Hamburg experts work on three-photon microscopy systems for neuroscience

Rapp OptoElectronic and Class 5 Photonics make the next step in commercial systems enabling three-photon microscopy for deep brain imaging. Joint measurements have been supported by the renowned University Medical Center Hamburg-Eppendorf (UKE) and show promising results in comparison to standard two-photon microscopy.

Multi-photon microscopy

Brain research is a hot topic. Researchers and scientists all over the world want to understand better how neurons are connected in the brain. Capturing neuronal dynamics in vivo at high speed and good resolution over a large brain volume is a long sought goal. Prof. Simon Wiegert at the University Medical Center Hamburg-Eppendorf (UKE) has been working in neuroscience for over 15 years and explains: ”In my lab, we are especially interested in the role of synapses in long-term information storage in the brain and we mainly use mouse models. Two-photon fluorescence microscopy is a powerful tool to look into organotypic hippocampal slice cultures or the upper layers of the brain in vivo, but access to deeper regions is unfortunately limited due to the brain’s turbidity. Under optimal conditions, we can reach depths of 500 µm, but interesting regions, such as the hippocampus, are buried deeper within the brain. Since there is no way around invasive surgery to access these regions it would be desirable to have access to a non-invasive imaging method giving access to deep brain regions.”
Nowadays, three-photon microscopy with infrared lasers at 1300 nm and 1700 nm is the technique enabling neuroscientists as Simon Wiegert to image deep brain tissue and neuronal activity in vivo. Required technologies are high-power lasers based on optical parametric chirped pulse amplification (OPCPA) and state-of-the-art laser scanning microscopes with specialized optics.

Local cooperation

Class 5 Photonics and Rapp OptoElectronic are two small-sized, high-tech companies from the Hamburg area in Germany, who both provide high-end, customized equipment for multi-photon microscopy.
While Class 5 Photonics provides the high-power, infrared laser system (White Dwarf OPCPA), Rapp OptoElectronic implements two-photon microscopes, e.g., the Movable Objective Microscope® (MOM®) of Sutter Instrument, according to the needs of scientists.
”We met each other at a scientific conference here in Hamburg and immediately realized the great potential of a local collaboration even before the Covid-19 pandemic started”, explains Luisa Hof-

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mann who works as a sales manager at Class 5 Photonics, ”We planned a first test integration of the White Dwarf OPCPA laser system with the MOM platform to directly measure a comparison between two-photon and three-photon imaging.” Excited about the potential of this plan, Simon Wiegert offered to contribute with brain slices comprising a fluorescent marker dye for three-photon excitation at 1300 nm.

Comparison to 2-photon microscopy

Figure 1 shows a first measurement result from a direct comparison of two-photon (920 nm) and three-photon (1300 nm) imaging of the dentate gyrus in coronal brain slices of the mouse dyed with Thy1-GFP. The two-photon image shows strong out-of-focus fluorescence, increasing the background intensity and reducing contrast, while the three-photon image has a remarkably higher contrast. For three-photon imaging, the range of fluorescence intensity is reduced in z-direction, providing a better sectioning capability at depth than the two-photon imaging.

