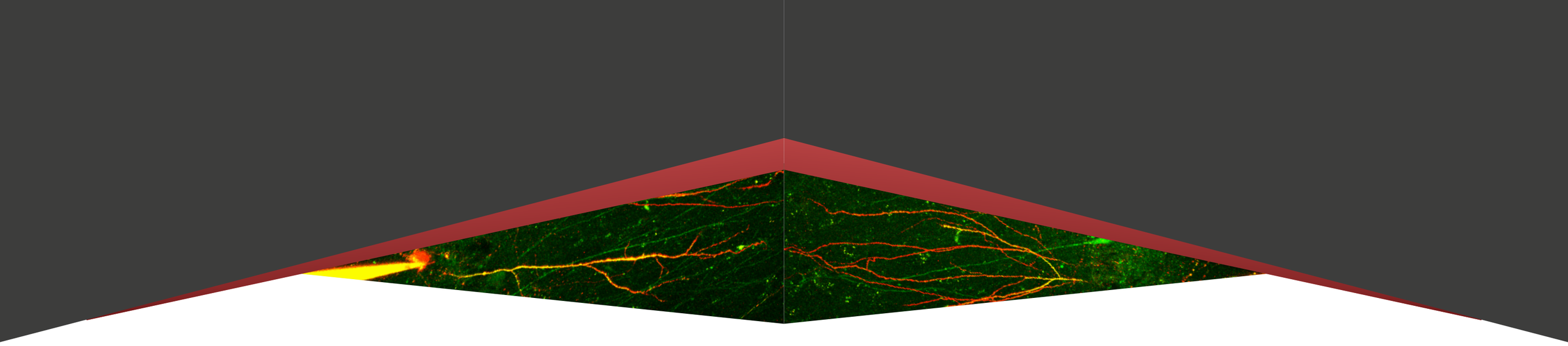
FEMTONICS

ADVANCED MICROSCOPY

# Two-Photon Microscopy

# About Femtonics



## Why Femtonics?

- Developed by scientists for scientists
- Instruments are designed to be easily upgradable
- Patented 2D and 3D scanning technology
- Specialized in custom-made imaging systems
- Choices of galvanometric, resonant or acousto-optic technology
- Ultrafast 3D random-access point scanning

## Mission

Our mission is to develop and manufacture custom imaging systems to keep up with the demanding scientific applications. Two-photon microscopy is one of the best choices for functional imaging in deep tissues in in vivo and in vitro applications. Femtonics was founded in 2005 by two scientists with roots in their academic and R&D environment. Today we are one of the most innovative manufacturers of two-photon laser scanning microscopes, introducing 3D acousto-optic (AO) microscopy to the research market and as well as manufacturing galvanometric and resonant scanner based imaging systems. Femtonics microscopes are modular, flexible, custom designed and upgradable: this ensures that it meets the researchers' needs and can address a wide variety of biological applications.

Our sales team will assist you to select the optimal microscope system for your application needs.

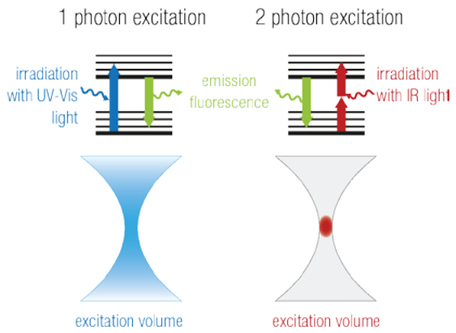
Share your thoughts, share your needs and we will find an innovative solution.

Tools for Science.

# Why two-photon microscopy?

Two-photon laser scanning microscopy is a powerful tool for fluorescence imaging and for studying physiological functioning in biological samples and offers many advantages over conventional imaging.

The concept of two-photon excitation was theoretically described by Maria Goeppert-Mayer, in 1931. This process is the simultaneous absorption of two photons in a single event resulting in the excitation of a molecule from one state to a higher energy state. After a short period of time the fluorophore relaxes back to its ground state by emitting a photon of light. The two photons have approximately half the energy and double the wavelength of the photon required for a single photon excitation quantum event to occur. Therefore, two-photon microscopy provides clear advantages in thick, highly scattering samples or living animal tissues/models.




The nonlinear dependence of photon absorption on the excitation light intensity eliminates background fluorescence and allows for efficient collection of both scattered and ballistic emitted photons. As an overall conclusion two-photon microcopy provides a very attractive solution to avoid photobleaching and phototoxicity in the specimen during three-dimensional, deep tissue imaging, while obtaining high spatiotemporal resolution.

Comparision of two-photon and confocal microscopy:

## Two-photon microscopy

Functional imaging in living animals and tissue samples up to 850 µm depth without photodamage

### IR laser

- less scattering
- deeper penetration
- single-point excitation
- less photodamage

### Non-descanned detectors

- high signal-to-noise ratio (SNR): non-descanned detectors combined with close coupled detectors for more effective photon collection

### 2D and 3D scanning possibilities

- galvanometric and resonant scanner based imaging
- acousto-optic deflector based ultrafast, 3D scanning method

## Confocal microscopy

Imaging in 100 µm thick slices or cell cultures

### Visible laser

- tissues scatter the visible light
- excitation in a focal cone
- photodamage and photobleaching throughout the tissue
- high background signal

### Pinhole and descanned detectors

- rejection of out of focus background fluorescence
- loss of emitted photons

### 2D scanning possibilities

- conventional galvanometric and resonant scanner based imaging methods

# The Femtonics two-photon product lines

Two-photon microscopy allows video-rate, real-time monitoring of cellular processes in living organs by revealing pathomechanisms at subcellular spatiotemporal resolution with minimal photodamage. Numerous patented technologies are applied to all our microscopes, providing ergonomic, modular, upgradable systems which can be optimized to meet the demands of different fields of the life sciences.

There are three distinct product lines that have application-specific benefits:

## Femto3D-AcoustoOptic

The Femto3D-AO two-photon microscope is our flagship microscope. This model incorporates all the latest features: it is a pioneer in 3D laser-scanning technology and can answer the most demanding neuroscience questions. The unique, patented acousto-optic scanner built into the microscope does not contain any mirrors or moving parts: the focal spot is positioned at very high speed and accuracy, independent of the distances involved. This provides fast scanning rates of up to 30 000 locations per second. This feature makes the microscope suitable for 3D random-access scanning modes, or for applications that require the capture of fast time-dependent events.

## FemtoSmart

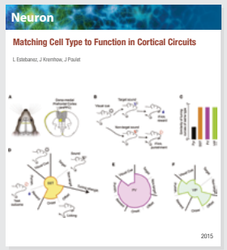
FemtoSmart features a large throat depth that offers plenty of room for small or large animal models, as well as behavioral equipment under the objective. The modularity of this microscope allows us to assemble the primary components, then recombine and upgrade the setup with various modules in order to meet the researcher's needs. The FemtoS-Galvo applies patented and flexible ROI scanning methods while the FemtoS-Resonant is capable of capturing images at 31 frames per second. The FemtoS-Dual microscope combines features of the two distinct microscopes providing the highest level of flexibility for versatile experimental protocols.

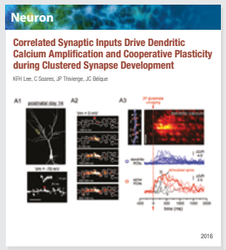
## Femto2D

Femto2D is our standard two-photon microscope product line. The innovative optomechanical design and the stable architecture support superior imaging techniques. The different models encompass modular galvanometric, resonant and dual scanner two-photon microscopes. Many extensions have been developed inspired by customer's research including photostimulation and fluorescent lifetime imaging modules. Femto2D series is highly suited for in vitro applications supporting electrophysiology studies. Complex, specifically designed microscopes have been built for various biological applications, and have been used around the world for more than 10 years.

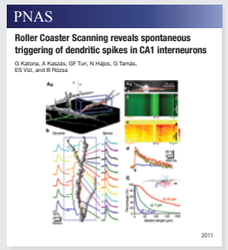
# Femtonics two-photon microscope

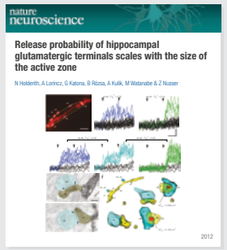
- a partner in the research process

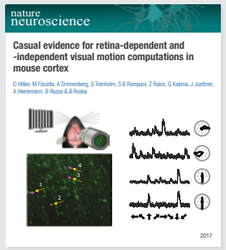


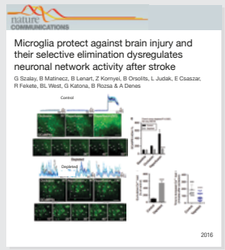







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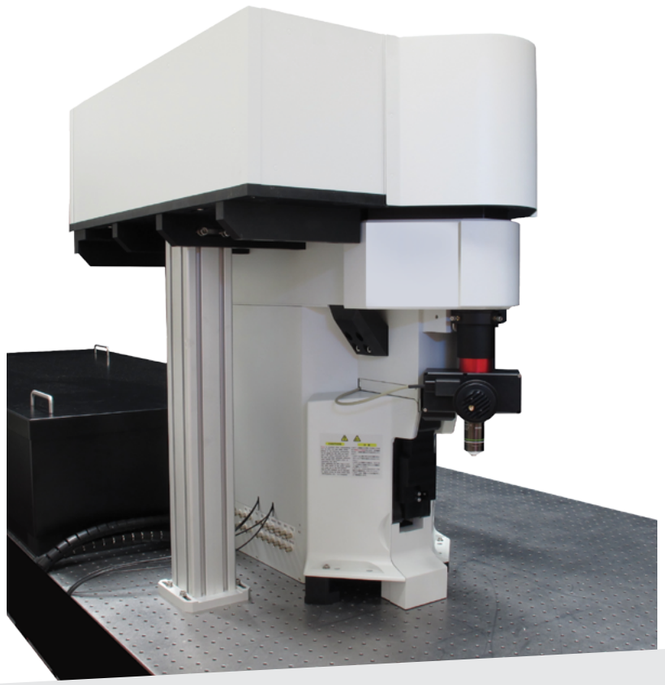
# Femto3D-AO

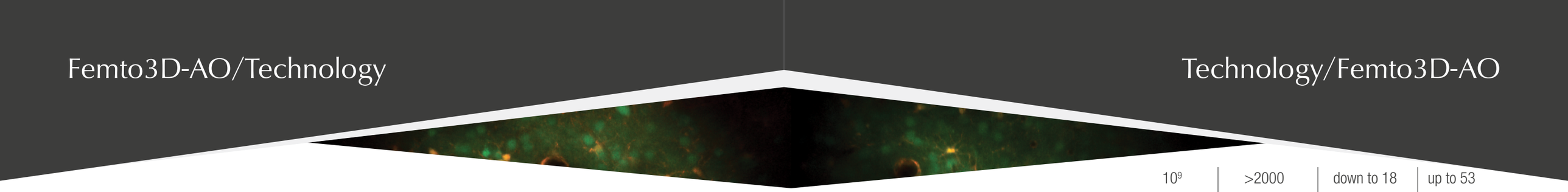


## Unique features for 3D imaging

- Over one billion faster scanning in 3D (30-50 kHz/ROI) than other models
- Fast in vivo and in vitro recording of over 2000 neurons, spines, dendrites in 3D
- Over 650 µm fast Z scanning range during in vivo recordings
- Preserved high spatial resolution (< 400 nm in the center)
- Over 500 µm × 500 µm × 700 µm scanning volume in vivo

## Unique technology

- Novel 3D scanning methods both for in vivo and in vitro
- Motion correction in behaving animals
- New-generation acousto-optics provides over 4-fold improved excitation
- Automatic beam stabilization
- Angular dispersion compensation
- Automatic wavelength tunability
- Multiple wavelengths
- Dynamic compensation for optical errors
- High-NA (1.0), wide-field objectives
- Separated Z unit to extend lateral scanning range
- Intelligent software control with renewed GUI



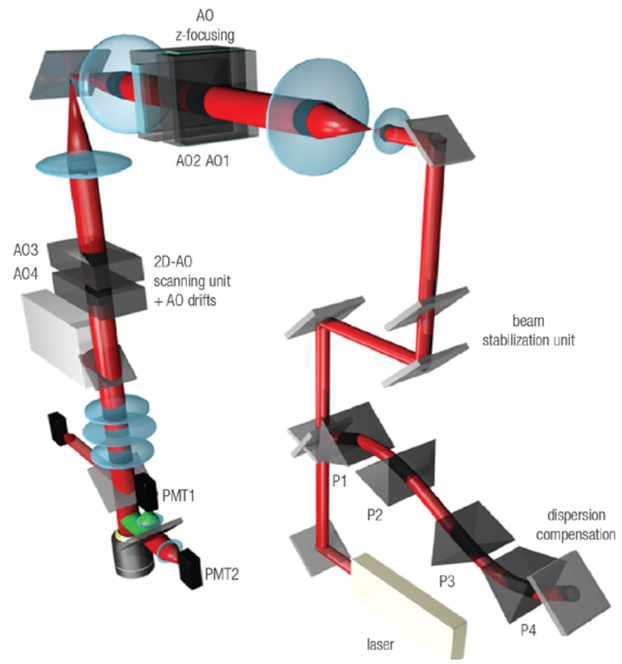
## Overview

The Femto3D-AcoustoOptic microscope is the first fast, 3D two-photon microscope on the market, using electrically tunable acousto-optic (AO) deflectors. AO scanners do not contain any slowly moving mechanical components (e.g. scanning mirrors), so are able to position the focal spot (lateral resolution <400 nm) at up to 53 kHz at any 3D location under the objective with <100 nm precision. This technical solution can increase measurement speed and signal-to-noise ratio (SNR) by several orders of magnitude in comparison to classical raster scanning (Szalay et al., Neuron, 2016; Katona et al., Nature Methods, 2012) because the preselected regions of interest (ROIs) can be precisely and rapidly targeted without wasting measurement time on unnecessary background areas or volume elements.

## One billion faster scanning speed

If we compare the relative gains in measurement speed ($v_{gain}$) and signal-to-noise ratio ($SNR_{gain}$) for 3D AO scanning relative to traditional raster scanning of the same sample volume, we can say that the $v_{gain} \times (SNR_{gain})^2$ is equivalent to the ratio of the total image volume to the volume covered by the pre-selected scanning points. This ratio can be very large, up to over $10^6$ per ROI, which makes 3D AO scanning suitable for precise multisite activity measurements, especially when ROIs are sparsely dispersed in the 3D volume (Szalay et al., Neuron, 2016).

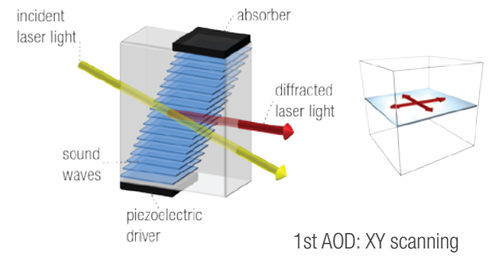
The single-point multi-photon excitation of 3D AO scanning technology enables whole-field detection of the scattered fluorescence photons required for deep-brain imaging.



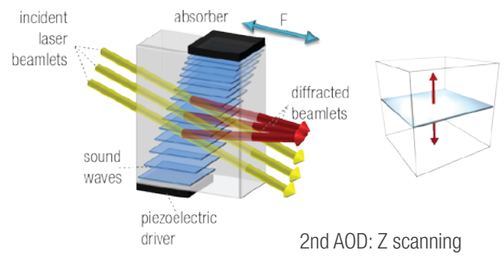

## Acousto-optic deflectors

| $10^9$ | >2000 | down to 18 | up to 53 |
|---|---|---|---|
| scanning volume ($\mu m^3$) | simultaneous measurement locations (ROIs) | multisite repetition rate (µs) | speed (kHz) |

In contrast to a traditional scanner, the microscope contains two pairs of AO deflectors (AODs) which are responsible for the X, Y, and Z focusing. The AODs control the optical beam spatially utilizing the interaction between sound and light waves. In imaging, AODs diffract laser beam through ultrasonically generated gratings. The sound wave induces pressure fluctuation in the AOD crystal and the evoked periodic change in the refractive index of the crystal results diffraction of the light beam similarly to a regular optical grating. By changing the sound frequency, the focus point also changes: its position depends on the parameters of the sound applied to the deflector.




1st AOD: XY scanning



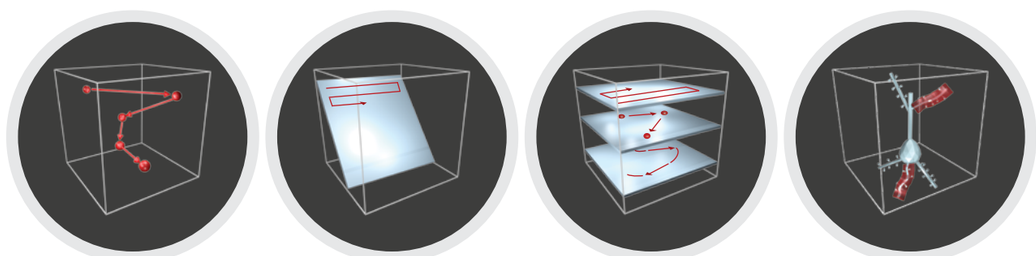

2nd AOD: Z scanning

## 3D AO imaging

Because the scanner does not contain any moving parts, positioning the focal spot during sparse labeling in 3D is stable, and independent of the travelling distance: this gives an extremely high scanning speed and image quality. Using the AO scanning technology in combination with an easy-to-use 3D data acquisition software package provides maximal flexibility in fast selection of ROIs, and a vast array of measurement possibilities which can be visualized and analyzed online and offline. Many 3D scanning patterns are available: these support imaging of neural networks, dendrites and even spines at high scanning speed and high SNR.

