FLUORESCENCE LIFETIME IMAGING WITHIN MICROFLUIDIC STRUCTURES USING A MAXIMUM LIKELIHOOD ESTIMATOR

Margarita A. Stapountzi[1,2] and Joshua B. Edel[1,2]

[1,2]Department of Chemistry and Institute of Biomedical Engineering, Imperial College London, South Kensington, London, United Kingdom

ABSTRACT

A novel technique is developed for visualizing hydrodynamic focusing within microchannels by using Fluorescent Lifetime Imaging (FLIM) along with a Maximum Likelihood Estimator (MLE) adapted from single molecule studies. As little as 10 photons are required to accurately determine fluorescence lifetime and build up a 2-D map of fluorescent lifetimes within a microfluidic device.

KEYWORDS: FLIM, MLE, microfluidics, hydrodynamic focusing

INTRODUCTION

FLIM is a powerful tool in biomedical research, and advances in detection technology along with the sensitivity of fluorescent molecules make it ideally suited for the study of biomolecular interactions. Unlike fluorescence intensity, which often depends on fluorophore concentration, detection efficiency, illumination intensity and uniformity, fluorescence lifetimes are not affected by such factors. Therefore, FLIM overcomes the artefacts arising from intensity measurements and provides improved precision and contrast in monitoring complex biological processes. Herein, we developed a novel technique for performing FLIM using a MLE. We demonstrate the feasibility of our detection technique using a microfluidic mixer.

THEORY

In microfluidics applications, whereby high sensitivity and high detection efficiency are required, hydrodynamic focusing may be applied as a sample confinement mechanism to ensure that the majority of the sample molecules traverse the probe volume and are subsequently detected (Fig.1). The side inlet flows are used for 'squeezing' the central sample flow into a narrow stream. By varying the side inlet flow rates, the dimensions of the focused stream can be made comparable to the dimensions of the detection volume. The width of the focused stream depends on the ratio α of the side inlet flow rate to the central inlet flow rate [1, 2].

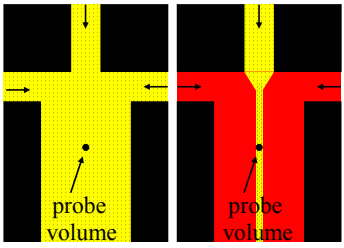

Fig.1. Hydrodynamic focusing used as a sample confinement mechanism.

EXPERIMENTAL

A laser scanning confocal microscope with an integrated home-built detection path was used for excitation and fluorescence detection (Fig.2). An avalanche

978-0-9798064-2-1/μTAS2009/$20

photodiode detector (APD) operating in single photon counting mode was used to achieve high detection sensitivity.

Analyte molecules within the detection probe volume were excited by a 466 nm pulsed diode laser operating at a repetition rate of 20 MHz and time correlated single photon counting (TCSPC) was used to acquire fluorescence lifetime data.

The 3inlet-1outlet microfluidic devices used, were fabricated with standard soft lithographic techniques having 20 µm channel depth and a width ranging from 20 to 120 µm. Two dye solutions with different lifetimes (Rhodamine 110 Cl and Acridine Orange, lifetimes 3.8 ns and 1.8 ns respectively) were delivered into the central and side inlet channels and hydrodynamic focusing was visualized by using FLIM and a MLE approach.

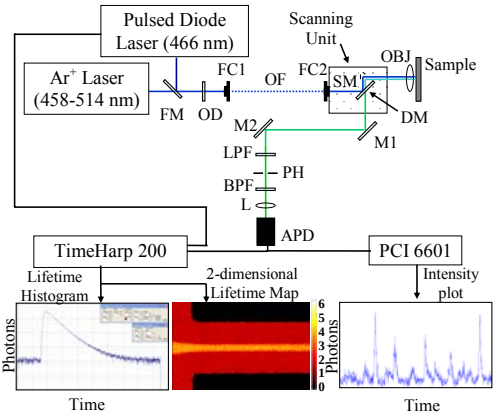

Fig.2. Schematic representation of the optical setup. (FM:flip-mount mirror, OD:neutral density filter, FC:fibre coupler, OF:optical fibre, SM: scanning mirror, DM:dichroic mirror, M:mirror, OBJ:objective, LPF:long pass filter, BPF:band pass filter, L:lens, PH:pinhole, APD:avalanche photodiode detector).

