Miniscope3D—A single-shot miniature three-dimensional fluorescence microscope

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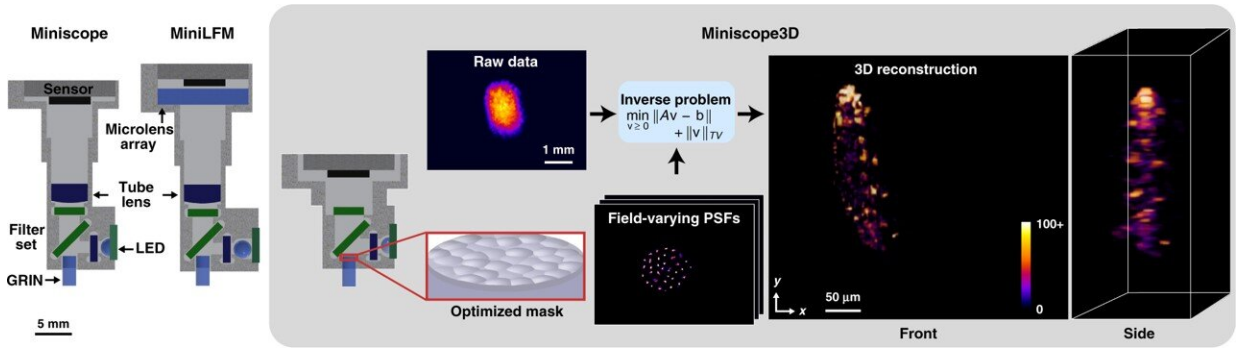


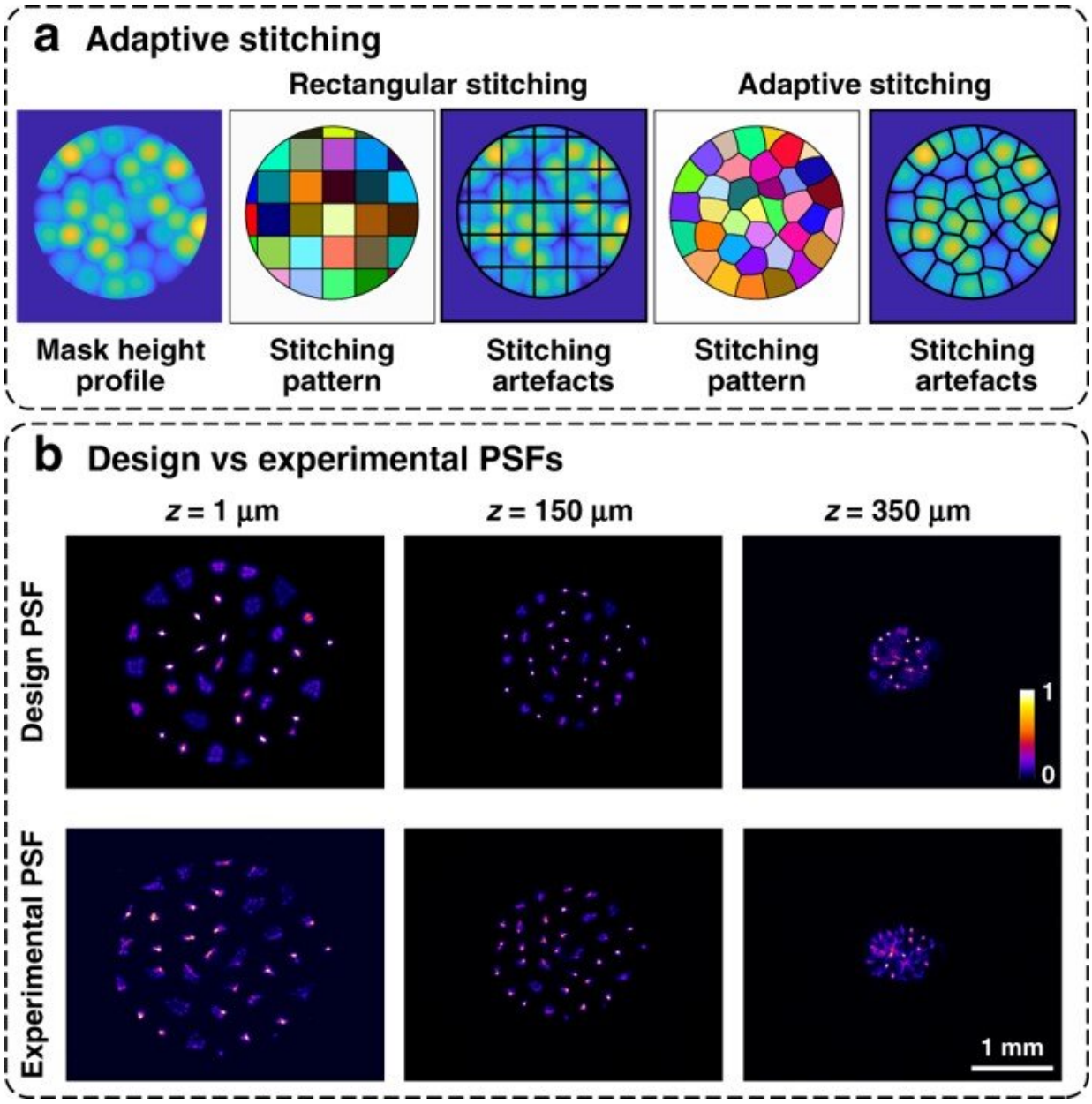
Miniscope3D system overview. Compared with previous Miniscope and MiniLFM designs, our Miniscope3D is lighter weight and more compact. We remove the Miniscope's tube lens and place a 55 µm thick optimized phase mask at the aperture stop (Fourier plane) of the GRIN objective lens. A sparse set (64 per depth) of calibration point spread functions (PSFs) is captured by scanning a 2.5 µm green fluorescent bead throughout the volume. We use this data set to pre-compute an efficient forward model that accurately captures field-varying aberrations. The forward model is then used to iteratively solve an inverse problem to reconstruct 3D volumes from single-shot 2D measurements. The 3D reconstruction here is of a freely swimming fluorescently tagged tardigrade. Credit: Light: Science & Applications, doi: 10.1038/s41377-020-00403-7

