

**Docket #:** S21-210

# **Temporally multiplexed one-photon and two-photon microscopy for neuroscience and spatial biological imaging**

Stanford researchers have developed a device that combines one-photon and two-photon microscopy using fast temporal multiplexing enabling 3D alignment between in vivo and ex vivo data for neuroscience and spatial biology applications.

High-resolution, three-dimensional fluorescence imaging promises rich information about biological function and structure with applications ranging from neuroscience to oncology to spatial genomics. While nonlinear multiphoton microscopy provides the resolution and depth information to produce 3D images of tissues at single-cell resolution, but it has a limited field of view, slow acquisition times, and must be performed in a controlled laboratory setting not compatible with many in vivo and functional imaging studies. On the other hand, one-photon fluorescence imaging can be used for in vivo imaging due to its portability and large field of view, but it produces a low resolution, low depth-of-field image only.

The invented device aligns one-photon imaging with multi-photon ex vivo images at the cellular level to obtain three-dimensional data at single-cell resolution during in vivo functional studies. The inventors demonstrate its utility in neuroscience applications by imaging functional neural ensembles in mice to determine neuron identity, the morphology of neurons, and fidelity of the time traces of genetically encoded calcium indicators expressed in these neurons. This technology will find use in functional neuroscience imaging such as this but also has applications in spatial biology, including spatial proteomics and transcriptomics.

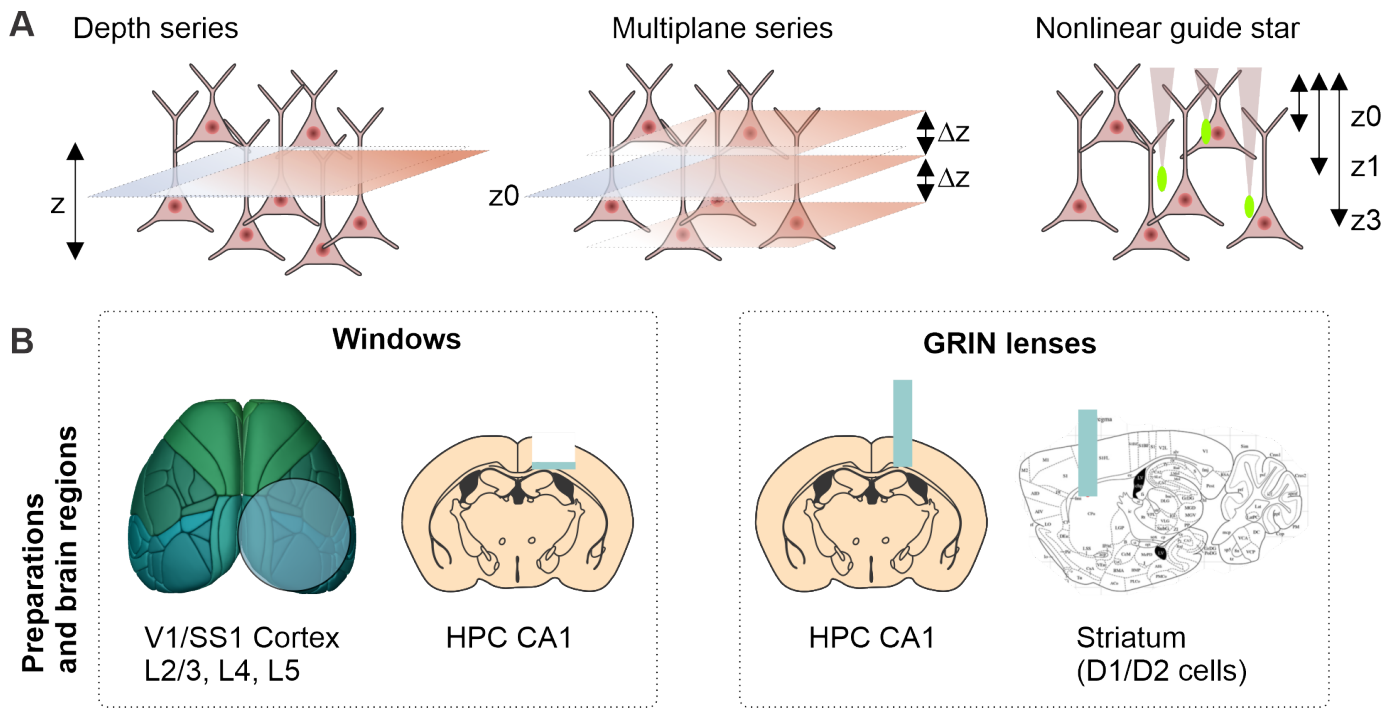


Figure (provided by inventors): Neuronal imaging in live mice with this technology

## Applications

- Alignment of widefield functional in vivo imaging and three-dimensional, single-cell resolution ex vivo imaging with applications in:
  - Functional neuroscience studies
  - Tumor cell mapping
  - Multi-omics spatial imaging

## Advantages

- Enables high-resolution 3D fluorescence imaging in settings where precise 3D microscopy is usually not possible (e.g., hospitals, live animals, patient's home)
- Combines advantages from both one-photon and two-photon imaging techniques
  - High-contrast
  - High-resolution
  - High signal-to-noise
  - Deep tissue penetration
  - Large field-of-view

## Patents

- Published Application: [WO2023114213](#)
- Published Application: [20250067672](#)

## Innovators

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