## Improved deep penetration with high SNR

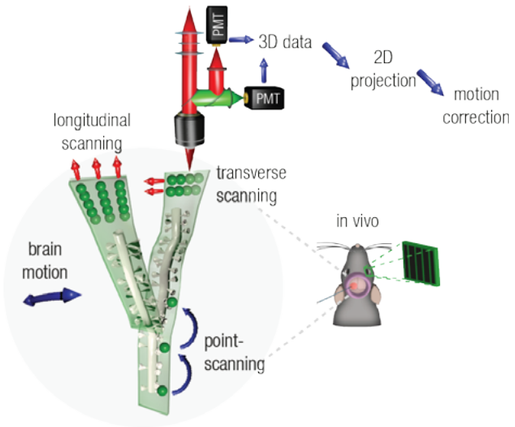
The Femto3D-AcoustoOptic microscope comes equipped with a travelling detector system, which is the patented feature of all Femtonics microscopes. This detector system is mounted on the objective arm and comprises commercial-grade GaAsP photomultipliers (PMTs) and special optics (lenses, dichroics, filters, etc.). This technical solution minimizes the distance between the objective and detectors and maximizes collection of photons from deep in the sample.



3D random access | arbitrary frame | all 2D scanning modes | Anti-mOtion scanning

## Anti-mOtion technology

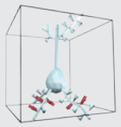
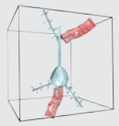
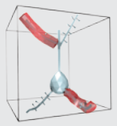
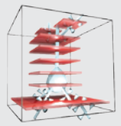
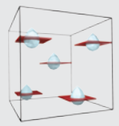
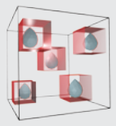
In contrast to traditional point-by-point scanning, here the Z scanning and XY scanning units of the microscope can drift the focal spot in 3D in any specified direction and at any specified speed. Therefore, we can extend individual scanning points to small surface or even volume elements (by using, for example, longitudinal or transverse scanning) to cover not only the interesting regions but also the neighboring areas. These surface and volume elements can be set parallel to the average direction of brain movement to preserve fluorescence information for motion correction caused by vessel pulsing, respiration, locomotion, or behavior.



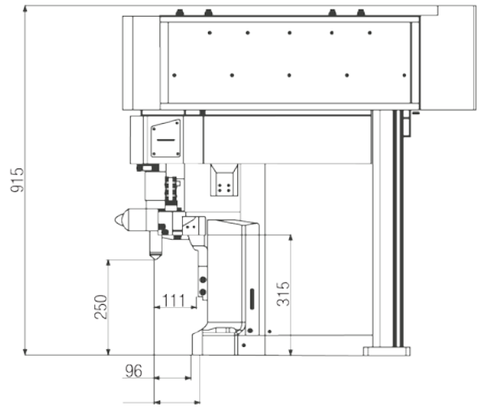

## Advanced scanning modes performed by Anti-mOtion technology

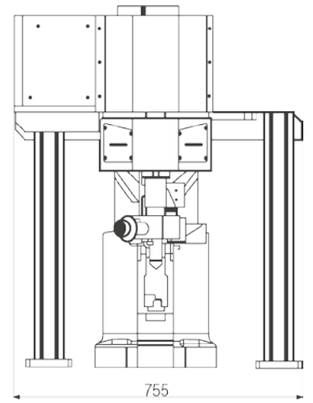
Advanced scanning modes can be performed by drifting the focal spot (3D DRIFT AO scanning, Szalay et al., Neuron, 2016). Depending on the size of the scanned area, the imaging speed reaches kHz range along surfaces and hundreds of Hz in volumes. The surface scanning methods are optimized for speed, while the methods based on volume imaging are optimized for large amplitude movements. Each scanning mode is useful for different neurobiological aims. Ribbon scanning, snake scanning, 3D multiple line scanning are optimal for different dendritic measurements, while chessboard scanning and multi-cube scanning are best for somatic recordings. In summary, we can preserve fluorescence information in the brain of behaving animals, and maintain the 10–1,000 Hz sampling rate necessary to resolve neural activity at the individual ROIs.

### ADVANCED SCANNING MODES

| | 3D MULTIPLE-LINE | RIBBON SCANNING | SNAKE SCANNING | MULTI-LAYER | CHESSBOARD | MULTI -CUBE |
|---|---|---|---|---|---|---|
| TECHNIQUES |  |  |  |  |  |  |
| SPEED | up to 30 kHz per spine | up to 3 kHz on a 50 µm long dendritic segment | up to 300 Hz on a 50 µm long dendritic segment | up to 3 kHz per 2D ROI | up to 3 kHz per 2D ROI | up to 300 Hz per volume |
| NUMBER OF SIMULTANEOUSLY SCANNED REGIONS | up to 1000 spines | up to an 1000 µm long dendritic segment | up to an 300 µm long dendritic segment | up to 300 regions | up to 300 regions | up to 30 volumes |
| BENEFITS IN NEUROSCIENCE DEMONSTRATED IN SZALAY ET AL 2016 | functional recording of over 150 spines | imaging of activity in over 12 spiny dendritic segments | dendritic imaging during large amplitude movements | imaging along the entire length of the cell | high speed somatic recordings, network imaging | imaging of somata during large amplitude movements |

## Dimensions




## Optional modules

Vessel pattern visualization by Green illumination

Before the two-photon measurement, green illumination can aid navigation on the surface of the organs under in vivo conditions, enabling high contrast visualization of structures such as blood vessels which provide orientation points in the tissue.

## In vitro extension

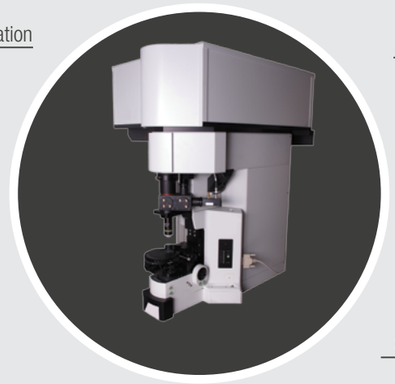
The frame and optical arrangement of the microscope makes it possible to equip it with an in vitro unit which allows measurements to be taken in slices of any organs, embryos, or cell cultures. See the detailed description in the Optional modules section.

Visual stimulation auditory stimulation

Optogenetic photostimulation

Uncaging

In vitro extension



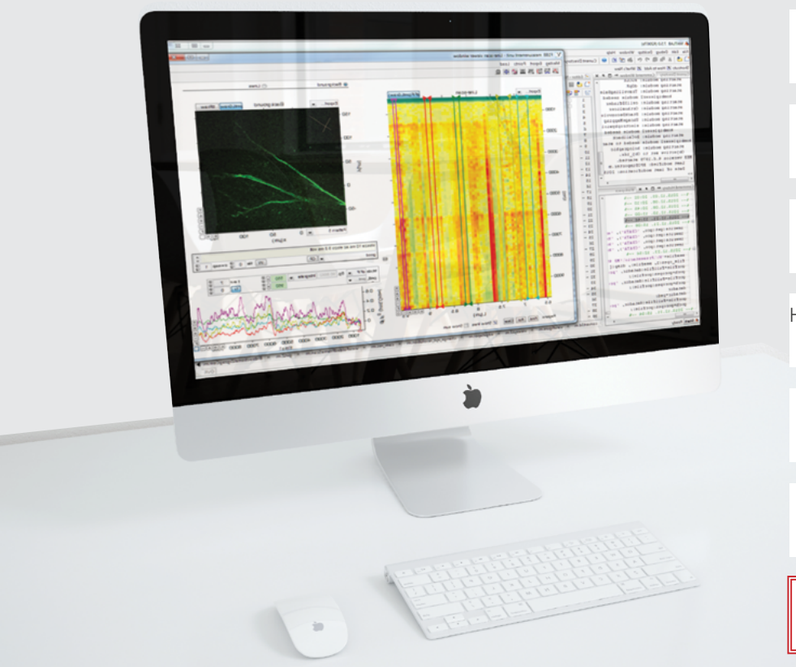
Behavior experiments (CS+/CS-)

Whole-cell electrophysiology
Local field potential (LFP)

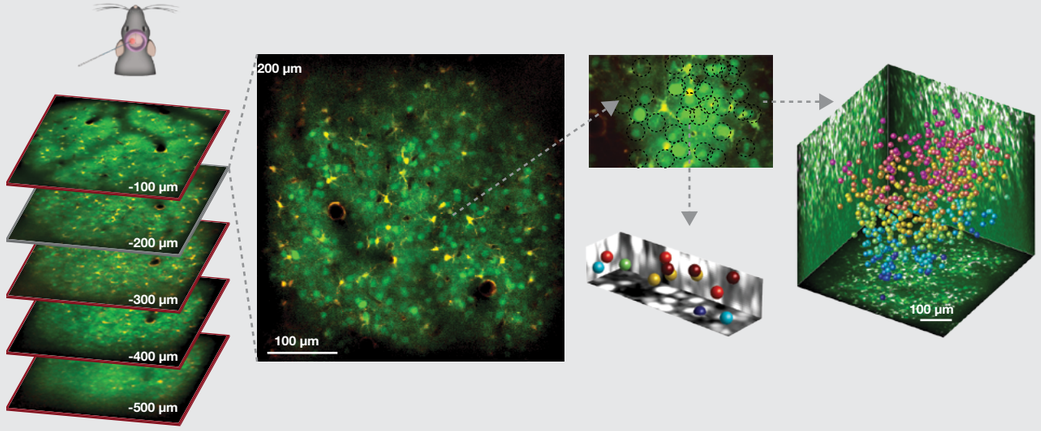
Virtual reality

Green illumination

Secondary 2D or 3D scan head

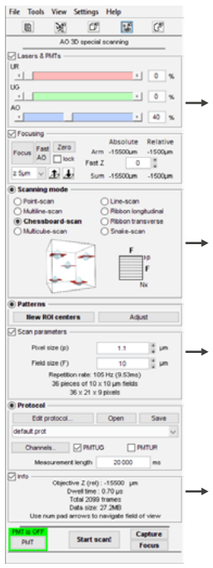


- Movement artefact correction of multi-ROI data
- ROI selection and handling
- Merging ROI sets on different measuremen units
- Calculation of background corrected relative fluorescence
- Handling and arithmetic of $Ca^{2+}$ traces in line with behavior signals
- AP detection
- Excel interface
- **Automatic motion correction and AP detection**




Automatic cell detection

## Easy-to-use user friendly GUI



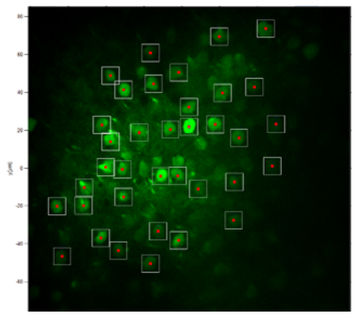

→ Laser intensity and PMT control

→ Scanning mode selection

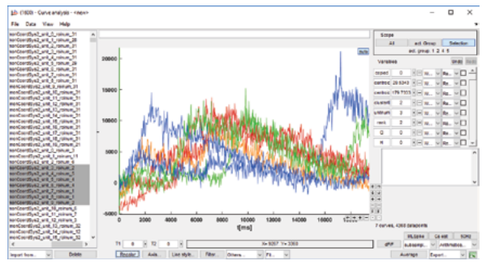
→ Scanning specific parameters

→ Measurement controls

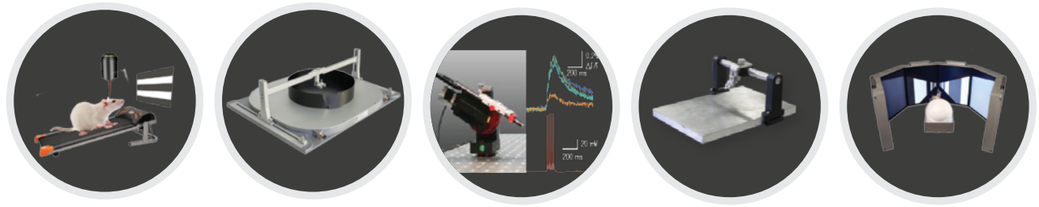
## Automatic ROI detection



## Semi-automatic tools for simultaneous analysis of multiple ROIs



## Integrated software tools for behavior and electrophysiology

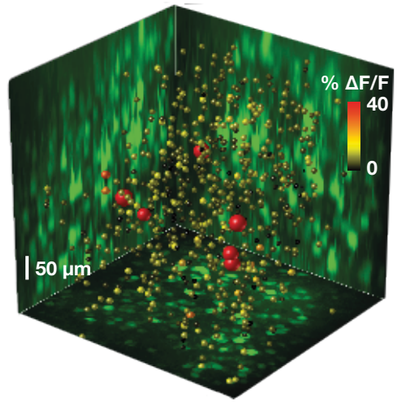


## Specification

- in vivo functional imaging down to over 700 µm depth
- 500 µm × 500 µm × 700 µm scanning volume in vivo (with a 20x obj.)
- 650 µm × 650 µm × 700 µm scanning volume in vitro (with a 20x obj.)
- available wavelength between 750 - 1100 nm
- integrated beam stabilization
- integrated dispersion compensation unit for the most effective excitation
- diffraction limited, submicrometer resolution in the center (~400 nm)
- two pairs of acousto-optic deflectors for XY and Z scanning
- switching between points in 3D without mechanical restrictions
- scanning speed up to 53 kHz to any points in 3D
- near simultaneous measurement of 2000 ROIs
- 3D scanning modes:
- random-access point, trajectory, stripe, tilted frame, volume scanning
- novel 3D scanning methods
  - ► for network measurements: chessboard and multi-cube scanning methods
  - ► for dendritic measurements: ribbon, 3D multiple-line, snake scanning methods
- Anti-mOtion scanning with many new scanning patterns for motion correction
- 2D scanning modes: point, line, frame, folded/multi-frame scanning, X-Y-Z stack
- ROI scanning and the optical system support high signal-to-noise ratio
- minimized optical path length by patented travelling detector system
- non-descanned, ultrasensitive GaAsP PMTs (>40% quantum efficiency)
- MATLAB based, integrated hardware, measurement control and analysis software
- parallel recording and analysis of electrophysiological data
- intelligent control of various sensory stimuli supporting behavioral studies
- optional modules: green lamp, epifluorescent unit, in vitro extension

## Random-access point scanning of neuronal networks in 3D

3D random-access point scanning is the fastest method to read-out neuronal activity because it enables multiple points, distributed in 3D, to be imaged simultaneously. Within a large scanning volume, it is approximately one million times faster than other frame-by-frame scanning methods. This imaging speed means that thousands of individual neurons (e.g. in different cortical layers) can be measured with microsecond resolution simultaneously, revealing the dynamics of neuronal networks.
See more Katona et al., Nature Methods, 2012.

