

## TriMScope

### Beam Multiplexer for Multifocal Multiphoton Microscopy

#### Description

LaVision BioTec's TriMScope is based on a patented beamsplitter that splits up an incoming laser beam into up to 64 beamlets which are scanned simultaneously in the object plane. This results in either 64 times brighter images or 64 times higher image acquisition rates compared to standard single beam multiphoton scanning microscopes. The foci in the object plane are aligned in a single line and the number of foci can be easily switched from 64 to 32, 16, 8, 4 and to a single beam. Image rates are up to 3500 Hz.

The TriMScope beam multiplexer is a compact and easy-to-use device that utilizes exclusively flat optics for dividing the incoming beam with high light efficiency, avoiding aberrations and producing equally intense foci in the sample. As the beamlets are inherently shifted with respect to each other by several picoseconds, there is no cross-talk in excitation. Spatial and axial resolutions are diffraction limited. In addition, line excitation allows convenient coupling of the fluorescence signal to the input slit of an imaging spectrograph yielding real-time spectral sectioning in the x-y and x-z planes.

#### microscopes

invert and upright microscopes from Leica, Nikon, Olympus and Zeiss

#### laser

ultrafast (fs, ps) Ti:Sapphire lasers from Spectra-Physics, Coherent, etc.

#### frame rate

The frame rate depends on the CCD camera.

frame rate: Imager Compact CCD camera  
>25 Hz in full frame (640x480) mode  
>50 Hz with 2x2 binning

#### scan rate

max. scan rate: 3.5 kHz in resonant mode

#### scan area/ field of view

The FOV depends on the CCD chip size and the magnification of the objective. The following values correspond to the LaVision Imager 3 CCD camera (array size 8.6 x 7.6 mm<sup>2</sup>).

100 x Objective: 86 x 76 μm<sup>2</sup>  
63 x Objective: 136 x 120 μm<sup>2</sup>  
20 x Objective: 430 x 380 μm<sup>2</sup>

#### scan area in resonant mode

In resonant mode, the scanner will only scan the fast direction which limits the FOV.

100 x Objective: 15 x 15 μm<sup>2</sup>  
63 x Objective: 25 x 25 μm<sup>2</sup>

#### optical throughput

> 75% within the TriM Scope

#### z-drive

objective driven via a precision stepper motor

speed: 10 Hz @ 0.9 μm step size  
min. step size: 25 nm

#### time multiplexing

time delay between adjacent beamlets: 680 fs

<b>inter-foci separation</b>	<p>100 x Objective: 240 nm          63 x Objective: 420 nm          40 x Objective: 600 nm</p>
<b>foci size</b>	<p>The focal spot size is diffraction limited and depends on the objective.</p> <p>63 x Objective: ca. 300 nm</p>
<b>polarization of adjacent foci</b>	<p>The polarization of foci alternates between S and P.</p> <p>Arrangement: s-p-s-p-s-p...</p>
<b>variable number of foci</b>	<p>The foci are arranged in a line. The number of foci can be set via software. The line dimension is proportional to the number of foci.</p> <p>number of foci: 1, 2, 4, 8, 16, 32, 64          point/line scan toggle switch</p>
<b>chirp compensation</b>	<p>optical dispersion of ultrashort laser pulses causes broadening of the laser pulses leading to a linear decrease in 2-photon induced fluorescence signal. TriMScope has a built-in prism based chirp compensation for optimal 2-photon excitation.</p> <p>chirp compensation: laser pulse width down to 60fs at the sample plane based on the original pulse width of 90fs.</p>
<b>intensity control</b>	<p>via motorized crossed polarizer arrangement at the input</p>
<b>dimensions</b>	<p>60 x 42 x 20 cm<sup>3</sup> ; 30 kg</p>
<b>software</b>	<p>for complete control of TriMScope and peripheral devices such as z-axis, laser, shutter, filter wheel, power monitor, imaging spectrographs, CCD and ICCD cameras, 4D data acquisition, processing and analysis, library of mathematical processing algorithms, real-time visualization and evaluation of multi-dimensional data sets, multi-exponential FLIM fitting routines, deconvolution routines for multi label experiments etc.</p>
<b>UPGRADE SPECTRAL IMAGING</b>	<p>The linear array of 64 foci lends itself for convenient coupling to the input slit of an imaging spectrograph allowing spectral sectioning and imaging.</p>
<b>UPGRADE FRAP</b>	<p>An additional laser can be coupled in the optical path which can be scanned via the XY scanner and used for FRAP studies, uncaging, optical tweezer, pump-probe experiments...</p>
<b>UPGRADE 3D FLIM</b>	<p>TriMScope in conjunction with LaVision's ultrahigh rep. rate, picosecond gated ICCD camera (PicoStar HR: 200ps gate width @ 110 MHz rep. rate) allows real-time 3D FLIM.</p>
<b>CUSTOMIZED SYSTEMS</b>	<p>We specialize in offering customized turn-key systems. Please feel welcome to contact us to discuss your special needs and requirements.</p>