Novel Sub 50 fs Lasers For Improved Multiphoton Microscopy

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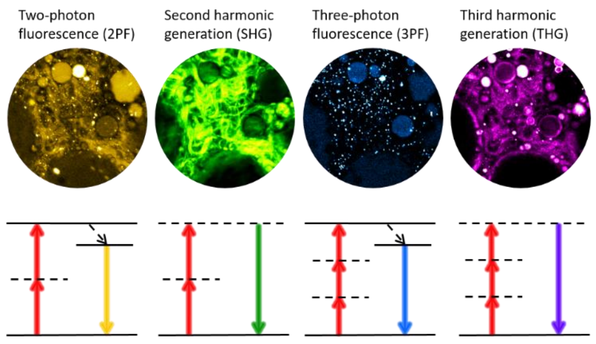
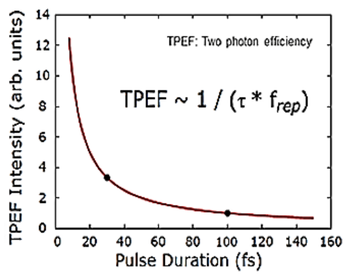
Introduction



Multiphoton microscopy allows for

- Label-free microscopy
- Higher contrast
- Deeper imaging
- 3D imaging
- Low photodamage



*Shorter pulses lead to increased two photon efficency

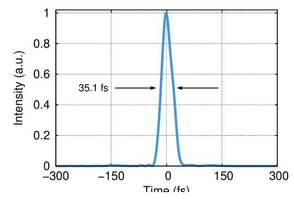
Novel Sub 50 fs Fiber Lasers

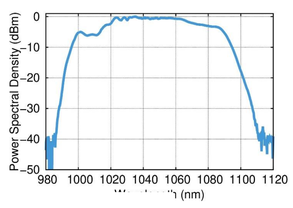
Specifications

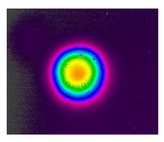
- Pulse duration: < 50 fs (typ. 40 fs)
- Repetition rate: 30 MHz
- Output power: 200 mW & 2 W
- Peak power: 166 kW & 1.6 MW
- Nearly perfect Gaussian beam profile

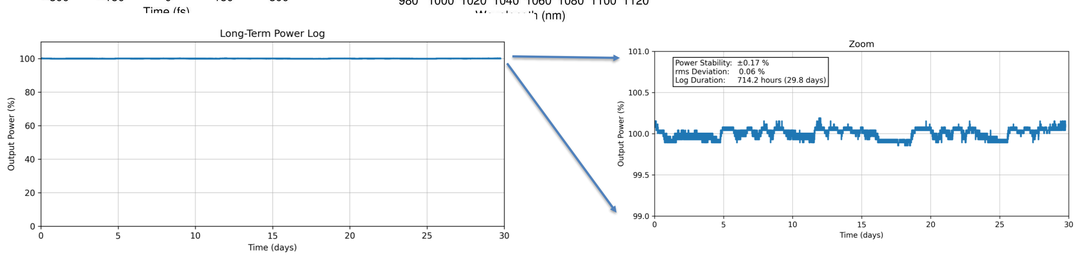
Features

- No water cooling, no fan
- Dispersion precompensation module integrated
- Remote controllable
- Active power stabilization - always at optimum
- No training / no installation required







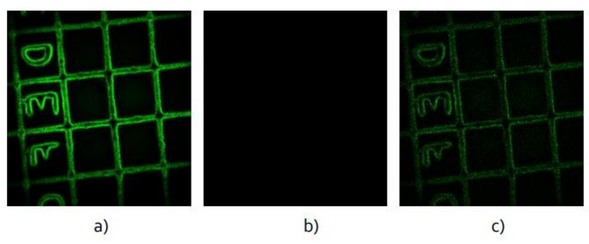


Advantages of Shorter Pulses

40 fs vs. 160 fs

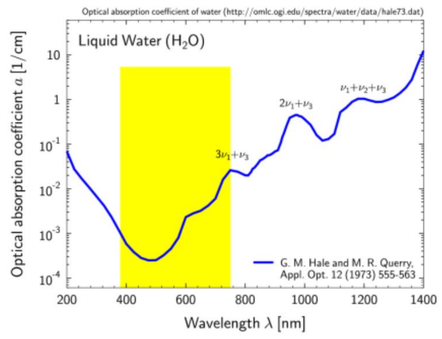
- ~4x higher efficiency (2 Photon) and > 16x higher efficiency (3 Photon) (same power)
- ~4x reduced thermal heating
- ~2x reduced photo induced damage (same peak power)
- Higher contrast
- Higher resolution
- Deeper scan depth

Pulse duration	Peak power (30 MHz)	Average power (for the same TPEF)
40 fs	16 kW	19 mW
160 fs	16 kW	96 mW



Third harmonics of a calibration grid (Ibidi) with 50 µm²
a) 4.7 mW full spectrum short pulse (sub 50 fs, VALO Femtosecond Series)
b) 6 mW with laser spectum limited to 10 nm (~ 160 fs)
c) Scaled up contrast for the 6 mW laser spectrum limited to 10 nm bandwidth

