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Improved depth range and resolution of inline holographic microscopy through fiber imaging bundles

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ABSTRACT

Inline holographic microscopy has recently been demonstrated through fiber imaging bundles, opening up the possibility of ultra-miniaturized microscopy probes. In order to minimize artefacts arising due to the multimode behaviour of the fiber bundle cores, a partially coherent light source was used: an LED coupled into a multimode fiber. However, partial coherence limits the maximum working distance between the bundle and the sample before the resolution begins to degrade. The resolution is also limited by the finite core spacing in the fiber bundle, leading to under-sampling of the finer details of the hologram. Here, we demonstrate and evaluate several techniques for improving the resolution and working range, including tailoring the source coherence and using multiple sources, demonstrating that we can achieve at least a two-fold improvement in performance.

Keywords: inline holography, holographic microscopy, endoscopic microscopy, fiber bundles

1. INTRODUCTION

Inline holography is a simple and versatile technique that allows for lensless transmission-mode microscopy. A diverging or collimated light source, typically a laser or spatially-filtered LED,[1] is partially scattered by the sample. Scattered and unscattered portions of the beam interfere on the plane of a camera, resulting in the collection of a digital hologram. Numerical propagation of the hologram back to the plane of the sample then allows an image to be recovered.

Recently, we have demonstrated that inline holograms can be captured through fiber imaging bundles.[2] These act as thin, flexible image conduits, transferring the hologram to a remote camera, offering the potential to perform holographic microscopy where it is difficult to place a camera. Imaging bundles have been widely used in endoscopy for many years, and more recently adapted to endoscopic microscopy, but are challenging to employ in coherent imaging applications such as optical coherence tomography[3] and holography[4] due to the multimode behaviour of the fiber cores which leads to speckle and other distortions. By using a partially coherent source - a fiber-coupled LED - these effects can be minimised for inline holography, leading to good quality imaging at short working distances.[2] However, the partial coherence then leads to a rapid drop in resolution with working distance, limiting the useful refocusing range to 1-2 mm.

1.1 Resolution and Working Distance in Fiber Bundle Inline Holography

The resolution of fiber bundle inline holography depends on four factors, all of which are related to the need to sample the interference pattern arising from light scattered at large angles, corresponding to the highest spatial frequencies and hence the finest details in the sample. Three of the factors also depend on the working distance (i.e. the distance of the object from the fiber bundle). The factors are:

- **Effective Numerical Aperture (NA):** The finite spatial extent of the fiber bundle limits the maximum angle of collected rays, as shown in Fig. 1(a). Note that this is distinct from the NA of the fiber cores, which is around 0.3 and not a limiting factor in general.

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- **Spatial Coherence:** The size of the illumination fiber core results in a geometrical blurring of the hologram, as shown in Fig. 1(b). The blurring diameter on the face of the bundle is given by bd/h where b is the core diameter, d is the sample to bundle distance, and h is the illumination fiber to sample distance.
- **Temporal Coherence:** Scattered and unscattered portions of the beam have an optical path length difference which increases with working distance, as shown in Fig. 1(c); if this significantly exceeds the coherence length of the source then interference is not obtained.
- **Core-core Spacing:** The bundle core spacing limits the sampling of the interference pattern arising from larger scattering angles. This part of the pattern has a higher spatial frequency pattern due to the way the planar surface of the bundle intersects with the spherical wavefront, as shown in Fig. 1(d).

