

Document Version

Final published version

Citation (APA)

Xia, F., Gevers, M., Fognini, A., Mok, A. T., Li, B., Akabri, N., Zadeh, I. E., Qin-Dregely, J., & Xu, C. (2022). Deep confocal fluorescence microscopy with single-photon superconducting nanowire detector. In M. A. Itzler, J. C. Bienfang, & K. A. McIntosh (Eds.), *Advanced Photon Counting Techniques XVI* Article 1208905 (Proceedings of SPIE - The International Society for Optical Engineering; Vol. 12089). SPIE. <https://doi.org/10.1117/12.2620775>

Important note

To cite this publication, please use the final published version (if applicable).
Please check the document version above.

Copyright

In case the licence states "Dutch Copyright Act (Article 25fa)", this publication was made available Green Open Access via the TU Delft Institutional Repository pursuant to Dutch Copyright Act (Article 25fa, the Taverne amendment). This provision does not affect copyright ownership.
Unless copyright is transferred by contract or statute, it remains with the copyright holder.

Sharing and reuse

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

Takedown policy

Please contact us and provide details if you believe this document breaches copyrights.
We will remove access to the work immediately and investigate your claim.

PROCEEDINGS OF SPIE

[SPIDigitalLibrary.org/conference-proceedings-of-spie](https://spiedigitallibrary.org/conference-proceedings-of-spie)

Deep confocal fluorescence microscopy with single-photon superconducting nanowire detector

Fei Xia, Monique Gevers, Andreas Fognini, Aaron Mok, Bo Li, et al.

Fei Xia, Monique Gevers, Andreas Fognini, Aaron T. Mok, Bo Li, Najva Akabri, Iman Esmaeil Zadeh, Jessie Qin-Dregely, Chris Xu, "Deep confocal fluorescence microscopy with single-photon superconducting nanowire detector," Proc. SPIE 12089, Advanced Photon Counting Techniques XVI, 1208905 (30 May 2022); doi: 10.1117/12.2620775

SPIE.

Event: SPIE Defense + Commercial Sensing, 2022, Orlando, Florida, United States

Deep confocal fluorescence microscopy with single-photon superconducting nanowire detector

Fei Xia^{1,2,3,*}, Monique Gevers³, Andreas Fognini³, Aaron T. Mok¹, Bo Li¹, Najva Akabri¹, Iman Esmaeil Zadeh^{3,4}, Jessie Qin-Dregely³ and Chris Xu¹

1 School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853, USA

2 Meinig School of Biomedical Engineering, Cornell University, Ithaca, New York 14853, USA

3 Single Quantum B.V., Molengraaffsingel 10, 2629 JD, Delft, the Netherlands.

4 Optics Research Group, ImPhys Department, Faculty of Applied Sciences, Delft University of Technology, Delft 2628 CJ, The Netherlands.

5 Current address: Laboratoire Kastler Brossel, École Normale Supérieure-Université PSL, CNRS, Sorbonne Université, Collège de France, Paris 75005, France.

**fx43@cornell.edu*

Abstract: Using short-wave infrared wavelength advantages, we demonstrate one-photon fluorescence confocal microscopy of adult mouse brains with penetration depths up to 1.7mm. This is achieved by labeling quantum dots with 1300 nm excitation and 1700 nm emission and detecting them with a single-photon superconducting nanowire detector.

Introduction

It has long been difficult to obtain high-quality images of turbid materials such as biological tissues because of scattering and absorption of light. An intriguing problem is high resolution, in vivo imaging of mouse brains. A standard tool to approach this goal is multiphoton microscopy (MPM) because of its spatially confined excitation and long excitation wavelength advantages. Recent studies have demonstrated the feasibility of imaging mouse brains via 2-photon microscopy [1] and 3-photon microscopy [2] at depths beyond 1-mm in vivo by leveraging the optimal wavelength windows for deep tissue penetration, 1.3 μm and 1.7 μm , determined through the effect of scattering and absorption of brain tissues in vivo [5]. Comparatively, fluorescence one-photon microscopy is a much cheaper and widely adapted technique in biology labs for imaging three-dimensionally resolved objects, but the short wavelengths used for excitation and emission (typically within the visible spectrum) limit the imaging depth. We demonstrate here confocal imaging of deep mouse

brain using excitation and emission wavelengths within the optimal window for deep mouse brain imaging, allowing us to push the penetration depth of 1-photon confocal fluorescence microscopy. Conventionally, wavelengths longer than 1100 nm can be detected either by nonlinear detection [9] or by InGaAs based detectors. However, both of these methods have low detection efficiency. In this study, we demonstrate *in vivo* fluorescence confocal microscopy of adult mouse brain vasculature at a depth of more than 1 mm using quantum dots with an excitation wavelength at 1300 nm and an emission wavelength >1600 nm, as well as a custom superconducting nanowire detector that is optimized for detection within the range 1300 nm - 2200 nm. At the surface of mouse brains, the maximum optical power is approximately 25 mW for deepest imaging. In this study, we demonstrate a very simple and robust long wavelength confocal microscopy method, which achieves a spatial resolution comparable to MPM or OCT and significantly deeper than previously reported fluorescence confocal imaging. Our experiments also open the possibility of imaging in multiple colors simultaneously with MPM at depth by leveraging the broader emission spectrum, which ranges from visible and near-infrared emission for multiphoton excitation and short-wave infrared emission for one-photon imaging.