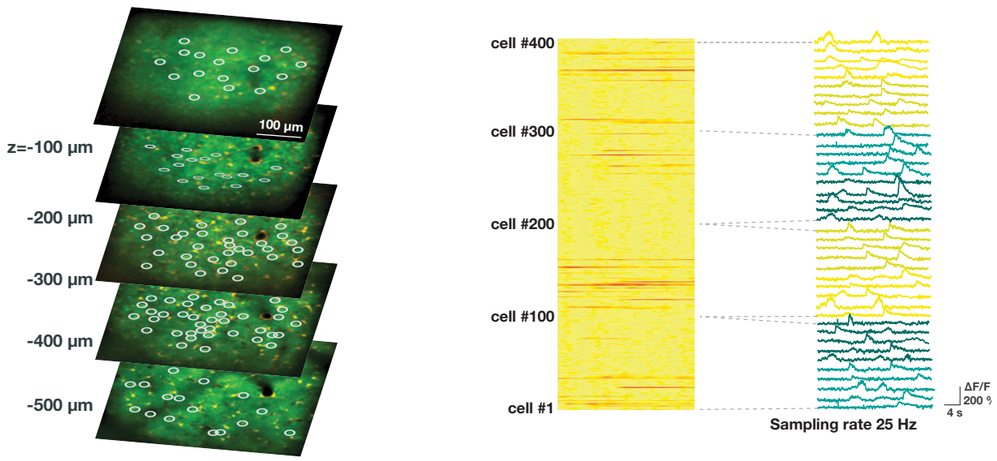



Visualization of the activity of aboved selected neurons measured simultanously in 3D

Automatic selection of neurons from a Z-stack

Fluorescence signals of the cells

Calcium transients of the most responsive cells

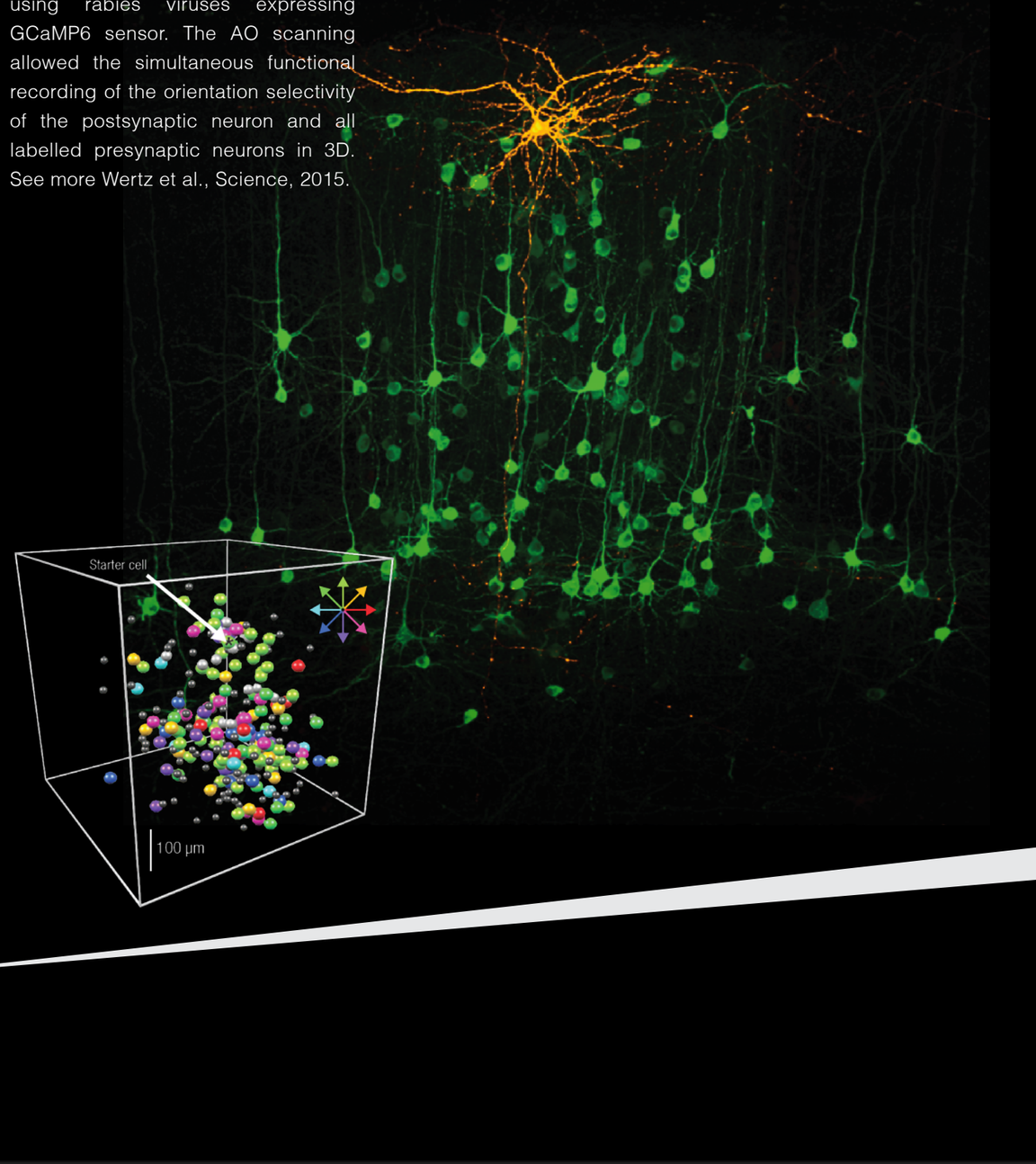



## Imaging of dendritic arbor with 3D Trajectory scanning

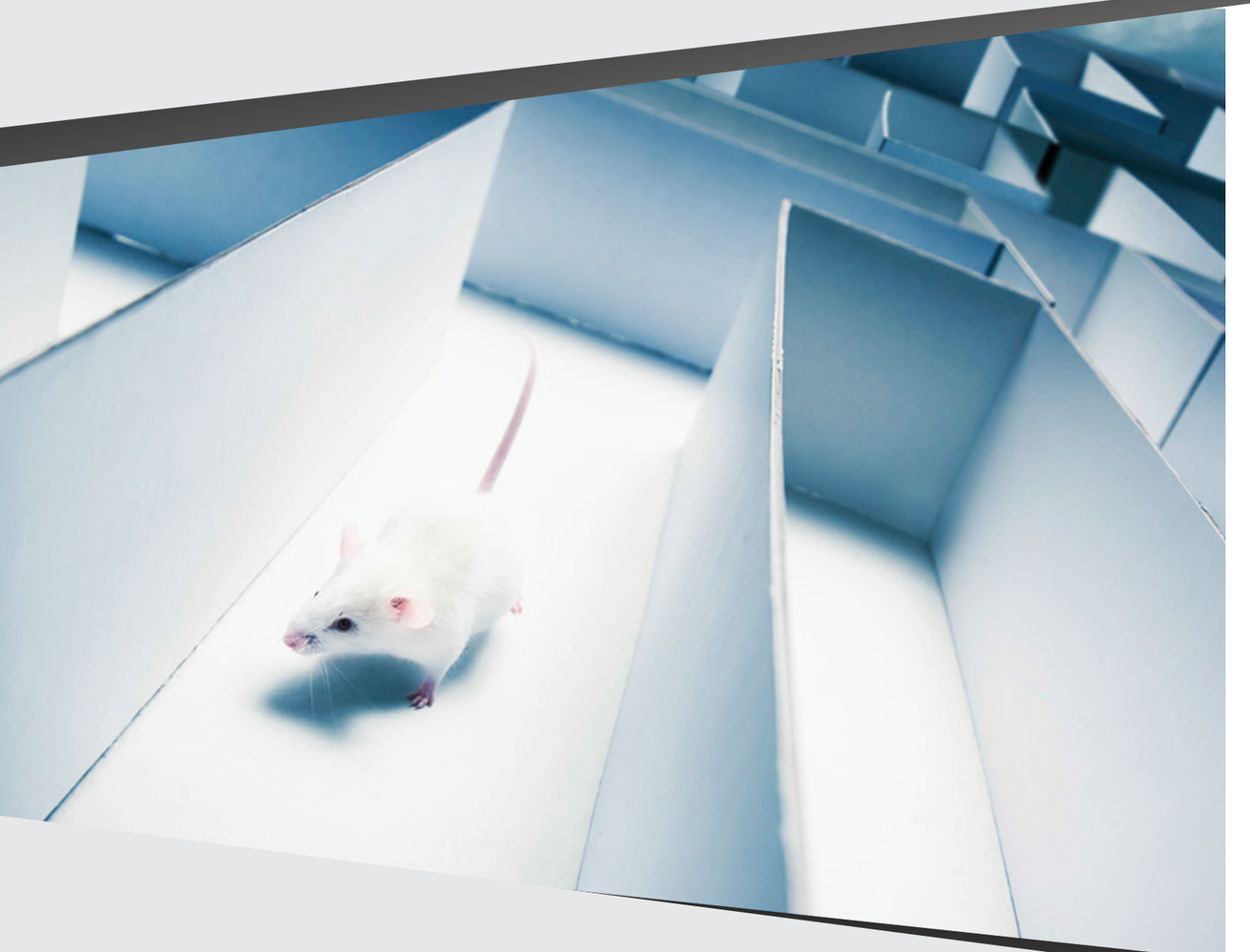
3D random-access point scanning extended by drifting the focal point along short 3D trajectories allows imaging without interruption at multiple dendritic branches. The sampling is continuous during the drift, so this scanning mode gives a more detailed spatial resolution without changing the overall scanning time: this remains as high as during the point scanning. As a result, the function of thin dendritic segments, or even spines and single action potentials can be revealed.
See more in Chiovini et al., Neuron, 2014.



Single-cell-iniciated, monosynaptically restricted, retrograde transsynaptic network tracing was performed with using rabies viruses expressing GCaMP6 sensor. The AO scanning allowed the simultaneous functional recording of the orientation selectivity of the postsynaptic neuron and all labelled presynaptic neurons in 3D. See more Wertz et al., Science, 2015.



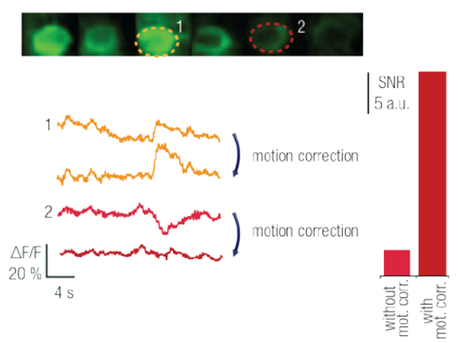
Applications/Femto3D-AO
Measurements in behaving animals



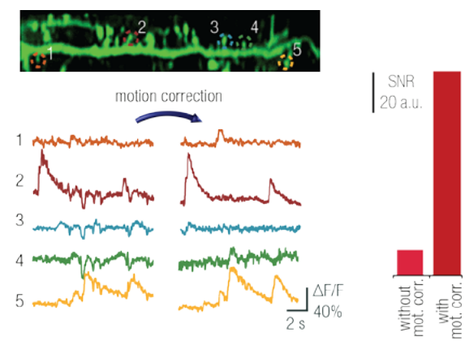
## Motion correction during behavior with Anti-mOtion technology

Our Anti-mOtion technology is an acousto-optic scanning method using drift scanning technology which has been successfully further developed for correcting those motions that appear during behavior. For scanning an enlarged area where fluorescent signals have been scattered due to motion, random-access points are extended to drifting lines (3D multiple-line) which are precisely fitted to each other by computer algorithms, resulting in surface or 3D volume elements. These elements cover not only the pre-selected ROIs but also the neighboring areas making it possible to preserve all fluorescent information during brain motion. This allows motion artifact and neuropil contamination to be eliminated. The implemented motion artifact elimination algorithm has been shown to increase the SNR by more than one order of magnitude in behaving animals. See also Szalay et al., Neuron, 2016.

Elimination of motion artifacts at somata



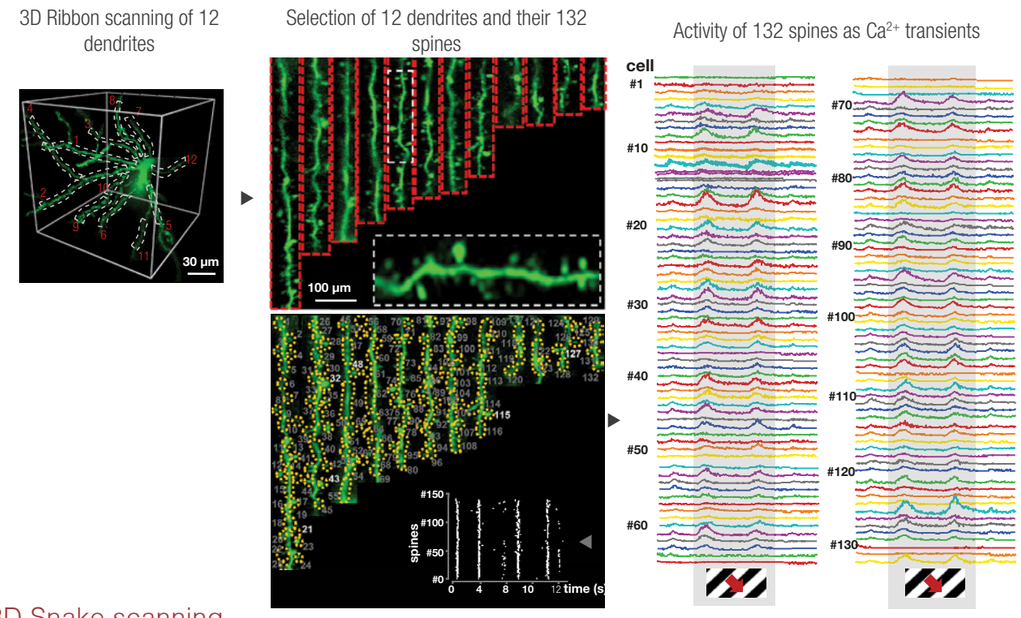

Elimination of motion artifacts at spines



## Imaging of dendrites and their spines in 3D in behaving animals

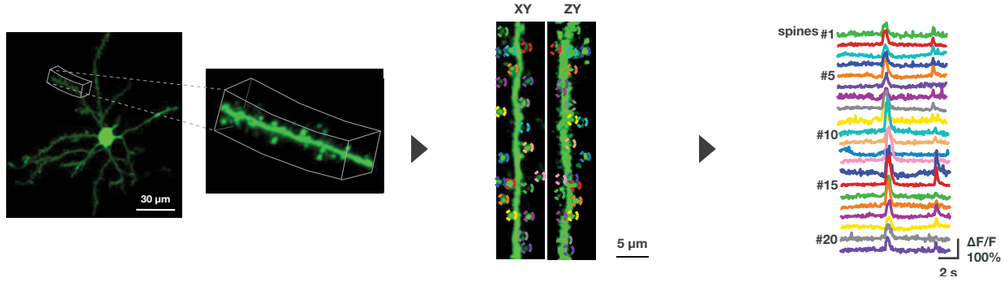
3D Ribbon scanning

Ribbon scanning is a surface extension of the 3D multiple-line scanning mode performed by Anti-mOtion technology. During imaging, the neighboring area around the trajectory is also captured by generating drifts either parallel or orthogonal to the trajectory. In this way, it is possible to follow the 3D curvature of one or more dendrites with their spines at the same time preserving fluorescent information during motion in behaving animals.




## 3D Snake scanning

3D snake scanning is a volume extension of ribbon scanning and contains the entire 3D environment of the dendrite. It therefore supports imaging of entire dendritic segments in larger animals, or during defined surgical or behavioral protocols, even when the amplitude of motion is very large. The figure shows fast snake scanning performed at 10 Hz in the selected dendritic region of a V1 pyramidal neuron. See also Szalay et al., Neuron, 2016.




3D Ribbon scanning of 12 dendrites

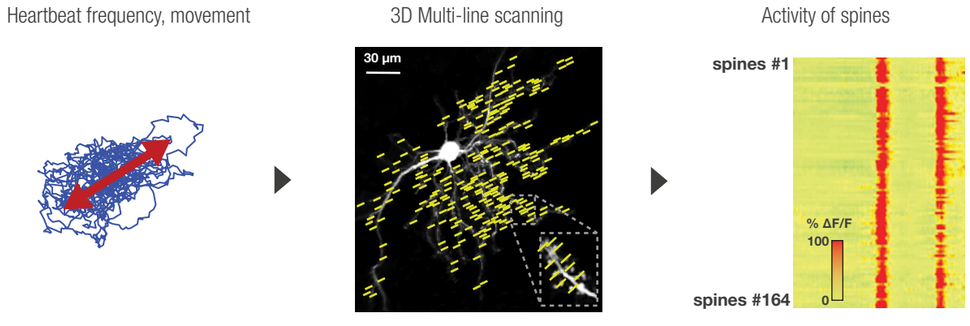
Projections of the dendrite after motion correction

Ca2+ responses from selected spines



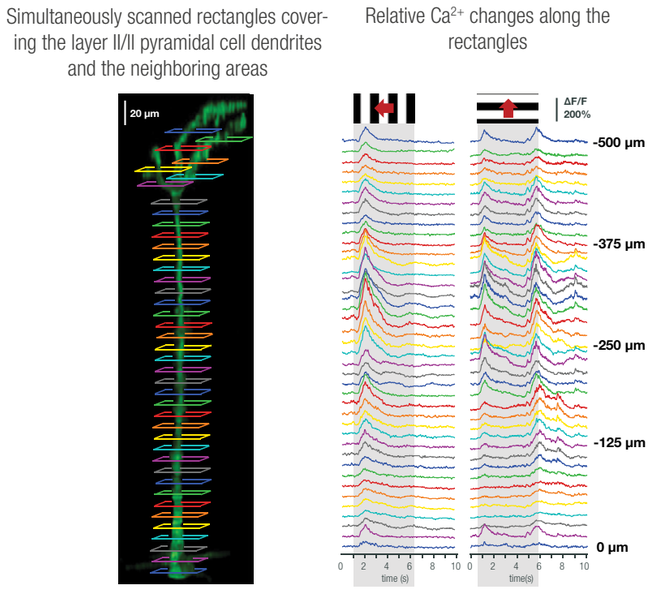
## 3D Multiple-line scanning

This method can be used for imaging over 100 spines in awake, behaving animals. In the figure, each scanning line is associated with one spine in a layer II/III pyramidal cell labeled with GCaMP6. The direction of the drift is set to be parallel to the average motion of the brain, which helps to maximally preserve fluorescence information to eliminate any motion artefacts. A total of 164 selected spines were examined simultaneously. See also Szalay et al., Neuron, 2016.




## 3D Multi-layer multi-frame scanning

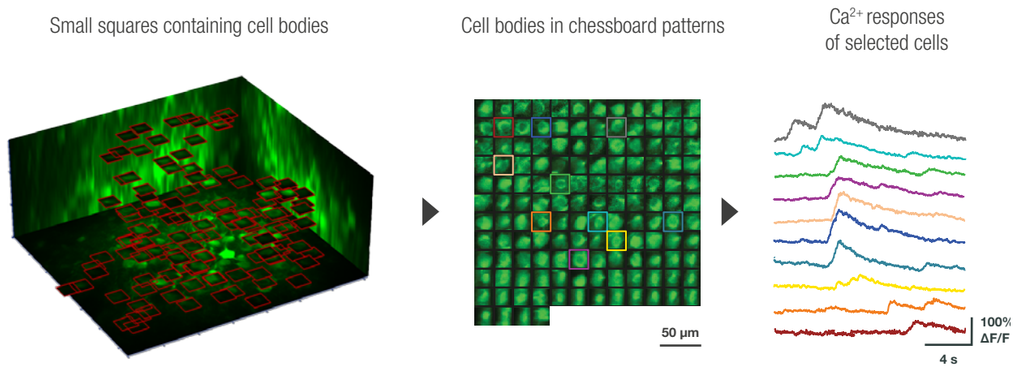
Imaging of multiple frames with different sizes and at any position in the scanning volume can be used to follow all events propagating along the cell. The figure shows imaging of the entire length of a pyramidal neuron, where the small scanned rectangles covering the areas around the cell enable us to record fluorescent signals and responses to visual stimuli. See also Szalay et al., Neuron, 2016.



