

4X Cloud Scanner

A beam multiplexer for 2-photon laser scanning microscopes generating a 4 foci line pattern in the specimen.

In microscopy, pixel size often exceeds the size of the focal spot of the excitation beam (Fig. 1a). This means, higher fluorescence intensities can be acquired without losing resolution if the whole area of a pixel is excited.

The LaVision BioTec 4x Cloud Scanner separates the single excitation beam both spatially and temporarily (Fig. 1b) and improves the overall performance of the TriM Scope II by:

- Higher frame rates
- Increasing image brightness
- Reducing photo toxicity and photo bleaching
- Working perfectly with resonant scanners

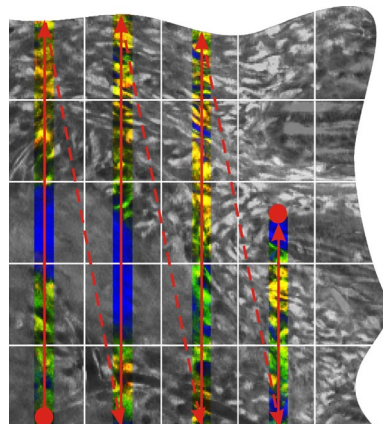


Fig. 1a: Standard single beam scanning¹

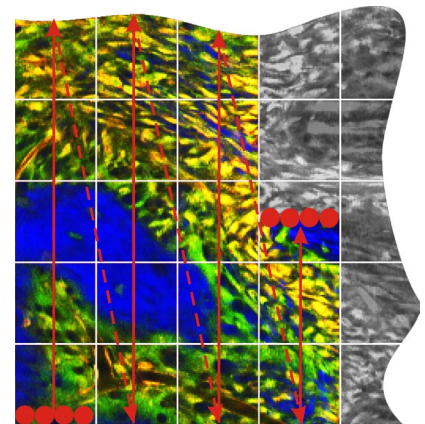


Fig. 1b: Cloud scanning

Idea behind Cloud Scanning

Many intravital 2-photon microscopy applications require the highest frame rates but do not depend on high resolution to deliver relevant information with respect to dynamic events (e.g. Calcium imaging). Under those imaging conditions the pixel size (1 to 2 μm) is much larger than the diffraction limited focus diameter (< 400 nm). Thus, only a fraction of the fluorophores within a single pixel will be excited by the single laser focus. Cloud scanning overcomes this drawback by generating a line pattern of 4 foci (< 2 μm @ 20x objective lens) which will excite all fluorophores within the pixel.

Integration into the TriM Scope II Scan Head

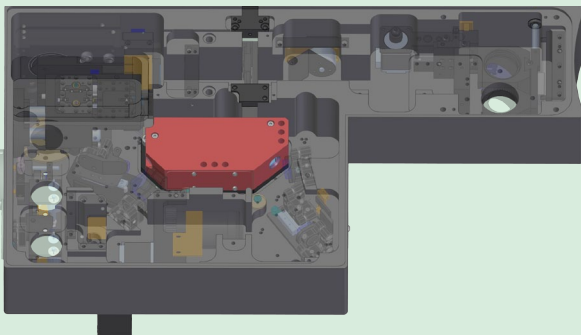


Fig. 2a: Cloud Scanner integration into the scanhead

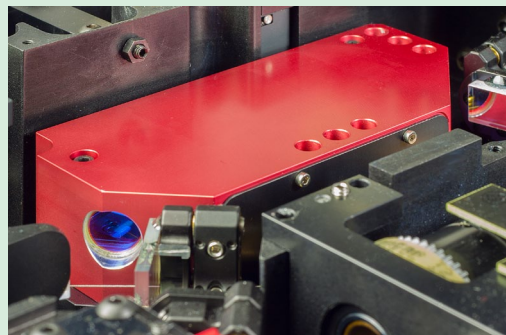
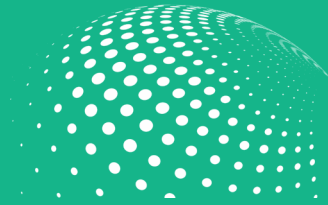


Fig. 2b: Cloud Scanner



The 4X Cloud Scanner is fully integrated into the TriM Scope II Scan Head and the LaVision BioTec ImSpector microscopy software. Each TriM Scope II including a 4X Cloud Scanner can be switched between single beam or Cloud Scanning mode by a single click in the ImSpector microscopy software. In standard single beam mode, the Cloud Scanner beam multiplexer is bypassed.

