Semi-random multicore fibre design for adaptive multiphoton endoscopy

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Abstract: This paper reports the development, modelling and application of a semi-random multicore fibre (MCF) design for adaptive multiphoton endoscopy. The MCF was constructed from 35 sub-units, each comprising 7 single mode cores, in a hexagonally close-packed lattice where each sub-unit had a random angular orientation. The resulting fibre had 385 single mode cores and was double-clad for proximal detection of multiphoton excited fluorescence. The random orientation of each sub-unit in the fibre reduces the symmetry of the positions of the cores in the MCF, reducing the intensity of higher diffracted orders away from the central focal spot formed at the distal tip of the fibre and increasing the maximum size of object that can be imaged. The performance of the MCF was demonstrated by imaging fluorescently labelled beads with both distal and proximal fluorescence detection and pollen grains with distal fluorescence detection. We estimate that the number of independent resolution elements in the final image – measured as the half-maximum area of the two-photon point spread function divided by the area imaged – to be ~3200.

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1. Introduction

The use of endoscopy to perform optical imaging in remote spaces is an established technique and there is continued interest to develop thinner endoscopes enabling access to smaller and more confined locations. One approach to this has been to utilise adaptive optics approaches in combination with optical fibres with multiple single (or few) mode cores [1] or a single multimode optical fibre [2] to perform scanning-spot imaging without the need for a distal lens or scanner. The desired intensity distribution in the focal plane is achieved through programmed interference of the radiation emerging from the optical fibre cores or modes. This requires that the radiation emerging from the cores is temporally coherent.

The single multimode optical fibre approach is compact, does not require specialised multicore optical fibres (MCF) and is well-suited to imaging fluorescence using single photon excitation [3]. However, modal dispersion in multimode fibre makes it challenging to transmit the ultrashort pulses required for multiphoton excitation, since the coherence length of the pulses is typically smaller than the spread in optical path lengths between modes, although this is possible by restricting the modes used to a small subset with similar optical path lengths [4].

The MCF approach was first demonstrated using ~500 cores of a commercially available 30,000 core fibre [1]. In this type of optical fibre the cores are packed closely to maximise the number of cores, and hence image pixel elements, for a given fibre diameter. Such fibres are deliberately manufactured in a way that the propagation constants of the cores varies between cores so as to reduce cross-talk between cores [5]. This is achieved by allowing the core diameter to vary between cores, which leads to some cores supporting a few modes at the wavelength used. The advantages of this type of fibre include a high mode area fill factor, and therefore a high fraction of the total light that can be concentrated into a single focal spot at the distal end of the fibre, and the absence of higher diffracted orders in the desired focal plane as there is no periodic arrangement of the cores. These benefits were also demonstrated in an elegant paper that employed short coherence length interferometry to correct modes with similar propagation constants to enable multiphoton excited fluorescence microscopy with proximal fluorescence detection through a similar commercially available MCF [6]. A disadvantage of the tightly-packed semi-random MCF is that ultrafast short coherence length light required for multiphoton fluorescence excitation means that only a fraction of the cores and/or modes within the cores in the bundle are sufficiently closely matched in path length to be utilised to generate a distal focus.

An alternative approach to MCF design is to use a more uniform core size with a similar propagation constant for all cores and to employ a larger core spacing to reduce cross-talk between cores. However, the greater core spacing reduces the mode area fill factor, which reduces the fraction of light that can be concentrated into the focal spot. In addition, the simplest MCF designs have a periodic array of fibre cores leading to a periodic array of focal spots in the desired focal plane, which limits the maximum size of object that can be imaged [7, 8]. This problem has recently been addressed through the use of a semi-random distribution of cores with a uniform core size [9], although this demonstration did not include proximal detection of the excited fluorescence. The semi-random core distribution led to the energy that would otherwise have been present in higher order diffracted spots being distributed more uniformly across the sample, thus increasing the maximum size of object that could be imaged. One advantage of utilising MCFs with a uniform core size is that it is relatively straightforward to measure – and therefore correct for – variations in optical path length between cores, enabling proximal measurement of and correction for the effect of fibre bending to be achieved [10].

