



Heriot-Watt University
Research Gateway

Microfluidic devices and biological lasers for biophotonic applications

Citation for published version:

MacKenzie, MD & Kar, AK 2019, 'Microfluidic devices and biological lasers for biophotonic applications', *Journal of Physics: Conference Series*, vol. 1151, no. conference 1, 012001. <https://doi.org/10.1088/1742-6596/1151/1/012001>

Digital Object Identifier (DOI):

[10.1088/1742-6596/1151/1/012001](https://doi.org/10.1088/1742-6596/1151/1/012001)

Link:

[Link to publication record in Heriot-Watt Research Portal](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Journal of Physics: Conference Series

General rights

Copyright for the publications made accessible via Heriot-Watt Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

Heriot-Watt University has made every reasonable effort to ensure that the content in Heriot-Watt Research Portal complies with UK legislation. If you believe that the public display of this file breaches copyright please contact open.access@hw.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

PAPER • OPEN ACCESS

Microfluidic devices and biological lasers for biophotonic applications

To cite this article: M D Mackenzie and A K Kar 2019 *J. Phys.: Conf. Ser.* **1151** 012001

View the [article online](#) for updates and enhancements.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

Microfluidic devices and biological lasers for biophotonic applications

M D Mackenzie* and A K Kar

Institute of Photonics and Quantum Sciences, Heriot Watt University, Edinburgh, EH14 4AS, United Kingdom

*Email: m.mackenzie@hw.ac.uk

Abstract. There is great potential for microfluidic devices in medical and civil applications. This talk will present work on ultrafast laser inscription for manufacturing microfluidic devices, temperature sensing, cell counting, 3D imaging of cells and biological lasers.

1. Introduction

This manuscript will present several microfluidic devices developed over the past few years using ultrafast laser inscription and selective chemical etching. Microfluidics as a field shows great promise for creating compact, robust, portable and low-cost devices which are suited to numerous applications. We have developed devices for use in temperature sensing, cell counting, 3D imaging amongst other applications.

2. Microfluidics

Microfluidics is an area of research focused on techniques to miniaturise medical and biological diagnostics with the aim of improving throughput, decreasing required sample volumes and reducing costs. It is an expanding field with an increasing number of publications each year [1]. Two of the most widely used methods of creating microfluidics are 1) lithography in Polydimethylsiloxane (PDMS) and 2) ultrafast laser inscription (ULI) followed by selective chemical etching. Our work makes use of the 2nd method of microfabrication as it allows for completely 3D fabrication, fast prototyping and does not require clean room facilities. While the devices presented in this manuscript are fabricated in fused silica glass it is possible to create devices in a variety of glasses, crystals and plastics [2-4].

Integrating both microfluidic channels and waveguides inside substrates allows for the fabrication of a wide variety of devices. This can be used for simple illumination of a sample for fluorescence excitation [5] or heating of a sample. Alternatively, it is possible to use waveguides to deflect [6], trap, count [7] and stretch cells through the use of counter propagating waveguides [8]. Many traditional optical components such as mirrors and interferometers can be miniaturized and integrated into microfluidics [9, 10] resulting in smaller more rugged diagnostic methods.

2.1. Ultrafast laser inscription

In ULI an ultrafast laser, with femtosecond scale pulse length, is focused into a material which is transparent to the laser wavelength. Due to the high irradiance at the focus the pulses are absorbed through multi photon processes leading to changes in material structure. This change can take multiple form such as refractive index change, both positive and negative, which can be used to fabricate



waveguides and nanocrack formation or weakening of the material which can be used to selectively etch microfluidic devices [11]. This is illustrated in figure 1. Both types of modification can be implemented in a single run by changing laser parameters. Our work presented below uses this technique to fabricate devices for a range of applications including temperature sensing, cell counting and cell imaging.

