

Fluid Dynamics of Orbitally Shaken Shallow Fluid Layers

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Declaration

I here declare that all material in this dissertation which is not my own work has been properly acknowledged.

Paola Alpresa Gutiérrez
Los Barrios, November 10, 2017

Abstract

Motivated by the pathological effect of blood flow on endothelial cells and its relevance in the progress of atherosclerosis, this thesis investigates the fluid dynamics of orbitally shaken shallow fluid layers in cylindrical containers. The study uses computational and analytical models to characterise flow in 33 case studies, which map the parameter space of the dimensionless numbers that govern the physics. A travelling wave characterises the flow and wave breaking and wall boundaries determine the multidirectional character of the shear stress field. A novel shear stress metric (transWSSmin) has been investigated by combining numerical simulations with laboratory experiments, and evidence is presented that transWSSmin correlates with endothelial permeability changes, implying that cells align close to the direction that minimises the transverse shear stresses instead of the mean flow direction, as widely accepted.

A potential flow theory has been shown to be a powerful tool to predict the surface amplitude up to the onset of the wave breaking, which allows for classification in non-breaking/breaking regimes based on an amplitude breaking threshold identified in the simulations. An improved analytical model for the prediction of the shear stress, the PT-Stokes WSS model, has been developed, which is a generalisation of the classic Stokes solution. The model consists of a Stokes boundary layer coupled with the potential flow, and thus takes into account the free surface and side walls. Even in highly nonlinear cases, the PT-Stokes model predicts shear stress within 50% accuracy except at resonant conditions. A strong correlation has been found between the bottom wall shear stress and the surface velocity, showing that the largest source of error is the surface velocity predictions by the potential flow model due to the omission of viscous effects. Finally, results are translated into recommendations and tools for the investigation of endothelial cells in orbital shakers.

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I will continue in Spanish so that my people can fully understand what I am writing, and at least then they will have something to read, since I doubt that they will dare read the rest of the thesis!

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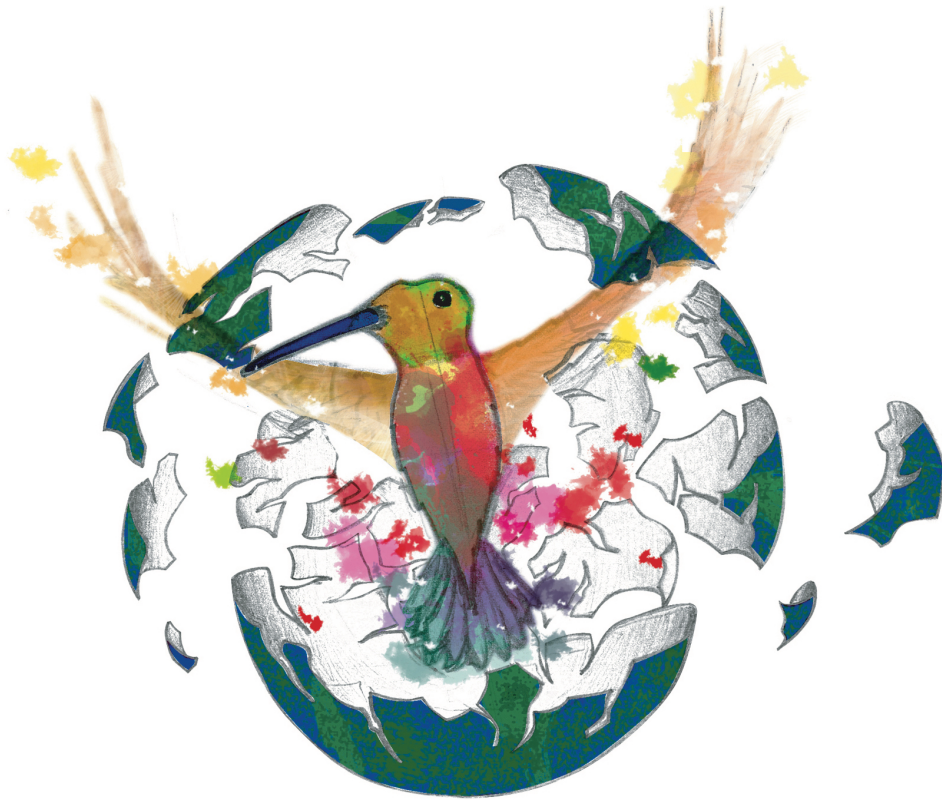
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‘Abril para sentir, abril para soñar, abril la primavera amaneció’ — *Carlos Cano*

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Nomenclature

The following list consists of abbreviations, dimensionless numbers, and symbols that are used frequently within the thesis. Their description is followed by the page number where the term is introduced or the definition is given.

Abbreviations

CFD	Computational Fluid Dynamics, page 46
CFL	Courant-Friedrichs-Lewy, page 54
HRIC	High-Resolution Interface Capturing, page 54
LDL	Low-density lipoprotein, page 32
MagMeanWSS	Magnitude of the temporal mean of the wall shear stress vectors, page 60
OSI	Oscillatory shear index, page 61
PT	Potential Theory, page 90
St	Stokes' model, page 114
TAWSS	Time-averaged wall shear stress, page 60
transWSS	Transverse wall shear stress, page 61
transWSS _{ϕ}	Transverse wall shear stress with respect to the direction ϕ , page 62
transWSSmin	Minimised transverse wall shear stress, page 62
VOF	Volume of fluid, page 53
WSS	Wall shear stress, page 38

Dimensionless Numbers

Γ	Shallowness, page 90
$\tilde{d}$	Dimensionless height, page 115
Bo	Bond, page 52
E	Eccentricity, page 90
F	Forcing, page 90
Re	Reynolds, page 90
We	Weber, page 52

Symbols

$(\mathbf{e}_x, \mathbf{e}_y, \mathbf{e}_z)$	Unit vectors in Cartesian coordinates, page 51
$(\tau_r, \tau_\theta, \tau_z)$	Wall shear stress vector in cylindrical coordinates, page 71
(τ_x, τ_y, τ_z)	Wall shear stress vector in Cartesian coordinates, page 60
(r, θ, z)	Cylindrical coordinates, page 89
(u, v, w)	Velocity vector in Cartesian coordinates, page 110
(u_r, u_θ, u_z)	Velocity vector in cylindrical coordinates, page 90
(x, y, z)	Cartesian coordinates, page 51
α_v	Surface velocity angle with respect to the x-axis at $t \bmod 2\pi/\omega = 0$, page 109
α_τ	WSS vector angle with respect to the x-axis at $t \bmod 2\pi/\omega = 0$, page 109
$\bar{\bar{\tau}}'$	Deviatoric stress tensor, page 51
$\bar{\tau}_w$	Temporal mean of the wall shear stress vectors, page 60
τ_w	Wall shear stress vector, page 38
Δh	Surface height amplitude, page 68
δ	Boundary layer characteristic thickness, page 110
ϵ_{1n}	Nth root of $J'_1(s)$, page 89
λ_{1n}	Nth root of $\partial J_1(\lambda_{1n}r)/\partial r$ at $r = R$, page 89
$\mathbf{f}_m$	Effective gravity vector ($\mathbf{f}_i + \mathbf{g}$), page 90

$\mathbf{f}_i$	Inertial force vector, page 51
$\mathbf{g}$	Gravity vector, page 51
$\mathbf{n}$	Unit surface normal vector, page 38
$\mathbf{v}$	Velocity vector, page 38
$\mathbf{v}_o$	Orbital shaker platform velocity vector, page 107
$\mathbf{v}_s$	Surface velocity vector, page 109
μ	Dynamic viscosity, page 53
ν	Kinematic viscosity, page 86
ω_{1n}	Natural frequency of the mode (1,n), page 89
ω	Speed of rotation of the orbital shaker, page 53
Φ	Flow potential, page 89
ϕ	Reference angle to calculate transWSS $_{\phi}$, page 62
ρ	Density, page 53
σ	Surface tension coefficient, page 52
$\sqrt{\frac{\nu}{\omega}}$	Viscous penetration depth, page 111
φ	Phase shift, page 98
ζ	Surface elevation relative to the static height, page 89
a	Radius of rotation of the orbital shaker, page 53
c	Volume of fluid function, page 53
d	Fluid static height, page 53
F_b	Analytical breaking forcing, page 100
F_u	Analytical uncovered-bottom forcing, page 100
g	Gravity constant, page 53
h	Surface height relative to the well bottom, page 68
J_1	Bessel function of the first kind, of order 1, page 89
p	Pressure, page 51
R	Radius of the well, page 53
r_p	Radial distance of the location of maximum surface velocity, page 100

S	Interface surface, page 52
s	Interface implicit function, page 52
T	Time period of a cycle, page 57
v_p	Phase velocity, page 100
V_s	Surface velocity magnitude, page 109
v_{max}	Maximum surface velocity, page 100
t	Time, page 51

Chapter 1

Introduction

This thesis presents an investigation of the fluid dynamics of orbitally shaken shallow fluid layers in cylindrical containers. This flow movement is extensively used in laboratories for multiple applications, such as cell and bacterial biofilm cultivation, and drug production. This study is motivated by the use of orbital shakers in applying shear stress to endothelial cells cultured in cylindrical wells which, in turn, is motivated by the shear dependence observed in the arterial disease atherosclerosis.

This chapter is organised as follows. The motivation for this thesis is outlined in Sections 1.1 and 1.2, starting with a description of atherosclerosis and the relationship between shear stress and endothelial cells; this is followed by a review of the research on *in vitro* studies of the effects of shear stress on endothelium. In Section 1.3, we discuss the possible methods used to investigate the fluid dynamics problem, and we summarise the approaches taken in this study. Section 1.4 presents the research aims and objectives, and the outline of the thesis is given in Section 1.5.

1.1 Atherosclerosis

According to the World Health Organization [147], an estimated 17.3 million people died from cardiovascular diseases in 2008, representing 30% of all global deaths. Cardiovascular diseases comprise any dysfunctionality related to the heart and the blood vessels: coronary artery dis-

ease, peripheral artery disease, cardiac dysrhythmias, etc.; however, the primary cause underlying cardiovascular morbidity is a blockage in an artery that supplies a vital organ provoked by a long-acting disease called *atherosclerosis*. Atherosclerosis starts in the early stages of human development and consists of the progressive accumulation of lipids, cells and fibrous proteins in specific locations in arterial walls; these accumulations develop into plaque-like lesions (*atheromata*, pl. for *atheroma*) located non-uniformly in the arterial system. Under certain circumstances, the atheroma is unstable and prone to rupture; when the rupture occurs, platelets are activated by the subendothelial connective tissue, creating a blood clot (thrombus) in the affected area, which may obstruct blood flow to an organ or tissue downstream, i.e., provoking an infarction. Additionally, part of the clot may break off and embolise in a smaller vessel downstream, also leading to infarction. Even without plaque rupture, atheromata may reduce blood flow sufficiently to cause ischaemia in organs supplied by the diseased vessel.

Atherosclerosis is a progressive disease. First understood as a passive mechanism in which some parts of the vascular system become more permeable to lipid molecules, over the years, more importance has been ascribed to (1) the behaviour of *endothelial cells* [113], which are the innermost layer of cells in the blood vessels, and to (2) the immune system [78]. Nowadays, the debate centres on whether atherosclerosis is an inflammatory process that promotes the absorption of lipids (“response-to-injury” theory [114]), or whether it is the penetration of lipids in the tissue that activates the immune system (“response-to-influx” theory [144]; “response-to-retention” theory [148])). In either case, the process is triggered by abnormal behaviour of the endothelial cells.

The endothelium is the monolayer of endothelial cells that separates the blood flow from the vessel tissue and determines what molecules or cells enter the wall from the lumen or leave the wall and enter the lumen. This layer acts as a selective membrane allowing the transport of specific blood-borne materials through the inter-cell junctions or by non-specific or receptor-mediated transport across the cell. The hallmark of atherosclerosis is an abnormal increment in disease-prone areas of the endothelial permeability to low-density lipoproteins (LDL), a macromolecule which in humans is the major cholesterol carrier. The mechanisms that account for this dysfunctionality of the cells are not fully understood.

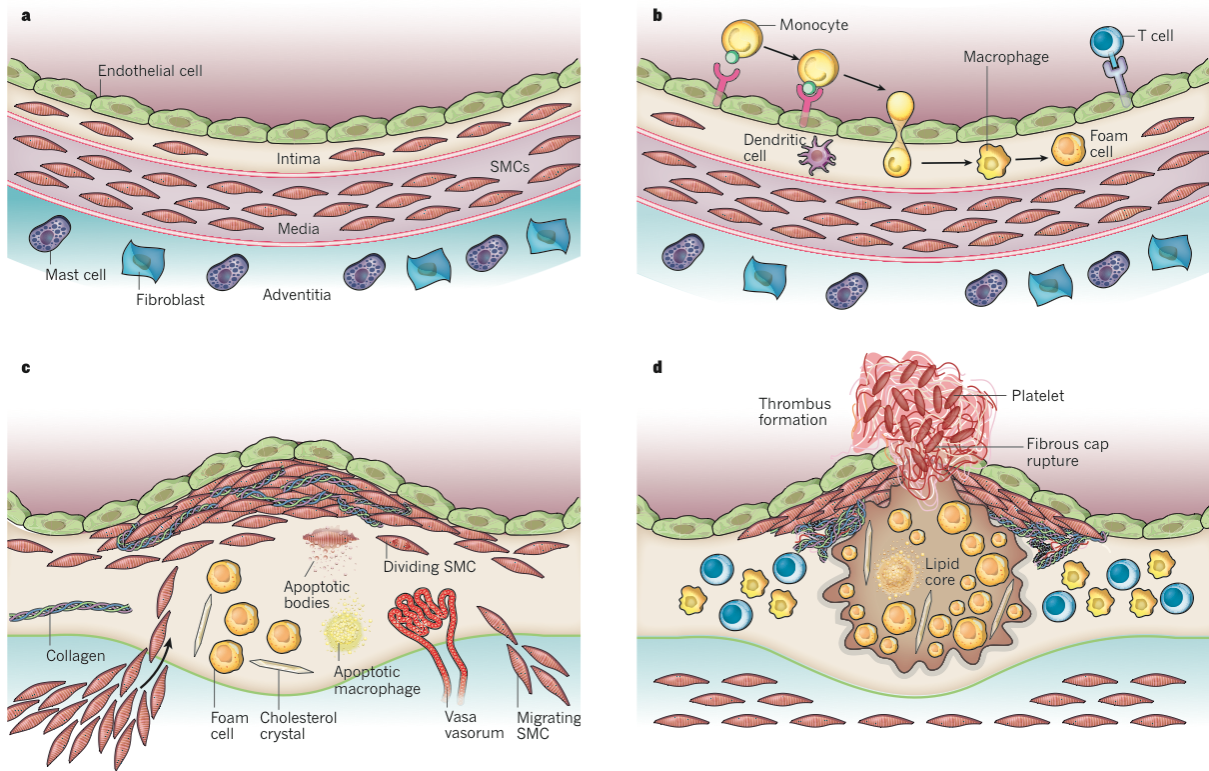


Figure 1.1: Stages in the development of atherosclerotic lesions [79].

Atherosclerosis is currently understood to involve the following set of key processes depicted in Figure 1.1: a) initial endothelial dysfunction; b) lipoprotein and monocyte (white blood cell) accumulation, monocyte differentiation into macrophages, foam cell formation by macrophage engulfment of lipids; c) smooth muscle cell migration, growth and cell division, collagen synthesis, cell apoptosis (programmed death); d) plaque rupture, necrotic material release and thrombus formation.

1.1.1 Local risk factors in atherosclerosis

A particular aspect of atherosclerosis is the highly localised distribution of the atheroma within the vasculature, known since the first recorded studies of *arteriosclerosis*, the general term to describe the hardening of the arteries, that can be dated to 1833 [82]. The spatial distribution of the lesions has been observed to vary between species and between and within vessels. In all cases, however, lesions are most frequent in areas of high curvature and arterial branching [22]. Also, the pattern of lesions has been demonstrated to strongly relate to age [144] (lesions in the aorta can already be observed at the fetal stage [120]). Notably, regions of high disease

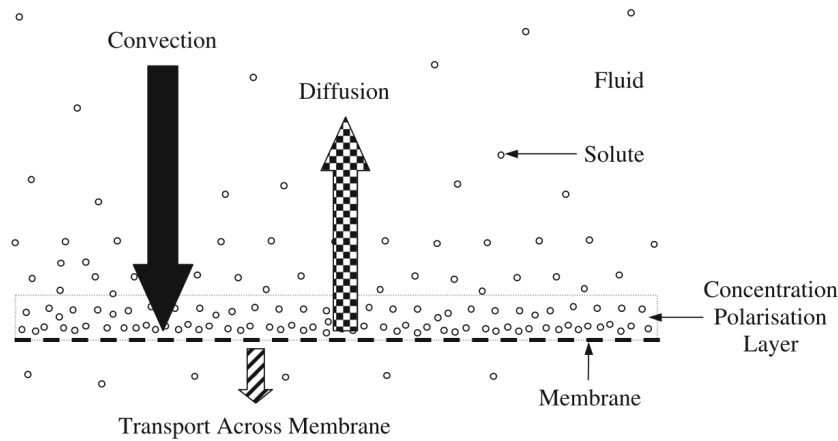


Figure 1.2: Sketch of non-biological transport of solutes and the characterisation of the concentration polarisation layer (from Vincent and Weinberg [134]).

probability are found close to regions of low probability [30]. Such a patchy nature represents a conundrum because most well-known risk factors (smoking, high blood pressure, hyperglycaemia, etc. [147]) should affect the entire vascular system homogeneously. *What are these powerful local risk factors that exacerbate or counterbalance the global ones?*

As previously described, the process of atheroma formation is associated with the transport of macromolecules across the endothelium. If we analyse a strictly physical (non-biological) transport of solutes in the arteries, it would depend upon the permeability of the endothelium (a semipermeable membrane), the blood flow dynamics — haemodynamics — (convection) and the plasma molecular concentration (solute diffusion), as we see in Figure 1.2.

Permeability was highlighted as a local risk factor at least as early as 1933, when Anitschkow observed that the incidence of lesions in rabbits coincided with an elevated uptake of plasma macromolecules [144]. Permeability to LDL is high in sites of high risk of atherosclerosis [98]. For example, rabbit aortic arch and branches present higher permeability than non-branch sites [117, 56, 127]. Similar results are observed in pig and dog aorta studies [39, 10, 122]. More recent studies investigating the uptake of macromolecules near aortic branches reveal a change in the permeability pattern with age that correlates with the age-related change observed in the lesions [118, 41, 5].

Haemodynamics could be a key factor in explaining local differences [96]: the arterial system has an irregular geometry which develops complex, spatially varying flow patterns in the blood flow (e.g., retrograde flow in the aorta [15, 14, 92], helical features in the aortic arch [64] and carotid artery bifurcations [88]). This was first postulated by von Rindfleisch [136] in 1872, who suggested that damage was caused to the vascular wall by the impingement of blood flow. Subsequently, many detailed studies of different parts of the vasculature have been conducted on the connection between lesions and flow. For example, Svindland [123] observed lesions in coronary bifurcations that coincided with regions of lower velocity; Sloop et al. [121] linked retrograde and antegrade flow with the observation of fatty streak patterns; and Moore and Ku [91] observed that atherosclerotic plaques occurred in regions of low and oscillatory near-wall velocities in a straight segment of the abdominal aorta.

The heterogeneous features of blood flow could have a significant effect on the *plasma solute concentration* through mixing, particularly in conjunction with the near-wall occurrence of a concentration polarisation layer, which consists of the accumulation of solutes rejected by the endothelial semipermeable membrane in the transendothelial flow influx [134]. Multiple experimental and theoretical studies connect concentration polarization of macromolecules to atherosclerotic flow features as reviewed in Vincent and Weinberg [134]. For example, experimental observations in canine carotid arteries showed that the concentration of LDL is higher at lower values of shear rate [139], and a similar connection between levels of LDL and low shear stress was observed in a simulation of the human aorta [74].

All three factors involved in a purely physical determination of transendothelial mass transport show local variations that correlate with atheroma distribution. However, the active role of the endothelial cells and their biological responses to mechanical forces, described in the next section, and the role of the immune system, which is controlled by shear-dependent gene expression [130, 34] and directly involved in the process of atherosclerosis [83, 78], are also likely to be critical, as are interactions between these biological processes (for example, the fact that inflammatory responses alter endothelial permeability [12, 13]). The interrelation between the transport factors in the vascular biological environment are sketched in Figure 1.3. In this study, our interest focuses on the effect of blood flow on transport, in particular through the effects of flow on the biological properties of endothelial cells.

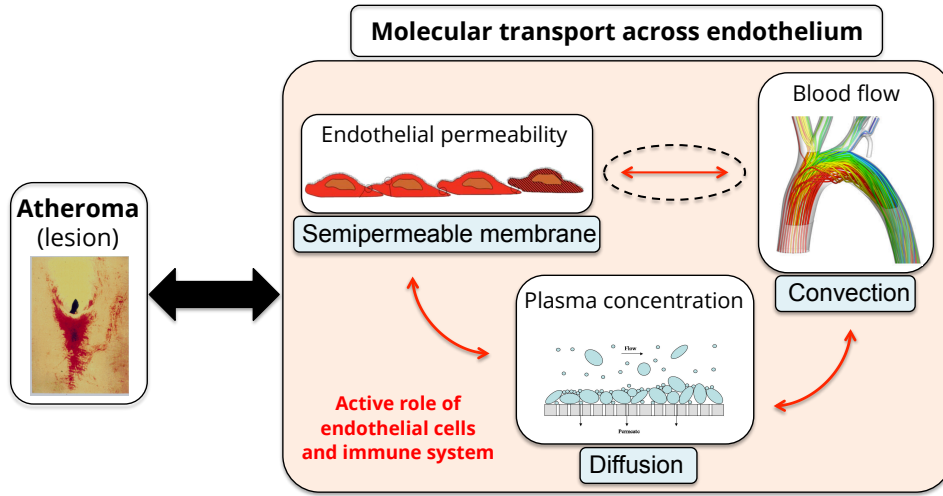


Figure 1.3: All three factors involved in a non-biological mass transport system show a local character that correlates with atheroma distribution, and at the same time, in an biological environment all factors are intercorrelated. In this work, we focus on the relationship between the endothelium and blood flow (composition of figures: atheroma [6], endothelium [124], blood flow [135], and concentration [150]).

1.1.2 The effect of blood flow on the endothelium

Early publications suggested that blood flow has a local influence by damaging the endothelium [136]. Over the years, this theory has been refined and evidence has been obtained that supports the more subtle proposition that endothelial cells are mechanosensitive [32, 27, 138]. Blood flow interacts with the arterial walls through pressure and viscous forces, and cells convert mechanical stimuli, such as shear stress and strain, into biochemical activity. In this section, we summarise some of the mechanisms by which flow can have an effect on two aspects of endothelium functioning: (1) modification of molecular transport, and (2) alteration of the cell morphology, these being the items of most relevance to our study. Nevertheless, it should be remembered that shear stresses also have an effect on endothelial gene expression and signalling pathways for multiple processes involved in atherosclerosis, such as coagulation, migration of leukocytes, smooth muscle proliferation and vasodilatation [94, 50, 101, 26, 25] (reviewed in Davies [32]).

The pathways that molecules take to cross the endothelium vary depending on their molecular characteristics. Molecules transport through differently sized intercellular gaps, and specific macromolecules can be captured by cell surface receptors and transported across the cell in vesicles (transcytosis). Lipid-soluble solutes, such as respiratory gases, can diffuse directly across

cell membranes but this transport is unregulated and not considered further in this review. Tarbell [124] reviewed the effect of flow on the different transport pathways and we briefly summarise some results: (1) proteins involved in intercellular tight junctions alter under shear stress [29]; when cells are linked by continuous junctions of this type (such as in the brain), the intercellular gap only allows transport of smaller molecules, but when this junction has breaks in it, some macromolecules (but not those as large as LDL) can transport through; (2) leaky junctions are associated with the division and death of cells, which have been reported to be inhibited under high levels of shear stress; this route allows for the transport of all macromolecules, including LDL; and (3) the formation of vesicles is influenced by changes in shear [33].

The effect of flow on the transport of molecules can be particularly complex if such transport involves multiple routes. Transport of LDL molecules, for example, occurs in regions of enhanced leaky junctions [143] and through vesicular transcytosis [98]; since LDL transport is impeded much more than water transport by the endothelial barrier, a concentration polarisation layer is observed in regions of low shear [35, 95] which, in turn, enhances endothelial LDL transport [52]. (Note that this effect is not present in *in vitro* experiments where there is no significant convection through the endothelium). Albumin, a macromolecule of smaller size than LDL, also transports through breaks in tight junctions; its endothelial permeability is observed to increase under different levels of shear [61]. The endothelial permeability of small solutes involved in atherosclerosis is various orders of magnitude higher than macromolecules; particularly in the case of oxygen [125], which is lipid soluble, meaning it also transports across the cell.

Endothelial cell morphology is observed to vary along the vasculature in correlation with the local flow patterns [112, 73, 99, 16]. For example, Reidy and Lowell Langille [112] observed that endothelial cells orientate their long axis with the direction of flow in the thoracic and abdominal aorta except in curved and bifurcated regions. *In vitro* experiments have also shown that cells elongate and orientate according to flow directionality [36, 40, 75, 109, 65] (see Figure 1.4). Furthermore, they have proved that morphological changes were time-dependent — for example, cells exposed to a physiological level of shear for 48 hours showed cell alignment in the flow direction, whereas no significant orientation was observed after 24 hours [36]. Cell orientation and shape may also be affected by cyclic strain [153].

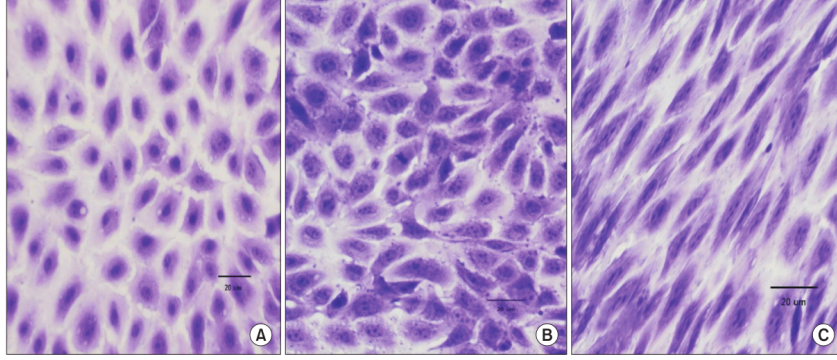


Figure 1.4: Cell orientation and shape under static conditions (A), flow with no predominant direction (B), and flow with a predominant direction (C) (from Kim [65]).

1.2 *In vitro* Studies of Effects of Shear Stress on Endothelium

1.2.1 Shear stress in the vasculature

The shear stress exerted by the blood on the endothelial cells, typically referred to as wall shear stress (WSS), has been postulated to be a key factor in the local distribution of atheroma through the mechanosensitive character of the cells (§§ 1.1.1 and 1.1.2). WSS, denoted $\boldsymbol{\tau}_w$, is a force vector per unit area — it has magnitude and direction. For an incompressible flow, it is defined by

$$\boldsymbol{\tau}_w = \mathbf{n} \cdot \mu(\nabla \mathbf{v} + \nabla \mathbf{v}^\top)|_{wall} \quad (1.1)$$

where μ is the dynamic viscosity of the fluid, $\mathbf{v}$ is the flow velocity, and $\mathbf{n}$ is the surface normal.

WSS will vary in space along the vessel wall and also in time due to the cardiac cycle (Figure 1.5). It is generally characterised as high and unidirectional in straight vessel sections and low or high and multidirectional in curved vessels and at branches and bifurcations [23]. The effect of flow-induced stress on the formation of atheroma has been a matter of controversy for many decades. In 1969, Fry [45] argued that atheromata occur in the vascular areas where high WSS is expected, whereas Caro et al. [22] proposed that early disease lesions were located in areas of low WSS, with high WSS having a disease-protective character. Later, Ku et al. [72] introduced the importance of the oscillatory nature of flow, and formulated the oscillatory shear

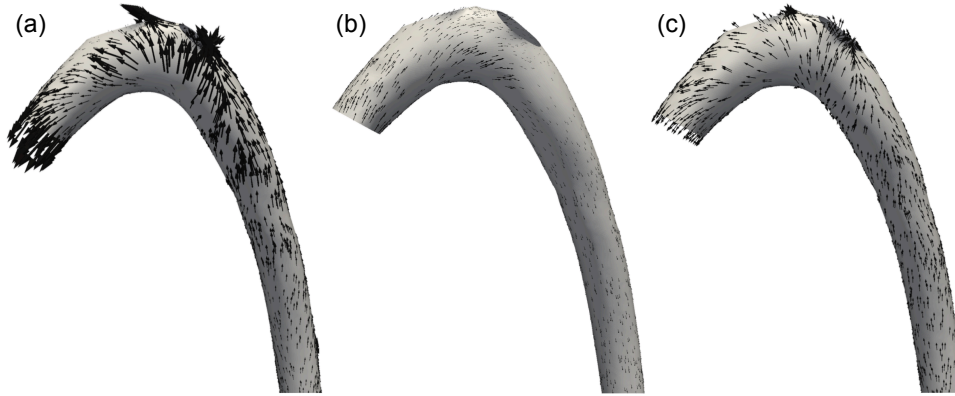


Figure 1.5: WSS vectors in a computational aorta at two different time steps (a) and (b), and time-averaged over the cardiac cycle (c) (from Mohamied [89])

index (OSI) metric (see § 2.4.3) to quantify pulsatility; OSI showed a positive correlation with plaque location. The current consensus links low and oscillatory shear to lesion-prone regions [23, 146]. However, Peiffer et al. [103] carried out a systemic review of the studies supporting the theory; this review revealed that, whilst the theory may be correct, the evidence was not conclusive.

There is a need for quantitative studies of multidirectional WSS since many studies reveal its importance but it is often poorly described as “disturbed flow” [28, 106, 27, 97, 141]. Recently, multiple metrics have been formulated to quantify multidirectionality [104, 93, 46, 2]. For instance, the transverse WSS (transWSS) metric [104] quantifies the time-averaged WSS perpendicular to the mean WSS direction, and correlates with the age-related change in the lipid deposit pattern between immature and mature rabbit aortas, which other classic metrics such as OSI are not capable of reproducing [104, 90]. *In vitro* experiments are important to investigate the endothelial cell responses to multidirectional shear stress.

1.2.2 *In vitro* experiments

In vitro experiments are an elegant way of investigating the effect of WSS on endothelium since they reduce significantly the number of variables in play. The study of transport across the endothelium cultured *in vitro* with the aim of assessing the correlation between endothelial permeability and the mechanical stress started more than thirty years ago [68, 18]. The advantages of *in vitro* experiments reside in isolating the endothelial behaviour from effects of subendothe-

lial tissues; measuring the permeability of the membrane to different plasma macromolecules and solutes; and controlling the mechanical forces exerted by the fluid. A potential disadvantage is that the cells are in a non-physiological environment and may have altered properties.

Over the years, multiple *in vitro* experiments have been carried out using different techniques. Regarding the mechanical forces exerted on the cells, two general classifications can be made, based on the duration of the experiment (acute or chronic) and on the instrument used to induce fluid motion in the plate (some examples are given in Figure 1.6). The instrument determines the magnitude and direction of τ_w , and some configurations limit the experiment duration.

A cone-and-plate apparatus (Figure 1.6[a]) consists of a cone that rotates with its axis of symmetry perpendicular to the plate where cells are cultured. τ_w is tangential and uniform over the plate, and its magnitude depends on the cone angle and the velocity of rotation [36]. Under certain conditions, there is also a radial component of τ_w , and an oscillatory WSS magnitude can be introduced if the symmetry axis is slightly tilted (wobbling cone) [33]. This experiment has been used, for example, to report that cells elongate and align in the direction of flow when exposed to sufficiently high levels of shear (0.8 Pa for 48 hours or 2 Pa for 24 hours) [36, 85], and that pinocytosis — a mechanism by which cells engulf liquid and molecules in small vesicles — is shear and time dependent [33].

In a parallel-plate flow chamber (Figure 1.6[b]), τ_w is unidirectional and has a constant magnitude along the plate that depends on the chamber height and inflow/outflow pressure difference, which can be varied to change the WSS magnitude [75]. Variations of this instrument can also produce a uniaxial oscillatory τ_w [55] or a WSS magnitude gradient along the long axis of the plate [131]. Furthermore, a backward-facing step (sudden expansion region) can be introduced in the flow chamber to produce a recirculation area where τ_w reverses and varies gradually in magnitude [129]. This flow feature is generally poorly described as a “disturbed flow” area. Parallel-plate flow chambers have been widely used, generally in acute experiments. For example, they have been used to show that the endothelial cell long axis aligns with the flow direction after a 72-hour flow exposure [75], and that a spatial shear stress gradient increases transendothelial macromolecular transport after a 5-hour exposure [106].

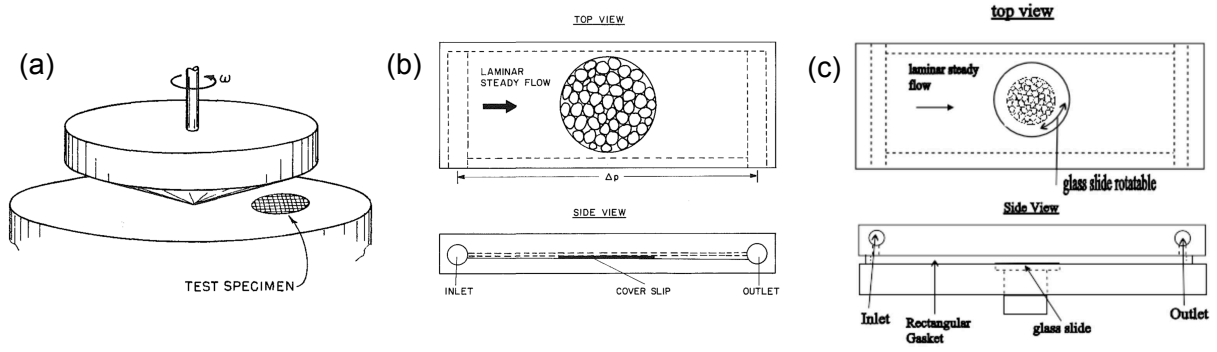


Figure 1.6: Three examples of instruments used to apply shear stress on endothelium *in vitro* experiments: (a) cone-and-plate [36]; (b) parallel-plate flow chamber [75]; (c) parallel-plate flow chamber plus rotatable plate [137].

Recently, Wang et al. [137] have developed a system consisting of a combination of a parallel-plate flow chamber and a rotatable plate on which cells are cultured (Figure 1.6[c]). With this technique, chronic shear was applied to elongate and align the cells in the direction of the flow, and then the cells were exposed to acute flow by increasing the rotatable-plate angle, yielding increasing levels of pro-atherosclerotic indicators [138]. This instrument has a high level of control over the flow but does not allow for chronic exposure to multidirectional flow.

This thesis focuses on orbital shaker experiments because of the multidirectional character of the WSS environment produced in some areas of such devices. The configuration is depicted in Figure 1.7: (a) cells are cultured in well plates and (b) placed on the orbital shaker device to be agitated; (c) the flow develops a wavy surface, causing (d) multidirectional WSS distributions to emerge in the well bottom with different profiles from centre to edge. The advantages of orbital shaker experiments are their simplicity, high throughput, easy access to the cell sample, and the possibility of chronic exposure. Their fluid dynamics configuration allows the development of different WSS scenarios by simply changing the volume of the medium or the frequency of rotation, as we will describe in more detail in the next section. Orbital shakers have been widely used to study the effect of flow on endothelial cell functions, such as gene expression [70, 71, 151, 65, 109, 25, 141], biochemical activity [77, 53, 3, 43], cell shape and alignment [31, 108, 24], and permeability [140, 48].

The directionality of the flow is a key factor in atherosclerosis [72, 101, 24, 138], and therefore it is fundamental to expose cells to multidirectional flow in order to be able to draw new conclusions. Special attention must be also paid to the difference in results under acute and

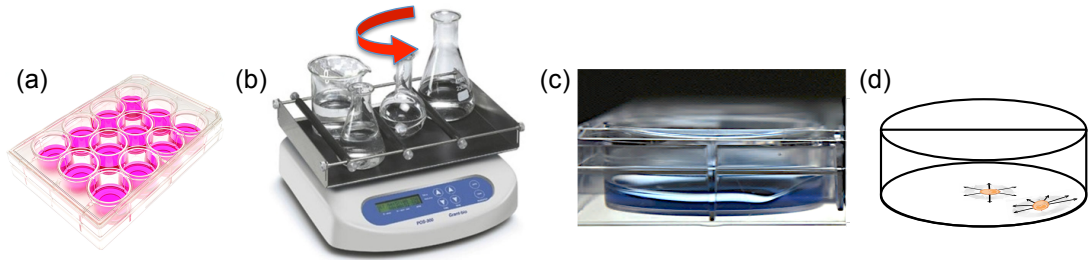


Figure 1.7: (a) A 12-well plate where cells are cultured [86]. (b) Orbital shaker apparatus consisting of a rotating platform [51]. (c) Snapshot of a fluid orbitally shaken. (d) Sketch of τ_w at the bottom of the well, at the centre and the periphery.

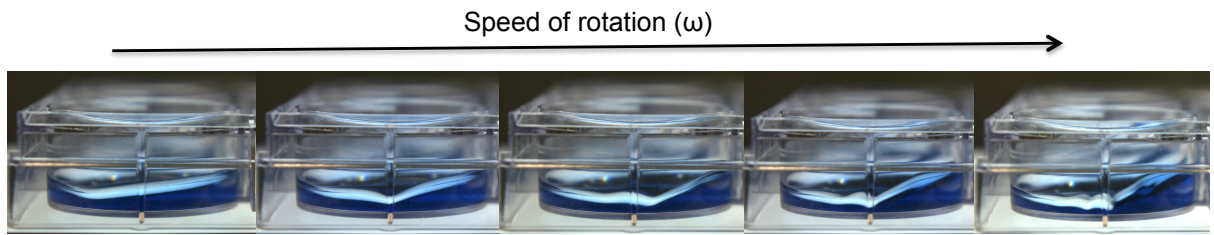


Figure 1.8: The orbital shaker wave steepens as the speed of rotation increases.

chronic exposure [140, 36]. Much attention has been paid to acute experiments in parallel-plate flow chambers with unidirectional and reversing flow, while chronically applied multidirectional motion experiments have been rarer and require further exploration, hence, the motivation for this study of orbital shakers.

1.2.3 Shear stress in orbital shakers

The investigation of effects of shear stress on orbital shaker systems has a limitation: the difficulty of directly measuring the shear stresses. That is why studies also have to rely on computational and analytical models. Determining WSS in orbital shakers by any means is difficult due to the complexity of the flow, which features a rotating wave that shows multiple behaviours depending on the experimental configuration. For example, in Figure 1.8 the wave is observed to steepen as the frequency of rotation is increased.

The first estimation of the WSS for orbital shakers was provided by Hubbe [58], and was based on the analytical solution of an infinite plate without limiting side walls that moves with a periodic translation (known as Stokes' second problem); it was proposed as an estimation of the maximum WSS. This solution, which we will refer to as Stokes' solution, has been widely

used by experimentalists [102, 70, 151, 3, 65] after it was published in Ley et al. [77]. The main limitations are that (1) it does not provide for the directionality nor spatial variation of the WSS, which is an important drawback considering that many studies [31, 65] use orbital shakers to show the difference in endothelial cell behaviour between the centre and periphery of the well; and (2) it does not include the dependence of the WSS on the well radius and the liquid height, where the latter is intrinsic to the calculation of τ_w . Salek et al. [116] have recently presented an analytical solution that approximately describes the free surface in highly shaken cases, but it does not give information about the WSS spatial variation.

Dardik et al. [31] directly measured WSS magnitude in orbitally shaken wells by using Doppler velocimetry; measurements were taken for increasing values of frequency of rotation f at two radial distances representing the centre and the periphery of the well. They noted differences between the WSS at the centre and periphery of the well for $f > 150$ rpm, and Stokes' solution overestimated their measurements in all cases except at the highest frequency $f = 210$ rpm. This method allows the measurement of WSS magnitude over time in one given direction and at one radial location, however, it is not a practicable way to produce a full WSS vector field. Its error has been measured as $\pm 10\%$ in a laminar flow duct [116]. The method was later used to validate computational models [126, 116].

Berson et al. [11] published the first quantitative map of WSS at the well bottom derived from a computational model; for the two cases presented, the WSS at the periphery was more than twice the WSS at the centre, and the maximum shear differed by 10% and 26%, respectively, from the simplistic Stokes' analytical estimation. Further computational studies [11, 108, 126, 116, 24, 43] have shown the richness of orbital shaker flows. An example is Figure 1.9 where (a) shows the complex pattern of the τ_w magnitude and the variation with the radial distance, and (b) represents the directionality of τ_w , demonstrating that cells would experience different WSS directions in different parts of the well. The pattern and average values of WSS change depending on the configuration parameters: well geometry, orbital radius and speed, and fluid volume and properties [11, 108, 126, 116, 24, 43]. The WSS in orbital shakers can attain a wide range of values with average values from 0.1 Pa to 1 Pa [126], and maximum values of 10 Pa [43], which are of the order of the values found *in vivo* for human and some animal models [145].