In this paper we extend the approach of Sivankutty et al. [9] by incorporating multiple single mode cores into each sub-unit of the final MCF to increase the number of MCF cores.
In addition, we fabricated a double-clad fibre that enables multiphoton fluorescence imaging with proximal fluorescence detection. We used the fibre developed to demonstrate multiphoton fluorescence imaging with proximal detection for the first time using a semi-random MCF with widely-spaced cores. In addition, we demonstrate that for multiphoton excitation it is possible to produce an image with a greater number of pixels than MCF cores, at the expense of a reduced signal to background ratio.

2. Methods

2.1 Fibre fabrication

The MCF was constructed in a 2-step process. In the first step, an initial stack was prepared consisting of 7 graded index germanium doped silica preforms (Draka–Prysmian) arranged in a hexagonal array as part of a close-packed array of pure silica rods, see the left hand side of Fig. 1. A total of 85 rods (78 silica, 7 germanium doped) with a diameter of 1.66 mm were used in the first stage and were arranged inside a silica tube with an internal diameter of 17 mm and an outer diameter of 20 mm. Air gaps within the stack were filled with smaller diameter pure silica rods as shown in Fig. 1. This initial stack was then drawn down to canes with an outer diameter of 1.33 mm.

In the second step, 55 of the 1.33 mm diameter canes produced in the first step were stacked inside a silica tube with an internal diameter of 10.9 mm and an outer diameter of 12.5 mm. The rotational orientation of each cane within the stack was random, and air gaps within the stack were filled with smaller diameter pure silica rods as shown in the right hand side of Fig. 1. This stack was then drawn down to a preform. To improve the fluorescence collection efficiency of the final fibre, the preform was inserted into a low index fluorine doped silica jacket tube (NA = 0.22, Heraeus) before being drawn to fibre. The final MCF had 385 cores and an outer diameter of 420 μm. The resulting minimum separations between cores within a sub-unit and between sub-units were calculated to be 9.0 μm and 13.4 μm respectively. The final diameter of the cores was 2.6 μm, resulting in single mode guidance and a measured inter-core cross-talk of < 5%. As we demonstrate below, this enables the MCF to be used to generate a scanning spot over a large field of view without having to consider the effect of cross-talk between cores.

![Fig. 1. Multicore fibre design. Left hand side, schematic of arrangement for the first step in the manufacture process. White circles show rods consisting of pure silica. Grey circles show rods consisting of graded index germanium doped silica preforms. Right hand side, schematic of the final multicore fibre consisting of 55 canes stacked together to provide a total of 385 cores – in the actual fibre the rotation of each cane is randomised. The outer cladding is not shown.](image-url)
2.2 Adaptive multiphoton endoscopy

The experimental setup used, see Fig. 2, is similar to that presented previously [8] and its description is reproduced here for completeness.

Excitation pulses of 100 fs duration were provided by a Ti:Sapphire mode-locked femtosecond laser (Tsunami 3941-M3BB, Spectra-Physics) with an 80 MHz repetition rate and a centre wavelength of 800 nm. The spectral bandwidth was 10.9 nm corresponding to a coherence length of 52 µm. For the low average power (<0.3 mW) in each optical fibre core used here, there was no measurable increase in spectral bandwidth after propagation through the MCF. Thus we expect negligible pulse broadening due to self-phase modulation but the pulses were broadened by group velocity dispersion (GVD), which is dominated by the material dispersion at 800 nm, resulting in an output pulse duration of 370 fs after propagation through the 30 cm long MCF.

