

Alpha³

Light Sheet Fluorescence Microscope

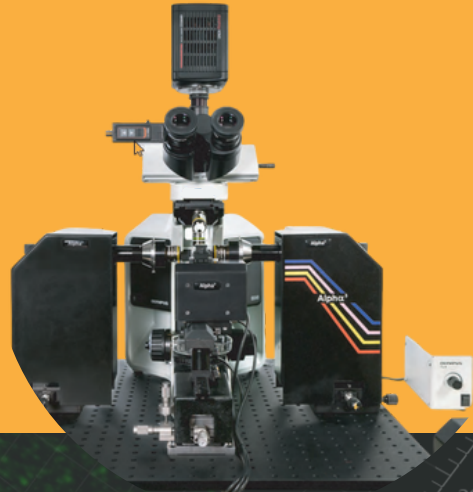
Modular Light Sheet Microscope

Alpha³ is a new generation of light sheet fluorescence microscope addressing the needs of high temporal resolution along with spatial high resolution to achieve qualitative and quantitative 3D imaging of fixed or live biological specimens.

From *in-vivo* imaging to large cleared samples, the Alpha3 microscope delivers unprecedented image quality while keeping the necessary flexibility and modularity expected for cutting-edge scientific research instruments.

Alpha³ features

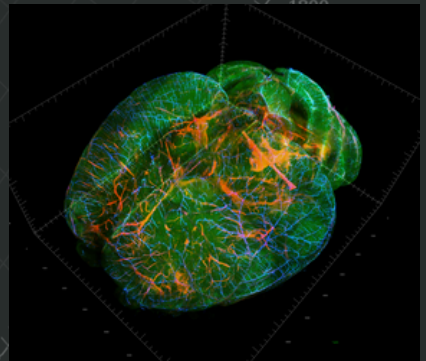
- From *in-vivo* imaging to large cleared samples
- Macro to micro view imaging, from whole organs at sub cellular resolution to very small specimen
- Compatible with all clearing solutions: aqueous buffers and organic solvents
- Multiple mounting accessories to accommodate sample nature and size
- Unique live observation of the optical section with eyepieces



Patented Smart Light Sheet Illuminators

The light sheet system architecture comprises dual illumination units, each integrating multi-directional light sheet, combined with wide field microscope detection, offering:

- Real-time laser focus sweeping for optimized sharpness on the entire field of view
- Stripe artifacts removal for absorbing or scattering specimen
- Sample perturbation-free and ultra fast acquisition thanks to synchronization with remote focal plane scanning
- Flexible imaging system with a large selection of detection objectives
- Modular microscopy system to easily adapt to experimental constraints



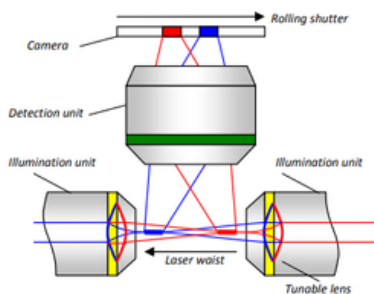
Pegasus cleared mouse brain, Lifeact-GFP / MARCKS td-tomato

Cutting Edge Light Sheet Technology

The advanced sharp optical sectioning and smart scanning functions drastically alleviate spatial and temporal resolution constraints for 3D image acquisition in light sheet microscopy.

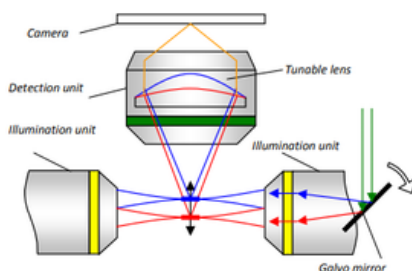
Alpha³ microscope, with its innovative light sheet illuminators and system architecture, broadens the possibilities of life science imaging, providing new quantitative and qualitative imaging capabilities.

Sharp Optical Sectioning



Real time lateral focus sweeping principle

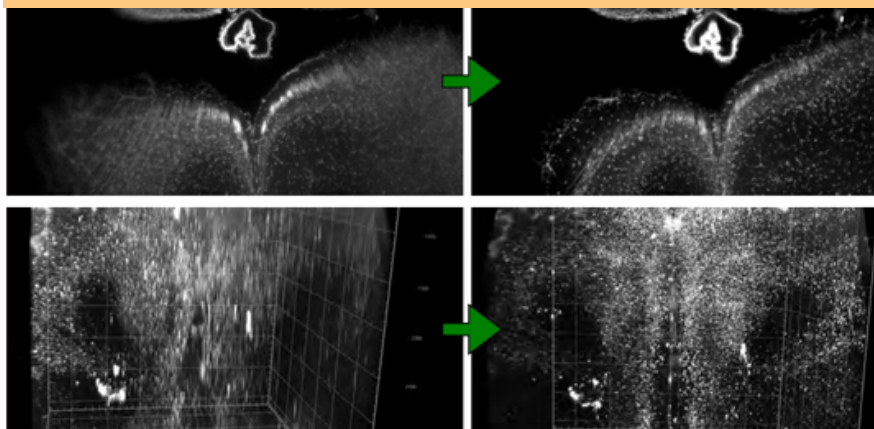
Smart 3D Scanning



Remote focal plane scanning principle

The multidirectional light sheet illuminators perform real time focus sweeping to extend the thinnest focus area over the entire field of view, while improving homogeneity for artefact-free imaging.

