2021 IEEE 18th International Symposium on Biomedical Imaging (ISBI)
April 13-16, 2021, Nice, France

2021 IEEE 18th International Symposium on Biomedical Imaging (ISBI) | 978-1-6654-1246-9/20/$31.00 ©2021 IEEE | DOI: 10.1109/ISBI48211.2021.9433950

MULTIVIEW VIRTUAL CONFOCAL MICROSCOPY THROUGH A MULTIMODE FIBER

Sakshi Singh, Simon Labouesse, Rafael Piestun

University of Colorado Boulder,
Department of Electrical, Computer, and Energy Engineering,
Boulder, Colorado 80309, USA

ABSTRACT

Confocal microscopy is a powerful imaging tool to obtain high-contrast images of thin sections within bulk tissue. However, tissue scattering limits its utility to only superficial depths. The use of multimode fibers as ultra-thin endoscopes is promising as a deep-tissue minimally invasive imaging tool. Here, we present a method to perform high signal-to-noise ratio (SNR) virtual confocal microscopy through a multimode fiber, by filtering the backscattered light through multiple coplanar virtual pinholes. The use of multiple pinholes helps generate multi-view perspectives of the object, which can be shift corrected and combined to boost the SNR levels. The improved SNR also allows exploiting the potential of confocal imaging for gain in resolution.

Index Terms— Confocal microscopy, imaging through scattering media, multimode fiber imaging, superresolution imaging

1. INTRODUCTION

Confocal microscopy has successfully enabled clinical applications for skin cancer diagnosis [1, 2], corneal imaging [3] and imaging in body cavities using fiber-optic catheters [4, 5]. However, the scattering nature of tissue prohibits its application in the deep tissue regime. Single mode fiber bundles have been employed [6] for some endoscopic applications however each single-mode fiber only provides one pixel of image information and the bundle cross-section scales rapidly with the number of fibers. Multimode fibers (MMFs) on the other hand, with their high bandwidth, excellent throughput and compact design possess the ideal characteristics of a minimally invasive single-fiber endoscope. However, they do not preserve spatial information due to mode dispersion as well as intermodal and polarization coupling.

Imaging through a stationary MMF is still possible, for instance by measuring its transmission matrix (TM) [7, 8, 9, 10], and using it to control the illumination scanning the object on the MMF's far end (distal). The back reflected signal from the object is then detected on the MMF's near (proximal) end to enable imaging.

Confocal imaging through MMFs has been demonstrated using a virtual confocal technique that backpropagates the reflected speckle pattern to the distal end to recover the confocal image [11, 12] or using optical correlation [13]. While these demonstrations show improved contrast and optical sectioning, they remain impractical for biological settings with a limited photon budget since the confocal effect improves inversely with the detection pinhole aperture, and hence the SNR levels. Moreover, when a large enough pinhole is employed to enable imaging with a feasible SNR, the gain in resolution inherent in confocal imaging with a small pinhole [14, 15] is lost.

Here, we propose a technique that we name Multiview Virtual Confocal Microscopy (MVCM) that enables high SNR confocal imaging through an MMF by employing multiple coplanar virtual pinholes to obtain different perspectives of the object. By re-shifting the different perspectives to a common axis and adding them together, we obtain a confocal image with a higher SNR.

The idea of employing multiple detection pinholes to enhance the SNR without losing the resolution or optical sectioning properties has been proposed for shift invariant systems and named pixel reassignment or image scanning microscopy [16, 17, 14]. Here we show that this principle is more general and can be adapted for imaging through complex media such as MMFs even when the images of the focal spots scanning the object are not directly measured [18]. The use of multiple pinholes helps preserve the ideal characteristics of confocal microscopy, without sacrificing the SNR.

In this report, we present simulation and experimental results comparing the SNR of the reconstructed images using the single pixel imaging (SPI) approach [10], single pinhole virtual confocal microscopy [11] and MVCM through an MMF.

2. METHOD

Virtual confocal microscopy through MMFs is performed by (a) measuring the fiber TM [7], (b) using the TM to generate controlled illuminations such as focal spots on the distal

We acknowledge support from National Science Foundation (Award 1548924

end of the MMF to scan the object, (c) collecting the proximal images reflected from the object on the proximal end of the MMF and (d) digitally backpropagating the proximal images to the distal plane using the TM to retrieve images of the scanning focal spots.

