3D REGION SCANNING

FEMTO 3D ATLAS

10^8 scanning volume (μm^3)

2000< simultaneous measurement locations (ROIs)

30 speed (kHz)

EFFICIENT SIMULTANEOUS ALL-OPTICAL ACCESS TO MULTIPLE REGIONS IN 3D

- FAST: Record fluorescence changes at up to 30 kHz in a 500 x 500 x 650 μm^3 volume.
- LARGE-SCALE: Simultaneous imaging of more than 2000 targets.
- FREE-FORM: Flexible control over the shape and 3D orientation of scanned regions.
- SHARP: Higher signal-to-noise ratio (SNR) compared to conventional microscopes.

The FEMTO3D Atlas is an ultrafast multiphoton imaging platform that fuses 3D fluorescence acquisition with 3D photostimulation. From sub-micrometer to behaving animal scale, Atlas gives you utmost flexibility for your all-optical experiments up to 850 µm deep in the sample. This unified architecture represents the highest value for scholars striving in cutting-edge life science research.

Main features making Atlas most suitable for competitive life science are detailed in our whitepapers:

3D REGION SCANNING for *in vivo* network, dendritic and spine imaging
Scan small regions of interest flexibly in 3D, with a kHz speed and high SNR.

3D ANTI-MOTION TECHNOLOGY for *in vivo* motion correction
Improve the imaging signal quality profoundly in behaving animals.

HIGH-SPEED ARBITRARY FRAME SCANNING with potential for voltage imaging
Rotate frames arbitrarily and scan them with 40 Hz without scanner noise.

3D PHOTOSTIMULATION for optogenetic studies
Perform simultaneous photostimulation and fast imaging in 3D.

Download these whitepapers at femtonics.eu!

Organoids, worms, flies or mammalian brains contain three dimensional (3D) networks of cells connected via fine processes. Visualizing such elaborate structures in action, in particular detecting fast temporal events occurring simultaneously at dispersed locations in 3D (e.g. across spines on a neuronal dendritic tree) can be done efficiently with the 3D region scanning modes of Atlas by confining scanning to target structures and skipping irrelevant parts of the specimen. In contrast, conventional imaging systems are bound to acquire data in consecutive 2D optical sections therefore they are not optimal for accessing 3D structures in tissues.

The concept of 3D region scanning implemented in Atlas microscopes is summarized in Figure 1. The first step is to find target structures in the specimen, e.g. neuronal cells in the brain. 3D volumetric data needs to be collected from the specimen and Atlas efficiently supports this step via fast z-stack acquisition. The second step in 3D region scanning is to define the locations and shapes of the target structures to be monitored at high speeds, up to kHz sampling rates. Atlas supports you in this workflow via automated cell detection and assisted manual selection of filament-like structures. Once the 3D locations and orientations of target regions are defined, fluorescence data can be acquired at a high-speed. The software tools provided with Atlas ensure immediately data visualization and analysis.