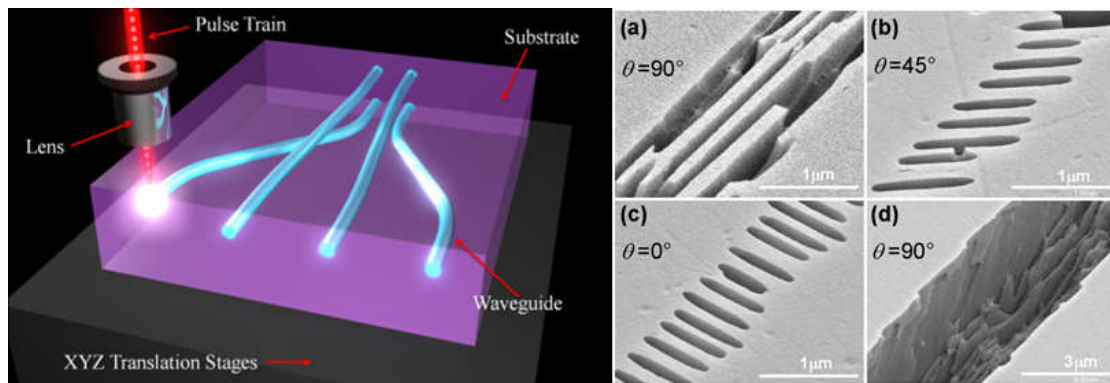


Figure 1. A femtosecond laser focused into a material can draw lines of modification for guiding light or create cracks for etching. (a)-(d) show that the orientation of these cracks can be controlled via laser polarisation with those aligned parallel to the line of modification etching faster than those perpendicular [12].

2.2. Temperature sensing

Temperature sensing in microfluidic devices is both crucial to device performance and difficult to measure. Traditional thermometers are likely to perturb the system they are monitoring so are not suitable on the scales we are interested in. Fluorescence thermometry is an alternative method of temperature sensing more suited to the scales required. The technique uses quantum dots [13] or fluorescent dye [14] which reacts to temperature changes, shifting in either wavelength or intensity. The change in the dye emission relative to temperature is calibrated by measuring its response at known temperature settings. The calibrated emission can then be used to non-invasively measure temperature. This has proven successful but demonstrations to date have either focused on static conditions or not studied the effects flow has on temperature distribution. To fully understand the dynamics of the devices we fabricate, predict how they will affect cells inside them and optimise our experiments we need to know how flow alters temperature distribution.

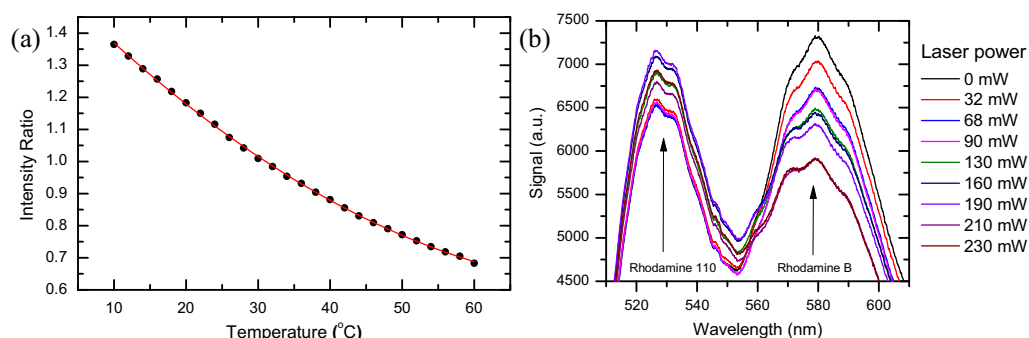


Figure 2. Two dye emission spectrum. (a) shows the response ratio of dye emission change as they are heated on a hot plate used for calibration. (b) shows the change in emission as the sample is heated using a laser through a waveguide. [15]

To investigate if it is possible to monitor the behaviour of microfluidic devices for a range of changing flow conditions a range of microfluidic devices were either fabricated, in fused silica via ULI or purchased, in PDMS. For our measurements we used two fluorescent dyes, Rhodamine B which is temperature sensitive and Rhodamine 110 which is not. Through the use of two dyes the Rhodamine 110 act as a calibration to remove influences other than temperature in the measurement. Figure 2 shows this in action where the ratio of the dye emission is first calibrated using a known temperature then the emission spectrum recorded as the sample is heated using a laser beam.

The technique was then applied to measure the temperature within an optical cell sorter, shown in figure 3(a). The evolution of temperature is shown in figure 3(b) where after switching on the temperature stabilises over ~ 5 s and it can be seen that for higher flow rates there is an increasing displacement of heating along the channel due to the flow.