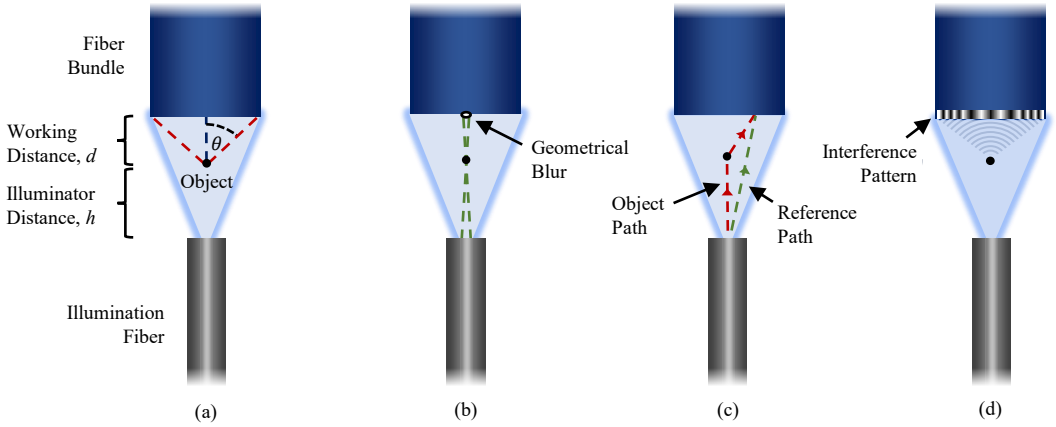


Figure 1. Illustration of limits to resolution in fiber bundle inline holography. (a) Numerical aperture, determined by fiber bundle diameter and increasing with working distance, limits largest collection angle, θ, and hence highest spatial frequency resolved. (b) Finite size of input fiber core (spatial coherence) results in geometrical blur which increases with working distance. (c) Optical path difference, which cannot be longer than the coherence length, increases with larger scattering angles, corresponding to higher spatial frequencies, and with working distance. (d) Larger scattering angles, corresponding to higher spatial frequencies, result in a higher spatial frequency interference pattern which must be sampled by bundle.

The bundle core diameter is also a limiting factor in principle, but since this is always smaller than the core pitch it has little practical impact. In practice, for very short working distances below 1 mm, the resolution is limited by the core spacing even when using high resolution bundles (3 µm core spacing) and LED light delivered via a 50 µm diameter core, 0.22 NA multimode fiber, as reported previously.[2] At longer working distances, the coherence length is the limiting factor when using an LED, assuming the illuminator to sample distance (h in Fig. 1) is kept large. Hence, to improve resolution at short working distances it is necessary to achieve denser sampling of the hologram, and to improve resolution at longer working distances the source bandwidth must be reduced.

2. METHODS

The experimental setup is shown in Fig. 2. Light is delivered from an optical source via an optical fiber, which may be single or multi-mode depending on the particular experiment. This is mounted a distance of $h = 40$ mm from the tip of a Fujikura high-density fiber bundle (FIGH-30-650S). This bundle has an imaging area diameter of approximately 600 µm, within which are approximately 30,000 cores with a typical pitch (core to core spacing) of 3 µm. The sample is always placed closer to the bundle than the illumination fiber, at a distance d which is between 0 and 2.5 mm in the experiments described here. The other end of the fiber bundle is imaged onto

a camera (FLIR Flea3 FL3-U3-13S2C-CS) via a microscope objective (Thorlabs RMS10X) and a tube lens (Thorlabs AC254-100-A-ML), resulting in approximately a 6X magnification. The camera pixel size of 3.6 µm is imaged onto the plane of the bundle with a magnified size of 0.64 µm, or approximately 5 camera pixels per core-spacing.