The laser beam was split into two arms of a Mach-Zehnder interferometer via a polarizing beam splitter (PBS), one part to be delivered to the sample via the MCF and the other providing a reference beam enabling optical path length variations between cores to be measured from interference fringes recorded by the camera (C). The relative intensity between the two beams was controlled by rotating the half-wave plate (HWP1). In the sample arm, the beam incident on the SLM (HSPDM512, Boulder Nonlinear Systems) was expanded so as to slightly overfill the active area of the SLM through a magnifying telescope (L3 and L4). We used a phase-only reflection SLM, which has 512 × 512 pixels and a pixel pitch of 15 µm. The SLM was then imaged onto a plane 20 µm in front of the proximal end of the MCF by two 4-f imaging systems (L5, L6 and L7, OL3) with an overall de-magnification
factor of 22.2. To minimize the intensity of light propagating through the cladding and to transmit the light through the cores of the MCF efficiently, a microlens array of focal length $f = 9.9$ mm was generated on the SLM, which resulted in an array of focal spots in the plane of the proximal end of the MCF, each with a numerical aperture of approximately 0.22, matching the spatial locations of the individual MCF cores. Thus the SLM could be used to control the phase of the light coupled into each fibre core. To further minimise the amount of excitation light travelling in the cladding of the MCF, binary amplitude modulation was implemented by applying a large phase-ramp to those areas of the SLM where an amplitude of zero was required. Light diffracted from these regions was blocked by an iris diaphragm (ID) placed between L5 and L6. A 30 cm length of MCF was used for all experiments. The distal end face of the MCF was imaged onto a scientific complementary metal oxide semiconductor camera (Zyla 10-tap sCMOS, Andor) through a 4-f imaging system (OL4 and L10) with a magnification of 16.6.

In the reference arm of the interferometer, a single mode fibre (SMF, 780HP, Thorlabs) of the same length (30 cm) as the MCF was used to match approximately the dispersion in the two arms. The sample and reference beams were recombined to generate interference fringes on the camera using a non-polarizing beamsplitter (BS). A polarizer (P) placed in front of the camera was used to select a linear polarization state for detection on the camera (C) and HWP2 was used to increase the amount of the reference beam passing P. The relative time delay between the sample and the reference beams was adjusted to maximize the contrast of the interference fringes using the mechanical delay stage (DS).

The procedure used to calibrate the system and the image processing procedure implemented in C++ used to determine the phase distribution at the distal end of the MCF were as described in [8].

To demonstrate two-photon excited fluorescence (TPEF) imaging, a sample was inserted at the focal plane of the wavefronts emerging from at the distal end of the MCF and the excitation spot was scanned using the SLM. TPEF from the sample was transmitted by the multimode inner cladding of the multicore fibre for proximal detection by PMT1 (PMH-100-0, Becker & Hickl GmbH) after the dichroic beamsplitter DB1 (Di01-R594-25x36, Semrock). TPEF could also be collected in transmission by a microscope objective (OL4) and reflection from the dichroic beamsplitter DB2 (Di01-R594-25x36, Semrock) to be detected by the photomultiplier tube PMT2 (PMH-100-1, Becker & Hickl GmbH). Band-pass (FF02-470/100-25, Semrock) and low-pass filters (FF01-680/SP-25, Semrock) located in front of the PMTs were used to block any residual light from the excitation laser.

3. Results

3.1 Simulations

The performance of the MCF design was simulated using MATLAB (Mathworks) by the following method. The simulation starts with the quantised phase distribution programmed onto the SLM, followed by scaling of the field by the magnification of the 4 lenses L5-L7 and OL3 to the focal plane of OL3, propagation of the field to the proximal end of the MCF using the angular spectrum of plane waves method, calculation of the coupling efficiency of light into each core using a Gaussian approximation to the core mode, randomisation of the linear input polarisation state by the fibre using the approach described by Eq. (5) of [8], propagation of the field output at the distal end of the MCF by a predefined distance to the focal plane and the calculation of the total intensity and total two-photon excitation signal from a randomly distributed population of absorption dipoles. The field emitted from each core of the distal end of the MCF was approximated by a circularly symmetrical Gaussian profile with a $1/e^2$ mode field diameter of 2.6 $\mu$m (measured from an image of the distal end of the actual fibre used with only a single core illuminated at the proximal end). The optical path lengths of the cores within the MCF were assumed to be random and were drawn from a uniform distribution in the range 0 to $\lambda$. Figure 3(a) shows the microlens profile programmed...
onto the SLM to correct for the path length variations for one simulated realisation of the MCF and to bring the light to a focus at a distance of 0.6 mm from its distal end. Figures 3(d) and 3(b) show the resulting intensity distribution at the distal end of the MCF and in the focal plane respectively.