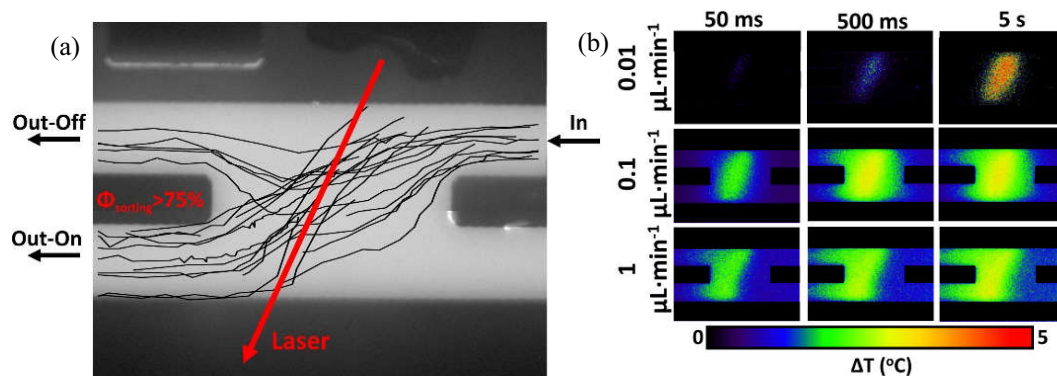


Figure 3. Microfluidic cell sorter. (a) particle tracking showing the sorting in action. (b) temperature for different flow velocities over time. [15]

2.3. Flow cytometry

The fabrication of microfluidic devices which mimic or exceed the capabilities of current experimental equipment has been one of the aims of the field since its beginning. Applying microfluidics to flow cytometers has the potential to decrease their cost and size opening up their use to every research laboratory and to clinical point of care uses. Demonstrations to date have mostly made use of PDMS due to its widespread availability and ability to fabricate highly defined channels. Demonstrations of imaging flow cytometers manufactured using PDMS have achieved throughputs of up to 20,000 cells per second [16]. We were interested in using ULI to fabricate an imaging flow cytometer in fused silica, taking advantage of the materials high durability to produce a more reusable device suitable for real world settings.

Our device is shown in figure 4, designed for both cells counting and 3D imaging. Multiple devices were fabricated in fused silica using ULI and selective chemical etching. For cell counting a camera is placed over the sample and images taken as cells flow past. This is then analysed in MATLAB to identify and count cells. Using red blood cells as a sample we were able to achieve a throughput of up to 4,700 cells per second [17].

To enable 3D imaging, we introduced an angle to the device channel which caused the cells to move through the focal plane of the objective as they moved across its field of view. This allowed us to capture multiple images through the cell. Using deconvolution and stacking the images it was possible to create a 3D model of the cell. To test our device, we first used microspheres with a known size and shape, spheres with a diameter $4.2 \pm 0.2 \mu\text{m}$. Our measurement produced the results shown in figure 5(a-d), a sphere with diameter $4.2 \pm 0.2 \mu\text{m}$. To test a more complex shape we used bovine sperm cell nucleus shown in figure 5(e). This produced the expected shape showing our method can be applied to more complex samples.

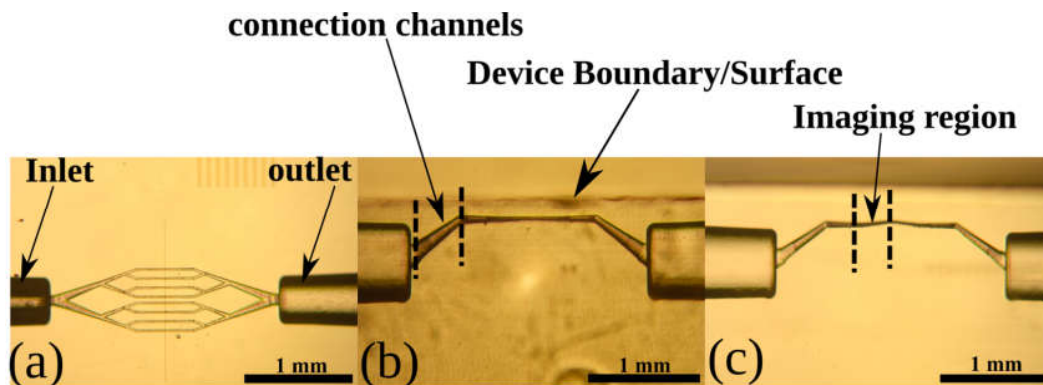


Figure 4. Microfluidic cell counter and imaging. (a) top view of the device showing multiple channels to enable multiplexing and continued operation if one becomes blocked, (b) size image of the device showing the channel moves to the surface for easier imaging, (c) angling the centre of the channel for 3D imaging. [18]

