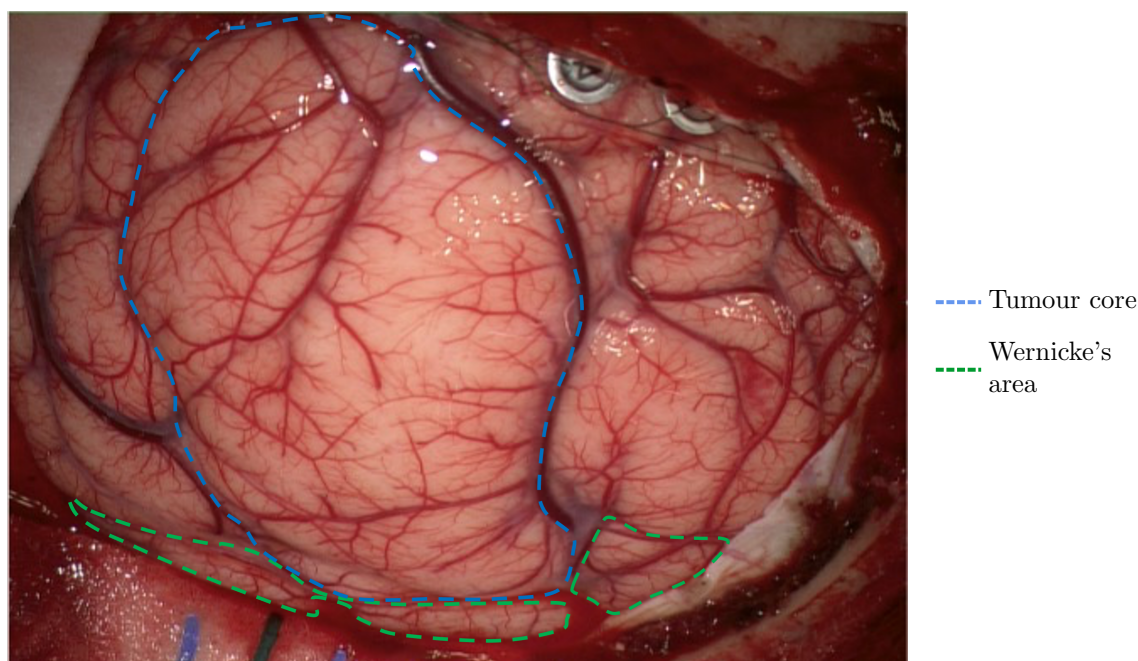


Figure 4.31: Case HS009 - intra-operative view. The image is flipped horizontally and seen from the bottom, to compare it with Figure 4.32.

This case seems to present more artefacts than expected, especially when looking at the marked peripheral halo of most sequences. Interestingly, we observed no significant fluctuation in the

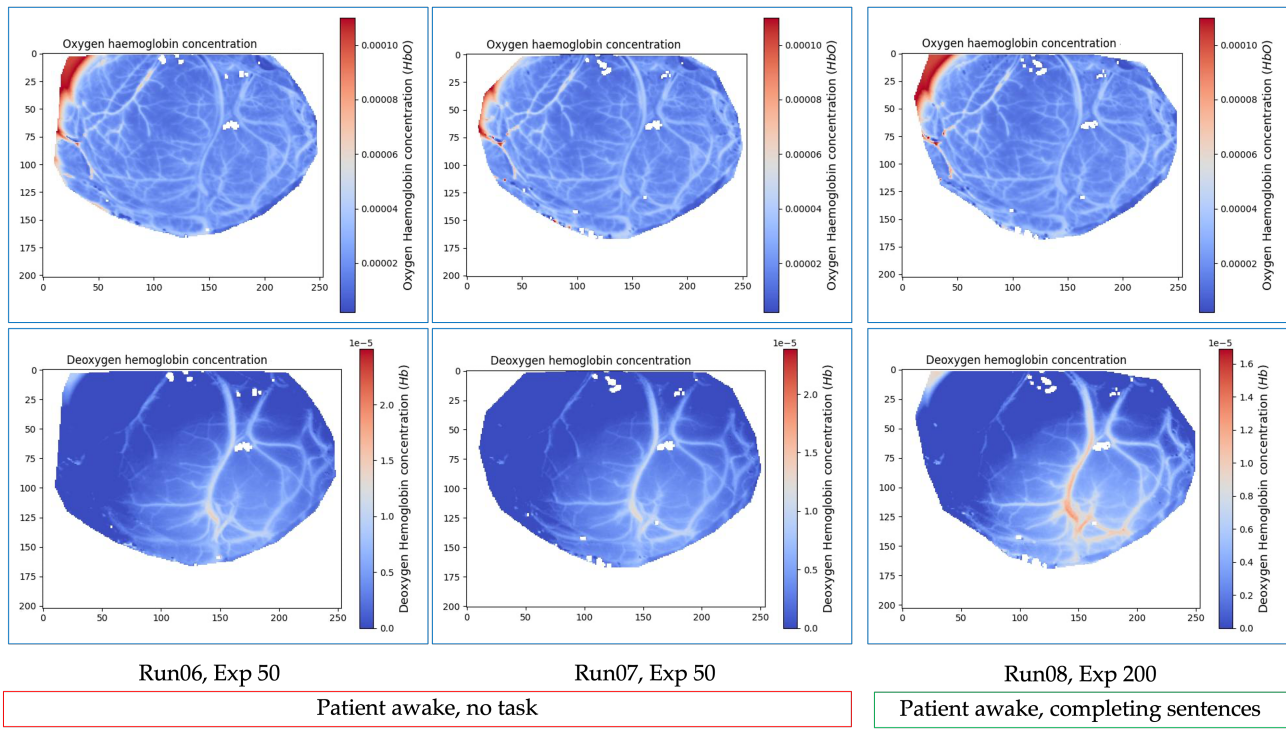


Figure 4.32: Case HS009 - Tumour exposed. Comparison between HbO and Hb concentrations during task executions.

THb between the several runs. Still, when computing the HbO and Hb sequences, a clear pattern of increased Hb was seen between the anaesthetized and the awake state (Figure 4.32) and between the silent mode and the speech mode (Figure 4.33). The same changes were not seen for Hb0 or oxygen saturation. This finding is in keeping with the signal seen during fMRI (BOLD) acquisitions [169] and will be analysed in the Discussion section

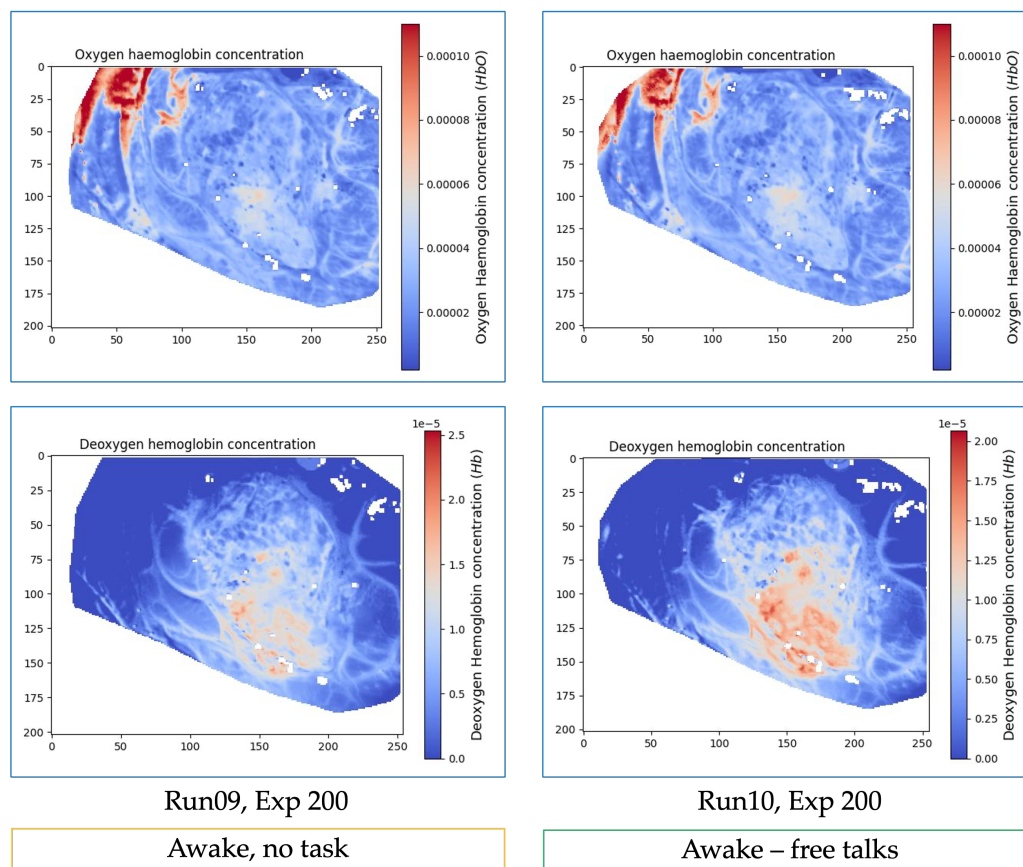


Figure 4.33: Case HS009 - Final cavity, comparison between HbO and Hb concentrations during task executions.

Case HS048

The following pictures show the findings on a case of a left-sided sub-central gyrus astrocytoma operated in awake surgery.

The tumour displaced the subcentral gyrus cranially and was seen in the centre of the surgical field.