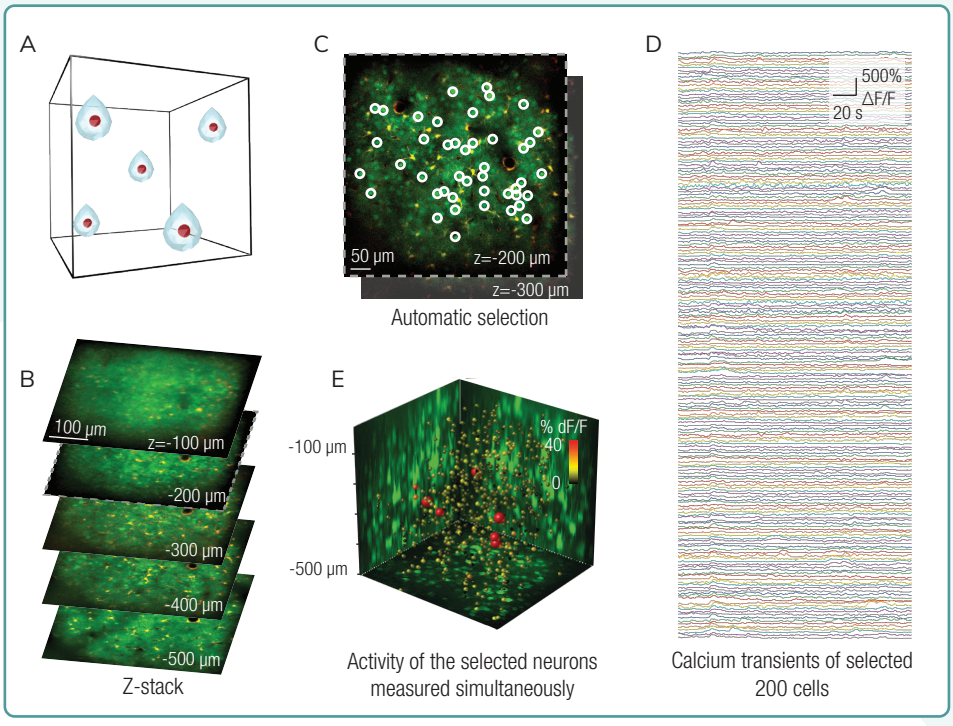


Figure 1. 3D region scanning available with the all-in-one Atlas multiphoton platform. **A**: *In living organisms, objects of interest are dispersed in 3D space. Blue objects represent target structures in which fast temporal changes of fluorescence need to be captured. Red spheres in each object illustrate the "random-access point scanning mode" of Atlas that can sample fluorescence changes in 3D at up to 30 kHz speed.* **B**: *The conventional approach to cover 3D structures is to collect a stack of planes via optical slicing. In Atlas, high-speed frame scanning supports stack acquisition. For this example, a 500 x 500 x 500 µm³ volume from the OGB1-AM loaded visual cortex of a mouse is shown.* **C**: *To define target locations for 3D region scanning, automatic cell detection methods are provided in the Atlas software.* **D**: *The result of 3D region scanning. Changes in fluorescence reflecting the activity of Ca^{2+} concentration of 200 cells as a function of time.* **E**: *Cellular activity visualized as color-coded spheres in the selected volume.*

When using Atlas 3D region scanning, the unprecedented speed and flexibility you gain for covering many structures are achieved without any compromise in signal quality. Figure 2 demonstrates that 3D region scanning yields superior signal quality compared to conventional scanners which waste considerable signal collection time outside of target regions.

