

LaVision BioTec *TriM Scope* - 2-Photon Microscopy with 64 laser beams

2-photon fluorescence microscopy features excellent sectioning capabilities even in dense and thick samples. However, the small amount of fluorescence generated by a single beam and bleaching at high excitation rates prevent real-time imaging as well as fast acquisition of 3D data stacks.

For most biological samples the time-averaged laser power is limited to a few mW in the focus [1]. This limitation can be overcome by multiplexing the excitation process [2,3].

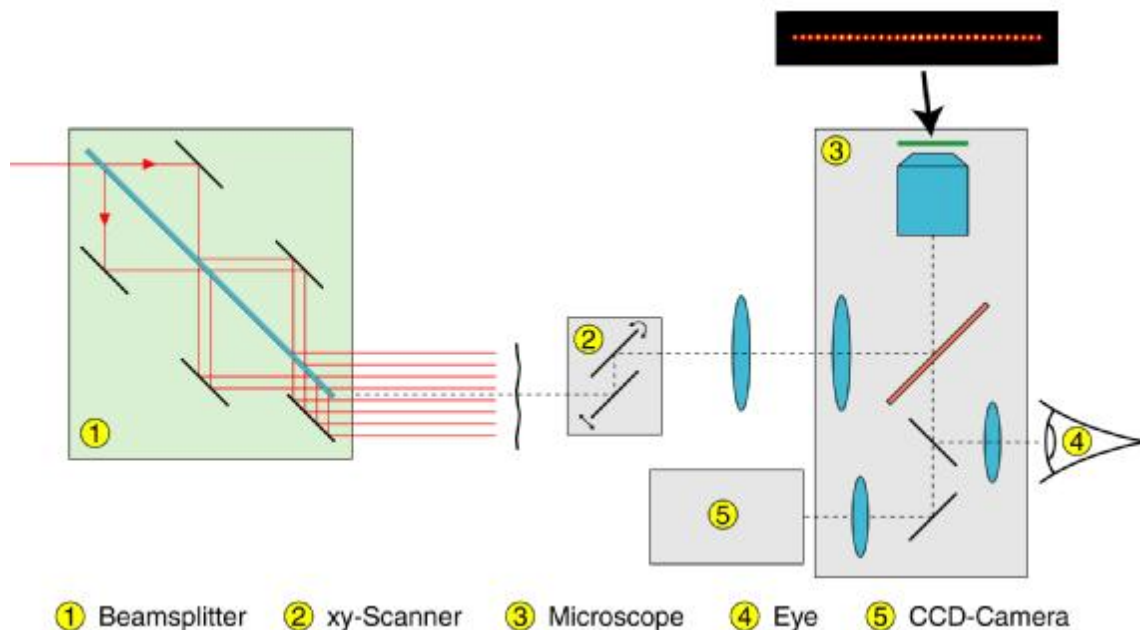


Figure 1: Principle of operation: an incoming laser beam is split up into up to 64 beams which are coupled into the microscope through intermediate optics and focused onto the sample by the objective lens. A $-xy$ -scanner scans all foci simultaneously in the object plane and enables together with the microscope z -drive the three-dimensional imaging process. The fluorescence can be viewed through the eyepieces or imaged onto the CCD-camera. The inset shows the 2-photon excited fluorescence of Rh6G solved in immersion oil generated by 32 beams.

LaVision BioTec's approach

LaVision BioTec has developed a novel multifocal multiphoton microscope that generates up to 64 times more fluorescence light while maintaining the sectioning capability of a single-beam system.

Core part of the setup is the patented beam-multiplexer which uses only flat optics minimizing aberrations thus providing diffraction limited resolution. A combination of

mirrors and a 50% beam splitting substrate divides the incoming beam of a pulsed Ti:Sa-laser (710 - 980 nm) into two sets of beams each consisting of up to 32 beamlets. The special setup of the beam-multiplexer generates two bundles of beamlets of opposite polarization, which are subsequently ordered into a line where polarization alternates between adjacent foci. The distance between two neighbouring foci can be adjusted by changing the intermediate optics or the objective lens. Typically the distance between two foci is $\approx 500\text{nm}$ for a 60x objective lens. The optical configuration features a throughput better than 75 %.

An important feature is the possibility to choose the degree of parallelization as scattering and wavefront distortion in dense samples demand for increased excitation power. LaVision BioTec implemented a method to easily switch the number of beams from 64 to 32, 16, 8, 4 and even to a single beam whereas the power per beam is doubled with each time the number is reduced by a factor of 2. Thereby the length of the line of foci is reduced while the spacing between adjacent foci remains constant.

A xy-beamscanner allows a free selection of the position and the size of the field of view.

The fastest scan mode is realized if the distance between neighbouring foci is in the order of the full-width at half maximum (FWHM) of the point-spread function (PSF) of a single focus. This results in a homogenous but still high-resolution line-illumination that enables scan-rates of up to 4 kHz with a resonant scanner. Therefore a rectangle with a width of $18\ \mu\text{m}$ can be scanned in less than 0.3 ms.

As a result LaVision BioTec's system achieves up to 64 times faster image acquisition speed in comparison to a single-beam scanning microscope enabling real-time observation of image planes deep inside the sample. The final image or 3D-stack acquisition time is at present only limited by the readout speed of the camera.

Literature

- [1] K. König, T. W. Becker, P. Fischer, I. Riemann: Puls-length dependence of cellular response to intense near infrared laser pulses in multiphoton microscopes, *Opt. Lett.* 24, 1999
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- [3] T. Nielsen, M. Fricke, D. Hellweg and P. Andresen: "High efficiency beam splitter for multifocal multiphoton microscopy", *J. of Micr.* 201, (2001)