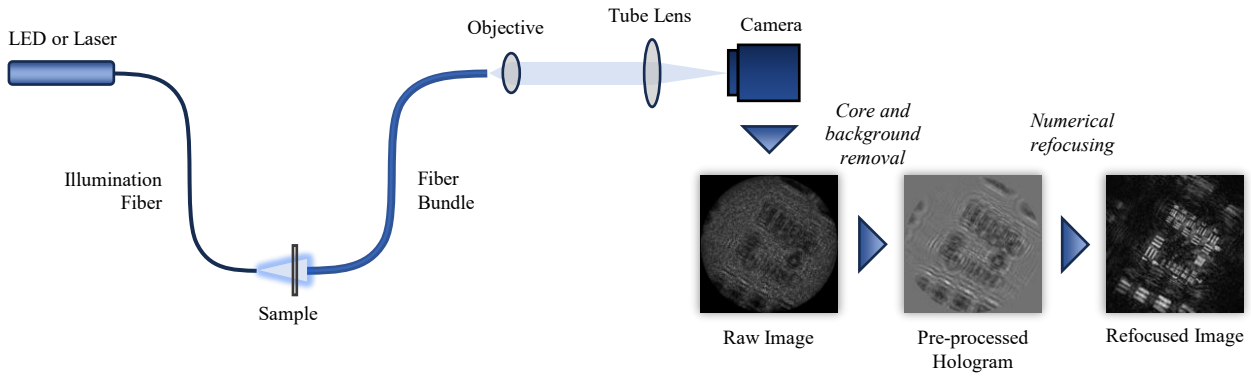


Figure 2. Schematic of experimental setup and illustration of processing pipeline.

To recover images, the raw hologram must first be processed to remove the pattern of fiber cores. This was performed using functions provided by the PyFibreBundle python package[5] which performs triangular linear interpolation between the cores. A one-time calibration is required to localise the cores and pre-compute the interpolation weights. Following this, reconstruction of each frame takes only 2 ms on a mid-range PC. Numerical refocusing is performed via the angular spectrum method[6] using the PyHoloscope python package. Using a GPU this is achieved in approximately 20 ms. Full details of the processing are available elsewhere;[7] the only difference here is that a Gaussian pre-filter was applied to the raw image prior to extracting the core intensities for interpolation. This reduces the impact of residual speckle when using higher coherence sources, by effectively averaging over the whole core.

To investigate how the resolution depends on the bundle core pitch, a method described previously[7] was used to synthesise a more densely sampled hologram by combining multiple holograms, each with a slight lateral shift of the sample. These images can be acquired rapidly using multiple offset fibers, each connected to their own LED,[7] but for the purposes of this study the sample was instead moved using a translation stage.

Two different optical sources were used. Firstly, as described previously,[2] a Royal Blue LED (455 nm central wavelength, 15 nm bandwidth) was butt-coupled to a 50 µm core diameter, 0.22 NA multi-mode fiber. Secondly, a 488 nm laser diode (Thorlabs LP488-SF20) was coupled to a single mode fiber. In addition to using this as a conventional laser, the drive current was reduced below the lasing threshold (to 19.5 mA), resulting in amplified spontaneous emission with an output power of 10 µm and a bandwidth (FWHM) of 5 nm. This is sufficient output power for inline holography, and is comparable to the output power of the fiber-coupled LED, but with decreased divergence due to the 0.12 NA of the single mode fiber.

3. RESULTS

The optical source coherence has a very significant impact on images captured through the fiber bundle. Fig. 3 compares images of the proximal end of the bundle when illuminated by the LED, the laser below the lasing threshold, and the laser above the lasing threshold (i.e. with large, medium and small bandwidth, respectively). For the LED and laser below threshold, the cores can clearly be seen, with minimal speckle or multimodal patterns. For the laser above threshold, a high degree of speckle is observed, such that individual cores cannot easily be resolved. This pattern changes with even small deformation of the fiber bundle. Offsetting the illumination fiber with respect to the bundle by 3 mm, so that rays strike the bundle at an angle of 3° at the centre, is

sufficient to remove part of the speckle noise. However, this has little impact on imaging, since scattered light from the sample will hit the bundle at a range of angles.