## Imaging of neuron populations during behavior

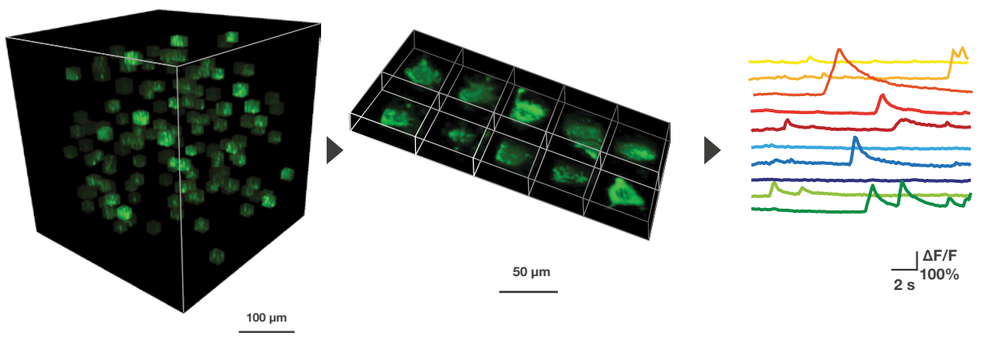
Chessboard scanning

Chessboard scanning is a planar extension of random-access point scanning, which extends scanning points localized in 3D to small squares by drifting the laser beam. The name, chessboard, is derived from the layout, which is generated by arranging all the squares side-by-side to get a chessboard like pattern containing the selected regions with somata and the surrounding areas. This pattern allows visualization of the somatal activity, handling and storing of the data and, importantly, correcting for motion to be carried out simultaneously.




## Multi-cube scanning

Multi-cube scanning is a spatially extended mode of chessboard scanning, where a Z dimension is added to the scanning squares to cover the entire volume of the somata. In this way, fluorescence information from somata is better preserved for motion correction.

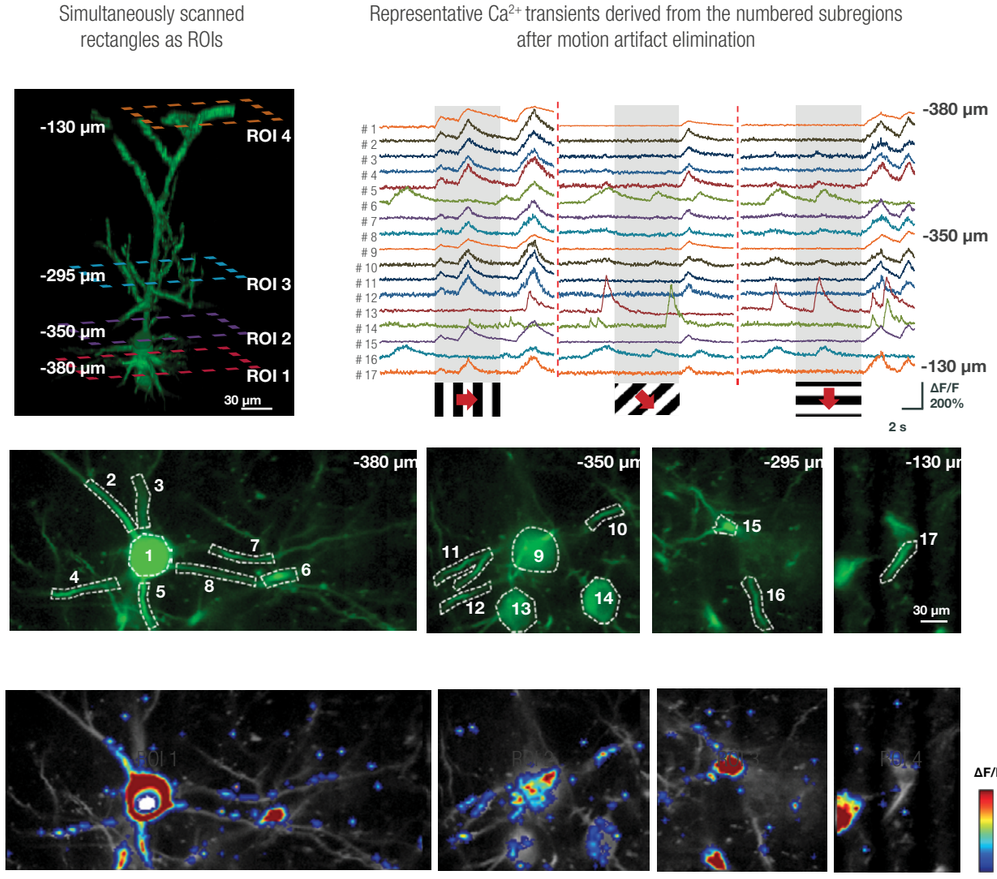



Small cubes containing cell bodies | Selected cubes containing cell bodies | Ca2+ responses of selected cells



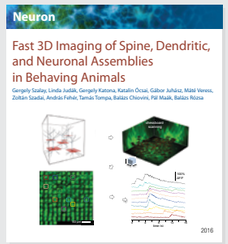
## Multi-layer, multi-frame scanning

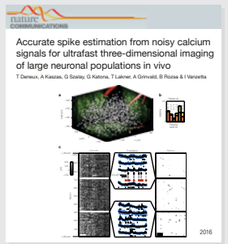
This scanning method allows not only dendrites, but also neuronal networks, to be imaged simultaneously at multiple planes faster than resonant scanning. The figure shows imaging of different specimens of a pyramidal neuron in vivo, where the small, scanned rectangles cover the apical dendrite across multiple layers. Motion compensation makes it possible to record fluorescent signals induced by responses to visual stimuli.

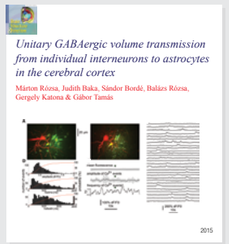


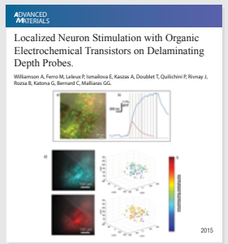

Offline subselection of the active parts of the cell from four different depths shown with color-coded relative Ca2+ changes

# Femto3D-AO/References



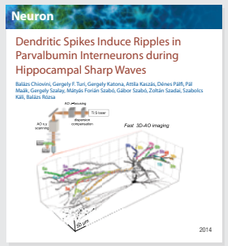











Szalay et al. Neuron, 2016
Deneux et al. Nat Commun, 2016
Rozsa et al. Brain Struct Funct, 2015
Williamson et al. Adv Mater, 2015
Boudhadfane et al. eLife, 2015
Wertz et al. Science, 2015
Chiovini et al. Neuron, 2014
Katona et al. Nat Meth, 2012


FEMTONICS
ADVANCED MICROSCOPY

# Femto3D-AO SCAN HEAD


Femto3D-AO SCAN HEAD

## The ultimate 3D scanning solution for your existing two-photon microscope

Extend your existing microscope to 3D imaging. The Femto3D-AO upgrade unit is a cost-effective solution which can easily be added to many existing microscopes.

### Key features

- Third generation acousto-optic scanner
- Involves all features of Femto3D-AcoustoOptic
- Wavelength tunable
- Available for a variety of microscopes

### The upgrade unit consists of

- acousto-optic scan head
- electronics
- measurement control and analysis software

The compact acousto-optic unit has the imaging parameters of the well-known Femto3D-AO microscope.

FEMTONICS
ADVANCED MICROSCOPY

# FemtoSmart

## Benefits

- Extremely large space under the objective
- For in vivo studies
- Field upgradability
- Patented imaging technologies
- Flexible scanning methods
- Maximal photon collection

## Features

- Elevated, column-based body
- XYZ positioning
- Tilting objective
- High level of modularity
- Galvanometric, resonant, or both scanning possibilities
- Travelling detector system
- 3P ready transmission range upon request

## Overview

The FemtoSmart series is the next step in Femtonics product evolution, fully customizable two-photon microscopes, which are primarily used in in vivo studies. Their special feature is the elevated body which can move in X, Y, and Z directions, providing ample room under the objective for optimal positioning of your sample. This feature makes them suitable for model organisms ranging from zebra fish larvae, through mice navigating in virtual reality to even non-human primates.

### Extreme positioning freedom

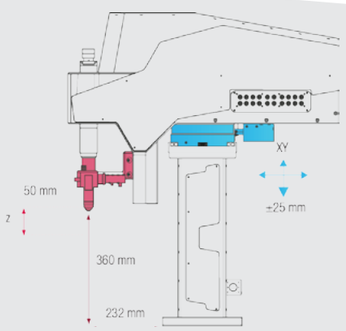
The column-based X-Y-Z moving body of the FemtoSmart houses the scanner unit, control circuits, and internal light path. The leg is 600 mm long and custom size is also available, and its footprint is only 250 mm × 250 mm; the distance between the objective and the surface is 360 mm. The design enables the objective to move in a 50 mm range in the Z-direction and the microscope body can also move in 50 mm range in the XY directions around the base. The Tilting objective, as an upgrade module, can further increase mobility.

### Superior imaging technology

The optomechanical design of FemtoSmart is based on the established technology of Femtonics. The fine-tuned optical construction allows imaging to a depth of 850 µm, and wavelength range from visible to the infrared regime allowing even 3p excitation. The high-quality scanners delivering the highest resolution currently available. Detection is performed by our patented travelling detector system, where the highest quality GaAsP PMTs and associated optical elements are mounted on the objective arm which helps to keep the photon collection ratio high.

### Modularity

The microscope's modular nature allows us to assemble the components, and recombine and upgrade the system to perfectly fit the customer's needs. Multiple lasers can be built into the microscope, either for imaging or photoactivation. Galvo and/or resonant scanner direct the beam. The detection is performed by multiple high-sensitivity GaAsP photomultipliers. The system can be equipped with a lot of optional modules enabling it to be adapted for a wide range of biological applications.






| 850 | 800×800 | 4.1 | 200 | 40 |
|---|---|---|---|---|
| µm deep | µm² FOV | fps 512×512 pixels 700×700 µm² | µs/point | lines/5 ms straight and curved lines |

## Overview

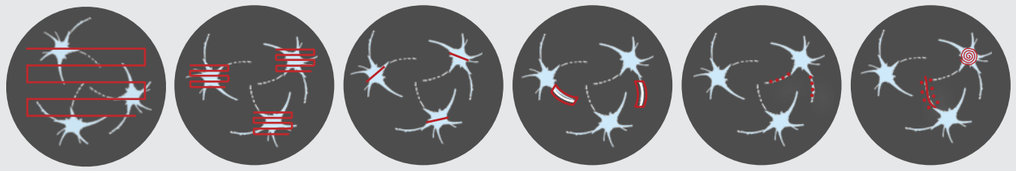
The FemtoS-Galvo is a galvanometric scanner-based two-photon microscope which enables in vivo and in vitro functional imaging to be focused on the region of interest (ROI). The scanner consists of two galvanometer-based motor-driven mirrors, meaning that the focal spot can be positioned where required. The high accuracy and this positioning freedom support flexible approaches for ROI creation. In contrast to sampling all pixels in images, ROI scanning (e.g. along a predefined line) restricts scanning to the regions which are relevant for the scientist, resulting in faster recording, and elimination of background noise. This helps to reveal cellular signaling and action potentials.

## Key features

- For in vivo functional imaging in deep tissues
- Each cell body, axon, dendrite and spine can be measured separately
- Flexible imaging modes, patented solutions for fast imaging only on the regions of interest
- High signal to noise ratio
- Intelligent control software

## Advanced 2D scanning methods

The advantages of the galvo scanner combined with the intelligent, user-friendly control software enables the user to use many scanning patterns covering the ROIs distributed across the field of view. These patterns have been developed based on the most frequent requests by neuroscientists. For example, multiple frame scanning focuses on cell bodies, multiple line scanning enables us to follow action potentials along dendrites, and random-access point scanning allows measurement or photostimulation of subcellular components of the highest temporal resolution. Many features of the software, such as real-time display, analysis functions, ΔF/F calculations and integrated parallel data acquisition of electrical recordings promote greater understanding of the physiological processes under the focus of your research.



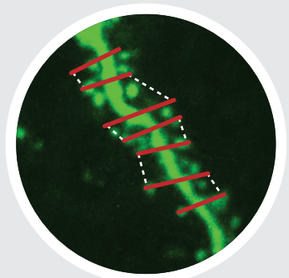
# FemtoS-Galvo/Technology

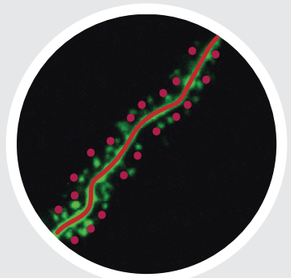
## Multiple line scanning

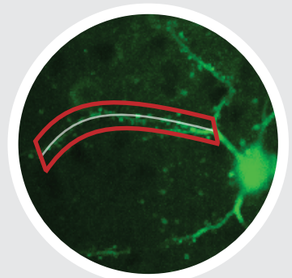
Multiple line scanning has been developed for researchers who want to resolve dendritic and even spine activity of neurons approaching near real-time measurement mode. During this scanning mode, the X and Y mirrors direct the laser beam flexible along straight lines or complex curves. The scanner spends most of its time collecting signals from these lines, while the intermediate sections between the lines are skipped. In this way, the scanning speed and the signal-to-noise ratio (SNR) of the signals sampled from the multi-site ROIs increases 3- to 4-fold compared to frame scanning.

## Photostimulus patterns

Uncaging, optogenetics, and other photostimulation techniques are also supported by our unique scanning patterns and their combinations. Random-access point scanning can be used for stimulation in femtoliter volumes near dendritic spines where the duration of the stimulation, can be set from microseconds to seconds precisely to the experiment. The evoked signals can be followed along the dendrite by line scanning near simultaneously with the photostimulation. The microsecond-scale switching time between the stimulation and imaging is achieved by using of a Pockels cell and gated detectors.







## Folded frame scanning

This patented method enables imaging of a confined area along a line, where the shape of the selected regions can be straight or curved. This advanced scanning method is useful for imaging single cell bodies in different regions of the specimen, or following events along winding dendrites with their protrusions, even while the tissue is moving.

## High signal-to-noise ratio

Subtle changes in the evoked signals can be revealed because of the following features:

- scanning only the relevant part of the field-of-view, and skipping the background, result in a very high signal-to-noise ratio,
- photon collection efficiency is enhanced thanks to our patented travelling detector system, which uses the shortest possible optical path,
- the most sensitive GaAsP photomultipliers available (quantum efficiency >40%) collect scattered photons.

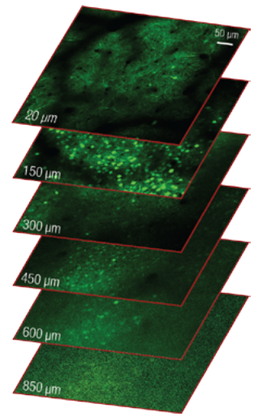
## Specification

- in vivo deep brain imaging down to 850 µm
- 800 µm × 800 µm FOV (with a 20x obj.)
- 2D scanning modes
  - point and random access point with 200 µs/point
  - free hand line and multiple line: 40 lines/5 ms
  - frame with 4.1 fps at 512 x 512 pixel, 750 µm x 750 µm
  - folded frame, multiple folded frame
- pixel dwell time adjustable: 0.5 µs - 10 ms, pixel-based averaging
- minimized optical path length by patented travelling detector system
- non-descanned, ultrasensitive GaAsP PMT (>40% quantum efficiency)
- high signal-to-noise ratio
- simultaneous detection of multiple wavelength
- custom-designed optical elements for maximal transmission efficiency
- MATLAB-based control software with analysis and upgrade possibilities
- ΔF/F, ΔG/R calculation
- parallel recording and analysis of electrophysiological data
- CMOS camera
- compatibility with extended IR wavelength range

# Applications/FemtoS-Galvo

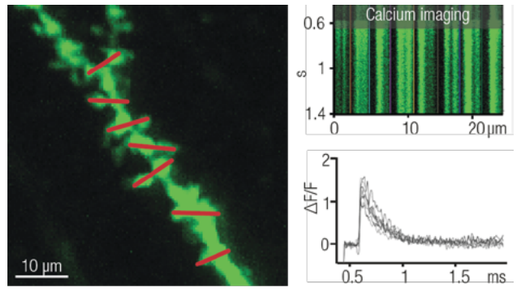
## Deep brain imaging

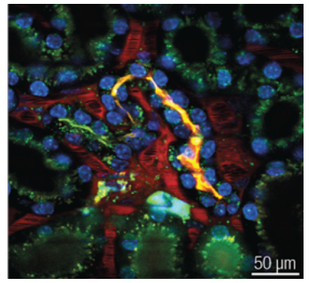
Thanks to two-photon laser technology and our optical developments, you can study cell bodies and dendrites at a high spatial resolution down to a depth of 850 µm with no photodamage. The 3D slicer module of the control software implements XZ or YZ sectioning and projection of the Z-stacks and enables 2D visualization of 3D stacks projected to any of the three axes.