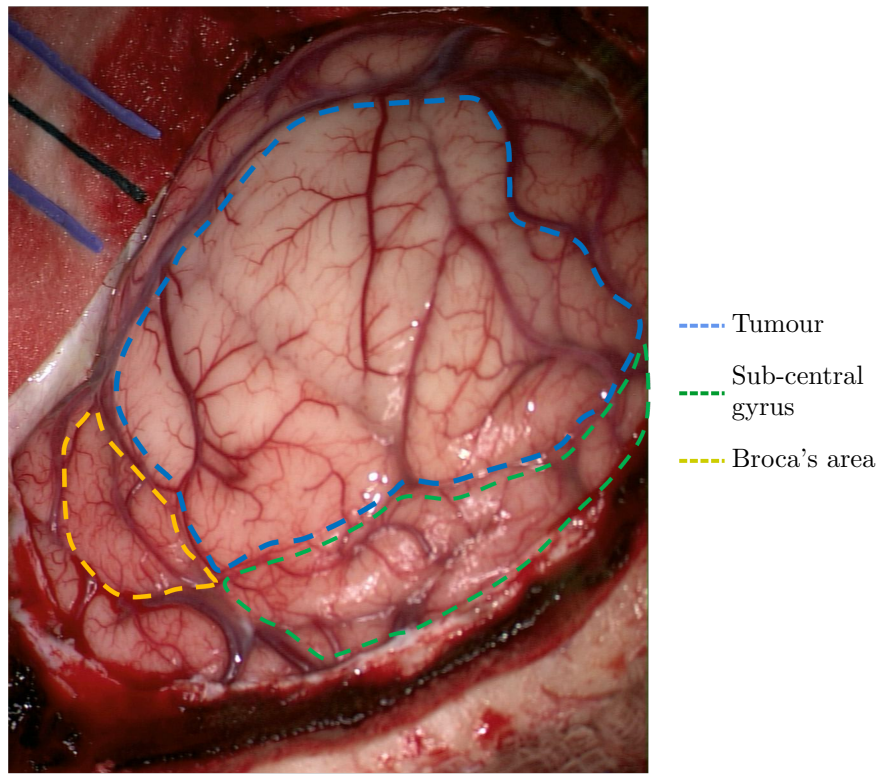


Figure 4.34: Case HS048 - intra-operative view. The image is flipped horizontally to compare it with 4.35.

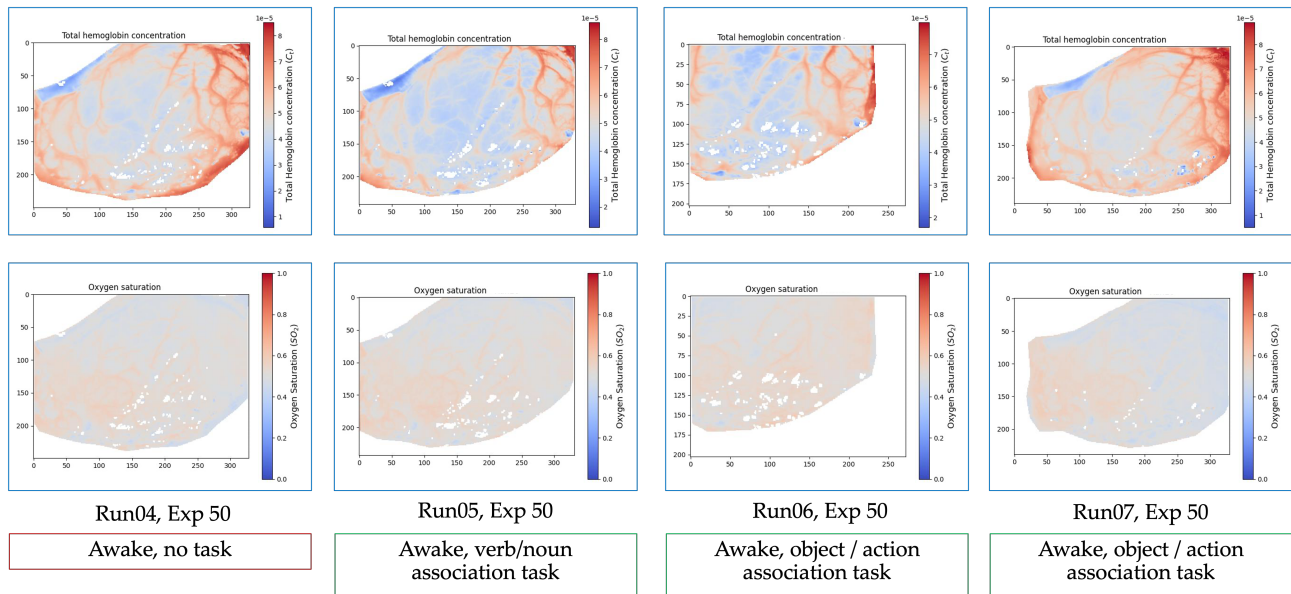


Figure 4.35: Case HS048 - THb concentrations and O2 saturation maps.

The observed changes are not localized but seem to affect the whole surgical field. The THb seems to fluctuate between acquisitions, and when different runs are compared, there is clearly an increase of both THb (more pronounced) and O2 (less pronounced) at Run07, when the patient is associating objects and actions. Theoretically, Run05 and Run06 are comparable to

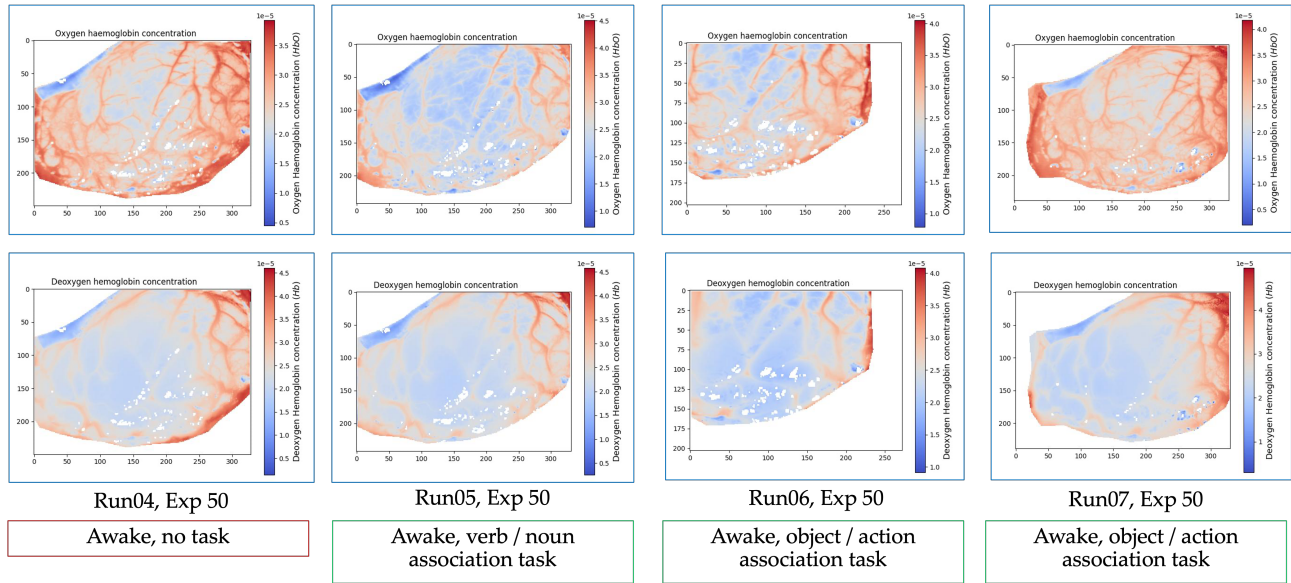


Figure 4.36: Case HS048 - Comparison between HbO and Hb concentrations during task executions.

Run07 in terms of exposure and tasks performed, which is counterintuitive when looking at the changed distribution.

4.4 Discussion

The study conducted at Imperial College Healthcare NHS Trust has succeeded in collecting the largest cohort of intra-operative hyperspectral images of brain tumours reported so far, more than doubling the current most extensive database [273], despite the challenge to recruit during a global pandemic.

The present section reflects on the study's main phases, findings, and limitations.

4.4.1 Set-up, recruitment and acquisitions: ex-post reflections

The hardware and software learning curve for image acquisition was user-friendly and quick to set up. The adaptor has provided good support for the available microscope, and the combination of relatively reliable software such as LabVIEW coupled with a structured database

has allowed clinicians to acquire images rapidly and in a way that was more often than not compatible with operative theatre time.

However, the current set-up cannot be used in a much more scaled study involving hundreds of patients and potentially multiple centres. Several patients consented but were eventually not recruited due to a few episodes of software crashing and operative theatre set backs. In the latter scenario, the delay resulted in a lack of time to set up the equipment. Moreover, with most of the NHS staff not being on the payroll for PAs dedicated to research activities, the team was often forced to sacrifice recruitment to provide adequate patient care. The long tail of the Covid19 pandemic has also caused significant disruptions in terms of operative theatre availability, which caused us to request an extension of the study (initially set up to finish in February 2023, eventually stopped in October) and to fall slightly short of our recruitment target (47 patients instead of the proposed 50).

For a more extensive multi-centric study, several steps are necessary. The first is to conduct further research and development in the lab. A more flexible camera set-up, with shortened acquisition times and intuitive software, are mandatory requirements. The second compulsory passage, equally important, is to apply for a grant proposal for more logistical support, both in the surgical theatre for data collection and at the South Kensington site for data analysis. The third step is to contact different research centres from similar catching areas and with comparable volumes to evaluate the practicality of a collaborative effort. More details on the points mentioned earlier are provided in Chapter 5, Future Developments.

