Femto3D-AO
ATLAS

Unique features for 3D imaging

- a novel extension for all upright microscopes
- ultra-stable design (integrated stabilization of the scanner), no need for adjustments
- compatible with a variety of microscopes
- all features of Femto3D-AO included
- third generation acousto-optic scanner
- user-friendly software for data acquisition and analysis
- over one million times faster scanning in 3D (30-50 kHz/ROI) than previously reported
- preserved high spatial resolution (<400 nm in the center)
- over 500 µm × 500 µm × 650 µm scanning volume in vivo
- automatic wavelength tuning (750-1050 nm)
- automatic beam stabilization
- dynamic compensation for optical errors
- fast in vivo and in vitro recording of over 2000 neurons, spines and dendrites in 3D
- motion correction in behaving animals
Overview

Femto3D-AO ATLAS is an acousto-optic scanner based two-photon microscope extension which can be added to existing upright microscopes to open their imaging capability to fast (>30 kHz) 3D functional imaging. It contains two pairs of AO deflectors (AODs) which are responsible for the X, Y, and Z focusing providing the superior in vivo imaging parameters of the well-known Femto3D-AO microscope. The acousto-optic scanner does not contain any slowly moving mechanical components (e.g. scanning mirrors), positioning of the focal spot is stable, independent of the travelling distance and the scanning speed is extremely high. The integrated electric stabilization of the 3D scanner provides long-term stability in measurements without the need for maintenance.

<table>
<thead>
<tr>
<th>10⁶</th>
<th>&gt;2000</th>
<th>18</th>
<th>53</th>
</tr>
</thead>
<tbody>
<tr>
<td>scanning volume (µm³)</td>
<td>simultaneous measurement locations (ROIs)</td>
<td>multisite repetition rate (µs)</td>
<td>speed (kHz)</td>
</tr>
</tbody>
</table>

3D AO imaging

The 3D AO imaging gives an extremely high scanning speed at up to 53 kHz at any 3D location under the objective with <100 nm precision and <400 nm lateral resolution. Using the AO scanning technology in combination with an easy-to-use 3D data acquisition software package provides maximal flexibility in fast selection of regions of interest (ROIs). The preselected ROIs can be precisely and rapidly targeted without wasting measurement time on unnecessary background areas or volume elements increasing further the measurement time and the signal-to-noise ratio (SNR). Many 3D scanning patterns are available, and a vast array of measurement possibilities can be visualized and analyzed online and offline: these support imaging of neural networks, dendrites and even spines at high scanning speed and high signal-to-noise ratio by several orders of magnitude in comparison to classical raster scanning (Szalay et al., Neuron, 2016; Katona et al., Nature Methods, 2012).
Acousto-optic deflectors

In contrast to a traditional scanner, AO deflectors (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.

One million faster scanning speed

If we compare the relative gains in measurement speed ($v_{\text{gain}}$) and signal-to-noise ratio (SNR$_{\text{gain}}$) for 3D AO scanning relative to traditional raster scanning of the same sample volume, we can say that the $v_{\text{gain}} \times (\text{SNR}_{\text{gain}})^2$ 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 (see Equations S82-87 in 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.

Anti-mOtion technology

Using Anti-mOtion technology, in contrast to traditional point-by-point scanning, 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. See more Szalay et al., Neuron, 2016.
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.
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, Anti-mOtion technology). 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**

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

- Movement artefact correction of multi-ROI data
- ROI selection and handling
- Merging ROI sets on different measurement units
- Calculation of background corrected relative fluorescence
- Handling and arithmetic of Ca²⁺ traces in line with behavior signals
- AP detection
- Excel interface

Automatic motion correction and AP detection

Automatic ROI detection

Easy-to-use user-friendly GUI

- Laser intensity and PMT control
- Scanning mode selection
- Scanning specific parameters
- Measurement controls

Semi-automatic tools for simultaneous analysis of multiple ROIs
Automatic cell detection

Integrated software tools for behavior and electrophysiology

Specification

- In vivo functional imaging down to over 650 µm depth
- 500 µm × 500 µm × 650 µm scanning volume in vivo (with a 20x obj.)
- Available wavelength between 750 - 1050 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 using Anti-mOtion technology for motion correction
  - For network measurements: chessboard and multi-cube scanning methods
  - For dendritic measurements: ribbon, 3D multiple-line, snake scanning methods
  - 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
  - MATLAB-based, integrated hardware, measurement control and analysis software
  - Intelligent control of various sensory stimuli supporting behavioral studies

Software/Femto3D-AO
TECHNICAL REQUIREMENTS

We will optimize the Femto3D-AO ATLAS to your existing system.