A miniature fluorescence microscope that weighs less while offering high resolution compared to existing devices will have a range of applications in systems biology. Existing miniature fluorescence

microscopes are a standard technique in life sciences, but they only offer two-dimensional (2-D) information. In a new report now on *Nature Light: Science & Applications*, Kyrollos Yanny, Nick Antipa and a team of scientists in the Joint Graduate Program in Bioengineering, Electrical Engineering and Computer Sciences at the University of California, Berkeley and the Universite libre de Bruxelles Belgium, developed a single-shot 3-D fluorescence microscope. They engineered the new device known as the Miniscope3D by replacing the tube lens of a conventional 2-D miniscope with an optimized multifocal phase mask at the objective's aperture stop. Using the device, Yanny and Antipa et al. optically recorded neural activity in free-moving animals and in long-term in situ imaging applications in incubators and within lab-on-a-chip devices.

Miniature fluorescence imaging and technical innovations

Miniature fluorescence microscopes are important in systems biology for optical recordings of neural activity in free-moving animals, long-term in situ imaging in incubators and medical devices. Such microscopes are also known as "miniscopes" and are made of 3-D printed parts, although offering 2-D fluorescence imaging alone. Single-shot methods can enable faster capture speeds and a temporal resolution limited by the camera frame rate. For example, a previously developed miniature light-field microscope (MiniLFM) can process neural activity with an optimized algorithm. In this work, Yanny et al. developed a 3-D miniscope to achieve higher resolution with lighter weight compared to existing techniques. The team tested the microscopic capabilities by imaging fluorescent resolution targets as well as freely swimming biological samples and mouse brain tissue. They validated the reconstructed outcomes in comparison with two-photon microscopy to understand limits of the new technique.

