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**DOI**

[10.1117/12.2620775](https://doi.org/10.1117/12.2620775)

**Publication date**

2022

**Document Version**

Final published version

**Published in**

Advanced Photon Counting Techniques XVI

**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>

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**SPIE.**

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

# Deep confocal fluorescence microscopy with single-photon superconducting nanowire detector

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**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  $\mu\text{m}$  and 1.7  $\mu\text{m}$ , 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.

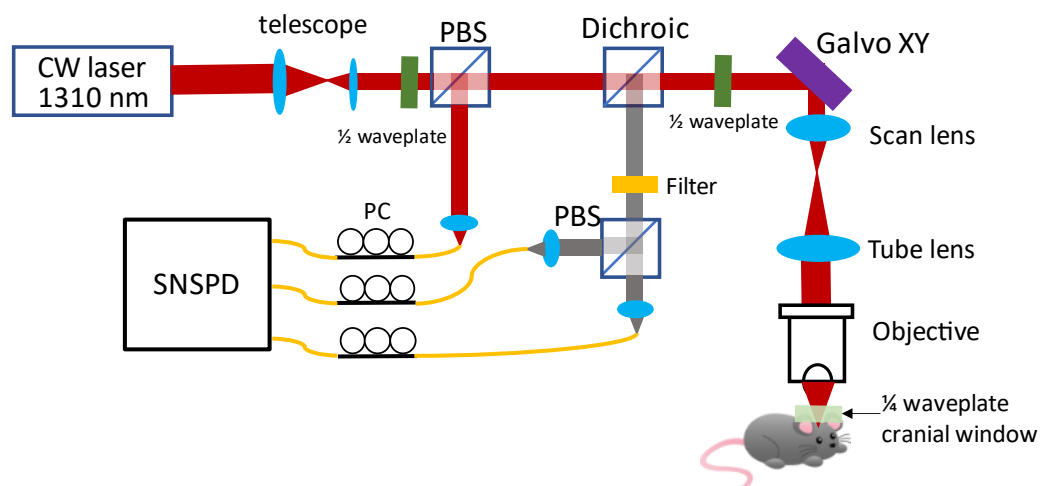


Fig. 1. Schematic illustration of the imaging system. PBS: polarizing beam splitter, PC: polarization controller, SNSPD: superconducting nanowire single-photon detector. The focal length for the scan lens and tube lens is 35 mm and 200 mm, respectively.

## 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 NH<sub>2</sub> 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  $\mu\text{m}$ , which is around  $\sim 5$  effective attenuation lengths in the mouse brain [5, 8].

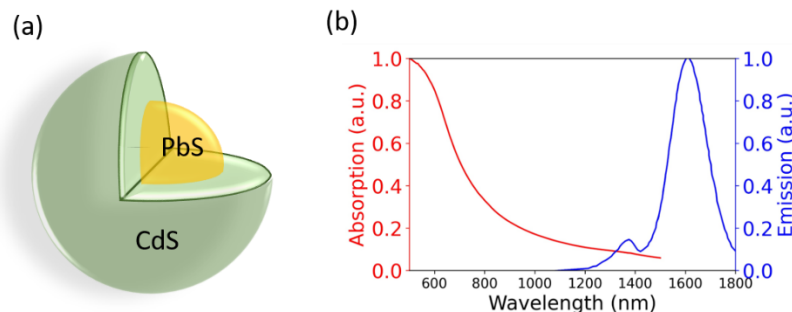


Fig. 2. NIR-IIb quantum dot probe. (A) Schematic structure of the PbS/CdS quantum dot. (B) Absorption and emission spectrum of the labeling agent. [7]

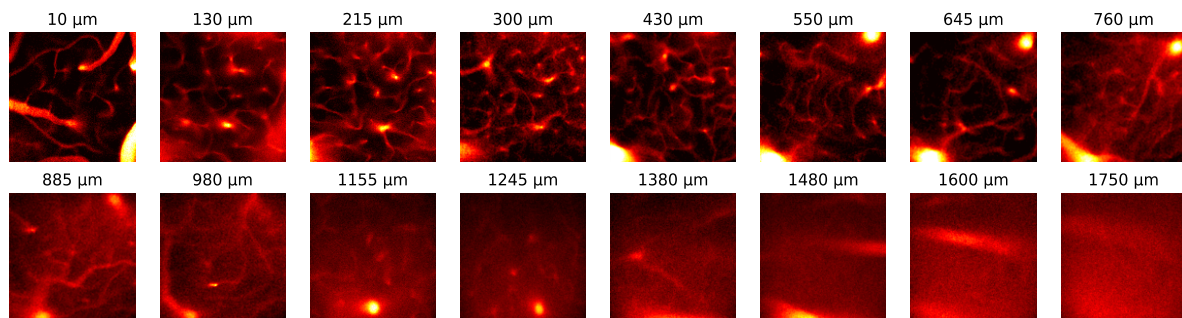


Fig 3. In vivo imaging of the adult mouse brain (8 weeks old) at various depths with PbS/CdS quantum dot. The signal is almost indistinguishable with the background at the depth of 1750  $\mu\text{m}$ . Scale bar: 50  $\mu\text{m}$ .

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).

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