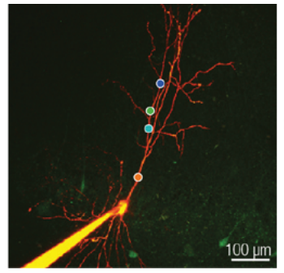


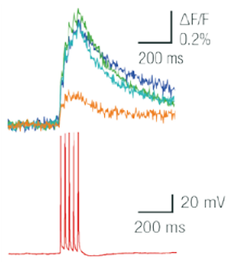

## Calcium imaging

The fast scanning speed on user-defined, separated regions ensures precise and repeatable measurements of rapid changes in the $Ca^{2+}$-level of neurons and their dendrites. The ratio-imaging software tool offers algorithms for eliminating background noise, and determinates the relative fluorescence changes, displaying them as transient curves as a function of time. The batch analysis tool contains efficient tools for analyzing entire multi-ROI measurement sets conveniently; it also implements the grouped analysis of imaging data at multiple regions.



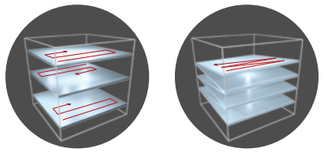

## Intravital imaging

The infrared excitation laser can penetrate thick specimens, enabling living cell behavior in intact tissues and organs to be visualized at high resolution for extended periods with no phototoxicity: essential for morphological studies. The metaprotocol module of the control software automates these measurements on a timescale of seconds by letting you run freely composed sequences of image acquisition. Figure shows renal morphology (red: blood vessels, green: tubule walls, blue: nuclei, yellow: tubular cavities).

## Parallel electrophysiology

Hardware and software tools help parallel two-photon imaging and aligned electrophysiological recordings. Precise triggering the external recording ensures time aligned measurement of the electrophysiology and imaging, providing different aspects for studying the neuronal cell and network activity. Software module helps automatic importing recorded traces for parallel analysis. Figure shows calcium imaging and patch-clamp recording in an OGB-1 and Alexa-594 filled hippocampal neuron.

# FemtoS-Resonant/Technology



## Overview

In the FemtoS-Resonant microscope, Femtonics combines high-speed and high-sensitivity imaging of living tissues by using a fast resonant scanner. Resonant-scanner-based raster scanning acquires images of the entire field-of-view ~5 times faster than galvanometric-based scanning: it is therefore the most appropriate choice for imaging the entire field-of-view at high frame rate.

## Key features

- For in vivo high-speed functional imaging in deep tissues
- Rapid image acquisition
- Long-term measurements
- Time-lapse imaging
- 3D volume scanning upgrade
- Intelligent control software

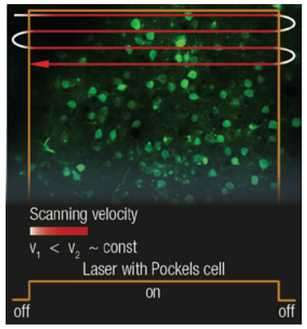
## Uninterrupted high-speed imaging

The resonant scanner consists of a fast oscillating mirror for x-axis deflection and a galvanometric mirror for y-axis sweep. Thanks to the 8 kHz oscillating speed of the fast x mirror, the microscope is capable of gathering images at 31 frames per second for hours.

| 850 | 600×600 | 31 | 500 |
|---|---|---|---|
| µm deep | µm² FOV | fps 512×512 pixels 600×600 µm² | fps 32×512 pixels |

## High scanning rate with no image distortion

The velocity of the resonant scanner is non-linear: the speed is different in the center and at the edges of the frame. In the microscope, Pockels cell limits the scanning range to that portion where the scanning velocity is near linear, avoiding photobleaching/photodamage at the two sides of the image. Scan electronics performs dynamic pixel dwelling for data linearization and to cancel out image distortion.




## Specification

- in vivo deep brain imaging down to 850 µm
- 600 µm × 600 µm FOV (with a 20x obj.)
- scanning modes
  - ► raster scanning with 31 fps at 512x512 pixels
  - ► and 500 fps at 512x32 pixels
  - ► 16 000 lines/sec (straight, parallel with x-axis)
  - ► 3D volume scanning with 3 Hz by Piezo objective positioner
- dynamic pixel dwell time to avoid image distorsion
- minimized optical path length by patented travelling detector system
- non-descanned, ultrasensitive GaAsP PMT (>40% quantum efficiency)

- high signal-to-noise ratio
- simultaneous detection of multiple wavelength
- custom-designed optical elements for maximal transmission efficiency
- C++-based control software with analysis and upgrade possibilities
- dedicated software efficiently handling large datasets
- nearly unlimited measurement times
- ΔF/F calculation
- parallel recording and analysis of electrophysiological data
- CMOS camera
- compatibility with extended IR wavelength range

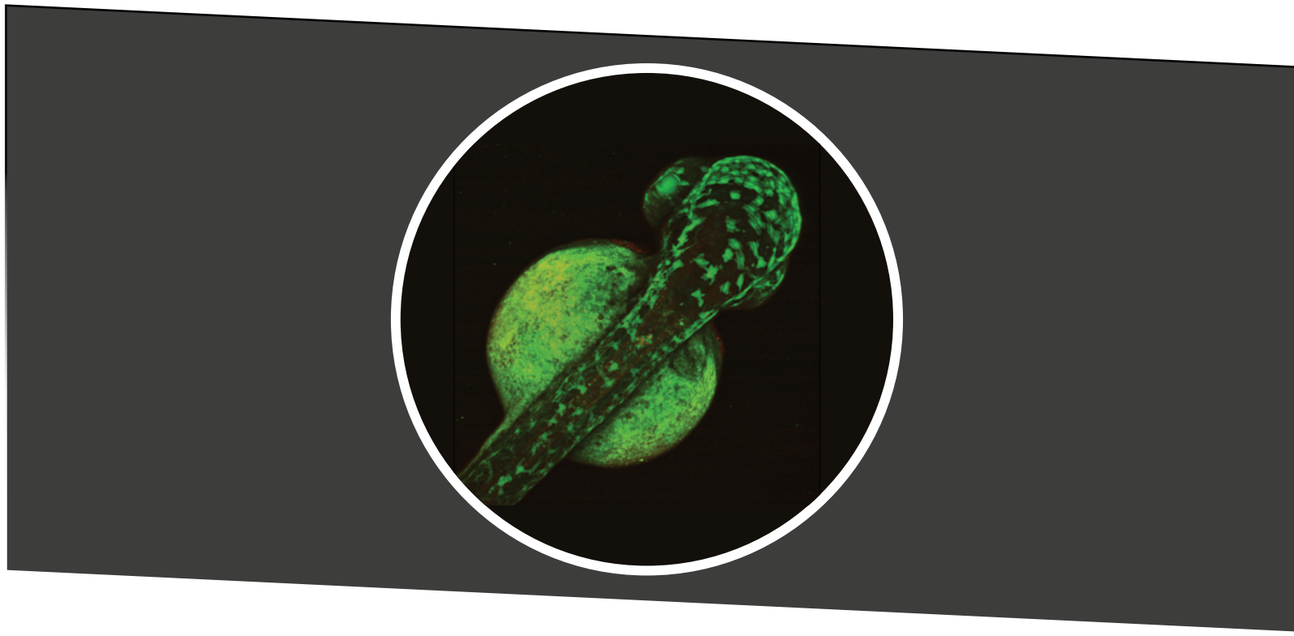
# Applications/FemtoS-Resonant

## 3D volume scanning

The fast XY-scanning, combined with fast Z-movement, ensures near real-time measurement of a 3D volume which enables us to study activity changes in 3D cellular networks or the morphology of organs. The fast Z movement can be performed by a Piezo objective positioner or a Liquid lens objective.

## Time-lapse imaging

While two-photon excitation ensures deep penetration and fine spatial resolution, the high frame scanning rate of the resonant scanner provides high temporal resolution. This feature means that the microscope is suitable for measuring rapid events in living cells, neuronal networks, or other circuits.



## Long-term measurements

The high frame scanning rate and the unlimited video streaming combined with the possibility of automated measurement, support long-term studies such as following learning processes, memory retrieval, associative learning, development of model organisms, etc. The middle figure shows an early stage of the development of a zebrafish embryo, whose ontogenesis was followed and recorded over a day from the low cell state.

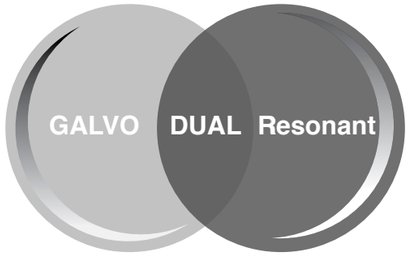
## Photostimulation

Full-field illumination from an LED light source enables cell populations to be stimulated in different locations of a specimen while the resonant scanner simultaneously images the evoked signals. The selective stimulation of the cells can be ensured by targeted expression of photosensitive molecules such as channelrhodopsin or halorhodopsin (optogenetics).

# FemtoS-Dual

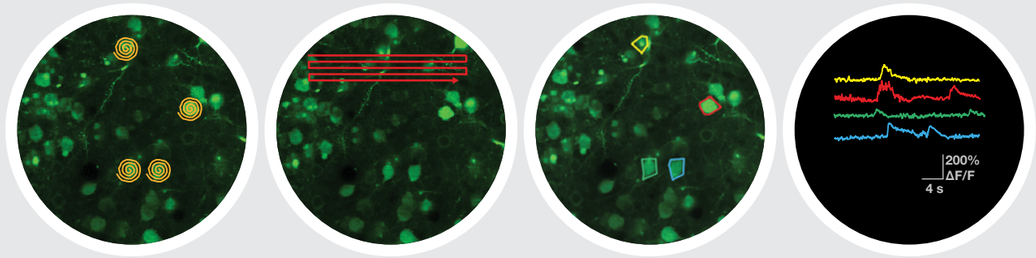
## Overview

The FemtoS-Dual microscope contains both galvanometric and resonant scanners, providing all advantages of the two types of imaging technique. For example, follow activity on a large field of view using resonant scanner, then zoom to selected ROIs or finish with a high-resolution z-stack using the galvo scanner.




## Photostimulation and imaging

Using the two scanners function in tandem is a perfect solution for photostimulation. The galvanometric scanner directs the laser to cells or subcellular components selectively. The selectivity is established by scanning along arbitrary line patterns placed on cell bodies or dendritic segments. With the resonant scanner, you can follow the changes in the cells of a neural network collecting imaging data simultaneously by high-speed frame scanning of the surrounding area. Timing of photostimulation and gating of the detectors are controlled by protocols designed on user-friendly GUI.




Stimulation along ROIs using spiral scanning pattern with galvo scanner on ChR2 expressing neurons

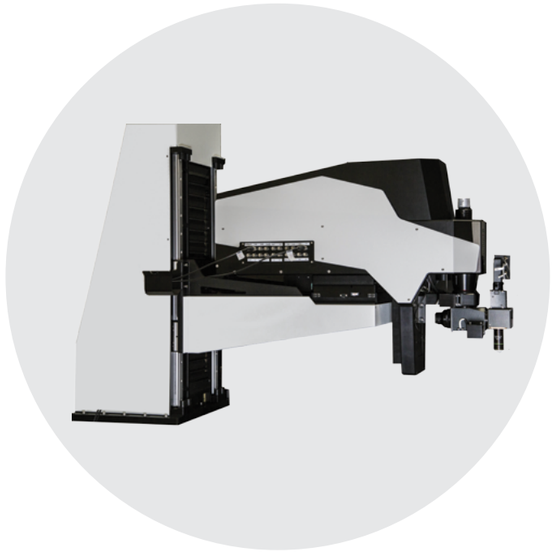
Full field imaging by resonant scanner

Offline ROI selection

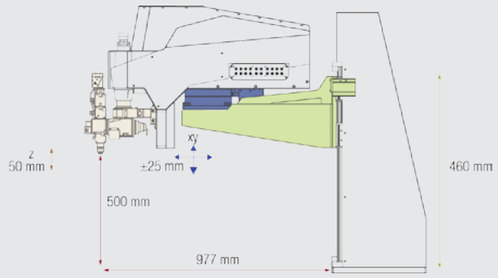
Calcium responses from the selected ROIs

# FemtoS-Bridge

The FemtoS-Bridge is a special edition of the FemtoSmart microscope which is designed to provide extreme freedom in positioning of the body. It involves all the advantages of the FemtoSmart series but it has been extended, making it particularly beneficial to studies which need a very large space for the sample or accessories by enabling functional brain imaging in head-fixed mice navigating in a large mobile cage.



## Unique features



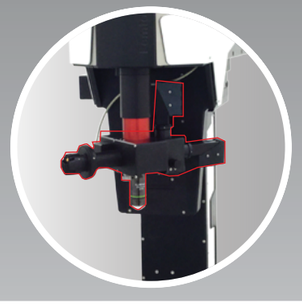

The foot is replaced with a lifting apparatus which moves the head vertically.

- Moving range: 50 cm
- Coarse Z adjustment: 1 mm
- Fine adjustments:
  - by the objective holding arm in a 50 mm range of the Z direction,
  - Piezo objective positioner for moving the objective with fast Z positioning in a range of a few hundred micrometers,
  - an XY actuator moves the body relative to the column in the X and Y directions (±25 mm range, step size: 1 µm),
  - our Tilting objective further increases accessibility to the lateral parts of the brain.

# FemtoSmart Product line/Optional modules

## Vessel pattern visualization

Green illumination



Green illumination from an LED light source allows high-contrast visualization of blood vessels, helping to navigate on the surface of any organs under in vivo conditions and position a patch pipette for bulk loading or patch clamping.

### Features

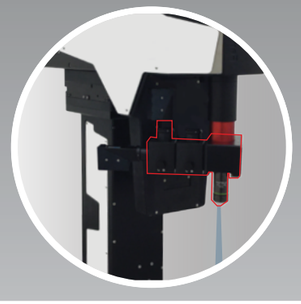
- excitation wavelength at 510-540 nm
- ~100 µm penetration depth
- 3 sec switching time between camera and two-photon modes
- detection by CMOS camera
- adjustable brightness, contrast and gamma parameters
- equipped on the objective arm
- built-in PMT protection during operation

### Benefits

- high-contrast visualization
- precise overlap in XYZ between camera and two-photon recorded field of view
- locate your chronic imaging spot within the craniotomy

## Full field photostimulation

LED light source



Full-field illumination using a selected LED source allows molecules and cells to be stimulated over the whole FOV homogeneously. Combine this module with gated detectors to achieve millisecond switching between stimulation and imaging.

### Features

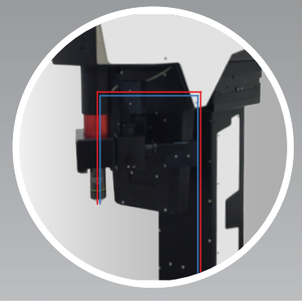
- VIS light stimulation by LED
- typical wavelengths: 430, 450, 480, 590 nm, others on special request
- precisely timed and highly repeatable impulses
- ~200 µm penetration depth
- PMT protection during the stimulation by built-in gating system
- software controlled
- equipped on the objective arm

### Benefits

- simultaneous stimulation over the entire FOV
- optimized for optogenetics studies: precise spatio-temporal activation of ChR2 and/or NpHR
- millisecond switching between stimulation and detection

## Uncaging/Optogenetics

Multiple beam path



The optomechanical design of the light path enables us to direct more beams into the microscope, utilizing the same light path. We offer secondary, fine-tuned laser sources for a wide range of biophotonics applications.

### Features

- additional IR or CW laser(s) coupled to the existing light path
- light path optimized for all wavelengths
- full optical engineering

### Benefits

- photostimulation with IR or visible light
- flexible stimulation patterns supporting stimulation of selected regions
- two-photon uncaging studies
- visible light optogenetics:
- ChR2 activation with 473 nm laser
- NpHR activation with 561 nm laser

# Optional modules/FemtoSmart Product line

## Free rotation of the objective

Tilting objective

The motorized tilting module rotates the objective, giving a higher level of freedom to reach the sample from different angles. The module also includes a Piezo objective positioner, ensuring additional movement of the objective in the Z direction.

### Features

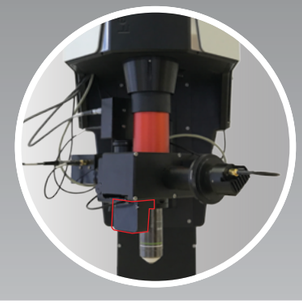
- 180° rotation around the horizontal axis
- 100° rotation around the vertical axis
- speed of rotation: 4°/sec
- unidirectional repeatability:
- < 0.02 mrad (~2 µm)
- Z movement with piezo:
- up to 400 µm

### Benefits

- flexible objective positioning with high precision
- highly stable in all positions
- minimized optical path to the detectors fixed on the tilting unit
- useful for in vivo experiments, intravital imaging, and deep brain imaging in rodents or even non-human primates

## Fast Z-stack and 3D imaging

Piezo objective positioner



A Piezo objective positioner enables the microscope to change the focal point by mechanically moving the objective. With this module the microscope is able to collect signals from different depths with up to 200 Hz, resolving activity in 3D samples.

### Features

- positions objectives in the Z direction with nanometer resolution
- travelling range up to 400 µm
- up to 200 Hz frequency in resonant mode
- millisecond step-and-settle
- any types of objectives can be attached
- no influence on the optical path

### Benefits

- 3D trajectory scanning using galvo scanner is able to collect signals from dendrites arbored in the 3D tissue fast enough to resolve biological activity
- 3D volume scanning using resonant or galvo scanners is able to collect signals from a 3D volume, revealing activity of the neural network or other cell groups
- fine positioning along the tilted axis when using tilting objective module

## Fast 3D imaging

Liquid lens objective

The Liquid lens objective contains a carefully chosen objective and a fast-focusing element which utilizes electrically controlled shape-changing membrane. This flexible membrane enables the objective to switch quickly between focal planes.

