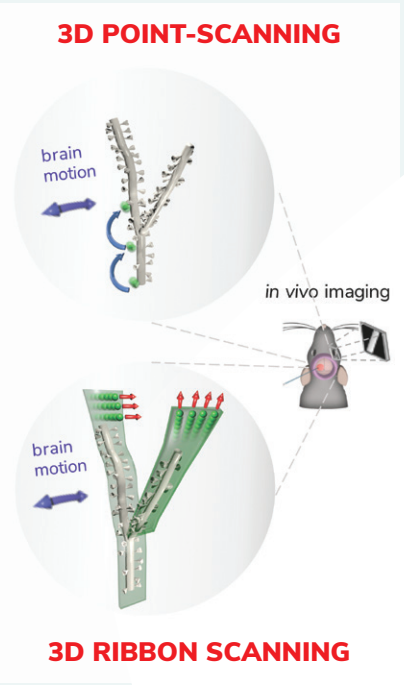
3D ANTI-MOTION TECHNOLOGY

FEMTO 3D ATLAS

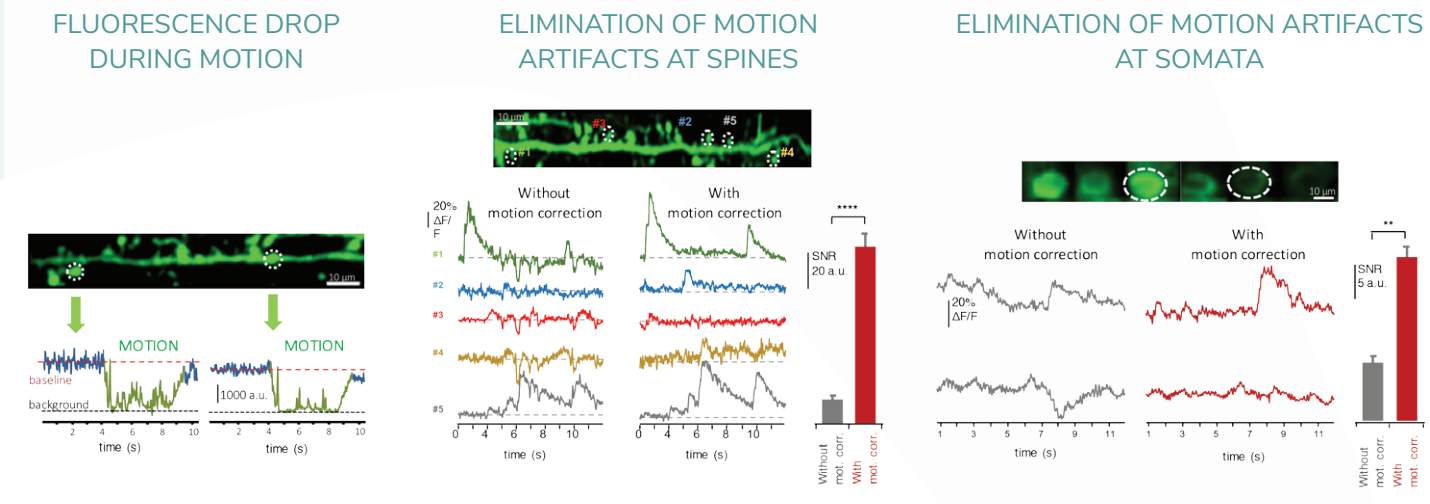
Temporal resolution is key to understand how the networks of neurons in the brain process information. Fluorescent probes with fast kinetics (GCaMP7f; Dana et al., 2019) and new voltage sensors (Chavarha et al., 2018) are sufficiently sensitive to resolve the temporal events at a microsecond scale, and the FEMTO3D Atlas, with its ultrafast scanning, keeps abreast of the newest methods. We developed the 3D anti-motion technology that is exceedingly useful for *in vivo* applications performed in behaving animal models, during which even a slight displacement of the tissue can degrade signal quality. The 3D anti-motion technology combined with new scanning methods united in FEMTO3D Atlas provides a flexible solution to gather motion-corrected data in large volumes at high framerates.