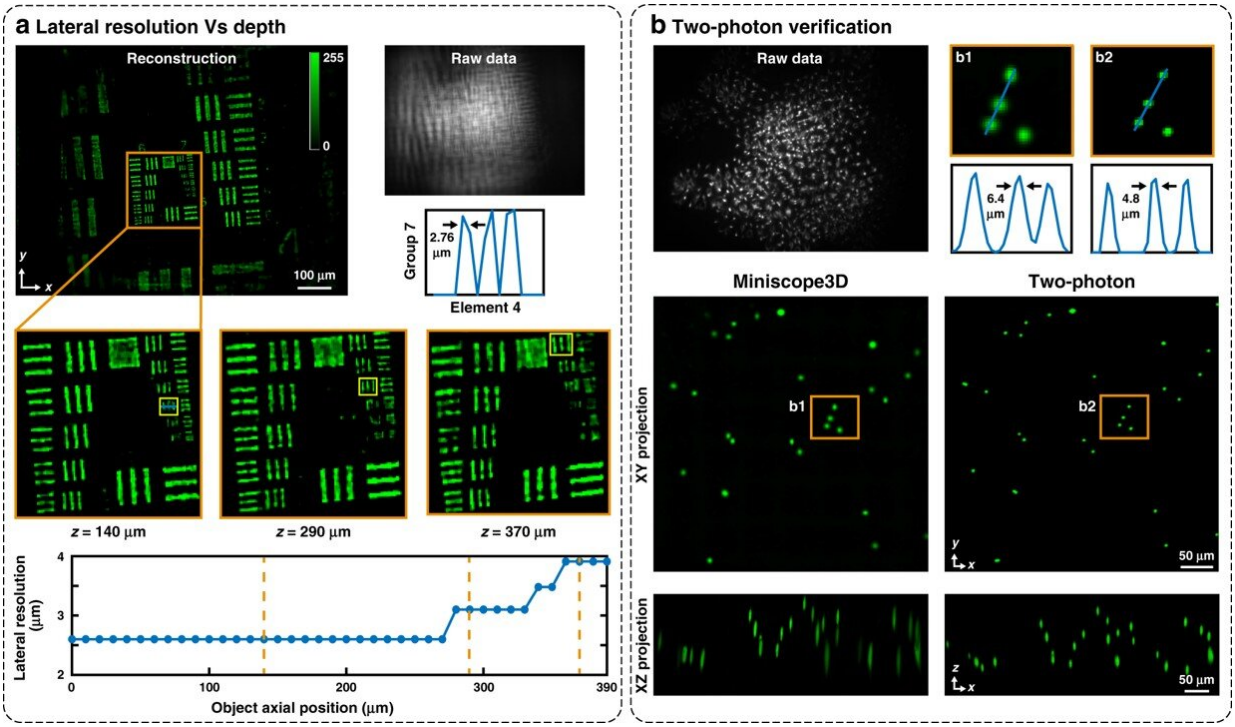


Phase mask fabrication with nanoscribe. (a) Rectangular stitching leads to seams (black lines) going through the many microlenses, whereas adaptive stitching puts the seams at the boundaries of the microlenses to mitigate artefacts. (b) Comparison between designed and experimental PSFs at a few sample depths, showing good agreement, with slight degradation at the edge of the volume. Credit: Light: Science & Applications, doi: 10.1038/s41377-020-00403-7