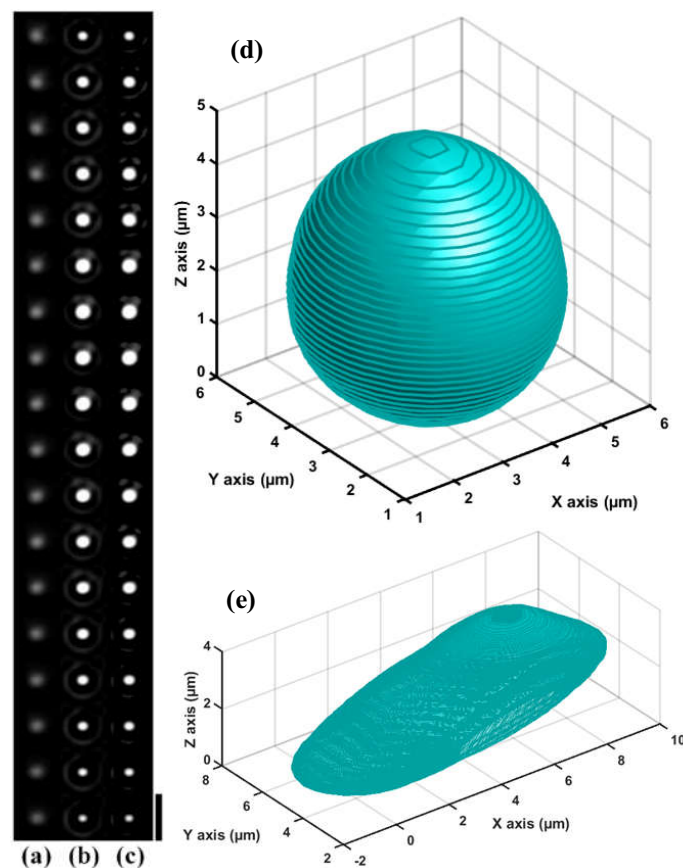


Figure 5. 3D imaging in a microfluidic device. (a) raw images of a microsphere at different focal depths, (b) & (c) the processed images, scale bar 10 μm . Stacking these gives a spherical shape as expected. The lower image is for bovine sperm cell nucleus which shows the expected shape. [18]

3. Living Laser

Using a biological cell containing a gain medium to form a ‘living’ laser is a novel design first demonstrated by Gather and Yun in 2011 using HEK293 cells containing the fluorescent protein eGFP [19]. They demonstrated that a laser cavity made from such an unconventional object was possible and that the cell was unharmed by the lasing process. Different cells produced different transverse modes indicating that it may be possible to gain structural or even functional information about the cells through the lasing process. This initial demonstration made use of a plane-plane laser cavity, further investigations have demonstrated the use of microspheres inserted into cells [20, 21] or cells within liquid droplets forming whispering gallery mode resonators [22].

To investigate the fabrication and potential applications of living lasers we formed a laser cavity containing HEK293 cells either expressing the fluorescent protein eGFP or stained with the fluorescent dye Calcein AM. Cells were placed in a plane-plane cavity of length $\sim 20 \mu\text{m}$ and pumped using femtosecond pulses. As the fluorescence lifetime of most fluorescent dyes and proteins is on the order of nanoseconds [23, 24] we used a shorter excitation pulse in order to create a large population inversion pre lasing. This has previously been shown to be successful for a 1D random laser containing the fluorescent protein DsRed [25].

We observed lasing behavior with a low threshold energy of $\sim 300 \text{ pJ}$ and lasing emission lasting up to 60s. To investigate if this could be extended we first confirmed that the cells were not being harmed by the lasing process using the viability indicator propidium iodide which confirmed the cells were still intact after lasing. The reduction in emission after 60s was due to photobleaching of the sample, a common issue in experiments using fluorescent stains. To reduce this, we used the antioxidant vitamin C. When this was added to the cells we saw an increase in lifetime of up to 140s.