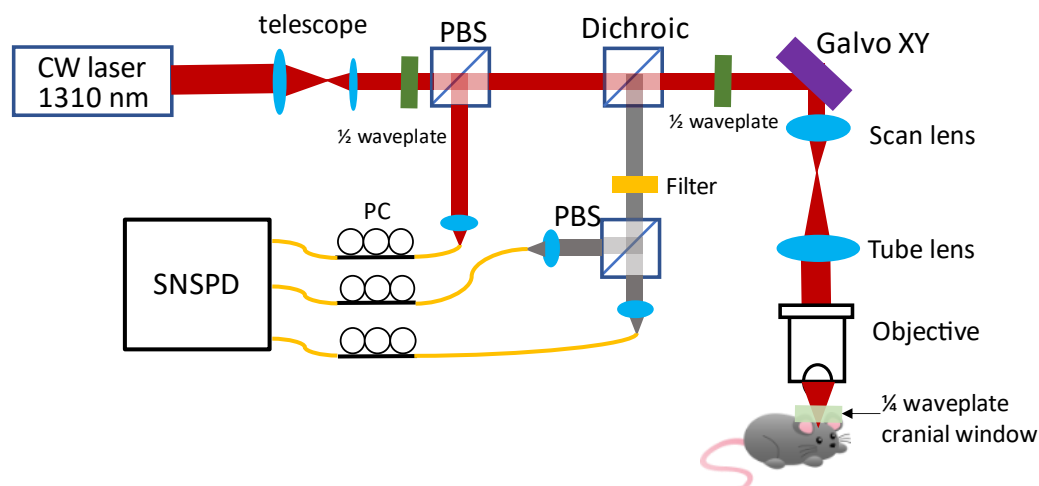


Fig. 1. Schematic illustration of the imaging system. PBS: polarizing beam splitter, PC: polarization controller, SNSPD: superconducting nanowire single-photon detector. The focal length for the scan lens and tube lens is 35 mm and 200 mm, respectively.

Methods

A custom laser scanning microscope was used with a high-numerical aperture objective (Olympus XLPLN25XWMP2, 25X, NA 1.0) and epi-collection of the signal, as shown in Fig. 1. In order to utilize the numerical aperture (NA), the back aperture of the objective is overfilled. Since superconducting nanowire single-photon detectors are particularly sensitive to polarization, the collected fluorescence signals were divided into two orthogonal polarizations, each controlled by a polarization controller, so that detection efficiency could be optimized. Additionally, using a polarization beam splitter and a 1/4-waveplate to separate illumination from backscattered light, we were able to monitor the reflectance signal from the brain [6]. We employed a continuous-wave (CW) diode laser as an illumination source at 1310 nm (FPL1053P from Thorlabs). The light collected by the epi-scanner is de-scanned and re-focused by the aspheric lens into the single-mode fibers (SMF-28), which also serve as confocal apertures. We acquired images and controlled stages using ScanImage running on MATLAB (MathWorks). Depending on the signal strength and required bandwidth, the current applied to the SNSPD was adjusted. A data acquisition card (NI PCI-6110, National Instruments) was used to convert analog signals to digital. The imaging was performed on C57BL/6J mice (8-16 weeks old) using NIR-IIb quantum dots as a retro-orbital injection injected into the mice at 2.2mm lateral and 2mm caudal from the bregma point. Quantum dots are inorganic and measure about 20 nm in size. It is functionalized with the NH₂ group and PEGylated. Because of this, it is aqueous soluble and highly biocompatible.

Main Results and Summary

We show in Fig. 3 that we were able to reach a signal-to-background ratio to about 1 at the depth of 1750 μm , which is around ~ 5 effective attenuation lengths in the mouse brain [5, 8].