The fraction of power in the central focal spot relative to the total power coupled into the multicore fibre was calculated to be 1.3%. Figure 3(c) shows a vertical line profile through the centre of the image shown in Fig. 3(b) and illustrates some of the higher order diffracted spots in the focal plane. Figure 3(e) shows the two photon excitation efficiency at each point in the focal plane. The fraction of two photon fluorescence originating from the focal spot (for the case of a uniform thin fluorescent sheet placed in the focal plane) was calculated to be 40%. Figure 3(f) shows a vertical line profile through the centre of Fig. 3(e) illustrating how the two photon excitation probability greatly reduces the effect of the higher order diffracted spots shown in Fig. 3(c).

Fig. 3. Simulation of the semi-random multicore fibre. (a) Phase profile of the simulated SLM. The false colour bar shows phase through the range $0..2\pi$. (b) Intensity distribution at the focal plane 0.6 mm from the distal tip of the multicore fibre. (c) Vertical line profile taken through the centre of (b). (d) Intensity distribution at the distal tip of the fibre. (e) Two photon excitation efficiency in the focal plane. (f) Vertical line profile taken through the centre of (e). (a&d) scale bar 50 $\mu$m. (b&e) scale bar 25 $\mu$m
3.2 Fibre characterisation

Fig. 4. (a) Reflected light image of the cleaved end of the MCF with additional illumination through the MCF to increase contrast of the cores, scale bar 100 \( \mu \text{m} \). (b) Scanning electron microscope image of the MCF cores.

The fibre fabricated is shown in Fig. 4 and the measured distance between cores within a sub-unit was 8.5 \( \mu \text{m} \), in reasonable agreement with the value expected from the design. The variation in optical path length between cores was measured to have a standard deviation of 59 \( \mu \text{m} \), which is only slightly larger than the coherence length of the source used of 52 \( \mu \text{m} \).

3.3 Adaptive multiphoton endoscopy

Fig. 5. Angular histograms of the output phases for 352 useable cores measured at the distal end of the MCF for 352 useable cores a) before and b) after phase correction. The number of cores whose phase values fall within each histogram bin are displayed on the radial scale and the phase angle in degrees is shown on the outer circumferential scale.

Correction for the optical path length variations was applied to the SLM using the method described previously [8] and the resulting distribution of the phases of the cores is shown in Fig. 5. Applying the correction reduced the circular variance of the phase histograms shown in Fig. 5 from 0.91 to 0.13. Adding curvature to the phase profile on the SLM generated a focused spot at 0.6 mm from the distal tip of the fibre, see Figs. 6(b) and 6(c), which was imaged using the camera.
The lateral full-width at half maximum of the focal spot was measured to be 3.3 μm. The total power output from the MCF was 90 mW of which 0.6 mW was calculated to be within the central focus, corresponding to 0.6% of the total transmitted power. This is lower than the simulated value of 1.3%, which we attributed to the path length variation between cores in the fibre being slightly larger than the coherence length of the source.

Were the MCF to consist of a single regular hexagonal lattice of cores with a separation of 8.5 μm, this would result in a hexagonal lattice of approximately equally bright spots separated by 57 μm in the focal plane 0.6 mm from the distal tip of the fibre. The corresponding higher-order spots can be seen in Fig. 6(c) as indicated with a yellow arrow. Due to the semi-random configuration of the cores, the brightest higher-order spot has a peak intensity that is 14% of the central spot and has 26% of the energy of the central spot.