### Features

- focuses with nanometer precision
- 200 µm focal range
- 10 ms focal-plane switching time
- fast settling time
- transmission 90% at 800 nm
- aperture 10 mm
- comes with a 40× magnification Nikon objective
- no mechanical perturbation

### Benefits

- fast switch between the focal planes
- 3D volume scanning: is able to collect signals from a 3D volume, revealing activity of the neural network or other cell groups
- imaging at multiple tissue depths near simultaneously

# Femto2D

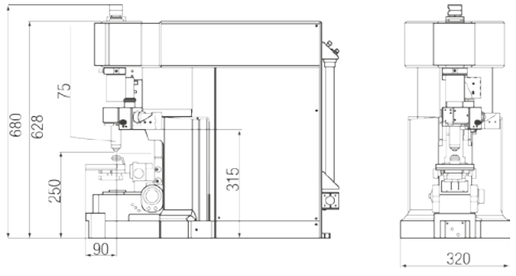
# Femto2D Product line

## Overview

Femto2D product line involves our standard, thoroughly field-tested two-photon microscopes. The different models encompass modular galvanometric and resonant based two-photon microscopes while many special extensions have been developed for supporting both in vitro and in vivo measurements.

## Classical microscope design with possibility for in vitro studies

Members of Femto2D series have highly stable frame which can be equipped with many generic extensions. Femto2D microscopes can be extended with a module for confocal imaging, holographic illumination, epifluorescent illumination, time correlated photon counting, furthermore with extensions of the FemtoS product line. All of the previously demonstrated in vivo imaging capability is implemented to these systems, while the in vitro extension can house PMTs for transmitted fluorescent detection. Scanning modes and optical properties are the same as in the corresponding FemtoS members.




### Femto2D-Galvo

All galvanometric based scanning modes and excellent optical properties of the FemtoS-Galvo had been established in this microscope. High accuracy and positioning freedom of the scanner are combined with the intelligent control software supporting flexible approaches for ROI creation.

### Femto2D-Resonant

The Femto2D-Resonant is the twin of the FemtoS-Resonant. Femtonics combines the high speed and high sensitivity imaging of living tissues in this scope, where the imaging speed reaches the 31 frames per second. The optomechanical quality is ensured by the established technology.

### Femto2D-Dual

The Femto2D-Dual microscope gives the ability to perform dual scanning using both galvo and resonant scanners in tandem. With galvo scanner you can zoom to tiny structures such as dendritic spines and jump quickly between these regions. In contrast, with resonant scanner you can capture images with high frame rate to follow rapid changes on the FOV.

# Specialized microscopes

The special feature of our company is that we manufacture unique, custom made microscopes based on client request. These microscopes are equipped with the newest techniques and patented solutions in the field of nonlinear scanning microscopy and ready to satisfy the specific needs.

## Spirit

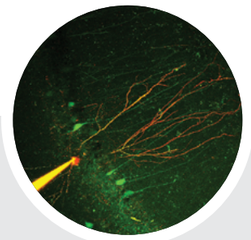
Spirit is designed for researchers who need to complement the two-photon microscopy research with confocal and FLIM imaging. Now, Spirit is working as a partner in molecular cellular studies, and studies of neuronal communication systems at synapses.

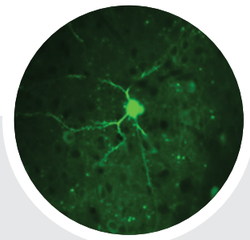
- Femto2D-Galvo microscope
- Confocal unit with three laser beams
- FLIM single photon counting system with hybrid detectors
- In vitro extension

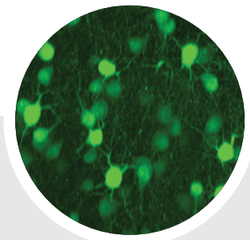
## Curiosity

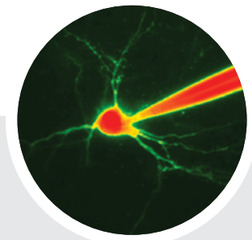
Curiosity has been developed primarily for optogenetics studies. The stimulation along various patterns in 3D is established by a SLM-based holographic system. Curiosity has reinforced a team who studies plasticity of cortical microcircuits in the living brain.

- Femto2D-Dual microscope
- Piezo objective positioner for volume scanning
- Holographic illumination
- Tilting objective
- In vitro extension









## Opportunity

Opportunity is designed to stimulate synthesized neurotransmitters by IR laser and follow their effect by fluorescence lifetime imaging (FLIM). Nowadays, Opportunity is contributing in studies of cellular mechanisms of epilepsy and other neurodegenerative diseases.

- Femto2D-Galvo microscope
- Multiple beam path extension for uncaging with IR laser
- Piezo objective positioner for volume scanning
- FLIM single photon counting system with hybrid detectors
- In vitro extension

## Stimulus

Stimulus is a fully equipped microscope specialized for photostimulation. It is capable of stimulating precisely along various patterns and imaging with high frame rate, thanks for the galvo and resonant scanners in dual configuration. Currently, Stimulus helps researchers performing uncaging and optogenetics studies.

- Femto2D-Dual microscope
- Multiple beam path extension for uncaging with IR laser
- Multiple beam path extension for optogenetics wtih 477 and 561 nm lasers for ChR2 and NpHR activation

## Multicolor full-field illumination

Epifluorescent Unit

Wavelength-specific full-field excitation is performed by powerful LED light sources built in a rotating unit above the objective. Fluorescent imaging can be achieved with a wide variety of scientific cameras.

### Features

- LEDs for customizable wavelentghs (430, 450, 480, 530, 590 nm, others on special request)
- wavelength-specific filters for excitation of narrow ranges
- additional emission filters for blocking the reflected light and filtering the emission spectra
- up to 5 sets of exchangeable filter cubes
- 100 µm penetration depth
- detection by CMOS camera
- installed into the light path above the objective

### Benefits

- homogenous excitation of the entire FOV in 100 µm depth
- excitation specified for GFP, YFP, RFP, mCherry, etc.
- cleared emission for bright signals

## 3D photostimulation

Holographic illumination

The holographic stimulation is an extremely flexible method which produces simultaneous illumination in variable shape and size of multiple regions in the three-dimensional sample by using diffractive spatial light modulator (SLM).

### Features

- 3D pattern activation in a 350×400×400 $\mu m^3$ stimulating volume
- simultaneous excitation in several depths with many ROIs by using SLM
- ROIs are defined as polygons with arbitrary shape and size
- high temporal resolution of the activation
- lateral resolution less than 3 µm, axial resolution less than 10 µm (with 20x objective at 1040 nm)
- wide laser wavelength range from the visible to the infrared regime

### Benefits

- photostimulation of neurons by activating light sensitive ion channels such as ChR2 or NpHR
- large illumination region allows activation of sufficient number of photoactive molecules

## Fluorescent Lifetime Imaging

FLIM extension

Time correlated single photon counting and the derived fluorescent lifetime imaging measures the time delay between each emitted photon and the laser pulse eliciting it. This time is not affected by fluorophore concentration and excitation intensity fluctuations, however provides intimate information about molecular interactions and dynamics.

### Features

- high efficiency hybrid GaAsP PMTs for photon counting
- no afterpulsing
- multiple channels for two separated wavelengths
- non-descanned design for high photon collection rate
- minimized optical path length by travelling detector system
- sub-picosecond temporal resolution
- parallel conventional integrating and photon counting modes

### Benefits

- absolute ion concentration measurement
- characterization of bio-physical properties

### Related article

Zheng et al, Neuron, 2015



## Confocal imaging

2 in1 solution: two-photon microscope with confocal mode: the confocal unit of Femtonics is designed for researchers who need to extend their two-photon microscopy research also with confocal imaging.

### Features

- stimulation with up to four laser lines
- available wavelengths: 405, 473, 488, 561, 640 nm
- high efficiency specific filters
- motorized pinhole wheel with four different pinhole sizes
- detection with up to three GaAsP PMTs
- simultaneous detection of three different wavelengths

### Benefits

- confocal imaging in living tissues
- multicolor imaging
- bright imaging with high SNR
- FRET

## In vitro extension

Imaging of acute brain slices or cultured tissues makes possible to study cells in a controlled environment outside of a living organism. Gradient contrast illumination eases camera guided patch-clamping while transmitted fluorescence detectors enhance signal collection and SNR.

### Features

- high sensitivity GaAsP PMTs for transmitted green and red fluorescence
- gradient contrast illumination with LED source operating at 840 nm
- oil condenser NA 1.4
- high quality cell visualization

### Benefits

- gradient contrast illumination helps to navigate in the sample
- patch-clamping and various electrophysiological studies
- transmitted detectors increase the SNR of fluorescence detection

## Dual perfusion chamber kit

The slice chamber is designed to maintain isolated, living tissues in healthy state during experiments. In contrast with the single perfusion, dual-superfusion of submerged slices increases the vitality of cells, since oxygen as well as other materials can freely and effectively diffuse with high flow rate from both sides into the thick slices.

### Features

- up to 800 µm thin slices
- kit contains:
  - unique dual perfusion
  - polycarbonate chamber
  - slice holder
  - bubble trap
  - dual-channel in-line solution heater
  - temperature controller
  - peristaltic pump

### Benefits

- increased oxygenation
- maintain acute slice preparations up to 800 µm thickness
- preserved physiological network oscillations during neurophysiological studies

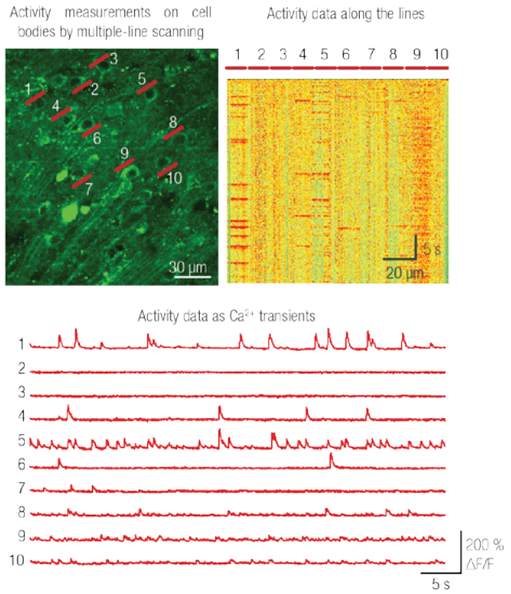
### Related article

Kerekes et al, Neurophotonics, 2014

# Applications

# Network Imaging

All sensations and behaviors are encoded in the dynamic activity patterns of neural networks. In other words, complex networks of many individual neurons respond to environmental features, visual or auditory stimuli, reward or punishment, etc. Neural networks extend over 3D space and, in most cases, cross many cortical layers of the brain. Two-photon microscopy and many new scanning methods have made it possible for neuroscientists to reach the deep regions of the brain (down to 850 µm) at a high spatiotemporal resolution, and study with optical methods the function of neuronal populations containing hundreds or even up to a few thousand identified cells in 3D.

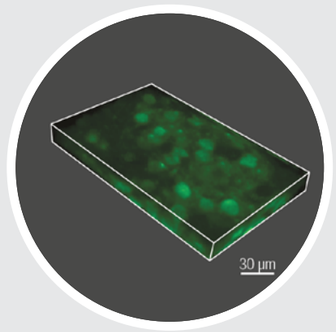



## Imaging of somata as ROIs in 2D
FemtoS-Galvo

The FemtoS-Galvo, with its flexible scanning patterns such as 2D random-access point scanning, 2D multiple-line scanning, and folded-frame scanning, supports the manual selection of individual cells in a 2D plane. By skipping measurement of the entire field, it is possible to maintain a high signal-to-noise ratio (SNR).
Using the TravellingSalesman software module, it is possible to determine the shortest pathway visiting defined points arbitrarily dispersed on the field of view. The short round-trip time results in a high measurement repetition speed, up to 100 Hz for about 30 cells.

## Fast scanning of the entire FOV or an entire volume
FemtoS-Resonant equipped with Piezo objective positioner

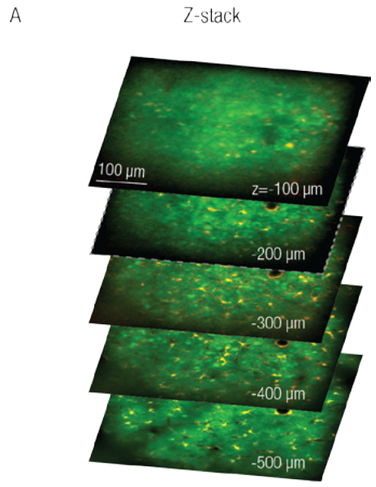
Fast-frame scanning based on a resonant scanner combined with fast Z-focusing performed by a Piezo objective positioner, is a well-known approach for studying three-dimensional neural networks. In this case, the entire field of view is imaged continuously by the fast scanner, while the objective positioner moves between planes. Different (cortical) layers might be recorded simultaneously this way, or the frames may be assembled to volumes resulting in a four-dimensional dataset.

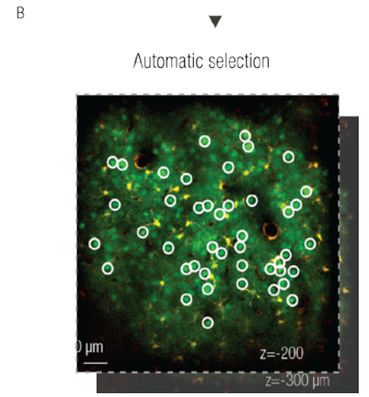


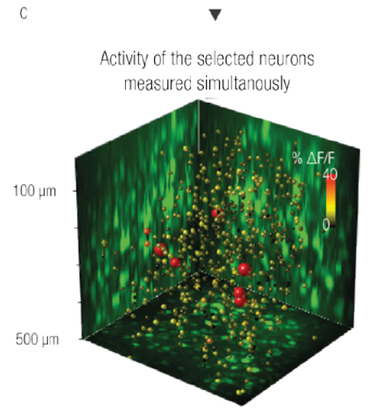

Network Imaging

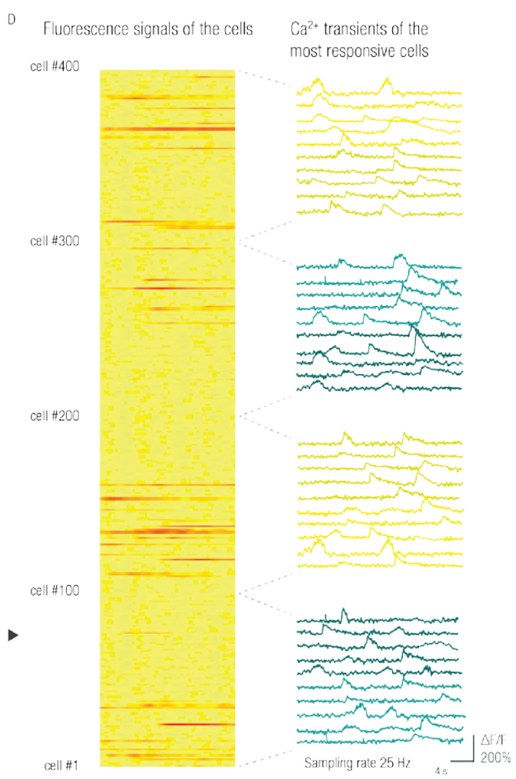
## The fastest imaging of cell populations in 3D
Femto3D-AcoustoOptic

The Femto3D-AO enables the spatial and temporal complexity of neuronal coding to be resolved by scanning distributed points as regions of interest (ROIs) in a large 3D volume. The Femto3D-AO is the fastest scanner available. Several thousand cells can be measured near-simultaneously by 3D random-access point scanning by restricting the imaging to sub-regions of the 3D volume. The Cell3DFinder software module finds cell centers in 3D image stacks automatically and can display them as a set of points. The figure (A-D) shows the changes in $Ca^{2+}$ concentration of 2000 cells from the visual cortex of a GCaMP-expressing mouse as a function of time.
See more Wertz et al., Science, 2015;
Katona et al., Nat Meth, 2012.



