Multimode fibre: a pathway towards deep tissue fluorescence microscopy

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ABSTRACT

Fluorescence microscopy has emerged as a pivotal platform for imaging in the life sciences. In recent years, the overwhelming success of its different modalities has been accompanied by various efforts to carry out imaging deeper inside living tissues. A key challenge of these efforts is to overcome scattering and absorption of light in such environments. Multiple strategies (e.g. multi-photon, wavefront correction techniques) extended the penetration depth to the current state-of-the-art of about 1000μm at the resolution of approximately 1μm. The only viable strategy for imaging deeper than this is by employing a fibre bundle based endoscope. However, such devices lack resolution and have a significant footprint (1mm in diameter), which prohibits their use in studies involving tissues deep in live animals. We have recently demonstrated a radically new approach that delivers the light in/out of place of interest through an extremely thin (tens of microns in diameter) cylindrical glass tube called a multimode optical fibre (MMF). Not only is this type of delivery much less invasive compared to fibre bundle technology, it also enables higher resolution and has the ability to image at any plane behind the fibre without any auxiliary optics. The two most important limitations of this exciting technology are (i) the lack of bending flexibility and (ii) high demands on computational power, making the performance of such systems slow. We will discuss how to overcome these limitations.

Keywords: multimode optical fibre, digital holography, fluorescence microscopy, micro-endoscopy, wavefront shaping

1. INTRODUCTION

Research in life sciences increasingly relies on obtaining high spatial resolution information from systems and processes that are deep inside biological tissues 1. This represents a major obstacle as most if not all modern imaging techniques fall into three categories - high resolution with very limited penetration depth 2, standard resolution with average penetration depth 3 or low resolution with exceptional penetration depth 4 - none of which are able to observe sub-cellular systems deeper than few millimetres. In recent years, multimode fibre (MMF) based endoscopes presented a possible solution to this problem. As opposed to bulky single mode fibre bundles, MMFs are thin and as such cause minimum mechanical damage upon insertion. Both imaging 5-10 and micromanipulation 11,12 techniques were demonstrated and showed great promise of the technology. The applications are based on the realisation that the input and output optical fields are related through the transformation matrix of the fibre. With the knowledge of the transformation matrix one can design an input field such that a desired output field is displayed at the other end of the fibre (beam-shaping) 12 and vice versa, one can use inverse transformation to realise imaging with the fiber 6. Both applications are realised using a hologram on a Spatial Light Modulator (SLM).

The two most important limitations of this exciting technology are (i) high demands on computational power, making the performance of such systems slow and (ii) the lack of bending flexibility. We will discuss how to overcome these limitations.
2. ULTRA-FAST BEAM SHAPING AT THE OUTPUT FACET OF MULTIMODE OPTICAL FIBRE

In the following we focus our attention on the ability to shape the light at the output facet of the fibre. The hologram generation procedure for arbitrary output field is computationally very intensive, which, until recently, restricted the fibre-based manipulation and potential structured illumination imaging techniques to pre-calculated holograms. Our previously published system\textsuperscript{13} dealt with the computational needs by harnessing the parallel power of modern GPUs and allowed real-time beam-shaping in multimode fibers. The system was capable of displaying on-demand oriented cube, made out of 120 points, at the distal end of the fiber at the refresh rate of 50 Hz. However, the key element of the system was an Acousto-Optic Deflector (AOD) that produced points at the distal end of the fiber in time-discrete intervals. The time-discretization of points removes the interference (present due to non-orthogonality of output points\textsuperscript{12}) but also significantly increases system complexity due to the presence of an AOD. Furthermore, only a limited number of points (120) was used for the output pattern as the fibre input fields would start to overlap in the SLM Fourier space for larger number of AOD deflections.

Here, we present a significantly simplified and improved system without the added AOD complexity. We remove the undesired interference effects computationally using the GPU accelerated Gerchberg-Saxton (GS) and Yang-Gu (YG) algorithms. The algorithms were previously implemented on a CPU platform\textsuperscript{12} restricting the use of the technology to the pre-calculated holograms. The GPU implementation is two orders of magnitude faster than the CPU implementation allowing video-rate image control at the distal end of the fiber virtually free of interference effects.

Figure 1 shows the experimental setup used to realise AOD-free light-shaping at the end of M\textsubscript{MF}. The system is used to measure the transformation matrix of the fibre, which is then used for subsequent beam-shaping. We have used a modified version of Yang-Gu and Gerchberg-Saxton algorithms\textsuperscript{13,14} and implemented the hologram generation on a GPU platform, which allows for massive parallelization of complex computations involved. This allowed real-time beam-shaping (target refresh rate of more than 50 Hz) for complex output patterns at the end of the fibre.