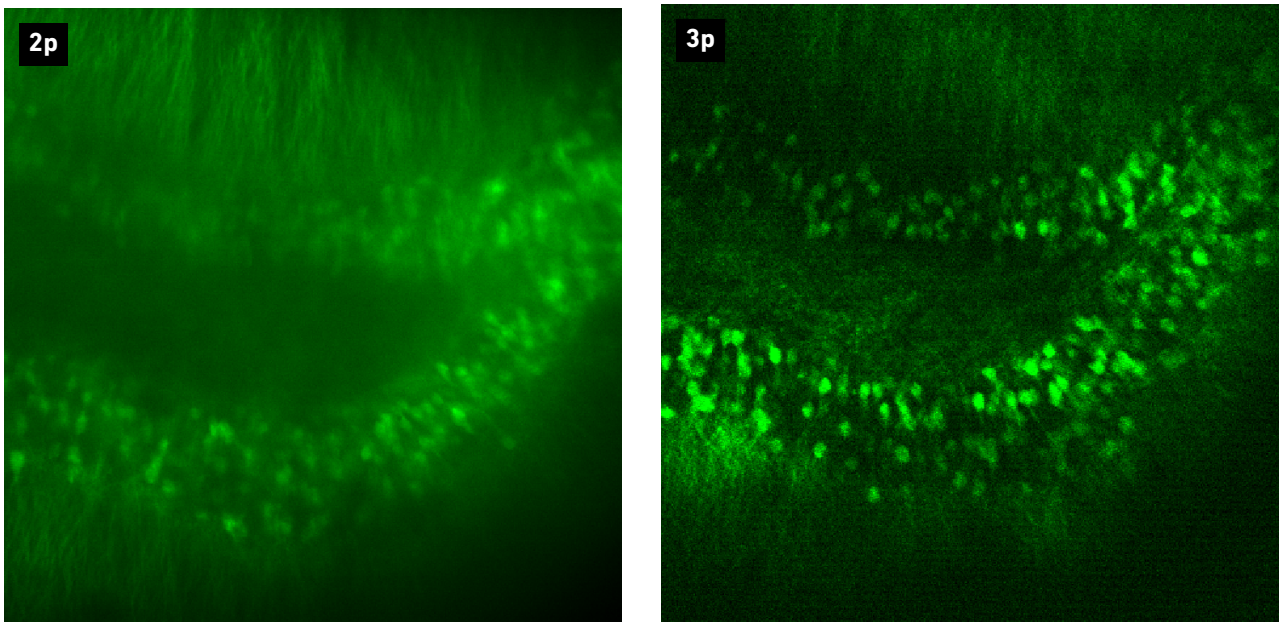

Figure 1: Two-photon image (2p) and three-photon image (3p) of the dentate gyrus region in the hippocampus of a mouse. Depth 500 µm. Measurements are the result of a joint campaign of Rapp OptoElectronic and Class 5 Photonics in Hamburg with sample preparation provided by the University Medical Center Hamburg-Eppendorf (UKE).

MOM upgradability from 2-photon to 3-photon microscopy

Florian Huhn, head of R&D at Rapp OptoElectronic, is fascinated. ”Our MOM platform has been originally designed for two-photon imaging. The integration of the White Dwarf OPCPA worked out smoothly and we had a strong three-photon signal in the image from the first day on. This clearly shows the versatility of our MOM and how easily it can be upgraded to a 3P system. Especially with an open two-photon microscope as the MOM, we have all the flexibility to combine the microscope

with a three-photon laser and optimize the optics for deep brain imaging. Three-photon microscopy has most advantages for deep imaging in vivo, and the MOM with its movable objective is optimally adapted to this setting."

3-photon laser system

The White Dwarf OPCPA is a laser system dedicated for three-photon microscopy providing the highest peak power for bio-imaging on the market. The peak power in the volume pixel (voxel) to be imaged ultimately defines the three-photon absorption and hence fluorescence signal response. This means for imaging even deeper, high pulse energies of a few µJ and short pulses below 50 fs at 1300 nm and 70 fs at 1700 nm are needed, as provided by Class 5 Photonics.
So far, even the brain of a mouse is still way too large and fast to be imaged as an entity, but both companies are determined to push the limits of deep brain imaging together with their partners and clients.

Products

White Dwarf OPCPA for 3p-microscopy

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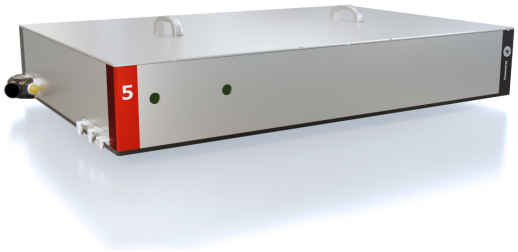

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