Let us consider an MMF with TM, T. If the incident fields are stored in a matrix, E_{in}, the object transfer function is represented by a matrix, O and the back-reflected speckles detected on the proximal side are stored in a matrix, E_p, then the system can be described using Eq. 1.

$$E^p = T'OTE^{in} \tag{1}$$

In the above equation, we have assumed reciprocity of the TM, which implies that the distal-to-proximal end TM is the transpose of the proximal-to-distal end TM, T. In order to recover images of the focal spot required for confocal imaging, we virtually backpropagate the proximal images, E_p, to the distal end by multiplying Eq. 1 with the inverse of the distal-to-proximal end TM, T'. Since the experimentally measured TM is singular and noisy, we find a Tikhonov regularized inverse of the TM [19, 11] to recover the digitally computed distal images of focal spots scanning the object, E_d^v.

For every focal spot scanned, each pixel of the digitally computed distal images acts as an independent virtual confocal pinhole providing a different perspective of the scanned part of the object. By appropriately re-shifting the confocal images obtained from the different virtual pinholes using the pixel reassignment algorithm [16, 20], the object is recovered.

3. EXPERIMENTAL SETUP

The experimental setup consisted of a 785 nm CW laser source and a liquid crystal spatial light modulator (SLM) for phase modulation. The SLM was used for TM calibration and later for generation of focal spots to sample the object on the distal end. The Fourier plane of the SLM was coupled into a step index MMF with a core diameter of $50\mu m$ and a numerical aperture of 0.2 using a microscope objective. The distal end of the fiber, where the object would be placed after calibration, was imaged using a 4-F system onto a CMOS camera.

4. RESULTS

We compared the SNR of the image reconstructed from various MMF imaging methods, both in simulation and experiments. We defined the distance between the peak of the scanning airy disk to its first zero crossing as 1 airy unit, which spans a 9 x 9 pinhole region in both simulation and experiment. In simulations, we model the TM of a complex medium as a Gaussian random distribution and add Gaussian noise with 5% of the signal variance to the proximal images. It should be noted that when using pinholes smaller than an airy unit for detection, shot noise would be the dominant noise. Nevertheless, the presented Gaussian noise limited reconstructions provide a representative comparison of the SNR in SPI, confocal microscopy and MVCM. The obtained single pixel image, single-pinhole confocal image and MVCM image are shown in Fig. 1 for a comparison.

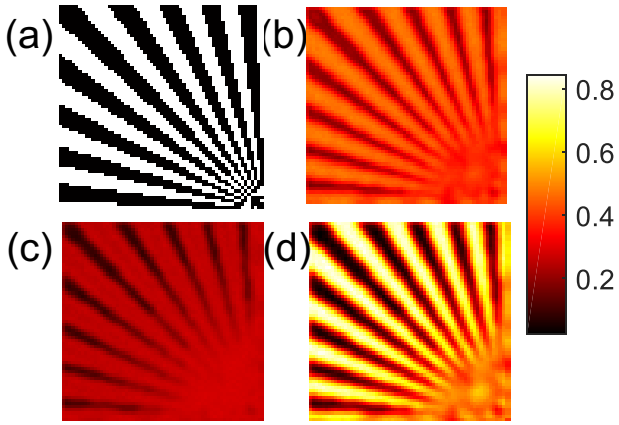

Fig. 1. Simulation of image reconstructions through complex media. (a) Binary object (ground truth). (b) Single pixel image reconstruction obtained by integrating the proximal images. (c) Confocal image using a single on-axis virtual pinhole. (b) MVCM reconstruction using 25 virtual pinholes. The common colorbar for (b), (c) and (d) is shown on the right.

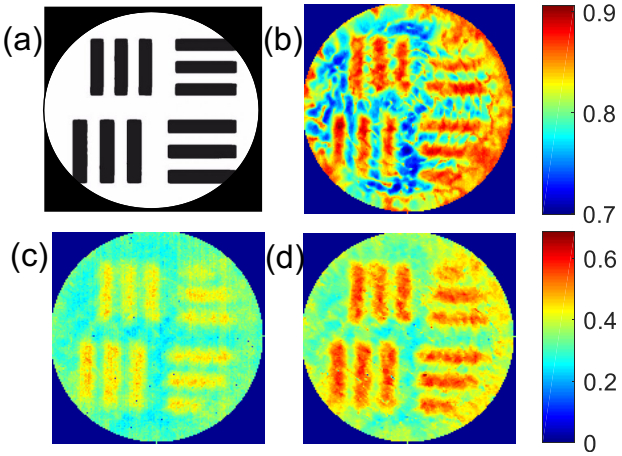

Fig. 2. Experimental image reconstructions of a USAF target through a multimode fiber. (a) Binary object (ground truth). (b) Single pixel image reconstruction obtained by integrating the proximal images. (c) Confocal image using a single on-axis virtual pinhole. (b) MVCM reconstruction using 25 virtual pinholes. The common colorbar for (c) and (d) is shown on their right.