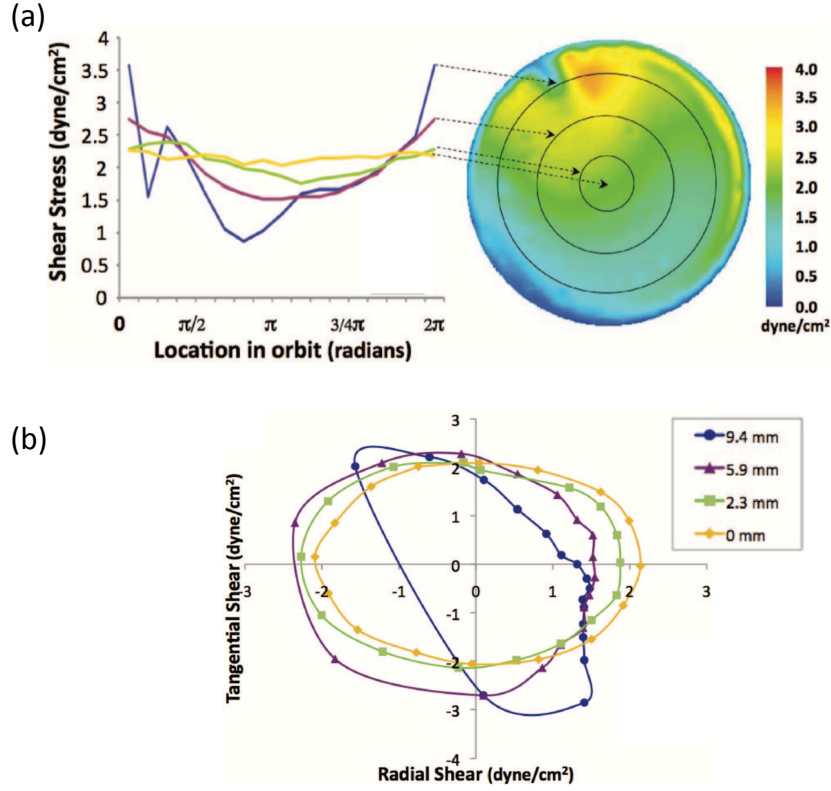


Figure 1.9: Results from a computational simulation described in Potter et al. [108]: (a) on the right, WSS map at the well bottom at a fixed time; on the left, the variation of WSS with the angle at different radii; (b) polar plot: each colour in the graph represents a different radius, and each point on the curve represents the tip of a vector plotted in equal time steps and connected following the cycle.

Salek et al. [116] compared their computational simulations to direct measurement of other problem variables: the velocity field and the free surface height. The velocity field was measured using high frame-rate particle imaging velocimetry (HFPIV) at a horizontal plane 2 mm above the well bottom; the HFPIV is only valid 5 mm from the side walls due to reflections. Results were in agreement with the flow streamlines and the velocity magnitude. The free surface height was measured using a digital camera mounted on the shaking platform and capturing about 14 frames per cycle of the (coloured) fluid in the well; the measured surface elevation was in agreement with the computational model.

These studies represent a significant advance in the understanding of the shear stress in orbital shakers. However, the currently employed tools have limitations: the analytical models are too simplistic and cannot provide reliable values of τ_w ; direct measurements are labour intensive and not suitable for reproducing complex vector fields; and the simulations are computationally

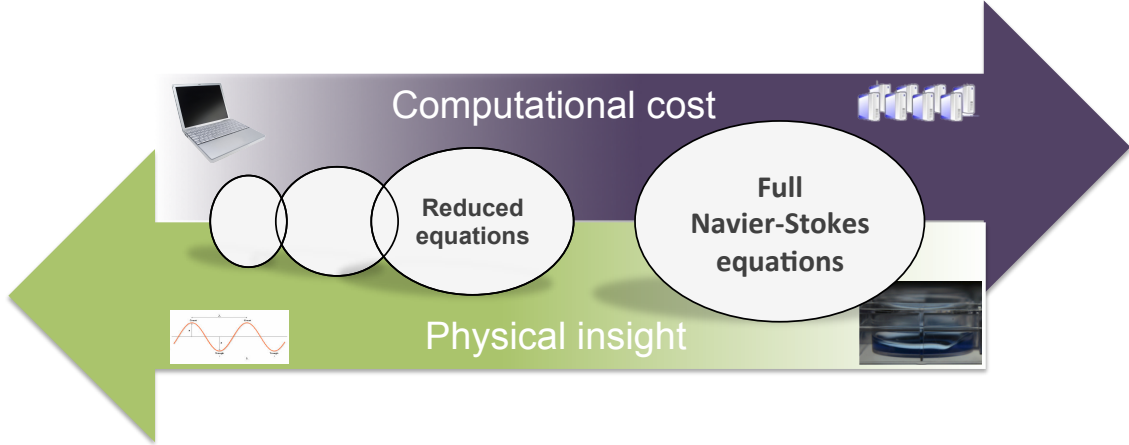


Figure 1.10: Sketch of advantages and inconveniences of the different approaches to tackle fluid dynamics equations. To the right, the computational cost of the model and the validity domain increase, as to the left, it is the physical insight that increases.

expensive, implying that the exploration of multiple cases is tedious. Therefore, there is a need to understand the fluid dynamics of orbital shakers in order to generate tools that accurately predict τ_w and to explore new shear stress scenarios for experimentalists.

1.3 Tackling the Fluid Dynamics of Orbital Shakers

Fluid dynamics problems are governed by the Navier-Stokes equations. Coupled with the mass conservation and potentially also enthalpy conservation equations, these are a complex system of equations that have no analytical solution in their general form. However, these equations can be solved numerically using computational methods, or they can be reduced to a simplified set of equations with analytical solutions using appropriate assumptions. Each approach has advantages and disadvantages, as we illustrate in Figure 1.10 in a simplified and didactic generalisation of the nature of problem. As we move to the right in the sketch, the domain of the validity of the model (symbolized by the circles) increases, which means that the model is capable of simulating a wider range of problems. However, the drawback is that the computational cost of the simulation increases notably. On the other hand, as we move in the sketch to the left, the physical insight offered by the model increases, that is, it presents an easier understanding of the flow features.

In this study, both numerical and analytical approaches will be used, albeit for different purposes. A potential flow analytical solution inspired by the theory of sloshing dynamics [59] will permit us to identify the dimensionless numbers that govern the fluid dynamics problem, to understand the flow behaviour in linear regimes, and to improve the current Stokes' estimation of WSS. The commercial computational fluid dynamics (CFD) solver STAR-CCM+ will provide a tool to study the detailed fluid features of the flow, to explore the orbital shaker configurations over a wide range of dimensionless numbers, and to validate the proposed analytical solutions.

1.4 Research Aims and Objectives

The overarching aim of this thesis is to further understanding of the fluid dynamics of orbitally shaken shallow fluid layers. This understanding will allow us to provide recommendations and tools on the use of orbital shakers for the study of endothelial cells and their role in atherosclerosis, and will pave the way for future improvement of the experiments.

The primary interest in the study of endothelial cells in orbital shakers is to have a precise map of the WSS experienced by the cells. The first specific research aim is to study in detail a typical case study to highlight the principal characteristics of the flow and review the ability of WSS metrics to capture the influence of flow on macromolecular transport and cell morphology. The second research aim is to explore the possibilities offered by the current configuration of the orbital shaker to reproduce different flow patterns and to classify the observed flow behaviour. The third aim is to improve the analytical estimates of the wall shear stress.

These aims have been achieved by targeting the following objectives:

1. Create a reliable computational setup to simulate orbital shaker flows and assess τ_w directionality in experiments.
2. Identify the dimensionless groups that govern the fluid dynamics of the problem and explore and classify flow behaviour in the dimensionless parameter space.
3. Develop new analytical models of WSS that can be used by experimentalists.
4. Produce recommendations and tools for endothelial cell experiments.

1.5 Outline

This thesis is organised as follows. Chapter 2 addresses the first research objective; it begins with a review of the biological significance of four established WSS metrics and introduces a novel metric (transWSSmin) derived from the observation of cell orientation. We present a robust computational model setup for the CFD commercial software Star-CCM+, which was the main tool used to simulate all case studies. The flow in a typical configuration used in our lab for endothelial experiments has been studied in detail, and the observed multidirectional WSS distributions have been compared to the change in endothelial permeability and orientation.

Chapter 3 addresses the second object of exploring the capacity of orbital shakers to provide different flow scenarios. We use a potential theory that serves to identify the dimensionless groups that govern the physical fluid dynamic problem. A set of 33 case studies was designed to cover the parameter space relevant to the endothelial cell experiments, presenting a wide range of flow behaviours. A regime classification of the cases based on the breaking of the free-surface wave follows from the analytical prediction of the height amplitude provided by the potential theory.

In Chapter 4, the potential theory and the simulations are used to develop and validate a new analytical WSS model, termed the PT-Stokes WSS model, which is a generalisation of the Stokes solution. Chapter 5 concludes by summarising the main results of the thesis, providing recommendations and tools for the investigation of endothelial cells in orbital shakers, and describing the possible routes for future work.

Chapter 2

Orbital Shaker Flow and Macromolecular Transport

2.1 Introduction

Progress on the understanding of the effect of wall shear stress on transendothelial transport has been particularly hindered by understanding of the overlying flow. Two main hurdles can be identified: (1) the difficulty of measuring the near-wall velocity profiles that are needed to calculate WSS; and (2) the complexity in quantifying the vectorial character of $\boldsymbol{\tau}_w$ (magnitude and directionality), which should be reduced to a scalar using mathematical metrics supported by the biological interpretation of how cells sense WSS, but this is often omitted in the biological literature. A rigorous analysis should include a trustworthy representation of the $\boldsymbol{\tau}_w$ field and a point-by-point statistical comparison of the biological observations with the relevant local WSS metric.

The aim of the chapter is to lay the grounds for the investigation of flow in orbital shakers using computational simulations. We will reduce the fluid dynamics to a model with seven configuration parameters and present a reliable configuration setup to implement the model in the commercial CFD software STAR-CCM+. The flow in a typical experimental configuration will be analysed in detail. Special emphasis will be made in describing WSS metrics — TAWSS, MagMeanWSS, OSI, transWSS, and the novel metric transWSSmin. The transWSSmin is a

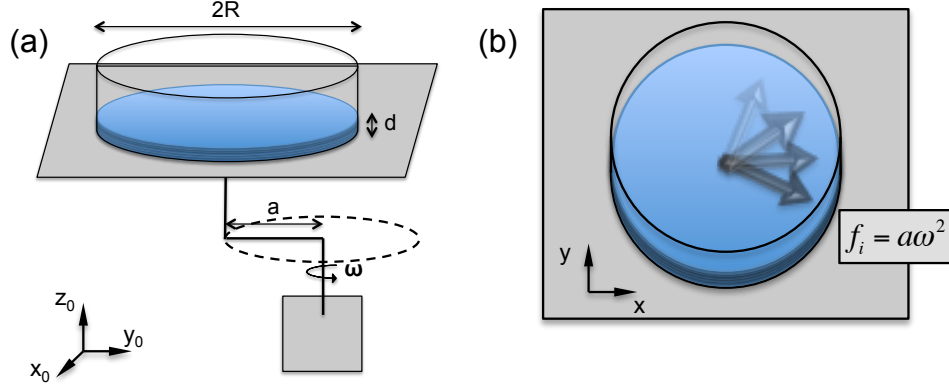


Figure 2.1: Sketch of an orbital shaker with a cylindrical container. (a) definition sketch of the system in the inertial reference frame (x_o, y_o, z_o) . (b) the system in an non-inertial reference frame (x, y, z) , where the shaker movement is represented by the inertial force $\mathbf{f}_i$.

novel metric that questions the prevailing theory that cells align with the temporal mean of τ_w [36, 40, 44, 75, 76], and will provide new insight regarding the alignment of endothelial cells with flow. A study of transendothelial transport of macromolecules in orbital shaker experiments was carried out by Dr. Mean Ghim as part of his PhD project. We will compare these results to the local distribution of the WSS metrics.

The chapter is organised as follows. Section 2.2 commences by describing the case setup, and Section 2.3 continues by introducing the computational model, which is validated in Section 2.3.1 with a mesh convergence and transient studies, and compared to other computational models in Section 2.3.2. Section 2.4 presents an analysis of the mathematical formulation and biological significance of four established WSS metrics and the novel metric transWSSmin; later, a set of analytical WSS distributions is used to demonstrate the difference between the transverse WSS-based metrics: transWSS and transWSSmin. Section 2.5 follows with a detailed description of the flow occurring in the orbital shaker (Table 2.1). Finally, in Section 2.6, we link the simulation to experiments and study the effect of flow on endothelial permeability and cell orientation. Conclusions are drawn in Section 2.7.

2.2 Problem Description

The orbital shaker experiment can be represented by a cylindrical container filled with a thin layer of liquid following an orbital translation (Figure 2.1[a]). The fluid dynamics problem can

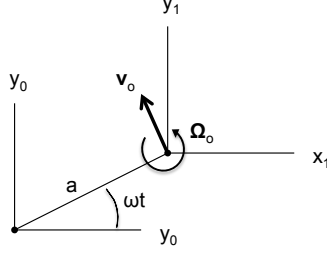


Figure 2.2: Sketch of the frames of reference: system 1 (non-inertial), system 0 (inertial).

be modelled as two Newtonian fluids (gas and liquid) separated by an interface, and its motion described by the continuity and Navier-Stokes equations:

$$\frac{\partial \rho_i}{\partial t} + \nabla \cdot \rho_i \mathbf{v}_i = 0 \quad (2.1)$$

$$\rho_i \frac{\partial \mathbf{v}_i}{\partial t} + \rho_i (\mathbf{v}_i \cdot \nabla) \mathbf{v}_i = \rho_i \mathbf{g} - \nabla p_i + \nabla \cdot \bar{\bar{\tau}}'_i + \rho_i \mathbf{f}_i \quad (2.2)$$

here given in the vectorial differential form, where subscript i denotes the gas (g) or liquid (l) phase, t is the time variable, $\mathbf{v}_i$ is the velocity vector, p_i is pressure, ρ_i is density, $\mathbf{f}_i$ is the inertial force, $\mathbf{g}$ is the gravity vector, and $\bar{\bar{\tau}}'$ is the deviatoric stress tensor

$$\bar{\bar{\tau}}'_i = \mu_i (\nabla \mathbf{v}_i + \nabla \mathbf{v}_i^T) - \frac{2}{3} \mu_i (\nabla \cdot \mathbf{v}_i) \mathbf{I} \quad (2.3)$$

where μ_i is the dynamic viscosity and $\mathbf{I}$ is the identity matrix.

We will adopt a moving reference frame in which the container is static (Figure 2.1[b]) and the inertial force $\mathbf{f}_i$ is thus non-zero. The general equation for the inertial force in the moving reference frame is [7]

$$\mathbf{f}_i = - \left(\frac{d\mathbf{v}_o}{dt} + \frac{d\mathbf{\Omega}_o}{dt} \times \mathbf{r}_o + \mathbf{\Omega}_o \times (\mathbf{\Omega}_o \times \mathbf{r}_o) + 2\mathbf{\Omega}_o \times \mathbf{v}_o \right) \quad (2.4)$$

where system 1 displaces with respect to system 0 as $\mathbf{r}_o = a (\cos(\omega t) \mathbf{e}_x + \sin(\omega t) \mathbf{e}_y)$, deriving a velocity $\mathbf{v}_o = a\omega (-\sin(\omega t) \mathbf{e}_x + \cos(\omega t) \mathbf{e}_y)$, where a and ω are the radius and speed of rotation of the orbital shaker platform, respectively, and $\mathbf{e}_x$ and $\mathbf{e}_y$ are unit vectors in the x and y -directions, respectively; and an angular velocity $\mathbf{\Omega}_o = 0$ because the axes of the systems remain parallel, i.e., $\mathbf{e}_{x0} \parallel \mathbf{e}_{x1}$ noted $\mathbf{e}_x$, and is the same for the y variable. Therefore,

$$\mathbf{f}_i = a\omega^2 (\cos(\omega t)\mathbf{e}_x + \sin(\omega t)\mathbf{e}_y). \quad (2.5)$$

The interface S between the fluids is a volume of infinitesimal thickness which can be considered a surface in its limit. At this surface, the velocity field must be continuous (kinetic boundary condition) and the dynamic forces must be at equilibrium (dynamic boundary condition). The kinetic condition implies that $\mathbf{v}_l|_S = \mathbf{v}_g|_S$, where S is defined by the implicit equation $s(\mathbf{x}, t) = 0$ and $\mathbf{x}$ are the space coordinates, the material derivative of which must be zero:

$$\frac{Ds}{Dt} = \frac{\partial s}{\partial t} + \mathbf{v} \cdot \nabla s = 0. \quad (2.6)$$

The dynamic boundary condition at S in the normal direction (scalar equation) and tangential directions (vectorial equation) to the surface is given by

$$\sigma \nabla \cdot \mathbf{n} = p_g - p_l + \mathbf{n} \cdot (\bar{\bar{\tau}}'_l - \bar{\bar{\tau}}'_g) \cdot \mathbf{n} \quad (2.7)$$

$$\mathbf{0} = \mathbf{n} \cdot (\bar{\bar{\tau}}'_g - \bar{\bar{\tau}}'_l) - [\mathbf{n} \cdot (\bar{\bar{\tau}}'_g - \bar{\bar{\tau}}'_l) \cdot \mathbf{n}] \mathbf{n} \quad (2.8)$$

respectively. Here σ is the surface tension coefficient and $\mathbf{n}$ is the surface normal pointing from gas to liquid.

The importance of surface tension forces is estimated by the Bond (Bo) and Weber (We) numbers, which are two dimensionless groups that arise in the nondimensionalisation of the normal component of the dynamic boundary condition and represent the ratio of the gravitational and inertial forces to the surface tension forces, respectively,

$$Bo = \frac{(\rho_l - \rho_g)g(2R)^2}{\sigma} \quad (2.9)$$

$$We = \frac{(\rho_l - \rho_g)U^2(2R)}{\sigma} \quad (2.10)$$

where, in the configuration of interest (Table 2.1), ρ_l is the liquid density ($\sim 1000 \text{kgm}^{-3}$), ρ_g is the gas density ($\sim 1 \text{kgm}^{-3}$), g is the gravitational acceleration ($\sim 10 \text{ms}^{-2}$), R is the well radius ($\sim 0.01 \text{m}$), U is the characteristic velocity (estimated by $\omega a \sim 0.1 \text{ms}^{-1}$), and σ is the surface tension between the two phases ($\sim 0.073 \text{Nm}^{-1}$). Hence, Bo and We are of the order of 10, meaning that the surface tension force is not dominant, and will be neglected in this thesis.

R (mm)	d (mm)	a(mm)	ω (s ⁻¹)	ρ (kg m ⁻³)	μ (Pa s)
11.05	2	5	15.7	997.6	0.001002

Table 2.1: Configuration parameters in the reference case: R is the well radius, d is the static medium height, a and ω are the radius and speed of rotation of the orbital shaker, respectively, and ρ and μ are the density and dynamic viscosity of the fluid, respectively.

The deviatoric stress tensors are, thus, considered equal and close to zero ($\bar{\tau}'_l|_S = \bar{\tau}'_g|_S \sim 0$) based on (2.7)-(2.8), their proportionality to the dynamic viscosity, and the fact that $\mu_g \ll \mu_l$. Therefore, this interface free of stress is named a *free surface* and, given the absence of surface tension, it also follows that $p_g = p_l$ at S .

The problem is thus described by seven parameters grouped in

- geometry of the well plate: fluid static height (d), well radius (R);
- forcing of the orbital shaker: radius (a) and speed (ω) of rotation of the orbital shaker;
- fluid medium properties: density (ρ) and dynamic viscosity (μ);
- physical constants: gravity (g).

In Chapter 3, the seven parameters will be combined into four dimensionless groups that govern the physical fluid dynamics problem, and a set of simulations is designed to map the dimensionless parameter space. In this chapter, the *reference case* with parameters' values given in Table 2.1 is used unless otherwise specified.

2.3 Computational Model

The movement of the medium covering the monolayer of endothelial cells was computed using the commercial CFD software STAR-CCM+ (v.9; CD-adapco [1]). STAR-CCM+ comprises an unstructured finite volume method for the spatial discretisation of the conservation equations (2.1)-(2.2). The two phases (gas and liquid) were modelled using a volume of fluid (VOF) method. The VOF scheme is an interface-capturing method [57] that tracks the gas-liquid interface using an extra conservation equation for a scalar field c that represents the volume fraction of liquid phase in the computational cell, that is, $c = 1$ for a computational cell containing liquid, $c = 0$ for gas, and $c \in (0, 1)$ if containing both. The VOF method can be implemented using different approaches [57, 63]. Star-CCM+ uses the Mahrenholtz and

Markiewicz [84] approach, which treats the fluid domain as a single-phase fluid, and only one set of mass and momentum conservation equations (2.1)-(2.2) is solved. The density and dynamic viscosity values of this single-phase fluid depend on the value of c and are given by

$$\rho = \rho_l c + \rho_g(1 - c) \quad (2.11)$$

$$\mu = \mu_l c + \mu_g(1 - c). \quad (2.12)$$

The liquid is modelled with the constant density and viscosity indicated in Table 2.1, and the gas is modelled as an ideal gas with a density of 1.1115 kg/m^3 and a constant dynamic viscosity of $18.688 \cdot 10^{-6} \text{ Pa s}$ at standard laboratory conditions. The free surface is not treated as a boundary, and there are no conditions to impose on it, although, it allows for the implementation of the surface tension force by modelling it as a body force [19]. The free surface is defined as the isosurface where $c = 0.5$.

The discretisation of the convective term in the conservation equation of c is delicate because the value c must be kept between zero and unity and the number of partially filled cells needs to be minimised as the fluid phases are assumed to be immiscible. Monotonicity could be ensured using a first-order upwind scheme, but it introduces excessive numerical diffusion and smears the interface. Furthermore, the amount of fluid of each phase that is convected across a cell face must not exceed the amount available in the donor cell. The High-Resolution Interface Capturing (HRIC) convective discretisation scheme [84] is used to adjust to this condition, which also allows for sharp interfaces. The scheme includes a correction based on the Courant-Friedrichs-Lewy (CFL) number and the interface orientation, which yields a mix between the HRIC and an upwind scheme. The CFL is a dimensionless number that compares the flow velocity U at the cell with a characteristic velocity derived from the cell's characteristic size Δl and the solver time step Δt , i.e., $\text{CFL} \sim \frac{U}{\Delta l / \Delta t}$. The properties of the VOF module include a custom lower and upper CFL threshold to control the blending of the schemes; for further details, see Mahrenholtz and Markiewicz [84] and the STAR-CCM+ User Guide [1].

The time solver chosen was implicit unsteady (second order backward Euler scheme) without turbulence model (Reynolds numbers are low). The problem was initiated from a static state, and the initial position of the free surface was set at the constant height d using the option *Flat*

VOF wave that is offered when activating the *VOF wave* module. The fluid motion was imposed as a time-dependent inertial force (2.5), and included in the gravity vector. Wall-type no-slip boundary conditions were imposed in the cylinder bottom and side walls, and outlet-type in the cylinder upper boundary. The resultant non-linear coupled algebraic equations are solved using a segregated flow solver, which solves the equations sequentially in an uncoupled manner where the linkage between the equations is achieved with a predictor-corrector approach [1]. The solver uses a second order upwind scheme to estimate the convective flux terms, and second order scheme for the estimation of the diffusive flux term of the momentum equation.

2.3.1 Mesh convergence and transient studies

In addition to the numerical method, the accuracy of a simulation depends on the resolution of the fluid domain, which is given by the number and size of the mesh cells that map the domain; this, in turn, determines the computational cost of the simulation. Therefore, it is necessary to carry out a mesh convergence study to find the mesh that resolves the domain accurately enough for the purpose of the study with minimal computational resources. This task consists of monitoring some key values of the problem and observing how they vary with the mesh resolution.

The reference-case fluid domain was implemented on a cylindrical butterfly mesh (22.1 mm diameter and 10 mm height) with a block-structured configuration to allow for an efficient distribution of the computational cells near the side walls. In this study, the cell size in the boundaries is critical because the determination of WSS depends on adequately capturing the boundary layer. In order to optimise the number of cells, the cell size increases geometrically from the wall boundaries toward the centre, both horizontally and vertically, as shown in Figure 2.3.

Given the periodicity of the external forcing, and according to former publications [11, 116], the fluid velocity field and free-surface shape are expected to be constant and rotating in synchrony at the orbital period after the initial transients damp out. Therefore, the mean and maximum values of the height, velocity and WSS are expected to reach a constant value, and were used as monitoring values. Under the initial setup of the computational model, mesh convergence was not attained: increasing the number of cells did not produce the expected convergence

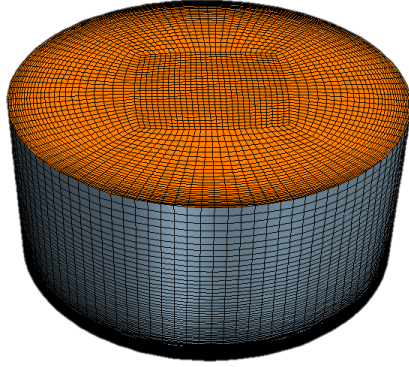


Figure 2.3: Cylindrical block-structured mesh with 291,600 computational cells. The cell size increases geometrically from the wall boundaries: edge to centre horizontally and bottom to top vertically.

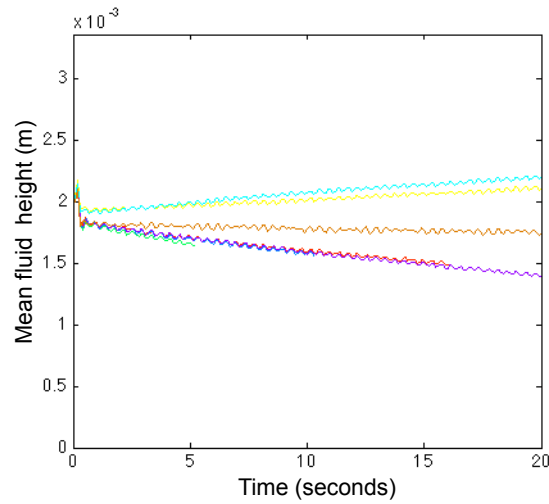


Figure 2.4: Mean fluid height (average free-surface elevation) as a function of time for multiple simulation configurations (not specified). The value should remain constant at 2 mm, however, there was a gain or loss of liquid volume under certain conditions.

trend. The trend of the mean surface height revealed that the volume of liquid was not being conserved: some configurations would have a gain in liquid volume and others would have a volume loss (see Figure 2.4).

The problem was related to the CFL number in the cells marked as interface by the VOF model. The VOF model uses this value to decide on the convective discretisation scheme. When tracking sharp interfaces, the CFL number must be kept below a setup threshold in order to ensure use of the HRIC scheme. To meet this criterion, the time step was lowered from 1 millisecond to 0.1 millisecond, and the average value of the CFL number changed from about 2 to under 0.5, the setup threshold for the HRIC scheme.

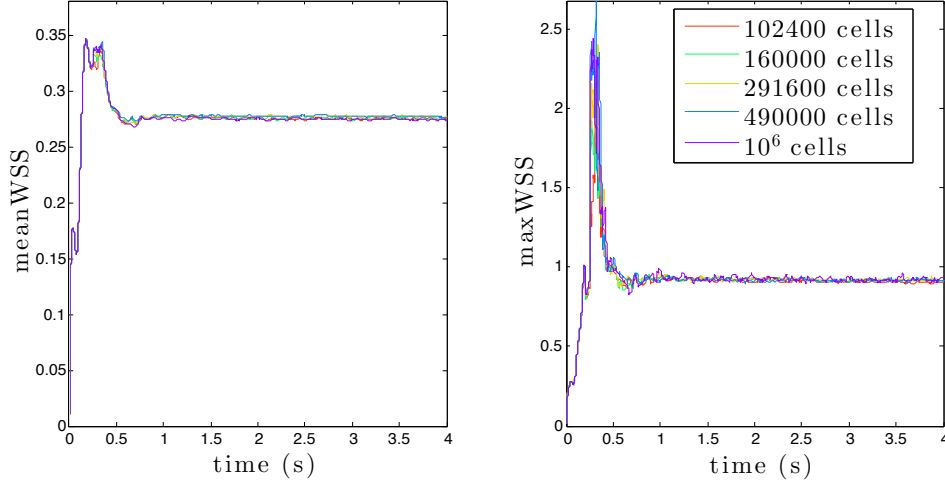


Figure 2.5: Mean and maximum WSS at the well bottom as a function of time for different mesh configurations.

Assuring that the criterion $CFL < 0.5$ was satisfied, the mesh convergence was observed. Figure 2.5 shows the trend of the mean and maximum WSS at the well bottom for meshes of 100K, 160K, 290K, 490K, and 1M cells. The fluid is set in motion from a static state and experiences a transient state before it reaches the periodic state after approximately 1 s for all meshes. This is equivalent to 2.5 rotations since the cycle period is $T = 0.4$ s; this result is similar to that reported by Berson et al. [11]. Once the steady state is reached, all meshes yielded similar results for the WSS values. In this study, we have used the mesh with 291,600 cells in order to obtain a high resolution near the side walls, and the results are reported in the tenth cycle ($t \in [4, 4.4]$ s) to ensure working at the periodic state.

2.3.2 Comparison with other computational models

To further validate the computational model, we have compared results in a configuration case presented in Salek et al. [116] with parameters $R = 17.5$ mm, $d = 2.08$ mm, $\omega = 10.5$ rad s $^{-1}$, $a = 9.5$ mm, $\mu = 10^{-4}$ Pa s, and $\rho = 998$ kg m $^{-3}$. Their results were obtained in the commercial CFD software FLUENT with a block-structured cylindrical mesh of 64,000 computational cells, and validated with experimental measurements of height, velocity and WSS. Figure 2.6 shows a comparison between Salek's and our computational model for the free surface and the bottom WSS maps. Both sets of simulation maps are in agreement. Small discrepancies can be observed between the WSS maps: in Salek's, the green area (WSS ~ 0.5 Pa) extends up to the boundaries

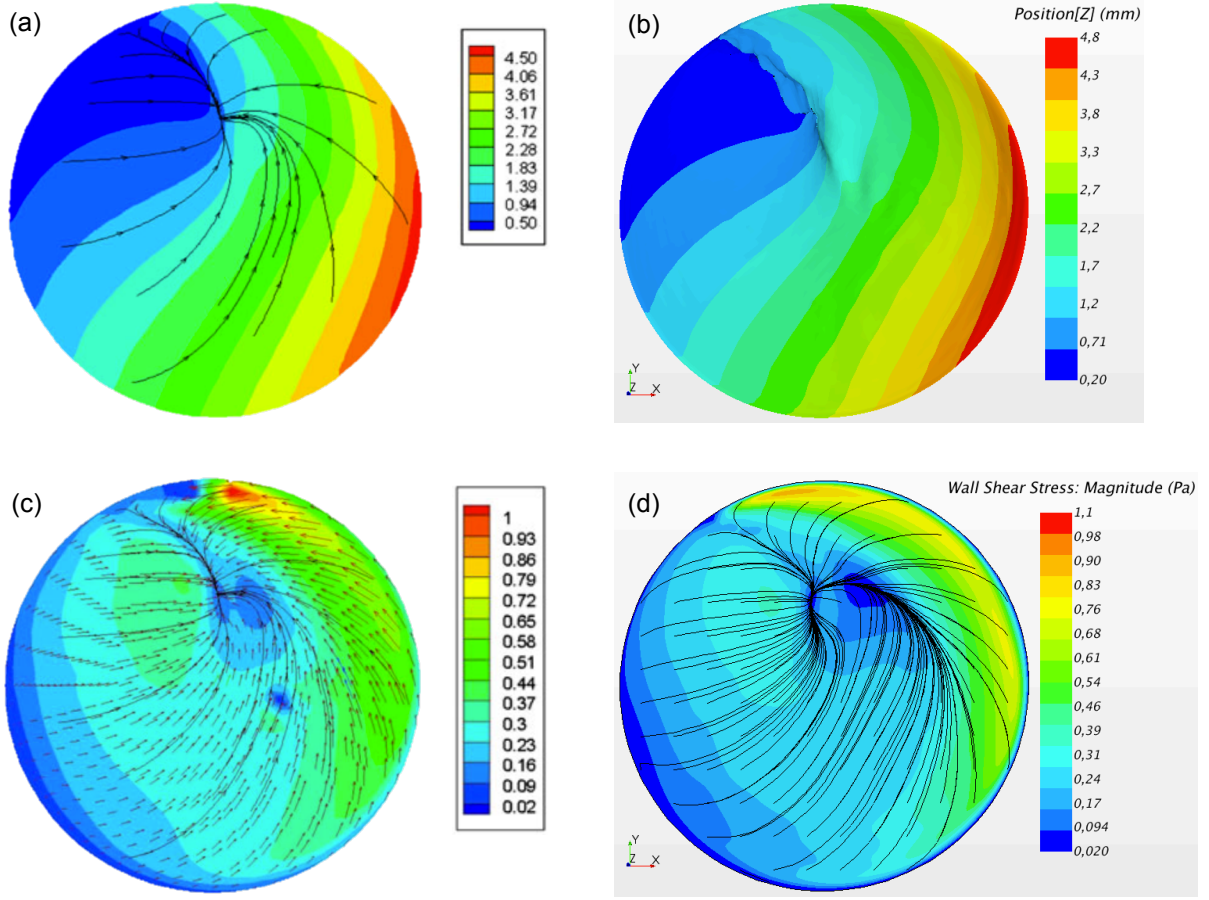


Figure 2.6: Height maps in mm (top) and WSS maps at the cylinder bottom in Pa (bottom) simulated with our computational model (right column) and from Salek et al. [116] (left column). The superimposed black lines represent the WSS “streamlines” defined in § 2.5.

in the top right of the well bottom (Figure 2.6[c]), whereas in our model, the WSS value is close to zero at all boundaries around the well bottom (Figure 2.6[d]). We argue that our solution gives the WSS value expected at the cylinder corner since velocities must be zero at the vertical wall, and speculate that the difference is caused by discrepancies in the imposition of the no-slip boundary conditions between our model and Salek’s. Figure 2.7 shows the time-averaged WSS (TAWSS) and the maximum WSS (maxWSS) over a cycle as a function of the radial distance. The differences are within the uncertainty of the experimental WSS measurements (10%), except around the maximum value of the maxWSS, which is the most sensitive value to the WSS discrepancies described at the boundaries.

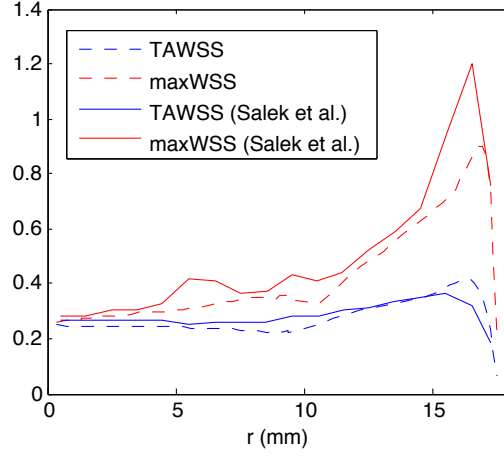


Figure 2.7: Time-averaged WSS (TAWSS) and maximum WSS (maxWSS) over a cycle as a function of the radial distance simulated with our computational model and from Salek et al. [116].

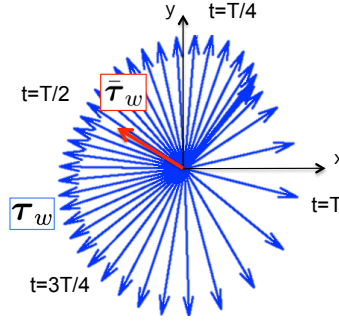


Figure 2.8: A multidirectional distribution of the WSS vector τ_w over a cycle. $\bar{\tau}_w$ is the temporal mean of τ_w , and t is time in a cycle of period T .

2.4 Wall Shear Stress Metrics

A quantitative analysis of the influence of blood forces on transendothelial transport needs adequate characterization of the WSS. The molecular transport is a spatial scalar field measured at an instant of time, whilst the WSS is a spatial vector field typically depending on time periodically. To correlate these two variables point-to-point, the vectorial and time-dependent character of the WSS needs to be contracted to a scalar field. This scalar field is called a WSS metric and there are several commonly used metric formulations. The WSS distribution over time can have a steady or oscillatory, and a unidirectional or multidirectional character (an example of a multidirectional WSS distribution over time is shown in Figure 2.8). The different metrics capture the WSS vector distribution in different manners, supported by the biological interpretation of how cells sense WSS.

In §§ 2.4.1-2.4.4, we describe the mathematical formulation and biological significance of four established WSS metrics: TAWSS, MagMeanWSS, OSI, and transWSS, and in § 2.4.5, we introduce a novel WSS metric: transWSSmin. Finally, in § 2.4.6, a set of ideal analytical $\boldsymbol{\tau}_w$ distributions are analysed to demonstrate the differences between the transverse WSS metrics.

2.4.1 Time-averaged wall shear stress (TAWSS)

TAWSS is the time average of the magnitudes of the instantaneous $\boldsymbol{\tau}_w$:

$$\text{TAWSS} = \overline{|\boldsymbol{\tau}_w|} = \frac{1}{T} \int_0^T |\boldsymbol{\tau}_w| dt = \frac{1}{T} \int_0^T \sqrt{\tau_x^2 + \tau_y^2} dt \quad (2.13)$$

where τ_x and τ_y are the x and y -components of the WSS, respectively, t represents time, and T is the period of the cycle. (Note that $\tau_z = 0$ due to the no-slip boundary condition and continuity equation). Biologically, TAWSS averages the amount of WSS experienced by the cell over a cycle, and it is normally quantified as low or high WSS in relation to the average values *in vivo*. The directionality of the $\boldsymbol{\tau}_w$ is ignored in this metric.

2.4.2 Magnitude of the mean wall shear stress vectors (MagMeanWSS)

MagMeanWSS is the magnitude of the temporal mean of $\boldsymbol{\tau}_w$ ($\bar{\boldsymbol{\tau}}_w$):

$$\text{MagMeanWSS} = |\bar{\boldsymbol{\tau}}_w| = \left| \frac{1}{T} \int_0^T \boldsymbol{\tau}_w dt \right|. \quad (2.14)$$

MagMeanWSS is not to be confused with TAWSS since $\overline{|\boldsymbol{\tau}_w|} \neq |\bar{\boldsymbol{\tau}}_w|$. For example, a pulsatile flow with $\boldsymbol{\tau}_w$ constant in magnitude c , and oriented in the positive axial direction for half of the cycle and in the opposite direction for the other half of the cycle would have a TAWSS = c and MagMeanWSS = 0; similarly, TAWSS = c and MagMeanWSS = 0 for an axisymmetric multidirectional flow of constant magnitude c ; and, conversely, TAWSS = MagMeanWSS if the flow is unidirectional. Biologically, MagMeanWSS determines if there is a prevailing WSS direction and what its intensity is. For instance, MagMeanWSS would be zero if the forces acting on the cell pull equally in opposite directions. This metric retains properties of both magnitude and direction, and ranges from zero (no dominant flow direction) to TAWSS (unidirectional flow).

2.4.3 Oscillatory shear index (OSI)

OSI expresses a ratio between the MagMeanWSS and TAWSS:

$$\text{OSI} = \frac{1}{2} \left(1 - \frac{|\int_0^T \boldsymbol{\tau}_w dt|}{\int_0^T |\boldsymbol{\tau}_w| dt} \right) = \frac{1}{2} \left(1 - \frac{\text{MagMeanWSS}}{\text{TAWSS}} \right). \quad (2.15)$$

This metric was introduced by Ku et al. [72] and later simplified in He and Ku [54] to measure the deviation of the cyclic WSS from the predominant direction. It can be understood as a normalised MagMeanWSS. It has the same biological significance as MagMeanWSS, but it does not consider the WSS intensity and scales inversely: it ranges from zero (unidirectional flow) to 0.5 (no dominant flow direction). This metric only retains directional properties.

2.4.4 Transverse wall shear stress (transWSS)

TransWSS is the time average of the $\boldsymbol{\tau}_w$ components perpendicular to the temporal mean direction $\frac{\bar{\boldsymbol{\tau}}_w}{|\bar{\boldsymbol{\tau}}_w|}$:

$$\text{transWSS} = \frac{1}{T} \int_0^T \left| \boldsymbol{\tau}_w \cdot \left(\mathbf{e}_z \times \frac{\frac{1}{T} \int_0^T \boldsymbol{\tau}_w dt}{\frac{1}{T} \left| \int_0^T \boldsymbol{\tau}_w dt \right|} \right) \right| dt = \frac{1}{T} \int_0^T \left| \boldsymbol{\tau}_w \cdot \left(\mathbf{e}_z \times \frac{\bar{\boldsymbol{\tau}}_w}{|\bar{\boldsymbol{\tau}}_w|} \right) \right| dt \quad (2.16)$$

where $\mathbf{e}_z$ is the vertical unit vector perpendicular to the well base. This metric was formulated in our lab [104]. TransWSS becomes undefined when $|\bar{\boldsymbol{\tau}}_w|$ is zero, that is, if $\text{MagMeanWSS} = 0$. For a unidirectional reversing or steady flow where $\bar{\boldsymbol{\tau}}_w \neq 0$, $\text{transWSS} = 0$. Biologically, the formulation of transWSS assumes that the cell is aligned in the direction of $\bar{\boldsymbol{\tau}}_w$ and averages the amount of transverse $\boldsymbol{\tau}_w$ relative to that direction (Figure 2.9). When there is no predominant direction, the cells are expected to orientate randomly and transWSS loses its significance. This metric retains both magnitude and directional properties.