The remote focal plane scanning technology combined with the dynamic illumination of light sheet planes allow for ultrafast Z-stacking and perturbation-free acquisition.



Cleared tissue image without focus sweeping (left) and with active focus sweeping (right)

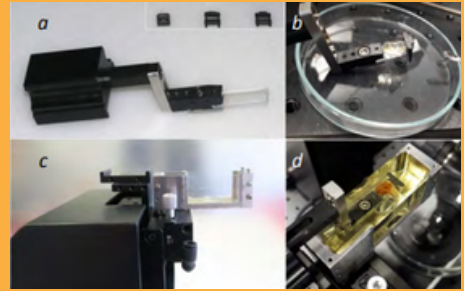
Flexible Sample Mounting

The chamber allows for easy sample insertion and observation with air or immersion objectives. As biological specimens are of different nature and size, multiple sample holders are provided to accommodate a large variety of fixed or live samples.

The architecture using a fluorescence microscope stand as a detection unit allows Alpha³ to flexibly adapt to any experiment setting. Complementary imaging capabilities can be easily added for multimodal microscopy.

Alpha³ features

- Multiple accessories provided for sample mounting to accommodate a large variety of specimens
- Chamber and sample holders highly resistant to corrosive media or clearing solutions
- Low volume chamber minimizes evaporation and use of costly clearing solutions
- Easy chamber access, allows addition of various experiment tools
- *In vivo* imaging environmental controls



Fast and reproducible sample mounting

- a) Typical mounting accessories for fixed samples;
- b) Chamber with magnetic handle;
- c) Cleared sample mounting on glass support;
- d) Chamber with sample filled with medium solution

Incubation enclosure

In order to maintain perfect physiological conditions of sensitive specimen, temperature, CO₂ and humidity may require precise controls. The optional H301 incubation enclosures cover every requirement and offer a new level of performance for incubation. The digital controllers associated with the incubation enclosures allow for optimal stability of the environmental parameters.

Thanks to the QtSPIM software, stacks of image data can be recorded for many hours or days for various applications such as developmental bio processes or stimuli response.



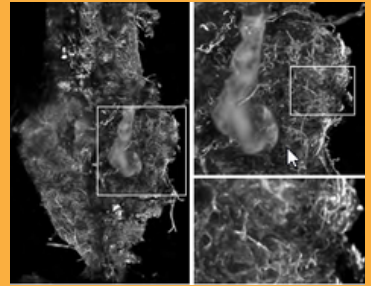
QtSPIM Acquisition Software

From sample mounting to image acquisition, Alpha³ performs seamless light sheet imaging. The QtSPIM software provides a clear and intuitive interface for collecting X, Y, Z, θ , T, λ images at maximum speed. Raw image data, along with their metadata, are saved in 16 bits TIFF format (compatible with open source or commercial software for further 3D display and analysis).

QtSPIM controls all image acquisition parameters. When paired with a workstation grade computer in an optimized configuration, the system is able to perform ultra-fast acquisition.

Alpha³ features

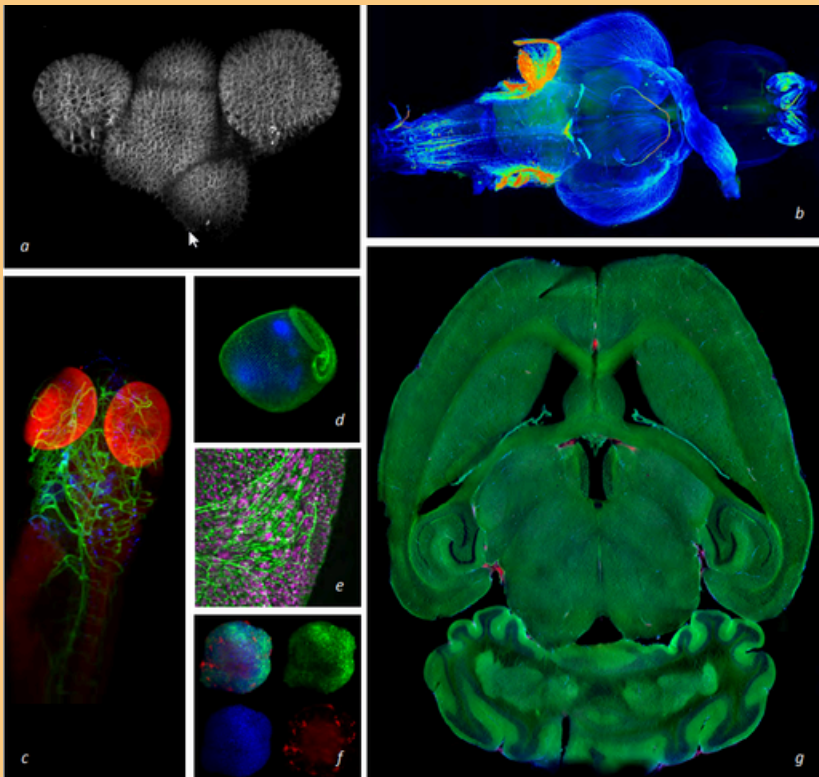
- Seamless X,Y,Z, θ ,T, λ image data acquisition
- Ultra-fast multichannel acquisition via a mult notch filter, completed by a large selection of emission filters
- Wide-field acquisition with sensitive high QE sCMOS camera
- Wide choice of detection objectives: LWD 2X to 60X air, dipping lenses, clearing objectives with correction collar for RI matching