3D ANTI-MOTION TECHNOLOGY FOR ACQUIRING CLEAN DATA FROM BEHAVING ANIMAL MODELS

When scanning distinct locations in your tissue samples via the 3D random-access point scanning mode of Atlas, temporal resolution can reach unprecedented speeds. However, because a living sample is rarely static, sampling targets with just a single point per object (*e.g. one point per dendritic spine, see Figure 1, top*) in a non-static tissue (awake, head-fixed animal model) will yield a suboptimal signal-to-noise ratio (SNR) in the recorded data. The 3D anti-motion technology applies the latest theoretical results implemented in our acousto-optic microscope control software to extend sampling points in 3D into a line along any specified direction (Drift scanning, Szalay et al 2016, Neuron). When many identical lines are precisely aligned, a free-form plane can be scanned in 3D (*see Figure 1, bottom*). During an *in vivo* experiment, the sample almost always exhibits motion artefacts due to vessel pulsation, respiration or limb movements. Scanning in ribbons or cubes assures that target features stay in the scanned regions, thereby drastically improving signal quality *(Figure 2)*.



1. Figure:

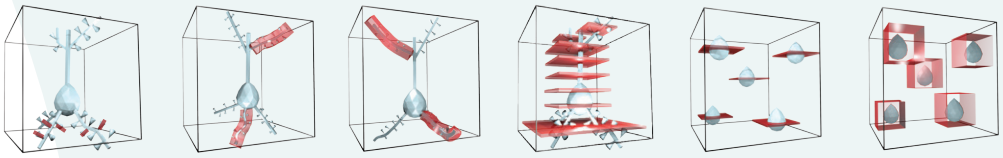


2. Figure: 3D anti-motion technology combined with additional algorithmic tools thereby increases the SNR by more than one order of magnitude in behaving animal models.

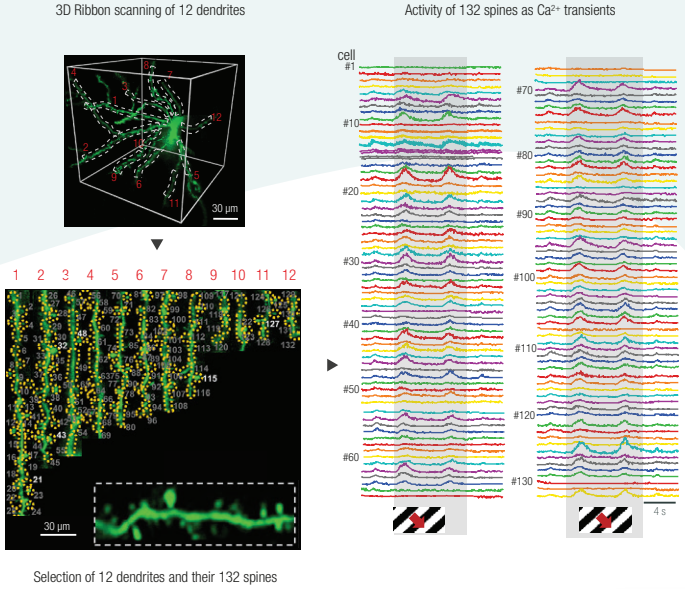
FEMTO 3D ATLAS

FLEXIBLE SCANNING MODES WITH 3D ANTI-MOTION TECHNOLOGY

The flexibility of FEMTO3D Atlas can serve a wide range of applications (Figure 3), from the smallest spatial scale – spine imaging – to experiments, in which "Chessboard" or "Multi-Cube" scanning strategies can cope with large motion artefacts, while still yielding high-quality fluorescence data. Users can easily tune to scanning modes, either to maximize the sampling rate, or to acquire data from a large number of cells. The measurement and analysis functions of the MES control software provide full flexibility in 3D scanning, and offer appropriate visualization and data selection tools to handle convoluted neuronal structures.

	3D MULTIPLE-LINE	RIBBON SCANNING	SNAKE SCANNING	MULTI-LAYER	CHESSBOARD	MULTI -CUBE
TECHNIQUES						
SAMPLING FREQUENCY	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

3. Figure



Selection of 12 dendrites and their 132 spines

EXAMPLE OF A MEASUREMENT WITH 3D RIBBON SCANNING

4. Figure: 3D ribbons encompassing twelve dendritic segments with their spines of a GCaMP6-labeled layer II/III pyramidal neuron measured within the brain of a living mouse. Activity triggered by visual stimuli was recorded from 132 selected spines and visualized in the form of classical Ca^{2+} transients.

For in-depth technical and application details, read the related article: Szalay et al., Neuron, 2016.

Femtonics Ltd. HQ
info@femtonics.eu | www.femtonics.eu

Femtonics Inc. USA
usa@femtonics.us

THINKING AHEAD FEMTONICS MICROSCOPY

WP-Atlas-3dantimot-201