4.4.2 Tumour identification: Main findings and limitations

The results provided are the starting point of this study, but just the beginning of a more structured, prolonged, and diversified data analysis phase. The dataset offers a remarkable range of opportunities for future research.

A more articulated analysis of this first batch of results focuses on three main factors that have played a significant role in the outcomes:

- acquisition/case-related;
- labelling-related;
- models-related.

Acquisition-related factors. As mentioned in the results section, the clinical pool of cases was heterogeneous regarding locations, surgeons operating, and approaches. These details represented both an advantage and a disadvantage. The main advantage was that our dataset is closer to a "real-world" scenario in a standard neurosurgical unit, where many different professionals can (and often do) choose a different approach or strategy for the same tumour. Therefore, the spectrum of the possible scenarios is quite variable and reflected by the hypercubes we have collected. The intuitive downside of this choice is the lack of "ideal-world" cases where the optimal illumination, exposure, and timing conditions are equally met. It was obvious from the first few cases that several factors were impacting the quality of the images acquired during phases 2 and 3, namely narrow surgical corridors, the presence of surgical instruments, the different angles of the light beam from the microscope, the blood pooling at the bottom of the cavity. This problem was often due to the surgeon's deliberate choice to opt for a small keyhole approach. While it can be argued that the volume of cases could not leave a margin of manoeuvre to cherry-pick the best tumours for our intents (something I have purposely tried to avoid by not limiting the tumour selection to specific locations or surgical approaches), examples such as HS017 or HS016, where the tumour was exposed together with a sizable portion of margins and normal brain, showed undoubtedly better final results than others. Another crucial point for further studies is the size of the dataset itself. These results confirm the necessity of a much wider sampling, for which a larger study with higher volumes is required (see Future Developments).

Labelling factors. The labelling process is amenable to refinements. I focused on minimizing the possibility of incorrect labelling by closely matching the anatomical landmarks and the areas of signal change identified on the intra-operative microscope snapshot with the corresponding regions seen on the MRI scan sequences. To our knowledge, this is the state-of-the-art reported

in the literature [310]. Despite this, I identified a degree of uncertainty in some specific passages. For example, the exact biological definition of "tumour margins" / "oedema" is unclear, even accounting for the known dynamic of tumour invasion [74, 75]. The altered brain tissue surrounding the lesion is neither tumour nor intact normal brain tissue, and its composition might vary from one patient to another despite showing the same signal change in the MRI scan. Moreover, cerebral oedema seen on T2 surrounding most tumours spreads through the white matter. Still, in many cases, it does not reach completely up the cortex by just a few millimetres, if not less, which leaves the open question of whether to segment the cortical surface with underlying oedema as normal grey or as "margins/alterd area", thus significantly impacting on the performance of the image analysis.

Another challenge warranting further reflection is when the labelling should stop segmenting blood vessels and enter the capillary bed. Capillaries are often considered as integral part of the organ texture, therefore by definition they should be included into the segmentation of the grey/white matter. However, on the hyperspectral images, they are expected to give a spectral signature similar, if not identical, to that of the normal blood and blood vessels. This would inevitably skew the results of the algorithm interpretation of the labelling system, categorizing an excessive number of "blood"/"blood vessels" pixels into the "grey matter" category, impacting the overall performance. I have chosen an exemplary approach by labelling only the main blood vessels and leaving the capillaries unlabelled. The possible limitation of this choice is confirmed by the relatively poor performance of the category "blood vessels", especially in HGG. In that sense, I plan to re-test all previously experimented models using different segmentation approaches and cross-check the new results with the ones reported here.

Model-related factors. The intra-patient algorithm analysis showed excellent metrics in all the cases analyzed. However, these results should be interpreted cautiously due to the intrinsic risk of over-fitting and excessive model reliability on the masks.

Regarding the inter-patient analysis, the results are more heterogeneous and sometimes unsatisfactory.

In meningiomas, the majority of the algorithms I have tested showed an acceptable performance

in identifying the tumour core when this is visible and show even more promising outputs for the most common classes, such as cortical grey matter, blood vessels, dura, and pooled blood outside the vessels. One critical finding in the meningioma cohort was the frequent mismatch between the tumour core and the surrounding dura. This is an interesting clinical point, as meningiomas are known to invade the dura. Still, at the same time, the dura often shows a similar signal compared to the tumour, especially in the proximity of the core. This aspect warrants further analysis, as it is one of the crucial diagnostic points for the successful surgical resection of intracranial meningiomas, as stressed in Chapter 2.

Although the HGG-IV's performance is inadequate, there is a broad margin for improvement, especially when considering the results of the most basic models.

On decision trees, random forests, SVM, or MLPC classifiers, the frequent lack of identification of the tumour core might be linked to several technical factors. Leaving aside surgical elements (such as the exposure), the settings of the several algorithms tested (the number of estimators on the random forest, the kernel chosen for the specific model), the pre-determined areas to sample (the number of pixels to sample), the decision to re-sample rather than ignore when the class was not adequately represented (ex: arachnoid), and the size of the chosen dataset are all elements that have presumably impacted on the performance. Several anatomical details might also play a role. Glioblastomas fade into normal brain tissue, and the interface can be so subtle that when a random sampling of the tumour and the nearby brain is performed during the validation phase, the model might mistakenly interpret a pixel in the tumour region as a false positive (or vice-versa) due only to the appearance of a very similarly looking area in the training set. I have attempted several different routes to improve the models' performance, including limiting the dataset to those cases with a more evident brain-tumour interface, changing the cases chosen for training, testing, and validation, changing the parameters of the models deployed, splitting the training/testing/validation sets with different partitions. However, I have not explored all the potential options, and the analysis is still ongoing.

The CNN performance has shown a markedly improved outcome on the inter-patient classification compared to the other models. However, this model's computational effort is exceptionally

demanding, and the results are still not optimal. For the CNN training, testing, and validation, we have included only ten cases for each histological type; therefore, there is ample margin for additional runs with different combinations and numbers.

4.4.3 Detection of haemodynamic changes

The detection of perfusion changes through MSI/HSI has shown exciting results but also posed new challenges.

A fundamental and necessary premise is that the expected fluctuations in oxygen perfusion would fall into an extremely narrow range. The reason is linked to the physiological features of the brain itself.

The clinical utility of these results is questionable at this stage. The fluctuations in brain perfusion are a known physiological process which has been studied for multiple medical applications. As discussed in Chapter 1, the specific detection of small changes in Hb and HbO is at the root of BOLD MRI imaging [320]. With all the technical limitations of the case (including an accuracy that is 75% at best [167, 169, 321]), BOLD can detect localized perfusion changes affecting active eloquent areas. Specifically, at the start of task execution, BOLD sequences detect a 1-2 seconds spike in Hb, which is compatible with an increased neuronal metabolism and a consequent increased oxygen extraction [169]. A subsequent reactive increase in cerebral blood flow washes away the Hb, causing an influx of HbO, which occurs with a further 2 to 6-second lag [169]. Therefore, the increase in Hb within the acquisition timeframe is quite promising for our results, as it reflects the physiological changes usually seen with fMRI. Our average acquisition time varied between 12 and 13 seconds, with deviations around 0.5 seconds. It is plausible that within the timeframe of the patient starting to execute a task and the camera acquiring the central wavelengths of the visible spectrum, I detected a more pronounced sensitivity to Hb changes (peak of absorption at 660nm) and less to HbO₂ changes (peak of absorption at 940nm). This result suggests that the rationale for this specific study point was correct: hyperspectral imaging can potentially detect haemodynamic changes related to neuronal activation, similarly to a fMRI. However, several limitations might have impacted these

results and warrant future data analysis.

Patient related factors. The human body and its adaptation to physiological changes is a never-ending field of research, and this section of the study showed exactly how limited our knowledge is still.

While the procedures relative to anaesthesia and its physical effects are well known, the specific physiological effects on a single individual can vary, and several variables are unknown.

A few factors might have impacted the system performance in the specific setting of the perfusion changes.

- *Heart rate.* The heart activity is known to produce three spikes of IntraCranial Pressure (ICP), physiologically: P1, corresponding to the systolic pulsation, P2, linked to the brain compliance (= the intracranial content "bouncing back" the pressure from the first wave), and P3, corresponding to the closure of the aortic valve [322]. While our system did consider the pulsation and the registration process aimed at eliminating the related artefacts, a more refined synchronization with the ICP waves may be required.
- *Respiratory rate.* An average human breathes between 12 and 18 times per minute. The fluctuation of systemic oxygen and CO₂ correspond to fluctuations of Hb and HbO, a phenomenon well known in the medical literature [323]. These fluctuations directly translate into brain perfusion and are impacted by systemic and local factors. While in all of our cases, the fluctuations of Hb and HbO are assumed to be above the threshold of 95% (evaluation based on the fact that no patient has gone into respiratory distress or has experienced hypoperfusion strokes), the specific delta of the variation for the single patient at the level of the target organ (the brain) is unknown and might have impacted.
- *Brain auto-regulation.* The brain maintains a constant perfusion level due to its intrinsic mechanism of auto-regulations provided by the arterioles branching from the anterior, middle, posterior cerebral, and basilar/vertebral arteries [324]. This phenomenon is challenging to quantify locally when a specific brain area requires an increased oxygen fraction,

especially when looking at minimal fluctuations. It might have impacted the performance of our model and will need additional research for a more measurable definition.

