High-resolution deep brain imaging up to a depth of 1.4 mm using White Dwarf OPCPA

A new microscopy technique developed at EMBL in Heidelberg enables highest imaging resolution for in vivo neuronal activity measurements deep in the brain, driven by a Class 5 Photonics White Dwarf OPCPA. Indirect adaptive optics (AO) in combination with three-photon microscopy reaches near-diffraction-limited resolution of very fine structures in the hippocampus up to a depth of 1.4 mm.

Three-photon microscopy for deep brain imaging

Multi-photon microscopy enables non-invasive imaging of cellular structures and neuronal activity within the brain. However, the most interesting brain regions, such as parts of the hippocampus in mice model systems, are below the maximum penetration depth of todays two-photon microscopes. To overcome this hurdle novel three-photon microscopy with near-infrared lasers at 1300 and 1700 nm is being implemented. The longer wavelengths are less diffracted in the brain tissue and the three-photon-absorption improves the signal-to-noise ratio by suppressed out-of-focus fluorescence.

Optical aberrations and adaptive optics microscope

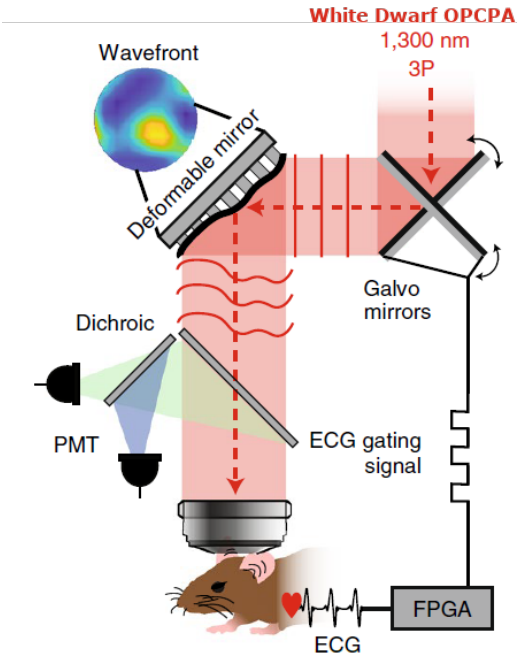

Figure 1: Custom-built three-photon microscope with adaptive optics (AO). [1]

Nevertheless, three-photon microscopy is still limited by optical aberrations due to tissue in-homogeneity, motion artifacts, refractive index mismatches and hence a decrease in imaging resolution at depths beyond 1 mm. Aberration correction based on adaptive optics (AO) is a key technology used in microscopy to restore performance of imaging systems and reduce distortions, similarly to laser guide stars used in astronomy.

Based on these principles the group of Robert Prevedel at EMBL in Heidelberg developed a custom-built adaptive optics (AO) microscope which integrates indirect, modal-based AO with three-photon microscopy [1]. The heart of the new development is a deformable mirror (DM) and an indirect, modal-based adaptive optics (AO) optimization algorithm to compensate not only the optical set-up but also tissue aberrations.

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This means, the deformable mirror iteratively corrects the wavefront in order to achieve a high fluorescence intensity and improved resolution for the imaged structure of interest. A couple of iterations already yield convergence of the feedback algorithm. Thanks to the high power of the White Dwarf OPCPA laser system, acquisition rates during wavefront optimization are around 25 Hz for a 40 x 40 μm^2 field of view (FOV). Hence, in about 250-450 ms an optimized waveform for AO is found. This is, for example, faster than somatic astrocyte GCaMP dynamics, hence making the method suitable for in vivo imaging in real time for slowly varying processes.

Results with near-diffraction limited resolution at 1.4 mm

The reliable day-to-day operation of the White Dwarf OPCPA and its excellent beam pointing stability allowed for fast and simple integration in to the adaptive optics (AO) set-up. The iterative AO approach yields substantial improvement in image quality and spatial resolution. Axial resolution is improved four times and the florescence signal is improved eight times when iteratively correcting tissue-induced aberration in comparison to solely correcting aberrations of the microscope itself. Hence, near-diffraction-limited performance is restored and fine dendritic processes in the hippocampus at up to 1.4 mm depth can be imaged.