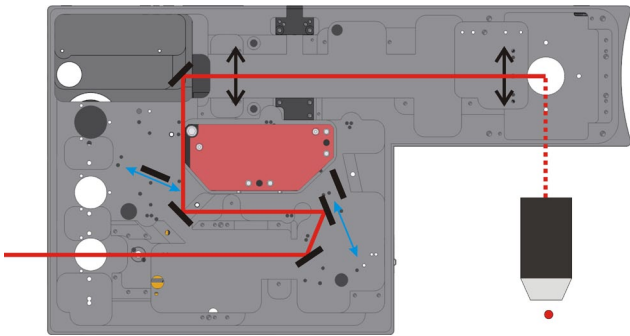


Fig. 3a: Standard single beam scanning

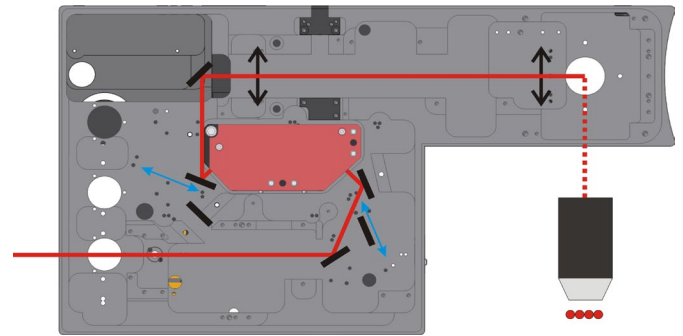
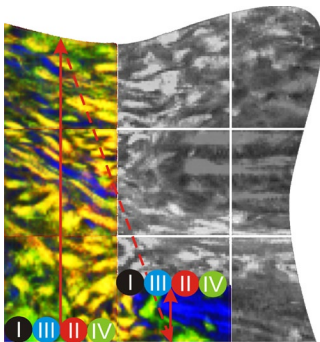


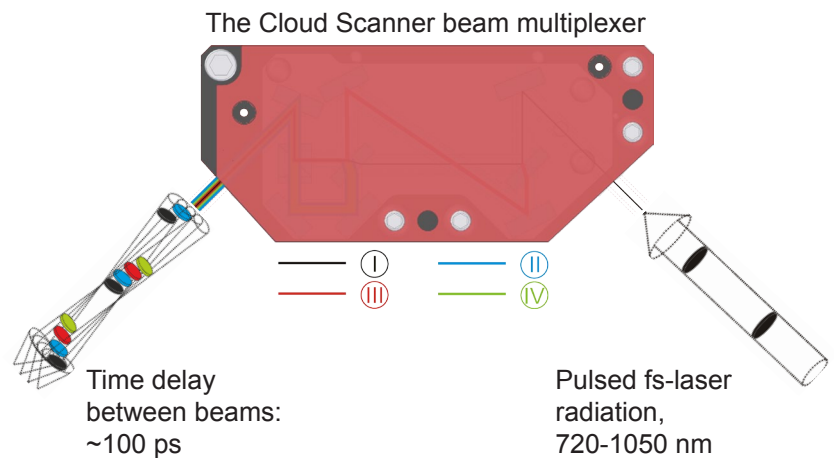
Fig. 3b: Cloud scanning

Optical Design

The Cloud Scanner beam multiplexer is a monolithic device that delivers four beams and is based on flat optics.



Fixed 4 foci line pattern, length <math>< 2 \mu\text{m}</math> when utilizing a 20x objective lens



The Cloud Scanner can be combined with the following options:

- Adaptive Optics Module
- Galvo and resonant scanners
- Targeted Path Scanning Module
- PI FOC^{®2} ultrafast z-stepper
- 2nd independent scanner option
- Doubleheader 2-Stands TriM Scope option

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1) Background images courtesy of Ann Haberman, Yale Medical School
 2) PI FOC[®] is a registered trademark of Physik Instrumente GmbH & Co. KG