Another crucial element to consider is that neuronal activity changes among different populations of neurons and that macro-areas are challenging to define, as explained in Chapter 1 - Introduction. Specifically, Broca's and Wernicke's areas, integral to speech production and comprehension, are activated almost immediately upon the decision to speak, typically within 100-200 milliseconds. This reflects a complex neural network encompassing motor planning for speech [325]. Similarly, for motor activities, the primary motor cortex initiates neuronal signals for muscle movements, with preparatory activity commencing approximately 100-200 milliseconds before movement - the exact timing varies based on movement complexity [326]. However, the relative changes in perfusion might happen at a different rate depending on the area examined, the specific patient's requirements, and the confounding factors of the tumour presence and the circulating anaesthetic drugs.

Model-related factors. As my review has shown, there is currently no available clinical standard to reliably identify the patterns of Hb and HbO changes in the brain, with a few groups having experimented with different protocols [259,304,310].

As mentioned at the beginning of this section, the most likely limitation of our findings lies in the acquisition method. While some promising results might be significant, a snapshot scanning technique coupled with NIR will be evaluated for future studies. This will provide an acquisition time comparable to that seen during fMRI (the current gold standard) and potentially remove structural artefacts linked to the lack of synchronicity between the camera acquisition and the physiological changes [169].

The proposed model based on the Beer-Lambert law is the most commonly adopted, but some model modifications could be implemented to refine the performances. For example, in this code, we have calculated the Differential Path-Length Factor (DPF) using the Monte Carlo simulation. However, this is just one of the many options available regarding theoretical calculation and direct/indirect measurements. Moreover, in this calculation, we have only accounted

for Hb and HbO as chromophores in a water medium. In contrast, in reality, lipids and flavo-proteins are known to be chromophores, too and might play a significant role in contributing to the final signal. The Beer-Lambert Law also assumes a linear absorption coefficient, which is not realistically comparable to biological tissues' properties. Finally, we did not consider the unequal light intensity of the source (the microscope) at different wavelengths. While not specified in the technical manual, the spectral emission profile of xenon light assembled on the Zeiss Pentero microscope should fall into a common standard from most surgical microscopes, which typically hold a smooth profile in the visible range [5]. Still, this might be a significant limitation of the model.

Our results confirm the possibility of detecting haemodynamic changes using hyperspectral imaging, but more work is required to refine the capacity to detect more subtle and locally limited changes, as with BOLD MRI.

4.5 Conclusions

Hyperspectral imaging technology is a promising tool warranting further research. It has the potential to become part of neurosurgical equipment and improve the intra-operative diagnostic capability of the operative team.

The study showed that hyperspectral imaging can successfully identify brain tumour tissue, normal cortex, and white matter and detect haemoglobin changes possibly related to brain physiological activity. However, the outputs produced are insufficiently reliable at this stage for a standardized clinical application, and further data analysis is desirable.

The hypercube dataset produced is suitable for public use worldwide and will serve as a benchmark for additional studies.

Chapter 5

Future developments

This project aims to contribute to the ambitious goal of expanding surgeons' visual capabilities during tumour resection. As detailed in the introduction section, two main elements of the surgical field are not visible to the naked eye: the tumour infiltration extent and the activation of functionally eloquent network hubs near the tumour itself.

A secondary but equally important goal was to engage in a more productive collaboration between Imperial College Healthcare NHS Trust and Imperial College London.

Specifically, the Hamlyn Centre of Robotics and the Department of Surgery and Cancer have been developing new optical and robotics technologies to enhance surgeons' intraoperative diagnostic and practical skills.

The neurosurgical department has not been actively engaged in this process for various logistical and historical reasons until now. Still, the present project and many others from different firms represent an excellent first step.

5.1 Introduction chapter and clinical series

This chapter summarizes the available evidence regarding the clinical and surgical management of brain tumours, discusses our clinical findings, and summarizes the road map of this project.

The results of the clinical series provided here served as a tool to identify areas that could benefit from further technological research. They helped audit our last five years of activity through a challenging period: the COVID-19 pandemic.

Due to time constraints, some aspects of this data collection have not been analysed, such as the service performances in terms of timing—e.g., the time between the first scan and the operation, the time between the surgery and the beginning of the adjuvant treatment, etc.

The plan for the future is to complete the audit by obtaining a more refined data assessment in specific areas (e.g., we need MRI tumour volumes for all cases), collecting the missing datasets, and retrospectively collecting data from different firms.

The purposes of such effort would be to:

- offer a comprehensive service evaluation;
- better characterize areas where the service could be improved with the current state of technology;
- report the results to the Trust and potentially the Clinical Commissioning Groups to foster more attention to the neuro-oncology service.

5.2 Intra-Operative UltraSound (IOUS) (Chapter 2)

In the context of the current literature evidence, our clinical results suggest that IOUS allows improved identification of tumour margins, increases the EOR, and could potentially benefit survival.

The main limitation of IOUS concerns the lack of visualization of fine haemodynamic activity to identify eloquent brain areas, a problem that some groups are addressing by introducing high-resolution, ultrasensitive ultrasound devices [227, 228]. Given the limitations expressed by the authors and the logistical issues encountered with our set-up, the decision was to focus

the IOUS section of this project more on the current limits of tumour detection and their improvement.

In the short to medium term, I would aim to restart a prospective data collection of intra-operative IOUS images, possibly directly comparing the visual information and volumetric calculation of the tumour with the MRI data. Unfortunately, this project is on stand-by due to logistical issues. Both Sonowand© IOUS machines have been dismissed from our department, as they are now obsolete. We are currently deploying a new generation Toshiba© machine for the same purpose. The resolution of this device is higher, and the quality of the image is much more refined. This machine is currently utilized for the FutureGB trial, and our Department is one of the recruiting centres [226]. However, this device lacks integration with the neuronavigation. A hypothesis is to buy a novel neuronavigation device that is potentially fully integrated with IOUS. This will require a significant financial investment, so at the moment, there isn't an established roadmap to restart clinical studies concerning IOUS integrated into neuronavigation to confirm our results on a larger dataset.

The long-term goal of IOUS research is to:

- better integrate the IOUS probes with robotics arms;
- produce and refine a software that would better highlight the tumour, its margins, and the artifacts to the surgeon.

The integration between IOUS and robotics is a project in its early stages. Still, we have already started to produce an automatic scanning path generation, as shown in Figure 5.1 [6, 327].

The preliminary results from this project have been pre-submitted to ArXiv.

Regarding the possibility of further technical development on IOUS in neuro-oncology, our main area of interest is the improved detection of tumour margins and brain parenchyma invasion.

We have applied several segmentation techniques and training/testing models to improve the identification of tumour margins [328] and reduce the possibility of artefacts [329].

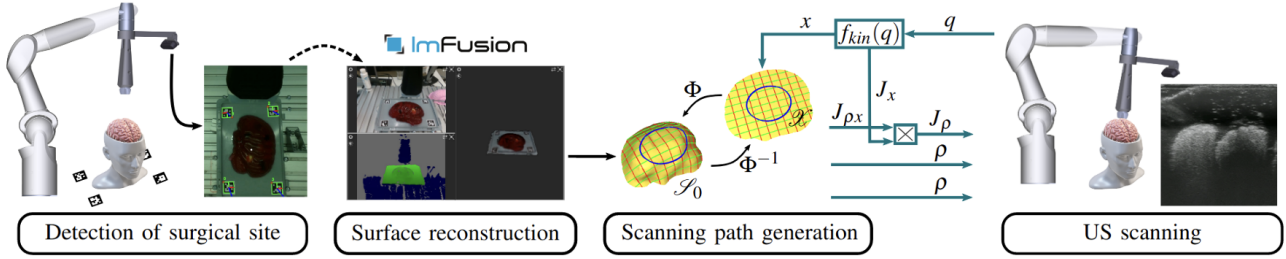


Figure 5.1: Workflow for an IOUS tissue scanning platform, showing how an IOUS can be linked to a robotic arm for automatic scanning - Picture taken from Dyck et al. [6]

However, a large dataset of IOUS images is required to train and test hypothetical software performing this task in real time, and a plan on how to obtain the desired software has been discussed for future research projects. Specifically, a potential development pipeline would be laid out as follows:

1. a large dataset of brain IOUS images is gathered, both from our series and publicly available databases;
2. an online, user-friendly segmentation tool is created;
3. trained members from international neurosurgical and neuroradiological societies are contacted to segment a random subset of images from our dataset;
4. the results of the segmentations are used to train and test the diagnostic algorithm;
5. software is produced to integrate the algorithm into the available IOUS devices so that, during the IOUS scanning of a brain tumour, the software produces a real-time visual probability map using different coloured masks with different intensities - as a theoretical example: intense green overlaid on top of the tumour would suggest close to 100% probability of tumour core, while a light green mask would indicate lower probability, same concept applied to intense yellow for a high likelihood of tumour margins / light yellow for low probability, and blue for normal brain tissue.

This project is in the preliminary phases of discussion with the Hamlyn and Imperial Healthcare teams, and the provisional plan is to start working on the first steps as early as January 2024.

5.3 Hyperspectral imaging as a clinical tool (Chapter 4)

Several limitations and areas of improvement emerged, which are partially addressed in the Discussion section of Chapter 4. The collection of intraoperative hyperspectral images represents a significant step forward in intraoperative imaging, building upon prior studies of deep learning approaches to hyperspectral data in brain tumours [273].

This study expands the largest available dataset and addresses specific operational challenges within a real-world neurosurgical setting. This sets the groundwork for advanced machine learning algorithms to be trained on a more diverse and representative set of data, which could lead to improved diagnostic capabilities and integration into surgical workflows.

