Generation of Chemical Movies using FTIR Spectroscopic Imaging of Segmented Flows

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ABSTRACT

We have previously demonstrated that FTIR spectroscopic imaging can be used as a powerful, label-free detection method for studying laminar flows. However, to date, the speed of image acquisition has been too slow for the efficient detection of moving droplets within segmented flow systems. In this paper, we demonstrate the extraction of fast FTIR images with acquisition times of 50 ms. This approach allows efficient interrogation of segmented flow systems where aqueous droplets move at a speed of 2.5 mm/s. Consecutive FTIR images separated by 120 ms intervals allow the generation of chemical movies at eight frames per second. The technique has been applied to the study of microfluidic systems containing moving droplets of water in oil and droplets of protein solution in oil. The presented work demonstrates the feasibility of using FTIR imaging to study dynamic systems with sub-second temporal resolution.

INTRODUCTION

Fourier transform infrared (FT-IR) imaging has been shown to be a powerful label-free and non-destructive chemical imaging method.¹ The contrast mechanism is based on the ability to quantify characteristic vibrations of the molecules under investigation and is therefore highly chemically specific. FT-IR imaging has been applied to a wide range of chemical and biological systems, including biomaterials such as skin, artery, bone,^{2, 3} cancerous tissues,^{4, 5} polymer, and pharmaceutical analysis.^{6, 7} With the use of focal plane array (FPA) infrared detectors, it is possible to measure ‘chemical images’ with all spectra acquired simultaneously.⁸ Briefly, an FPA detector incorporates thousands of individual infrared detectors laid out in a two-dimensional array. Each of the individual detectors collects chemical information from a localised area of the sample in the form of an absorption spectrum. These small detectors (or pixels) together form a large field of view image that provides the spatial distribution of various components within the sample. FPA detectors have been used successfully to extract spatially resolved chemical information from dynamic systems exhibiting relatively slow processes (ranging from seconds to minutes) such as chemical changes in live cells,⁹ diffusion of chemicals in polymers and polymer processes,¹⁰⁻¹³ hair fibres,¹⁴ lipid bilayer,¹⁵ biological tissues^{16, 17} and the dissolution of pharmaceutical formulations.¹⁸⁻²¹

In recent years the concept of miniaturization has been applied to a range of chemical and biological problems in the fields of proteomics, genomics, drug discovery, high-throughput screening and medical diagnostics.²²⁻²⁴ Of particular note has been the development of microfluidic technology, which has been motivated by the fact that physical processes can be more easily controlled, accelerated and exploited

when system dimensions are reduced to a micron or sub-micron scale.²⁵ The application of FT-IR imaging in microfluidic environments has recently been demonstrated.²⁶⁻²⁸ FT-IR imaging provides a direct method of simultaneously monitoring multiple analytes within laminar flows without the need of adding labels. For example, Chan and co-workers have reported the study of mixing of PEG and water²⁸ and the formation of HDO from D₂O and H₂O in a laminar flow environments.²⁷

Unfortunately, conventional FT-IR imaging methods are too slow to capture information rich chemical images of picoliter-sized droplets moving within an segmented flow and as previously discussed have to date only been applied to the investigation of continuous flows. Accordingly the ability to increase image acquisition rates would significantly broaden the applications of FT-IR imaging and allow the capture and interrogation of fast chemical processes. More specifically, in the case of segmented flow microfluidics, rapid FT-IR imaging would directly enable the quantitative analysis of reactive processes occurring within moving droplets without the need for analyte labelling.

FTIR images measured with an FPA detector are typically acquired in continuous scanning (or rapid-scan)^{29, 30} mode. As part of this process a number of co-adding interferograms are scanned, averaged and then processed before the next measurement is started. This data processing task produces a time lag of approximately 40 s between image acquisitions while the detector does not collect any data, therefore severely limiting attainable temporal resolution. Significantly higher acquisition rates are required to image faster or more transient processes. To achieve such a “chemical movie” requires an increase in imaging rate of at least two to three orders of magnitude. This is achievable if only single wavelength measurements are required and a tuneable, narrow band infrared source such as a quantum cascade laser is available. For examples, the frame rate of a modern 64 x 64 FPA detector is ~3775 Hz with an

associated integration time as low as 500 μ s which can capture infrared images at every 0.26 ms. Video-rate single wavelength molecular imaging using stimulated Raman has been also demonstrated with 512 x 512 pixel image measured in 37 – 150 ms.³¹