The centre core of each sub-unit is arranged in a regular hexagonal lattice with a spacing of 31 μm. This corresponds to a hexagonal lattice of spots separated by 15 μm in the focal plane 0.6 mm from the distal end of the fibre. These spots are indicated by the green arrow in Fig. 6(c). The brightest of these spots has a peak intensity of 8% of the central spot and has 14% of the energy of the central spot.

To approximately simulate multiphoton excitation, we numerically squared the image shown in Fig. 6(b) and, accounting for the fact that this image only includes half of the total signal due to polariser P (see Fig. 2) and considering the case of a uniform thin fluorescent object placed in the focal plane, we estimate 19% of the total two-photon signal to originate from the central spot, compared to 40% from the simulation. Again, we attribute this difference to the path length variation between cores in the fibre being slightly larger than the coherence length of the source.

Fig. 6. Intensity distribution in the object plane 0.6 mm away from the distal end of the MCF recorded with the camera a) before and b&c) after correction for an applied focal length of 0.6 mm. a-c) are all to the same scale, bar 50 μm. In a) & c) the intensity is displayed with a gain of 10 × relative to b). d) Line profile taken along the yellow line indicated in c).
Figure 7 demonstrates the ability of the new MCF to image an increased field of view compared to that expected from an MCF comprising a regular hexagonal lattice of cores. Fluorescent beads (3.55 µm diameter, Light Yellow, Spherotech, Inc.) were imaged using both proximal and distal detection of the fluorescence and for a 50 ms pixel dwell time. The signal to background ratio (SBR) measured from Fig. 7(b) was 5.9.

Imaging of pollen grains (Mixed Pollen Grains Slide, Carolina) with distal detection was also demonstrated with a 50 ms pixel dwell time, see Figs. 7(d) and 7(e). It was not possible to image the pollen grains proximally owing to the lower signal level for this detector.

For a multicore fibre with a core-to-core spacing of 8.5 µm and a focal distance of 0.6 mm, the maximum size of object that can be imaged is 56 µm due to the spacing between the hexagonal lattice of excitation spots. Using the semi-random multicore fibre presented here, we have demonstrated the ability to image a field of view 117 µm across. The FWHM of the single-photon illumination intensity distribution at 0.6 mm from the distal end of the fibre without phase correction was measured to be 235 µm from Fig. 6(a) and this corresponds to a two-photon illumination FWHM of 166 µm. Therefore a field of view of this diameter could potentially be imaged using this MCF at the chosen focal distance of 0.6 mm.

In Fig. 7 we demonstrated imaging over a square field of view with side 117 µm corresponding to an area of $1.4 \times 10^4 $ µm$^2$. Using the measured point spread function (PSF) FWHM of 3.3 µm gives a one-photon half-maximum PSF area of 8.6 µm$^2$ and a two-photon half-maximum PSF area of 4.3 µm$^2$. Therefore a crude estimate of the number of independent resolution elements in the image is the ratio of these two areas, yielding $\sim$3200, which is greater than the number of cores used (352) by a factor of 9. This increase in the number of resolution elements comes at the cost of the background present in the image that originates from the energy in the higher diffracted orders of the focused spot.

**4. Discussion**

In the semi-random MCF design, much of the energy that would otherwise be concentrated into sharp higher diffracted orders of the focused spot – in the case of a regular array of cores – is spread across the field of view as a background speckle pattern, see Figs. 6(b) and 6(c).
Using this image, we estimated that 19% of the two-photon signal originates from the central focal spot for the case of a uniform thin fluorescent object.

The effect of the residual background on the image signal to background ratio (SBR) depends on the distribution of fluorophores within the area illuminated. For a field of view with a single point feature, 19% of the recorded fluorescence will be distributed over the area of the PSF and the remaining 81% will be distributed over the whole illuminated area. Therefore the predicted signal-to-background ratio in this case is given by

$$SBR_{\text{point}} = \frac{\varepsilon A_{\text{illum}}}{(1-\varepsilon)A_{\text{PSF}}},$$ (1)

where $\varepsilon$ is the fraction of two-photon signal from the central focal spot, $A_{\text{PSF}}$ is the area of the PSF and $A_{\text{illum}}$ is the area illuminated. For the case of an object of uniform brightness occupying fraction $\phi$ of the area illuminated with features larger than the size of the PSF, Eq. (1) becomes

$$SBR_{\text{thin object}} = \frac{\varepsilon}{(1-\varepsilon)\phi}. $$ (2)