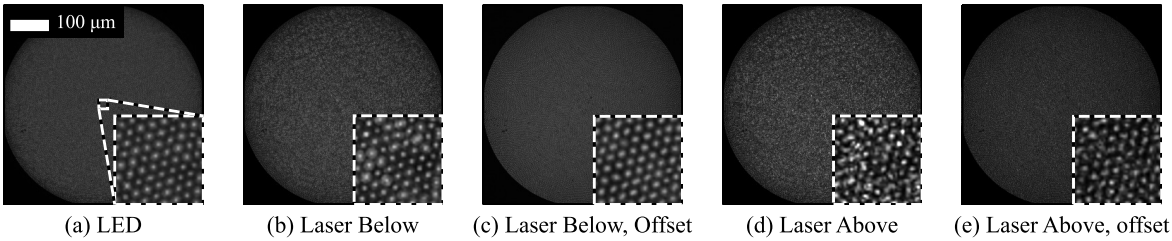


Figure 3. Images of bundle with no processing, showing effect of different optical sources on visible core pattern. (a) Royal Blue LED via 50 µm core fiber. (b) 488 nm laser diode below threshold via single mode fiber. (c) as (b) but with fiber laterally offset by 3 mm. (d) 488 nm laser diode above threshold via single mode fiber. (e) As (d) but with fiber laterally offset by 3 mm.

A United State Air Force (USAF) target was imaged using the LED, the laser above threshold, and the laser below threshold. In each case the exposure was adjusted so that the hologram used the full well-depth of the camera. Holograms and refocused images acquired using the LED are shown for working distances of 0.5 and 2.5 mm in Fig. 4(a) and (b). At 0.5 mm the resolution is limited by the bundle core spacing, and hence this shows the best resolution that can be obtained without using the shifting technique. Group 7 Element 7 (G7E7) is resolved, indicating a resolution of better than 4.2 µm. At a working distance of 2.5 mm, the refocused hologram acquired with the LED has a resolution of only 8 µm. In comparison, the refocused holograms acquired with the laser below and above threshold, shown in Fig. 4(c) and (d) respectively, maintain a resolution of 5.5 µm at the 2.5 mm working distance. The hologram with the laser above threshold is also considerably noisier due to the impact of speckle.

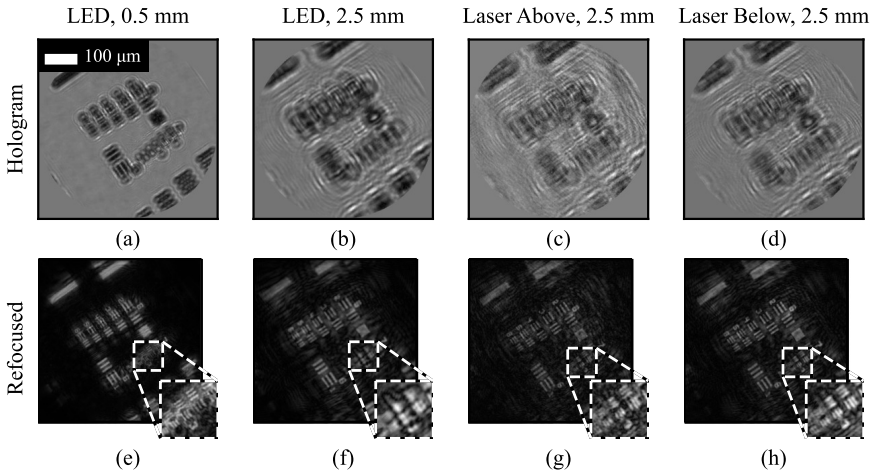


Figure 4. Holograms and numerically refocused images of a USAF resolution target. The left column (a,e) is at a working distance of 0.5 mm, where the resolution is limited by the fiber core spacing. The other columns are at a working distance of 2.5 mm, where the resolution for the LED image is limited by coherence. The insets show a zoom on Group 7 by a factor of 2. The brightness of the insets was also increased by a factor of 2 to more clearly show the line patterns.

Fig. 5 shows the results of applying the shifted-images resolution enhancement technique to holograms of a high resolution USAF target, acquired using the laser below threshold. There is a visible improvement in resolution at all four working distances, showing that when using the laser below threshold, resolution is primarily

limited by the core spacing even at a working distance of 2 mm, and not by the coherence length.