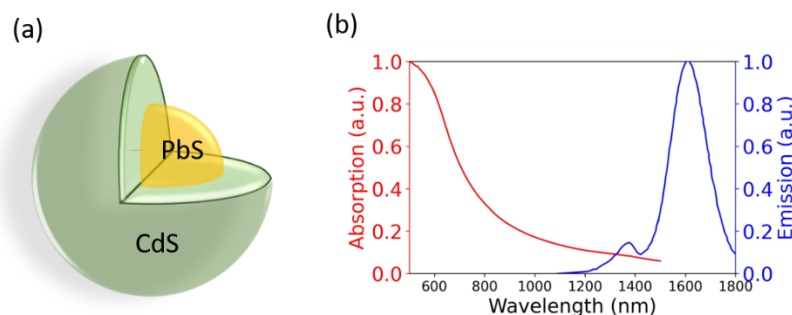


Fig. 2. NIR-IIb quantum dot probe. (A) Schematic structure of the PbS/CdS quantum dot. (B) Absorption and emission spectrum of the labeling agent. [7]

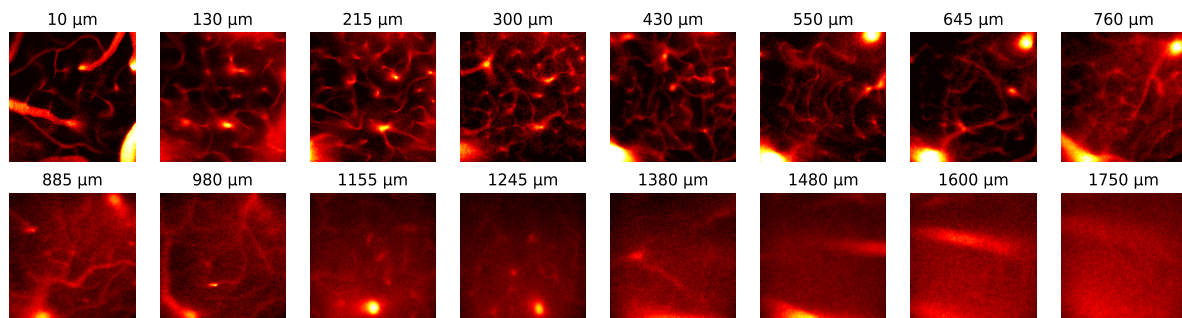


Fig 3. In vivo imaging of the adult mouse brain (8 weeks old) at various depths with PbS/CdS quantum dot. The signal is almost indistinguishable with the background at the depth of 1750 μm . Scale bar: 50 μm .

To summarize, using NIR-IIb quantum dots and a single-photon superconducting nanowire detector, we demonstrate in vivo deep brain fluorescence confocal microscopy at 1310 nm and beyond 1600 nm. We achieved in vivo fluorescence confocal imaging of adult mouse brain at more than 1.0 mm depth. Our work may inspire future research into SWIR fluorescent probes and detectors for in vivo imaging.

Acknowledgement

This work was funded by National Science Foundation (DBI-1707312).

References

- [1] Kobat, Demirhan, Nicholas G. Horton, and Chris Xu. "In vivo two-photon microscopy to 1.6-mm depth in mouse cortex." *Journal of biomedical optics* 16.10 (2011): 106014.
- [2] Horton, N. G., Wang, K., Kobat, D., Clark, C. G., Wise, F. W., Schaffer, C. B., & Xu, C. (2013). In vivo three-photon microscopy of subcortical structures within an intact mouse brain. *Nature photonics*, 7(3), 205-209.

- [3] Chong, S. P., Merkle, C. W., Cooke, D. F., Zhang, T., Radhakrishnan, H., Krubitzer, L., & Srinivasan, V. J. (2015). Noninvasive, in vivo imaging of subcortical mouse brain regions with 1.7 μm optical coherence tomography. *Optics letters*, 40(21), 4911-4914.
- [4] Yamanaka, M., Teranishi, T., Kawagoe, H., & Nishizawa, N. (2016). Optical coherence microscopy in 1700 nm spectral band for high-resolution label-free deep-tissue imaging. *Scientific Reports*, 6(1), 1-8.
- [5] Wang, M., Wu, C., Sinefeld, D., Li, B., Xia, F., & Xu, C. (2018). Comparing the effective attenuation lengths for long wavelength in vivo imaging of the mouse brain. *Biomedical optics express*, 9(8), 3534-3543.
- [6] Xia, F., Wu, C., Sinefeld, D., Li, B., Qin, Y., & Xu, C. (2018). In vivo label-free confocal imaging of the deep mouse brain with long-wavelength illumination. *Biomedical Optics Express*, 9(12), 6545-6555.
- [7] Zhang, M.; Yue, J.; Cui, R.; Ma, Z.; Wan, H.; Wang, F.; Zhu, S.; Zhou, Y.; Kuang, Y.; Zhong, Y.; Pang, D. W.; Dai, H. Bright Quantum Dots Emitting at $\sim 1,600$ Nm in the NIR-IIb Window for Deep Tissue Fluorescence Imaging. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, 115 (26), 6590–6595.
- [8] Xia, F., Gevers, M., Fognini, A., Mok, A. T., Li, B., Akbari, N., ... & Xu, C. (2021). Short-wave infrared confocal fluorescence imaging of deep mouse brain with a superconducting nanowire single-photon detector. *ACS Photonics*, 8(9), 2800-2810.
- [9] Xia, F., Sinefeld, D., Li, B., & Xu, C. (2017). Two-photon Shack–Hartmann wavefront sensor. *Optics letters*, 42(6), 1141-1144.