For the experimental demonstration, we calibrated the distal-to-proximal end TM separately by placing a mirror on the distal end and collecting the back-reflected proximal images while scanning focal spots on the mirror. In order to avoid difficult alignment, we implement a one-time independent calibration of the distal-to-proximal TM. This separate calibration also allows flexibility in designing the detection system, which could be useful for various other endoscopic systems that use separate excitation and detection pathways to enhance throughput [21, 22, 23].

The object in the field of view of the MMF consisted of the fourth and fifth elements of the 7th group in the USAF 1951 resolution target, which have a resolution of 181- and 203-line pairs/mm respectively.

The comparison of image reconstruction for various techniques is summarized in Fig. 2. In both simulation and experimental results, we find an increase in contrast from the single-pixel imaging to the single-pinhole confocal and further to the MVCM images. Moreover, a considerable improvement in the SNR is apparent in MVCM with respect to the single pinhole confocal images.

5. CONCLUSION

We demonstrate high SNR confocal imaging through MMF that uses multiple virtual pinholes to improve the SNR without compromising the resolution and optical sectioning capabilities of conventional confocal imaging. These advantages come at the cost of an added computational complexity of $O(N_{pinholes}N_{in}N_{il})$, where $N_{pinholes}$ is the number of pinholes used, N_{in} is the number of proximal detector pixels and N_{il} is the number of focal spots scanned. However, all the computation is done offline and does not affect the data capture speed. Furthermore, the method can be easily generalized to other complex media with different excitation and detection pathways. The improved SNR also paves the way towards adaptation of the technique for fluorescence imaging by measuring the multi-spectral TM of the MMF [24, 25]. Overall, MVCM offers an attractive solution for minimally-invasive high contrast imaging in the deep tissue regime when the photon budget is limited.

6. COMPLIANCE WITH ETHICAL STANDARDS

This study does not involve human or animal subjects and no ethical approval was required.

7. ACKNOWLEDGEMENTS

The authors would like to acknowledge the National Science Foundation (Award No. 1548924) for funding this research.

8. REFERENCES

[1] Milind Rajadhyaksha, Melanie Grossman, Dina Esterowitz, Robert H Webb, and R Rox Anderson, "In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast," *Journal of Investigative Dermatology*, vol. 104, no. 6, pp. 946–952, 1995.

[2] Salvador González and Zeina Tannous, "Real-time, in vivo confocal reflectance microscopy of basal cell carcinoma," *Journal of the American Academy of Dermatology*, vol. 47, no. 6, pp. 869–874, 2002.

[3] I Jalbert, F Stapleton, E Papas, DF Sweeney, and M Coroneo, "In vivo confocal microscopy of the human cornea," *British Journal of Ophthalmology*, vol. 87, no. 2, pp. 225–236, 2003.

[4] Ralf Kiesslich, Martin Goetz, Juergen Burg, Manfred Stolte, Ekkehard Siegel, Markus J Maeurer, Steven Thomas, Dennis Strand, Peter R Galle, and Markus F Neurath, "Diagnosing helicobacter pylori in vivo by confocal laser endoscopy," *Gastroenterology*, vol. 128, no. 7, pp. 2119–2123, 2005.

[5] Pei-Lin Hsiung, Jonathan Hardy, Shai Friedland, Roy Soetikno, Christine B Du, Amy P Wu, Peyman Sahbaie, James M Crawford, Anson W Lowe, Christopher H Contag, et al., "Detection of colonic dysplasia in vivo using a targeted heptapeptide and confocal microendoscopy," *Nature medicine*, vol. 14, no. 4, pp. 454–458, 2008.

[6] Vivien Szabo, Cathie Ventalon, Vincent De Sars, Jonathan Bradley, and Valentina Emiliani, "Spatially selective holographic photoactivation and functional fluorescence imaging in freely behaving mice with a fiberscope," *Neuron*, vol. 84, no. 6, pp. 1157–1169, 2014.

[7] SM Popoff, G Lerosey, R Carminati, M Fink, AC Boccara, and S Gigan, "Measuring the transmission matrix in optics: an approach to the study and control of light propagation in disordered media," *Physical review letters*, vol. 104, no. 10, pp. 100601, 2010.