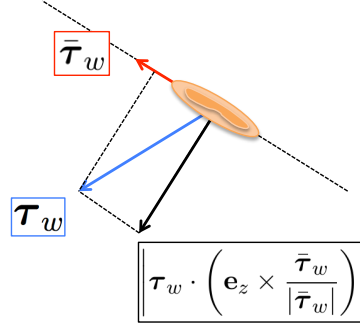


Figure 2.9: Biological interpretation of transWSS. The cell is assumed to align in the direction of $\bar{\tau}_w$ (red), and the transWSS metric averages the transverse components (black) of τ_w (blue) relative to that direction.

2.4.5 Novel metric: minimised transverse wall shear stress (transWSSmin)

TransWSSmin is a generalisation of transWSS and is defined as the minimum transWSS $_{\phi}$ calculated with respect to all possible reference directions ϕ :

$$\text{transWSSmin} = \min_{\phi \in [0, \pi]} \frac{1}{T} \int_0^T \underbrace{\left| \tau_w \cdot \begin{pmatrix} \mathbf{e}_z \times \begin{pmatrix} \cos \phi \\ \sin \phi \\ 0 \end{pmatrix} \end{pmatrix} \right|}_{\text{transWSS}_{\phi}} dt. \quad (2.17)$$

Figure 2.10 exemplifies the mathematical formulation: for a given τ_w distribution (blue arrows, Figure 2.10[a]), ϕ sweeps all possible angles ($\pm 90^\circ$ from $\bar{\tau}_w$) and is used as a reference direction to calculate transWSS $_{\phi}$ (Figure 2.10[b]), hence, transWSSmin = min(transWSS $_{\phi}$). For the τ_w distribution plotted in Figure 2.10(a), we observe in Figure 2.10(b) that the value transWSS $_{\phi}$ is different in all directions, and that transWSSmin (green star) is different from the metric transWSS (red star).

Biologically, transWSSmin represents the transverse WSS that the cells would experience if they aligned in the direction where they sense the minimum transverse forces, instead of the direction of $\bar{\tau}_w$ (see Figure 2.11), and the metric's value indicates the average amount of transverse τ_w relative to that direction.

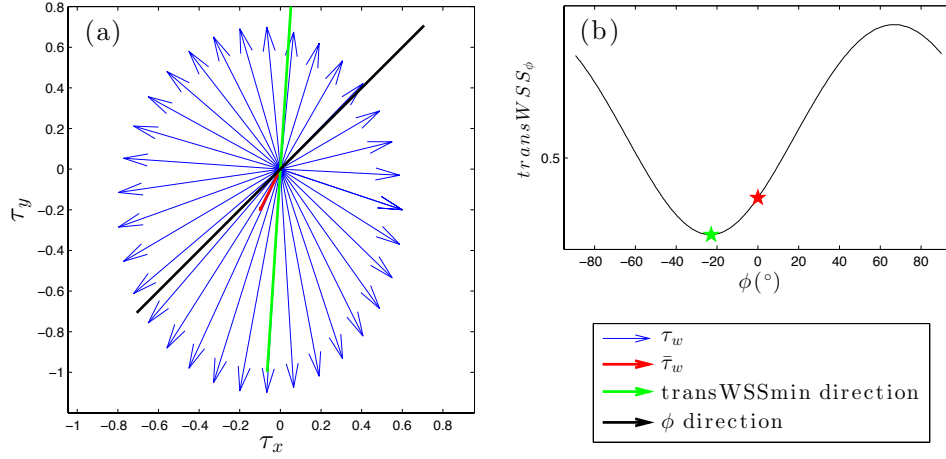


Figure 2.10: Mathematical interpretation of transWSSmin. (a) shows an example of a τ_w distribution over time; the red arrow represents the temporal mean of τ_w ; the black line is the reference direction ϕ to calculate transWSS_ϕ ; and the green line is the direction in which transWSS_ϕ is a minimum. (b) shows the values of transWSS_ϕ as a function of ϕ , where ϕ is measured from the direction of $\bar{\tau}_w$ (red star); the green star marks the ϕ value where transWSS_ϕ is a minimum.

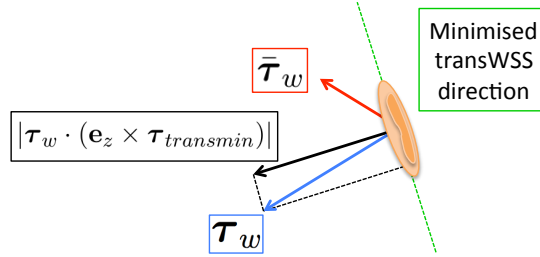


Figure 2.11: Biological interpretation of transWSSmin. The cell is assumed to align in the direction where it senses the minimum transverse WSS (green line), instead of the direction of $\bar{\tau}_w$ (red), and the transWSSmin metric averages the transverse components (black) of τ_w (blue) relative to that direction.

2.4.6 Demonstrating the differences between the transverse WSS metrics

We now analyse a set of ideal analytical τ_w distributions — not necessarily realistic — to illustrate the mathematical difference between the new transWSSmin metric and the earlier transWSS metric. The τ_w distributions have been mathematically defined with trigonometric functions for a generic period T

$$\begin{aligned}\tau_x(t) &= k_1 \cos\left(\theta_i + (\theta_f - \theta_i)\frac{t}{T}\right) \\ \tau_y(t) &= k_2 \sin\left(\theta_i + (\theta_f - \theta_i)\frac{t}{T}\right)\end{aligned}\tag{2.18}$$

where k_1 and k_2 are constants that determine the instantaneous τ_w magnitude and the eccentricity of the distribution contour, θ_i and θ_f are constants that determine the range of τ_w directionality, and t is the time variable $\in [0, T)$. We have studied the distribution cases summarised in Table 2.2. Figure 2.12 shows these distributions in the left column and the corresponding transWSS_ϕ plot in the right column, as explained in Figure 2.10. The values of the metrics defined in §§ 2.4.1-2.4.5 are summarised in Table 2.3, but the focus is made on describing the differences between transWSS and transWSSmin .

Figure 2.12(a)-(d) shows a set of τ_w distributions with a circular contour (constant magnitude of τ_w). Distribution (a) has no predominant direction ($\text{MagMeanWSS} = 0$), thus transWSS is undefined; on the other hand, the value of transWSS_ϕ is constant in all directions, thus there is not a preferred direction to minimise the transverse τ_w components (transWSSmin is undefined). Distribution (c) has a predominant direction ($\text{transWSS} = 0.64$), however transWSS_ϕ is constant in all directions, and therefore transWSSmin is again undefined. Distribution (b) has the peculiarity that the transWSSmin direction is perpendicular to $\bar{\tau}_w$ and transWSS coincides with the maximum of transWSS_ϕ , while in distribution (d), they fall in the same direction.

Figure 2.12(e)-(i) shows a set of τ_w distributions with an elliptical contour ($k_1 = 0.5$ and $k_2 = 1$, thus, the maximum τ_x and τ_y are 0.5 and 1, respectively). In this case, transWSSmin is defined in all distributions. Distribution (e) has no predominant direction ($\text{MagMeanWSS} = 0$), thus transWSS is undefined; however, transWSS_ϕ is minimised in the direction of the long axis of the contour. In distribution (f), $\bar{\tau}_w$ and the transWSSmin direction differ by 36° , and in distribution (h) they are perpendicular and transWSS coincides with the maximum of transWSS_ϕ . In distributions (g) and (i), they point roughly in the same direction.

τ_w distribution	k_1	k_2	θ_i	θ_f
(a)	1	1	0	2π
(b)	"	"	"	$3\pi/2$
(c)	"	"	"	π
(d)	"	"	"	$\pi/2$
(e)	0.5	1	0	2π
(f)	"	"	"	$3\pi/2$
(g)	"	"	"	π
(h)	"	"	$\pi/2$	$3\pi/2$
(i)	"	"	0	$\pi/2$

Table 2.2: Analytical model parameters of τ_w distributions displayed in Figure 2.12.

	TAWSS	MagMeanWSS	OSI	transWSS	transWSSmin
(a)	1.00	0.00	0.50	undef	undef
(b)	1.00	0.30	0.35	0.72	0.55
(c)	1.00	0.64	0.18	0.64	undef
(d)	1.00	0.90	0.05	0.37	0.37
(e)	0.77	0.00	0.50	undef	0.32
(f)	0.77	0.24	0.35	0.46	0.30
(g)	0.77	0.64	0.09	0.32	0.32
(h)	0.77	0.32	0.29	0.64	0.32
(i)	0.77	0.71	0.04	0.24	0.22

Table 2.3: WSS metrics for the analytical τ_w distributions displayed in Figure 2.12 (normalised units).

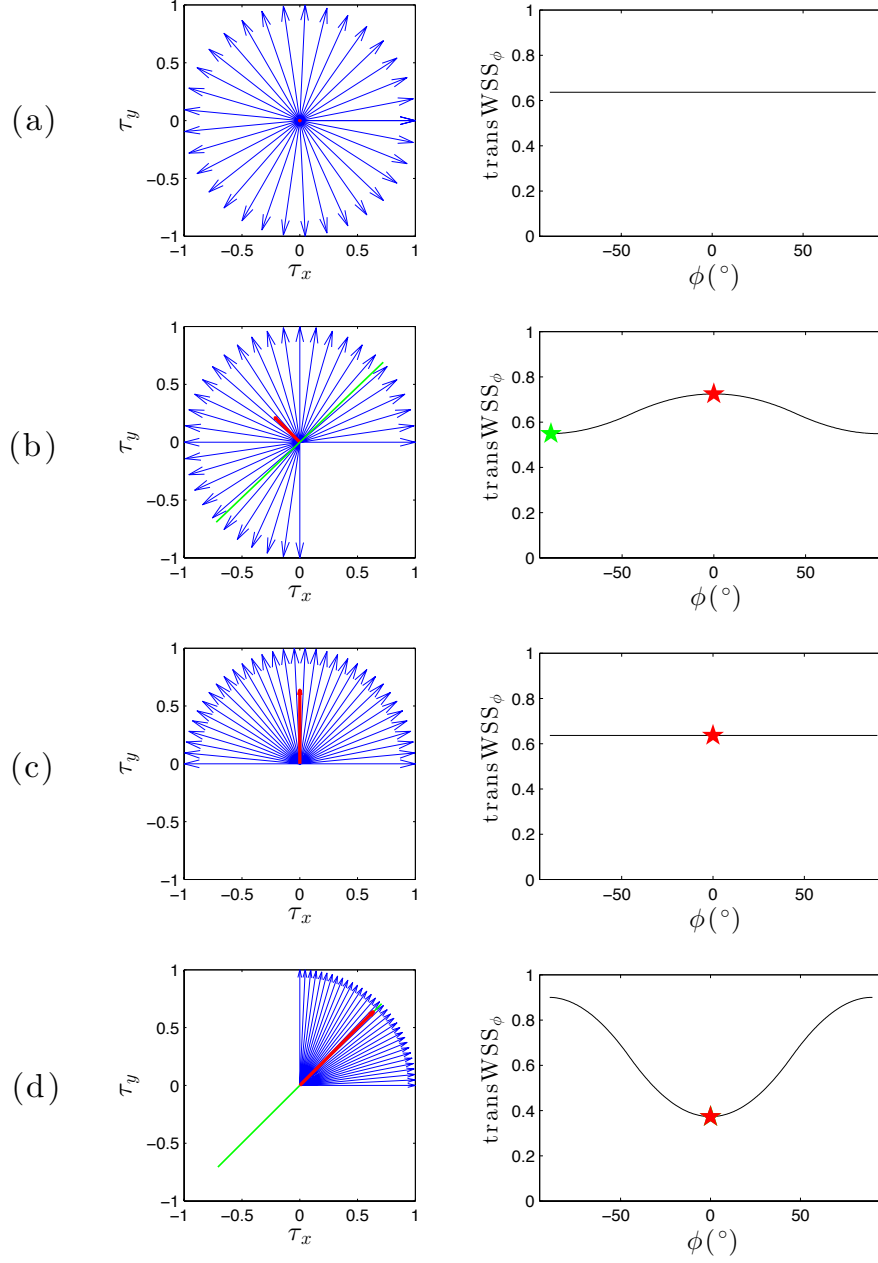


Figure 2.12: Circular (a)-(d) and elliptical (e)-(i) contours of ideal analytical τ_w distributions over a full cycle in normalised units (2.18). In the left column, blue arrows represent the τ_w distribution, the red arrow is $\bar{\tau}_w$, and the green line indicates the direction that minimises transWSS_ϕ . In the right column, the black line is transWSS_ϕ as a function of ϕ , the red and green stars indicate the position of the transWSS and transWSSmin , respectively, if defined.

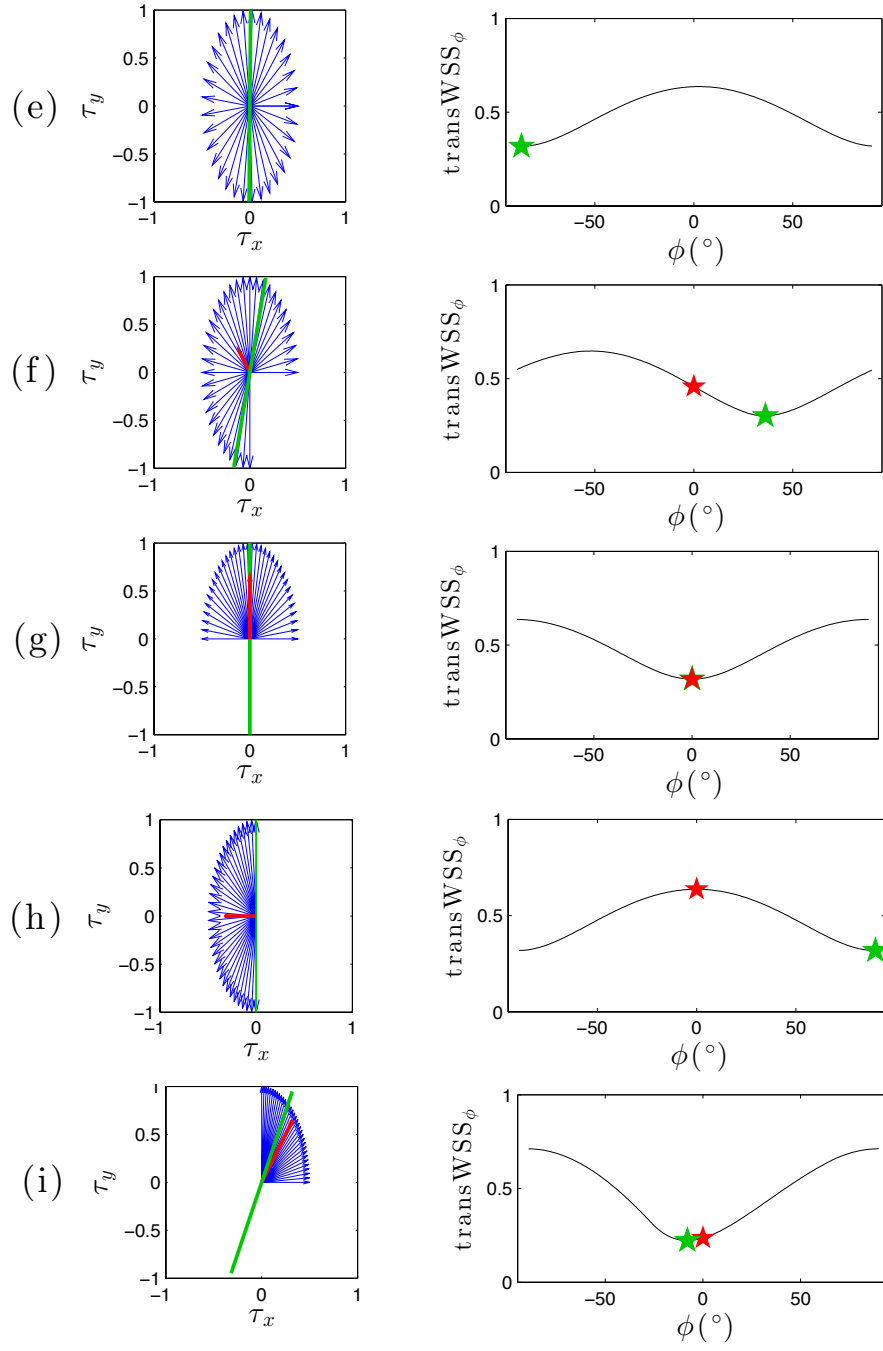


Figure 2.12: Continued.

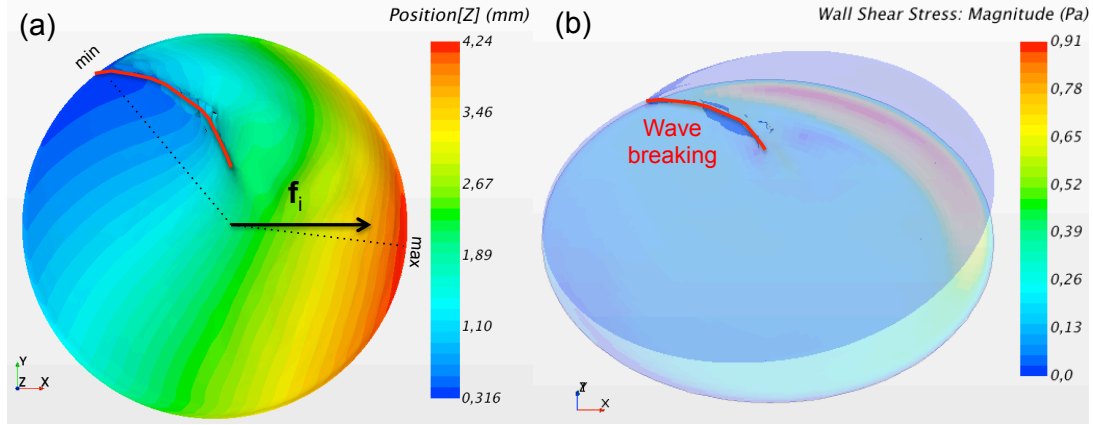


Figure 2.13: (a) Height h map showing the location of the minimum and maximum h , and the direction of the inertial force $\mathbf{f}_i$. (b) A perspective view of the bottom WSS map with a transparent free surface showing the location of the wave front (red line).

2.5 Flow Description and Wall Shear Stress Characterisation

This section describes the simulation results for the reference case given in Table 2.1. The main flow feature is a travelling wave which characterises the free surface and the underlying velocity field (Figure 2.13). The wave is single crested with a dimensionless amplitude $\Delta h/(2R) = 0.17$, where $\Delta h = \max(h) - \min(h)$, and h is the fluid surface height relative to the well bottom. The maximum of the wave is located at the edge of the well practically in the direction indicated by the inertial force, whilst the minimum height (trough) is shift 50° degrees clockwise with respect to the opposite direction of the inertial force (Figure 2.13[a]). The wave breaking in the simulation has been identified visually as the region where the surface elevation changes rapidly (see red lines in Figure 2.13). It occurs before the trough and covers a narrow curved region that extends from the edge where the minimum is located, almost to the centre of the well. As we will explore in detail in Chapter 3, wave breaking is a phenomenon that occurs when the amplitude reaches a value above a critical level; this threshold depends on the nature of the fluid problem, and we have identified $\left. \frac{\Delta h}{2R} \right|_b = 0.7 \frac{d}{R}$ for the orbital shaker. In this case, $\frac{\Delta h}{2R} = 1.35 \left. \frac{\Delta h}{2R} \right|_b$, therefore, the wave is expected to break, as the simulation clearly shows.

The wave has a periodic motion enforced by the shaker platform movement and travels around the well at the rotational speed of the orbital shaker ω . The velocity field moves in synchrony with the wave, therefore, the velocity field — but not the fluid particles — also rotates at the rotation speed ω of the shaker. As a consequence, the WSS pattern also remains constant and

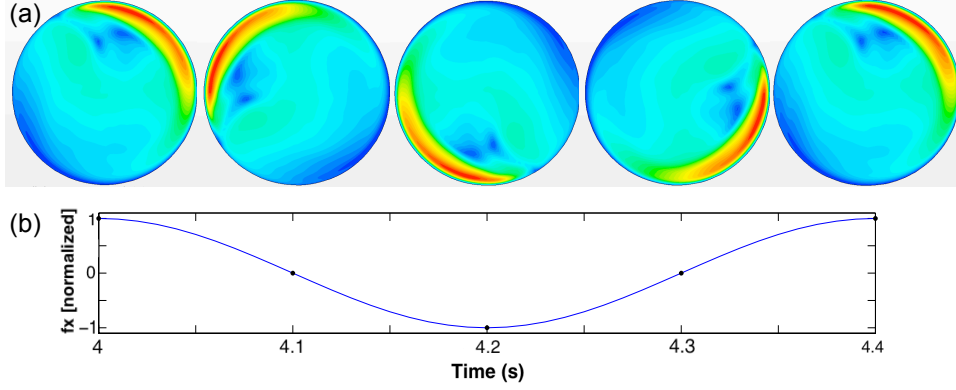


Figure 2.14: (a) WSS maps show the same pattern albeit rotated at five different time steps over the cycle. (b) Inertial force x-component f_x as a function time for a complete cycle.

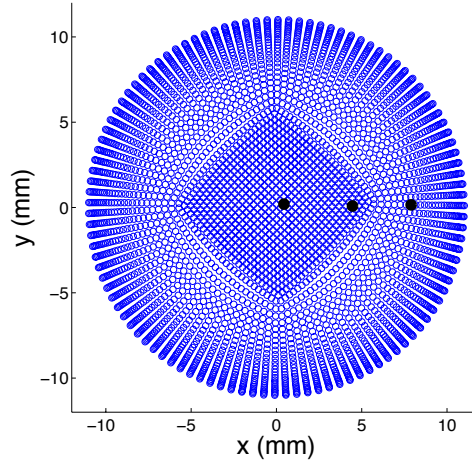


Figure 2.15: Blue marks are all the grid points of the computational domain at $z=0$. The selected points to study WSS periodicity are marked in black.

rotates in synchrony with the wave, as shown in Figure 2.14. Thus, all information about h , $\mathbf{v}$, and $\boldsymbol{\tau}_w$ is contained in a single instantaneous field.

Three points from the computational domain (Figure 2.15) have been chosen to observe the periodicity of the WSS in Figure 2.16. The WSS plots for the three sample points are displayed in rows. Columns 1 and 2 show the x and y -components of the WSS (τ_x and τ_y , respectively) as a function of time for the duration of eleven periods starting at $t = 0$; the discontinuous lines are the normalised velocity components of the well platform: x -component u is proportional to $\sin(\omega t)$ and y -component v to $\cos(\omega t)$. Column 3 shows the instantaneous WSS vector $\boldsymbol{\tau}_w$ over a cycle, what reproduces the local WSS distribution. Last, we have highlighted in red the time interval $[4.27, 4.37]$ s, the quarter of the cycle where $\boldsymbol{\tau}_w$ changes direction more rapidly. In the following, we describe the observations for each sample point:

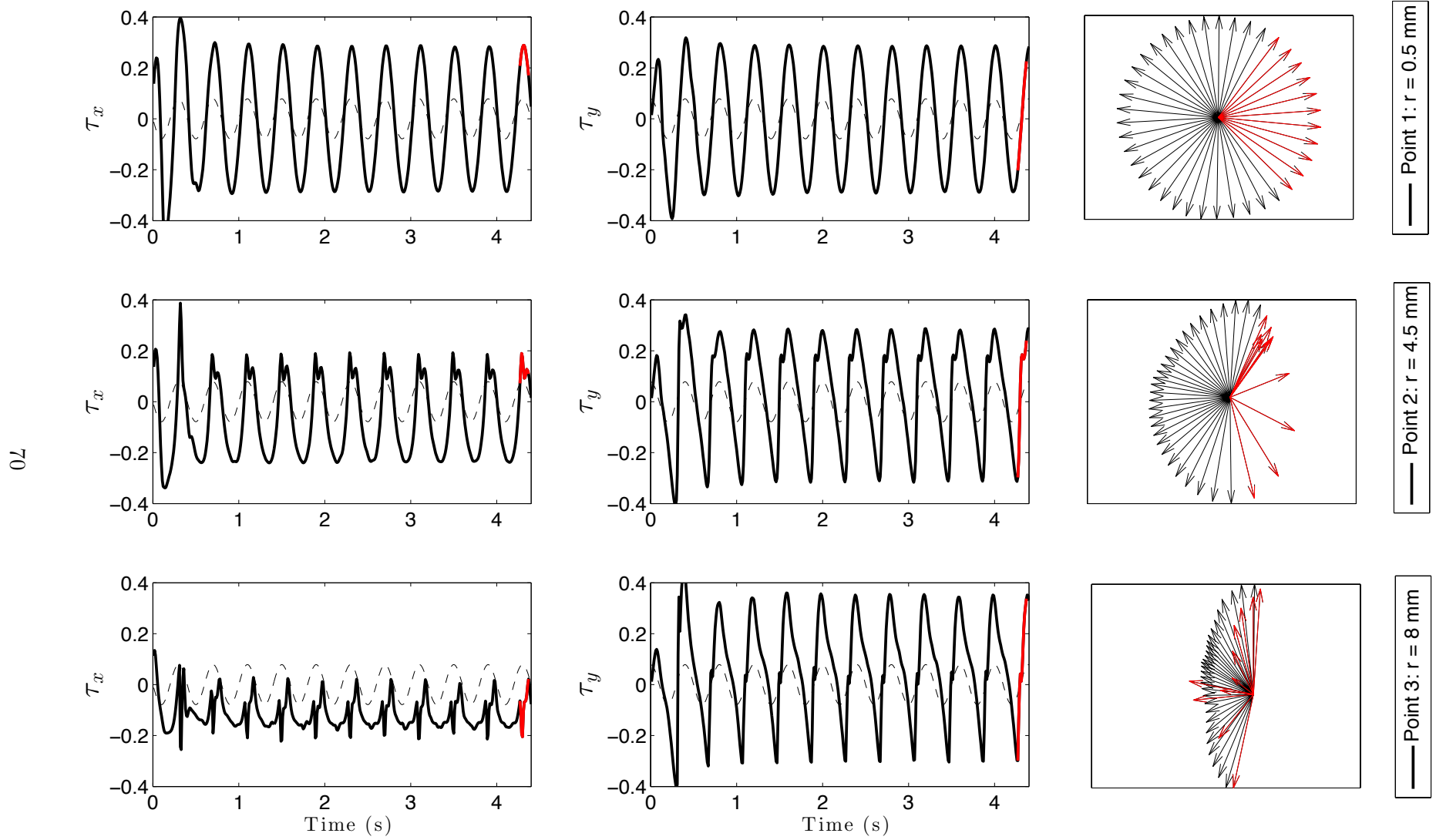


Figure 2.16: WSS distribution for the reference case (Table 2.1) at three different locations. Left column: τ_x at three different locations from the initiation of the simulation and for eleven rotations; centre column: idem for τ_y ; and right column: τ_w distributions over the cycle $t \in [4.27, 4.37]$ s. Each row shows a sample point marked in Figure 2.15. The time interval $[4.27, 4.37]$ s has been marked in red.

Point 1 (top row) represents approximately the centre of the well; at that location both WSS components follow a sinusoid curve in synchrony with the velocity of the well. As a result, the WSS distribution describes a curve close to a circle. Moving from the centre, in point 2 (middle row), the WSS curves are periodic, however, the sinusoid functions are disturbed and the amplitude of τ_x has decreased; the resultant WSS distribution is a disturbed circle and is not axisymmetric. Further towards the edge, in point 3 (bottom row), both WSS components are still periodic, but the difference between the two components is notable: τ_x remains negative for almost the whole cycle, i.e., pointing to the centre of the well, while τ_y still oscillates to both sides, with slightly higher positive values than in point 2. These disturbances occur mainly over the interval of the cycle marked in red. The three WSS distributions analysed are multidirectional, however, the τ_w patterns are manifestly different; this is due to the confinement of the liquid and the occurrence of the breaking wave.

A crucial result of the orbital shaker flow is the periodicity of the WSS vectors, which implies that all points in the domain at the same radial distance from the centre have equal WSS distribution over a cycle, albeit with shifted phases, i.e., the movement is axisymmetric over a period of time with respect to the well's axis of symmetry. Therefore, the WSS components referred to as x and y in Figure 2.16 are equivalent to the radial τ_r and tangential τ_θ components, respectively, at different locations of the well at equal radial distances (disregarding the phase shift). Furthermore, this result implies that integrating in time over a full cycle is equivalent to integrating in the θ coordinate for 2π radians, using polar coordinates in both cases.

Under wave breaking, a bifurcation line originates where τ_w changes direction more rapidly. This bifurcation line (red line in Figure 2.17) is observed clearly where the τ_w “streamlines” converge. (The τ_w streamlines refer to the lines tangent to the τ_w vector by analogy with the velocity streamlines). A second bifurcation line is also observed approximately perpendicular to the former (red discontinuous line). These lines can also be identified in an instantaneous plot of the τ_w field at the well bottom (Figure 2.18): the arrows show the τ_w vectors; the three circles indicate the radial distance of the points plotted in Figure 2.16; the thick red lines depict the bifurcation lines; and the thin red lines indicate the interval [4.27,4.37] s. In Figure 2.16, the τ_w vectors in the interval [4.27,4.37] s change more rapidly, showing that the breaking wave is the main cause of the skewness and variability of the profiles. A detailed study of the fluid

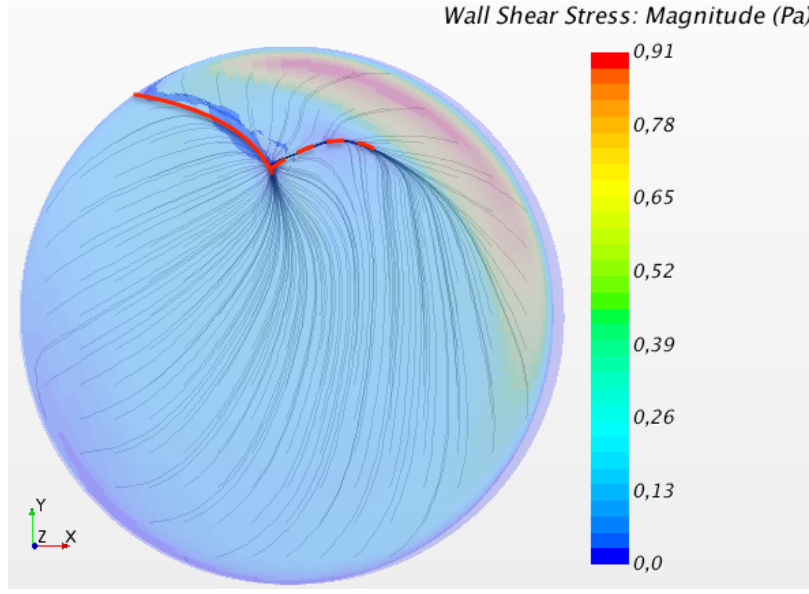


Figure 2.17: Bottom WSS map with τ_w “streamlines” superimposed. The bifurcation lines are sketched in red.

structure that results in the bifurcation line is beyond the scope of this thesis. We note from Salek et al. [116] that a semi-torus vortex develops in the bulk of the fluid, directing the flow from the free surface along the side walls to the bottom wall, giving rise to the bifurcation line where the streamlines converge; and from Weheliye et al. [142], we note the identification of two different secondary motion regimes with a transition region.

Figure 2.19 shows the WSS distributions along the radial distance at the cycle [4,4.4) s; for each distribution, the direction of the temporal mean WSS and the direction that minimises transverse WSS have been plotted. The distributions observed can be compared with the analytical distributions studied in § 2.4.6. In the centre ($r < 3$ mm), the distributions present a quasi-circular profile, as in Figure 2.12(a); in these cases, there is no predominant direction ($\bar{\tau}_w$ is undefined) and the transverse flow is the same in all directions (transWSSmin is undefined). Nevertheless, the fact that the distributions are not perfectly circular yields a result for the directions, as plotted, but they are not relevant in this radial interval. At the very centre, the distribution is perfectly axisymmetric, based on the periodicity of the movement. Distributions at 3–7 mm resemble Figure 2.12(b) or (f), where the direction of $\bar{\tau}_w$ and the minimised transverse WSS differ from 40° to 90° . Distributions at 7–8.5 mm resemble Figure 2.12(h), where $\bar{\tau}_w$ and the minimised transWSS are perpendicular. In the interval 8.5–11 mm, the distributions evolve into a quasi-unidirectional reversing profile, to which the closest analytical profile would

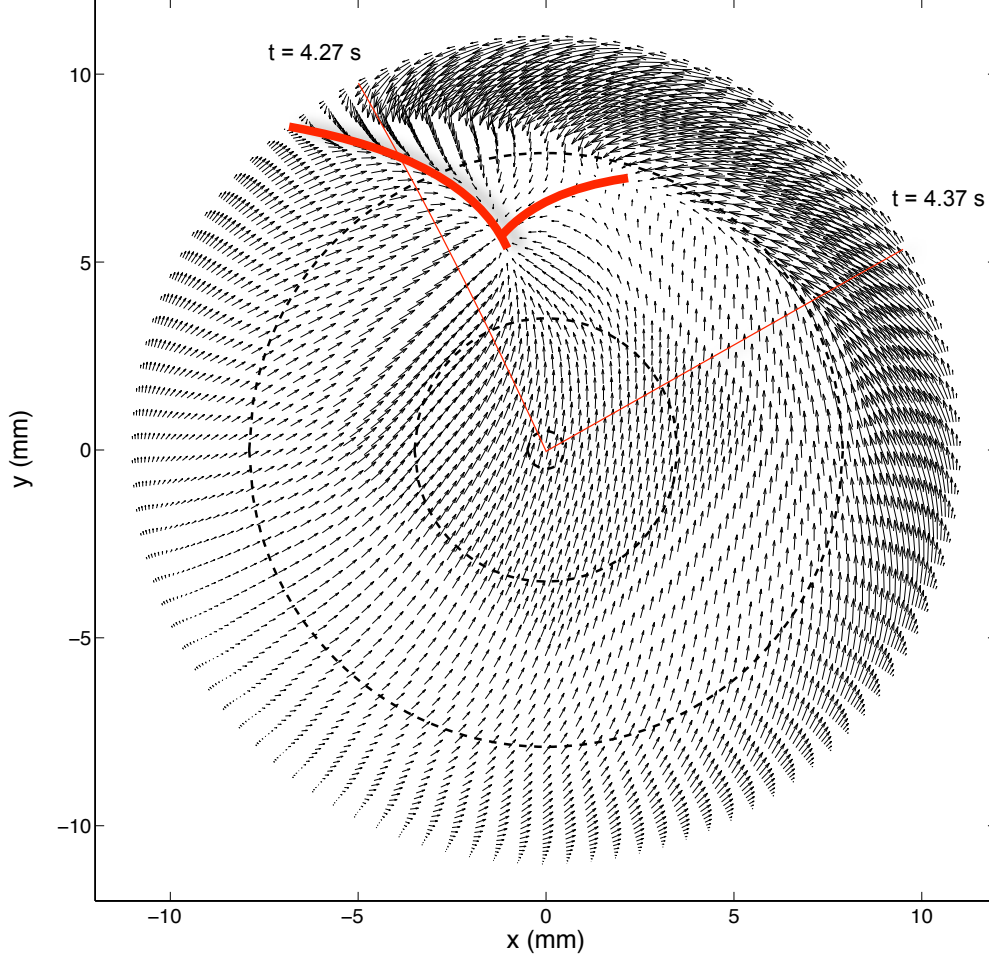


Figure 2.18: τ_w field at the well bottom. The black circles indicate 0.5, 3.5 and 7.9 mm radial distances, and the red curve is the sketched bifurcation line.

be Figure 2.12(e), where $\bar{\tau}_w$ and the minimised transWSS fall in the same longitudinal direction. The values of transWSS and transWSSmin also differ along the radial distance, as shown in Figure 2.20. In the centre, transWSS_ϕ is approximately constant for all ϕ , thus transWSS and transWSSmin are practically equal, in agreement with the quasi-circular distributions observed in this radial interval. Interestingly, $\text{transWSS} = \max(\text{transWSS}_\phi)$ in the radial interval 2–7 mm. Closer to the edge, transWSS_ϕ shows a large variability, while the tendency of transWSS and transWSSmin are rather similar. These plots demonstrate the multiple τ_w distributions — from axisymmetric-multidirectional, to asymmetric-multidirectional and quasi-unidirectional reversing — offered by the orbital shaker simultaneously in a single cell culture environment, in which the differences between the transverse metrics are not negligible.

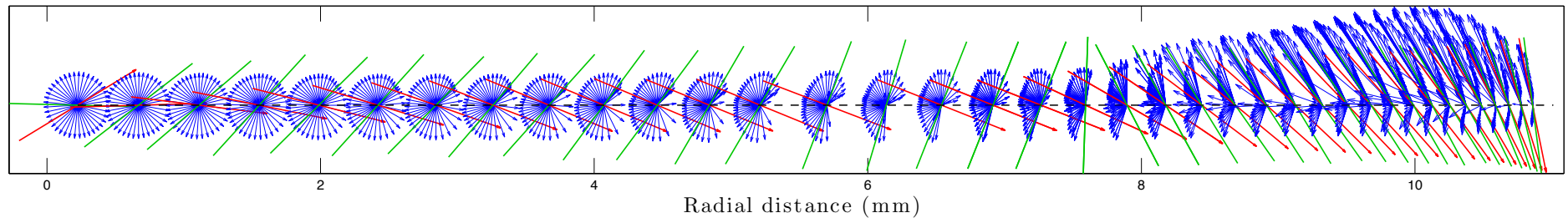


Figure 2.19: WSS distributions along the radial direction (blue arrows) with corresponding temporal mean WSS $\bar{\tau}_w$ (red arrows) and the direction-minimising transverse WSS (green line).

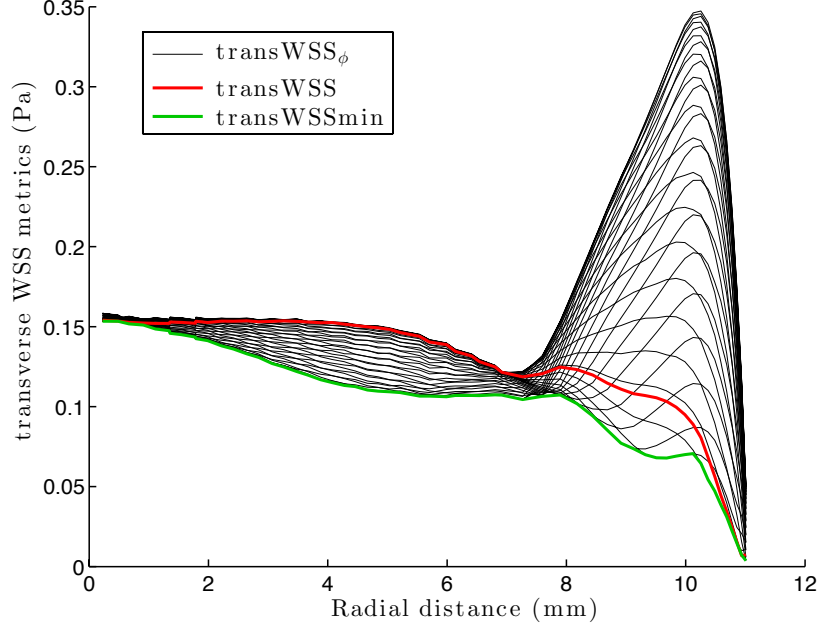


Figure 2.20: Transverse WSS metrics described in §§ 2.4.4 and 2.4.5 along the radial distance.

Figure 2.21 is known as a polar plot, which is a different way of presenting the τ_w distributions of Figure 2.19. Each colour in the graph represents a different radius, and each point on the curve represents the tip of a vector plotted in equal time steps and connected following the cycle. The advantage of the polar plot is that τ_w distributions at different radii can be superimposed, making it easier to compare. In Figure 2.21, the distributions can be observed to vary from the centre to the edge, in agreement with the analysis of Figures 2.16 and 2.19. There is no preferred direction at the centre of the well: τ_w rotates spending equal time in each direction, and with equal magnitude at each time (circular distribution). With increasing distance from the well centre, the influence of the liquid confinement becomes noticeable, and τ_w changes direction rapidly as the breaking wave travels through (bifurcation line jump). Nearer to the edge, the wall forces the vectors to align in the tangential direction, becoming close to a uniaxial distribution.