QtSpim user interface

Applications From In Vivo Imaging to Clearing

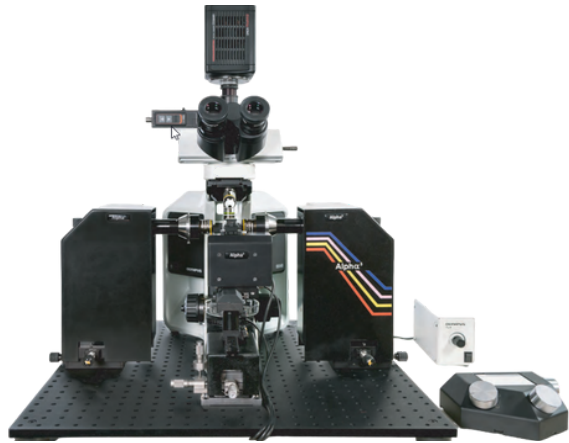
Typical applications of Alpha³ include in toto imaging of small animal models such as whole mouse embryos, morphogenesis and embryogenesis of model organisms: C.elegans, Drosophila, Zebra fish, live imaging of cell cultures, functional imaging of neuronal activity, fluorescence imaging of marine organisms or plant developmental biology.



- a) *In vivo* Arabidopsis Meristems, GFP membrane staining at 40x0.8NA;
- b) Cleared Zebrafish Brain in iDisco, 2 channels at 10x0.6NA;
- c) Cleared Zebrafish in MD media, kdr and Vmat2 staining at 10x0.6NA;
- d) Fixed Stentor in PBS, GFP cilia and DAPI polynuclei at 10x0.3NA;
- e) Fixed Drosophila Egg in PBS, 2 channels at 40x0.8NA;
- f) Fixed Spheroid in PBS, 3 channels, at 20x0.5NA;
- g) Cleared Mouse Brain in Pegasus, Lifeact-GFP and MARCKS td-tomato staining, at 4x0.28NA.

Specifications

Laser source	Laser combiner with up to 4 or 6 laser lines, Excitation wavelength from 405 nm to 785 nm Output power from 25 to 250 mW Fibered connection to light sheet generator
Light sheet generator	Single or dual smart illuminators. Multi-directional light sheet with real time focus sweeping and fast 3D scanning capabilities Minimum light sheet thickness 2 μm , constant thickness across the whole field of view with focus sweeping
Chamber & mounting accessories	Standard and large chamber available, dimensions : W 20/30 mm, L 70/90 mm, H 25/33 mm; Volume < 15/60 ml For sample size from μm to cm range, highly resistant to various corrosive medium, clearing agents, sea water Multiple sample mounting accessories: molds, cover slips, glass supports to accommodate different sample sizes and natures Optional Incubation enclosure for temperature, CO2 and humidity controls
Volume scanning	Z motorized stage: range 15 mm, precision 0.1 μm , acquisition speed 40 fps Optional: XY motorized stages for tiling: X range 15 mm, Y range 15 or 25 mm, precision 0.1 μm Optional: ultra fast 3D scanning module (75 images/second)
Detection unit	Fluorescence microscope stand comprising objective turret 1 or 2 positions, eyepieces, video port, optional 2X magnification changer, transmitted light, motorized filter wheel with up to 6 emission filters, multi-notch rejection filter
Detection lenses	Objective Magnification from 2X to 60X: air, water dipping lenses, clearing objectives with correction collar RI 1.33 - 1.56 Large selection of long Working Distance, high Numerical Aperture objectives, e.g. 10X NA 0.6, WD 8 mm, 25X NA 0.95 WD 8 mm
Image sensor	sCMOS 2048 x 2048 pixels, format 13 x 13 mm, 6.5 μm square pixels., USB3 / CameraLink Interface
Software	QtSPIM software for multi-dimensional image acquisition: Z-stacks, XY, Tiling, rotation, multichannel and time lapse acquisition. Easy export of raw images (16 bit TIFF) with metadata, compatible with open source or commercial 3rd party software
PC	QtSPIM software for multi-dimensional image acquisition: Z-stacks, XY, Tiling, rotation, multichannel and time lapse acquisition. Easy export of raw images (16 bit TIFF) with metadata, compatible with open source or commercial 3rd party software
Dimensions	Microscope breadboard format W 600 mm x L 600 mm



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