In the images of fluorescent beads shown in Figs. 8(a)-8(c), the beads occupy approximately 10% of the FOV. However, the FOV imaged (square of side 117 $\mu$m) was smaller than the illuminated FOV (disc with two-photon FWHM of 166 $\mu$m) and there were no additional beads outside the field imaged. Hence, we estimate $\phi \approx 6\%$, which gives an estimated $SBR_{\text{thin object}} = 3.7$.

The measured SBR from Fig. 7(b) was 4.9. In this image the assumptions of Eq. (2) are not well met, i.e. the beads are not uniformly bright and they are of a similar size to the PSF. Nevertheless, the simple calculation based on the measured PSF and the value measured from the image of fluorescent beads are in reasonable agreement.

We now set out to develop simple relationships linking the number of resolution elements and the SBR to the physical parameters of the MCF for two-photon fluorescence. Consider a bundle with $n_{\text{cores}}$ identical cores. At each point in the focal plane, the field is given by the coherent superposition of the fields from all cores. In the absence of phase correction, the field amplitude from each core has equal magnitude $u_0$ and random polarization with Stokes vectors uniformly distributed on the surface of the Poincaré sphere [8]. The resulting superposition also has random polarization but can be resolved onto any pair of orthogonal polarization components that are statistically independent each with a field amplitude that is Rayleigh distributed, giving a field intensity for each polarization component that is exponentially distributed [11] with mean $\langle I_{\text{speckle}} \rangle = \frac{2}{3} u_0^2 n_{\text{cores}}$.

Now consider the situation with phase correction. Our system is only able to correct the phase for one polarization component. If, for this polarization, the phases of the cores are set to be perfectly in-phase for a focal point on axis, the resulting constructive superposition will have an expected field amplitude at the focal point of $U_{\text{locus}} = \frac{2}{3} u_0 n_{\text{cores}}$, where the factor 2/3 arises from the random polarization in the cores [8] giving an intensity $I_{\text{locus}} = \frac{4}{3} u_0^2 n_{\text{cores}}^2$.

Moving away from the axis, at an angle $\theta$, the contribution from each core acquires an extra phase $2\pi\delta x_i/\lambda$ due to the increased pathlength (using the small angle approximation), where $x_i$ is the position of the core within the bundle (in the direction of the tilt). Close to the axis, the effect of the tilt phase is to reduce the amplitude resulting from the superposition giving a well-defined focal spot. However, as we move further off-axis, the random positioning of the cores results in an increasing random component to the phases, tending to a uniform random distribution over $[0, 2\pi]$ for the ideal case of a fully randomized bundle. The resulting superposition then reverts to the exponential distribution of intensity of the uncorrected
situation. For the orthogonal, uncorrected polarization component, the statistics remain unchanged across the focal plane. Note that in our system, the positioning of the cores is only partially random, with the centres of canes still located on a hexagonal lattice. The resulting correlations are responsible for the residual higher-order diffraction peaks apparent in Fig. 6(c) (see green arrow) which will give a higher background fluorescence than the ideal case.