The τ_w magnitude can be examined over one cycle for each radial distance from the well centre, which, given the periodicity of the WSS vector demonstrated, is equivalent to plotting WSS against the azimuthal coordinate over a 2π -interval. Figure 2.22(a) shows WSS curves for different distances; the arrows connect the different radii to the circle of the same radius in Figure 2.22(b), which shows the WSS map truncated according to the limited ability of the

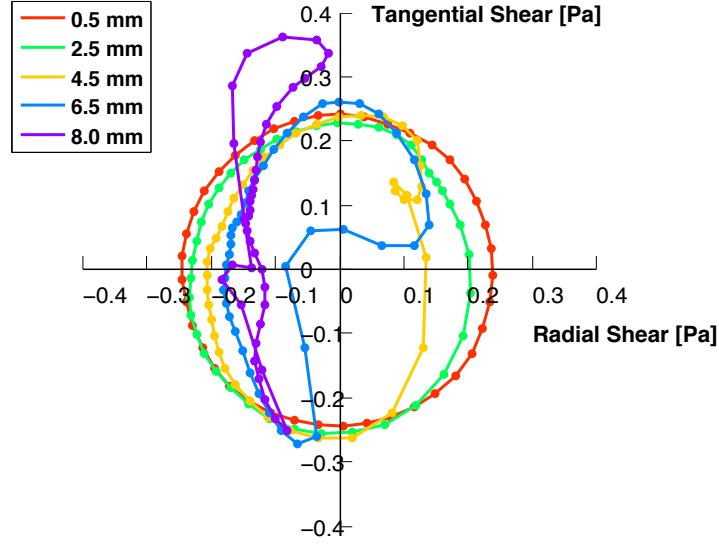


Figure 2.21: Polar plot of magnitude and direction of the instantaneous WSS vector during one cycle. Each colour in the graph represents a different radius, and each point on the curve represents the tip of a vector plotted in equal time steps and connected following the cycle.

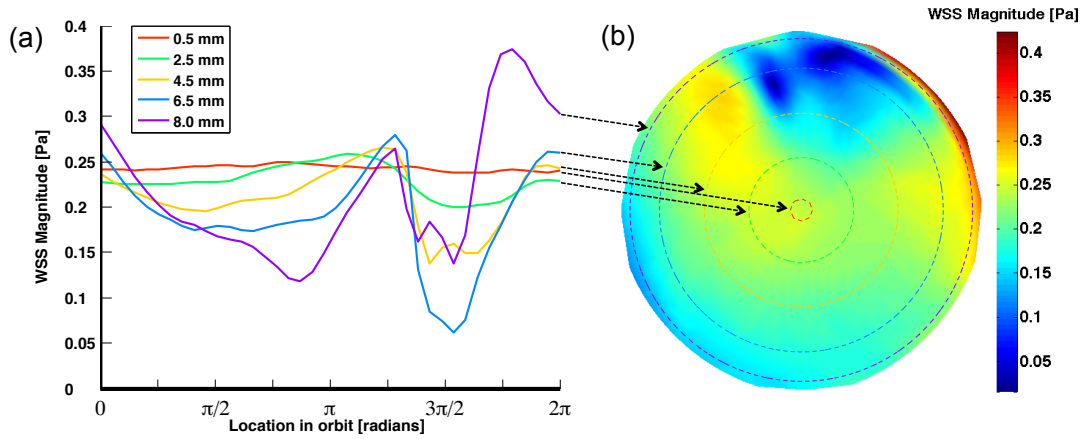


Figure 2.22: (a) WSS magnitude for different radii throughout one cycle (2π radians). The arrows indicate the radial position for each curve. (b) Map of the instantaneous wall shear stress (WSS) magnitude acting on the base of the well. The radius of the map has been truncated so that only the area of the well covered by the scanning plate reader is shown.

scanning plate reader used to measure fluorescence levels in the laboratory experiments, which does not cover the region close to the well side walls. Again, the temporal pattern of WSS can be observed to change upon moving from the centre to the edge of the computational domain. At the centre, the magnitude is approximately constant over time, but a double-peaked oscillation with increasing maximum is observed further away from the centre, which coincides with the location of the bifurcation lines depicted in Figure 2.17.

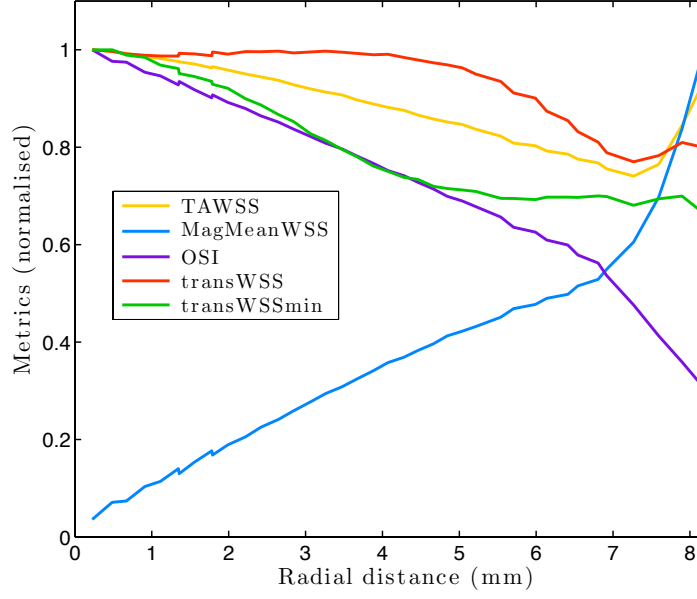


Figure 2.23: WSS metrics described in § 2.4 for the reference case (Table 2.1).

Finally, the five WSS metrics described in § 2.4 are plotted in Figure 2.23 as a function of the radial distance r up to radius covered by the scanning plate reader used in the laboratory experiments. TAWSS decreases with r up to 7.5 mm, coinciding with the second bifurcation line, where WSS reaches a local minimum. MagMeanWSS increases with r from a value close to zero (the metric cannot be computed at the exact centre of the well, where $\text{MagMeanWSS} = 0$, because there is no computational point with $x = y = 0$). Figure 2.19 shows that the distribution shape changes gradually with r from a circular to a non-axisymmetric profile, therefore, MagMeanWSS increases gradually but with a change in its tendency at $r \sim 7\text{mm}$. OSI decreases with r since by definition its tendency is inverse to MagMeanWSS and TAWSS^{-1} , therefore, it also shows a change in the tendency at $r \sim 7\text{mm}$. TransWSS shows a flat profile up to $r \sim 5\text{ mm}$, then decreases at 5–7.3 mm, and again remains flat from 7.3 mm up to the plotted radius. In contrast, transWSSmin decreases with r up to $r \sim 5\text{ mm}$, and then remains practically constant.

2.6 Macromolecular Transport: Cell Permeability and Alignment

The flow results were used to assess the effect of flow on transendothelial macromolecular transport in orbital shaker experiments. All the laboratory experimental work and post-processing was carried out by Dr. Mean Ghim as part of his PhD project [49]. The case explored is the reference case, and this section includes from Dr. Mean Ghim’s work the following results: permeability maps in Figure 2.24, permeability histograms in Figure 2.25, and experimental cellular alignment values in Figure 2.26; for further details and results, see Ghim et al. [48].

In the experiments, the transendothelial transport of three macromolecules of different sizes was assessed in the reference case under static ($\omega = 0$) and shear conditions for seven days each (chronic conditions). The method used to assess molecular transport is based on Dubrovskiy et al. [38] and its description is beyond the scope of this work. Briefly, monolayers of endothelial cells are grown on top of a biotinylated gelatin in twelve-well plates, and covered with a medium containing the tracers. The tracers all consist of avidin (a biotin-binding protein) and a fluorescent label of varying size. When the tracers cross the endothelium, they bind to the substrate, and can be visualised by en face confocal microscopy, or measured by a fluorimetric plate reader. This method allows for visualization of the cell morphology, identification of the transport pathways and measurement of transendothelial permeability. Here, the discussion of macromolecular transport focuses on the observation of the change in permeability and cell orientation due to the flow conditions. The experimental configuration is summarised in Table 2.1 and the resultant flow and WSS metrics are described in § 2.5.

Figure 2.24 shows permeability maps in arbitrary (but self-consistent) relative fluorescence units (RFU) — this unit is related to the fluorescence of the tracer used to detect the macromolecules. The macromolecules analysed have increasing sizes, comparable to those of albumin, high-density lipoprotein (HDL), and LDL and are termed small, medium, and large macromolecules for clarity. For the static condition (Figure 2.24[a,c,e]), permeability presents a practically uniform pattern for the three macromolecules. For the sheared maps (Figure 2.24[b,d,f]), permeability presents a fairly axisymmetric pattern in the small and medium macromolecules, increasing at the centre of the well and decreasing towards the edge; for the large macromolecule, permeability decreased nearly uniformly across the well.

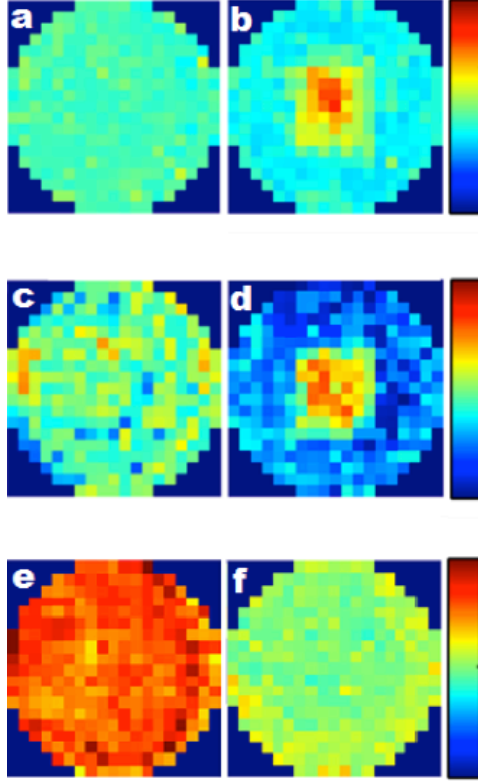


Figure 2.24: Maps of permeability for the small ([a] static, [b] shear), medium ([c] static, [d] shear) and large ([e] static, [f] shear) macromolecules. Intensity values are in arbitrary but self-consistent units.

The axisymmetric character of the results is in agreement with the flow periodicity demonstrated in § 2.5. Thus, permeability has been plotted in a histogram taking the average value over concentric rings of approximately 1 mm radial thickness (Figure 2.25). The relative increment in the permeability at the centre region was greater for the small macromolecule than the medium macromolecule, however, flow had an overall greater relative effect on the permeability of the medium size macromolecule. For the medium macromolecule, permeability decreased by almost half from 1.8–2.8 mm to the edge. For the small macromolecule, permeability decreased slightly from 2.8–3.7 mm to the edge. For the large macromolecule, the permeability decreased significantly and nearly uniformly at all radii.

The effect of shear on permeability along the radial distance can be compared to the local WSS distribution using the WSS metrics described in § 2.4 and computed in Figure 2.23. Results are truncated according to the limited ability of the scanning plate reader, which cannot measure fluorescence close to the well side walls. TAWSS, OSI, transWSS, transWSSmin, and the inverse

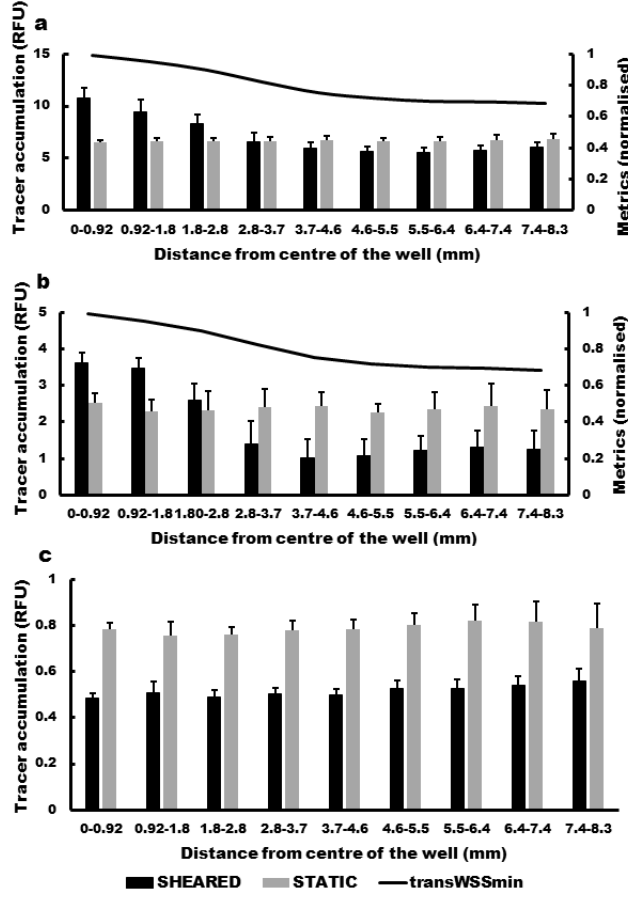


Figure 2.25: Histogram showing permeability for the small (a), medium (b) and large (c) macromolecules. The plotted metric transWSSmin is the one that shows the best correlation out of the five explored metrics.

of MagMeanWSS showed a similar radial trend to the permeability in the cases of the small and medium macromolecules, however, transWSSmin showed the best correlation (correlation coefficients were $r = 0.97$ and $r = 0.94$ for the small and medium macromolecules, respectively). For the large macromolecule, none of the metrics matched the flat permeability profile. In Ghim et al. [48], we explain how each of the three macromolecules uses a different transendothelial transport pathway; the small and medium macromolecules are transported through paracellular routes (inter-cell junctions), while the large macromolecules appear to be transported across the cells perhaps via some vesicular process. A possible explanation of the uniform reduction in permeability of the large macromolecule is that cells exposed to a certain flow condition release a solute mediator that spreads uniformly in the medium, causing the same effect on all cells simultaneously independently of the flow they are experiencing locally.

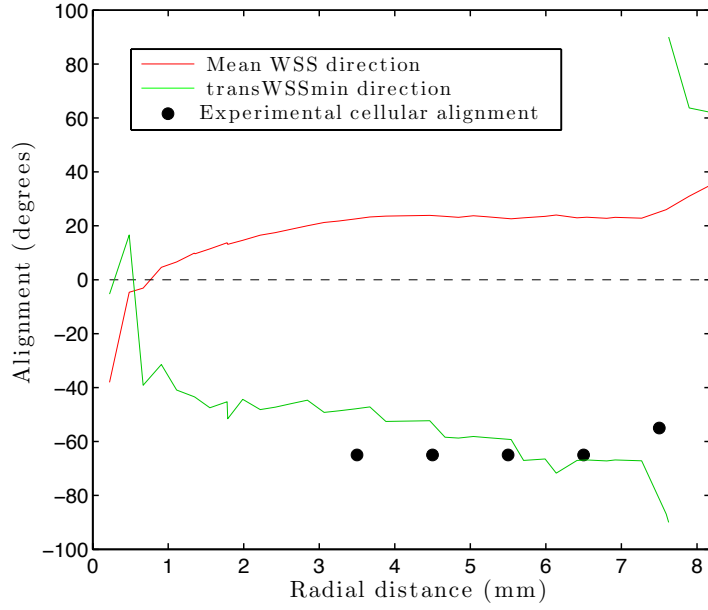


Figure 2.26: Cells alignment. Data show the direction of the temporal mean WSS $\bar{\tau}_w$, the minimising transverse WSS, and the observed cells' orientations. These angles are defined positive clockwise from the radial axis — line connecting the centre of the well to cell or the computational point — angles between -90 and 90 degrees. (Data from the three locations nearest the centre of the well were excluded since cells showed random orientations).

The biological significance of transWSSmin involves the hypothesis of cells aligning in the direction that minimises the transverse WSS (§ 2.4.5). The cell orientation has been investigated to test the hypothesis opened up by the significant correlation between permeability and transWSSmin. To determine cell orientation, an ellipse was fitted to the cell shape, and its long axis assumed to be the cell orientation. The direction connecting the centre of the well with the ellipse centroid was used to measure the angle in polar coordinates. Under static conditions (data not shown), cells were mainly randomly orientated although there was a tendency to gradually align in the circumferential direction (along the edge). Under shaken conditions, cells were randomly orientated up to 3 mm, and orientated in a direction about 65° for the rest of the scanned area. These observations and the results from the directions of the temporal mean WSS and the minimum transverse WSS (Figure 2.19) are plotted in angles in Figure 2.26. The absence of a significant cell orientation at 0–3 mm coincide with the absence of a direction that minimises transWSS or a predominant WSS direction, as described in § 2.5. In the rest of the well, cells appear to be aligned in the direction that minimises transWSS, differing about 90° from the direction of the temporal mean WSS $\bar{\tau}_w$.

2.7 Conclusions

In this chapter, we have established the orbital shaker fluid dynamics model that will be used throughout this thesis. In the model, the liquid is a shallow fluid layer confined by a cylindrical container; the interface is a free surface where the surface tension has been neglected; the flow is regarded from a non-inertial frame of reference where the well remains static and the orbital translation is represented by an inertial force. Furthermore, we have described the computational tool in which the model has been implemented. We have used the commercial CFD software STAR-CCM+ with a cylindrical block-structured mesh with 291,600 computational cells, and the VOF method to simulate both the gas and liquid phases.

We presented a novel WSS metric: the transWSSmin. A detailed description of the mathematical formulation and the biological significance of transWSSmin and four other established metrics (TAWSS, MagMeanWSS, OSI, and transWSS) was provided. The transWSSmin metric is a variation of the transWSS metric formulation [104] considering that cells align in the direction that minimises the transverse WSS instead of the direction of $\boldsymbol{\tau}_w$ temporal mean. A set of analytical WSS distributions demonstrated that the direction that minimised the transverse WSS and the temporal mean are generally different, thus, yielding different values of transWSS and transWSSmin and allowing for a new interpretation of cell orientation.

The flow in the reference case was studied in detail using the computational method. After the transients, we observed a periodic wave travelling around the well at the speed of rotation of the orbital shaker, causing the WSS vector field to rotate synchronously; as a consequence, all points in the domain at the same radial distance from the centre have equal WSS distributions over a cycle, albeit with shifted phases. Wave breaking occurred at the free surface, which caused a rapid change in the WSS directionality in the well bottom. WSS varied in magnitude and direction: near the centre, the WSS magnitude was practically constant over the cycle with an axisymmetric WSS distribution. Closer to the edge, the WSS magnitude featured a local minimum-to-maximum change when crossing the bifurcation line, and the distribution became asymmetric with a predominant tangential direction. Lastly, the five metrics studied were plotted as a function of the radial distance, presenting a general decreasing trend towards the edge with the exception of the MagMeanWSS which has an increasing trend.

Finally, we showed experimental evidence consistent with the influence of flow on the endothelial permeability to three differently sized macromolecules using an orbital shaker device. The flow has a different effect on the transendothelial transport of each of the macromolecules studied, which also changes gradually from centre to edge for the small and medium-sized macromolecules. Examination of endothelial cell orientation showed that the long axis of the cells was not aligned with the direction of the temporal mean WSS — as widely accepted [36, 40, 44, 75, 76] — but instead, cells align close to the direction that minimises the forces acting across the long axis, i.e., the direction that minimises transverse WSS. The transWSSmin metric, designed specifically to quantify this direction and the average transverse WSS experienced with respect to that direction, was strongly correlated to the radial change of permeability of the small and medium sized macromolecules, while the transport of the large macromolecule appears to be influenced by other factors not identified in this work.

Chapter 3

Wave-Breaking Regime Classification

3.1 Introduction

In orbital shaker studies that focus on the fluid dynamics [116, 111], there is agreement on the need for a systematic approach to characterise the flow regimes, and determine how the different operating parameters influence shear stress, mixing and aeration conditions needed in the different applications where this flow motion is used [4, 149, 81, 69]. There are some thorough parametric studies regarding mixing [128], power input [67] and energy dissipation rates [8], the latter also including a study of oxygen transfer rates. Other studies characterise the free surface and the velocity fields: Kim and Kizito [66] remark on the importance of the secondary flow in the mixing process, and connect the height aspect ratio to the free surface deformation and the mean velocity; Weheliye et al. [142] describe this secondary flow as counter-rotating vortices, observing different vortical structures in different operating regimes, and derive various dimension scaling laws for the height amplitude; and Reclari et al. [111] study the occurrence of wave breaking in deep cylindrical containers, and investigate mixing and aerations using particle trajectories as proxies.

An understanding of flow regimes starts with the identification of appropriate dimensionless groups. Restricting attention to cylindrical containers, as in the case of the well plates used in

endothelial experiments, and ignoring surface tension effects, the problem has seven parameters (§ 2.2): the fluid height d , the well radius R , radius a and speed ω of rotation of the orbital shaker, the fluid's density ρ , the fluid's dynamic viscosity μ (or, equivalently, the kinematic viscosity $\nu = \mu/\rho$), and the gravitational acceleration g . Taking guidance from inviscid solutions [59, 66, 111] (see § 3.2 for details), four independent dimensionless groups can be identified:

$$F = \frac{a\omega^2}{g} \quad E = \frac{a}{R} \quad \Gamma = \frac{d}{R} \quad Re = \frac{R^2\omega}{\nu}. \quad (3.1)$$

Here, F is the dimensionless forcing, E represents the eccentricity, Γ is the shallowness of the fluid layer and Re is the Reynolds number. Table 3.1 shows the value of the dimensionless groups F , E , Γ , and Re for a large number of experimental, computational, and combined orbital shaker studies, in applications including endothelial cells, bacterial biofilms, cells in suspension, stem cells, protein degradation, mixing, and aeration. In the endothelial cell studies, Γ is constrained by the availability of standard well sizes and the restrictions on the fluid height needed for aeration, operating typically in shallow water conditions ($\Gamma \ll 1$). Other applications explore multiple size containers for which Γ has a wider variability, especially in applications of cells in suspension, where there is an interest in scaling-up the problem to process larger volumes and no restriction on the fluid height is imposed. The dimensionless forcing F can be varied over a continuum because orbital shakers allow tuning of the frequency over a given range. At high forcing, the well bottom can become exposed or shear can attain too high a level, which can damage the cells and proteins or prevent the cells from attaching to the wall, thereby placing an upper limit on the value of F . The eccentricity E typically takes discrete values because experiments are carried out in commercial orbital shakers that have a fixed orbital forcing radius a , with some exceptions [67, 142, 111]. The Reynolds number Re typically is low in endothelial cell, protein degradation and stem cell applications ($Re < 10^4$), but increases notably in cells in suspension and mixing where larger containers are used. Table 3.1 shows that there is an enormous spread in the dimensionless parameters used for the different applications.

The aim of this chapter is to provide insight into the flow regimes that occur in orbital shakers with cylindrical containers at small Γ , i.e., shallow fluid layers. Specifically, the regimes will be identified by carrying out a large number of numerical simulations at $\Gamma = 0.18$. We will show that for the portion of the parameter space covered, the effect of Re on velocity and surface

elevation is limited, and the focus will be on analysing the E - F parameter space. In classifying the regimes, it turns out that the potential flow theory — despite not including any viscous effects — is a powerful tool that can be used to predict the liquid height amplitude and the onset of breaking.

The chapter is organised as follows. In Section 3.2, the potential flow theory is introduced. Section 3.3 contains details of the numerical simulations and a description of the 33 case studies. Section 3.4 explores the applicability of the inviscid potential flow solutions to the (viscid) simulations, and it will be demonstrated that the theory predicts the surface elevations excellently and the velocities reasonably for low-forcing cases. In Section 3.5, the validity of the potential theory to predict height amplitude and phase shift is explored at higher forcing and for a wide range of E . In Section 3.6, the effect of viscosity (Re) — not considered in the potential theory — is shown to be small. In Section 3.7, all case studies are systematically analysed as a function of E and F , and a criterion for the onset of breaking is identified. Finally, in Section 3.8, the operating regimes of the endothelial cell experiments considered in Table 3.1 are observed in the E - F parameter space. Concluding remarks are made in Section 3.9.

Type	Reference	R (cm)	d (cm)	a (cm)	f (rpm)	F	E	Γ	Re	Application	Other
E	Ley et al. [77]	0.78-1.75	0.21-0.26	0.43	50-150	0.01-0.11	0.25-0.55	0.12-0.34	42-641	EC	
E	Kraiss et al. [70]	1.75	ND	1.4	200	0.63	0.8	ND	855	EC	[71] [151] [53]
E	Dardik et al. [31]	1.75	0.21	0.95	60-210	0.04-0.48	0.56	0.12	1924-6735	EC	[3] [65]
CE	Warboys et al. [140]	1.2	0.33	0.5	150	0.13	0.42	0.28	2262	EC	[108]
C	Thomas et al. [126]	Same values as Dardik et al. [31] but computational									
CE	Chakraborty et al. [24]	2.5	0.2	0.95	50-150	0.03-0.24	0.38	0.08	3272-9817	EC	[25]
C	Salek et al. [116]	1.75	0.2-0.4	0.95	100-200	0.11-0.42	0.54	0.12-0.24	3207-6414	EC, BF	[69](E)
CE	Warboys et al. [141]	1.75	0.31	1.0	210	0.49	0.57	0.18	6735	EC	
CE	Filipovic et al. [43]	1.75	0.21	3.8	100-200	0.42-1.7	2.16	0.12	3207-6414	EC	
CE	Velasco et al. [133]	1.75	0.2	0.95	120	0.15	0.54	0.11	3848	EC	
E	Gardner and Tatterson [47]	2.3	1.5-4.5	0.95	0-90	$\sim 0-0.09$	0.41	0.56-1.96	0-5000	MX	
CE	Kim and Kizito [66]	4.35	2-4	2	60	0.08	0.46	0.46-0.92	743	MX	
CE	Zhang et al. [152]	8.6	5.4-12.9	5	110	0.68	0.58	0.75-1.5	85196	CS	
E	Tissot et al. [128]	5-1.4	5-2.8	1.25-2.5	80-140	0.11-0.55	0.09-0.5	0.62-2.55	26179-301898	CS, MX	
CE	Barrett et al. [8]	0.781	0.4-1	2	120-250	0.32-1.4	2.56	0.53-1.34	766-1597	CS, MX	
CE	Bai et al. [4]	0.7275	0.6	0.8	100-300	0.09-0.8	1.1	0.83	554-1663	PD	
E	Klöckner et al. [67]	12.5	20.4	2.5-10	40-160	0.02-1.43	0.1-0.4	1.63	65450-261799	CS	
C	Discacciati et al. [37]	7.2	10	2.5	85-125	0.2-0.44	0.35	1.39	46144-67858	CS	
E	Weheliye et al. [142]	5-6.5	3-7	1.5-5	60-140	0.03-0.55	0.12-0.5	0.46-1.4	15700-61900	MX	
E	Lim et al. [80]	1.75	ND	0.95	5-40	$\sim 0-0.02$	0.54	ND	160-1283	SC	
E	Reclari et al. [111]	7.2-14.35	4.5-15	0.2-6.5	20-200	0.001-2.91	0.01-0.9	0.31-2.08	10857-431283	MX, AER	

Table 3.1: Overview of the orbital shaker studies reviewed in the literature (only cylindrical containers considered). Notation used: E=experimental, C=computational, EC=endothelial cells, ND=no data, SC=stem cells, BF=bacterial biofilms, CS=cells in suspension, MX=mixing, PD=protein degradation, AER=aeration.

3.2 Potential Flow Theory

Analytical predictions of the surface elevation and the velocity field can be obtained by assuming that (1) the liquid phase is incompressible and irrotational; (2) the boundary conditions on the container wall are stress-free; and (3) the kinematic boundary condition at the free surface can be linearised [59, 66, 111]. By virtue of irrotationality, a flow potential Φ can be introduced which is linked to the velocity field by $\mathbf{v} = \nabla\Phi$. The continuity equation (2.1) in an incompressible flow thus reduces to the Laplace equation $\nabla^2\Phi = 0$. The Laplace equation can be solved using separation of variables to obtain a solution of the potential Φ , from which the surface elevation ζ can be derived using the boundary conditions, resulting in

$$\Phi(r, \theta, z, t) = -a\omega \sin(\omega t - \theta) \sum_{n=1}^{\infty} \left[\frac{2R}{(\epsilon_{1n}^2 - 1)} \frac{\omega^2}{(\omega_{1n}^2 - \omega^2)} \frac{J_1(\lambda_{1n}r)}{J_1(\epsilon_{1n})} \frac{\cosh[\lambda_{1n}(z)]}{\cosh(\lambda_{1n}d)} \right] \quad (3.2)$$

$$\zeta(r, \theta, t) = \frac{a\omega^2}{g} \cos(\omega t - \theta) \left[r + \sum_{n=1}^{\infty} \frac{2R}{(\epsilon_{1n}^2 - 1)} \frac{\omega^2}{(\omega_{1n}^2 - \omega^2)} \frac{J_1(\lambda_{1n}r)}{J_1(\epsilon_{1n})} \right] \quad (3.3)$$

where Φ is the flow potential; ζ is the surface elevation relative to the static height d ; r , θ , and z are cylindrical coordinates; $J_1(r)$ is the Bessel function of the first kind, of order 1; ϵ_{1n} is the n th root of $J'_1(s)$ (where the prime denotes differentiation with respect to the argument); $\lambda_{1n} = \epsilon_{1n}/R$; and ω_{1n} is the natural frequency of the mode (1,n) given by the dispersion relationship $\omega_{1n}^2 = g\lambda_{1n} \tanh(\lambda_{1n}d)$. The derivation of this solution is explained in detail in Appendix A.

The solution (3.2)-(3.3) and the dispersion relationship can be made dimensionless by a change of variables $\tilde{r} = r/R$, $\tilde{z} = z/R$ and $\tilde{t} = \omega t$, leading to

$$\frac{\zeta(\tilde{r}, \theta, \tilde{t})}{2R} = F \cos(\tilde{t} - \theta) \left[\tilde{r} + \sum_{n=1}^{\infty} \frac{1}{(\epsilon_{1n}^2 - 1)} \frac{F}{E\epsilon_{1n} \tanh(\epsilon_{1n}\Gamma) - F} \frac{J_1(\epsilon_{1n}\tilde{r})}{J_1(\epsilon_{1n})} \right] \quad (3.4)$$

$$\begin{aligned} \frac{u_r(\tilde{r}, \theta, \tilde{z}, \tilde{t})}{a\omega} &= -2 \sin(\tilde{t} - \theta) \sum_{n=1}^{\infty} \left[\frac{\epsilon_{1n}}{(\epsilon_{1n}^2 - 1)} \frac{F}{E\epsilon_{1n} \tanh(\epsilon_{1n}\Gamma) - F} \frac{J'_1(\epsilon_{1n}\tilde{r})}{J_1(\epsilon_{1n})} \frac{\cosh(\epsilon_{1n}\tilde{z})}{\cosh(\epsilon_{1n}\Gamma)} \right] \\ \frac{u_\theta(\tilde{r}, \theta, \tilde{z}, \tilde{t})}{a\omega} &= 2 \cos(\tilde{t} - \theta) \sum_{n=1}^{\infty} \left[\frac{1}{\tilde{r}} \frac{1}{(\epsilon_{1n}^2 - 1)} \frac{F}{E\epsilon_{1n} \tanh(\epsilon_{1n}\Gamma) - F} \frac{J_1(\epsilon_{1n}\tilde{r})}{J_1(\epsilon_{1n})} \frac{\cosh(\epsilon_{1n}\tilde{z})}{\cosh(\epsilon_{1n}\Gamma)} \right] \\ \frac{u_z(\tilde{r}, \theta, \tilde{z}, \tilde{t})}{a\omega} &= -2 \sin(\tilde{t} - \theta) \sum_{n=1}^{\infty} \left[\frac{\epsilon_{1n}}{(\epsilon_{1n}^2 - 1)} \frac{F}{E\epsilon_{1n} \tanh(\epsilon_{1n}\Gamma) - F} \frac{J_1(\epsilon_{1n}\tilde{r})}{J_1(\epsilon_{1n})} \frac{\sinh(\epsilon_{1n}\tilde{z})}{\cosh(\epsilon_{1n}\Gamma)} \right] \end{aligned} \quad (3.5)$$

where the solution was expanded into its velocity components in cylindrical coordinates (u_r, u_θ, u_z) using $\mathbf{v} = \nabla\Phi$ and the dimensionless groups are those introduced in (3.1), i.e.,

$$F = \frac{a\omega^2}{g} \quad E = \frac{a}{R} \quad \Gamma = \frac{d}{R} \quad Re = \frac{R^2\omega}{\nu}.$$

The dimensionless forcing F represents the ratio between the inertial force per unit mass and the gravitational acceleration. A physical interpretation of F is that it represents the inclination of a free surface at rest under the influence of the effective gravity $\mathbf{f}_m = \mathbf{f}_i + \mathbf{g}$, where $\mathbf{f}_i$ is the inertial force (2.5) and $\mathbf{g}$ the gravity vector. The surface will be perpendicular to $\mathbf{f}_m$, implying that its angle α follows from the ratio of the inertial force and the gravitational force, i.e., $\tan \alpha = a\omega^2/g = F$. The eccentricity E represents the ratio between the orbital forcing radius a and the cylinder radius R ; the name was chosen by analogy to in the mathematical theory of conic sections. The shallowness Γ represents the ratio between the vertical and the horizontal scales of the static liquid volume, and as the name implies, it determines if the fluid is in the shallow or deep water regime. For completeness, we have added the Reynolds number Re to the dimensionless groups, which represents the ratio between the inertial force derived from the local acceleration and the viscous forces. This parameter does not feature in the potential flow solution because of the assumption of irrotationality.

Shown in Figure 3.1 is the prediction of the potential theory of the dimensionless surface amplitude $\Delta h^{\text{PT}}/(2R)$, where $\Delta h^{\text{PT}} = \max(\zeta) - \min(\zeta)$ ($h = d + \zeta$) (3.4). The portion of the parameter space shown in Figure 3.1 roughly covers those of the studies in Table 3.1, with the shallowness Γ ranging from very shallow ($\Gamma = 0.05$) to moderately deep ($\Gamma = 1$). It is clear that the parameter space has a formidable complexity due to the resonance frequencies of the various wave modes which occur when $\omega = \omega_{1n}$, i.e., $E = \frac{\coth(\epsilon_{1n}\Gamma)}{\epsilon_{1n}}F$; this equation represent a set of straight lines through the origin with positive slope, where the height amplitude Δh^{PT} is unbounded (dark red lines). It is thus not surprising that the flow structure in the orbital shaker experiments depends notably on the problem parameters [11, 108, 126, 116, 24, 43].

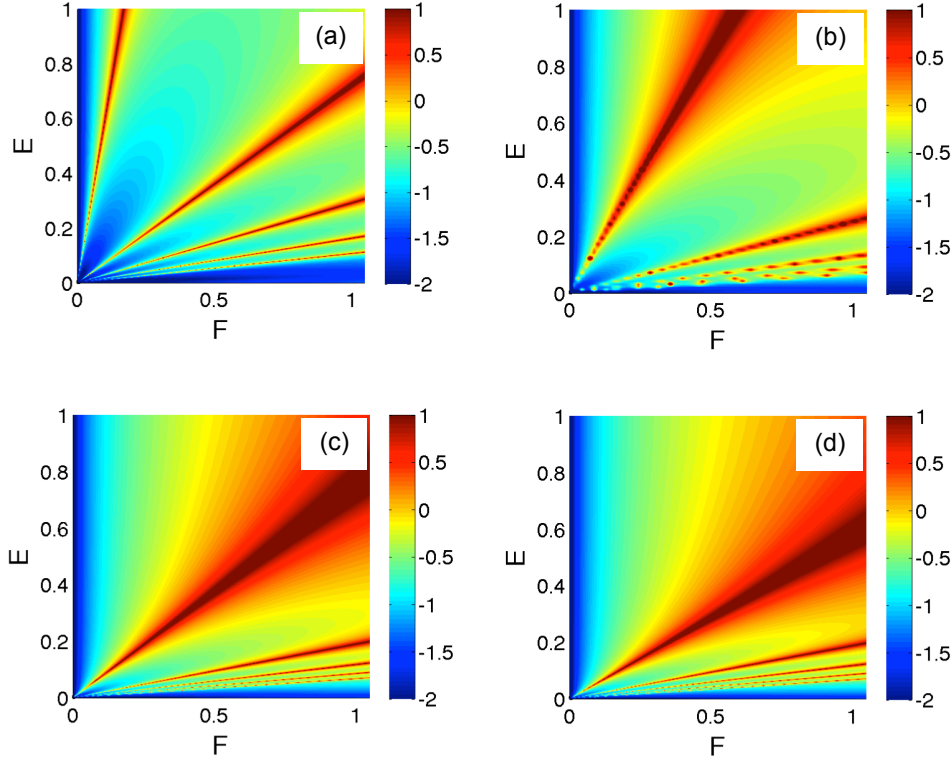


Figure 3.1: Contour plot of dimensionless surface amplitude predicted by the potential theory $\Delta h^{\text{PT}}/(2R)$ (logarithmic scale). (a) $\Gamma = 0.05$. (b) $\Gamma = 0.18$. (c) $\Gamma = 0.5$. (d) $\Gamma = 1$.

3.3 Simulation Details

For this study, 33 case studies were designed and simulated using the computational model described in § 2.3, the parameters of which are displayed in Table 3.2. The reference case simulation (*Re4*), which was analysed in detail in Chapter 2, has parameters $d = 2$ mm, $R = 11.05$ mm, $a = 5$ mm, $\omega = 15.7$ rad s⁻¹, $\rho = 997.6$ kg/m³, and $\mu = 1.002 \cdot 10^{-3}$ Pa s, which corresponds to $F = 0.13$, $E = 0.45$, $\Gamma = 0.18$, and $Re = 1918$. This is a strongly forced case, in which a travelling wave with substantial breaking is observed (§ 2.5). This case was rigorously validated through a mesh convergence study in § 2.3.1, with particular emphasis on accurately capturing the free surface and the boundary layers. The mesh is cylindrical block structured with 291,600 cells (Figure 2.3).

The 33 case studies were designed such that the mesh chosen for the reference simulation could be used for all other cases, which avoids having to remesh and carry out additional convergence studies. Thus, the shallowness Γ was kept constant at a value of 0.18, since its variation would require modifying R or d . By modifying the gravitational acceleration g — which is

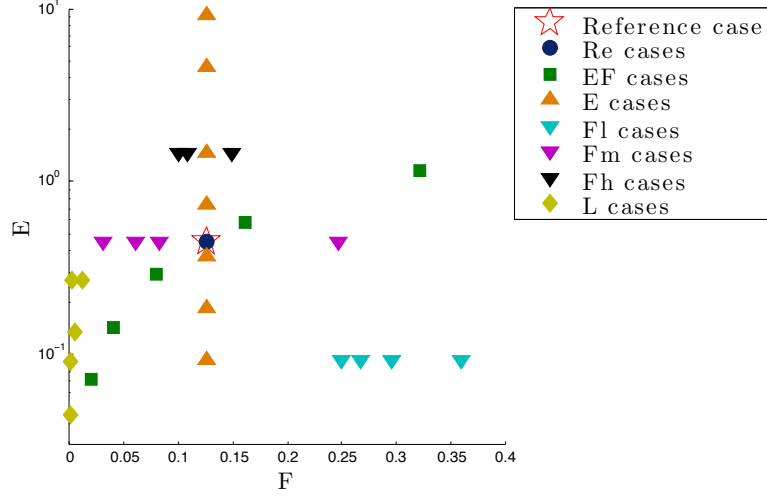


Figure 3.2: Case studies in the E - F parameter space. Different combinations of symbols and colours correspond to different sets of simulations, as described in the text and the legend. The reference case $Re4$ is represented with a red star.

straightforward to do in computational studies — it was possible to vary the dimensionless group F while keeping the values of the other groups constant. Table 3.2 presents all dimensionless group values adopted for the simulations. Figure 3.2 summarises the total of the 33 case studies analysed in a E - F plot. The simulations have been grouped into sets, the names reflecting the dimensionless groups that vary in the set:

- Re cases: vary Re with respect to the reference case;
- EF cases: vary E and F with respect to the reference case, keeping constant the ratio $E/F = 0.36$;
- E cases: vary E with respect to the reference case;
- Fl , Fm , and Fh cases: vary F at different values of constant E (low, medium, high); and
- L cases are low-forcing cases ($F \ll 1$), where Re , E and F vary simultaneously. These cases were carried out to compare the (viscous) simulations with the (inviscid) potential theory in the linear regime, results of which will be discussed in § 3.4.