[8] Ioannis N Papadopoulos, Salma Farahi, Christophe Moser, and Demetri Psaltis, "High-resolution, lensless endoscope based on digital scanning through a multimode optical fiber," *Biomedical optics express*, vol. 4, no. 2, pp. 260–270, 2013.

[9] Tomáš Čižmár and Kishan Dholakia, "Exploiting multimode waveguides for pure fibre-based imaging," *Nature communications*, vol. 3, no. 1, pp. 1–9, 2012.

[10] Antonio M Caravaca-Aguirre and Rafael Piestun, "Single multimode fiber endoscope," *Optics express*, vol. 25, no. 3, pp. 1656–1665, 2017.

[11] Damien Loterie, Salma Farahi, Ioannis Papadopoulos, Alexandre Goy, Demetri Psaltis, and Christophe Moser, "Digital confocal microscopy through a multimode fiber," *Optics express*, vol. 23, no. 18, pp. 23845–23858, 2015.

[12] Szu-Yu Lee, Brett Bouma, and Martin Villiger, "Confocal imaging through a multimode fiber without active wave-control," in *2019 IEEE Photonics Conference (IPC)*. IEEE, pp. 1–2.

[13] Damien Loterie, Sebastianus A Goorden, Demetri Psaltis, and Christophe Moser, "Confocal microscopy through a multimode fiber using optical correlation," *Optics letters*, vol. 40, no. 24, pp. 5754–5757, 2015.

[14] CJ R SHEPPARD, "Super-resolution in confocal imaging," *Optik (Stuttgart)*, vol. 80, no. 2, pp. 53–54, 1988.

[15] M Bertero, P Brianzi, and ER Pike, "Super-resolution in confocal scanning microscopy," *Inverse Problems*, vol. 3, no. 2, pp. 195, 1987.

[16] Claus B Müller and Jörg Enderlein, "Image scanning microscopy," *Physical review letters*, vol. 104, no. 19, pp. 198101, 2010.

[17] Colin JR Sheppard, Shalin B Mehta, and Rainer Heintzmann, "Superresolution by image scanning microscopy using pixel reassignment," *Optics letters*, vol. 38, no. 15, pp. 2889–2892, 2013.

[18] Alexander Jesacher, Monika Ritsch-Marte, and Rafael Piestun, "Three-dimensional information from two-dimensional scans: a scanning microscope with postacquisition refocusing capability," *Optica*, vol. 2, no. 3, pp. 210–213, 2015.

[19] SM Popoff, Geoffroy Lerosey, Mathias Fink, Albert Claude Boccara, and Sylvain Gigan, "Controlling light through optical disordered media: transmission matrix approach," *New Journal of Physics*, vol. 13, no. 12, pp. 123021, 2011.

[20] Colin JR Sheppard, Marco Castello, Giorgio Tortarolo, Takahiro Deguchi, Sami V Koho, Giuseppe Vicidomini, and Alberto Diaspro, "Pixel reassignment in image scanning microscopy: a re-evaluation," *JOSA A*, vol. 37, no. 1, pp. 154–162, 2020.

[21] Sophie Brustlein, Pascal Berto, Richard Hostein, Patrick Ferrand, Cyrille Billaudeau, Didier Marguet, Alistair Muir, Jonathan Knight, and Hervé Rigneault, "Double-clad hollow core photonic crystal fiber for coherent raman endoscope," *Optics express*, vol. 19, no. 13, pp. 12562–12568, 2011.

[22] D Yelin, BE Bouma, SH Yun, and GJ Tearney, "Double-clad fiber for endoscopy," *Optics letters*, vol. 29, no. 20, pp. 2408–2410, 2004.

[23] Salma Farahi, David Ziegler, Ioannis N Papadopoulos, Demetri Psaltis, and Christophe Moser, "Dynamic bending compensation while focusing through a multimode fiber," *Optics express*, vol. 21, no. 19, pp. 22504–22514, 2013.

[24] Mickael Mounaix, Daria Andreoli, Hugo Defienne, Giorgio Volpe, Ori Katz, Samuel Grésillon, and Sylvain Gigan, "Spatiotemporal coherent control of light through a multiple scattering medium with the multispectral transmission matrix," *Physical review letters*, vol. 116, no. 25, pp. 253901, 2016.

[25] Antoine Boniface, Ivan Gusachenko, Kishan Dholakia, and Sylvain Gigan, "Rapid broadband characterization of scattering medium using hyperspectral imaging," *Optica*, vol. 6, no. 3, pp. 274–279, 2019.