We are now in a position to calculate the expected fluorescence response from the focal plane. On axis, for $n_{\text{cores}} >> 1$, the field is dominated by the corrected linear polarization component, which has intensity $I_{\text{focus}}$. If we assume randomly oriented dipole fluorophores, the two-photon excitation is proportional to $\left(\frac{\lambda}{4} I\right)^2$, where the factor of $1/3$ results from the mean squared projection of a randomly oriented unit dipole onto a linearly polarized field with unit amplitude. Thus the expected two-photon excitation at focus from the corrected linear polarization component is $S \propto \left(\frac{\lambda}{4} I_{\text{focus}}\right)^2 = \left(\frac{1}{4} \mu_{\parallel}^2 n_{\text{cores}}^2\right)^2 = \frac{16}{81} \mu_{\parallel}^4 n_{\text{cores}}^4$. Away from the axis, the polarization is random and may be resolved into orthogonal linear components that are parallel and perpendicular to the dipole of a randomly oriented fluorophore. Only the parallel component will interact with this dipole and will result in an exponentially distributed intensity with mean $\left(\frac{\lambda}{4} I_{\text{speckle}}\right)^2$. The factor of $2/3$ results from the mean squared projection of a randomly oriented unit dipole onto a plane. The two-photon excitation is proportional to the second moment of the intensity distribution, which for exponentially distributed intensities is $\langle I^2 \rangle = 2 \langle I \rangle^2$. Thus, for points away from the focal spot, the expected two-photon excitation is $B \propto \left(\frac{\lambda}{4} I_{\text{speckle}}\right)^2 = 2 \left(\frac{1}{4} \mu_{\parallel}^2 n_{\text{cores}}^2\right)^2 = \frac{1}{3} \mu_{\parallel}^4 n_{\text{cores}}^2$.

For a single point object, the signal-to-background in the image is then simply the ratio of the on-axis to off-axis 2-photon intensities, i.e.

$$SBR_{\text{point}} = \frac{S}{B} = \frac{81 n_{\text{cores}}^2}{81 n_{\text{cores}}^2} = \frac{n_{\text{cores}}^2}{10}. \quad (3)$$

The ratio of fluorescence from the focal spot to the total fluorescence signal $\varepsilon$ in the image is

$$\varepsilon = \frac{A_{\text{PSF}} S}{A_{\text{PSF}} S + A_{\text{FOV}} B} = \frac{S}{S + n_{\text{res}} B} = \frac{n_{\text{cores}}^2}{n_{\text{cores}}^2 + 10 n_{\text{res}}}, \quad (4)$$

where $A_{\text{PSF}}$ is the area of the focal spot, $A_{\text{FOV}}$ is the total area of the image and $n_{\text{res}}$ is the number of image resolution elements in the image, given by the ratio of image to the focus spot areas, i.e.

$$n_{\text{res}} = \frac{A_{\text{FOV}}}{A_{\text{PSF}}}. \quad (5)$$

For a thin extended object of uniform brightness occupying fraction $\phi$ of the area illuminated with features larger than the size of the focal spot, the signal to background ratio is then

$$SBR_{\text{thin object}} = \frac{A_{\text{PSF}} S}{\phi A_{\text{FOV}} B} = \frac{n_{\text{cores}}^2}{10 \phi n_{\text{res}}}. \quad (6)$$

The maximum field of view of the image is limited by the divergence of the field emitted from the cores in the bundle. We consider that the mode in a single core is described by a Gaussian with a $1/e^2$ radius of $w_0$. The $1/e^2$ divergence of the field $\theta_{1/e^2}$ from this core is given by the relationship $\theta_{1/e^2} = \lambda/(\pi w_0)$. For two-photon fluorescence, the square of intensity is the
relevant quantity, which falls to half maximum for \( \theta_{\text{IM}} = \frac{1}{2} \sqrt{2 \ln 2} \), giving a FWHM of the field of view

\[
\text{FOV} = 2f \tan \theta_{\text{IM}} = \sqrt{\ln 2} \frac{f \lambda}{\pi W_0},
\]

where \( \lambda \) is the wavelength and \( f \) is the distance at which the focus is formed from the distal tip of the MCF.

Assuming uniform illumination from all the cores in a circular bundle MCF gives a diffraction limit for the FWHM of the focal spot for two-photon excitation,

\[
\delta = 0.74 \frac{f \lambda}{D},
\]

where \( D \) is the diameter of the MCF.

This gives a crude estimate of \( n_{\text{res}} \), the maximum number of independent resolution elements possible,

\[
n_{\text{res}} = \frac{A_{\text{FOV}}}{A_{\text{PSF}}} = \left( \frac{\text{FOV}}{\delta} \right)^2 = 0.13 \frac{D^2}{W_0^2},
\]

which is independent of \( n_{\text{cores}} \).