In conducting the simulations, the flow experiences initial transients during which the flow adjusts into its forced motion. In the fully developed forced solution, the spatial profiles of velocity and surface elevation remain constant and are rotating with an angular velocity ω ; thus, a single snapshot at $t \bmod 2\pi/\omega = 0$ provides all the required information. All simulations are

	Parameters				Dimensionless groups			
Code	a (mm)	$\frac{\omega}{2\pi}$ (s ⁻¹)	g (m/s ²)	ν (m ² s ⁻¹)	E	F	Re	Γ
<i>Re1</i>	5	2.5	9.81	8.0 10 ⁻⁶	0.45	0.13	240	0.18
<i>Re2</i>	"	"	"	4.0 10 ⁻⁶	"	"	480	"
<i>Re3</i>	"	"	"	2.0 10 ⁻⁶	"	"	959	"
Re4	5	2.5	9.81	1.0 10⁻⁶	0.45	0.13	1918	0.18
<i>Re5</i>	"	"	"	0.5 10 ⁻⁶	"	"	3836	"
<i>EF1</i>	0.8	2.5	9.81	1.0 10 ⁻⁶	0.07	0.02	1918	0.18
<i>EF2</i>	1.6	"	"	"	0.15	0.04	"	"
<i>EF3</i>	3.2	"	"	"	0.29	0.08	"	"
<i>EF4</i>	6.4	"	"	"	0.58	0.16	"	"
<i>EF5</i>	12.8	"	"	"	1.16	0.32	"	"
<i>E1</i>	1.02	2.5	2	1.0 10 ⁻⁶	0.09	0.13	1918	0.18
<i>E2</i>	2.04	"	4	"	0.18	"	"	"
<i>E3</i>	4.08	"	8	"	0.37	"	"	"
<i>E4</i>	8.15	"	16	"	0.74	"	"	"
<i>E5</i>	16.31	"	32	"	1.48	"	"	"
<i>E6</i>	50.97	"	100	"	4.61	"	"	"
<i>E7</i>	101.93	"	200	"	9.22	"	"	"
<i>Fl1</i>	1.02	2.5	1.01	1.0 10 ⁻⁶	0.09	0.25	1918	0.18
<i>Fl2</i>	"	"	0.94	"	"	0.27	"	"
<i>Fl3</i>	"	"	0.85	"	"	0.30	"	"
<i>Fl4</i>	"	"	0.70	"	"	0.36	"	"
<i>Fm1</i>	5	2.5	40	1.0 10 ⁻⁶	0.45	0.03	1918	0.18
<i>Fm2</i>	"	"	20	"	"	0.06	"	"
<i>Fm3</i>	"	"	15	"	"	0.08	"	"
<i>Fm4</i>	"	"	5	"	"	0.24	"	"
<i>Fh1</i>	16.31	2.5	40	1.0 10 ⁻⁶	1.48	0.10	1918	0.18
<i>Fh2</i>	"	"	37	"	"	0.11	"	"
<i>Fh3</i>	"	"	27	"	"	0.15	"	"
<i>L1</i>	0.5	0.67	9.81	1.0 10 ⁻⁶	0.05	0.0009	511	0.18
<i>L2</i>	1	"	"	"	0.09	0.0018	"	"
<i>L3</i>	3	0.5	"	"	0.27	0.0030	383	"
<i>L4</i>	1.5	1	"	"	0.14	0.0060	767	"
<i>L5</i>	3	"	"	"	0.27	0.0120	"	"

Table 3.2: Case studies analysed in this chapter. The omitted parameters are kept constant in all cases: $\rho = 997.6$ kg/m³, $d = 2$ mm, and $R = 11.05$ mm. The reference case *Re4* is indicated in a bold font.

analyses after ten orbits (unless otherwise specified) to ensure the periodic state. An impression of the large variety observed in the fluid behaviour is shown in Figure 3.3, which displays the surface elevation of some of the case studies in the E - F parameter space; the behaviour observed varies from cases simply showing a flat tilted surface to others showing multiple crests, wave breaking and some extreme cases with part of the bottom of the container exposed.

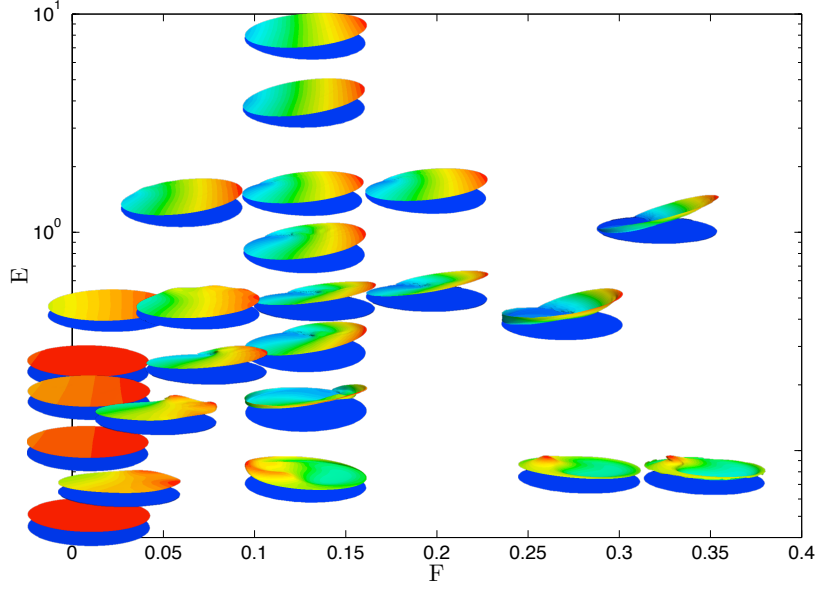


Figure 3.3: 3D surface elevation perspective of various flow cases sketched in the E - F parameter space. Colours represent surface elevation in relative units.

3.4 Validation of Potential Theory

The aim of this section is to verify the extent to which the potential theory can describe flow behaviour in orbitally shaken shallow fluid layers in cylindrical containers. Indeed, as Re is relatively low for many studies (Table 3.2), it is not unreasonable to ask to what extent an inviscid theory can describe the fluid behaviour. Furthermore, we will consider low-forcing F cases (L cases) to remain in a situation in which one could expect linear theory to be valid.

Figure 3.4 displays the mean velocity at the surface as a function of time for the $L5$ case. Simulations were started from a static state at $t = 0$, and the sudden acceleration excites multiple modes. For an inviscid fluid, these modes would remain in existence indefinitely, but viscous effects will damp all the initial modes until only the forced response of the system remains. As it can be seen in Figure 3.4, a constant velocity is reached ($< 1\%$ difference between maximum and minimum) within six shaking periods. All data presented in the remainder of this section are taken from the forced response. Figure 3.5 shows the maps of height and velocity magnitude at $z = 1$ mm for $L5$ at $t = 6T$ (T is the rotation period $2\pi/\omega$); the height map is well characterised, and the elliptic shape of the velocity is well predicted by the potential model at the bulk of the liquid, but there is a discrepancy in the velocity scale (similar results are observed in the other L cases).

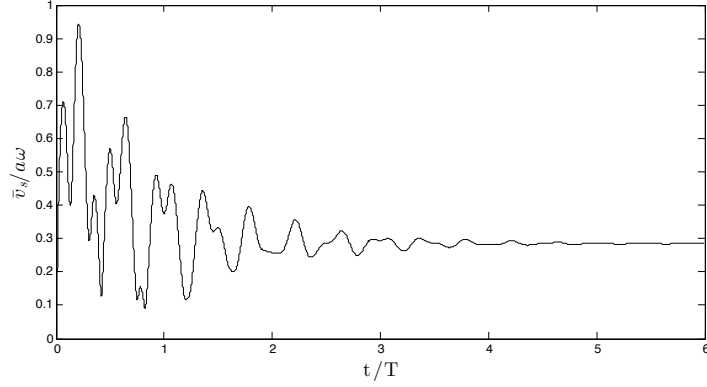


Figure 3.4: Mean surface velocity $\bar{v}_s$ in the $L5$ case as a function of time showing the damping of the not forced modes during the transient. T is the rotation period $2\pi/\omega$.

Cases	Height Amplitude ($\frac{\Delta h}{2R}$)		Max. Velocity at $z=1\text{mm}$ ($\frac{v_m}{a\omega}$)		Mean Velocity at $z=1\text{mm}$ ($\frac{\bar{v}}{a\omega}$)	
	Simulation	Potential	Simulation	Potential	Simulation	Potential
$L1$	0.001	0.001	0.055	0.042	0.038	0.034
$L2$	0.002	0.002	0.055	0.042	0.038	0.034
$L3$	0.003	0.003	0.031	0.023	0.021	0.019
$L4$	0.006	0.006	0.130	0.100	0.084	0.079
$L5$	0.013	0.013	0.128	0.100	0.083	0.079
$L5FS$	0.014	0.013	0.128	0.100	0.086	0.079

Table 3.3: Comparison of the surface elevation and velocity values for the L cases in the computational and the potential model.

The surface elevation amplitude and the maximum and mean velocities are summarised in Table 3.3 for all L cases, as well as the predictions from the potential theory. The surface elevation agrees within 1% for all cases, while there is a discrepancy in the velocity fields: the maximum velocity is consistently underestimated by 20%, and the mean velocity is underestimated with a discrepancy that ranges from 5% (higher F) to 10% (lower F). We also carried out simulation $L5$ with free-slip boundary conditions (case $L5FS$ in Table 3.3), but apart from requiring much longer to damp all initial modes except the forced solution (over 40 oscillations), both velocity and surface amplitude are indistinguishable from the no-slip case (besides the boundary layer at the walls). We thus conclude that the differences in the velocity amplitudes are due to viscous effects in the bulk of the liquid.

The discrepancies between the simulations and the potential theory can be understood using the conservation of mechanical energy. This equation can be derived from the momentum equation, and represents the balance between the time rate-of-change in kinetic energy and the work done per unit time by the surface and volume forces. Making use of the periodicity of the movement,

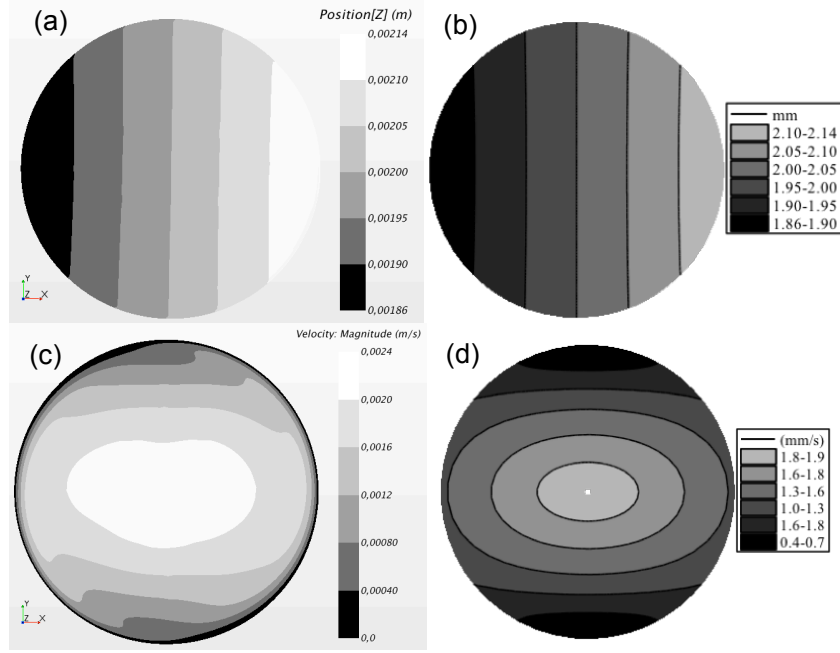


Figure 3.5: (a)-(b): surface elevation in the computational and potential theory models, respectively, in $L5$ case. (c)-(d): velocity magnitude at $z=1\text{mm}$ in the computational and potential theory models, respectively, in $L5$ case.

and taking into account other properties of the problem under consideration (see Appendix B for a derivation), the mechanical energy equation can be reduced to a balance between the viscous dissipation rate and the work per unit time done by the inertial forces:

$$\int_{V(t)} \bar{\bar{\tau}}' : \nabla \mathbf{v} d\Omega - \int_{V(t)} \rho \mathbf{f}_i \cdot \mathbf{v} d\Omega \quad (3.6)$$

where $\bar{\bar{\tau}}'$ is the deviatoric stress tensor, $\mathbf{v}$ is the velocity vector, ρ is density, $\mathbf{f}_i$ is the inertial force, and $V(t)$ is the integration volume. The dissipation rate ($\bar{\bar{\tau}}' : \nabla \mathbf{v}$) is always positive, i.e., there is always a loss of mechanical energy that is transformed into internal energy. For the potential flow theory, $\bar{\bar{\tau}}' = 0$ by definition due to the assumption of irrotationality. Thus, there is no viscous dissipation and the velocity field must be such that the work done by the inertial forces is zero ($\int_{V(t)} \rho \mathbf{f}_i \cdot \mathbf{v} d\Omega = 0$), i.e., the dot product of the inertial force (2.5) and the velocity (3.5) must cancel out in the fluid volume at each instant of time. Indeed, the potential flow satisfies the equation because the symmetry in the velocity field ensure that work is zero upon integration over the symmetric volume.

The potential theory has been demonstrated to predict the surface elevation accurately while underestimating the velocity field. The conservation of energy sheds light on the origin of the

discrepancies in the velocity scales. The viscosity term introduces a fundamental difference between the two models: the viscous dissipation rate causes an unavoidable loss of mechanical energy in the viscous problem, which leads to a different distribution of the fluid field to the inviscid problem. Equation (3.6) shows that the term feeding the viscous dissipation is the work per unit time done by the inertial forces. We note two possible origins of this work: a symmetry breaking of the velocity magnitude field, and a lag between the volume and velocity field symmetry planes. However, this analysis is beyond the scope of this study.

3.5 Surface Amplitude and Phase Shift

Having established that the potential theory describes the surface elevation well for situations with low F , we study the extent to which the theory describes cases with high F . We will use the E cases to explore the predictions for highly forced cases, in which F is large but constant and the eccentricity E varies in a range of two orders of magnitude (see Table 3.2). An attractive feature of the E cases is that it crosses a resonant line between $E1$ and $E2$.

In the potential theory, the maximum surface elevation occurs at $r = R$, $\theta = 0$ and the minimum at $r = R$, $\theta = \pi$ (with identical deviation from the static height d) in all cases at $t \bmod 2\pi/\omega = 0$, with the exception of $F1$ cases and $E1$ for which the secondary modes are of such magnitude that they change the location of the maximum. Therefore, the amplitude Δh^{PT} is given by

$$\frac{\Delta h^{\text{PT}}}{2R} = \frac{\zeta(r = R, \theta = 0, t = T)}{R} = F \left[1 + \sum_{n=1}^{\infty} \left(\frac{2}{\epsilon_{1n}^2 - 1} \frac{1}{\frac{E}{F} \epsilon_{1n} \tanh(\epsilon_{1n} \Gamma) - 1} \right) \right] \quad (3.7)$$

predicting a dependence of $\frac{\Delta h^{\text{PT}}}{2R}$ on F , $\frac{E}{F}$, and Γ .

Shown in Figure 3.6(a) are the amplitudes $\Delta h/2R$ as observed in the E cases as a function of $\frac{\omega_{11}}{\omega} = \sqrt{\frac{E}{F} \epsilon_{11} \tanh(\epsilon_{11} \Gamma)}$, a dimensionless parameter proportional to $E^{1/2}$ in the E cases where F remains constant. The potential model prediction, as indicated by the dashed line, estimates the height amplitude correctly except for the cases $E2$ and $E3$; in these cases, the shaking frequencies approach the resonant frequency (assuming that the natural frequencies of the inviscid potential model are also the resonant frequencies in the viscous model), where the potential model predicts an infinite amplitude due to the absence of viscosity.

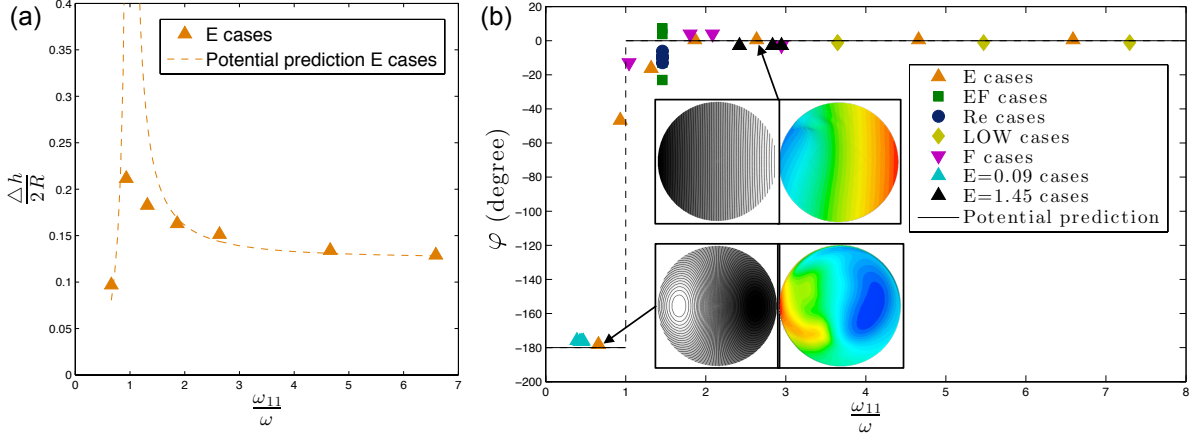


Figure 3.6: (a) Dimensionless amplitude $\frac{\Delta h}{2R}$ against the dimensionless parameter $\frac{\omega_{11}}{\omega}$, where ω_{11} is the first natural frequency of the mode $m = 1$, for the E cases; the orange discontinuous line represents the potential prediction. (b) The indicator phase shift φ against the dimensionless parameter $\frac{\omega_{11}}{\omega}$ for all cases; the black line represents the potential prediction. Colour maps show the surface elevation in the simulations versus grey scaled maps of the potential theory for the cases indicated.

Another characteristic observed in crossing the resonant line is that the location of the maximum surface elevation shifts from an angle 0° to π in the x-axis direction. The indicator *phase shift* φ has been defined as the angle between the line connecting the centre of the well with the maximum surface height and the direction of the inertial force ($\varphi \in [-\pi, \pi]$). Figure 3.6(b) shows φ as a function of $\frac{\omega_{11}}{\omega}$. The potential theory predicts very well the change in the indicator by π when crossing the first natural frequency ω_{11} of the mode $m = 1$ (Fl cases and $E1$). The prediction differs somewhat for cases at $\omega \sim \omega_{11}$, which is expected because viscous effects will create strong differences near the resonance frequency.

3.6 Effect of Reynolds Number

As observed by Reclari [110], increasing the viscosity ($Re \downarrow$), decreases the area of the breaking regions (see Figure 3.7); however, contrary to what they observed in breaking waves, the amplitude decreases with viscosity. The change in amplitude is small (difference $\sim 10\%$ between $Re1$ to $Re5$), but the tendency is to initially increase with Re , and later the amplitude remains the same for $Re4$ and $Re5$. Their arguments are 1) that viscosity increases the resistance to agitation when the wave is not breaking, thereby decreasing the amplitude; and 2) that viscosity reduces the area of dissipation of energy when the wave is breaking, thereby increasing the

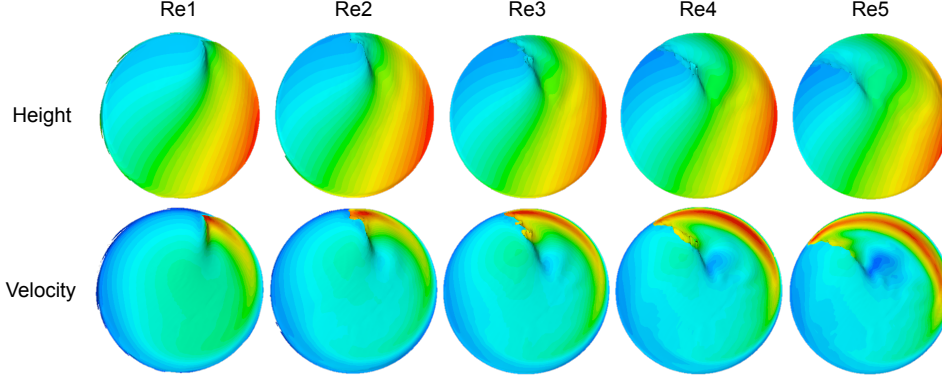


Figure 3.7: Effect of Re on the flow behaviour for Re cases. On the top row, the surface height map, and on the bottom, the velocity maps at the free surface.

amplitude [110]. We argue that a combination of both phenomena takes place when the wave is breaking, resulting in a small change in amplitude.

The phase shift φ , defined in the previous section, remains practically constant at -10° ; however, the position of the minimum surface height varies from $+140^\circ$ to $+80^\circ$ with increasing viscosity. We leave this finding as an observation, considering that it would be necessary to undertake an in-depth study of the structure of the velocity secondary flows to explain this effect, and indicating its possible connection to the effect of dissipation of mechanical energy introduced in § 3.4.

3.7 Transition to Breaking

In this section we will focus on regime classification, making use of the fact that the potential theory provides a good description of the expected surface elevation with the exception of cases in which the system is close to resonance. In particular, we will describe a criterion for wave breaking.

The closest study of wave breaking in orbitally shaken cylindrical fluid layers was carried out by Reclari et al. [111], who found that potential theory predicted the surface amplitude accurately for single-crested non-breaking waves. Their experimental observations show a change of behaviour when the wave breaks; this change occurs at amplitudes $\frac{\Delta h}{2R}$ between 0.35 and 0.45. They argue that the theory of gravity waves establishes a limit in the wave steepness beyond which no gravity waves can exist and breaking is expected; in the context of an orbital shaker,

this limit translates to $\frac{\Delta h}{2R} \cong 0.443$, supporting their findings. However, this result is not valid in the study of small Γ , where the wave is expected to be perturbed if $\frac{\Delta h}{2R} > \frac{2d}{2R} = \Gamma$ (uncovered bottom), e.g., in this study if $\frac{\Delta h}{2R} > 0.18$. Indeed, the wave-breaking literature typically separates the study of wave breaking between shallow and deep water [42].

In shallow water, many studies are inspired by the criterion established by Miche [87] for one-dimensional, periodic, irrotational waves over a flat bottom given by $H_{max} = \frac{\beta_{max}}{\kappa} \tanh(\kappa d)$, where H_{max} is the maximum height measured from the static height d , κ is the wavenumber, and $\beta_{max} = 0.88$ is a constant. In the current work, this implies $H_{max} = \frac{\Delta h}{2}$, $\kappa = \frac{2\pi}{L} = \frac{2\pi}{2\pi R} = 1/R$, and thus the breaking criterion for shallow water is $\frac{\Delta h}{2R} = \beta_{max}\Gamma$. This theory can be used as a reference in shallow water given that the breaker is similar to that of a solitary wave [42], but β_{max} need not be 0.88 in this problem due to the inertial forces and the fact that the Reynolds number is limited. The Miche criterion is derived from the fact that wave breaking takes place when the particle velocity approaches or reaches the phase velocity v_p , which translates in the orbital shaker problem considered herein into the breaking criterion $v_{max} \sim v_p$, where v_{max} is the maximum velocity at the free surface, $v_p = r_p\omega$, and r_p is the radial distance of the location of maximum surface velocity.

Similarly to Reclari et al. [111]’s findings, we observed that the potential theory predicted the amplitude correctly up to the forcing value where breaking is predicted. This analysis has been carried out in the simulation sets where $\frac{\Delta h^{PT}}{2R}$ (3.7) only depends on F , i.e., in Fl, m, h cases and also EF cases since $\frac{E}{F}$ is constant. In these cases, there is thus a unique positive correspondence between the predicted amplitude and the forcing. This allows the definition of two analytical limits: i) the *uncovered-bottom* forcing F_u when the amplitude prediction is equal to double the static height $\frac{\Delta h}{2R} = \Gamma$, then for $F > F_u$ the bottom would be uncovered and the analytical prediction is not physical; and ii) the *breaking* forcing F_b when the dimensionless amplitude prediction is equal to the theoretical prediction of breaking $\frac{\Delta h}{2R} = 0.88\Gamma$, then for $F > F_b$ the wave is expected to break. (Note that F_u and F_b change in each simulation set, and that $F_b < F_u$ always). Figure 3.8 shows the amplitude over the potential amplitude against the forcing non-dimensionalised by F_b . There, it is observed that for cases with $F < 0.9F_b$, there is a discrepancy of under 10% in the potential prediction, while for cases with $F > F_b$, the potential model overestimates the amplitude.

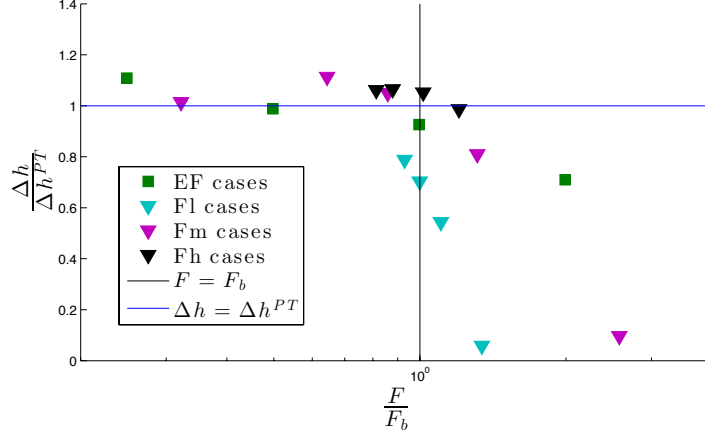


Figure 3.8: Surface height amplitude Δh over the potential theory amplitude prediction Δh^{PT} against the forcing F non-dimensionalised by the predicted breaking forcing F_b .

All cases have been classified visually in the simulations as *breaking* or *non-breaking* based on the presence of the breaking discontinuity in the free surface (as described in § 2.5), and as *covered* or *uncovered bottom* based on the number of vertical computational cells at the lowest point of the free surface. Cases with $F > F_b$ are observed to be indeed breaking, and thus the reduced amplitude with respect to the prediction is explained by the extra dissipation of energy due to the breaking. The exceptions are the *Fl* cases, where the prediction is not accurate even in the non-breaking cases; in this simulation set, the first symmetric mode is not predominant, and the potential theory predicts the predominance of the second symmetric mode. However, while this is true, contrary to the potential prediction, the solution is clearly not antisymmetric: $\zeta_{max} > \zeta_{min}$, probably due to the excitation of other modes not contemplated in the potential model used. On the other hand, no analytical conclusions can be drawn with respect to the uncovered bottom limit because the validity domain of the potential theory reaches its limit at $F = F_b$, thus at $F < F_u$.

In Figure 3.9(top), all case studies have been plotted in diagrams of dimensionless amplitude $\frac{\Delta h}{2R}$ against the maximum surface velocity over the phase velocity $\frac{v_{max}}{v_p}$. On the top left, the points have been coloured according to the simulation set colour codes (Figure 3.2), and can be linked to the bottom figures which represent the height maps arranged left to right from the lowest to highest amplitude within each simulation set. Two black discontinuous lines represent the two theoretical breaking limits for amplitude and velocity: $\frac{\Delta h}{2R} = 0.88\Gamma$ and $v_{max} = v_p$. On the top right, the same figure has been represented colour-coded based on the

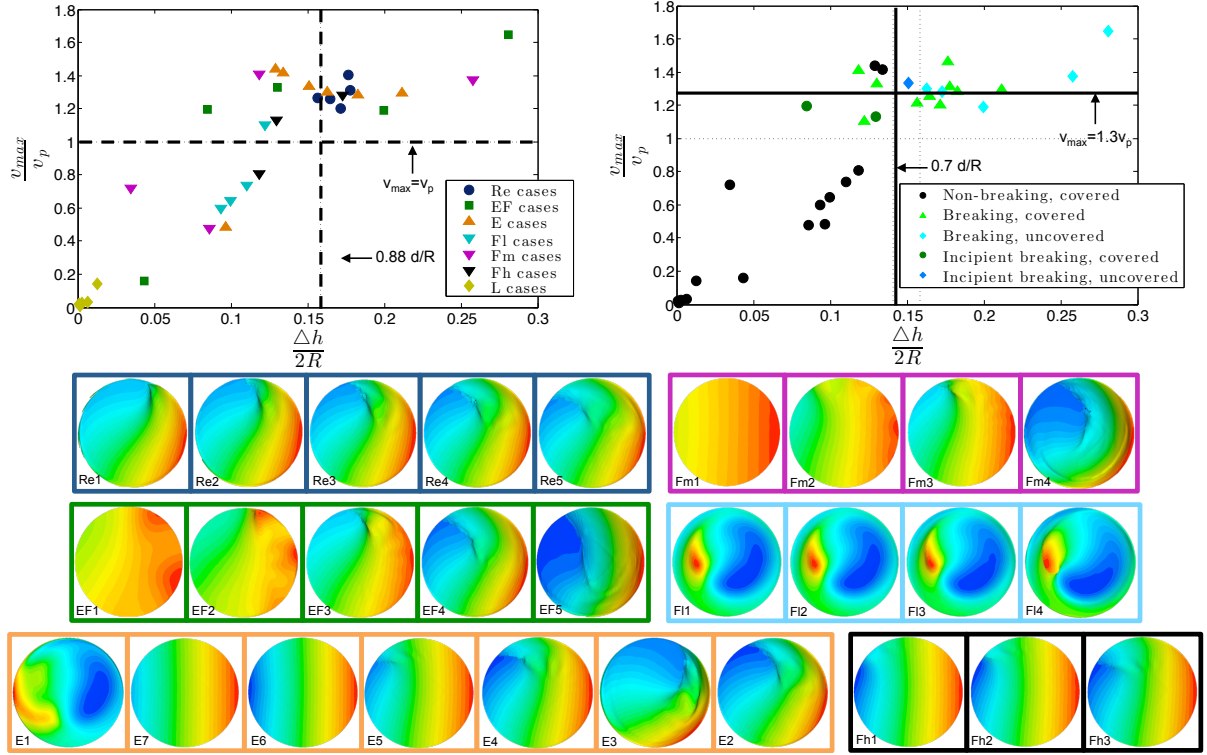


Figure 3.9: Plot top left: dimensionless amplitude $\frac{\Delta h}{2R}$ against the maximum surface velocity normalised by the phase velocity ($\frac{v_{max}}{v_p}$) with the points coloured according to the simulation set colour codes depicted at the bottom. Plot top right: same coordinates as before but with the points coloured according to the breaking and covered bottom classification. In both plots, the black lines represent the breaking limits introduced in the text. Multiple bottom maps: surface elevation maps of the multiple simulation sets, the maps are arranged left to right from the lowest to highest amplitude within each simulation set, in order to make it possible to connect with the points in the top plots.

non-breaking/breaking and uncovered/covered-bottom classification. The axes are the same in both top figures, therefore, the points can be connected one-to-one, and linked to the height maps. We observe that the breaking occurs before the theoretical limit of solitary waves $\frac{\Delta h}{2R} = 0.88\Gamma$, and the breaking limit for this problem has been identified at $\frac{\Delta h}{2R} = 0.7\Gamma$. Interestingly, the maximum velocity in the breaking cases has a common tendency to $\sim 1.3v_p$ ($EF5$ seems to be outside the rule presumably because the maximum velocity is no longer at the breaking of the wave, but has extended over the surface, affecting the definition of v_p through r_p).

All observations made come together in Figure 3.10. There, all case studies have been plotted in the E - F space and colour-coded based on the non-breaking/breaking and uncovered/covered-bottom classification. In addition, each point in the E - F space corresponds to a unique value of the potential theory height amplitude $\Delta h^{PT} = \zeta_{max} - \zeta_{min}$ (3.3) with $\Gamma = 0.18$, as was shown in

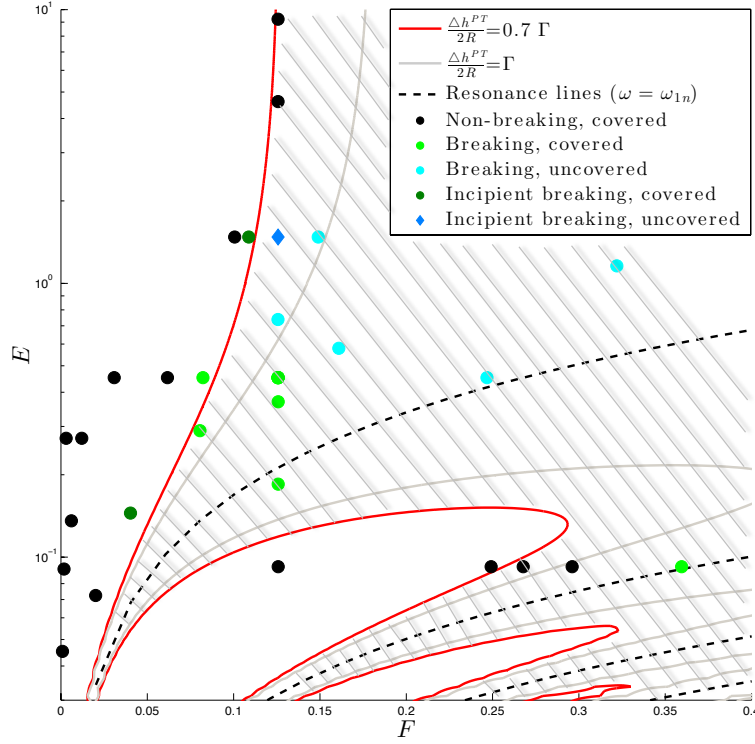


Figure 3.10: Wave-breaking regime classification. All 33 simulation cases in the E - F space coloured according to the breaking and covered-bottom classification. The red line is a contour under the condition $\frac{\Delta h^{\text{PT}}}{2R} = 0.7 \Gamma$, the breaking limit introduced in the text. The discontinuous black lines represent the lines where $\frac{E}{F} \epsilon_{1n} \tanh(\epsilon_{1n} \Gamma) = 1$, i.e., the resonance lines where $\omega_{1n} = \omega$. The dark green and blue points ($EF2$, $Fh2$, and $E5$) are cases where the breaking is incipient. Finally, the striped area represents the wave-breaking regime region.

Figure 3.1 for multiple Γ . The latter allows drawing of the breaking limit identified as the curve where $\frac{\Delta h^{\text{PT}}}{2R} = 0.7\Gamma$ (red line), the potential prediction of uncovered bottom as the curve where $\frac{\Delta h^{\text{PT}}}{2R} = \Gamma$ (grey line), and the resonance lines as the curve where $\frac{E}{F} \epsilon_{1n} \tanh(\epsilon_{1n} \Gamma) = \left(\frac{\omega_{1n}}{\omega}\right)^2 = 1$ (discontinuous black lines). These lines separate the E - F space into multiple regions. The wave-breaking regime region corresponds to the marked (striped) area, which is limited by the breaking threshold $\frac{\Delta h^{\text{PT}}}{2R} > 0.7\Gamma$, and all cases that fall in this region are expected to have a breaking wave. Indeed, the breaking threshold adequately separates the breaking regime with the exception of the Fh cases, where, as previously noted, the potential theory model used in this study does not predict the amplitude accurately. On the contrary, the predicted uncovered-bottom line does not delimit a region where the cases have uncovered bottom. As remarked before, this is due to the fact that the wave breaks before it reaches the uncovered-bottom amplitude threshold, and the potential theory fails to predict the amplitude in the breaking regime.

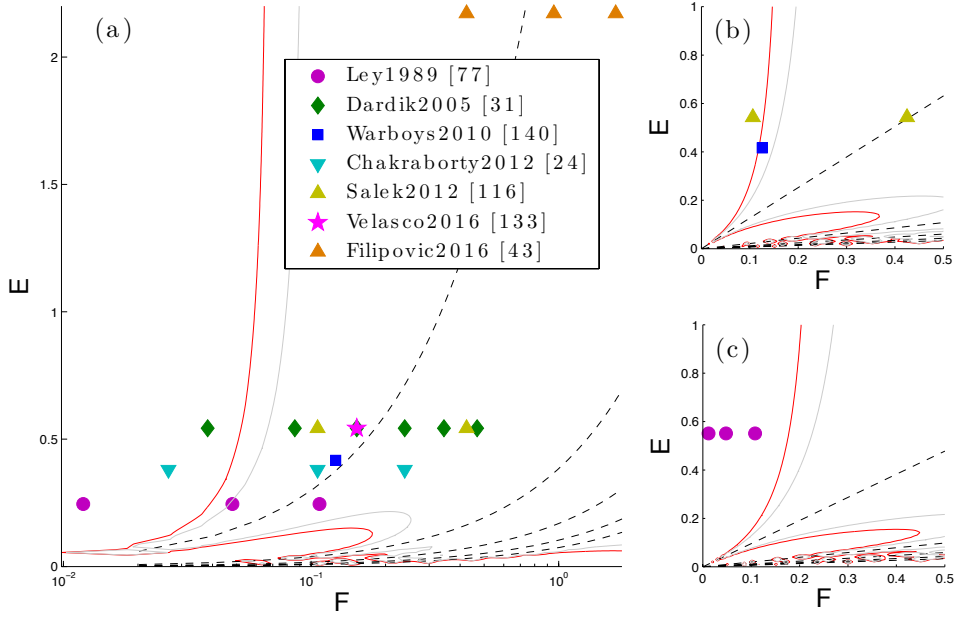


Figure 3.11: Operating regimes in endothelial studies from Table 3.1 in E - F space. (a) $\Gamma = 0.1$. (b) $\Gamma = 0.25$. (c) $\Gamma = 0.35$. The red contour represents the breaking limit $\frac{\Delta h}{2R} = 0.7\Gamma$. The grey contour represents $\frac{\Delta h}{2R} = \Gamma$. The discontinuous black lines represent the lines where $\frac{E}{F}\epsilon_{1n}\tanh(\epsilon_{1n}\Gamma) = 1$, i.e., the resonance lines where $\omega_{1n} = \omega$.

3.8 Operating Regimes in Endothelial Cells Studies

The endothelial cells studies summarised in Table 3.1 have been plotted in the E - F space for an approximated value of Γ . All cases contemplated operate at the shallow water regime ($\Gamma < 1$), and most cases operate beyond the identified breaking limit $\frac{\Delta h}{2R} = 0.7\Gamma$. All computational studies report the travelling wave [140, 24, 25, 133, 108, 126, 116, 43], but they do not analyse the occurrence of wave breaking. Only Salek et al. [116] show the height maps of their cases, which corroborate our results: the two reported cases at $\Gamma = 0.12$ are beyond the breaking limit in the E - F space, and their height maps show a clearly breaking region; the case at $\Gamma = 0.24$ and $F = 0.11$ is located in the non-breaking region in the E - F space, and the height map shows an almost flat surface with an incipient breaking, similar to our cases *Fh2* and *E5*; the case at $\Gamma = 0.24$ and $F = 0.42$ is located in the breaking region close to a resonant line, and the height map shows a breaking region of long radial length and a partially uncovered bottom.

3.9 Conclusions

Increasingly, orbital shakers are being used for *in vitro* studies of endothelial cells to understand how the wall shear stress τ_w is related to the physiological and pathological function of these cells and its role in atherosclerosis. These experiments use specific conditions of τ_w magnitude and directionality, however, there are few guidelines on how to choose the parameter configuration to achieve the required conditions. Using a combination of potential theory and numerical simulations, we have shed light on the characterisation of the flow by carrying out a regime classification. This classification is based on the onset of wave breaking, which is a crucial event to the multidirectional character of τ_w in orbital shakers.

Our initial consideration of the potential theory as a model for this forced free-surface flow leads to the identification of four dimensionless groups which are key to classifying the case studies (Table 3.2). These dimensionless groups are (i) the dimensionless forcing F , (ii) the eccentricity E , (iii) the shallowness Γ , and (iv) the Reynolds number Re . In this study, we have fixed $\Gamma = 0.18$ in order to focus on the shallow water regime typically adopted in endothelial studies to obtain an adequate level of oxygen in the medium. We demonstrate that, in spite of the simplified linear and inviscid potential theory used, the analytical solution predicts the amplitude correctly up to the onset of breaking. Thus, this allows for delimiting theoretically a breaking region in the E - F space (Figure 3.10) based on the breaking threshold $\frac{\Delta h}{2R} = 0.7\Gamma$ identified observationally in the simulations (Figure 3.9), which is specific for shallow water. The classification is valid for the wide range of behaviour observed in the case studies. The prediction did not hold for the cases where the second modes became dominant, opening the way for improvement of the potential theory.

To conclude, we extract some recommendations to help select the appropriate operating regime in the case of endothelial cell experiments: (i) the region around the breaking threshold should provide a rich environment to experiment with different WSS patterns depending on the geometry of free surface breaking; and (ii) it is recommended to keep away from the resonant lines and areas of higher surface amplitude as this can lead to the bottom of the well becoming uncovered, leading to cell damage.