Experimental Section

FTIR spectroscopic imaging. An infrared spectrometer (IFS66s, Bruker Optics, Germany) equipped with a macro chamber attachment (IMAC, Bruker Optics) was employed for all experiments. A horizontal macro transmission accessory was used to allow the microfluidic device created on the transmission cell to be probed in a horizontal position. Further details about this accessory can be found elsewhere.³² Through this optical system no magnification is expected. A 64x64 focal plane array detector (40 μ m per pixel) was used for imaging, generating an imaging area of 2.56 mm x 2.56 mm with a lateral spatial resolution of 40 μ m. Spectra were measured at 16 cm^{-1} resolution.

Microfluidic device fabrication. Microfluidic devices were fabricated using a droplet-on-demand microdroplet printing system (AutoDrop®, MicroDrop). The system consists of a xyz control unit, a heated dispensing nozzle head with heated reservoir, an xyz position robotic arm and built-in computer control. Paraffin (mp 58-62 °C, Sigma-Aldrich) was melted by heating to 90°C in the reservoir. The nozzle head was subsequently maintained at 90°C during the deposition process. Paraffin walls were created by printing wax drops (approximately 50 μ m diameter) adjacent to each other with a separation of 50 μ m. Moreover, each microfluidic device is fabricated by direct printing onto a CaF_2 infrared transparent window. Further details concerning the fabrication process can be found elsewhere.²⁸

Segmented flow microfluidics. Picoliter-sized droplets were generated by introducing water through a wide input channel (240 μm wide, 25 μm height) and a continuous oil phase (FC40, source) via a narrower input channel (140 μm wide, 25 μm height) at a standard “T” junction (140 μm wide, 25 μm height). The joined stream then enters a serpentine region (240 μm wide, 25 μm height) with two 90° and three 180° turns before exiting the device. The speed and size of individual droplets are controlled by the volumetric flow rates of both the water and oil streams. A CaF_2 liquid cell (Omni Cell, Specac Ltd, UK) with 25 μm spacers was used as the substrate. The device was connected to poly(ethylene) tubing for injection of liquids into the device. Precision syringe pumps (PHD 2000, Harvard Apparatus) were used to deliver the fluids into the channel. Deionised water and oil (Sigma Aldrich) were used as model fluids in this demonstration. Lysozyme (Sigma Aldrich) was dissolved in deionised water to create the 2.7 mM solution.

Results and discussion

Previous attempts to perform FT-IR imaging of fast dynamic systems have adapted the approach often used in time-resolved infrared spectroscopy.³³ In such experiments, a step scan spectrometer is used to record images at specific time points of a repeating event. Using this technique, time resolved FT-IR images³⁴ or linear images³⁵ can be achieved with millisecond temporal resolution. However, this approach relies on reproducing identical events and requires a precise timing of the measurement process. A second approach to achieve high temporal resolution is to operate in “kinetic scan” mode. In a “kinetic scan” mode, instead of generating a number of interferograms for averaging, each scan is taken as an individual measurement with minimum time lag in between. Temporal resolution in this case is defined by the speed of the interferometer and the actual time taken to perform each scan. In such

a scanning mode, all data processing is performed at the end of the experiment. Accordingly, the time taken per scan is determined by the detector frame rate (which is also a function of the number of pixels used), spectral resolution and the spectral range. As noted, when using a 64 x 64 FPA detector, the frame rate is approximately 3775 Hz. For a spectral resolution of 8 cm^{-1} and a spectral range of 3940 cm^{-1} in an imaging measurement, the total number of data points collected in the interferogram will be 1110 when using single sided backward-forward collection mode (the fastest scanning method) or 1776 when using double sided backward-forward collection mode (the most efficient scanning method offered by the spectrometer used in this study).

