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Time-lapse imaging of myelin dynamics by Higher Harmonic Generation Microscopy



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Higher Harmonic Generation Imaging



New ultra broadband femtosecond lasers

Specifications

0.8 -

0.2

-0.8

- Spectral bandwidth: 990 -1140 nm (@-10dB)
- Pulse duration: typ. 30 fs
- Repetition rate: 30 MHz
- Output power: 3 W

Features

- No water cooling, no fan
- Dispersion pre-compensation module integrated
- Remote controllable
- Active power stabilization always at optimum



- SHG:
- visualizing connective tissues, collagen, cytoskeletal structures and fibrillar proteins
- THG:
 - imaging of interfaces, lipid rich structures, cell membranes and organelle boundaries
- 2PE/3PE:
- Imaging of cell cytoplasm and elastin^{1,2}

Objective transmission

Most microscope objectives are not transparent for 3rd Harmonic of 1030 nm - but for 3rd Harmonic above 1080 nm

• Simultaneous excitation of 2 and 3 photon processes!



Nearly perfect Gaussian beam profile (M² < 1.25)
 No training / no installation required







Higher Harmonic Generation Imaging of physiologically viable human brain tissue

Three-dimensional cutaway from a z-stacked THG image. Volumetric rendering ($100 \times 100 \times 100 \mu m$) image highlighting cortical grey matter, GM, showcasing the technique's capability to provide detailed structural insights.

(B) Comparative analysis of myelin imaging using THG (left) against LFB staining (right) of cortical tissue. Insets highlight myelinic swellings visible in both THG and LFB images, with THG's sensitivity to lipid bodies (red arrow in THG inset) and swelling (white arrowheads, purple arrows in LFB staining).



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Subtle alterations in myelin morphology during THG imaging.

(A) Multi-harmonic overlayed micrograph of a myelinated axons in human cortical slice culture (DIV12). Lipofuscin in neuronal soma is broadly fluorescent across SHG (red at 525nm) and 2PF (blue at >580 nm) channels (arrowhead).

(B) Irregularities in myelin sheath develop quickly (blue and pink arrows)
following 1 mM sodium azide and 0.2 mM glutamate stimulation, resulting in
the development of myelin swellings during 2-hour acquisitions (white arrow).
Time Lapsed z-stacks were drift corrected and the axon in A (yellow outline)
was traced and straightened in ImageJ.

(C) Post-stimulation, myelin membrane swelling (ab- and adaxonal) becomes distinct (black arrowhead) as shown in the profile plot (white dotted line in B). Scale bars in A are 20 μ m. Scale bar in B is 10 μ m. For more information, see **ref 3**.



µm from axonal axis

References

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Summary

- New class of ultrabroadband femtosecond lasers allow for simultaneous excitation of 2 and 3 photon processes (Higher Harmonic Imaging)
- High peak power Low average power allows for long term imaging
- Low laser power (5 mW) preserves the tissue viability over extended periods during THG microscopy
- Prolonged THG imaging is able to detect experimentally induced subtle alterations in myelin morphology.



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