Chapter 4

PT-Stokes Wall Shear Stress Model

4.1 Introduction

As we saw in Chapter 3, orbital shaker flows are of notable complexity partly due to the free surface which often presents a discontinuity in the form of a breaking wave. Hence, to predict the shear stress field produced by the flow becomes a particular challenge. Many experimental studies estimate WSS magnitude using the Stokes second problem extended solution [119]:

$$\tau_w^{\text{St}} = \rho a \omega^{3/2} \sqrt{\nu} \quad (4.1)$$

where a and ω are the radius and speed of rotation, respectively, and ρ and ν are the fluid density and kinetic viscosity, respectively. The classic Stokes second problem configuration consists of a half-space bounded by a plate that oscillates uniaxially in its plane as $\mathbf{v}_o = A \cos(\omega t) \mathbf{e}_x$, where A and $\mathbf{e}_x$ are the oscillation amplitude and direction, respectively, and t represents time. Since the governing equations are linear, the theory can be extended to a plate oscillating in two perpendicular directions out of phase by $\pi/2$ [119]: $\mathbf{v}_o = A [-\sin(\omega t) \mathbf{e}_x + \cos(\omega t) \mathbf{e}_y]$ (orbital shaker platform velocity), the solution of which yields the wall shear stress prediction given in (4.1). The main difference between Stokes' second problem and the orbital shaker configuration are the boundary conditions: the orbital shaker has a finite liquid height and side walls whereas the Stokes problem is unbounded and assumes that the fluid is unperturbed in the far field. As a result, Stokes' solution is unable to capture the spatial dependence of $\boldsymbol{\tau}_w$ observed in orbital shakers [116] — fundamental information needed for the study of endothelial cells —

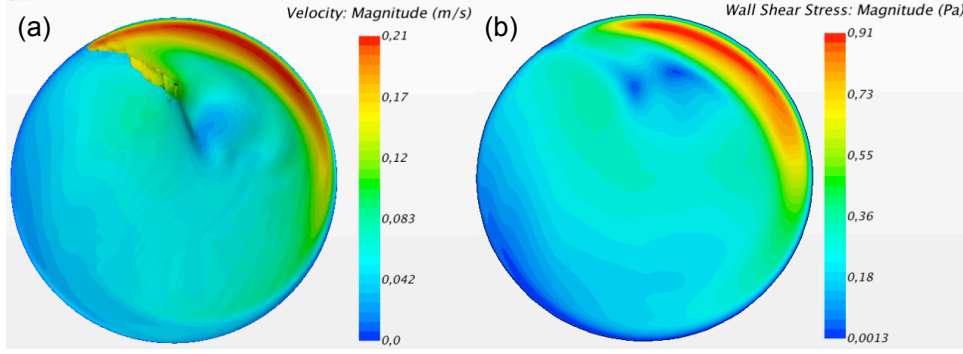


Figure 4.1: The surface velocity $\mathbf{v}_s$ magnitude map (a) and τ_w magnitude map at the well bottom (b) for the reference case ($Re4$) present similar patterns

and, thus, does not take into account the configuration parameters' fluid height d and well radius R , which have been proved in Chapter 3 to have a notable influence on the emerging flow. As such, Stokes' solution can only be expected to be valid in the centre of the orbital shaker (away from the side walls).

The validity of the τ_w^{St} estimation has been investigated using numerical simulations by different authors. Thomas et al. [126] tested its validity at the centre of the well for six cases with increasing speed of rotation ω ; τ_w and τ_w^{St} magnitudes followed similar trends, increasing with ω , but Stokes predicted notably higher values of WSS in all cases. Salek et al. [116] observed that, at a low frequency, doubling the fluid height d doubled the mean τ_w , and that at a high frequency, Stokes correctly predicted the value at the centre but there was high variability from centre to the edge (four times greater). In fact, Chakraborty et al. [24] suggested that Stokes' solution is only suitable in a particular domain of dimensionless numbers; these numbers describe the relative importance of the viscous to inertial terms, the fluid height to the viscous boundary layer thickness, the gravity waves to forced ones, and the free surface angle. For instance, Chakraborty et al. [25] reported agreement to within 1% in the centre of the well for a case with a viscosity an order of magnitude higher than the typical experimental value, while in this chapter, we examined the Stokes solution for cases at very low forcing, for which (4.1) severely overpredicted the observation by a factor of 10, proving the limitations of the model and the need for improvement.

The aim of this chapter is to carry out a detailed investigation of τ_w in orbitally shaken shallow fluid layers, and to present an improved model of the Stokes solution that takes into account

the boundary geometry of the orbital shaker problem. The crux of the model is to link τ_w to the surface velocity $\mathbf{v}_s$; these are strongly correlated as can be seen from Figure 4.1. The novel model, which we will refer to as the PT-Stokes WSS model, is a generalisation of the extended Stokes model, in which the surface velocity is predicted by potential theory (PT).

The chapter is organised as follows. In Section 4.2, the theory for the PT-Stokes WSS model is presented. Section 4.3 contains the simulation case studies used as observations to compare with the model. Section 4.4 explores the validity of the PT-Stokes WSS model for the low-forcing cases, demonstrating that the boundary layer profile and τ_w magnitude are predicted correctly (with the exception of the edge), and the τ_w angle is adequately predicted at the centre region. In section 4.5, the model applicability to higher-forcing cases is explored, and a good correlation is found for the values at the centre region and intermediate regions with the exception of resonant cases. In Section 4.6, the Stokes and PT-Stokes WSS models are compared, and the five metrics described in § 2.4 are predicted using the PT-Stokes WSS model for the reference case. Section 4.7 contains the main conclusions of the study.

4.2 Theory

We develop a novel WSS model that is a generalisation of the extended classic Stokes model that takes into account the orbital shaker finite boundaries. The flow near the bottom is represented by a Stokes bottom boundary layer that relates τ_w to the outer velocity, and the outer velocity is represented by the potential flow theory surface velocity prediction $\mathbf{v}_s^{\text{PT}}$. As a result, we will show that τ_w is related to $\mathbf{v}_s^{\text{PT}}$ in magnitude and direction by

$$\tau_w = \rho V_s^{\text{PT}} \sqrt{\nu \omega} \quad (4.2)$$

$$\alpha_\tau = \alpha_v^{\text{PT}} + 45^\circ. \quad (4.3)$$

where ρ and ν are the fluid density and kinetic viscosity, respectively, V_s is the surface velocity magnitude, and α_τ and α_v are the τ_w and $\mathbf{v}_s$ angles with respect to the x-axis at $t \bmod 2\pi/\omega = 0$.

4.2.1 Relationship of the wall shear stress to the surface velocity

A mathematical derivation is presented in this section describing the velocity field in the orbital shaker bottom boundary layer, which yields the relationship between $\boldsymbol{\tau}_w$ and $\mathbf{v}_s$. The boundary layer is the near-wall region where viscous effects are significant and the velocity field adapts from the velocity at the wall (no-slip condition) to a external value. The flow field can be described by the Navier-Stokes equations in a non-inertial frame of reference where the well remains static (§ 2.2). These equations can be simplified under the following assumptions: convective terms are negligible compared to inertial terms and the vertical velocity is smaller than the horizontal (shallowness assumption, $\Gamma = \frac{d}{R} \ll 1$, where d is the fluid height at rest and R the well radius). Taking into account the viscosity terms in the near field, the equations simplify to

$$\frac{\partial u}{\partial t} = -\frac{1}{\rho} \frac{\partial p}{\partial x} + \nu \frac{\partial^2 u}{\partial z^2} + a\omega^2 \cos(\omega t) \quad (4.4)$$

$$\frac{\partial v}{\partial t} = -\frac{1}{\rho} \frac{\partial p}{\partial y} + \nu \frac{\partial^2 v}{\partial z^2} + a\omega^2 \sin(\omega t) \quad (4.5)$$

where u and v are the velocity components in the x and y -directions, p is pressure, ρ and ν are the fluid density and kinetic viscosity, respectively, and a and ω are the radius and speed of rotation of the well, respectively. The trigonometric terms on the right-hand side of the equations are the inertial forcing terms; they derive from the acceleration (2.5) caused by the circular translation of the platform on which the well stands: $u_o = -a\omega \sin(\omega t)$ and $v_o = a\omega \cos(\omega t)$. The momentum equation in the vertical direction has been omitted; consistent with the assumption of shallowness, it provides the hydrostatic pressure distribution in the z -direction.

The boundary layer is assumed to be unbounded in the horizontal direction ($\Gamma \ll 1$). In the vertical direction, the liquid is bounded by the bottom wall at $z = 0$ with no-slip conditions; at the upper limit, the outer velocity is assumed constant and equal to that at the free surface $\mathbf{v}_s$, which is assumed sufficiently far away (far field) from the viscous influence ($h \gg \delta$, where h is the free surface height and δ is the boundary layer characteristic thickness). Therefore,

$$\text{at } z = 0 : u = 0, v = 0 \quad (4.6)$$

$$\text{at } z = h : u = V_s \cos(\omega t + \alpha_v), v = V_s \sin(\omega t + \alpha_v). \quad (4.7)$$

Here, V_s and α_v are the surface velocity magnitude and angle with respect to the x-axis at $t \bmod 2\pi/\omega = 0$, respectively. Note that the model assumes a sinusoidal variation on the surface velocity direction and a constant magnitude; this is true at the centre of the well, but as we move to the edge, the influence of the side walls will affect the velocity temporal distribution. Also, the spatial gradient of the surface velocity is assumed small compared to the time variation ($\frac{V_s^2/R}{V_s\omega} = \frac{V_s(\sim a\omega)}{\omega R} \sim \frac{a}{R} = E \ll 1$).

The pressure gradient can be calculated in the far field region ($z \gg \delta$) where the velocity field $\mathbf{v} = \mathbf{v}_s$ is independent of z , and under these circumstances, (4.4) and (4.5) simplify to

$$-\frac{1}{\rho} \frac{\partial p}{\partial x} \Big|_{z \gg \delta} = -V_s \omega \sin(\omega t + \alpha_v) - a \omega^2 \cos(\omega t) \quad (4.8)$$

$$-\frac{1}{\rho} \frac{\partial p}{\partial y} \Big|_{z \gg \delta} = V_s \omega \cos(\omega t + \alpha_v) - a \omega^2 \sin(\omega t). \quad (4.9)$$

Since we have derived a hydrostatic pressure distribution inside the boundary layer, the horizontal pressure gradients are independent of z and thus equal to those calculated in the far field. Hence, equations (4.8) and (4.9) can be substituted back into (4.4) and (4.5):

$$\frac{\partial u}{\partial t} = \nu \frac{\partial^2 u}{\partial z^2} - V_s \omega \sin(\omega t + \alpha_v) \quad (4.10)$$

$$\frac{\partial v}{\partial t} = \nu \frac{\partial^2 v}{\partial z^2} + V_s \omega \cos(\omega t + \alpha_v). \quad (4.11)$$

These equations have the following analytical solution applying the boundary conditions (4.6)-(4.7):

$$u(z, t) = V_s \cos(\omega t + \alpha_v) - V_s \cos \left(\omega t + \alpha_v - \sqrt{\frac{\omega}{2\nu}} z \right) e^{-\sqrt{\frac{\omega}{2\nu}} z} \quad (4.12)$$

$$v(z, t) = V_s \sin(\omega t + \alpha_v) - V_s \sin \left(\omega t + \alpha_v - \sqrt{\frac{\omega}{2\nu}} z \right) e^{-\sqrt{\frac{\omega}{2\nu}} z}. \quad (4.13)$$

Here, $\sqrt{\frac{\nu}{\omega}}$ is a diffusive length scale commonly referred to as the viscous penetration depth [9], and the boundary layer characteristic thickness δ is expected to be of this order of magnitude.

The velocity magnitude V and the angle α with respect to the far field velocity $\mathbf{v}_s$ are given by

$$\frac{V(z)}{V_s} = \sqrt{1 + e^{-\sqrt{\frac{\omega}{2\nu}}z} - 2 \cos\left(-\sqrt{\frac{\omega}{2\nu}}z\right) e^{-\sqrt{\frac{\omega}{2\nu}}z}} \quad (4.14)$$

$$\alpha(z) = \arctan\left(\frac{v}{u}\right) - (\omega t + \alpha_v) \quad (4.15)$$

as depicted in Figure 4.2(a)-(b); both V and α are independent of t . The velocity profile has its maximum velocity at $V_{max} = 1.07V_s$ located at $z_{max} = 3.2\sqrt{\nu/\omega}$. The velocity direction changes 45° with z from the far field direction to the bottom zero velocity. This change in the velocity direction draws a spiral-like distribution in the velocity projection on the plane $\{x, y\}$ (see Figure 4.2[c]); this spiral is known as the Ekman spiral in atmospheric and ocean dynamics [132], there, a boundary forms where the viscous effect becomes comparable to the inertial Coriolis forces. The spiral originates from zero velocity at the bottom, and develops clockwise with z up to the far-field velocity $\mathbf{v}_s$. The profile is fully developed around $z = 5.8\sqrt{\nu/\omega}$, where $V = 1.01V_s$ and $\alpha = 0.99\alpha_s$. The spiral shape is invariable in time and rotates with frequency $\omega/2\pi$.

The WSS vector $\boldsymbol{\tau}_w$ is obtained using (4.12) and (4.13):

$$\frac{\tau_x}{\rho} = \nu \left. \frac{\partial u}{\partial z} \right|_{z=0} = V_s \sqrt{\frac{\nu\omega}{2}} (\cos(\omega t + \alpha_v) - \sin(\omega t + \alpha_v)) \quad (4.16)$$

$$\frac{\tau_y}{\rho} = \nu \left. \frac{\partial v}{\partial z} \right|_{z=0} = V_s \sqrt{\frac{\nu\omega}{2}} (\cos(\omega t + \alpha_v) + \sin(\omega t + \alpha_v)). \quad (4.17)$$

Deriving the magnitude and direction of $\boldsymbol{\tau}_w$, we obtain the coveted relationship between $\boldsymbol{\tau}_w$ and $\mathbf{v}_s$:

$$\tau_w = V_s \sqrt{\rho\mu\omega} \quad (4.18)$$

$$\tan \alpha_\tau = \frac{\tau_y}{\tau_x} = \frac{(\cos \alpha_v + \sin \alpha_v)}{(\cos \alpha_v - \sin \alpha_v)} \Rightarrow \alpha_\tau = \alpha_v + 45^\circ. \quad (4.19)$$

The boundary layer model predicts that the magnitude of $\boldsymbol{\tau}_w$ is proportional to V_s , and that the angle between $\boldsymbol{\tau}_w$ and $\mathbf{v}_s$ is 45° , with $\boldsymbol{\tau}_w$ tangent to the spiral at its origin (see Figure 4.2[c]).

The relationship between the characteristic length $\sqrt{\frac{\nu}{\omega}}$ and surface height h will influence the suitability of the model. If $h \ll \sqrt{\frac{\nu}{\omega}}$, viscous effects would extend up to the free surface,

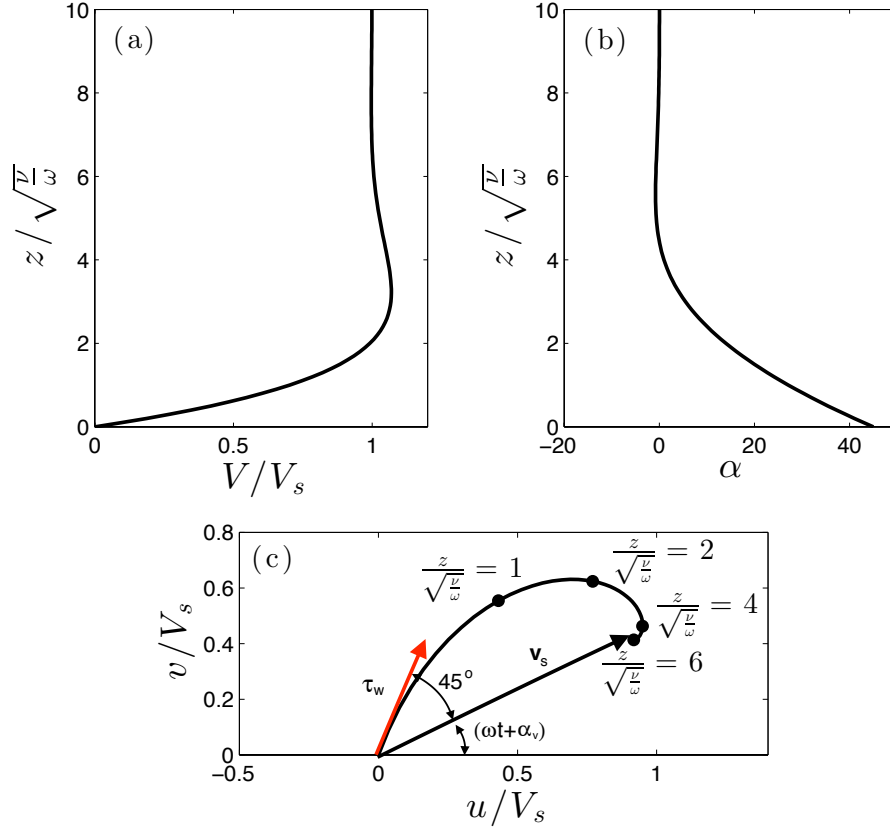


Figure 4.2: Boundary layer profile. (a) Velocity magnitude V as a function of z . (b) Angle α of the velocity $\mathbf{v}$ with respect to $\mathbf{v}_s$ as a function of z . (c) Ekman spiral.

affecting the inviscid character of the flow outside the boundary layer; on the contrary, if $h \gg \sqrt{\frac{\nu}{\omega}}$, the free surface velocities are undisturbed and the model is expected to be a good approximation.

This derivation of the boundary layer velocity field is an alternative way of deriving the extended Stokes solution [119] (the commonly used WSS model). The Stokes solution is the particular case where the velocity in the far field is quiescent due to the hypothetical unbounded problem configuration. Thus, if regarded from a non-inertial frame of reference where the plate is static, the fluid in the far field is predicted to move homogeneously as $\mathbf{v}_s = -\mathbf{v}_o = A [\sin(\omega t)\mathbf{e}_x - \cos(\omega t)\mathbf{e}_y]$. In the present formulation, this is equivalent to $V_s = a\omega$ and $\alpha_v = -\pi/2$; and Stokes' solution can be obtained from (4.12) and (4.13), and the commonly used Stokes WSS prediction (4.1) from (4.18). On account of this, the boundary layer is referred to as a Stokes boundary layer.

4.2.2 PT-Stokes wall shear stress model

The PT-Stokes WSS model is a generalisation of the Stokes model where the velocity in the far field is assumed to behave as a potential flow confined by the orbital shaker boundaries (free surface and side walls) instead of the quiescent flow assumed by Stokes. In Chapter 3, we showed that the potential theory provides a reasonably good description of the behaviour of orbital shakers, suggesting a surface velocity (in cylindrical coordinates)

$$u_{rs}^{\text{PT}}(r, \theta, t) = -2a\omega \sin(\omega t - \theta) \sum_{n=1}^{\infty} \left[\frac{\epsilon_{1n}}{(\epsilon_{1n}^2 - 1)} \frac{1}{\left(\frac{\omega_{1n}}{\omega}\right)^2 - 1} \frac{J_1'(\epsilon_{1n} \frac{r}{R})}{J_1(\epsilon_{1n})} \frac{\cosh(\epsilon_{1n} \frac{h}{R})}{\cosh(\epsilon_{1n} \frac{d}{R})} \right] \quad (4.20)$$

$$u_{\theta s}^{\text{PT}}(r, \theta, t) = 2a\omega \cos(\omega t - \theta) \sum_{n=1}^{\infty} \left[\frac{1}{\frac{r}{R}} \frac{1}{(\epsilon_{1n}^2 - 1)} \frac{1}{\left(\frac{\omega_{1n}}{\omega}\right)^2 - 1} \frac{J_1(\epsilon_{1n} \frac{r}{R})}{J_1(\epsilon_{1n})} \frac{\cosh(\epsilon_{1n} \frac{h}{R})}{\cosh(\epsilon_{1n} \frac{d}{R})} \right] \quad (4.21)$$

where h is the free surface elevation relative to the well bottom; $J_1(r)$ is a Bessel function of the first kind, of order 1; ϵ_{1n} is the n th root of $J_1'(s)$ (where the prime denotes differentiation with respect to the argument); $\lambda_{1n} = \epsilon_{1n}/R$; and ω_{1n} is the natural frequency of the n th mode given by the dispersion relationship $\omega_{1n}^2 = g\lambda_{1n} \tanh(\lambda_{1n}d)$. The vertical velocity u_{zs}^{PT} has been neglected.

In Cartesian form, (4.20) and (4.21) are expressed as

$$u_s^{\text{PT}} = u_{rs}^{\text{PT}} \cos \theta - u_{\theta s}^{\text{PT}} \sin \theta \quad (4.22)$$

$$v_s^{\text{PT}} = u_{rs}^{\text{PT}} \sin \theta + u_{\theta s}^{\text{PT}} \cos \theta. \quad (4.23)$$

On the other hand, Stokes' model assumes a quiescent far field, which in the non-inertial frame of reference is given by

$$u_s^{\text{St}}(t) = a\omega \sin(\omega t) \quad (4.24)$$

$$v_s^{\text{St}}(t) = -a\omega \cos(\omega t). \quad (4.25)$$

Stokes and the potential theory's surface velocity predictions are not in agreement. For instance, at the centre of the well at the given instant $t = 0$ and assuming $h = d$:

$$\mathbf{v}_s^{\text{St}}(t = 0) = -a\omega \mathbf{e}_y \quad (4.26)$$

$$\mathbf{v}_s^{\text{PT}}(r = 0, \theta = 0, t = 0) = a\omega \sum_{n=1}^{\infty} \left[\frac{\epsilon_{1n}}{(\epsilon_{1n}^2 - 1)} \frac{1}{\left(\frac{\omega_{1n}}{\omega}\right)^2 - 1} \frac{1}{J_1(\epsilon_{1n})} \right] \mathbf{e}_y. \quad (4.27)$$

	V_s	$\omega t + \alpha_v$	
Stokes' solution (St)	$\sqrt{(u_s^{\text{St}})^2 + (v_s^{\text{St}})^2} = a\omega$	$\omega t - \pi/2$	(4.28)
Potential Theory (PT)	$\sqrt{(u_s^{\text{PT}})^2 + (v_s^{\text{PT}})^2}$	$\arctan\left(\frac{v_s^{\text{PT}}}{u_s^{\text{PT}}}\right)$	(4.29)

Table 4.1: Surface velocity magnitude V_s and x-axis angle ($\omega t + \alpha_v$) predicted by the analytical models.

Typically, $\frac{\omega_{1n}}{\omega} > 1$ giving a positive summation factor; hence, the predicted velocities at the centre have an opposite direction. This discrepancy highlights the limitations imposed by the Stokes model in the far field velocity. The surface velocities predicted by each model in the PT-Stokes model notation are summarised in Table 4.1.

Equations (4.18) and (4.19) together with the surface velocity prediction of the potential theory (4.29) in Table 4.1 form the PT-Stokes WSS model for shallow orbital shakers that will be validated in this chapter.

4.3 Simulations

This study uses, for the validation of the PT-Stokes WSS model, the simulation case studies introduced in detail in § 3.3. The cases display a wide range of flow behaviours including wave breaking, multi-crest waves, and some cases in which the bottom is uncovered, as unveiled in Chapter 3. The simulation configurations are summarised in Table 4.2 using the dimensionless groups:

$$F = \frac{a\omega^2}{g} \quad E = \frac{a}{R} \quad \Gamma = \frac{d}{R} \quad Re = \frac{R^2\omega}{\nu}. \quad (4.30)$$

As explained in § 3.2, the dimensionless forcing F represents the ratio between the inertial force per unit mass and the gravitational acceleration. The eccentricity E represents the ratio between the orbital forcing radius a and the cylinder radius R . The shallowness Γ represents the ratio between the horizontal and the vertical scales of the cylinder. The Reynolds number represents the ratio between the local acceleration and the viscosity forces.

A dimensionless height $\tilde{d} = \frac{d}{\sqrt{\frac{\nu}{\omega}}}$ has been included in the table and represents the ratio between the fluid height at rest and the viscous penetration depth (Chakraborty et al. [25] name it the

Stokes number). This dimensionless number characterises whether the bottom viscous effects reach the free surface ($\tilde{d} < 1$), presumably affecting the model validity; it can be rewritten using the dimensionless groups as $\tilde{d} = \Gamma Re^{1/2}$.

All simulations can be considered to be in the shallow water regime ($\Gamma \ll 1$). The dimensionless height $\tilde{d}$ is 7.9 in most cases, hence, the model assumption ($d \gg \sqrt{\nu/\omega}$) is satisfied fairly well. For the Re cases, $\tilde{d}$ varies from 2.8 ($Re1$) to 11.2 ($Re5$); the $Re1$ case is the most extreme case, and the viscous effects are expected to reach the free surface.

4.4 Validation of the PT-Stokes Wall Shear Stress model

The aim of this section is to verify in detail the extent to which the PT-Stokes WSS model predicts τ_w in orbitally shaken shallow fluid layers in cylindrical containers. This validation is carried out in two parts: first, the suitability of the boundary layer model is validated by observing in the simulations the velocity profile, the boundary layer characteristic thickness and the relationship of τ_w to $\mathbf{v}_s$; second, the analytical surface velocities and the τ_w predicted by the PT-Stokes WSS model are compared to the simulation observations. The low-forcing cases (L cases), where $F \ll 1$ (see Table 4.2), are used in order to limit the non-linear effect that especially affects the behaviour of the free surface. For each case, the domain has been sampled radially and azimuthally as shown in Figure 4.3. The sample points have been divided into three regions: centre, $r < 0.2R$ (purple points); intermediate, $0.4R < r < 0.6R$ (orange points); and edge, $r = 0.96R$ (green points). As we described in § 2.5, the flow forced response is periodic, thus, for a point at a given radial distance, sampling in the azimuthal direction is equivalent to sampling in time.

4.4.1 Relationship of the wall shear stress to the surface velocity

In the boundary layer model, the velocity field is characterised by the diffusive length scale $\sqrt{\frac{\nu}{\omega}}$. At this z level, the theory (4.14) predicts that $V(z = \sqrt{\frac{\nu}{\omega}}) = 0.7V_s = 0.66V_{max}$; by means of the inverse operation, the characteristic thickness boundary layer δ has been defined as the z level where the observed velocity is 0.66 of the observed maximum velocity ($V(z = \delta) = 0.66V_{max} \mapsto \delta$). Figure 4.4 shows a comparison of δ to the theoretical value $\sqrt{\nu/\omega}$ at each sample point.

Case	E	F	Re	Γ	$\tilde{d}$
$Re1$	0.45	0.13	240	0.18	2.8
$Re2$	"	"	480	"	4.0
$Re3$	"	"	959	"	5.6
$Re4$	0.45	0.13	1918	0.18	7.9
$Re5$	"	"	3836	"	11.2
$EF1$	0.07	0.02	1981	0.18	7.9
$EF2$	0.15	0.04	"	"	"
$EF3$	0.29	0.08	"	"	"
$EF4$	0.58	0.16	"	"	"
$EF5$	1.16	0.32	"	"	"
$E1$	0.09	0.13	1981	0.18	7.9
$E2$	0.18	"	"	"	"
$E3$	0.37	"	"	"	"
$E4$	0.74	"	"	"	"
$E5$	1.48	"	"	"	"
$E6$	4.61	"	"	"	"
$E7$	9.22	"	"	"	"
$Fl1$	0.09	0.25	1981	0.18	7.9
$Fl2$	"	0.27	"	"	"
$Fl3$	"	0.30	"	"	"
$Fl4$	"	0.36	"	"	"
$Fm1$	0.45	0.03	1981	0.18	7.9
$Fm2$	"	0.06	"	"	"
$Fm3$	"	0.08	"	"	"
$Fm4$	"	0.24	"	"	"
$Fh1$	1.48	0.10	1981	0.18	7.9
$Fh2$	"	0.11	"	"	"
$Fh3$	"	0.15	"	"	"
$L1$	0.05	0.0009	511	0.18	4.1
$L2$	0.09	0.0018	"	"	"
$L3$	0.27	0.0030	383	"	3.5
$L4$	0.14	0.0060	767	"	6.3
$L5$	0.27	0.0120	"	"	"

Table 4.2: Summary of all simulation cases analysed in this study. The configuration is given in the dimensionless groups defined in (4.30). In addition, the dimensionless height $\tilde{d}$ is given, which provides an indicator of the validity of the model.

At the centre region, $\delta \sim \sqrt{\nu/\omega}$; as we move to the edge, the scatter increases, denoting a discrepancy in the velocity profile shape. Interestingly, δ has a lower limit of $0.6\sqrt{\nu/\omega}$.

The velocity profile observed has been plotted in the vertical direction normalised by the characteristic thickness δ up to the free surface for each point in the different regions (Figure 4.5); this allows for comparison to the theoretical profile shape (4.14) (discontinuous line). In the

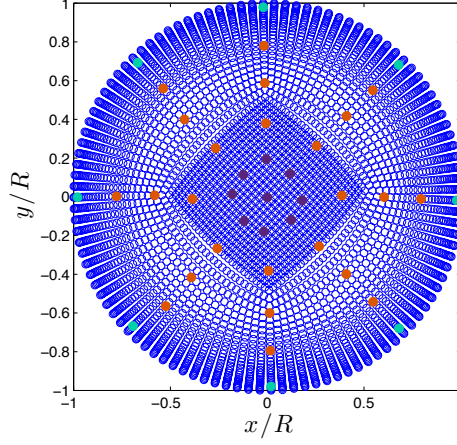


Figure 4.3: Mesh points at a z -constant plane. The sample points are divided into three regions: centre (purple points); intermediate (orange points); and edge (green points).

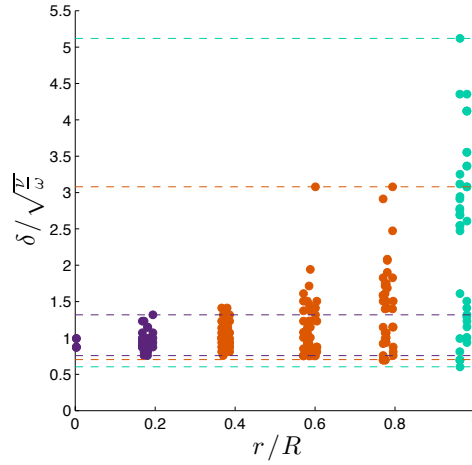


Figure 4.4: Boundary layer characteristic thickness δ normalised by the characteristic penetration depth $\sqrt{\nu/\omega}$ for each sample point for the L cases.

centre region, the theoretical profile correctly describes the velocity shape except for some points where the maximum peaks at the free surface instead of $z = 3.2\delta$ as predicted by the theory. As we move to the edge, the scatter increases. In the intermediate region, the theoretical profile correctly describes the velocity slope at the bottom with very few exceptions; however for $z > \delta$, the profiles show multiple behaviours. In the edge region, most profiles have a shape that differs from the theoretical prediction. The latter is caused by the influence of the side walls, which is not considered in the theoretical boundary layer model.

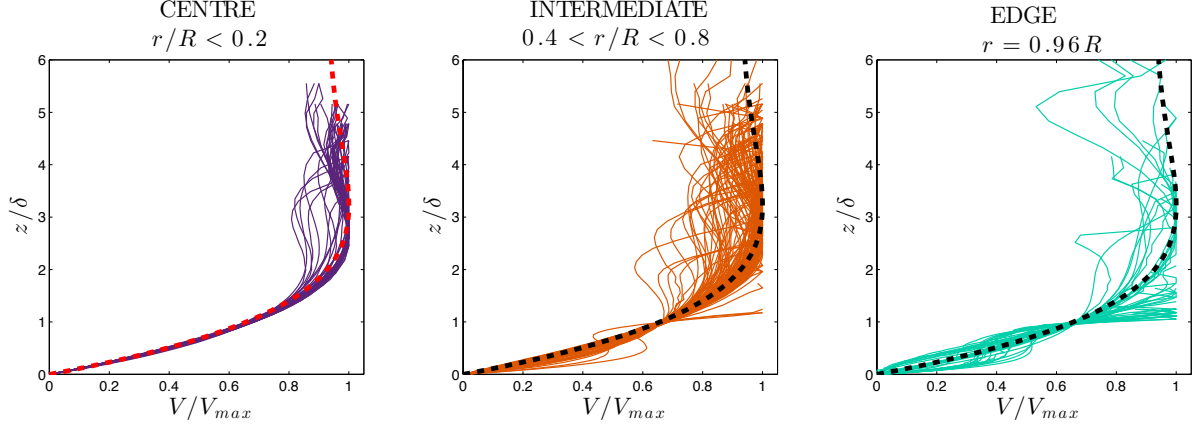


Figure 4.5: Velocity magnitude profiles in the vertical direction z normalised by the boundary layer characteristic thickness δ at the different regions for the L cases. The discontinuous line (red or black) is the theoretical profile (4.14).

A linear regression with the equation $x = y$ has been used to fit the paired data prediction-observation (X_p, X_o) . Here, the observation has been taken as the dependent variable [107], from where the coefficient of determination $r^2 = 1 - SS_{res}/SS_{tot}$ can be calculated, where SS_{res} is the sum of the regression squared residuals $\sum(X_o - X_p)^2$, and SS_{tot} is the total sum of the squares in the dependent variable $(n-1)\sigma(X_o)$; here, n is the number of pairs, and σ represents the variance. The coefficient r^2 is used as a measure of the goodness of the correlation between observation and prediction. Note that r^2 can be negative because the constant term of the fitting curve has been omitted; $r^2 < 0$ indicates a fit that is poorer than just fitting to the mean.

The Ekman spiral (Figure 4.2[c]) connects τ_w to $\mathbf{v}_s$ in magnitude and direction by $\tau_w = \rho V_s \sqrt{\nu \omega}$ and $\alpha_\tau = \alpha_v + 45^\circ$, respectively. Figure 4.6 shows the values observed in the simulations in the abscissa against the theory prediction in the ordinate. The τ_w magnitude (Figure 4.6[a-c]) is predicted well in the centre and intermediate regions ($r^2 = 0.97$ and 0.91 , respectively); in the edge region, the correlation decreases, outlining the influence of the side walls as observed in the boundary layer profiles. The observed τ_w angle in the centre region (Figure 4.6[d]) remains practically constant at 125° , but the prediction varies between $+80^\circ$ and $+145^\circ$. The points' scattering increases in the intermediate region and further towards the edge (Figure

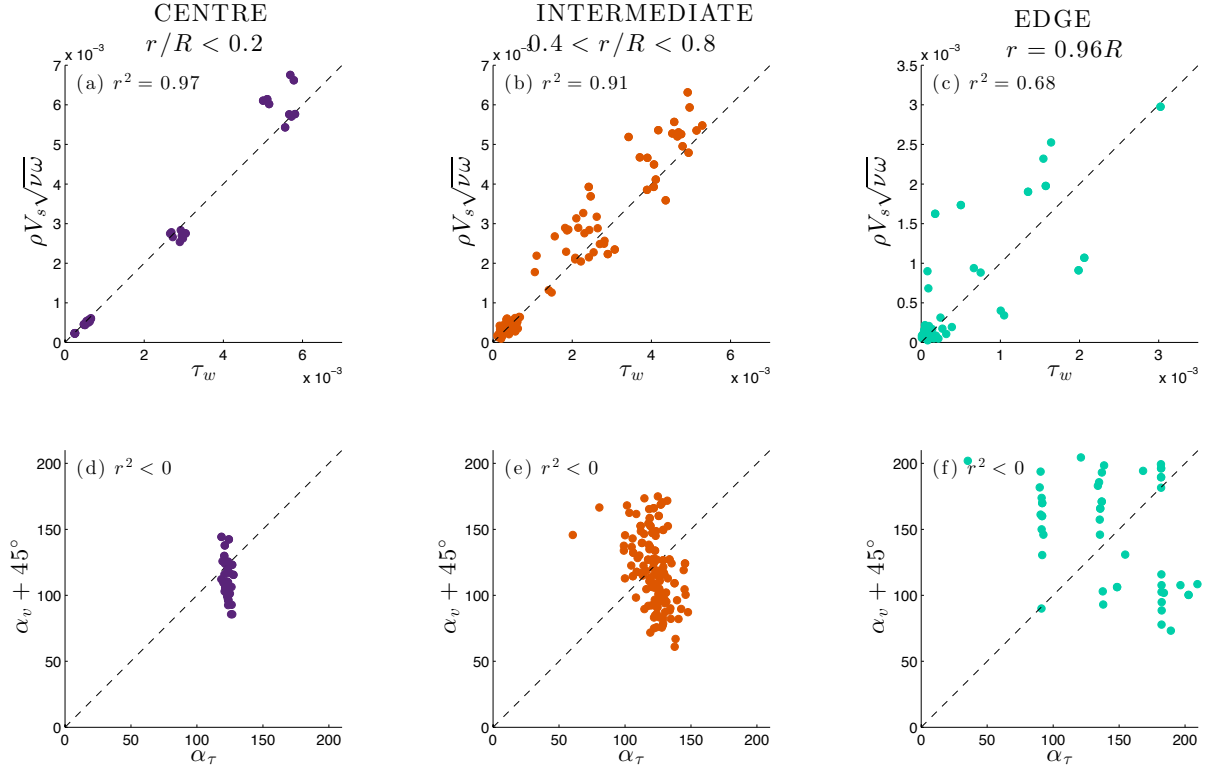


Figure 4.6: Relationship of τ_w to $\mathbf{v}_s$ for L cases in the different well regions. (a)-(c) observed τ_w against the prediction $\rho V_s \sqrt{\nu \omega}$ (values presented in Pa); (d)-(f) observed α_τ against the prediction $\alpha_v + 45^\circ$ (values presented in degrees). r^2 is the coefficient of determination. The discontinuous line marks the linear relationship of proportionality one.

4.6[e][f]) ($r^2 < 0$). The discrepancy in the centre and intermediate regions can be attributed to disturbances near the free surface, where, despite the perfectly flat surface, the velocity vectors are deviated from the linear solution and point in the direction of a subtle bifurcation line; these disturbances vanish gradually from the free surface to the inner fluid. A case with a flat surface but at higher F ($Fm1$) has been studied for comparison, and contrarily, it presents a linear behaviour in the centre region, what highlights that a peculiar behaviour occurs for the very low-forcing cases near the free surface. At the edge, the discrepancy is mainly caused by the side walls.

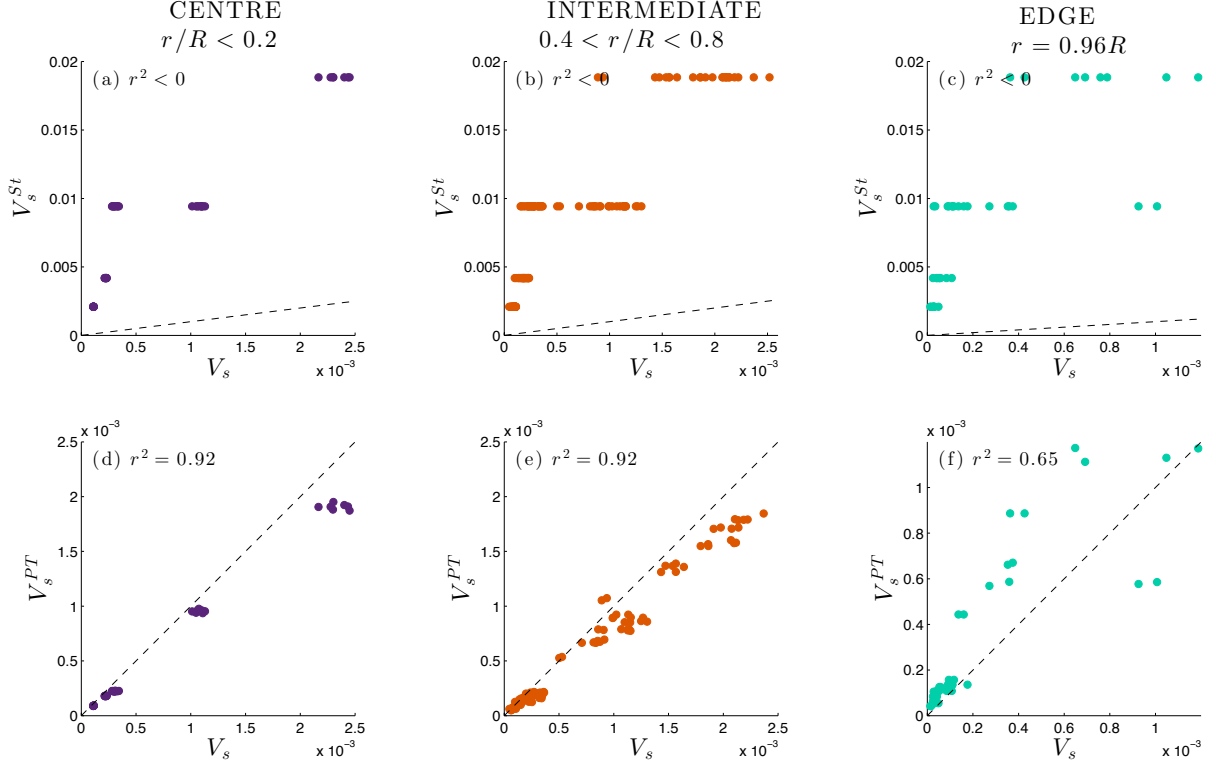


Figure 4.7: Velocity predictions compared to observations for L cases in the different well regions (values presented in m/s). (a-c) V_s against the velocity predicted by Stokes's model V_s^{St} (4.28). (d-f) V_s against the velocity predicted by the potential theory V_s^{PT} (4.29). r^2 is the coefficient of determination. The discontinuous line marks the linear relationship of proportionality one.