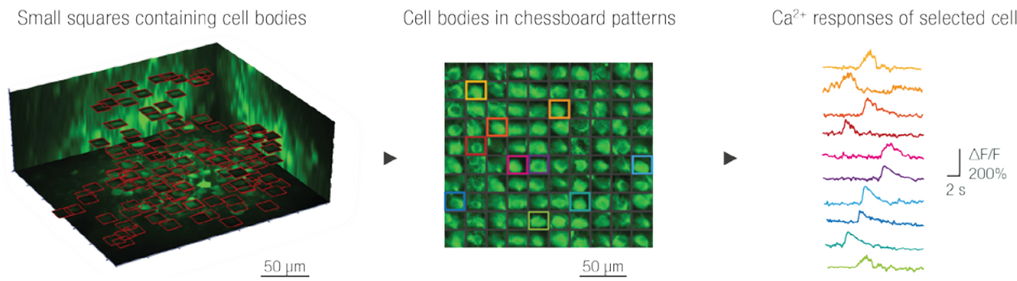



# Network Imaging

## Imaging of multiple somata distributed in 3D in behaving animals

Femto3D-AcoustoOptic

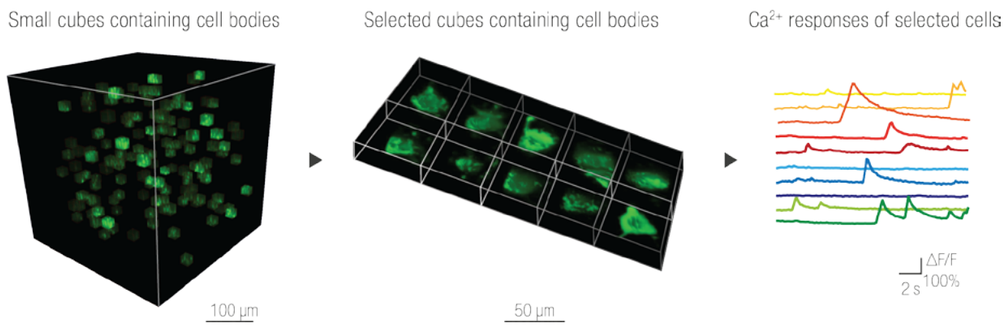
Chessboard scanning is a planar extension of random-access point scanning using the Anti-mOtion technology, where random-access points are extended to small squares by drifting the laser beam. These squares can be located anywhere in a near cubic millimeter volume, and include the somata with the surrounding area. Up to 300 somata can be measured simultaneously. The chessboard arrangement of the squares helps to visualize and analyze soma activity, and correct for motion artefacts. The figure shows in vivo neuronal activity, $Ca^{2+}$ transients from 100 neuronal somata from the mouse V1 region, labeled with GCaMP6. See also Szalay et al., Neuron, 2016.

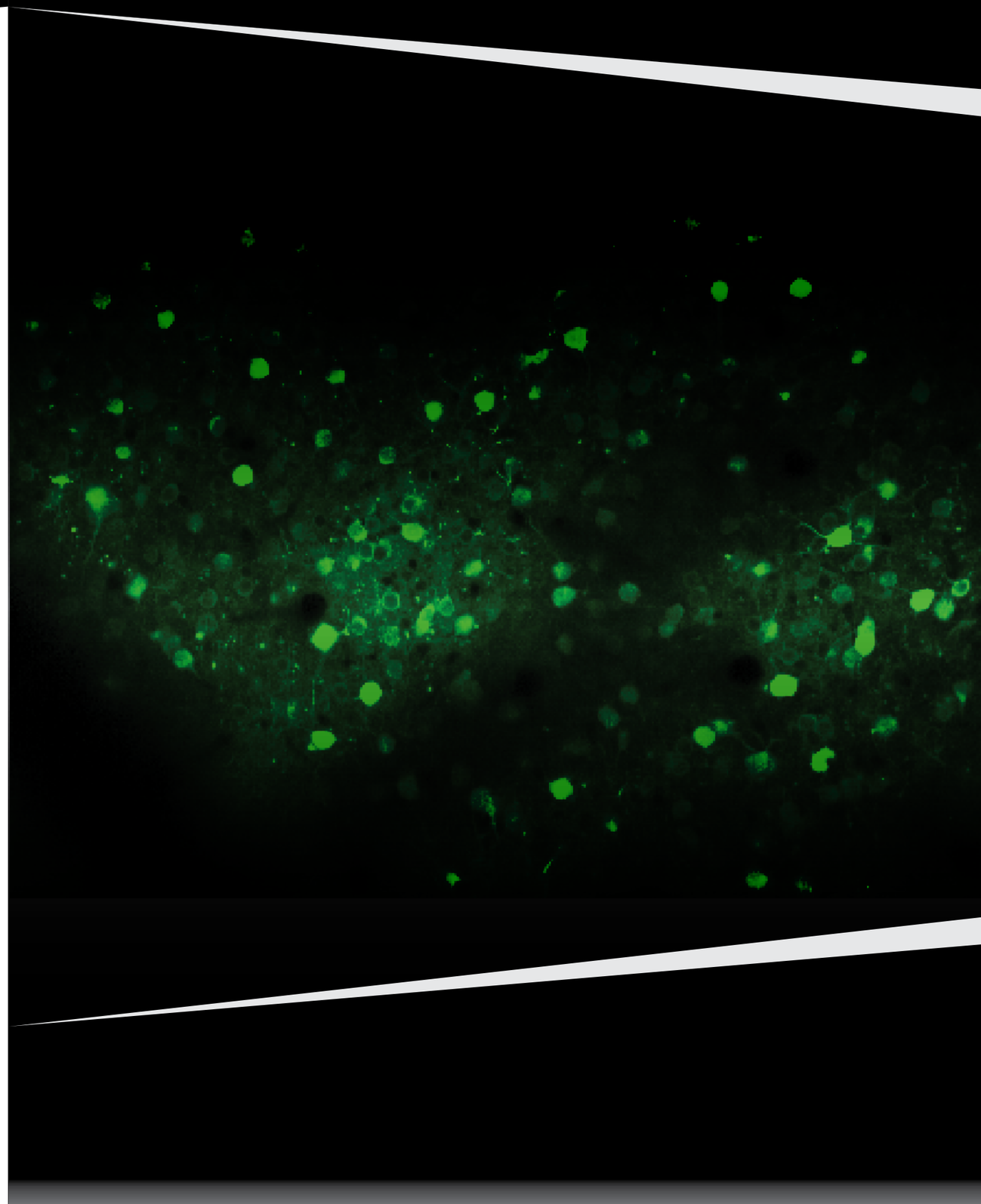



## Imaging of multiple somata during movement

Femto3D-AcoustoOptic

Multi-cube scanning is a spatially extended mode of chessboard scanning, where a Z dimension is added to the aforementioned squares to cover the whole extent of the somata, therefore preserving all fluorescent information even during large amplitude movements.



# Dendritic Imaging

Dendrites and dendritic spines are made of thin, sensitive processes and are therefore difficult to study. Using two-photon laser scanning technology, however we are able collect signals from femtoliter volumes of deeper regions of the brain, while at the same time avoiding phototoxicity. In addition, the spatially confined scanning of axons, dendrites, and spines as regions of interest (ROIs) makes it possible to detect even sub-threshold signals because of the high signal-to-noise ratio (SNR). There are several configurations which make it possible to visualize dendritic arborization and perform functional measurements under in vitro and in vivo conditions in 2D and 3D samples.

## High scanning speed along dendrites and spines in 2D

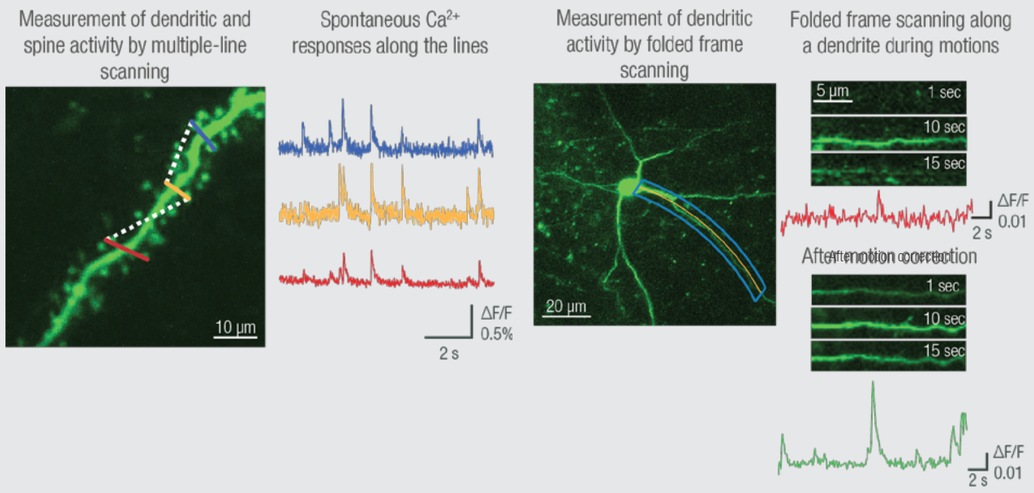
FemtoS-Galvo

The adaptable X and Y mirrors of the galvanometric scanner, coupled with the special electronic boards from Femtonics, can follow the tortuous protrusions of the dendritic arbor precisely using the 2D multiple-line scanning method. By limiting the scanning to the spines and omitting the space between them, both the scanning speed (up to 2 kHz) and the SNR can be increased.

## In vivo dendritic measurements

FemtoS-Galvo

Using folded-frame scanning, an area along a pre-selected line can be imaged. The selected regions can take many shapes, from areas around straight lines to complex bent curves. This advanced scanning method is useful for following events along curved dendrites with spines, and can also be advantageous for dendritic measurements in behaving animals where motion artefacts are a common problem. The images are corrected for motion offline by the control software, as long as the dendrite remains in the scanned area.





# Dendritic Imaging


## The fastest imaging of 3D dendritic arborization

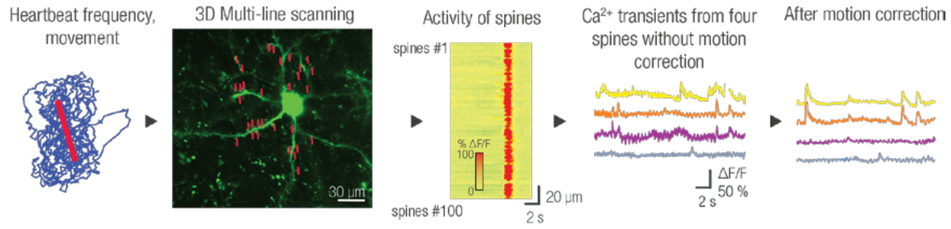
Femto3D-AcoustoOptic

With the Femto3D-AcoustoOptic microscope events less than a millisecond apart can be separated, and therefore propagation speed of regenerative activity determined at multiple sites of the dendritic tree. 3D multiple-line scanning is an advanced version of random-access point scanning: the scanning points are extended by drifting the focal point along lines, providing more detailed spatial resolution without changing the overall scanning time. See also Katona et al., Nat Meth, 2012, Chiovini et al., Neuron, 2014.

## Motion correction during 3D imaging of spines

Femto3D-AcoustoOptic

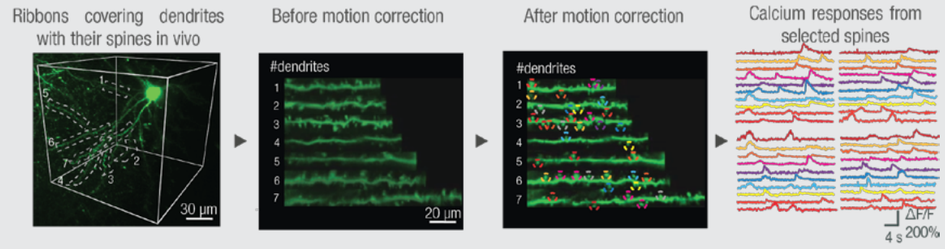
The Femto3D-AcoustoOptic 3D multiple-line scanning method can be used not only for fast dendritic imaging, but also for imaging spines in awake, behaving animals. In the figure, each scanning line is associated with one spine in a layer II/III pyramidal cell labeled with GCaMP6. The direction of the drift is set to meet the average trajectories calculated from brain motion, helping to eliminate the motion artefacts. A total of 100 pre-selected spines were examined simultaneously, and four representative $Ca^{2+}$ transients are shown before and after motion correction, demonstrating the improved SNR. See also Szalay et al., Neuron, 2016.




## 3D dendritic imaging in behaving animals

Femto3D-AcoustoOptic

An extension of the 3D multiple-line scanning performed by Anti-mOtion technology is 3D ribbon scanning, which makes it possible to image ribbon shaped surfaces containing dendrites and the neighboring areas. Figures show 3D ribbons encompassing seven dendritic segments with their spines of a GCaMP6-labeled layer II/III pyramidal neuron measured within the brain of a living mouse. The seven ribbons were projected into a 2D image ordering dendrites above each other for better visualization, and activity was recorded from 40 selected spines and visualized in the form of classical $Ca^{2+}$ transients. See also Szalay et al., Neuron, 2016.

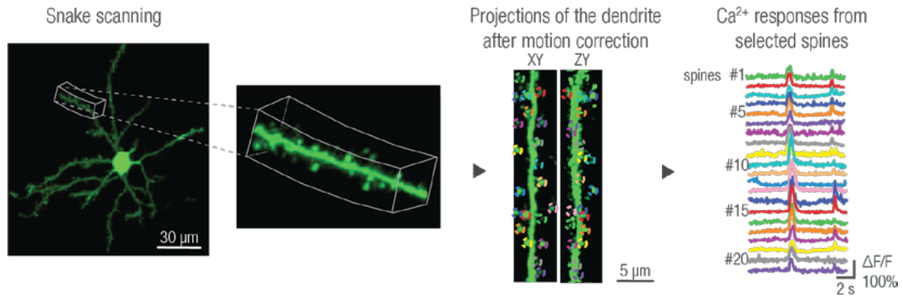


# Dendritic Imaging

## 3D dendritic imaging in behaving animals

Femto3D-AcoustoOptic

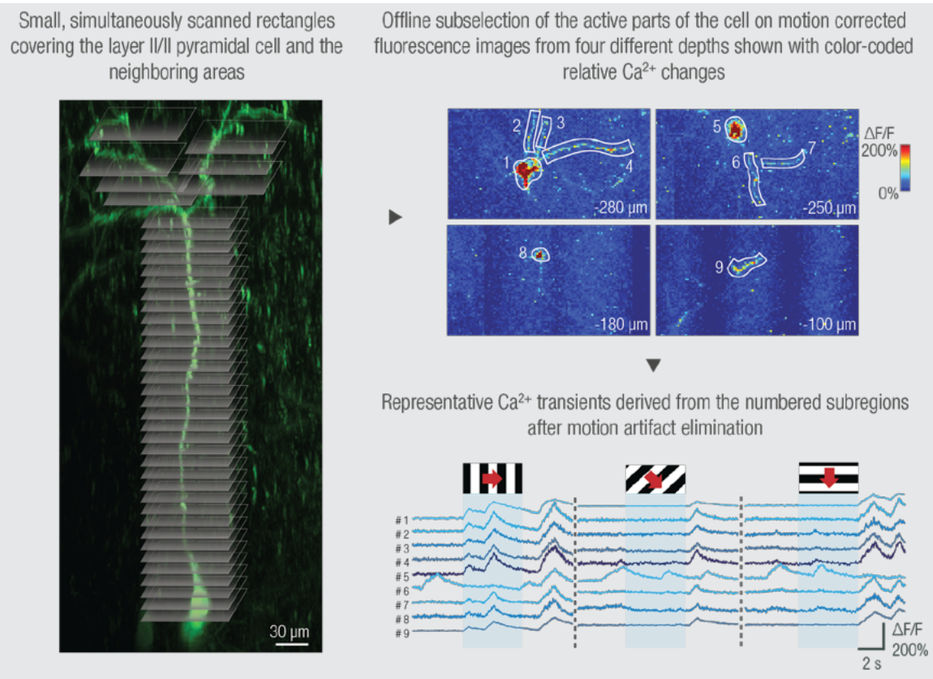
3D snake scanning is a volume extension of ribbon scanning and contains the entire 3D environment of the dendrite. It therefore supports imaging of dendrites in larger animals, or behavioral protocols, where the amplitude of motion can be large. Figure shows fast snake scanning performed at 10 Hz in the selected dendritic region of a V1 pyramidal neuron. See also Szalay et al., Neuron, 2016.




## The fastest imaging of 3D dendritic arborization

Femto3D-AcoustoOptic

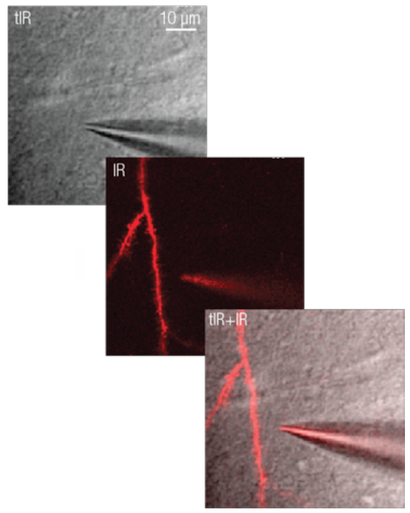
Imaging multiple frames with different sizes at any position in the scanning volume can be used to follow all events propagating along the cell. The figure shows imaging of the entire length of a pyramidal neuron in vivo, where the small scanned rectangles cover the apical dendrite across multiple layers. Motion compensation enables us to record fluorescent signals and responses to visual stimuli while the animal is running on a treadmill. See also Szalay et al., Neuron, 2016.




# Electrophysiology

Two-photon $Ca^{2+}$ imaging and electrophysiological data acquisition provide excellent tools for the study of neural cell and network activity. Using the two methods simultaneously, it is possible to follow potential fluctuations and relate them to changes in their $Ca^{2+}$ levels. While the two-photon $Ca^{2+}$ imaging technique shows events in multiple small areas (distinguishable parts of dendrites and spines) at high spatial resolution (<1 µm) but low temporal resolution (>100 ms), electrophysiology can cover changes over larger areas (cells or extensive areas) at low spatial resolution (~150 µm), and the changes can be followed considerably faster (<1 ms).