3. BENDING DYNAMICS OF MULTIMODE FIBRE

The transformation matrix of the fibre is valid only for conformation of the fibre for which it was measured. Any changes in conformation of the fibre, such as bending, will lead to change of transformation matrix of the fibre and subsequent loss of imaging capability. We have recently shown\textsuperscript{15}, that it is possible to predict transformation matrix of the bent fibre from the transformation matrix of the straight fibre. This removes the necessity to empirically measure the transformation matrix every time the fibre conformation is changed, and also without the need to access the distal end of the fibre.
Figure 1. Linearly polarised light (CrystaLaser CL532-075-S) passes through half-wave plate and optical isolator (Thorlabs IO-3-532-LP). This configuration prevents back reflections into the laser and at the same time allows control of power in the system. The second half-wave plate together with the polarising beam splitter was used to control the splitting of power between the two fiber coupling optical pathways. The optimal coupling into the Polarisation Maintaining Fiber (PMF) (Thorlabs P1-488PM-FC-2) was achieved by using $L_1 = 100$ mm plano-convex lens and $L_2 = 8$ mm aspheric lens. Coupling into the single-mode fiber (SMF) (Thorlabs P1-405B-FC-5) was realised using a dielectric mirror $M_1$ and aspheric lens $L_3 = 8$ mm. Both SMF and PMF were cleaved at the coupling site at an angle of approximately 10 degrees to remove the power oscillations in the system due to SMF and PMF acting like resonators. Without the angled cleave, the optical power in the system oscillated on average by 10 percent making the measurement of the transformation matrix inaccurate. The output polarisation of PMF was aligned with the polarisation axis of the SLM (BNS HSPDM512-(480-540mm)-DVI) and the light was collimated onto the SLM by $L_4 = 60$ mm. The light from the SLM passed through $L_5 = 100$ mm and all the light except the first order was filtered on the iris. Telescope consisting of lenses $L_6 = 50$ mm and $L_7 = 8$ mm, yielding a slightly higher NA than the NA of the MMF (Thorlabs FG050UGA), was used for the coupling into the MMF. The quarter-wave plate transformed the linearly polarised light into circularly polarised light. This step ensures that the input field is decomposed into real-eigenmodes of the straight fiber, which then propagate through the fiber without coupling into other polarisation. The selected mode-basis works also very well for bended fibers, but the larger the bending the stronger the coupling to the opposite circular polarisation modes, which ultimately leads to stronger background noise during beam-shaping. Light exiting the MMF was collected by a microscope objective (MO) (Olympus PlanN 20x/0.40) and the circularly polarised light was transformed back to a linearly polarised light using another quarter-wave plate. The light was then re-combined with the collimated output $L_8 = 8$ mm from SMF on the non-polarising beam splitter (NPBS). Lens $L_9 = 150$ mm was used to image the pattern resulting from interference of SMF reference and MMF beam. CCD (Basler piA640-210gm) was used for transformation matrix measurement. SMF beam was blocked for subsequent beam-shaping applications.
Figure 2a shows bending configurations of the fibre that we have tested. Figures 2b shows the theoretically predicted change of phase of the fibre eigenmodes (plotted in the mode pyramid used in\textsuperscript{15}) due to bending and Figure 2c shows the same for experimentally measured phase changes. The two clearly match each other. This means that the changes in transformation matrix can be theoretically predicted by simply observing the configuration of the fibre. For more details on how we calculated the phase change of the modes in bent fibre, please see the supplementary information in\textsuperscript{15}.

The imaging of USAF 1951 target for the maximally bent fibre (case V in Figure 2a) is clearly not optimal as can be seen on Figure 3a. But after applying the theoretical correction to transformation matrix, the imaging quality is significantly improved (Figure 3b).

![Figure 2. (a) Fibre conformations tested. (b) Bending of the fibre changes phase of the output eigenmodes (especially those with low orbital angular momentum value) (theoretical prediction of the output phases); (c) experimentally measured change of phase of fibre output eigenmodes. The comparison shows a very good agreement between theory and experiment.](image-url)
Figure 3. (a) Imaging of the USAF 1951 target using the bent fibre (conformation V from Figure 2a) and empirically measured matrix for the straight fibre. (b) Imaging using the same bent fibre with the empirically measured matrix of the straight fibre, however, this time with theoretical corrections to eigenmode phases included.

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7A  Biocompatible Materials II  
   Peggy P. Chan, Swinburne University of Technology (Australia)
Introduction

In December 2013, the United Nations declared 2015 as the International Year of Light (IYL), recognizing the immense importance of light-based technologies in our lives, for our futures, and for the development of humankind.

In December 2015, the SPIE Micro+Nano Materials, Devices, and Applications symposium and the new Australian Institute for Nanoscience (AaN) at the University of Sydney’s Camperdown campus offered the opportunity to celebrate the culmination of the IYL and heightened global awareness of the importance of light-based technologies, including nanoscience.

The SPIE symposium is an interdisciplinary forum for collaboration and learning among top researchers in all fields related to nano- and microscale materials and technologies. This 2015 event took place over 4 days, 6-9 December, and included both oral and poster presentations with a focus on nanostructured and biocompatible materials, medical and biological micro/nanodevices, micro/nanofluidics and optofluidics, nanophotonics for biology and medical applications, plasmonics, and solar cell technologies and fabrication.

The University of Sydney is Australia’s first university with an outstanding global reputation for academic and research excellence. Located close to the heart of Australia’s largest and most international city, the Camperdown campus features a mixture of iconic gothic-revival buildings and state-of-the-art teaching, research, and student support facilities. The University of Sydney attracts many of the most talented students in Australia drawn by its range of quality degrees and strong track record of research programs. The University’s academics are leaders in their disciplines nationally and internationally, driving major research initiatives.

Sydney is Australia’s truly international city and one of the world’s most iconic and livable cities in the world, with plenty of open space, famous beaches, glittering harbour, waterways and bushland, great climate and vibrant culture rich of entertainment, cultural activities, and sporting events. Sydney is at the heart of Australia’s economy, and is ranked first in the Asia Pacific in terms of intellectual capital and innovation. Sydney offers a safe and secure environment for individuals and families, with world-class health care, education, transport and telecommunications with a multicultural environment as over a third of Sydney’s population was born overseas.

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