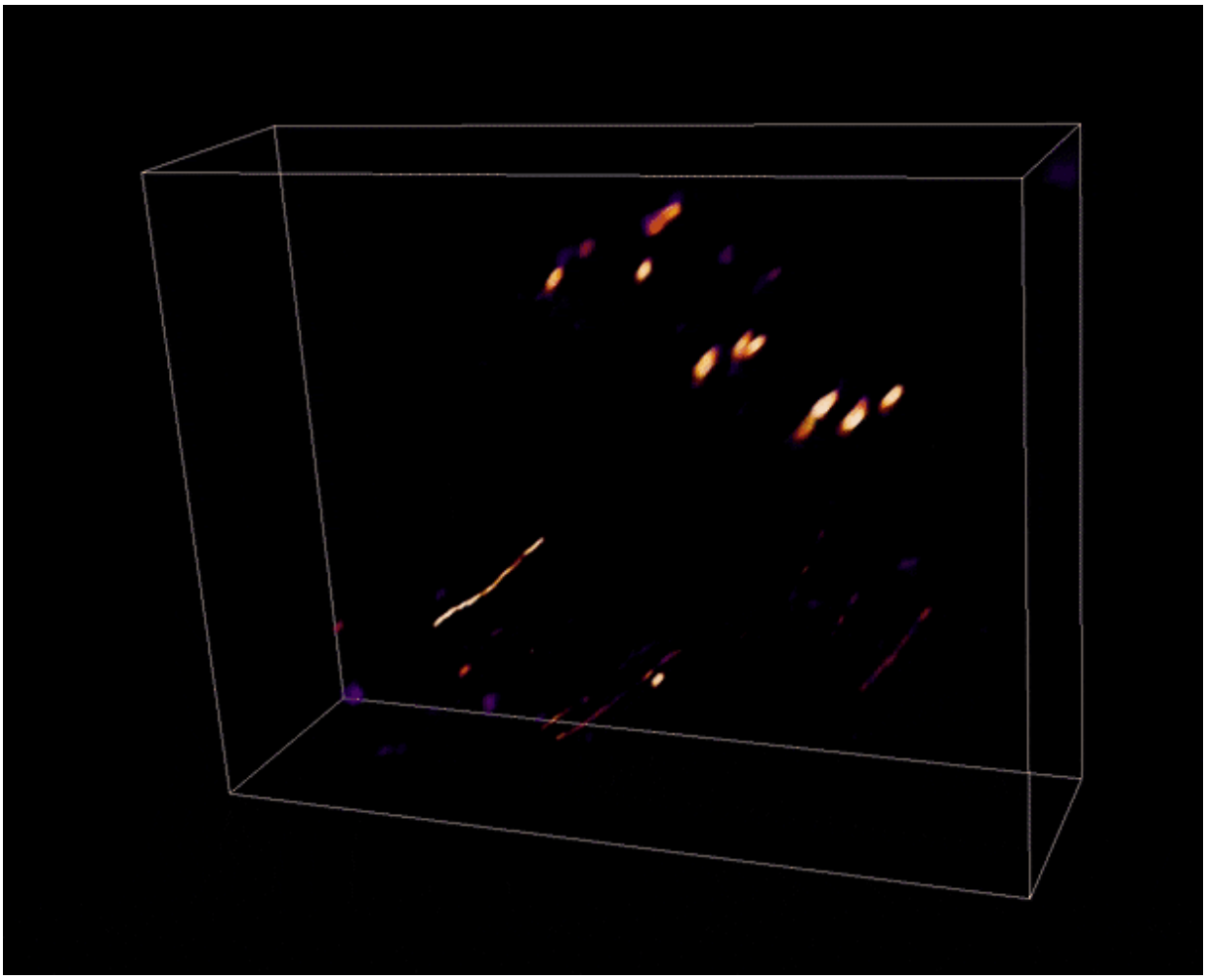
To achieve high-quality imaging in a small, low-weight device, Yanny et al. placed the phase mask (where light passing through the mask will undergo a phase-shift proportional to the thickness of the material) in Fourier space to reduce computational burden and improve compactness. They added 3-D capabilities to the 2-D miniscope at the cost of a small loss in lateral resolution and lower signal-to-noise ratio. The algorithm united the optical theory with compressed sensing to fabricate the optimized phase masks. The technique facilitated a new miniature 3-D microscope architecture with higher resolution, open-source designs, higher-quality fabrication and an efficient calibration scheme or reconstruction algorithm.