4.4.2 PT-Stokes wall shear stress model

The surface velocities have been compared to the Stokes solution (4.28) (Figure 4.7[a]-[c]) and the potential theory (4.29) (Figure 4.7[d]-[f]). The Stokes solution V_s^{St} severely overpredicts the surface velocity magnitude by a factor of 10, while the potential theory velocity magnitude V_s^{PT} yields a remarkably better prediction in the centre and intermediate regions ($r^2 = 0.92$) — V_s is underestimated (30% on average) due to the absence of the viscous effects in the potential theory, as discussed in § 3.4. The surface velocity direction α_v has also been analysed (data not shown). The Stokes model predicts a constant $\alpha_v^{\text{St}} = -90^\circ$, while the observed α_v varies from 15° to 160° along the free surface. Focusing at the centre region, where the Stokes solution is expected to work better, α_v varies from 40° to 100° , an average angle being $\bar{\alpha}_s = 72^\circ$, almost the opposite direction to α_v^{St} . The potential theory predicts the angle notably better: α_v^{PT} in the centre region has an approximately constant value of 90° . The greater variability in the

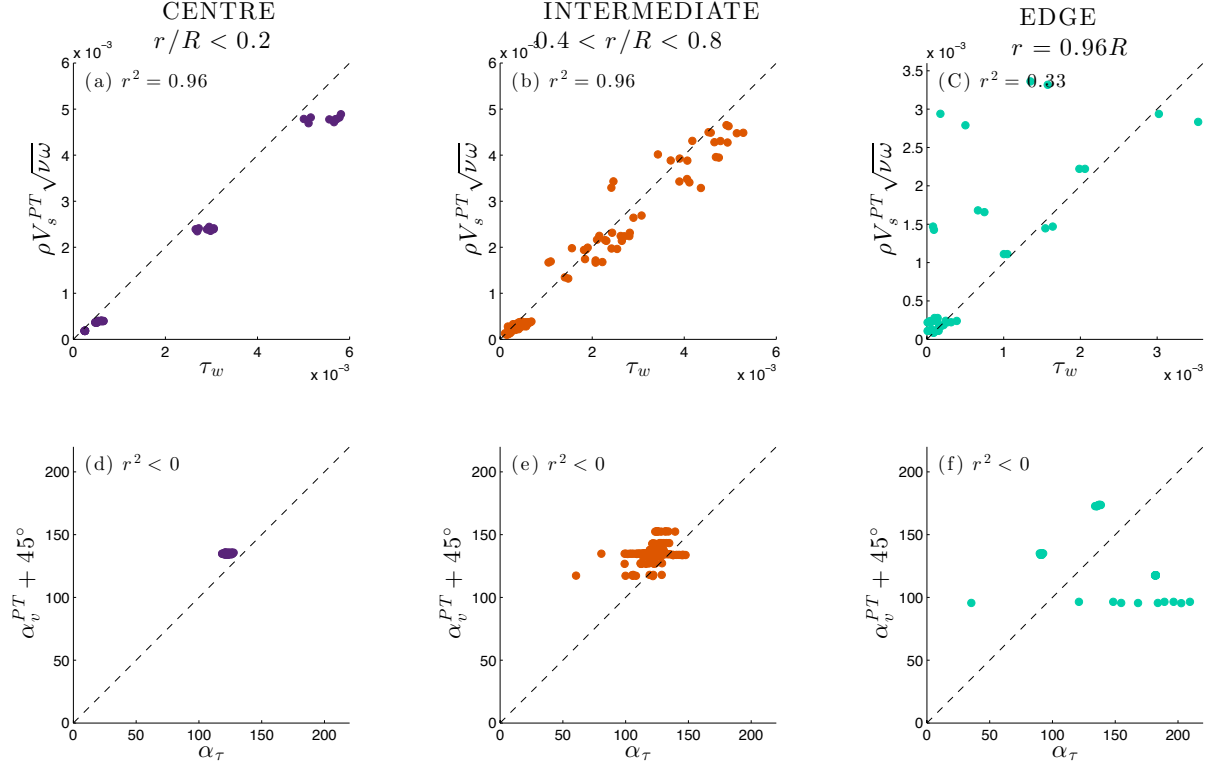


Figure 4.8: τ_w prediction by the PT-Stokes model compared to observations for L cases in the different well regions. (a-c) Observed τ_w against the prediction $V_s^{PT} \sqrt{\rho \mu \omega}$ (values presented in Pa). (d-f) Observed α_τ against the prediction $\alpha_v + 45^\circ$ (values presented in degrees). r^2 is the coefficient of determination. The discontinuous line marks the linear relationship of proportionality one.

observed angle is due to disturbances observed near the free surface. In fact, the potential theory correctly predicts the velocity direction some computational cells below the free surface.

Lastly, we have compared the observed τ_w and α_τ to the PT-Stokes model prediction $\rho V_s^{PT} \sqrt{\mu \omega}$ and $\alpha_v^{PT} + 45^\circ$, respectively. The magnitude τ_w (Figure 4.8[a]-[c]) shows a very good correlation in the centre and intermediate regions ($r^2 = 0.96$). As expected from the results in the potential theory velocity prediction, τ_w is underestimated by 30% on average. Interestingly, the angle prediction (Figure 4.8[d]-[f]) is improved as compared to the direct comparison (Figure 4.4[d]-[f]); in particular, the dispersion decreases substantially at the centre. This result corroborates that disturbances of unknown origin are occurring near the free surface for the L cases, which do not follow the expected linear behaviour.

4.5 Applicability to Highly Forced Cases

The aim of this section is to explore the applicability of the PT-Stokes WSS model to highly forced cases using the case studies summarised in Table 4.2, in which the dimensionless forcing F and eccentricity E take a wide range of values.

Firstly, a qualitative schematic overview of the spirals observed at the centre point ($x = y = 0$) for each case study in the E - F - Re parameter space is presented in Figure 4.9. Each framed spiral is depicted by the velocity vectors $\mathbf{v}$ (black arrows) in the $\{x, y\}$ plane projection from $z = 0$ to $z = h$, and $\boldsymbol{\tau}_w$ (red arrow) at the bottom wall (see § 4.2.1 for the description of the theoretical Ekman spiral). The spirals are framed using a colour code based on the breaking classification used in Chapter 3 (§ 3.7): non-breaking (black), breaking and covered bottom (green), and breaking and uncovered bottom (blue). Furthermore, some of the cases are pinned with a star to denote that they have distinctive features, and to connect them with the points in Figure 4.10; there are grouped under the following characteristics:

- Group (a) (black frame, yellow star) corresponds to low-forcing cases (L cases), where a wider spiral than the theoretical prediction is observed due to the disturbances observed in the velocity pattern near the free surface (see § 4.4.1).
- Group (b) (black frame, red star) corresponds to cases with very high E , where the spiral looks similar to the theoretical Ekman spiral but the velocity rotates about 10° and increases in magnitude in the region close to the free surface.
- Group (c) (supplementary window, green frames) corresponds to the Re cases, where only viscosity is varied, and the spiral is observed to develop as the viscosity decreases.
- Group (d) (blue frame, black star) corresponds to a case with very high F , where a large breaking area is observed in the free surface.
- Group (e) (green and blue frame, red star) corresponds to cases close to a resonance line, where the potential model overpredicts the surface and velocity amplitudes due to the lack of viscosity to damp resonant frequencies.

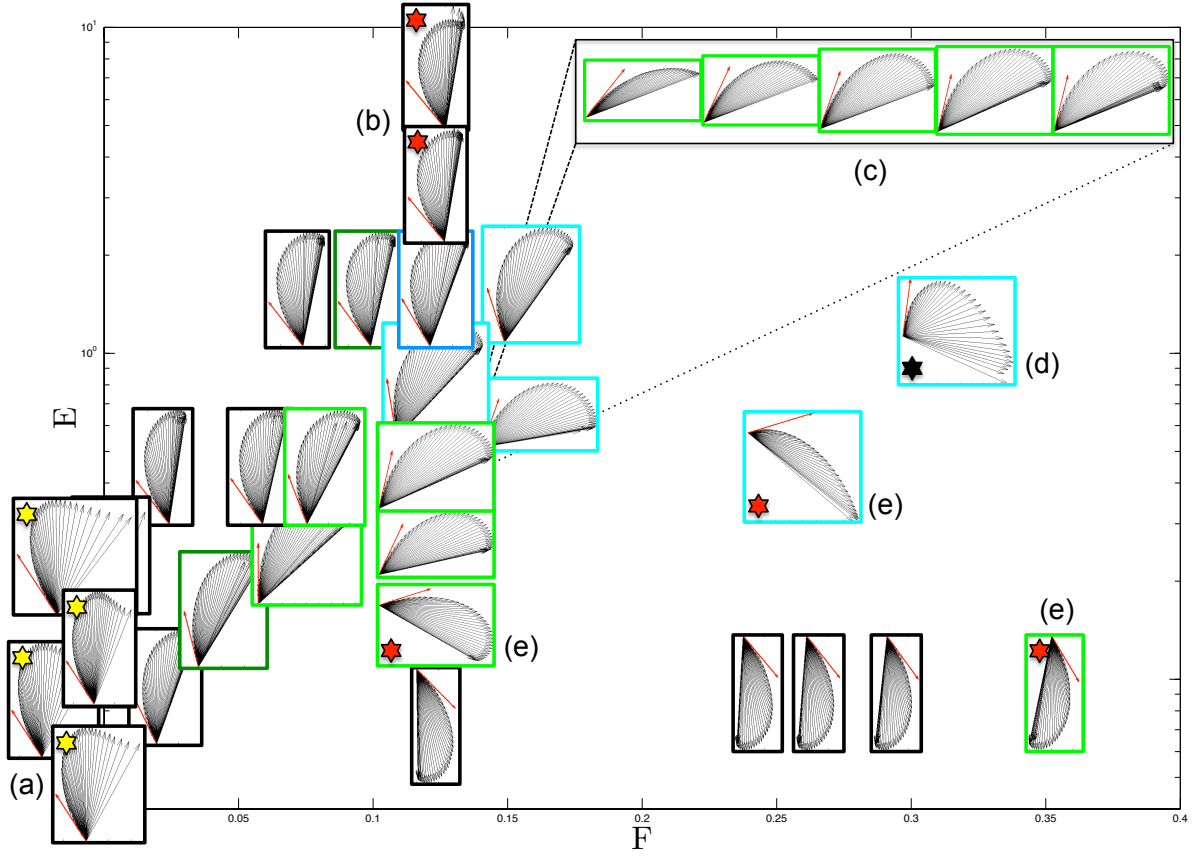


Figure 4.9: Ekman-like spirals at the well centre for all case studies. Each frame represents a velocity projection (black arrows) in the horizontal plane $\{x, y\}$ at the centre ($x = y = 0$) for all 33 simulations in a E - F - Re parameter space; the red arrow represents τ_w . The frames are colour coded as non-breaking and covered (black), breaking and covered (green), and breaking and uncovered (blue). Some of the cases are pinned with a black, red or yellow star denoting cases with distinctive features (to connect them with Figure 4.10), and they are grouped with letters (a) to (e).

A quantitative analysis of the boundary layer thickness δ , the relationship of τ_s to $\mathbf{v}_s$, and the PT-Stokes WSS model magnitude are presented in Figure 4.10 for all case studies in the centre, intermediate and edge regions. The focus below is on explaining the behaviour in the centre region.

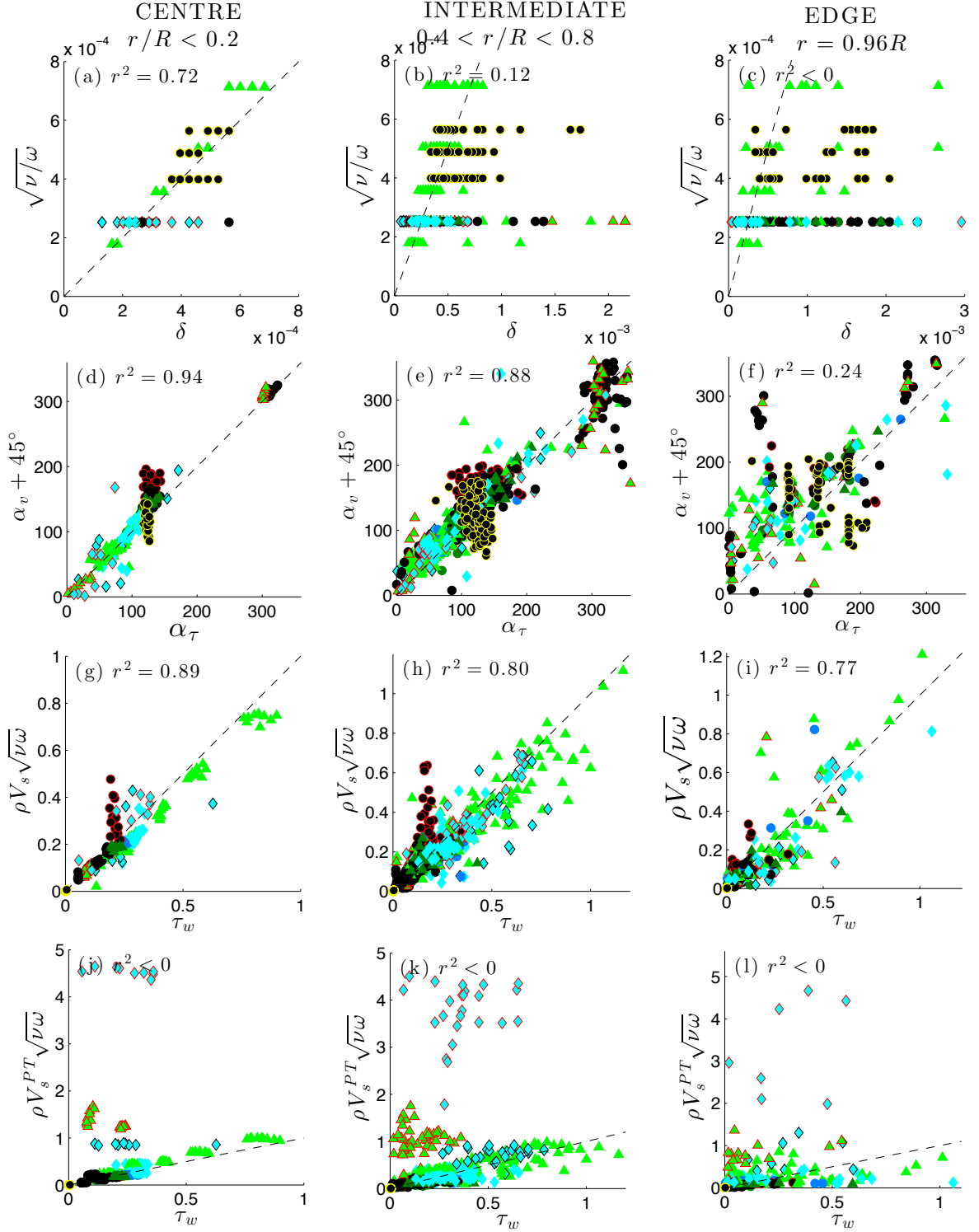
The boundary layer thickness δ shows a good correlation with the viscous penetration depth $\sqrt{\nu/\omega}$ (Figure 4.10[a]) except for the following cases: the severely breaking cases (blue-black, group d); and the very high E cases (black-red, group b), where points with $\delta > 10^{-3}$ have been omitted because the overestimation of δ is due to the relative increment of the velocity magnitude in the region near the free surface.

The WSS angle α_τ is well predicted from α_v (Figure 4.10[d]) except for the following cases: low-forcing cases (black-yellow, group a) experience a rapid change of direction in the velocity vector at the surface (see spirals in Figure 4.9), opening up the spiral and leading to angles between $\mathbf{v}_s$ and $\boldsymbol{\tau}_s$ greater than 45° ; cases with high E (black-red, group b) have a peculiar behaviour at the free surface despite the expected linear behaviour of the flat surface; and cases with large breaking areas (blue-red, blue-black) show large scattering.

The WSS magnitude τ_w is well predicted from V_s (Figure 4.10[g]) except for the following cases: very high E cases (black-red, group b) — as previously noted — show a peculiar behaviour in the free surface, and it has been verified that the relationship of $\boldsymbol{\tau}_s$ to $\mathbf{v}_s$ improves a couple of computational cells underneath the free surface; and very highly forced cases (blue-black, group d) show a dispersive behaviour even at the centre region.

The PT-Stokes WSS model predicts τ_w magnitude well (Figure 4.10[j]) except for the following cases: very highly forced cases (blue-black, group d), which are in a very non-linear regime; and the cases close to the resonance lines (green-red and blue-red, group e), where the potential prediction overestimates the velocity magnitude due to the lack of viscosity to damp the resonant frequencies. The correlation is poor ($r^2 < 0$), however, if the coefficient of determination is calculated omitting the resonant cases, the correlation improves notably ($r^2 = 0.4$).

The PT-Stokes WSS model is not able to predict the α_τ angle for the highly forced cases (data not shown). The potential theory model used to predict the surface velocity is linear and inviscid, and cannot reproduce the surface velocity angles in the highly force regime where wave breaking alters the linearity of the free surface (a potential and inviscid flow can only satisfy the boundary conditions at a stationary free-surface plane [9], p.366).



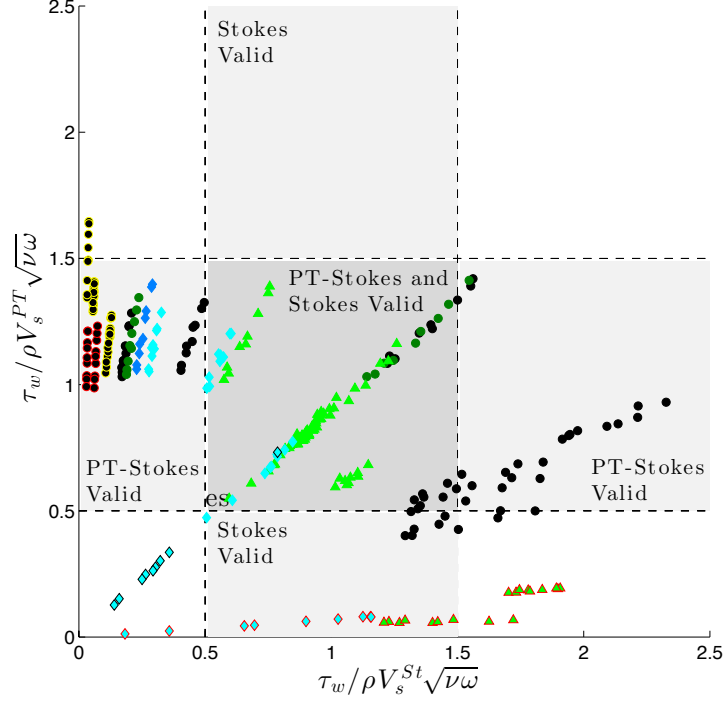


Figure 4.11: Comparison of τ_w observation to prediction ratio in the Stokes and PT-Stokes models for all cases in the centre region. The valid grey shadowed areas are regions where the discrepancy between observation and model specified is $< 50\%$. The marker's code is the same as in § 4.5.

4.6 Prediction of Wall Shear Stress Metrics

The PT-Stokes model is a notable improvement on the Stokes WSS model, as Figure 4.11 demonstrates. We have plotted the centre region points for all case studies in a graph with the observed τ_w magnitude over Stokes' prediction in the abscissa, and τ_w over PT-Stokes' prediction in the ordinate. The validity domain of each of the models, defined as the region where the discrepancy between observation and model is $< 50\%$, is depicted as light grey shaded areas, and a dark grey shaded area where they overlap. The new model's validity domain covers practically the same cases as the Stokes' solution and extends its validity to all non-breaking or incipient breaking cases (black, dark green, and dark blue markers); the exceptions are the resonant cases (blue-red diamonds and green-red triangles), which are partly captured by the Stokes solution but overpredicted by the PT-Stokes model due to the absence of viscosity, as previously discussed. The very highly forced case (blue-black diamonds) is overpredicted by both solutions.

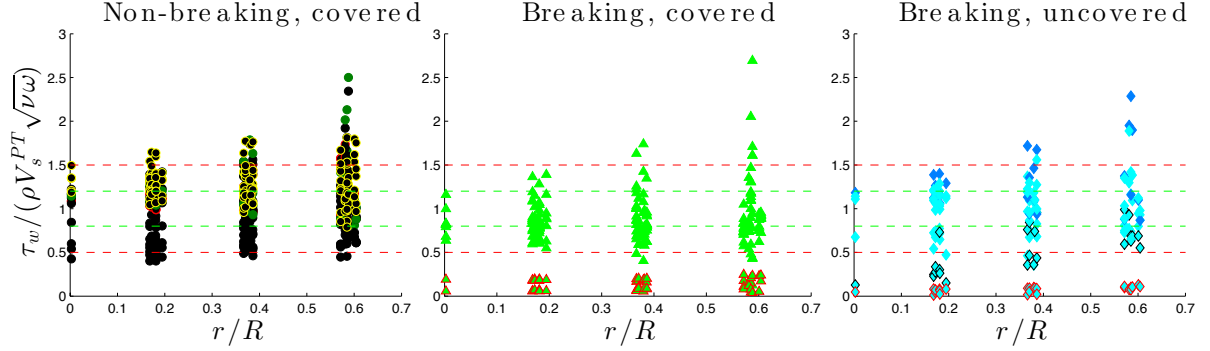


Figure 4.12: τ_w observation to prediction ratio in the PT-Stokes model for the three different regimes identified in Chapter 3.

The PT-Stokes WSS model provides a spatial prediction of τ_w . Figure 4.12 shows the observation to prediction ratio as a function of the normalised radial distance. The PT-Stokes model estimates τ_w within a 50% discrepancy up to the radial distance $r = 0.5R$, with the exception of the resonant and very highly forced cases.

The PT-Stokes WSS model also provides a spatial prediction of α_τ which unlocks the possibility of calculating WSS metrics including those which take into account directionality. Six WSS metrics (see § 2.4 for definition) have been plotted in Figure 4.13 for the reference case Re_4 , which compares the computational model, the Ekman spiral relationship (the observed WSS to observed surface velocity relationship), and the PT-Stokes model. For the TAWSS, the PT-Stokes model captures the metric tendency up to $r = 0.7R$, and for the maxWSS, a similar flat profile is predicted also up to $r = 0.7R$. For the metrics including directionality, the PT-Stokes model fails to represent MagMeanWSS, and thus the metric OSI; for the transverse WSS metrics, the PT-Stokes model captures the metrics' tendency. We observe that the profiles of the WSS metrics in the PT-Stokes model are smooth, because the linear potential model used does not capture the non-linear effects of the free surface. However, the prediction of the metrics is notably improved using the Ekman spiral relationship, pointing out that the relationship of τ_w to $\mathbf{v}_s$ is a good approximation for most of the domain, and that the potential theory needs to be refined to include viscous effects. Finally, in Appendix C, Figure C.1 shows the plot of the five WSS metrics studied for all case studies, however, it has been beyond the scope of this thesis to extend this analysis to all cases.

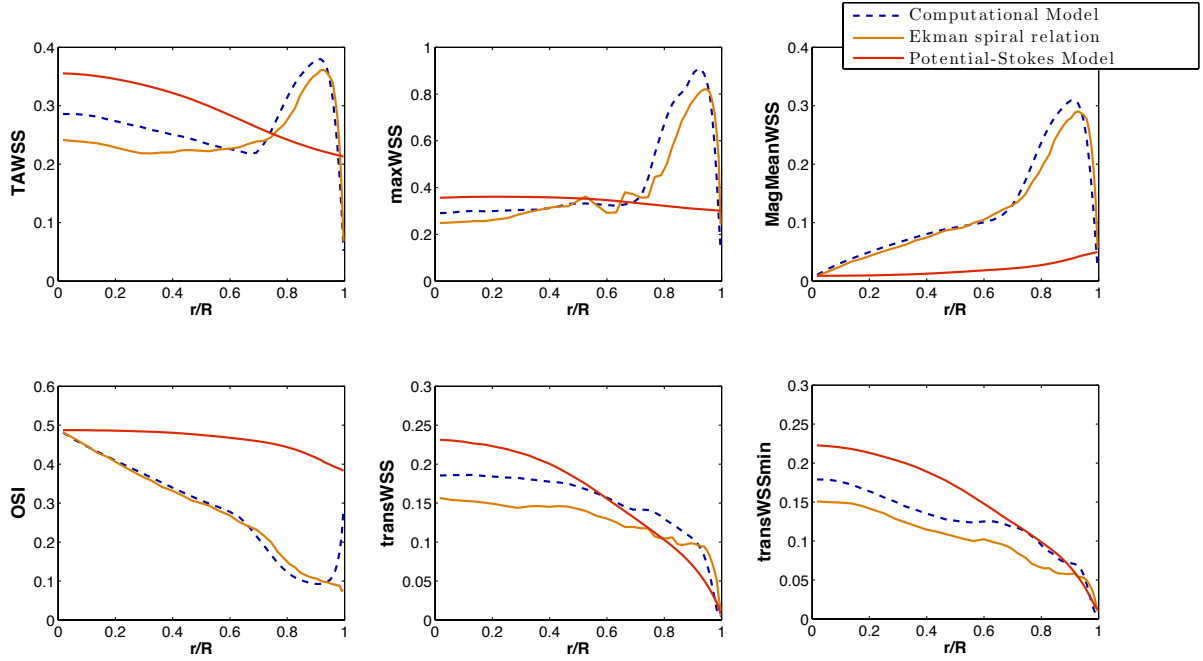


Figure 4.13: WSS metrics (§ 2.4) observed (computational model) and predicted (Ekman spiral relationship and the PT-Stokes model).

4.7 Conclusions

We have presented an improved Stokes WSS model for orbitally shaken shallow fluid layers that predicts WSS in magnitude and direction, which is referred to as the PT-Stokes WSS model. The model considers that the orbital shaker fluid behaves as a potential flow with a Stokes viscous boundary layer at the bottom wall. The Stokes boundary layer has a characteristic diffusive length $\sqrt{\nu/\omega}$ (viscous penetration depth), and the horizontal velocity profile takes a spiral-like shape (bottom Ekman spiral) that relates the WSS vector $\boldsymbol{\tau}_w$ to the outer velocity field $\mathbf{v}_s$ (4.18)-(4.19). Beyond the boundary layer, the fluid velocity is modelled by the potential flow theory $\mathbf{v}_s^{\text{PT}}$. The main assumptions of the model are the layer shallowness ($d \ll R$, where d is the fluid height at rest and R the well radius) and a high static height to boundary layer thickness ratio ($\delta \ll d$). The PT-Stokes WSS model is summarised in equations (4.2), (4.3), and (4.29).

The model has been validated using low-forcing cases (L cases). The observed boundary layer features a characteristic thickness δ , the vertical profile is similar to the model prediction $\sqrt{\nu/\omega}$ in the centre and intermediate regions, and the spiral relationship of the observed τ_w to the observed $\mathbf{v}_s$ correlates strongly in magnitude. For the outer field, the Stokes and potential analytical solutions of the velocity field have been compared to the observed velocity. Stokes' solution fails to predict the low-forcing cases, whereas the potential theory is in good agreement away from the side walls, where viscosity becomes relevant. Finally, the PT-Stokes WSS model shows a strong correlation for the L cases away from the side walls, since, close to the edge, the boundary layer model assumptions are not satisfied; for instance, the presence of the side wall introduces a vertical velocity component neglected in the model and its presence also impedes the assumed periodic constant character of the surface velocity (4.7).

Furthermore, the model's applicability to highly forced cases was examined using 33 numerical simulations, which cover a wide range of flow behaviours from linear to breaking regime, violent forcing with uncovered bottom, and resonant cases. Our observations at the centre of the well reveal that all cases show an Ekman-like spiral, with distinctive features at peculiar cases. In particular, the influence of viscosity in the ratio height to boundary layer thickness can be observed in the Re cases, where the spiral is observed to develop as the viscosity decreases. The relationship of observed τ_w to observed $\mathbf{v}_s$ shows remarkable results, with few exception of extreme cases (very high E and F). We demonstrate that the PT-Stokes WSS theory predicts τ_w magnitude adequately, with the exception of cases close to resonant frequencies, where the potential theory overpredicts the velocity magnitude due to the absence of viscous damping.

This theory represents a notable improvement to the prediction of WSS for shallow orbital shaker flows. The PT-Stokes WSS model has a broader validity region than the Stokes model: it predicts τ_w within a 50% discrepancy at the well half-radius inner area ($r < 0.5R$) in all cases examined, except for those close to the resonant line, and very highly forced cases. The most important feature of new WSS model is the relationship of τ_w to the surface velocity $\mathbf{v}_s$ in magnitude and direction, which has been shown to be valid up to the edge of the well and for most case studies, and that it reproduces the WSS metrics correctly for a typical endothelial experimental configuration.

The wavy non-linear nature of the flow complicates the characterisation of the orbital shaker fluid dynamics problem, which the linear and inviscid potential theory cannot capture. However, it is a notable advance to understand that τ_w magnitude and directionality is linked to the behaviour of the free surface. The potential model can be refined by expanding on the secondary modes [111, 115], and by adding viscosity to the irrotational flow solution to cope with the free-surface curvature and the viscous damping [59, 115, 62]. Also, it is important to understand the occurrence of the secondary flows (toroidal vortex) [116, 66, 142] and its relation to the free surface, the wave breaking front and the velocity bifurcation line.

Chapter 5

Conclusions

The aim of this thesis was to further understanding of the fluid dynamics of orbital shakers by exploring the four-dimensional phase space and the development of theories to explain the findings.

This chapter is organised as follows. Section 5.1 contains a summary of the work presented in concluding the thesis. Section 5.2 provides recommendations and tools for the investigation of endothelial cells in orbital shakers. In Section 5.3, we discuss the possible further routes to continue this investigation. Finally, Section 5.4 contains the list of publications in progress.

5.1 Summary of Results

The data collection in this thesis was carried out with the commercial CFD software STAR-CCM+. Mesh convergence and transient state studies were carried out to ensure that the meshing of the domain was appropriate to the problem and that simulations were analysed at the periodic state. A computational method was used to simulate the flow that occurs in a typical experimental configuration (*reference case*), which was described in detail in Chapter 2. The *transWSSmin* metric, a novel wall shear stress metric that captures multidirectionality, was formulated and investigated in Chapter 2. This metric challenges the currently accepted theory which states that cells align in the predominant direction of the flow — meaning the temporal mean of τ_w . Based on the mathematical formulation of the transWSS metric, the biological significance of transWSSmin suggests that cells align in the direction in which they experience the minimum transverse WSS. It might be assumed that these directions (temporal

mean and minimised transverse WSS) are similar. However, we have produced a set of analytical τ_w distributions that makes explicit their differences. It was shown that the direction that minimises the transverse WSS generally differs from the direction of the temporal mean, and in some cases these directions are even perpendicular to each other. Furthermore, the relevance of transWSSmin in the study of endothelial cells was demonstrated by showing that there is a significant correlation between transWSSmin and the endothelium permeability and that the cells align closely to the direction that minimises the transverse WSS in the experimental reference case.

We then sought to explore and develop analytical models to further the insight of flow behaviour in orbital shakers. In Chapter 3, we introduced a *potential flow theory* which also served as the basis of the new WSS model developed in Chapter 4. The potential theory is a linear and inviscid model for sloshing dynamics [59], extended to the application of the orbital shaker experiments by Reclari [110]. This theory was used to infer the four dimensionless numbers that govern the physics of the problem: (i) the dimensionless forcing F , (ii) the eccentricity E , (iii) the shallowness Γ , and (iv) the Reynolds number Re . We have worked with constant $\Gamma = 0.18$, which corresponds to the reference case Γ value, with an emphasis on endothelial cell experiments. We designed 33 case studies to sweep the E - F - Re parameter space to explore flow behaviours and the validity domain of the potential theory (Table 3.2). The flow structured differently, significantly showing multiple free-surface shapes. The ability of the potential theory to predict the surface height and the velocity field was assessed using the computational results of the low-forcing cases, where the fluid behaviour is practically linear (§ 3.4). The surface was predicted within 1% accuracy while the velocity was underestimated by up to 20%. An analysis of the conservation of mechanical energy (Appendix B) revealed that the latter discrepancy was due to the unavoidable loss of mechanical energy caused by viscous stresses in the bulk of the liquid, which is not considered in the inviscid potential model.

A notable result of this thesis is the analytical prediction of the *wave-breaking regime* derived in § 3.7. The potential theory was also used to predict the surface amplitude in highly forced cases. Results showed that the predictions were accurate except in cases where wave breaking or resonance occurred. The occurrence of breaking was studied by observing the free surface in the computational simulations, which allowed us to adjust the theoretical amplitude threshold

formulated by Miche [87] for one-dimensional periodic waves to the particular geometry of the orbital shaker; the incipient breaking appeared at amplitudes $\Delta h/2R = 0.7\Gamma$. The resonant frequencies were inferred from the potential theory. The amplitude was overestimated in the breaking and resonant cases due to the model's inability to account for the energy dissipation introduced by the breaking and viscous damping. The validity domain of the potential theory extends up to the observed wave breaking, and was therefore used to estimate and classify the case studies in non-breaking and breaking regimes, as displayed in Figure 3.10. This study reinforces the value of combining analytical and computational tools.

With the analytical insight gained in Chapter 3, a novel model to predict WSS, the *PT-Stokes WSS model*, was derived in Chapter 4. The new model is a generalisation of the classic Stokes solution where the boundary conditions in the far field have been modified to take into account the finite boundaries of the orbital shaker (free surface and side walls). The Stokes description of the boundary layer was coupled to the potential flow behaviour observed in the bulk of the liquid. The main feature of the model is the Ekman spiral that characterises the boundary layer; this spiral connects τ_w to the velocity in the far field, approximated as the surface velocity. This is a hallmark of the fluid field which is observed in all cases. Despite the limitations of the potential theory to model non-linear regimes raised in Chapter 3, the PT-Stokes estimates τ_w magnitude within a 50% accuracy in all case studies, except for the resonant cases, whose occurrence can be anticipated by the model. However, the prediction of τ_w directionality needs to be improved, as highlighted by the imprecise prediction of the WSS metrics in the reference case (Figure 4.13). However, the Ekman spiral relationship of τ_w to the surface velocity shows a very strong correlation, which provides further evidence that surface velocity predictions are the dominant source of error.

The WSS estimation provided by the PT-Stokes model is a notable improvement over the commonly used Stokes solution, and provides a valuable tool for experimentalists. Figure 5.1 illustrates the validity domains, represented by circles, of the different models explored to obtain WSS. The PT-Stokes model has extended the validity domain of the classic Stokes solution; while still not covering the same domain as the CFD model, this study has established a strong correlation between the WSS and the surface velocity, which could allow for further extension of the validity domain of analytical models.

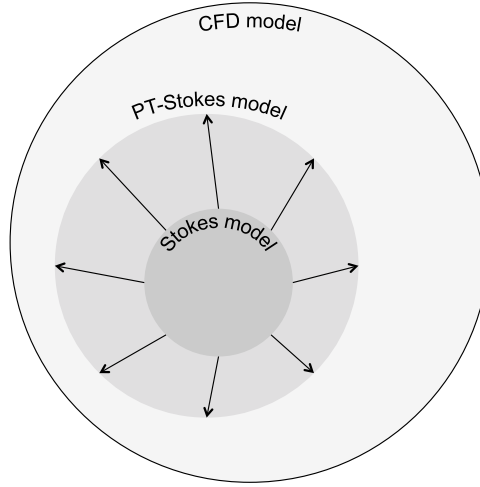


Figure 5.1: Sketch of the validity domains, represented by circles, of the different investigated models.

5.2 Recommendations for Endothelial Experiments

Computational model and setup

The generation of a computational model for free-surface flow with a breaking wave is not straightforward. The commercial CFD software STAR-CCM+ and its module VOF wave has proved to be a reliable tool to simulate the fluid dynamics of orbital shakers (§ 2.3). We list some recommendations for a successful model setup using this tool:

Meshing. We recommend a cylindrical block-structured mesh, which allows for the refinement of the computational cells next to the wall boundary layers. A study of the appropriate number of computational cells is provided in § 2.3.1: we used a mesh with 291,600 cells for the work presented in this thesis, but a coarse mesh of 100,000 cells is also satisfactory; nevertheless, a mesh convergence study is always recommended. Two theoretical findings are of use to design the mesh: (1) the bottom boundary layer thickness can be estimated as $\delta = \sqrt{\nu/\omega}$, and the same relationship is expected to hold for the side walls. We recommend that a minimum of five computational cells are used in a δ distance from the wall boundaries (a sufficient resolution in the boundary layer is very important in this problem because it is directly connected to the calculation of τ_w); (2) the potential theory prediction of the surface height provides an estimation of the maximum height reached by the liquid, which can be used to adjust the height of the cylindrical computational domain; the value is conservative since the amplitude is

accurately estimated in the linear cases and overestimated in the highly forced cases.

VOF wave module and time step. We recommend monitoring the total liquid volume as a function of time to ensure convergence of the solution. As explained in § 2.3.1, the VOF model is not conservative and was leading to a mass loss due to too high a CFL number. We recommend that mesh refinement is accompanied by the refinement of the time step to maintain a CFL number under 0.5.

Inertial forces. We have modelled the orbital motion of the orbital shaker as an inertial force in a non-inertial system (see Figure 2.2). This inertial force can easily be implemented in the computational model by modifying the gravity vector as $\bar{g} = \{a\omega^2 \cos(\omega t), a\omega^2 \sin(\omega t), -g\}$ (in Cartesian coordinates).

Operating regime

We have focused our study in the shallow water regime ($\Gamma = \frac{d}{R} < 1$) since endothelial cells experiments are normally carried out with a shallow fluid layer to ensure that the cells are not hypoxic (deprived from an adequate level of oxygen).

In Chapter 3, we classified the case studies in a parameter space in *breaking* and *non-breaking* regimes (Figure 3.10). Cases are predicted to have a breaking wave when their amplitude Δh is greater than $1.4d$, where d is the static fluid height (this criterion is the dimensional form of $\frac{\Delta h}{2R} > 0.7\Gamma$). The potential theory provides an estimation of the height amplitude which is valid till the incipient breaking of the wave, and is thus valid for determining the breaking regime. This amplitude estimation for typical cases (maximum at the edge of the well pointed to by the inertial force) is given in (3.7), however, it is recommended that the amplitude is estimated using the general form $\Delta h = \max(\zeta) - \min(\zeta)$, where ζ is given in (3.3). In § D.1, we have included a Matlab routine that calculates the potential amplitude, and determines the validity of the estimation and the occurrence of breaking.

We have also identified the resonance lines in the parameter space, i.e., the operating condition under which the operating frequency ω is equal to the natural frequency $\omega_{1n} = \sqrt{\frac{g}{R}\epsilon_{1n} \tanh(\epsilon_{1n} \frac{d}{R})}$ (approximately the resonant frequency also for the viscous problem); the physical consequence

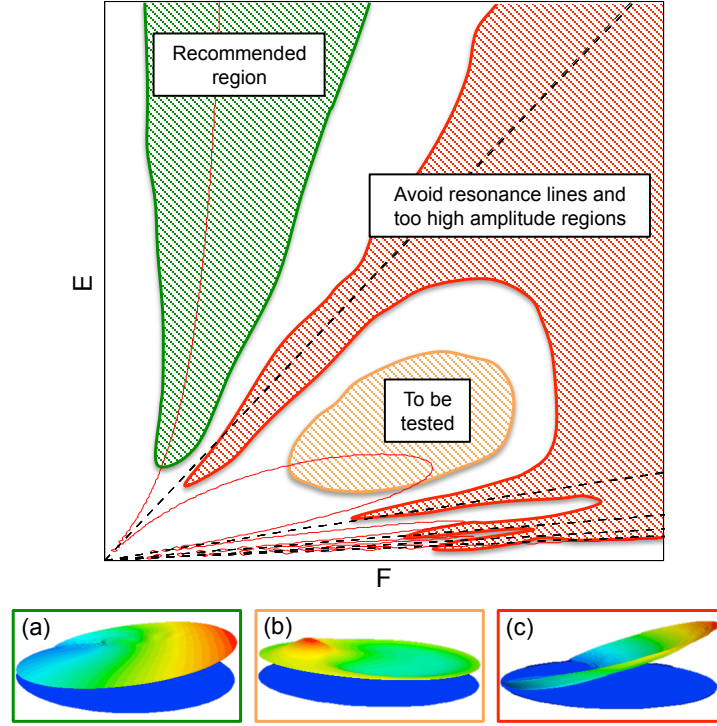


Figure 5.2: Sketch of recommendations in the operating regimes in a generic E - F space. The red line is the breaking limit threshold and the discontinuous black lines are the resonance lines. Figures (a)-(c) show an example of the free surface in each of the identified regions matching their colours.

of this operating condition is that the wave amplitude is especially amplified. In § D.2, we have included a Matlab routine that calculates the natural frequencies of the experimental configuration.

As we concluded in Chapter 3, these findings lead to recommendations about the operating regime for endothelial cell experiments. In Figure 5.2, we have illustrated these recommendations: the green area is the recommended operating regime, which corresponds to the region around the breaking threshold that allows the investigation of different WSS vector fields depending on the geometry of the free surface breaking area (a); the orange area is an unknown experimental regime within which the surface presents a double peak (b) that may be of interest for testing new WSS scenarios; the red area is the operating regime to avoid, which corresponds to the region near the resonance lines and has too high an amplitude, as observed in (c); this can lead to the bottom of the well becoming uncovered, leading to cell damage.

Analytical wall shear stress predictions

The PT-Stokes WSS model (Chapter 4) was shown to be a marked improvement over the Stokes WSS model. It predicts τ_w magnitude with a 50% discrepancy within the central circular area of radius $R/2$, i.e.,

$$\tau_w = \tau_w^{\text{PT-Stokes}}(1 \pm 0.5) \text{ for } r < 0.5R \quad (5.1)$$

except for the resonant cases, as shown in Figure 4.12.