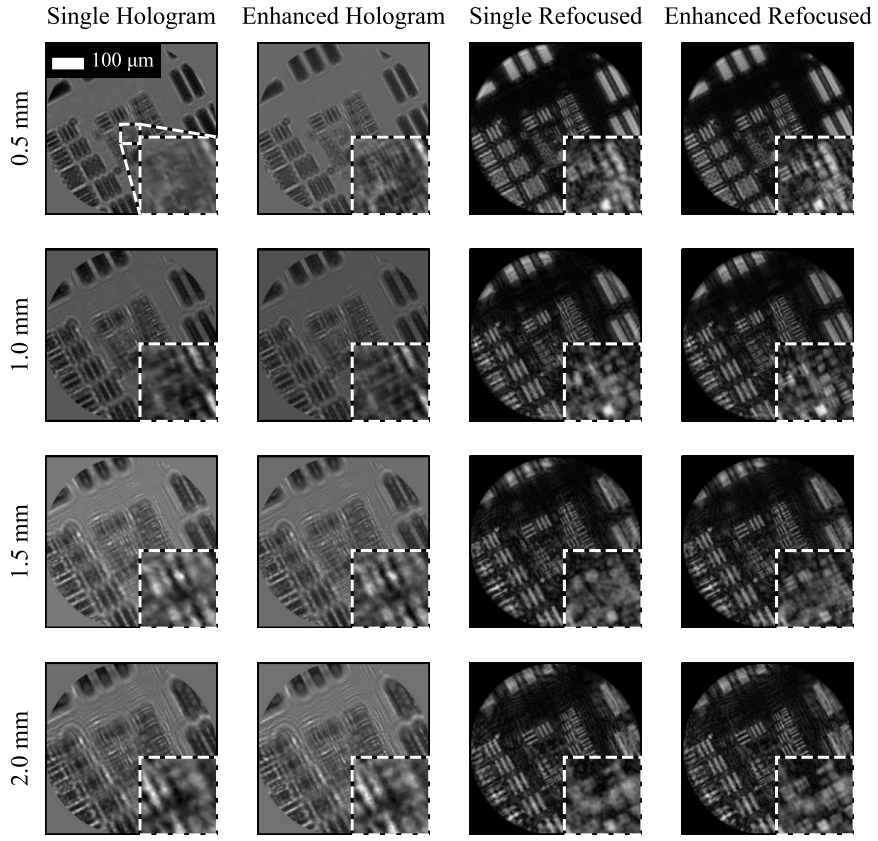


Figure 5. Multi-shifted-image resolution enhancement of holograms and refocused images of USAF resolution target, using laser below threshold. Rows show different working distances. Even at a working distance 2 mm there is an observable improvement in resolution. The insets shows a 4X zoom on Group 8.

4. DISCUSSION AND CONCLUSIONS

These preliminary results demonstrate that is possible to improve the resolution and/or maximum working distance of fiber bundle inline holography by (a) synthesising a denser core sampling via multiple shifted holograms, and (b) increasing the source spatial and temporal coherence. At shorter working distances, a 15 nm bandwidth LED coupled into a 50 µm core, 0.22 NA multimode fiber is sufficiently coherent such that it is not the limiting factor in resolution, and a two-fold improvement in resolution can be obtained using shifted holograms. Beyond 1 mm the source coherence is now the dominant effect. Reducing the bandwidth to 5 nm and using an effective pinhole size (single mode fiber mode field diameter) of approximately 3.5 µm maintains a resolution of approximately 4 µm to a working distance of 2.5 mm. Even at 2 mm, some small improvement in resolution can be obtained using multiple shifted holograms. Reducing the bandwidth, and increasing the coherence length, also increases noise due to speckle, and further work is required to fully explore the parameters space and optimise the temporal coherence for imaging at a given working distance.

5. ACKNOWLEDGEMENTS

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