In the current study, the spectrometer is set to operate at of 75% of the maximum frame rate (to improve on the stability on the data read-out), thus yielding a scanning time of 400 ms and 630 ms per scan. An object (in this case a droplet) speed of less than 3 projected pixels per scan would not cause significant blurring of the captured image because the image sharpness is limited to between 2 and 3 pixels due to other optical aberrations caused by, for example, imperfect mirrors. Since each projected pixel has a size of $40 \times 40\text{ }\mu\text{m}^2$, the maximum droplet speed under these conditions without causing image blurring is between 190 and 300 $\mu\text{m/s}$. Infrared microscope can also be used with this device to improve this spatial resolution but resulting in a much smaller imaged area, hence reducing the maximum speed of studied moving droplets. Importantly, since the most informative part of the infrared spectrum is its fingerprint region between 1000 and 1800 cm^{-1} , a reduction of the measured spectral range will increase the scanning speed without a significant compromise in accessible chemical information. Indeed the use of a low pass filter will easily reduce the spectral range to between 1000 and 1974 cm^{-1} . In turn, this will reduce the scanning time four fold to between 100 and 160 ms per scan. Another way of increasing imaging speed is to collect at lower spectral resolution. This is suitable when spectral bands are well

separated. When spectral resolution is reduced from 8 to 16 cm^{-1} , the number of data points needed is also halved and therefore the scanning time can be further reduced to between 50 and 80 ms. Accordingly, the maximum droplet speed that can be captured without significant blurring increases to 2.4-1.5 mm/s.

To demonstrate imaging of picoliter droplets within segmented flows using FT-IR spectroscopy, a microfluidic device was fabricated by wax printing (Figure 1a). Consecutive FT-IR images of water droplets flowing inside the microfluidic channel (with a field of view of 2.56 mm x 2.56 mm) are shown in Figure 1b. The generated images are based on the water bending mode absorption at 1640 cm^{-1} . As observed, it is possible to capture sharp spectroscopic images at 120 ms time intervals with 50 ms per scan. The time interval is found to be larger than the scanning time and a time lag of 70 ms in between each scan is observed. Nevertheless, from calculation of the displacement of individual water droplets between images, droplets were seen to move at a speed of approximately 2.5 mm/s. These images were subsequently used to generate chemical movies at ten frames per second (**see electronic supplementary material as Web Enhanced Object: chemical movie.avi**). Note that 4096 spectra (in the range of 1000 and 1974 cm^{-1}) are measured in each image frame and spectra can be extracted from within each droplet providing chemical information at high temporal resolution.

The system was also characterised using aqueous droplets formed from a dilute (2.7 mM) solution of the protein lysozyme. To define the location of the protein solution droplet in the channel, the water band at 1640 cm^{-1} is monitored and used to construct the image shown in Figure 2a. Imaging of lysozyme contained within the droplets shown in Figure 2b and generated using the characteristic lysozyme amide II band at $\sim 1558 \text{ cm}^{-1}$. For reference, the corresponding spectrum extracted from a lysozyme solution

droplet is shown in Figure 2c. As shown, despite the fact that each image is collected using a single scan (in 80 ms), it is still possible to detect the presence of the protein in a moving droplet at low solute concentrations.

Conclusions

In conclusion, the presented method demonstrates the imaging of fast dynamic processes, exemplified by droplet flow within microfluidic channels. Chemical images with a scanning time of 50 ms and a temporal resolution of 120 ms are reported. These images can potentially be used to produce chemical videos of fast reactions²⁴ which cannot be captured using conventional approaches. Importantly, and unlike conventional time-resolved step scan measurements, the method utilises a commercially available continuous scan spectrometer, does not require the establishment of a repeating event or the use of a reversible reaction.

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Figure 1a. Left: Schematic diagram of the microfluidic device which is created by wax printing on a CaF_2 substrate (the window of the transmission cell). Middle: Visible image of the resultant wax channel created by the wax printing. The square with dotted line shows the area where FTIR images are captured. Right: FTIR image of the wax channel with an imaging area of 2.56 mm x 2.56 mm. Orange/red represent the area where wax is present, blue represent the absence of wax.

Figure 1b. Ten consecutive snapshots of the water droplets flowing in the microfluidic channel at a speed of ~ 2.5 mm/s using the FTIR imaging system. Images are created using the water band at 1640 cm^{-1} . The time of measurement taken is indicated at the bottom of each image in seconds with the first measurement as time zero. The size of the imaged area is $2.56\text{ mm} \times 2.56\text{ mm}$.

Figure 2. A snapshot of the dilute protein solution droplet (lysozyme) flowing in the wax-printed microfluidic channel captured using the FTIR imaging system. a) Image created using the water band at 1640 cm⁻¹. b) Image created using the protein amide II band at 1558 cm⁻¹. The size of the imaged area is 2.56 mm × 2.56 mm. c) Extracted spectrum measured from a protein solution droplet (black line). The spectrum measured from a water droplet extract from Figure 1b is also inserted for comparison purpose (grey line).

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