Characterizing the computational microscope and investigating the mouse brain

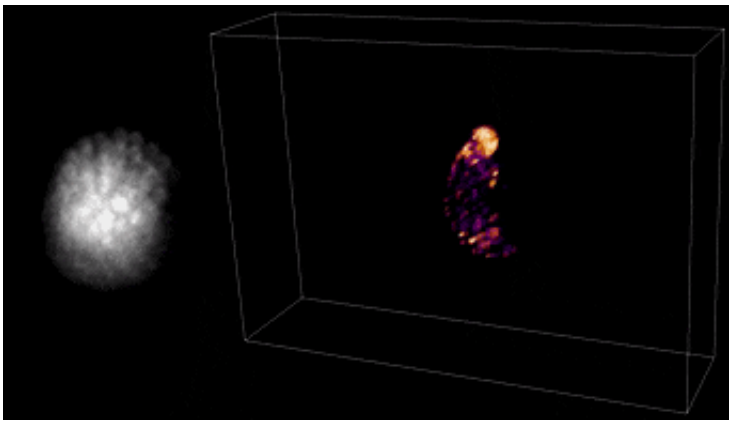
The team tested the performance of the computational microscope using samples of increasing complexity to capture 3-D dynamic recordings. They measured the lateral resolution at different depths by imaging a fluorescent resolution target. They then validated the accuracy of their results using two-photon microscopy. For example, the Miniscope3D could accurately recover all reconstructed images of the 3-D fluorescent bead sample post-processing. They showed the potential of the method using neuro-biological samples where green fluorescent protein tagged regions expressed sparse populations of neurons throughout the sample. The reconstructed images obtained from different parts of the hippocampus showed dendrites running across the surface alongside individual cell bodies. When Yanny et al. next investigated dynamic samples of free-swimming, green-dyed tardigrades (also known as water bears), the reconstructed images showed the efficiency of Miniscope3D imaging to track freely moving biological organisms at high resolution in space-time.



Experimental characterization. (a) Reconstructions of a fluorescent USAF target at different axial positions to determine the depth-dependent lateral resolution. We recover a 2.76 μm resolution across most of the 390 μm range of depths, with the worst case of 3.9 μm (dashed orange lines mark the inset locations, and yellow boxes in the insets indicate the smallest resolved groups). Note that the resolution target has discrete levels of resolution that result in jumps in the data and that resolution here refers to the gap between bars, not the line-pair width. (b) Reconstruction of a 160 μm thick sample of 4.8 μm fluorescent beads compared with a two-photon 3D scanning image (maximum intensity projections in the yx and zx planes are shown). Our system detects the same features, with a slightly larger lateral spot size. Credit: Light: Science & Applications, doi: 10.1038/s41377-020-00403-7



Reconstruction of GFP-tagged neurons in 300 μm thick optically cleared mouse brain slice demonstrating single neuron resolution and clearly resolved dendrites running across the volume axially. Credit: Light: Science & Applications, doi: 10.1038/s41377-020-00403-7



3-D reconstruction of freely swimming tardigrades. (Left) Raw Data. (Right) Reconstruction of freely moving SYBR-green stained tardigrades. Credit: Credit: Light: Science & Applications, doi: 10.1038/s41377-020-00403-7

Applications and accessibility of the device

Most applications of Miniscope3D will be similar to 3-D microscopy and MiniLFM (miniature light-field microscopy), which is considered the gold standard for single-shot miniature 3-D fluorescence imaging. Compared to MiniLFM, however, the new Miniscope3D method offered multiple improvements including multifocal lenses, best—case lateral resolution and a 10-fold increase in the useable measurement volume. The improved performance arrived in a hardware package smaller than the MiniLFM with lighter weight to freely observe moving organisms. The method further enabled experimental reconstruction with or without scattering for mouse brain tissue at single neuron resolution. The team will optimize existing limits of the device including scattering, for further applications.

By building upon a popular open-source miniscope platform, Yanny et al. provided accessibility for the Miniscope3D design. In this way, Kyrollos Yanny, Nick Antipa and colleagues provided a 3-D prototype as

an opportunity to upgrade the 2-D miniscopes currently in use across 450 laboratories. The experimental results were in good agreement with the theoretical design and analysis to serve as a useful framework for customized single-shot 3-D systems.

More information: Kyrollos Yanny et al. Miniscope3D: optimized single-shot miniature 3-D fluorescence microscopy, *Light: Science & Applications* (2020). DOI: 10.1038/s41377-020-00403-7

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