However, one of the main limitations—stemming from image acquisition variability and potential mislabelling during segmentation—aligns with broader challenges in surgical imaging. Moreover, current methods in HSI are still evolving, and identifying areas where models struggled (e.g., glioblastoma margins) reflects the broader issues in spectral tissue differentiation discussed in recent literature.

Gathering more data is essential for model refinement. Despite being a large dataset, the results of this project suggest that a larger pool of hypercubes could refine the training and testing process, often optimized to a limited availability of data but still not ideal [330]. In the meantime, several more targeted and resource-efficient improvements are being evaluated to bridge some identified gaps [265].

In the first instance, applying automated image pre-processing tools could ensure image quality and consistency, independent of variations in surgical conditions [331]. The variability of the surgical field (both during the same case or between different cases) is a technical problem that not only impacts the quality of the registered image but also affects the labelling and the training/testing process.

For example, the number of classes to be segmented for labelling is debatable. Too many classes would increase the computational complexity while potentially causing overfitting and

class imbalance, as seen, for example, with the "arachnoid" label. Conversely, too few classes pose the risk of loss of information, reduced accuracy, and partially reduced generalization. Algorithms correcting for lighting variations, surgical instrument interference, and blood pooling, for example, through real-time filtering techniques or immediate post-acquisition processing, could standardise data acquisition and improve model robustness without requiring additional surgical time or resources.

Implementing classification models that account for context-specific information—such as patient physiological data or tumour heterogeneity—could improve classification accuracy. For example, combining intraoperative imaging data with preoperative MRI or CT scans via multimodal image fusion techniques, as demonstrated by different groups [238, 290], would enable real-time adaptive models tailored to individual patients. This would optimize tumour margin detection even in challenging cases like glioblastomas or meningiomas.

Regarding the limitations in detecting haemodynamic changes, exploring hybrid approaches that integrate BOLD-fMRI principles into hyperspectral analysis might enhance sensitivity. However, this is a more challenging point, as fMRI itself shows limited accuracy [167]. Several authors have pointed out that the most significant discrepancy between the reflectance spectra of HbO and Hb lies within the range of NIR, which can be used both for the detection of tumour margins [332, 333] and haemodynamic changes [334, 335]. In both cases, the integration with fluorescence also seems to enhance the model's accuracy. In our case, basing the assessment on these preliminary results, it is plausible that a combination of snapshot scanning and integration of HSI with NIR might provide more accurate results. However, a slightly tangential approach could be attempted by integrating the hyperspectral signal with fluorescence emitted by more specific chromophores that could also serve as neuronal activation markers, such as flavoprotein. If effective, this solution would provide direct visualization of the neuronal activity, removing the challenges of inferring it from haemodynamic changes, and it has been explored recently by other groups [336]. Possible future projects would look at integrating our current set-up with near-infrared and fluorescent cameras, which would require the implementation of the theatre settings in terms of exposure, acquisition times, and scanning technology.

Examining the results reported in Chapter 4, more specific areas of interest for further research and development are:

1. **advancing hypercube analysis algorithms** - there is a need for further development in the algorithms analysing the hypercubes; this includes refining their metrics and analysis speed, with the ultimate goal of creating a comprehensive system that offers direct hyperspectral intra-operative readings to surgeons;
2. **increasing case volume for enhanced accuracy** - an increased number of cases should be incorporated to refine the overall accuracy and reliability of the image analysis models;
3. **enhancing user experience and efficiency** - future studies or even trials should aim for a more intuitive and user-friendly setup so that:
 - the logistical issues in the operating theatre can be reduced to a minimum;
 - the hypercube acquisition is as rapid as possible, better if near-real time for the human perception.

5.3.1 Ongoing projects and immediate future

As mentioned in the Discussion section of Chapter 4, the data analysis of the current dataset is far from being completed.

We are refining the analysis of the gliomas, meningiomas, and grade subsets.

The most promising results have been obtained with the cohort of meningiomas and gliomas, especially utilizing the Convolutional Neural Network, but a few points remain to be addressed.

First, the analysis on HGG-IV was only performed on 10 patients out of 26 available. This was due to pressing deadlines and the intense computational demand of the algorithm itself.

A new state-of-the-art processor has recently been acquired to augment the calculation power. The immediate plan is to process all the HGG-IV using CNN, experimenting with different

labelling methods and hyperparameter settings. We are also approaching the LGG series, which remains unprocessed by CNN.

The image analysis on the cases where haemodynamic changes were expected is also incomplete. It must be implemented by adding additional chromophores to the model and considering different options to estimate the differential path length. Further research into the physiological values to be considered in the model will also be considered, including the possibility of a literature-based, more accurate mathematical modelling of the perfusion dynamics of the brain during awake and asleep surgery.

We are also evaluating further software developments to apply the codes developed by different groups to our series and cross-check the results obtained [264, 273].

5.3.2 Long term developments

A new hyperspectral camera has been bought, adapted and calibrated to the Leica microscope. Dr Alfie Roddan has conducted this preliminary, crucial set-up work.



Figure 5.2: Cubert Ultris S5/SR5 hyperspectral camera module

The hardware and software development carried out during the first months of 2024, coupled with the analysis and writing up mentioned in Section 6.3.1, will provide the baseline for a more expanded study on the application of multispectral imaging.

We foresee needing to recruit a more significant number of cases on a preliminary estimation based on the data presented here, epidemiological factors [2], and logistical issues. According to the Clinical Commissioning Group report, Imperial NHS Healthcare holds a catching area population between 1,500,000 and 2,000,000 people [337]. Considering the incidence of glioblastoma in the UK (3-4 cases/100,000 [338]) and the volume of referrals diverted to surgical treatment in our Trust (as per Chapter 1), it is likely that a multi-centric study would be preferable.

In the case of HGG and meningiomas, the outcome metrics of the CNN for HGG-IV could greatly benefit from an expanded dataset. This is a critical population of tumours to treat, and the identification of the margins is crucial. The expanded sampling size should include all the possible locations, surgical strategies, approaches, intra-operative scenarios, and set-up settings for model training, testing, and validation.

LGG and anaplastic gliomas are even more problematic, as they affect younger people, they are even less common, and their macroscopic features are the most challenging to distinguish from the rest of the surrounding brain. Hyperspectral imaging could significantly enhance the surgeon's performance. This subgroup of patients also shows an improved prognosis in response to treatment. However, because these tumours are so sporadic, obtaining the high volumes required to train the model adequately will be challenging.

Finally, regarding detecting haemodynamic changes, the algorithm to detect perfusion changes can be applied to any tumour surgery, in any location. However, as stressed in the Discussion section - Chapter 4, the signal might be impacted by several physiological and model-related factors, including the presence of the tumour itself, the task/stimulation administered, and the anaesthetic choices. To correct those factors, we would not only plan to recruit patients where awake surgery and neurophysiological monitoring are not applied, but we would also consider recruiting patients with different neuropathological conditions, including trauma, stroke and neurovascular.

A significant recruitment-related problem arises when applying the power calculation model discussed in Materials and Methods, Chapter 4 [313]. For an average accuracy of 80% obtained

with the CNN, as reported in the Results section, the assumed effect size (δ) impacts the estimated population to recruit for a new study. As minor diagnostic effects are challenging to detect without a large population, for an estimated 5% effect size, the number of patients to recruit would be six thousand. Changing the effect size to 10% would only marginally improve the sample size (1,500). Both these estimates are logistically unrealistic due to the epidemiological considerations and the challenges of setting up a study with such a large population (see below).

For a 15% effect, a more realistic (still significant) sample of 600 patients is calculated. There are, however, important limitations to consider, even with this number. As discussed above, the results presented are provisional, and more work on fine-tuning hyperparameters is required. It is possible that the discussed metrics cannot be significantly improved by working on the processing algorithms, which would make the diagnostic advantage of HSI not sufficiently appealing for the efforts of a large study. Recruiting hundreds of patients for a clinical trial is notoriously challenging, especially in a multi-centric setting [339], as this would require personnel and coordination between recruiting centres over a few years. The funding issue is directly related to the trial setup, which might make it problematic to arrange for a study on a technology dedicated to a specific aspect of a rare disease.

We will spend most of the future months working on this project's results and discussing the details of a possible multicentric study, including the target of patients to recruit and different sample calculation models, which might be more appropriate for the specifics of our research question, will be evaluated [340, 341]. At the same time, we will aim to assess the availability of grants to apply to while perfecting the acquisition hardware and software to make it user-friendly, rapid, and scalable for a multicentric study. Scrupulous attention will be dedicated to the data collection tool, database organization, and anonymization process so that the whole pipeline will be as effective as possible, considering the requirement of personal data protection laws.

5.3.3 Final summary: the bigger picture

The two technologies discussed in this project are part of a broader technological development landscape for neurosurgery and surgery in general.

Considering the current research and development brewing environment, inside and outside the UK, the next 10-20 years will radically change how surgeons perform diagnostic and therapeutic procedures.

This project is a first step toward participating in this change and contributing to pushing the boundaries of neuro-oncological surgery forward. The goal is to maximize the effect of radical resection and minimize the rate of complications.

Imperial's future teamwork should focus on similar projects, exploring the different optical and spectroscopy modalities for enhanced intra-operative visualization, diagnosis, and molecular profiling. Implementing robotic surgery, another booming research field, would be an integral part of this landscape, effectively contributing to developing "the operative theatre of 2040."