The PT-Stokes model formulation is summarised in equations (4.18), (4.19) and (4.29). In § D.3, we have included a Matlab routine with the equations coded for easy access. This routine provides the PT-Stokes WSS prediction when the operating frequency is not close to its natural frequency ($|\frac{\omega_{1n}}{\omega} - 1| > 0.1$).

5.3 Future work

In this section, we discuss some of the possible routes to extend this work.

Computational model

Endothelial experiments are carried out in multi-well plates of different sizes. In this work, we have taken as reference the twelve-well plate, which has a radius of 11.05 mm. Under these conditions, the Bond (Bo) and Weber (We) numbers, which represent the relevance of the surface tension forces, are of order 10 (§ 2.2); therefore, the surface tension forces are not negligible although not dominant, and these forces have been omitted in the computational model. A further study could analyse if these forces introduce any significant change in the WSS pattern. Also, a computational model with surface tension could simulate smaller sized wells, such as 96-microwell plates, where $Bo, We < 1$, and the surface forces cannot be neglected.

The computational model setup has been validated with a thorough mesh convergence analysis (§ 2.3.1). This demonstrates that the computational model is well posed, but it does not confirm that the problem modelled (§ 2.2) and the actual experimental configuration are exactly the same. There could be some discrepancies between the models; for example, experiments could

be taking place in a slightly tilted (with respect to the gravity direction) orbital shaker platform, and this would introduce an asymmetry in the problem configuration; also, cells are cultured on top of a gel which is observed to solidify forming a curved surface, instead of the flat surface modelled; moreover, the fluid properties may be modified over time due to an exchange of solutes with the gel. These modifications could be easily implemented in the computational model to observe if they significantly influence WSS patterns. Furthermore, the computational and experimental configurations could be compared using direct measurement methods such as those found in Salek et al. [116]: a Doppler velocimetry to measure WSS, a HFPIV to measure velocity, and a high-speed camera to measure surface height. Other methods to measure height are Shadowgraph and Schlieren imaging [20, 100], which are based on refractive index changes in the free surface. Pressure at the well bottom could also be measured but the sensitivity of the pressure sensors may not be within the problem pressure differences, which are small due to the small vertical scale used. These techniques would confirm whether some physical conditions have not been taken into account.

In Chapter 4, it was discovered that there is a strong correlation between τ_w and the surface velocity. This result highlights the importance of correctly characterising the free surface. One of the factors for the correct characterisation of the free surface is the mesh resolution. We have chosen a structured mesh which adapts to refine near the walls, while the bulk of the domain has a lower resolution, and that includes most of the free surface. The convergence study shows that the mesh was sufficient but a refined mesh may be needed if surface tension is included. An efficient way to improve the resolution of the free surface could be to set up a dynamic mesh, that is a mesh that refines based on a given criterion, which in this case would be the value of the VOF function. Refining the mesh at the free surface may resolve the surface velocity disturbances reported in § 4.5.

Analytical models

We have made progress in understanding the physics of orbital shaker flows and created an analytical framework that guides future work. The potential theory model used in this work has proven to be a useful tool to predict the free surface, velocity field, and WSS, although, the absence of viscous damping causes errors in the surface velocity of resonant cases. As

noted by Joseph et al. [62], the potential theory can describe viscous fluids, since a potential flow is an irrotational flow and not necessarily an inviscid flow, as sometimes misunderstood. A way of tackling the problem is a Helmholtz decomposition of the flow into rotational and irrotational components of the velocity field. Ibrahim [59] derives the exponential decay in the amplitude due to viscous damping at the bottom, the side walls, and at the free surface, and the change in the natural frequency when considering the surface tension; this bounds the resonant cases to more realistic values. Considering viscosity also allows the imposition of stress at the boundaries, which would improve the potential solution at the side walls and permit the representation of curved free surfaces. A weakly non-linear potential solution including other excitation modes was described by Reclari et al. [111]. It captures the multi-crest waves that we observed in several cases. Furthermore, the dissipation caused by wave breaking could be explored as another source of energy damping.

The PT-Stokes WSS model could be improved via the potential theory model, which has been previously addressed, and via the boundary layer model. We have identified two aspects in which the boundary layer model could be extended. (1) In the boundary layer upper boundary conditions, we assumed a periodic variation in the surface velocity direction and a constant magnitude (4.7); as we therein remark, this is true at the centre of the well, but as we move to the edge, the influence of the side walls would skew the temporal velocity distribution. Other more realistic profiles could be implemented such as an elliptic profile; we have observed that this profile affects the shape of the Ekman spiral. (2) We have assumed no lateral limiting walls in the boundary layer model (although they are considered in the potential model) based on the hypothesis of shallowness ($\Gamma \ll 1$); they could be included to give a better prediction of the velocity profiles near the edge in deeper wells.

Computational-analytical models

An important part of the work carried out during the first year of this PhD (but not included in the thesis) was dedicated to the study of a shallow water model. The shallow water equations were implemented in the solver Nektar++ [21], but an analytical model of the vertical boundary layer profile was needed to take into account the viscous terms. The results were not fully successful due to a lack of understanding of the fluid dynamics of the problem, and in particular,

of the boundary layer profile. The findings made in this work have provided the understanding necessary to continue this line of research. We briefly recapitulate the work carried out with the pertinent modifications.

In the shallow water model, the vertical component of the velocity is neglected, and the Navier-Stokes and continuity equations are depth-integrated. As a consequence the pressure is hydrostatic and the viscous term is reduced to τ_w . We used the conservative form of the equations in cylindrical coordinates:

$$\frac{\partial h \bar{u}_r}{\partial t} + \frac{1}{r} \frac{\partial r h \bar{u}_r^2}{\partial r} + \frac{1}{r} \frac{\partial h \bar{u}_r \bar{u}_\theta}{\partial \theta} - \frac{h \bar{u}_\theta^2}{r} + g h \frac{\partial \zeta}{\partial r} + \frac{\tau_r}{\rho} = h a \omega^2 \cos(\omega t - \theta) \quad (5.2)$$

$$\frac{\partial h \bar{u}_\theta}{\partial t} + \frac{1}{r} \frac{\partial r h \bar{u}_r \bar{u}_\theta}{\partial r} + \frac{1}{r} \frac{\partial h \bar{u}_\theta^2}{\partial \theta} + \frac{h \bar{u}_r \bar{u}_\theta}{r} + \frac{g h}{r} \frac{\partial \zeta}{\partial \theta} + \frac{\tau_\theta}{\rho} = h a \omega^2 \sin(\omega t - \theta) \quad (5.3)$$

$$\frac{\partial h}{\partial t} + \frac{1}{r} \frac{\partial r h \bar{u}_r}{\partial r} + \frac{1}{r} \frac{\partial h \bar{u}_\theta}{\partial \theta} = 0 \quad (5.4)$$

where r , θ , and t are the space and time variables, h is the free-surface height, $\bar{u}_r$ and $\bar{u}_\theta$ are the radial and tangential depth-averaged velocities, g is the gravity acceleration, ρ the fluid density, and in the orbital shaker, the shear stress at the bottom $\tau_r = \frac{\partial u_r}{\partial z} \Big|_{z=0}$ and $\tau_\theta = \frac{\partial u_\theta}{\partial z} \Big|_{z=0}$.

An assumption has to be made about the u_r and u_θ vertical profiles to calculate the τ_w components. In our previous work, we theorised a parabolic profile, as in a Hagen-Poiseuille flow, however, we have demonstrated in Chapter 4 that the velocity profile can be represented by a Stokes boundary layer for most of the domain (4.12)-(4.13).

Equations (5.2)-(5.4) form a set of non-linear hyperbolic second order partial differential equations, which do not have an exact solution in their general form. They were implemented in the in-house CFD solver Nektar++. The hyperbolic character of the equations allows for discontinuous solutions (referred to as shock waves), and the solver became unstable under these discontinuities, which appeared at high forcing. A shock-capturing method was implemented to stabilize the system, which consisted of an artificial diffusion term that activated when the surface slope reached a certain threshold [105].

The results obtained using the parabolic velocity profile assumption were compared for a case computed by Salek et al. [116](Figure 5.3). The surface height maps were in agreement, but the WSS maps disagreed in the pattern and order of magnitude. We expect that introducing the Stokes boundary layer profile would produce a notable improvement in τ_w .

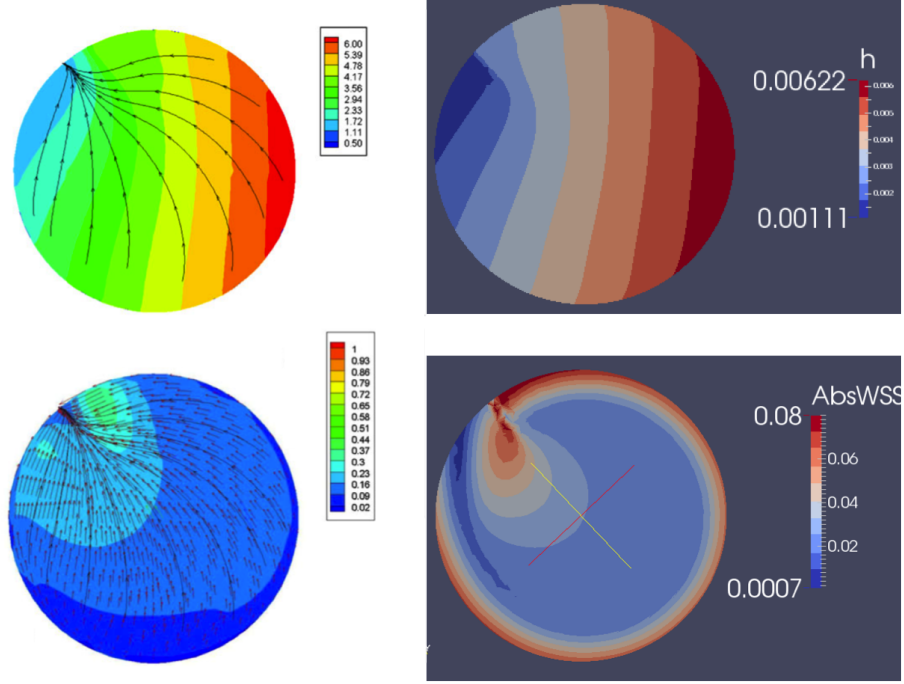


Figure 5.3: Height maps (top) and WSS maps in Pa (bottom) from Salek et al. [116](left column) and generated with Nektar++ using the shallow water model (right column).

Case studies and orbital shaker configuration

We have focused our investigation in the shallow water regime at a constant value $\Gamma = 0.18$. Other Γ values could be explored to further utilise the new theories developed herein, such as the values identified as operating regimes in Figure 3.11. For Γ values close to or greater than 1, the flow regime starts to be considered deep water and the analytical models described in this thesis may not be valid. For example, we anticipate that the breaking condition $\frac{\Delta h}{2R} = 0.7\Gamma$ becomes independent of Γ in deep water; Reclari et al. [111] provide a breaking threshold $\frac{\Delta h}{2R} = 0.443$. The transition from one limit to the other may be around $\Gamma = 0.63$, where both limits are equal.

Furthermore, the configuration of the orbital shaker could be modified to generate different WSS metrics scenarios. The geometry of the well could be modified by adding a cylindrical rod in the middle to decelerate the flow (Figure 5.4), which could provide a region similar to the periphery but with lower WSS magnitude values. The orbital translation could be changed from circular to elliptical, which would give a defined $\text{transWSS}_{\min}$ value at the centre, while the transWSS would remain undefined.

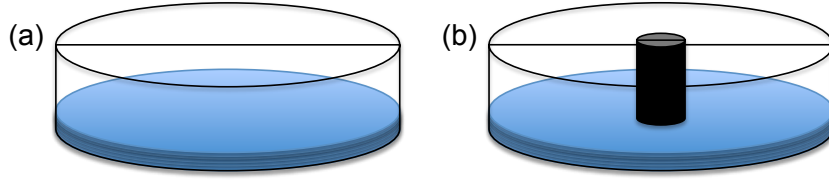


Figure 5.4: Sketch of the classical well configuration (a) and the suggested alternative configuration adding a rod at the centre (b).

General remarks

The orbital shaker has been proven, in § 2.5, to be useful to exert a variational flow effect from centre to edge on endothelial cells. Although the novel transWSSmin metric presents the best correlation with the endothelial permeability and explains coherently cell alignment, the other metrics have a rather similar radial gradient. We hope that the methods, observations and future work presented in this thesis help in designing an experiment that allows for more distinguishable profiles between the classic metrics (TAWSS, MagMeanWSS and OSI, where the latter correlates with the two former), the transWSS metric, and the new metric transWSSmin. For instance, in Appendix C, the five metrics studied (§ 2.4) have been plotted in an E - F parameter space for all case studies. Generally, the trend of the metrics remains rather linked, however, there are some cases that would be worth exploring experimentally. Also, it may be worth using a scanner plate that could cover the well bottom fully, since discrepancies between the metrics are pronounced close to the edge. It is worth mentioning the relevance of the breaking wave in producing variable τ_w distribution. It would be of interest to study the relationship between the occurrence of breaking and the origin of the secondary flows, and the relationship of the latter to the variation in the τ_w distribution and repercussions on the metric profiles. All in all, future research should aim to find the ideal orbital shaker experiment to explore the transverse WSS metrics.

5.4 Publications Submitted and in Preparation

Corresponding with Chapter 2, Chapter 3 and Chapter 4, three manuscripts for publication are in progress:

Visualisation of three pathways for macromolecule transport across cultured endothelium and their modification by flow,

M. Ghim, **P. Alpresa**, S. Yang, S. Braakman, S. Gray, S. Sherwin, M. van Reeuwijk, and P. D. Weinberg

AJP-Heart and Circulatory Physiology, July 2017.

Orbitally shaken shallow fluid layers. Part 1. Regime classification,

P. Alpresa, S. Sherwin, P. D. Weinberg, and M. van Reeuwijk

Physics of Fluids (submitted).

Orbitally shaken shallow fluid layers. Part 2. An improved wall shear stress model,

P. Alpresa, S. Sherwin, P. D. Weinberg, and M. van Reeuwijk

Physics of Fluids (submitted).

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Appendix A

Potential Theory

This appendix presents the derivation of the potential theory introduced in Chapter 3, taken from the classic potential flow solution under sinusoidal excitation in sloshing dynamics [59], and the extended orbital translation solution presented by Reclari [110].

A.1 Problem formulation

The fluid is assumed incompressible and irrotational, and the velocity can thus be represented by $\mathbf{v} = \nabla\Phi$, where Φ is the potential function. The boundary conditions at the wall do not permit no-slip conditions because of the irrotationality of the flow, and free-slip boundary conditions are thus imposed. Furthermore, the kinematic and dynamic boundary conditions at the free surface can be linearised to first order considering this surface a fluid surface and applying Bernoulli equation, respectively, and neglecting viscous and surface tension forces. Under these conditions, the continuity and boundary conditions take the form

$$\nabla^2\Phi = 0 \tag{A.1}$$

$$\frac{\partial\Phi}{\partial r} = 0 \quad \text{at } r = 0 \text{ and } r = R \tag{A.2}$$

$$\frac{\partial\Phi}{\partial z} = 0 \quad \text{at } z = 0 \tag{A.3}$$

$$\frac{\partial\Phi}{\partial t} - a\omega^2 r \cos(\omega t - \theta) + g\zeta = 0 \quad \text{at } z = d + \zeta(r, \theta, t) \tag{A.4}$$

$$\frac{\partial\Phi}{\partial z} = \frac{\partial\zeta}{\partial t} \quad \text{at } z = d + \zeta(r, \theta, t) \tag{A.5}$$

where r , θ and z are cylindrical coordinates in a non-inertial frame with origin at the centre of the well bottom, d is the fluid height, R is the well radius, a and ω are the orbital radius and frequency of rotation, respectively, g is the gravitational acceleration, and ζ is the surface elevation relative to the static height d .

The conditions at the free surface (A.4) and (A.5) are combined to eliminate ζ dependence, resulting in

$$\frac{\partial^2 \Phi}{\partial t^2} + g \frac{\partial \Phi}{\partial z} = -a\omega^3 r \sin(\omega t - \theta) \quad \text{at } z = d + \zeta(r, \theta, t). \quad (\text{A.6})$$

A.2 Homogeneous solution

Laplace's equation (A.1) can be solved using separation of variables. The solution consists of Bessel functions in the radial direction, harmonic functions in the azimuthal direction, and exponential functions in the vertical direction [60]. Taking into account the boundary conditions at $r = 0$, $z = 0$, and the free surface, the general solution is

$$\Phi(r, \theta, z, t) = \sum_{m=0}^{\infty} \sum_{n=1}^{\infty} [\alpha_{mn}(t) \cos m\theta + \beta_{mn}(t) \sin(m\theta)] J_m(\lambda_{mn}r) \frac{\cosh(\lambda_{mn}z)}{\cosh(\lambda_{mn}d)} \quad (\text{A.7})$$

where J_m are the Bessel functions of the first kind, of order m ; α_{mn} and β_{mn} are time-dependent functions. The Bessel functions of the second kind have been dropped because they do not have a finite value at $r = 0$. The solution in $\sinh(\lambda_{mn}z)$ in the axial variable is not considered because it does not satisfy the boundary condition (A.3). The coefficients λ_{mn} are obtained from satisfying the boundary condition (A.2):

$$\frac{dJ_m(\lambda_{mn}r)}{dr} = 0 \quad \text{at } r = R \quad (\text{A.8})$$

therefore, λ_{mn} is the n th root of $J'_m(\lambda_{mn}r)$ for $r = R$; in its dimensionless form, $\epsilon_{mn} = \lambda_{mn}R$ is the n th root of $J'_m(s)$ given in Table A.1.

The natural frequencies of the system ω_{mn} are obtained assuming a periodic solution in time and an arbitrary phase in of the form $\alpha_{mn} = a_{mn} \cos \omega_{mn}t$ and $\beta_{mn} = b_{mn} \sin \omega_{mn}t$. Substituting into equation (A.6), ignoring the forcing, and assuming $d \gg \zeta$, the result is

$$\omega_{mn}^2 = g\lambda_{mn} \tanh(\lambda_{mn}d). \quad (\text{A.9})$$

ϵ_{mn}	n=1	n=2	n=3	n=4	n=5
m=0	3.8317059702	7.0155866698	10.173468135	13.323691936	16.470630051
m=1	1.8411837813	5.3314427735	8.5363163663	11.706004903	14.863588634
m=2	3.0542369282	6.7061331941	9.9694678230	13.170370856	16.347522318
m=3	4.2011889412	8.0152365983	11.345924310	14.585848286	17.788747866
m=4	5.3175531260	9.2823962852	12.681908442	15.964107038	19.196028800

Table A.1: The n th roots of the derivative of the Bessel function of the first kind J_m , of order m .

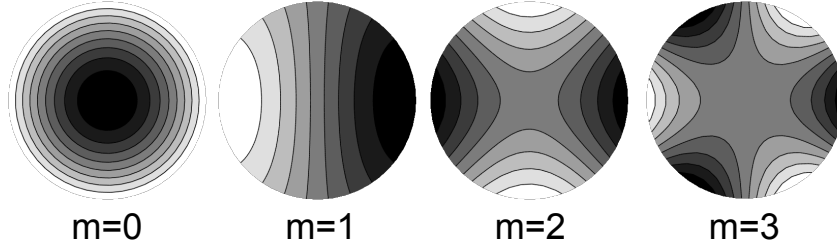


Figure A.1: Surface elevation natural modes (A.11) at $n = 1$.

Hence, the homogeneous solution is

$$\Phi(r, \theta, z, t) = \sum_{m=0}^{\infty} \sum_{n=1}^{\infty} [a_{mn} \cos(\omega_{mn}t) \cos m\theta + b_{mn} \sin(\omega_{mn}t) \sin(m\theta)] J_m(\lambda_{mn}r) \frac{\cosh(\lambda_{mn}z)}{\cosh(\lambda_{mn}d)} \quad (\text{A.10})$$

where a_{mn} and b_{mn} are constants to be determined from initial conditions.

A.3 Particular solution

Having obtained the homogeneous solution, the response to the forcing can be determined. Reclari [110] analyses the natural modes of the free surface. Introducing (A.10) into (A.4) at $t \bmod 2\pi/\omega = 0$ and ignoring the forcing term, the surface elevation of the mode (m, n) scales as:

$$\zeta(r, \theta)_{mn} \propto \sin(m\theta) J_m(\lambda_{mn}r) \quad (\text{A.11})$$

depicted in Figure A.1 for the radial model of order $n = 1$. The forcing term determines which natural modes are excited. The orbital shaker moves the liquid laterally; it has a non-axisymmetrical effect, thus exciting the first mode with that characteristic ($m = 1$ in Figure A.1). Hence, the potential solution (A.7) used in this study is restricted to the terms $m = 1$ [110].

To find the particular solution, the forcing on the right-hand side of (A.6) has to be expressed in terms of Bessel functions. A Dini expansion [17] has been used in order to obtain r in terms of $J_1(\lambda_{1n}r)$ with the result

$$r = \sum_{n=1}^{\infty} c_n J_1(\lambda_{1n}r) \quad (\text{A.12})$$

where $c_n = \frac{2R}{(\epsilon_{1n}^2 - 1)J_1(\epsilon_{1n})}$ Reclari [110].

Introducing (A.12) and (A.7) into the boundary condition (A.6) and assuming $d \gg \zeta$ gives

$$\begin{aligned} & \sum_{n=1}^{\infty} [\ddot{\alpha}_{1n}(t) + g\lambda_{1n} \tanh(\lambda_{1n}d)\alpha_{1n}(t) + a\omega^3 c_n \sin \omega t] J_1(\lambda_{1n}r) \cos \theta \\ & + [\ddot{\beta}_{1n}(t) + g\lambda_{1n} \tanh(\lambda_{1n}d)\beta_{1n}(t) - a\omega^3 c_n \cos \omega t] J_1(\lambda_{1n}r) \sin \theta = 0 \end{aligned} \quad (\text{A.13})$$

which can only be satisfied if

$$\begin{aligned} \ddot{\alpha}_{1n}(t) + \omega_{1n}^2 \alpha_{1n}(t) &= -a\omega^3 c_n \sin \omega t \\ \ddot{\beta}_{1n}(t) + \omega_{1n}^2 \beta_{1n}(t) &= a\omega^3 c_n \cos \omega t \end{aligned} \quad (\text{A.14})$$

where ω_{1n} was defined in (A.9). The structure of these equations represents that of two forced harmonic oscillators for the functions $\alpha_{1n}(t)$ and $\beta_{1n}(t)$. The particular solutions of (A.14) are

$$\begin{aligned} \alpha_{1n}(t) &= -\frac{a\omega^3 c_n}{\omega_{1n}^2 - \omega^2} \sin \omega t \\ \beta_{1n}(t) &= \frac{a\omega^3 c_n}{\omega_{1n}^2 - \omega^2} \cos \omega t. \end{aligned} \quad (\text{A.15})$$

Substituting (A.15) and the derived results into (A.7), the particular solution is given by

$$\Phi(r, \theta, z, t) = -a\omega \sin(\omega t - \theta) \sum_{n=1}^{\infty} \left[\frac{2R}{(\epsilon_{1n}^2 - 1)} \frac{\omega^2}{(\omega_{1n}^2 - \omega^2)} \frac{J_1(\lambda_{1n}r)}{J_1(\epsilon_{1n})} \frac{\cosh[\lambda_{1n}(z)]}{\cosh(\lambda_{1n}d)} \right]. \quad (\text{A.16})$$

The surface elevation can be obtained from (A.4):

$$\zeta(r, \theta, t) = \frac{a\omega^2}{g} \cos(\omega t - \theta) \left[r + \sum_{n=1}^{\infty} \frac{2R}{(\epsilon_{1n}^2 - 1)} \frac{\omega^2}{(\omega_{1n}^2 - \omega^2)} \frac{J_1(\lambda_{1n}r)}{J_1(\epsilon_{1n})} \right]. \quad (\text{A.17})$$

Appendix B

Mechanical Energy Equation

The conservation of mechanical energy equation is obtained by taking the dot product of the conservation of momentum equation and the velocity vector. Equation B.1 represents its integral form over the full fluid volume, which is dependent on time

$$\frac{d}{dt} \int_{V(t)} \rho \frac{v^2}{2} d\Omega = \int_{V(t)} (-\mathbf{v} \cdot \nabla p + \mathbf{v} \cdot (\nabla \cdot \bar{\bar{\tau}}')) d\Omega + \int_{V(t)} \rho \mathbf{f}_m \cdot \mathbf{v} d\Omega \quad (\text{B.1})$$

where $\mathbf{v}$ is the velocity vector and v its magnitude, ρ density, p pressure, $\bar{\bar{\tau}}'$ deviatoric stress tensor, $\mathbf{f}_m$ mass forces, t time variable, $V(t)$ the fluid volume of integration, and $d\Omega$ the differential volume.

The mass forces are conservative: $\mathbf{f}_m = \mathbf{f}_i + \mathbf{g} = -\nabla U$, where $\mathbf{f}_i$ is the inertial force (2.5) and $\mathbf{g}$ the gravity vector, and derive from the potential function U where

$$U = U_i + U_g = -a\omega^2 r \cos(\theta - \omega t) + gz \quad (\text{B.2})$$

where U_i and U_g are the potential functions of $\mathbf{f}_i$ and $\mathbf{g}$, respectively. Using the product rule and Gauss' theorem, and taking into account the incompressibility of the fluid ($\nabla \cdot \mathbf{v} = 0$), the pressure, viscous and gravity terms can be partially rewritten as surface integrals. Moreover, the gravity force is independent of time, and, according to the Reynolds transport theorem (B.3), the variation of the potential function depends only on the boundary fluxes.

$$\frac{d}{dt} \int_{V(t)} U_g d\Omega = \int_{V(t)} \frac{\partial U_g}{\partial t} d\Omega + \int_{\Sigma(t)} U_g \mathbf{v} \cdot \mathbf{n} d\sigma. \quad (\text{B.3})$$

Equation B.1 can be rewritten

$$\underbrace{\frac{d}{dt} \int_{V(t)} \rho \left(\frac{v^2}{2} + gz \right) d\Omega}_{(I)} = \underbrace{\int_{\Sigma(t)} -p \mathbf{v} \cdot \mathbf{n} d\sigma}_{(II)} + \underbrace{\int_{\Sigma(t)} \mathbf{n} \cdot \bar{\bar{\tau}}' \cdot \mathbf{v} d\sigma}_{(III)} - \underbrace{\int_{V(t)} \bar{\bar{\tau}}' : \nabla \mathbf{v} d\Omega}_{(IV)} + \underbrace{\int_{V(t)} \rho \mathbf{f}_i \cdot \mathbf{v} d\Omega}_{(V)}. \quad (\text{B.4})$$

Analysing term-by-term in the no-slip model:

- (I) *Derivative of the kinetic and potential energy:* the velocity field and the surface elevation rotate as a solid body around the centre of the well, thus, the integral over the volume is constant and its derivative zero. This value was monitored in the simulation to confirm this result.
- (II) *Pressure rate of work:* at the wall, the velocity is zero, and consequently the integral over these boundaries is also zero. At the free surface, the pressure is constant because surface tension forces have been neglected. This term can thus be manipulated and rewritten as $-p_{air} \int_{\Sigma(t)} \mathbf{v} \cdot \mathbf{n} d\sigma$, proportional to the integral form of the continuity equation for a liquid, thus, zero.
- (III) *Viscous rate of work:* at the wall, the velocity is zero, and consequently the integral over these boundaries is also zero. At the free surface, the viscous tensor is equal on both sides of the boundary because the surface tension forces have been neglected, i.e., $\bar{\bar{\tau}}'_{liquid} = \bar{\bar{\tau}}'_{air}$. This term is negligible at a free surface.
- (IV) *Viscous dissipation:* the integrand of this term can be demonstrated to always be positive [9]; known in mechanics as Rayleigh's dissipation function, it represents a loss of mechanical energy that contributes entirely to incrementing the internal energy.
- (V) *Inertial force rate of work:* in general, different from zero.

Hence,

$$\int_{V(t)} \bar{\bar{\tau}}' : \nabla \mathbf{v} d\Omega \simeq \int_{V(t)} \rho \mathbf{f}_i \cdot \mathbf{v} d\Omega. \quad (\text{B.5})$$

Appendix C

Wall Shear Stress Metrics in the E - F - Re Parameter Space

This appendix presents in Figure C.1 the WSS metrics for all case studies in the E - F - Re parameter space. For each case, the five metrics described in § 2.4 have been plotted, normalised by its maximum — as in Figure 2.23 but for the whole radial length of the well. These metrics are TAWSS (yellow), MagMeanWSS (blue), OSI (purple), transWSS (red), and transWSSmin (green). Each plot has been colour-framed after the simulation sets coding described in § 3.3, and are schematically distributed in the E - F - Re space to enable visualisation of all plots.

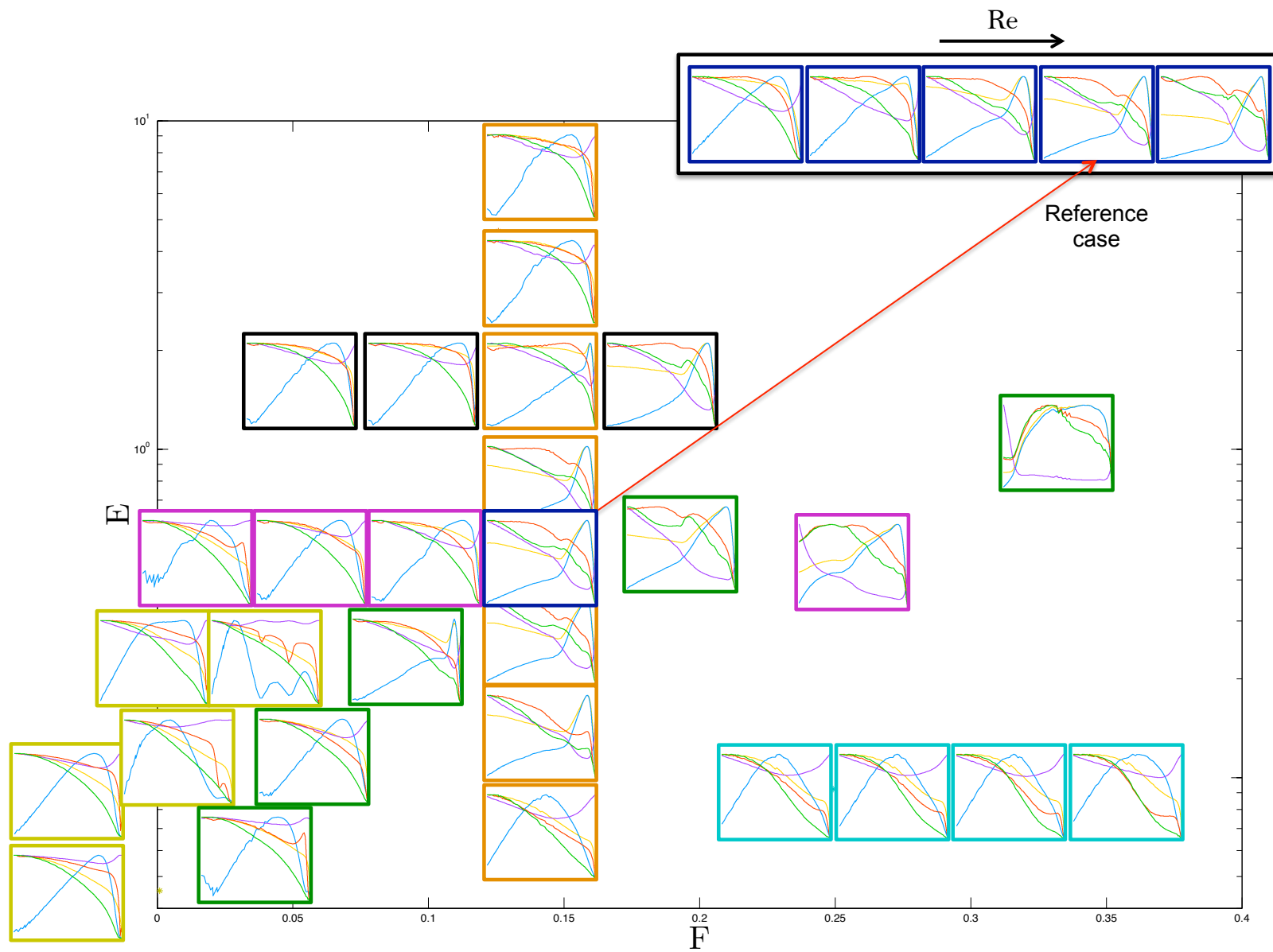


Figure C.1: WSS metrics in all case studies.

Appendix D

Matlab Codes

D.1 Amplitude and breaking

This routine provides the surface height amplitude predicted by the potential theory (§ 3.2) for a given orbital shaker configuration, and the prediction of wave breaking based on the results of Chapter 3. It also evaluates the relevance of the secondary modes in the chosen configuration, since the potential theory used fails to accurately reproduce these cases.

```
%% Matlab code to predict amplitude and wave breaking
% based on the results from Chapter 3.
clear all
close all

%% DATA INPUT (use coherent unit system)

% Choose orbital shaker configuration
d=0.002;      % fluid height at rest
R=0.01105;   % cylinder radius
a=0.005;     % orbital radius
om=2*pi*150/60; % orbital speed
g=9.81;      % gravity constant

%% START

% Breaking threshold
breaking_amplitude=1.4*d;

% Domain resolution
ndomain=200;

% Polar coordinates
Rv=linspace(0,R,ndomain);
THv=linspace(0,2*pi,ndomain);
```

```

% Time
t=0; %the amplitude is independent of time

% Bessel roots
e1n=[1.8411837813 5.3314427735 8.5363163663 11.706004903 14.863588634];

% Domain mapping height
for i=1:length(THv)

    for j=1:length(Rv)

        ri=Rv(i);
        thj=THv(j);

        % Summatory for height

        % Initialization values
        sumheight=0;

        for n=1:5

            om1(n)=sqrt(e1n(n)*g/R*tanh(d*e1n(n)/R)); % Natural frequencies

            sumheight=(2*R/(e1n(n)^2-1))*(1/((om1(n)/om)^2-1))*...
                (besselj(1,e1n(n)*ri/R)/besselj(1,e1n(n)))+sumheight;

        end

        % Fluid Height at ri,thj
        heightPT(i,j)=d+(a*om^2/g)*cos(om*t-thj)*(ri+sumheight);

    end

end

% Surface height amplitude
height_amplitudePT=max(max(heightPT))-min(min(heightPT));

% Surface amplitude to breaking amplitude ratio
amplitude_ratio=height_amplitudePT/breaking_amplitude

% Wave breaking prediction
if (amplitude_ratio>1.1)
    disp([char(10),'Wave breaking predicted.',char(10),'The potential prediction of the surface
        height amplitude is thus not accurate',char(10)])
elseif (0.9<=amplitude_ratio && amplitude_ratio<=1.1)
    disp([char(10),'Amplitude within 10% of the breaking threshold.',char(10),'Possible wave
        breaking.', char(10), 'The surface height amplitude is ',num2str(height_amplitudePT),'
        in the chosen units',char(10)])
else
    disp([char(10), 'The surface height amplitude is ',num2str(height_amplitudePT),' in the
        chosen units',char(10)])
end

% Are secondary modes relevant?
if ((om1(2)+om1(1))/2<om)
    disp([char(10),'Secondary modes relevant.',char(10),'Therefore, the potential solution is not
        reliable.',char(10)])
end

```


D.2 Natural frequencies and resonance

This routine calculates the natural frequencies of the flow as predicted by the (inviscid) potential theory (§ 3.2) for a given orbital shaker configuration. It also evaluates if the operating frequency is close to any of these frequencies, which are assumed to be also the resonant frequencies for the (viscous) computational model, and such case configuration is thus referred to as a resonance case (Chapter 3).

```
%% Matlab code to detect if operanting regime near resonant frequency
% based on the results from Chapter 3.
clear all
close all

%% DATA INPUT (use coherent unit system)

% Choose orbital shaker configuration
d=0.002;      % fluid height at rest
R=0.01105;    % cylinder radius
a=0.005;      % orbital radius
om=2*pi*150/60; % orbital speed
g=9.81;       % gravity constant

%% START

% Bessel roots
e1n=[1.8411837813 5.3314427735 8.5363163663 11.706004903 14.863588634];

% Initialization values
c=0; % for frequency counting

for n=1:5

    % Natural frequencies
    om1(n)=sqrt(e1n(n)*g/R*tanh(d*e1n(n)/R)); % Natural frequencies
    disp(['Natural frequency m=1 and n=',num2str(n),': ',num2str(om1(n))])

    % Natural frequency to operating frequency ratio
    ratio_frequencies(n)=om1(n)/om;

    c=c+1; % frequency counting

    % Resonant operating regime prediction
    if (0.9<ratio_frequencies(n) && ratio_frequencies(n)<1.1)
        disp([char(10),'Operating regime within 10% of the resonant mode m=1 and n=',num2str(n),
            char(10)])
    elseif (c==5)
        disp([char(10),'Not operating near a resonant frequency (for m=1,n<6)',char(10)])
    end
end

end
```

D.3 Wall shear stress prediction by the PT-Stokes WSS model

This routine provides the WSS magnitude at the well bottom at a given point as predicted by the PT-Stokes WSS model (4.2), (4.3), and (4.29), for a given orbital shaker configuration and fluid properties. The prediction is not provided if the operating frequency is close to a natural frequency ($|\frac{\omega_{1n}}{\omega} - 1| < 0.1$), since the PT-Stokes model overpredicts the WSS in these resonant cases due to the absence of viscous damping in the potential model.

```
% Matlab code to predict wall shear stress (WSS) at a given well bottom point
% based on the PT-Stokes WSS described in Chapter 4.
% This routine only provides a WSS prediction if the operation frequency
% is not too close to a resonant frequency [abs(om1(m)/om-1) > 0.1]
clear all
close all

%% DATA INPUT (use coherent unit system)

% Choose orbital shaker configuration and fluid properties
d=0.002;      % fluid height at rest
R=0.01105;    % cylinder radius
a=0.005;      % orbital radius
om=2*pi*150/60; % orbital speed
nu=1e-6;      % fluid kinetic viscosity
rho=1000;     % fluid density
g=9.81;       % gravity constant

% Choose a point in the well bottom domain in polar coordinates
ri=1e-10; % Use ri=1e-10 for the centre point
thj=0;

% Choose time
t=0;

%% START

% Initialization values
sumheight=0;
sumr=0;
sumth=0;
sumz=0;

% Bessel roots
e1n=[1.8411837813 5.3314427735 8.5363163663 11.706004903 14.863588634];

% Free surface height at chosen point
% Summatory for height
for n=1:5

    om1(n)=sqrt(e1n(n)*g/R*tanh(d*e1n(n)/R)); % Natural frequencies
```

```

sumheight=(2*R/(e1n(n)^2-1))*(1/((om1(n)/om)^2-1))*...
    (besselj(1,e1n(n)*ri/R)/besselj(1,e1n(n)))+sumheight;
end

% Height at chosen point
heightPT=d+(a*om^2/g)*cos(om*t-thj)*(ri+sumheight);

% Velocity evaluated at the free surface
zk=heightPT;

% Summatory for velocity
for n=1:5

    sumr=(2*R/(e1n(n)^2-1))*(1/((om1(n)/om)^2-1))*...
        *((besselj(0,e1n(n)*ri/R)*e1n(n)/R-besselj(1,e1n(n)*ri/R)/ri)/besselj(1,e1n(n)))*...
        *cosh(e1n(n)*zk/R)/cosh(e1n(n)*d/R)+sumr;

    sumth=(2/(e1n(n)^2-1))*(1/((om1(n)/om)^2-1))*(R/ri)*...
        *(besselj(1,e1n(n)*ri/R)/besselj(1,e1n(n)))*cosh(e1n(n)*zk/R)/cosh(e1n(n)*d/R)+sumth;

    sumz=(2*e1n(n)/(e1n(n)^2-1))*(1/((om1(n)/om)^2-1))*...
        *(besselj(1,e1n(n)*ri/R)/besselj(1,e1n(n)))*sinh(e1n(n)*zk/R)/cosh(e1n(n)*d/R)+sumz;

end

% Surface velocity in cylindrical coordinates at chosen point
Ur=-a*om*sin(om*t-thj)*sumr;
Uth=a*om*cos(om*t-thj)*sumth;
Uz=-a*om*sin(om*t-thj)*sumz;

% Surface velocity in Cartesian coordinates at chosen point
U=Ur*cos(thj)-Uth*sin(thj);
V=Ur*sin(thj)+Uth*cos(thj);
W=Uz;

% Surface velocity magnitude and angle at chosen point
velocityPOT=sqrt(U^2+V^2+W^2);
anglevelPOT=atan2(V,U)*180/pi;

% PT-Stokes WSS prediction if not close to a resonant frequency
if (abs(om1/om-1)>0.1)

    % WSS magnitude and angle predicted by PT-Stokes
    WSSMagPTStokes=rho*velocityPOT*sqrt(nu*om);
    WSSangPTStokes=anglevelPOT+45;
    disp([char(10),'The WSS magnitude predicted by the PT-Stokes model is ',num2str(
        WSSMagPTStokes),' in the chosen units',char(10)])

else

    disp([char(10),'Operating regime within 10% of a resonant mode.',char(10),'Therefore, the PT
        -Stokes WSS model not reliable.',char(10)])

end

```