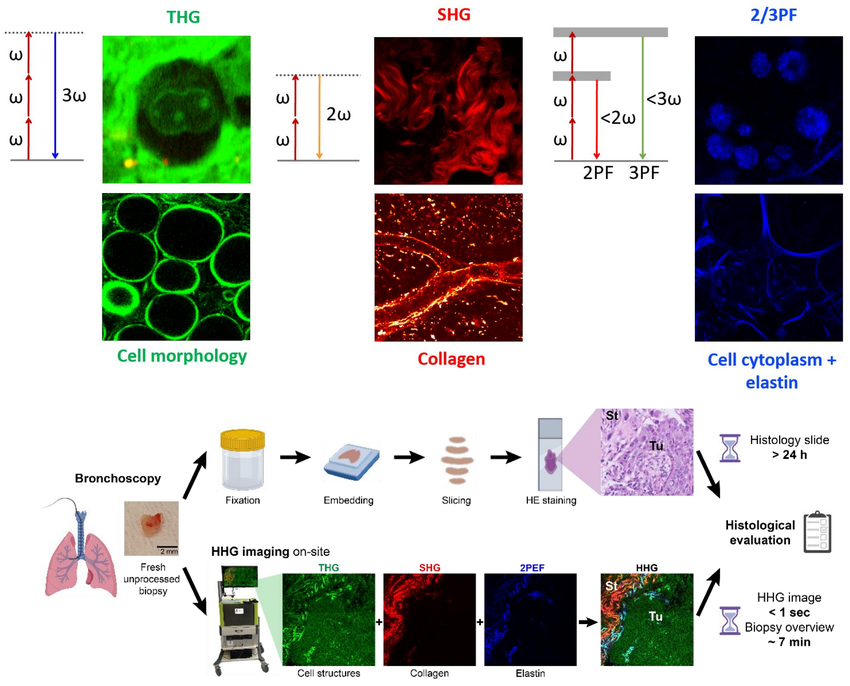
How to Choose a Laser for SHG / THG / 2PF / 3PF



- Longer wavelengths to avoid scattering in tissue
- Wavelengths outside of absorption regions – espescially avoiding water absorption
- Wavelength ≥ 1050 nm to be able to have epi-detection of THG
- Shortest pulses (below 50 fs) to enhance nonlinear effects overcoming linear absorption (heating)
- Use of high peak power and low average power to avoid photo bleaching

→ Sub 50 fs lasers at 1050 nm for SHG /THG /2PF /3PF

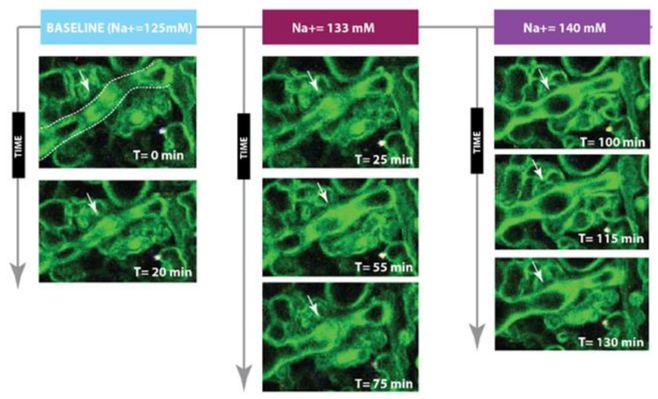
Higher Harmonic Generation Microscopy for Instant Pathology [1]



- SHG and Multiphoton Fluorescence signals have Molecular Origin/Specificity
- THG is generated at all density variations: 3D Phase-Contrast Microscopy
- HHG Microscopy allows for lable free and real- time images of histopathology quality

Longer Imaging Time Due to Reduced Photodamage [2]

Analyzing braintissue from obduction of MS patient



- Using very low average power and high peak power lasers allow for following dynamics of individual neurons over hours
- The measuring light does not induce changes
- The perfusion solution can be changed: adding salt or drugs
- The swelling increases when increasing the salt concentration

References

[1]L. Van Huizen, J. Daniels, T. Radonic, J. Von Der Thüsen, K. Kalverda, M. L. Groot, J. Annema, " Higher harmonic generation microscopy for instant on-site pathologic feedback of lung biopsies" European Respiratory Journal Sep 2022, 60 (suppl 66) 3791

[2] M.L. Groot, F. van Mourik, N. Meijns, Y. Ma, O. Prochnow, "Optimization of higher harmonic generation microscopy for acute tissue imaging", Proc. SPIE PC12384, Multiphoton Microscopy in the Biomedical Sciences XXIII, PC123840X (15 March 2023)

Acknowledgement

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Summary

New class of femtosecond lasers allow for drastically improved results

- Sub 50 fs lasers are ideal for nonlinear and multiphoton microscopy applications
- Very easy to use due to integrated dispersion precompensation module
- Signal efficiency can be drastically improved by using shorter pulses
- Lower average power reduces heating and photo damaging effects
- High peak power and low average power allow for long time imaging of living cells

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