Appendix A

Publications and supportive work

This PhD thesis has been compiled in the broader context of working for the Brain Tumour Research Centre of Excellence at Imperial College of London and Imperial College Healthcare NHS Trust.

When the project started in 2018, the Centre consisted mainly of a translational pipeline, with clinicians and lab scientists coordinating efforts to expand the lines of research on brain tumours.

This collaboration has been implemented over the past few years, and our team has expanded by actively opening new multidisciplinary projects to be conducted in partnership with the Hamlyn Centre of Robotics at Imperial College of London, the Neuroradiology Department at Imperial College Healthcare NHS Trust, and multiple other centres outside Imperial, including Oxford University.

The following list provides the publications from this thesis and other projects in which the candidate played an active role.

- Giulio Anichini, Zepeng Hu, Maria Leiloglou, Iani Gayo, Neekhil Patel, Nelofer Syed, Dipankar Nandi, Kevin O'Neill, and Daniel Elson, 2022. **Multispectral analysis for intra-operative characterization of brain tumours, margins of resection, and eloquent areas activation—preliminary results.** Neuro-Oncology 2022, 58, p.14. [342]

- Giulio Anichini, Islam Shah, Dominic Edward Mahoney, Neekhil Patel, Lillie Pakzad-Shahabi, Olga Fadeeva Da Costa, Nelofer Syed, Richard Perryman, Adam Waldman, Kevin O'Neill. **3D ultrasound-augmented image-guidance for surgery of glioblastomas – A quantitative analysis focused on the extent of resection.** Surgical Neurology International, 2024.
- Sophie Camp, Stamatia Giannarou, Mou-Cheng Xu, Giulio Anichini, Luke Dixon, Kevin O'Neill, Dipankar Nandi. **AI-Guided Surgical Resection in Neurosurgery Using Intraoperative Ultrasound.** Surgical Life, ASGBI, 59(93-96) [328].
- Alistair Weld, Luke Dixon, Giulio Anichini, Michael Dyck, Alex Ranne, Sophie Camp, Stamatia Giannarou. **Identifying Visible Tissue in Intraoperative Ultrasound Images during Brain Surgery: A Method and Application.** arXiv preprint arXiv:2306.01190, 2023. [329]
- Michael Dyck, Alistair Weld, Julian Klodmann, Alexander Kirst, Giulio Anichini, Luke Dixon, Sophie Camp, Stamatia Giannarou, Alin Albu-Schäffer. **Collaborative Robotic Ultrasound Tissue Scanning for Surgical Resection Guidance in Neurosurgery** arXiv preprint arXiv:2301.08174. [327]
- Michael Dyck, Alistair Weld, Julian Klodmann, Alexander Kirst, Giulio Anichini, Luke Dixon, Sophie Camp, Stamatia Giannarou, Alin Albu-Schäffer. **Automated robotic intraoperative ultrasound for brain surgery** arXiv preprint arXiv:2301.08174. [6]
- Khan S, Anichini G, Mian A, Kareem H, Syed N, O'Neill K. **Tonsillar Carcinoma Spreading Metastases to Central Nervous System: Case Report and Literature Review.** J Neurol Surg Rep 2021 Jan;82(2):e11-e16.
- Hajji, Nabil, Juan Garcia-Revilla, Manuel Sarmiento Soto, Richard Perryman, Jake Symington, Chad C. Quarles, Deborah R. Healey et al. **Arginine deprivation alters microglial polarity and synergizes with radiation to eradicate non-arginine-auxotrophic glioblastoma tumors.** The Journal of Clinical Investigation 132, no. 6 (2022) [343];

- Syed, Nelofer; Crook, Timothy; Dubey, Sudhir; Potharaju, Mahadev; Limaye, Sewanti; Ranade, Anantbhushan; Anichini, Giulio ; Patil, Darshana; Datta, Vineet; Datar, Rajan. **Profiling of circulating glial cells for accurate blood-based diagnosis of glial malignancies.** International Journal of Cancer, 2023 - ACCEPTED, ahead of print;
- Inglese, Marianna, Shah Islam, Matthew Grech-Sollars, Giulio Anichini, James Davies, Azeem Saleem, Matthew William, Kevin O'Neill, Adam Waldman, and Eric O. Aboagye. **Quantitative multiparametric 18F-FPIA PET/MRI for the characterization of primary brain gliomas: a pilot study.** [344];
- O'Neill, Kevin, Nelofer Syed, Timothy Crook, Sudhir Dubey, Mahadev Potharaju, Sewanti Limaye, Anantbhushan Ranade et al. **Detection of Circulating Glial Cells Enables Non-Invasive Diagnosis of Glial Malignancies.** medRxiv (2022): 2022-07. [345];
- Anichini, Giulio, Pooja Fulmali, Kevin O'Neill, Vineet Datta, Timothy Crook, and Nelofer Syed. **Accurate identification of glial malignancies from peripheral blood** In Neuro-oncology, vol. 24, pp. 155-155. Journals dept, 2001 evans rd, cary, nc 27513 usa: oxford univ press inc. [346];
- Shahabi, Lillie, Kerlann Le Calvez, Seema Dadhania, Catherine Blake, James Wang, Manasi Ingle, Giulio Anichini et al. **Real-world experience with Temozolomide and Sativex in patients with recurrent High Grade Gliomas.** Neuro-oncology 21, no. Supplement 4 (2019): iv13-iv14 [347].
- Giulio Anichini, Maria Leiloglou, Zepeng Hu, Kevin O'Neill, Daniel Elson. **Hyper-spectral and multispectral imaging in neurosurgery: a systematic updated literature review and metanalysis.** European Journal of Oncology, 2023 [348]
- Hajji, Nabil, Juan Garcia-Revilla, Manuel Sarmiento Soto, Richard Perryman, Jake Symington, Chad C. Quarles, Deborah R. Healey et al. **Arginine deprivation alters microglial polarity and synergizes with radiation to eradicate non-arginine-auxotrophic glioblastoma tumors.** The Journal of Clinical Investigation 132, no. 6

(2022) [343];

- Syed, Nelofer; Crook, Timothy; Dubey, Sudhir; Potharaju, Mahadev; Limaye, Sewanti; Ranade, Anantbhushan; Anichini, Giulio; Patil, Darshana; Datta, Vineet; Datar, Rajan. **Profiling of circulating glial cells for accurate blood-based diagnosis of glial malignancies.** International Journal of Cancer, 2023.
- Inglese, Marianna, Shah Islam, Matthew Grech-Sollars, Giulio Anichini, James Davies, Azeem Saleem, Matthew William, Kevin O'Neill, Adam Waldman, and Eric O. Aboagye. **Quantitative multiparametric 18F-FPIA PET/MRI for the characterization of primary brain gliomas: a pilot study."** [344];
- O'Neill, Kevin, Nelofer Syed, Timothy Crook, Sudhir Dubey, Mahadev Potharaju, Sewanti Limaye, Anantbhushan Ranade et al. **Detection of Circulating Glial Cells Enables Non-Invasive Diagnosis of Glial Malignancies.** medRxiv (2022): 2022-07. [345];
- Anichini, Giulio, Pooja Fulmali, Kevin O'Neill, Vineet Datta, Timothy Crook, and Nelofer Syed. **Accurate identification of glial malignancies from peripheral blood** In Neuro-oncology, vol. 24, pp. 155-155. Journals dept, 2001 evans rd, cary, nc 27513 usa: oxford univ press inc. [346];
- Shahabi, Lillie, Kerlann Le Calvez, Seema Dadhania, Catherine Blake, James Wang, Manasi Ingle, Giulio Anichini et al. **Real-world experience with Temozolomide and Sativex in patients with recurrent High Grade Gliomas.** Neuro-oncology 21, no. Supplement 4 (2019): iv13-iv14 [347].
- Verónica Mientes, Giulio Anichini, Ji Qi, Kevin O'Neill, Olga M. Conde, Daniel S Elson **PoLambRization: A multispectral polarimetric atlas of the lamb brain.** Journal of Biomedical Optics, 2024

Appendix B

Python codes

B.1 Code for single hypercube classification

Written, modified, and executed by Giulio Anichini.

```
import os
import numpy as np
from PIL import Image
from sklearn.ensemble import RandomForestClassifier
from sklearn.svm import SVC
from sklearn.tree import DecisionTreeClassifier
from sklearn.neural_network import MLPClassifier
from sklearn.cluster import KMeans
from sklearn.model_selection import train_test_split
from sklearn.metrics import confusion_matrix
import matplotlib.pyplot as plt
from matplotlib.lines import Line2D
from matplotlib.colors import ListedColormap
folder_path = "/Users/Giulio/Desktop/LGG/HS009/Field01/Run04_reg"
```

```

file_names = sorted([file for file in os.listdir(folder_path) if
    file.endswith('.png') and not file.startswith('mask')])
data = []
print("Loading images...")
for file_name in file_names:
    with Image.open(os.path.join(folder_path, file_name)) as img:
        img_gray = img.convert('L')
        data.append(np.array(img_gray))
print("All images loaded.")
data = np.stack(data, axis=-1)
mask_path = os.path.join(folder_path, '/Users/Giulio/Desktop/LGG/
    HS009/Field01/mask.png')
with Image.open(mask_path) as mask_img:
    mask = np.array(mask_img)
kmeans = KMeans(n_clusters=7, n_init=10)
mask_flat = mask.reshape(-1, 3)
kmeans.fit(mask_flat)
cluster_centers = kmeans.cluster_centers_
cluster_centers = np.uint8(cluster_centers)
custom_cmap = ListedColormap(cluster_centers / 255.0) # Create
    custom colormap from cluster centers
labels_flat = kmeans.predict(mask_flat)
labels = labels_flat.reshape(mask.shape[0], mask.shape[1])
X = data.reshape(-1, data.shape[2])
y = labels.reshape(-1)
X_train, X_temp, y_train, y_temp = train_test_split(X, y, test_size
    =0.4, random_state=42)
X_test, X_validate, y_test, y_validate = train_test_split(X_temp,
    y_temp, test_size=0.5, random_state=42)