Figure 2 shows dendrites and spines in the hippocampus of the in vivo mouse brain (EGFP–Thy1(M)) through a cranial window with full wavefront correction of the microscope and brain tissue. The yz image shows a depth of 1.243 to 1.423 mm. The zoomed image shows an orthogonal view along a dendrite which can be clearly resolved, demonstrating the near-diffraction-limited resolution of the developed microscope.

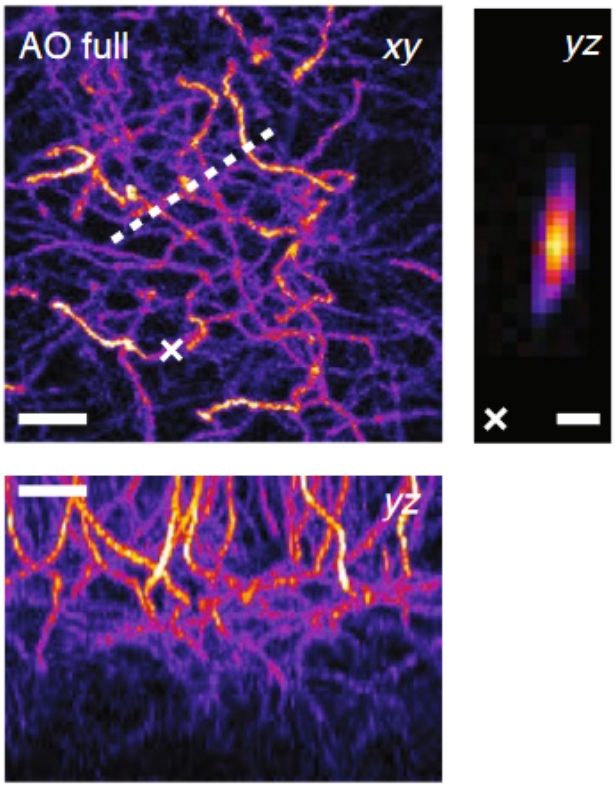

Figure 2: Fine dendritic processes in the hippocampus can be resolved with the newly developed microscope at EMBL Heidelberg: Scale bar is 20 µm on left pictures showing a depth of 1.243 to 1.423 mm on yz plot; on zoomed region scale bar is 2 µm showing the cross-section of a dendrite. [1]

Hence, three-photon microscopy in combination with adaptive optics (AO) paves the way for in vivo studies of sub-cellular structures and physiology with minimal invasiveness despite large depths. This opens neuroscientists new possibilities to further study the brain and its functionality.

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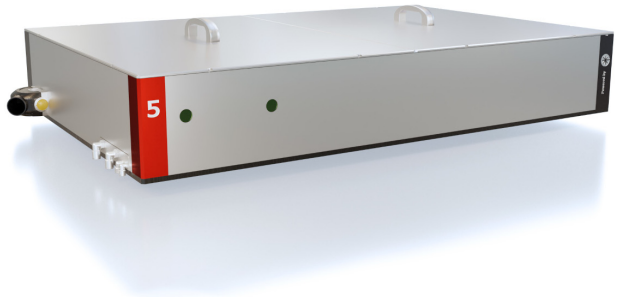
3-photon laser system

For imaging in the hippocampus with the custom-built microscope the White Dwarf WD-1300 laser system from Class 5 Photonics is used. Relatively high pulse energies > 2 µJ with low repetition rates of 500 kHz are required at 1300 nm in order to reach into the deep tissue while not damaging the sample and staying in safe margins.
Dispersion compensation of the custom-built microscope was based on a highly transmissive compressor inside the laser system to reach optimal compression at the sample plane.



The White Dwarf OPCPA is a robust laser system specially designed for three-photon microscopy at high performance parameters providing:

- > 5 W at 1300 nm
- repetition rates up to 10 MHz
- short pulse durations < 50 fs at sample
- excellent beam pointing stability
- robust and stable design with power stability < 1 % rms
- pumped by Coherent Monaco 60 W industrial femtosecond laser

Try the White Dwarf in your lab!

The great success of our previous demo users motivates us to our next demo roadshow in 2022. Achieve immediate, new scientific results in Neuroscience with the White Dwarf by testing and trying out our high performance laser system specially designed for 3-photon microscopy at 1300 and 1700 nm.

Request more information and reserve your trial slot here!

References

[1] L. Streich, J.C. Boffi, L. Wang, and et al. High-resolution structural and functional deep brain imaging using adaptive optics three-photon microscopy. Nat Methods, 18:1253–1258, 2021.