THORLABS

BERGAMO®

RAPID VOLUMETRIC IMAGING WITH BESSEL BEAMS

In-vivo volumetric imaging of neuronal activity requires both submicron spatial resolution and millisecond temporal resolution. While conventional methods create 3D images by serially scanning a diffraction-limited Gaussian beam, an alternative Bessel-beam-based multiphoton imaging technique relies on an axially elongated focus to capture volumetric images. The excitation beam's extended depth of field creates a 2D projection of a 3D volume, effectively converting the traditional 2D frame rate into a 3D volumetric rate.

To highlight the power of this technique, Figure 1 shows a 300 x 300 µm scan of a Thy1-GFP-M mouse brain slice imaged with Bessel (left) and Gaussian (right) scanning. 45 optical slices taken with a Gaussian focus are vertically stacked to generate a volume image, while the same structural features are visible in a single Bessel scan taken with a 45 µm-long focus. This indicates a substantial gain in volume-imaging speed, making this technique suitable for investigating sparsely labeled samples *in-vivo.*

In partnership with Howard Hughes Medical Institute and Prof. Na Ji (UC Berkeley), Thorlabs is now offering a Bessel beam module for our Bergamo® multiphoton laser-scanning microscopes.

Capturing Volume Information in a Single Scan

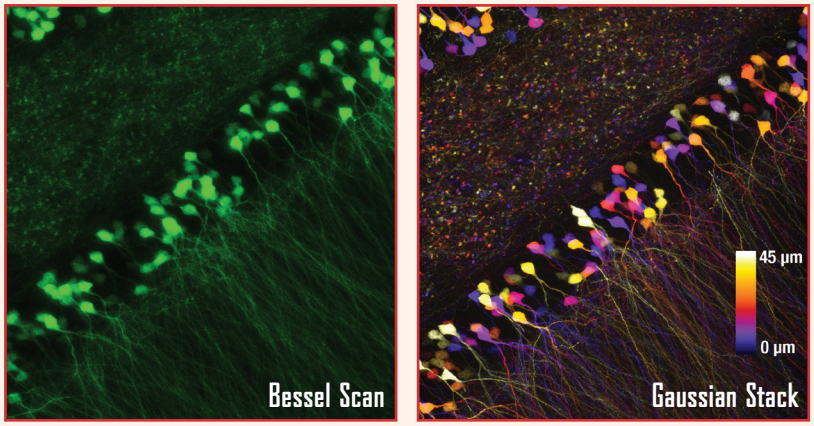


Figure 1. A single Bessel scan (left) captures the same structural information obtained from a Gaussian volume scan created by stacking 45 optical sections (right), reducing the total scan time 45-fold. The images show a brain slice scanned over a 300 µm x 300 µm area. Scan depth for the Gaussian stack is indicated by the scale bar.[b]

As demonstrated in Ji's pioneering work,[a] this rapid Bessel-beam-based imaging technique has synaptic resolution, capturing the Ca^{2+} dynamics and tuning properties of dendritic spines in mouse and ferret visual cortices. The Bessel beam module has also been used to successfully measure GCaMP6s expression in the subesophageal zone of a fly brain (Figure 2), synchrony of inhibitory neuron activity in the visual cortex of a mouse, and the network dynamics of reticulospinal neurons in the hindbrains of zebrafish larvae.

Extended Focus for *In-Vivo* Studies

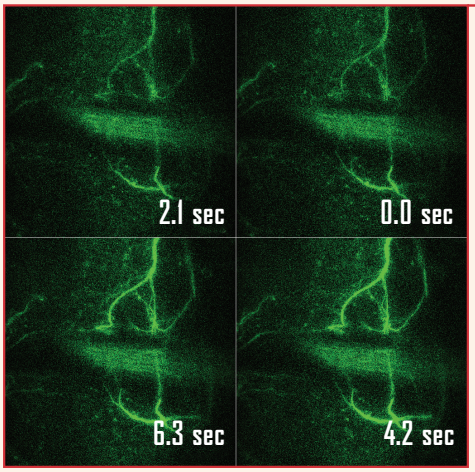
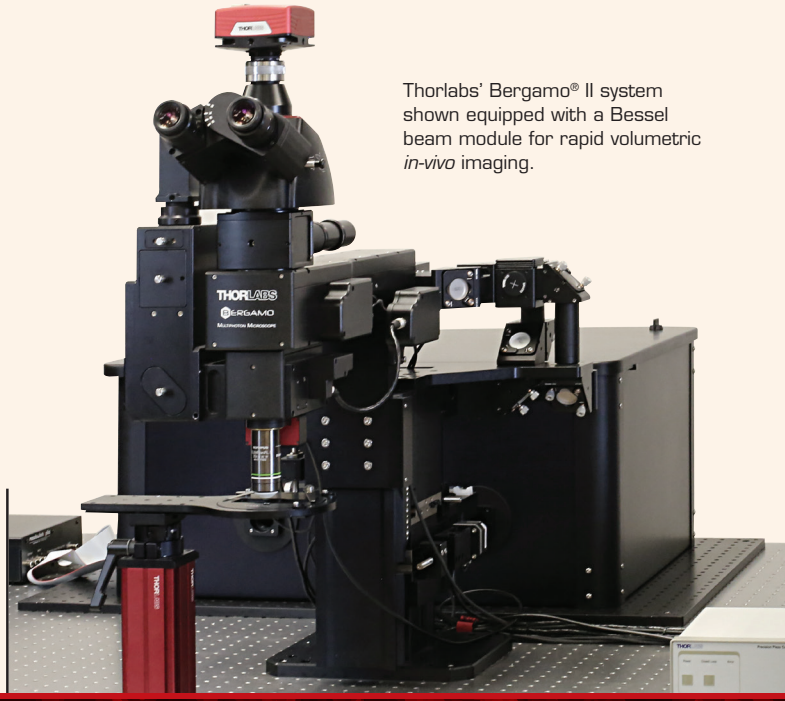


Figure 2. GCaMP6s expression in two neuron pairs arborized within the subesophageal zone of a fly brain. Ca^{2+} transients are captured by XY-scanning a 30 µm Bessel focus at a rate of 30 frames per second. A single Bessel scan corresponds to an XY-scan with no translation in the Z-direction.[c]



Thorlabs' Bergamo® II system shown equipped with a Bessel beam module for rapid volumetric *in-vivo* imaging.

a. R. Lu *et al.*, "Video-rate volumetric functional imaging of the brain at synaptic resolution," Nat. Neurosci. **20**, 620 - 628 (2017).
b. Sample Courtesy of Qinrong Zhang, PhD and Matthew Jacobs; Ji Lab, Department of Physics, UC Berkeley
c. Sample Courtesy of Zepeng Yao; the Scott Lab, Department of Molecular & Cell Biology, UC Berkeley