## Dendritic patch-clamp



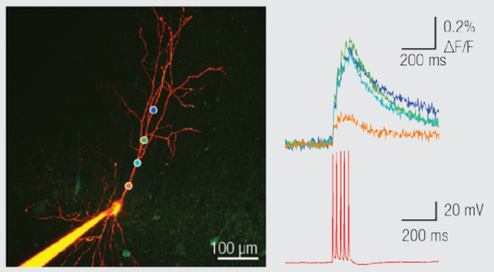

With intracellular patch-clamp recording it is possible to observe and manipulate membrane potential fluctuations in neurons. All Femtonics microscopes can be equipped with electrophysiological devices, and the following settings facilitate the experiments:

- automatic software module moves the tip of the pipette to the selected dendritic location,
- pipette movement and distance to target location can be monitored in real-time,
- guide points can be positioned by the user according to the scanned transmitted or two-photon images,
- real-time transmitted infrared and two photon images can be shown separately or in overlay,
- only one simple calibration step is required before using a new recording pipette.

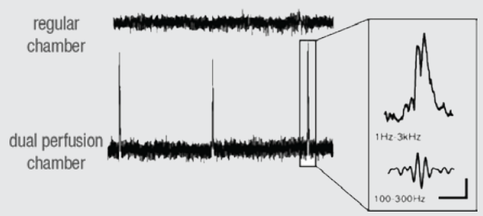
## Processing of electrophysiological data

The electrophysiology software module acquires electrophysiology signals using the A/D converters of the microscope. The telegraphing function communicates the conversion parameters from the amplifier to the A/D converters managed by the control software. Electrophysiology signal acquisition time is aligned with the imaging data. The electrophysiology GUI also includes a seal test window.

Simultaneous $Ca^{2+}$ imaging and patch-clamp recording in an OGB-1 and Alexa-594 filled hippocampal neuron.




Representative local field potentials recorded in a regular and in dual perfusion chamber. Sharp wave oscillations could be observed only in the dual perfusion chamber. see more Chiovini et al, Neurochem Res, 2010




## Dual-perfusion chamber kit

This slice chamber is designed to maintain isolated, living tissues in a healthy state during experiments. In contrast with single perfusion, dual-superfusion of submerged slices increases the vitality of cells, since oxygen and other materials, for example, can freely and effectively diffuse from both sides into the thick slices at a high flow rate.

# Behavior studies

Behavioral processes are paired with patterns of brain activation. The neural basis of a behavioral trait such as visuomotor learning, memory retrieval, associative learning, or spatial navigation can be mapped by controlled, multiform experimental manipulation. This can be a change in stimulus information, task instruction, or reward and punishment which alter the underlying pattern of brain activation. Monitoring or evoking behavioral data, and parallel imaging of neuronal circuits in a living animal's brain can reveal the connections between changes at the cellular and behavioral levels.

## Anti-mOtion technology

### Femto3D-AcoustoOptic

Our Anti-mOtion scanning technology enables cell activity to be captured while an animal is moving in virtual reality and performing tasks. The acousto-optic drift scanning technology is useful for correcting tissue motions caused by behavior. To preserve signals, scanning points are extended to drifted lines which are precisely fitted to each other resulting surface or 3D volume elements. These elements cover not only the pre-selected ROIs but also the neighboring areas giving an opportunity to preserve all fluorescent information during motions and decrease the artefacts by more than one order of magnitude in behaving animals. See more Szalay et al, Neuron, 2016.

## Large space under the objective

### FemtoSmart

The FemtoSmart product line was primarily developed to perform in vivo imaging. This system's special feature is the elevated body which can move in X, Y, and Z dimensions: it offers plenty of space under the objective for free and easy positioning of the sample. This feature supports functional behavioral studies of model organisms with different weights and sizes, from flies to even non-human primates, while they are moving in a virtual-reality environment.

## Software-controlled triggering of stimuli

Different forms of stimulation can be triggered or driven directly using the analog and digital output signals of the microscope, planned and controlled from the measurement control software. Visual, auditory, tactile, whisker or odor stimuli can be generated this way. The Stim Visual software module of the control software enables videos or images for visual stimulus to be loaded and played sequentially, and the stimuli synchronized with the evoked neural responses.

## Behavior experiment control

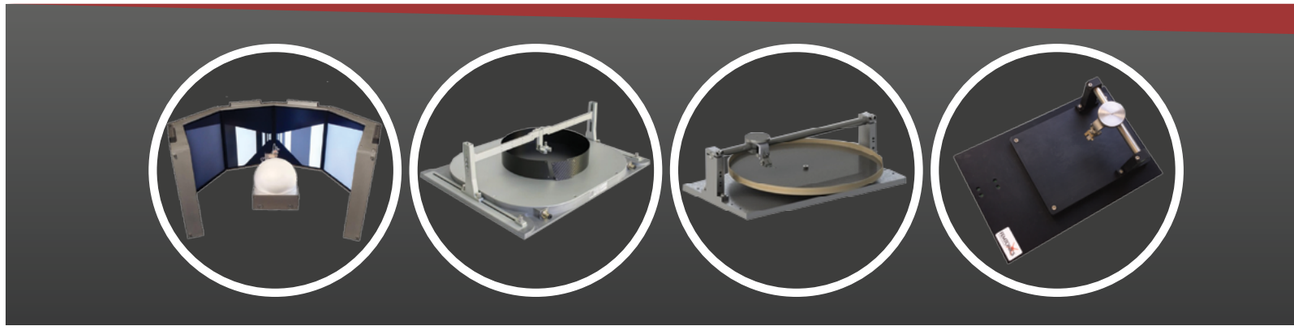
Bpod is an open-source behavior-recording system and real-time environment controller for rodent experiments. Using high-level programming environments (MATLAB/Python), it provides a low-latency closed-loop link between behavioral events, stimulus delivery, and stimulation. Using its built-in liquid reward delivery system, it can be used to power go/no-go discrimination, two alternative forced choice, and CS/US behavioral paradigms. Bpod data acquisition is fully synchronized with the microscope, and the behavioral events can be precisely aligned to the recording of $Ca^{2+}$ transients.

# Virtual reality systems

Virtual Reality (VR) systems enable head-fixed but free-to-move rodents to enter a VR environment and perform complex behaviors, and provide an easily controllable experimental protocol for investigating cognition, navigation, learning, memory, and operant conditioning.

Phenosys JetBall-TFT consists of a TFT surround monitoring system focusing on 200° around the animal, and a spherical treadmill which make unobscured field or maze designs possible. This device can be coupled with optional operant devices, and allows a restrained animal to navigate in virtual space.

Neurotar Mobile HomeCage provides a real and familiar moving VR environment. A head-fixed, awake rodent walks freely on the flat-floored, air-lifted cage that moves according to the animal's locomotion, while exploring and navigating during in vivo recordings and imaging experiments.



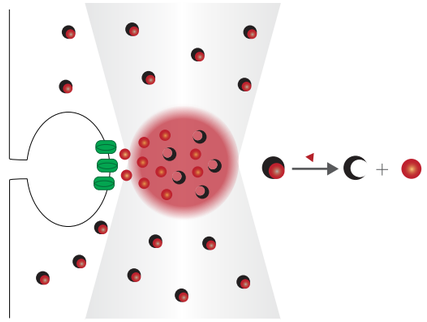
Femto-Gramophone is an affordable, single-dimension VR system. The animal runs on a spinning disk while the background on the coupled screen changes according to the speed of the mouse. The Femto-Gramophone device can be coupled with multiple operant devices such as a water reward, air puff or visual stimulation. The animal's speed and the two-photon $Ca^{2+}$ signal can be recorded simultaneously using a built-in module in our main measurement software.

Head holder stands and head plates fix the rodent's head in different positions, enabling precise measurements in the brain. Head holders with different dimensions are available for mice and rats. For anesthetized rodents, we offer a heating pad coupled to the holder, or for behaving animals a stand that ensures access to jetballs, treadmills, or other devices.

Designed under collaboration available on request.

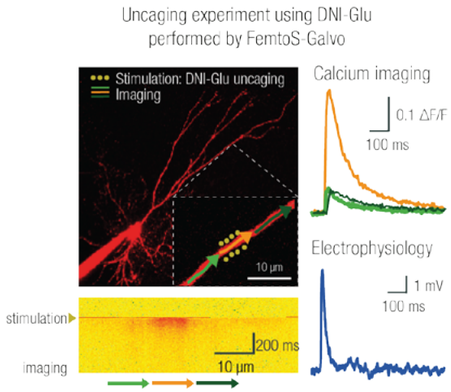
# Photostimulation/Uncaging

Uncaging means the activation of biochemically masked (‘caged’) molecules via photolysis, which mimics the physiological release of bioactive compounds. This technique is widely used in neuroscience, where the bioactive molecule is usually glutamate or another neurotransmitter. Using two-photon photostimulation, made possible by the secondary laser beam in the FemtoS-Galvo, very precise release of these compounds can be elicited in extremely small volumes. Two-photon imaging is a powerful opportunity to follow the changes evoked in dendrites or spines, even the distribution of receptors on the neurons can be investigated.



## In vivo uncaging at well-defined points

FemtoS-Galvo equipped
with a Multiple beam path extension




The accuracy of the excitation point, and the highly flexible scanning patterns, mean that the FemtoS-Galvo is the best choice for uncaging experiments. The secondary laser beam, essential for the stimulation, is coupled to the existing light path. Thus the stimulation and the imaging can be performed near simultaneously using the galvo scanner. Multiple-point scanning (yellow points on the figure) is used for stimulation around spines, while multiple-line scanning (arrows) makes high-speed imaging along the dendrites possible. See also Katona et al., PNAS, 2011, Chiovini et al., Neuron, 2014.

## Compounds for uncaging experiments FemtoChemistry

Femtonics Chemistry designs and develops new caged neurotransmitters for frontier neuroscience research. The main product is a glutamate derivative, but custom-synthesized compounds can also be generated to meet customers’ specific needs.

### DNI-GLU

This dinitro-indoline-masked form of glutamate releases the bioactive glutamate more rapidly than any other commercially available compound. It was developed for high-quantum yield requiring less irradiation for release. In other words, its effective concentration is lower than other caging scaffolds. DNI-Glu is a compound developed in-house, only available from Femtonics. See also Chiovini et al., Neuron, 2014.

### Features

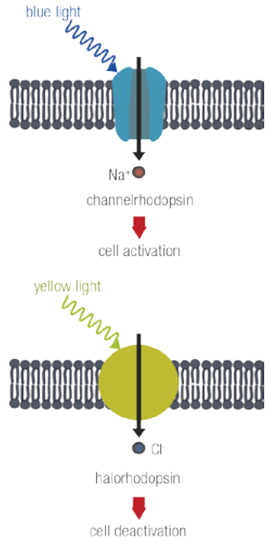
- Photostimulation with two-photon laser
- High quantum-yield
- Effective at low concentration

### Benefits

- Seven-fold higher quantum yield than any other caged form
- High excitatory postsynaptic potential and high $Ca^{2+}$ transient as a response to the photorelease
- Less illumination is sufficient to elicit the same response as with alternative compounds
- Elicits large transients or regenerative activity

# Optogenetics/Photostimulation

The essence of optogenetics is introducing light-activated recombinant ion channels such as channelrhodopsin (ChR2) or halorhodopsin (NpHR) into excitable cells. Light activation of these molecules leads to an influx of ions which induces turning neurons on or off selectively. Halorhodopsin and channelrhodopsin together enable multicolor optical activation, silencing, and desynchronization of neural activity, creating a powerful neuroengineering toolbox.




## Stimulation and imaging

The photostimulation can be induced using visible or infrared light, while imaging is performed by a femtosecond IR laser. Switching between the stimulation and imaging is done at a sub-millisecond scale. Importantly the detectors are protected during the stimulation by a built-in gating system.

## Full-field illumination

FemtoS-Resonant equipped
with LED light source

The entire FOV can be stimulated with the LED source above the objective. LEDs are available at different wavelengths, exciting ChR2 at 473 nm or NpHR at 561 nm. The light impulses are precisely timed and highly repeatable. The FemtoS-Resonant microscope follows the changes over the whole field of view at a resolution of 31 frames per second.

## Stimulation along ROIs

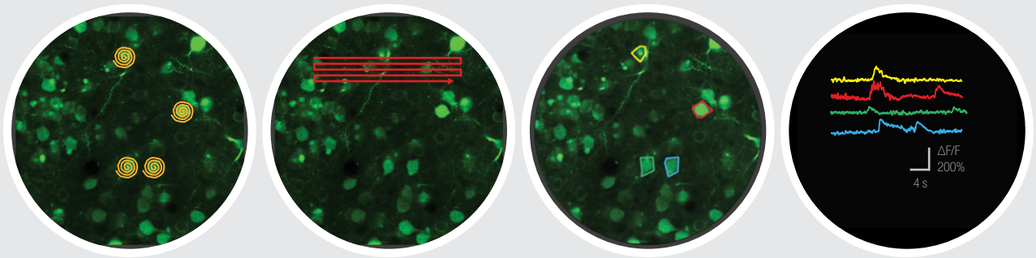
FemtoS-Galvo equipped
with Multiple beam path extension

To stimulate cells or subcellular components selectively, the best solution is using the FemtoS-Galvo to steer the laser beam rapidly through optimized scanning patterns, such as a spiral, zigzag, etc. We offer a continuous laser tuned to 473 or 561 nm for ChR2 or NpHR activation, respectively. Precise two-photon activation of these molecules is also a viable option.

## Simultaneous photoactivation and imaging

FemtoS-Dual

The FemtoS-Dual microscope contains a galvo and a resonant scanner which function in tandem to combine the advantages of both the galvo and resonant microscopes. This is the best solution for simultaneous photostimulation and high-speed imaging.




Stimulation along ROIs using spiral scanning pattern with galvo scanner on ChR2 expressing neurons

Full field imaging by resonant scanner

Offline ROI selection

Calcium responses from the selected ROIs

# Software

# MESc

## Measurement control and data analysis software

## Overview

The purpose of the MESc measurement control and data analysis software is to drive the newer generations of Femtonics two-photon microscopes with high data recording rates. MESc is under active development for performing cellular and network imaging measurements supporting more and more features and Femtonics hardware.

## Key features

- Measurement configuration and control
- Live data display and processing
- Data exporting into standard formats
- MESc Application Programming Interface
- A live connection to MATLAB
- Convenient and fast measurement data processing
- Custom measurement protocols of arbitrary complexity

## Explore MESc

Download MESc from our website for free and test it without a time limit.

femtonics.eu/software/sw-download

## Measurement control

- Integrated control of all hardware units: focusing, resonant and galvo scanners, PMTs, Pockels cells, light path actuators, auxiliary digital and analog channels, pipette manipulators, XY(Z) stages, etc.
- Live preview mode with on-the-fly parameter adjustment
- Real-time measurement data display with live analysis functions
- Digital lab-book philosophy: entire measurement series with different types of measurements can be saved into one .mesc file, including per-measurement metadata
- Unlimited measurement time, unlimited video streaming to the hard drive
- Pervasive auto-save and rescue

## Data analysis

- Composite display of color-coded multi-channel data
- Polygonal and rectangular ROIs for intensity change calculations
- Recorded imaging data exportable to common image and video formats
- Electrical recordings exportable to common spreadsheet software

## New in 2017

- Resonant Z-stack acquisition with the objective Z positioner
- Time series and Z stack recording with galvanometric-based scanning
- Dual scanning: resonant-scanner-based imaging with simultaneous galvanometric scanner based photostimulation
- Tilting objective control
- Analog and digital electrical input and output, optionally synchronized to imaging
- MESc Application Programming Interface (API) for no-limit custom data processing and measurement control

# FEMTONICS Software

Performance, stability, ease of use, flexibility

## MESc Application Programming Interface

The MESc API is a set of clearly defined instructions that can be given to MESc from various software systems like MATLAB or Python. It lets the experimenter perform data analysis with external tools and control measurements with a freely designable protocol.

| MESc | API | High Level Programming Language |
|---|---|---|
| Standalone measurement control application | Application Programming Interface | |

### Reliable

- Standalone C++ application Omitting third party layers is a key for stability

### Efficient

- Optimized for high data
- throughput
- Controls all microscope
- functionality

### Easy to use GUI

Beautiful, state of the art design

### Process data

- Access complex data with all metadata
- Efficient for large file I/O
- Works also during measurements
- No version compatibility issues

### Control measurements

- Two-way communication
- Query and set hardware states
- Control the creation of measurement units

### Easy to code

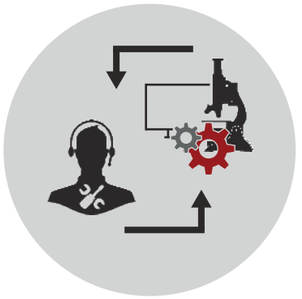
- Quick code development
- Advanced IDEs with debugging
- Extensive math and other libraries
- Script and automate
- Control complex measurement sets
- Algorithmic decision making during measurement

# Femtonics Support

Our mission is to make readily available high quality technical support pre and post microscope selection. We can enhance your work by custom optimizing the software and/or the hardware. Femtonics support team consists of mechanical, optical and software engineers as well as application scientists who are ready to provide technical advice and technical guidance with the microscope which starts at production and continues through assembly, installation and maintenance.

## Remote technical support

Femtonics provides remote technical support with each newly installed Femtonics microscope in which we include an internet camera and a microphone along with remote control software. This allows our technical team, with customer's permission, to remotely test the hardware, to modify the software and to find solutions to any unexpected issues. This is like having your own technical support person next to you proving to have a high customer satisfaction. We aim to keep your system healthy, up-to-date and operating at the highest level of its performance.



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Worldwide Distributors

Femtonics Ltd. HQ
H-1094 Tuzolto utca 59. Budapest, Hungary
info@femtonics.eu

Femtonics Inc. USA
319 Derby St. Salem, MA 01970 , USA
usa@femtonics.us