RESULTS AND DISCUSSION

Fluorescence intensity and lifetime images illustrate that artefacts arising from intensity measurements such as edge effects and fluorescence intensity variations occurring at solution boundaries are removed when using FLIM (Fig.3).

Experimentally measured fluorescence lifetimes are described by Poissonian and multinomial statistics therefore for a high number of photon counts, this converges to Gaussian statistics. Under high photon count conditions, the fluorescence lifetime is typically extracted by a least squares (LS) fitting approach. When determining a fluorescence lifetime with less than 2000 photons, the conventionally used LS approach is not appropriate as the error originating from assuming a Gaussian distribution becomes significant. Therefore, a MLE previously developed in our lab [3, 4] defined by multinomial statistics was used for fluorescence lifetime determination. The MLE equation used herein is given by:

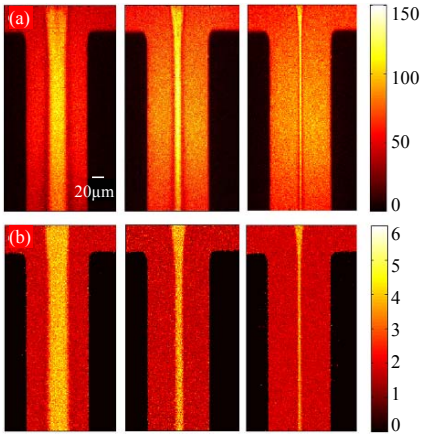

Fig.3. 2-D intensity (a) and lifetime (b) maps within the microfluidic device. The images shown are for ratio α=1,4,7 from left to right.

$$\frac{N}{1-e^{-\omega/\tau}}-\frac{Nk}{e^{k\omega/\tau}-1}=\sum_{i=1}^{k} in_i\left(\frac{i-\sum_{j=1}^{i} jr_j e^{j\omega/\tau}}{\sum_{j=1}^{i} r_j e^{j\omega/\tau}}\right) \quad (2)$$

where n_i is the number of photon counts in channel i, k is the number of channels, $N=\sum n_i$ is the total number of counts for a given decay and τ is the fluorescence lifetime. The instrument response function corresponds to r_j for each bin j, recorded with a time resolution ω and was determined by using the picosecond lifetime dye Rose Bengal. As little as 10 photons were used herein to accurately extract the fluorescence lifetime for each pixel and produce 2D fluorescence lifetime maps.

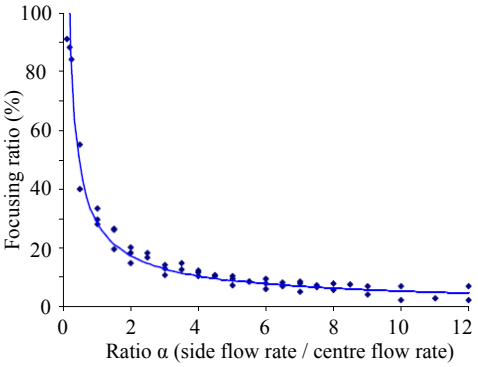

Fig.4. Focusing width as percentage of outlet channel width with respect to ratio α.

Fig.4 shows how the hydrodynamic focusing depends on ratio α. Increasing α up to 7 reduces the width of the focused stream while beyond that point there is not significant change. Values of α above 12 were not achievable in the current system as the side stream began to enter the central inlet and focusing was lost. For the device studied here, the optimum focussed width was 6.12 μm (5.1% of the outlet channel width) for ratio α=7.

CONCLUSION

We have shown by using our home-built optical setup and custom-written MLE that we can construct high resolution two-dimensional fluorescence lifetime maps of the flow within microchannels. FLIM provides improved contrast and eliminates artefacts arising from fluorescence intensity measurements. Fluorescence lifetimes were successfully calculated using as little as 10 photons indicating that our technique is a powerful tool for single molecule studies that require extraction of information from a typically low amount of collected photons.

ACKNOWLEDGEMENTS

This work was in part supported by the Engineering and Physical Sciences Research Council (EPSRC).

REFERENCES

[1] Knight, J.B., et al., Physical Review Letters, 1998. **80**(17): p. 3863-3866.
[2] Hertzog, D.E., et al., Analytical Chemistry, 2006. **78**(13): p. 4299-4306.
[3] Edel, J.B., et al., Journal of Physical Chemistry B, 2007. **111**(11): p. 2986-2990.
[4] Srisa-Art, M., et al., Phys Rev Lett, 2008. **101**(1): p. 014502.