For the MCF described in the experimental work above, the \( 1/e^2 \) radius of the fibre mode was 1.3 \( \mu \text{m} \), the diameter of the inner cladding was \( D = 293 \mu \text{m} \) and there were \( n_{\text{cores}} = 352 \) useable cores. These parameters give \( n_{\text{res}} = 6600 \) for the maximum number resolution elements with a corresponding value for \( c = 0.65 \). The experimentally estimated values from our system of around 3200 resolution elements and \( \epsilon = 0.19 \) are comparable, but smaller. We also estimated the theoretical signal-to-background for an extended object. A uniform object with \( \phi = 0.06 \) (as estimated for the fluorescent bead images of Fig. 7), gives \( \text{SBR}_{\text{res,ideal}} = 31 \) for an ideal random bundle. The experimentally estimated value of 3.7 is again smaller. There are several reasons for this. First, for the working distance \( f = 0.6 \text{ mm} \) used here, the finite divergence of the fibre cores is not negligible compared with the numerical aperture defined by the diameter of the MCF bundle, resulting in a focal spot with a larger FWHM (increasing the single-photon FWHM from the naive theoretical calculation of 1.7 \( \mu \text{m} \) to the measured value of 2.3 \( \mu \text{m} \), which would reduce the number of resolution elements by approximately one half) and a lower peak intensity, with reduced signal to background contrast. Second, that the cores are not completely randomly positioned further increases the background compared with the ideal random bundle. Third, it is also worth noting that the 2-photon signal is very sensitive to residual uncorrected phase. For example, a residual circular variance of 0.13 in the corrected phases such as observed in Fig. 5) would reduce the peak amplitude in the focus by approximately 13\%, resulting in a reduction of the peak 2-photon signal of nearly one half.

In the future, a polarisation-maintaining, semi-random multicore fibre would combine the advantages of the MCF presented here and the one used in [8]. However, the challenge is to find a way to introduce a random rotation for each cane and simultaneously induce birefringence in all cores of the cane in a manner that ensures that the fast axis of all cores within the MCF are aligned in the same direction. Further work is needed to find a practical solution to this challenge.

5. Conclusions

In this paper we have extended the approach of Sivankutty et al. [9], who presented a semi-random MCF fibre constructed from a hexagonally close-packed array of fibres where the core of each individual fibre was off-centre within its cladding and was incorporated into the
bundle with a random rotation. Our work demonstrated an MCF constructed from sub-units consisting of seven single mode cores in a hexagonal close-packed array. These sub-units were incorporated into the bundle with a random rotation. Producing the MCF required manual stacking of rods in the first step and canes in the second step, which are both highly labour intensive. Our approach of using multiple cores within each sub-unit enables the total number of cores in the final MCF to be increased without the fabrication process becoming prohibitively complex.

We demonstrated a semi-random MCF consisting of 385 cores – which is an increase by a factor of ~2.7 over previous work [9] – that was double-clad for proximal detection of two-photon excited fluorescence. Using our MCF with a focal length of 0.6 mm provided a single-photon illumination FWHM of 235 μm, compared to an illumination FWHM of 120 μm for a focal length of 0.5 mm in previous work [9]. The peak signal in the higher diffracted orders was less than 14% of that in the central focal spot and we estimated that 19% of the two-photon signal originated from the central focal spot. We demonstrated two-photon imaging with proximal fluorescence detection using a semi-random MCF with widely-spaced cores for the first time using a sample of commercially available fluorescent beads, and the final image had approximately 3200 resolution elements and a field of view of 117 μm. We presented a theoretical model that provides relationships describing how the number of resolution elements and the SBR in the final two-photon image scale for an ideal randomized MCF.

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**Data sharing statement**

The raw image data from this work is available under an open source licence from Imperial College London’s OMERO server at https://omero.bioinformatics.ic.ac.uk/omero/webclient/?show=project-4752.