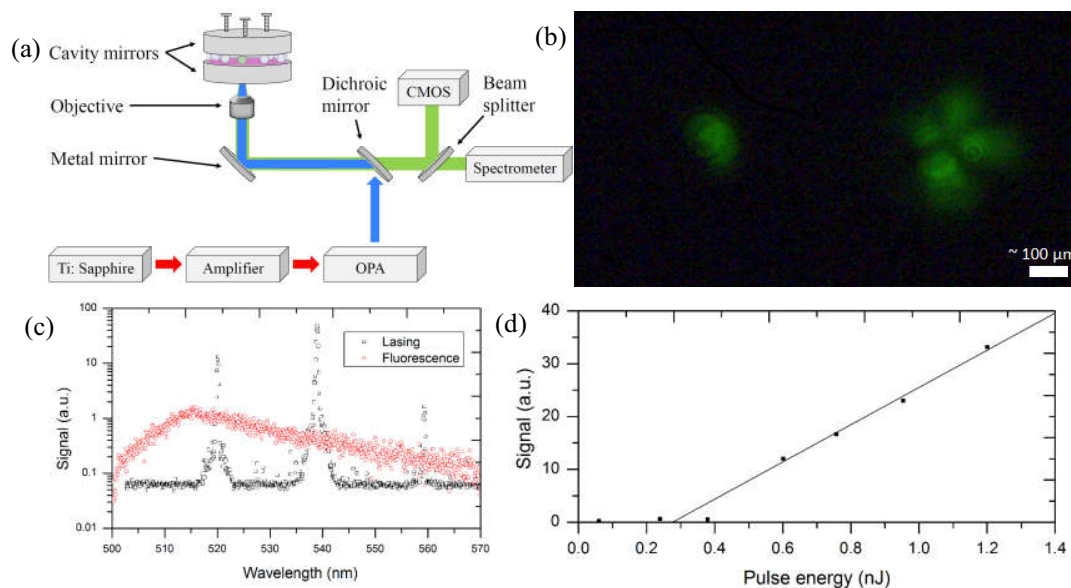


Figure 6. Cell laser. (a) experimental setup, (b) transverse lasing modes from the cells, (c) laser emission spectrum showing longitudinal modes, (d) emission vs pump energy. [26]

4. Conclusion

We have presented research in this manuscript focusing on microfluidics and biophotonics. The variety of devices can be attributed to the versatility of the ULI fabrication method. Other talks will present work by the NLO group using the same method for the fabrication of lasers in multiple glasses and microfluidics in different materials. As our work is ongoing we aim to continue the work presented here and integrate it into ongoing and future work.

