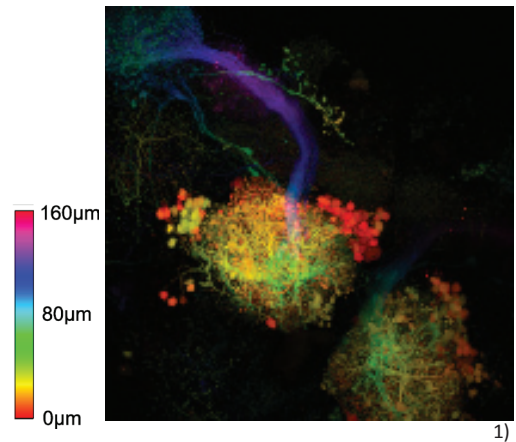


Microscopes



Two-Photon iMIC – „Large-volume-in-vivo-scope“

The Two-Photon Advantage

When it comes to fluorescence imaging of living or freshly excised tissue, two-photon excitation (TPE) microscopy has two major advantages over confocal microscopy: The combination of increased penetration depth and reduced tissue damage. Penetration depth is dependent on the scattering properties of the sample, but usually you can expect to reach at least four times the depth that a confocal microscope reaches in the same sample.



Two-Photon iMIC with Yanus IV and GaAsP PMT module

1) *Drosophila* brain whole mount, courtesy of Laurence Lewis. GH146 neurons expressing GCaMP. Colour codes for depth. Bar = 50µm.

As TPE evokes fluorescence only in the focal plane, and because far-red light is less harmful for biological tissues, there is much less chance for tissue damage.

Another merit of two-photon excitation is that many different dyes can be excited simultaneously without changing the laser wavelength. Thus, TPE avoids the illumination-wavelength-dependent focus error that can lead to artifacts in confocal microscopy with multiple dyes.

The Two-Photon iMIC is equipped to entirely harvest the TPE advantages: The novel VoiceCoil z-Drive allows faster and more precise steps than piezo-designs, and it has a longer travel range. The Yanus IV laser scanner, also used in Stefan Hell's lab for STED microscopy, excels in large scan fields as well as fast frame rates. For fluorescence detection we use GaAsP detector modules with 40% quantum efficiency in combination with collecting lens systems. You can get two detection channels, each with up to three dichroic/filter blocks in a motorized slider for multi-colour scans.

Two-Photon iMIC

Components

- TILL iMIC
- Yanus IV digital scan head
- One or two hand-selected GaAsP detector modules with 40% quantum efficiency
- Olympus 60x NA 1.2 water immersion objective
- Olympus 10x NA 0.4 objective
- All filters included
- LED-Epi-illumination and PCO 1400 camera
- MCU power and real-time control unit
- TOPTICA fiber laser
- Laser beam conditioning and laser safety
- National Instruments AD card and connector module, 16bit

Features

- Freely programmable laser scanner
- Scan control by DSPs, accelerations based on physical model of the mechanical system
- Free scan field rotation
- Arbitrary scan tracks

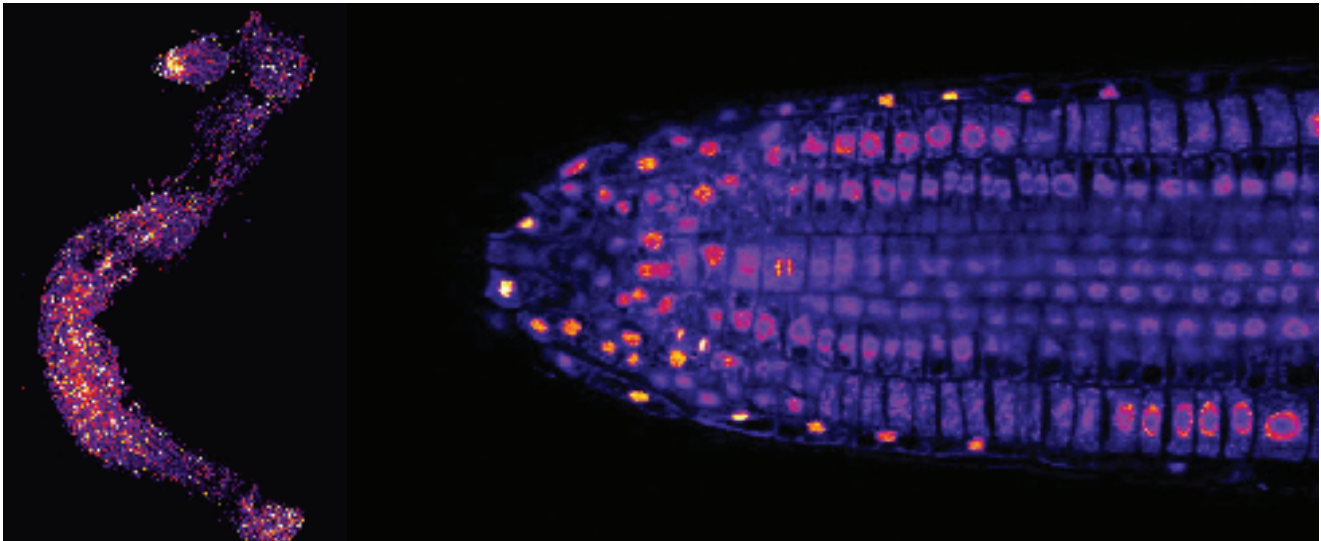
- Line scan, Spot scan, Spiral scan
- 4MHz sampling speed, variable oversampling
- Line speeds from 200 μ s (bi-directional) to several s

Performance (60x objective):

- Fast time lapse: e.g. 46.5fps at 10 μ m x 10 μ m, 100 x 100 pixel, 1 μ s/pixel
- Large images: e.g. 300 μ m x 300 μ m, 4000 x 4000 pixel, 0.5 μ s/pixel, 8 seconds
- Tested for long continuous scans, e.g. with 9.4fps at 30 μ m x 30 μ m, 100 x 100 pixel, 10 μ s/pixel, >1000 frames

Colibri LaserScan Software

The Colibri LaserScan open source software offers full control over the basic setup, including the motorized microscope and the scan head and also provides the flexibility of including further components, such as cameras, into the system.



Arabidopsis thaliana, DAPI stained (false colours); left: stitched overview image; right: root tip

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