```

```

print(" Training classifier ...")
#clf = DecisionTreeClassifier(max_depth=5, random_state=42)
#clf = RandomForestClassifier(n_estimators=50, random_state=42)
clf = MLPClassifier(alpha=1, max_iter=1000, random_state=42)
#clf = SVC(kernel='rbf', verbose=True)
clf.fit(X_train, y_train)
print(" Classifier trained.")
print(" Predicting labels ...")
predicted_labels = clf.predict(X_test)
predicted_image = clf.predict(X)
predicted_image_rgb = np.array([cluster_centers[label] for label in
    predicted_image])
predicted_image_rgb = predicted_image_rgb.reshape(data.shape[0],
    data.shape[1], 3)
predicted_image_path = os.path.join(folder_path, 'predicted_image.
    png')
Image.fromarray(predicted_image_rgb).save(predicted_image_path)
print(f"Predicted image saved as {predicted_image_path}")
cnf_matrix = confusion_matrix(y_test, predicted_labels)
classes = ["bkg", "core", "cortex", "arachnoid", "vessels", "margins",
    "blood"]
for i, class_name in enumerate(classes):
    TP = cnf_matrix[i, i]
    FP = cnf_matrix[:, i].sum() - TP
    FN = cnf_matrix[i, :].sum() - TP
    TN = cnf_matrix.sum() - TP - FP - FN
    sensitivity = TP / (TP + FN) if (TP + FN) != 0 else 0
    specificity = TN / (TN + FP) if (TN + FP) != 0 else 0
    accuracy = (TP + TN) / (TP + TN + FP + FN)

```

```

    print(f"{class_name}:")
    print(f"Sensitivity: {sensitivity:.2f}")
    print(f"Specificity: {specificity:.2f}")
    print(f"Overall Accuracy: {accuracy:.2f}\n")
sorted_indices = np.argsort(y_test)
fig, (ax1, ax2) = plt.subplots(1, 2, figsize=(18, 5))
ax1.imshow(y_test[sorted_indices].reshape((int(data.shape[0] * 0.2),
    data.shape[1])), cmap=custom_cmap, aspect='auto')
ax1.set_title('Original Mask – Test Data (Grouped)')
ax2.imshow(predicted_labels[sorted_indices].reshape((int(data.shape
    [0] * 0.2), data.shape[1])), cmap=custom_cmap, aspect='auto')
ax2.set_title('Classified Image (Grouped)')
colors = [custom_cmap(i / len(cluster_centers)) for i in range(len(
    cluster_centers))]
legend_elements = [Line2D([0], [0], color=colors[i], lw=4, label=
    classes[i]) for i in range(len(cluster_centers))]
ax1.legend(handles=legend_elements, loc='center left',
    bbox_to_anchor=(-0.3, 0.5))
plt.tight_layout()
plt.show()

```

B.2 Code written for inter-patients classification

This is the code used to classify the set of gliomas. The code for classifying meningiomas is identical, except for the different file paths.

Written by Giulio Anichini. Revised and corrected by Dr Hu Zepeng.

Executed and modified (hyperparameters tuning, sampling, selection of datasets for training, validation and testing) by Giulio Anichini.

```
import numpy as np
import os
import cv2
from imblearn.over_sampling import SMOTE
from sklearn.svm import SVC
from sklearn.ensemble import RandomForestClassifier
from sklearn.tree import DecisionTreeClassifier
from sklearn.neural_network import MLPClassifier
from sklearn.metrics import accuracy_score, precision_score,
    recall_score
from sklearn.metrics import confusion_matrix
data_path = '/Users/Giulio/Desktop/HGGSUP/'
tra_pathlist = ['HS001', 'HS012', 'HS017', 'HS019', 'HS020', 'HS026',
    ', 'HS029', 'HS030']
val_pathlist = ['HS037']
test_pathlist = ['HS016']
num_1 = int(5000/10)
num_2 = int(5000/14)
num_3 = int(5000/13)
num_4 = int(5000/15)
num_5 = int(5000/14)
num_6 = int(10000/13)
num_7 = int(5000/5)
num_1v = int(4000/4)
num_2v = int(2000/6)
num_3v = int(4000/5)
num_4v = int(5000/4)
num_5v = int(5000/6)
num_6v = int(4000/5)
```

```

num_7v = 2000

def rgb_to_2D_label(label):
    label_seg = np.zeros(label.shape, dtype=np.uint8)
    label_seg[np.all(label == bkg, axis=-1)] = 0
    label_seg[np.all(label == ara, axis=-1)] = 1
    label_seg[np.all(label == blood, axis=-1)] = 2
    label_seg[np.all(label == cort, axis=-1)] = 3
    label_seg[np.all(label == core, axis=-1)] = 4
    label_seg[np.all(label == margins, axis=-1)] = 5
    label_seg[np.all(label == vessel, axis=-1)] = 6
    label_seg[np.all(label == white, axis=-1)] = 7
    label_seg = label_seg[:, :, 0] # Just take the first channel,
    no need for all 3 channels
    return label_seg

def standar(image):
    mean_val = np.mean(image, axis=2)
    std_dev = np.std(image, axis=2)
    normalized_cube_zscore = (image - mean_val[:, :, np.newaxis]) /
        std_dev[:, :, np.newaxis]
    return normalized_cube_zscore

def normal(image):
    min_val = np.min(image, axis=2)
    max_val = np.max(image, axis=2)
    normalized_image = (image - min_val[:, :, np.newaxis]) / (
        max_val[:, :, np.newaxis] - min_val[:, :, np.newaxis]
    )
    return normalized_image

def createData(pathlist, train=True):
    xData_train_list, yLabels_train_list = [], []

```

```

reference_shape = None
for train_path in pathlist:
    image_paths = os.listdir('/Users/Giulio/Desktop/HGGSUP/' +
                               train_path)
    image_paths = [ip for ip in image_paths if ip != '.DS_Store'] # Ignore .DS_Store
    print(train_path, ': ', image_paths)
    for img_path in image_paths:
        image_base_path = '/Users/Giulio/Desktop/HGGSUP/' +
                           train_path + '/' + img_path
        image_path = os.path.join(image_base_path, 'org/')
        mask_path = os.path.join(image_base_path, 'mask.png')
        if not os.path.exists(image_path) or not os.path.exists(
            mask_path):
            print(f"Hypercube or mask not found for {
                  image_base_path}. Skipping.")
            continue
        img_dataset = []
        mask_dataset = []
        img_nms = os.listdir(image_path)
        img_nms = [inm for inm in img_nms if inm != '.DS_Store']
        # Ignore .DS_Store
        print(img_nms, len(img_nms))
        img1 = cv2.imread(image_path + img_nms[0], 0)
        img1 = img1[:, :, None]
        for k in range(1, len(img_nms)):
            img2 = cv2.imread(image_path + img_nms[k], 0)
            img2 = img2[:, :, None]
            img1 = np.concatenate((img1, img2), axis=-1)

```

```

img1 = normal(img1)
img_dataset.append(img1)
mask_path = '/Users/Giulio/Desktop/HGGSUP/' + train_path
           + '/' + img_path + '/mask.png'
mask = cv2.imread(mask_path, 1)
mask = cv2.cvtColor(mask, cv2.COLOR_BGR2RGB)
mask_dataset.append(mask)
img_dataset = np.array(img_dataset)
mask_dataset = np.array(mask_dataset)
print(img_dataset.shape)
print(mask_dataset.shape)
labels = []
for i in range(mask_dataset.shape[0]):
    label = rgb_to_2D_label(mask_dataset[i])
    labels.append(label)
labels = np.array(labels)
print(labels.shape)
X = img_dataset[0]
y = labels[0]
labeled_points = []
for i in range(class_num):
    p = np.argwhere(y == i)
    labeled_points.append(p)
print(train_path+' '+img_path)
n = len(labeled_points)
print(n)
for i in range(1, n):
    c = labeled_points[i]
    print('class number:      ', i)

```

```

print('c:      ', c.shape)
if c.shape[0] > 0:
    if i == 1:
        if train:
            patch_num = num_1
        else:
            patch_num = num_1v
        selected_indices = np.random.choice(c.shape
            [0], patch_num, replace=True)
        xData = np.zeros((patch_num, X.shape[2]))
        yLabels = np.zeros((patch_num))
        patchIndex = 0
        for k in range(patch_num):
            j = selected_indices[k]
            xData[patchIndex, :] = X[c[j][0], c[j]
                ][1], :]
            yLabels[patchIndex] = y[c[j][0], c[j]
                ][1]] - 1
            patchIndex = patchIndex + 1
        nan_mask = np.isnan(xData).any(axis=1)
        X_without_nan = xData[~nan_mask]
        Y_without_nan = yLabels[~nan_mask]
        xData_train_list.append(X_without_nan)
        yLabels_train_list.append(Y_without_nan)
    if i == 2:
        if train:
            patch_num = num_2
        else:
            patch_num = num_2v