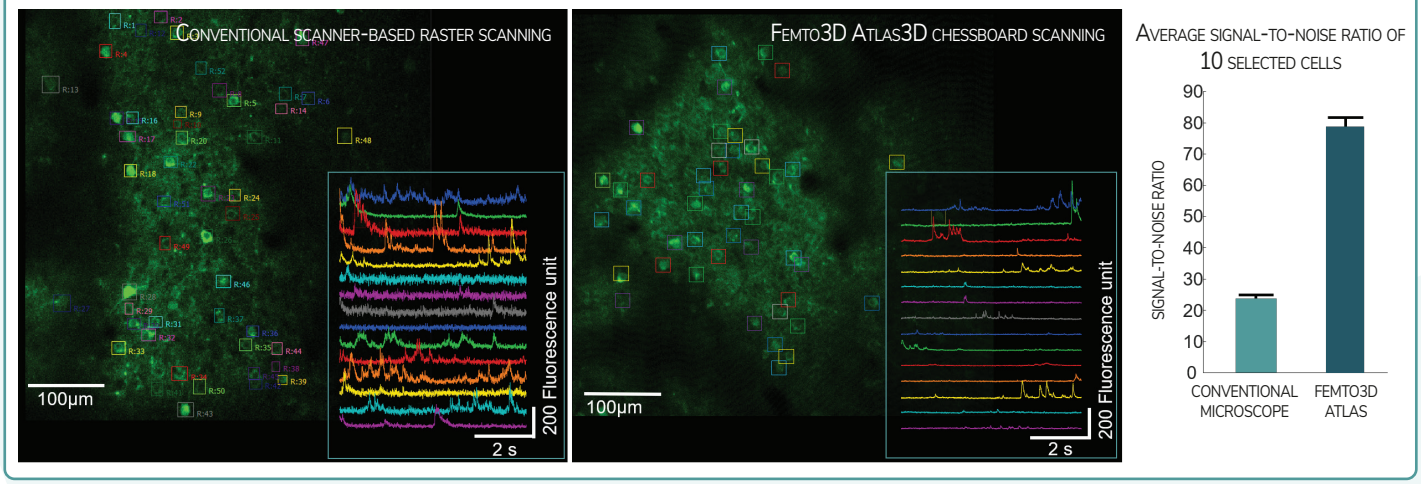


Figure 2. In addition to the speed and flexibility in monitoring living structures, 3D region scanning also yields superior signal quality. Cells from the same cortical region of a GCaMP6s expressing mouse were recorded with a conventional resonant-galvo based microscope (left) and with the Atlas 3D region scanning mode (right). Note the large improvement in overall noise level when activity is monitored with Atlas 3D region scanning (right) compared to signals acquired from the same region using a conventional microscope. Both microscopes were set up to scan 50 regions in a 500 x 500 µm² field of view from the visual cortex at a 31 Hz scanning speed. For each microscope, signal-to-noise ratio was estimated from the standard deviation of fluorescence changes of 10 selected cells.

DIMENSIONS OF CHOICE FOR 3D REGIONS: FROM POINTS TO LINES, SHAPES AND VOLUMES

3D random-access point scanning powered by the acousto-optic (AO) technology is the foundation of the flexibility of Atlas, making experimental strategies possible, that have been out of reach so far due to the limitations of conventional 2D microscopes (Figure 3). In random-access point scanning mode, Atlas can jump across a series of submicron-sized locations requiring only a very short amount of time (30 µs) to reposition the focal point to the next point, achieving up to one million times faster data collection in 3D than traditional plane-by-plane scanning methods. Atlas now implements "drift scanning" modes that recover this short time during jumps to extend points into 3D lines. Aligned lines can form free-form 3D surface or volume scan patterns, depending on the structure of target features to be scanned. Drift-based scanning modes bring highly efficient and flexible options for your experimental needs. Using drift-based 3D scanning modes allows you to record continuously along intricate structures with the option of eliminating motion artifacts often compromising signal quality in *in vivo* experiments (3D anti-motion technology).

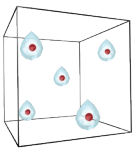
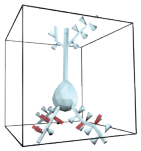
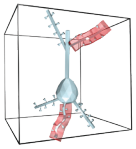
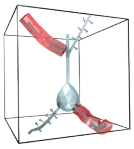
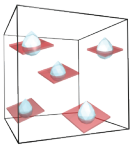
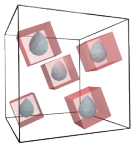
	3D RANDOM-ACCESS POINT	3D TRAJECTORY AND MULTIPLE-LINE	3D RIBBON	3D SNAKE	3D CHESSBOARD	3D MULTI -CUBE
TECHNIQUES						
SPEED	30 kHz per point	up to 30 kHz per spine	up to 3 kHz on a 50 µm long dendritic segment	up to 300 Hz on a 50 µm long dendritic segment	up to 3 kHz per 2D ROI	up to 300 Hz per volume
NUMBER OF SIMULTANEOUSLY SCANNED REGIONS	2000 < cells	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 30 volumes
BENEFITS IN NEUROSCIENCE	3D network imaging in large cell populations	dendritic imaging without interruption and recording activity of over 150 spines	imaging of activity in over 12 spiny dendritic segments	dendritic imaging during large amplitude movements	high speed somatic recordings, network imaging	imaging of somata during large amplitude movements

Figure 3. New experimental strategies enabled by the FEMTO3D Atlas

In-depth technical and application details can be found in Katona et al 2012, Nature Methods; Szalay et al 2016, Neuron; Wertz et al 2015, Science. For more information, contact our Application Specialist Team – they will be glad to answer any further questions.

Femtonics Ltd. HQ | info@femtonics.eu | www.femtonics.eu
Femtonics Inc. USA | usa@femtonics.eu

THINKING AHEAD
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MICROSCOPY