The Femto3D-AO ATLAS system

- 3D acousto-optical scan head
- automatic beam stabilization unit
- control electronics and workstation
- measurement control and analysis software

Femto3D-AO ATLAS can be attached to

- any upright third-party microscope with a camera port (C-mount) on the top*
- or to a Femto-SMART two-photon microscope
- or to a Femtonics 2p platform
- or to many custom-made microscopes*
*for more compatibility information please contact us

Required components

- **recommended laser types:** MaiTai HP*, Chameleon Ultra II*, Chameleon Discovery*, Insight X3*, Fidelity HP. (*Additional prism compressor module is required for these tunable two-photon lasers.)
- **components for detection**
  - option #1, PMT connection KIT: it divides PMT signals between the host microscope and the Femto3D-AO ATLAS, pre-amplifier is needed.
  - option #2, PMT detector KIT: it adds two more external detectors to the existing microscope without interfering with the existing system.
- **optical table**
- **external optical pathway** optimized for the existing setup

Accessories

- **Femtonics 2p platform:** Femto3D-AO ATLAS provides full functional 3D microscope with the Femtonics 2p platform which is required if there is no other existing host microscope.
- **prism compressor** (not required for some lasers)
- **camera**
- **blackout box**
Neuronal network imaging
3D random-access point scanning

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.

Imaging of dendritic arbor
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. As a result, the function of thin dendritic segments, or even spines can be revealed.
Imaging of dendrites and their spines in 3D in behaving animals

3D Ribbon scanning

Ribbon scanning is a surface extension of the multiple line scanning mode performed by Anti-mOtion technology, where 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. See also Szalay et al., Neuron, 2016.

3D Snake scanning

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 during defined surgical or behavioral protocols, even when the amplitude of motion is very large. The figure shows snake scanning performed at 10 Hz in the selected dendritic region of a V1 pyramidal neuron.
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.
3D 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.

Simultaneously scanned rectangles as ROIs

Representative Ca$^{2+}$ transients derived from the numbered subregions after motion artifact elimination

Offline subselection of the active parts of the cell from four different depths shown with color-coded relative Ca$^{2+}$ changes
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 somata 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.
Unitary GABAergic volume transmission from individual interneurons to astrocytes in the cerebral cortex

Márton Rózsa, Judith Baka, Sándor Bordé, Balázs Rózsa, Gergely Katona & Gábor Tamás

2015

DVANCED MATERIALS

Localized Neuron Stimulation with Organic Electrochemical Transistors on Delaminating Depth Probes.


2015

Dendritic Spikes Induce Ripples in Parvalbumin Interneurons during Hippocampal Sharp Waves

Balázs Chiovini, Gergely F. Turi, Gergely Katona, Attila Kaszás, Dénes Pálfi, Pál Maák, Gergely Szalay, Mátyás Forián Szabó, Gábor Szabó, Zoltán Szadai, Szabolcs Káli, Balázs Rózsa

Neuron

Sensitization of neonatal rat lumbar motoneuron by the inflammatory pain mediator bradykinin

Mouloud Bouhadfane, Attila Kaszás, Balázs Rózsa, Ronald M Harris-Warrick, Laurent Vinay, Frédéric Brocard

2015

eLIFE

Single-cell-initiated monosynaptic tracing reveals layer-specific cortical network modules


2015

Nature Methods

Techniques for life scientists and chemists

Fast two-photon in vivo imaging with three-dimensional random-access scanning in large tissue volumes

G Katona, G Szalay, P Maak, A Kaszas, M Veress, D Hillier, B Chiovini, ES Vizi, B Roska & B Rozsa

2012

Accurate spike estimation from noisy calcium signals for ultrafast three-dimensional imaging of large neuronal populations in vivo

T Deneux, A Kaszas, G Szalay, G Katona, T Lakner, A Grinvald, B Rozsa & I Vanzetta

2016

Neuron

Fast 3D Imaging of Spine, Dendritic, and Neuronal Assemblies in Behaving Animals

Szalay et al.

2016

Neuron

Deneux et al.

2016

Nat Commun

Rozsa et al.

2015

Brain Struct Funct

Williamson et al.

2015

Adv Mater

Bouhadfane et al.

2015

eLife

Wertz et al.

2015

Science

Chiavini et al.

2014

Neuron

Katona et al.

2012

Nat Meth