References

- [1] Sackmann E K, Fulton A L and Beebe D J 2014 The present and future role of microfluidics in biomedical research *Nature* **507** 181-9
- [2] Choudhury D, Rodenas A, Paterson L, Jaque D, Kar A K and Ieee 2013 3D Microfabrication in YAG Crystals by Direct Laser Writing and Chemical Etching *2013 Conference on Lasers and Electro-Optics Pacific Rim (Cleo-Pr)* **2**
- [3] Jo B H, Van Lerberghe L M, Motsegood K M and Beebe D J 2000 Three-dimensional micro-channel fabrication in polydimethylsiloxane (PDMS) elastomer *Journal of Microelectromechanical Systems* **9** 76-81
- [4] Choudhury D, Rodenas A, Paterson L, Díaz F, Jaque D and Kar A K 2013 Three-dimensional microstructuring of yttrium aluminum garnet crystals for laser active optofluidic applications *Applied Physics Letters* **103** 041101
- [5] Vazquez R M, Osellame R, Nolli D, Dongre C, van den Vlekkert H, Ramponi R, Pollnau M and Cerullo G 2009 Integration of femtosecond laser written optical waveguides in a lab-on-chip *Lab on a Chip* **9** 91-6
- [6] Keloth A, Paterson L, Markx G H and Kar A K 2015 Three-dimensional optofluidic device for isolating microbes *SPIE Photonics West*
- [7] Paie P, Bragheri F, Vazquez R M and Osellame R 2014 Straightforward 3D hydrodynamic focusing in femtosecond laser fabricated microfluidic channels *Lab on a Chip* **14** 1826-33
- [8] Bellini N, Vishnubhatla K C, Bragheri F, Ferrara L, Minzioni P, Ramponi R, Cristiani I and Osellame R 2010 Femtosecond laser fabricated monolithic chip for optical trapping and stretching of single cells *Optics Express* **18** 4679-88
- [9] Crespi A, Gu Y, Ngamsom B, Hoekstra H, Dongre C, Pollnau M, Ramponi R, van den Vlekkert H H, Watts P, Cerullo G and Osellame R 2010 Three-dimensional Mach-Zehnder interferometer in a microfluidic chip for spatially-resolved label-free detection *Lab on a Chip* **10** 1167-73
- [10] Cheng Y, Sugioka K, Midorikawa K, Masuda M, Toyoda K, Kawachi M and Shihoyama K 2003 Three-dimensional micro-optical components embedded in photosensitive glass by a femtosecond laser *Optics Letters* **28** 1144-6
- [11] Choudhury D, Macdonald J R and Kar A K 2014 Ultrafast laser inscription: perspectives on future integrated applications *Laser & Photonics Reviews* **8** 827-46
- [12] Hnatovsky C, Taylor R S, Simova E, Bhardwaj V R, Rayner D M and Corkum P B 2005 Polarization-selective etching in femtosecond laser-assisted microfluidic channel fabrication in fused silica *Optics Letters* **30** 1867-9
- [13] Vetrone F, Naccache R, Zamarron A, de la Fuente A J, Sanz-Rodriguez F, Maestro L M, Rodriguez E M, Jaque D, Sole J G and Capobianco J A 2010 Temperature Sensing Using Fluorescent Nanothermometers *Acs Nano* **4** 3254-8
- [14] Jaque D and Vetrone F 2012 Luminescence nanothermometry *Nanoscale* **4** 4301-26
- [15] del Rosal B, Sun C, Yan Y, Mackenzie M D, Lu C, Bettiol A A, Kar K and Jaque D 2014 Flow effects in the laser-induced thermal loading of optical traps and optofluidic devices *Optics Express* **22** 23938-54
- [16] Schonbrun E, Gorthi S S and Schaak D 2012 Microfabricated multiple field of view imaging flow cytometry *Lab on a Chip* **12** 268-73
- [17] Jagannadh V K, Mackenzie M D, Pal P, Kar A K and Gorthi S S 2015 Imaging Flow Cytometry With Femtosecond Laser-Micromachined Glass Microfluidic Channels *Ieee Journal of Selected Topics in Quantum Electronics* **21** 6
- [18] Jagannadh V K, Mackenzie M D, Pal P, Kar A K and Gorthi S S 2016 Slanted channel microfluidic chip for 3D fluorescence imaging of cells in flow *Optics Express* **24** 22144-58
- [19] Gather M C and Yun S H 2011 Single-cell biological lasers *Nature Photonics* **5** 406-10
- [20] Humar M and Yun S H 2015 Intracellular microlasers *Nature Photonics* **9** 572
- [21] Schubert M, Steude A, Liehm P, Kronenberg N M, Karl M, Campbell E C, Powis S J and

- Gather M C 2015 Lasing within Live Cells Containing Intracellular Optical Microresonators for Barcode-Type Cell Tagging and Tracking *Nano Letters* **15** 5647-52
- [22] Jonas A, Aas M, Karadag Y, Manioglu S, Anand S, McGloin D, Bayraktar H and Kiraz A 2014 In vitro and in vivo biolasing of fluorescent proteins suspended in liquid microdroplet cavities *Lab on a Chip* **14** 3093-100
- [23] Berezin M Y and Achilefu S 2010 Fluorescence Lifetime Measurements and Biological Imaging *Chemical Reviews* **110** 2641-84
- [24] Patel H, Tscheka C and Heerklotz H 2009 Characterizing vesicle leakage by fluorescence lifetime measurements *Soft Matter* **5** 2849-51
- [25] Drane T M, Bach H, Shapiro M and Milner V 2013 *Frontiers in Ultrafast Optics: Biomedical, Scientific, and Industrial Applications Xiii*, ed A Heisterkamp, *et al.* (Bellingham: Spie-Int Soc Optical Engineering)
- [26] M. D. Mackenzie C H, R. Duncan and A. Kar 2015 Femtosecond Pumped Biological Laser *Photonics West, SPIE BiOS*