```



```

selected_indices = np.random.choice(c.shape
[0], patch_num, replace=True)
xData = np.zeros((patch_num, X.shape[2]))
yLabels = np.zeros((patch_num))
patchIndex = 0
for k in range(patch_num):
    j = selected_indices[k]
    xData[patchIndex, :] = X[c[j][0], c[j]
    ][1], :]
    yLabels[patchIndex] = y[c[j][0], c[j]
    ][1]] - 1
    patchIndex = patchIndex + 1
nan_mask = np.isnan(xData).any(axis=1)
X_without_nan = xData[~nan_mask]
Y_without_nan = yLabels[~nan_mask]
xData_train_list.append(X_without_nan)
yLabels_train_list.append(Y_without_nan)
if i == 3:
    if train:
        patch_num = num_3
    else:
        patch_num = num_3v
    selected_indices = np.random.choice(c.shape
[0], patch_num, replace=True)
    xData = np.zeros((patch_num, X.shape[2]))
    yLabels = np.zeros((patch_num))
    patchIndex = 0
    for k in range(patch_num):
        j = selected_indices[k]

```

```

        xData[patchIndex, :] = X[c[j][0], c[j]
                                ][1], :]
        yLabels[patchIndex] = y[c[j][0], c[j]
                                ][1]] - 1
        patchIndex = patchIndex + 1
    nan_mask = np.isnan(xData).any(axis=1)
    X_without_nan = xData[~nan_mask]
    Y_without_nan = yLabels[~nan_mask]
    xData_train_list.append(X_without_nan)
    yLabels_train_list.append(Y_without_nan)

    if i == 4:
        if train:
            patch_num = num_4
        else:
            patch_num = num_4v
        selected_indices = np.random.choice(c.shape
                                             [0], patch_num, replace=True)
        xData = np.zeros((patch_num, X.shape[2]))
        yLabels = np.zeros((patch_num))
        patchIndex = 0
        for k in range(patch_num):
            j = selected_indices[k]
            xData[patchIndex, :] = X[c[j][0], c[j]
                                      ][1], :]
            yLabels[patchIndex] = y[c[j][0], c[j]
                                    ][1]] - 1
            patchIndex = patchIndex + 1
    nan_mask = np.isnan(xData).any(axis=1)
    X_without_nan = xData[~nan_mask]

```

```

        Y_without_nan = yLabels[~nan_mask]
    xData_train_list.append(X_without_nan)
    yLabels_train_list.append(Y_without_nan)
    if i == 5:
        if train:
            patch_num = num_5
        else:
            patch_num = num_5v
        selected_indices = np.random.choice(c.shape
            [0], patch_num, replace=True)
        xData = np.zeros((patch_num, X.shape[2]))
        yLabels = np.zeros((patch_num))
        patchIndex = 0
        for k in range(patch_num):
            j = selected_indices[k]
            xData[patchIndex, :] = X[c[j][0], c[j]
                ][1], :]
            yLabels[patchIndex] = y[c[j][0], c[j]
                ][1]] - 1
            patchIndex = patchIndex + 1
        nan_mask = np.isnan(xData).any(axis=1)
        X_without_nan = xData[~nan_mask]
        Y_without_nan = yLabels[~nan_mask]
    xData_train_list.append(X_without_nan)
    yLabels_train_list.append(Y_without_nan)
    if i == 6:
        if train:
            patch_num = num_6
        else:

```

```

        patch_num = num_6v
        selected_indices = np.random.choice(c.shape
            [0], patch_num, replace=True)
        xData = np.zeros((patch_num, X.shape[2]))
        yLabels = np.zeros((patch_num))
        patchIndex = 0
        for k in range(patch_num):
            j = selected_indices[k]
            xData[patchIndex, :] = X[c[j][0], c[j]
                ][1], :]
            yLabels[patchIndex] = y[c[j][0], c[j]
                ][1]] - 1
            patchIndex = patchIndex + 1
        nan_mask = np.isnan(xData).any(axis=1)
        X_without_nan = xData[~nan_mask]
        Y_without_nan = yLabels[~nan_mask]
        xData_train_list.append(X_without_nan)
        yLabels_train_list.append(Y_without_nan)
    if i == 7:
        if train:
            patch_num = num_7
        else:
            patch_num = num_7v
        selected_indices = np.random.choice(c.shape
            [0], patch_num, replace=True)
        xData = np.zeros((patch_num, X.shape[2]))
        yLabels = np.zeros((patch_num))
        patchIndex = 0
        for k in range(patch_num):

```

```

        j = selected_indices[k]
        xData[patchIndex, :] = X[c[j][0], c[j]
                                ][1], :]
        yLabels[patchIndex] = y[c[j][0], c[j]
                                ][1]] - 1
        patchIndex = patchIndex + 1
    nan_mask = np.isnan(xData).any(axis=1)
    X_without_nan = xData[~nan_mask]
    Y_without_nan = yLabels[~nan_mask]
    xData_train_list.append(X_without_nan)
    yLabels_train_list.append(Y_without_nan)

\begin{lstlisting}
    return xData_train_list, yLabels_train_list
def compute_metrics(y_true, y_pred, classes):
    confusion = confusion_matrix(y_true, y_pred)
    num_classes = confusion.shape[0]
    label_names = {
        0: 'ara',
        1: 'blood',
        2: 'cort',
        3: 'core',
        4: 'margins',
        5: 'vessel',
        6: 'white',
    }
    for class_label in range(num_classes):
        TP = confusion[class_label, class_label]
        FN = np.sum(confusion[class_label, :]) - TP
        FP = np.sum(confusion[:, class_label]) - TP

```

```

    TN = np.sum(confusion) - (TP + FP + FN)
    sensitivity = TP / (TP + FN)
    specificity = TN / (TN + FP)
    accuracy = (TP + TN) / (TP + TN + FP + FN)
    NPV = TN / (TN + FN)
    PPV = TP / (TP + FP)
    label_name = label_names[class_label]
    print(f"Class {label_name} - sensitivity: {sensitivity},
          specificity: {specificity}, accuracy: {accuracy}, NPV: {
            NPV}, PPV: {PPV}")
if __name__ == "__main__":
    class_num = 8
    root_dir = 'data/'
    a = int('3C', 16) # 3C with base 16. Should return 60.
    bkg = '#000000'.lstrip('#')
    bkg = np.array(tuple(int(bkg[i:i + 2], 16) for i in (0, 2, 4)))
        # 60, 16, 152
    ara = '#000080'.lstrip('#')
    ara = np.array(tuple(int(ara[i:i + 2], 16) for i in (0, 2, 4)))
        # 132, 41, 246
    blood = '#008000'.lstrip('#')
    blood = np.array(tuple(int(blood[i:i + 2], 16) for i in (0, 2,
        4))) # 226, 169, 41
    cort = '#800000'.lstrip('#')
    cort = np.array(tuple(int(cort[i:i + 2], 16) for i in (0, 2, 4))
        )
    core = '#800080'.lstrip('#')
    core = np.array(tuple(int(core[i:i + 2], 16) for i in (0, 2, 4))
        ) # 155, 155, 155

```

```

margins = '#808000'.lstrip('#')
margins = np.array(tuple(int(margins[i:i + 2], 16) for i in (0,
    2, 4)))
vessel = '#008080'.lstrip('#')
vessel = np.array(tuple(int(vessel[i:i + 2], 16) for i in (0, 2,
    4)))
white = '#808080'.lstrip('#')
white = np.array(tuple(int(white[i:i + 2], 16) for i in (0, 2,
    4)))
xtrain_list, ytrain_list = createData(pathlist=tra_pathlist)
print('xtrain_list: ', len(xtrain_list), ' ytrain_list:
    ', len(ytrain_list))
xval_list, yval_list = createData(pathlist=val_pathlist, train=
    False)
print('xval_list: ', len(xval_list), ' yval_list: ', len
    (yval_list))
Xtrain = xtrain_list[0]
ytrain = ytrain_list[0]
for i in range(1, len(ytrain_list)):
    Xtrain = np.concatenate((Xtrain, xtrain_list[i]), axis=0)
    ytrain = np.concatenate((ytrain, ytrain_list[i]), axis=0)
smote = SMOTE(random_state=42)
Xtrain_smote, ytrain_smote = smote.fit_resample(Xtrain, ytrain)
Xval = xval_list[0]
yval = yval_list[0]
for i in range(1, len(yval_list)):
    Xval = np.concatenate((Xval, xval_list[i]), axis=0)
    yval = np.concatenate((yval, yval_list[i]), axis=0)
print(Xtrain.shape, Xval.shape)

```

```
for i in range(8):
    print(str(i), ':', np.sum(ytrain == i))
for i in range(8):
    print(str(i), ':', np.sum(yval == i))
#model = RandomForestClassifier(n_estimators=50, random_state
    =42, verbose=True) # Initialize classifier once
#model = DecisionTreeClassifier(max_depth=5, random_state=42)
#model = SVC(kernel='rbf', verbose=True)
model = MLPClassifier(alpha=1, max_iter=1000, random_state=42)
model.fit(Xtrain_smote, ytrain_smote) # Train on the validation
    data
y_pred = model.predict(Xval)
classes = ['blood', 'cort', 'core', 'margins', 'vessel', 'white
    ']
matrix = compute_metrics(yval, y_pred, classes)
accuracy = accuracy_score(yval, y_pred)
precision = precision_score(yval, y_pred, average='weighted')
recall = recall_score(yval, y_pred, average='weighted')
print(f"Validation Accuracy: {accuracy}")
print(f"Validation Precision: {precision}")
print(f"Validation Recall: {recall}")
print(matrix)
```


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