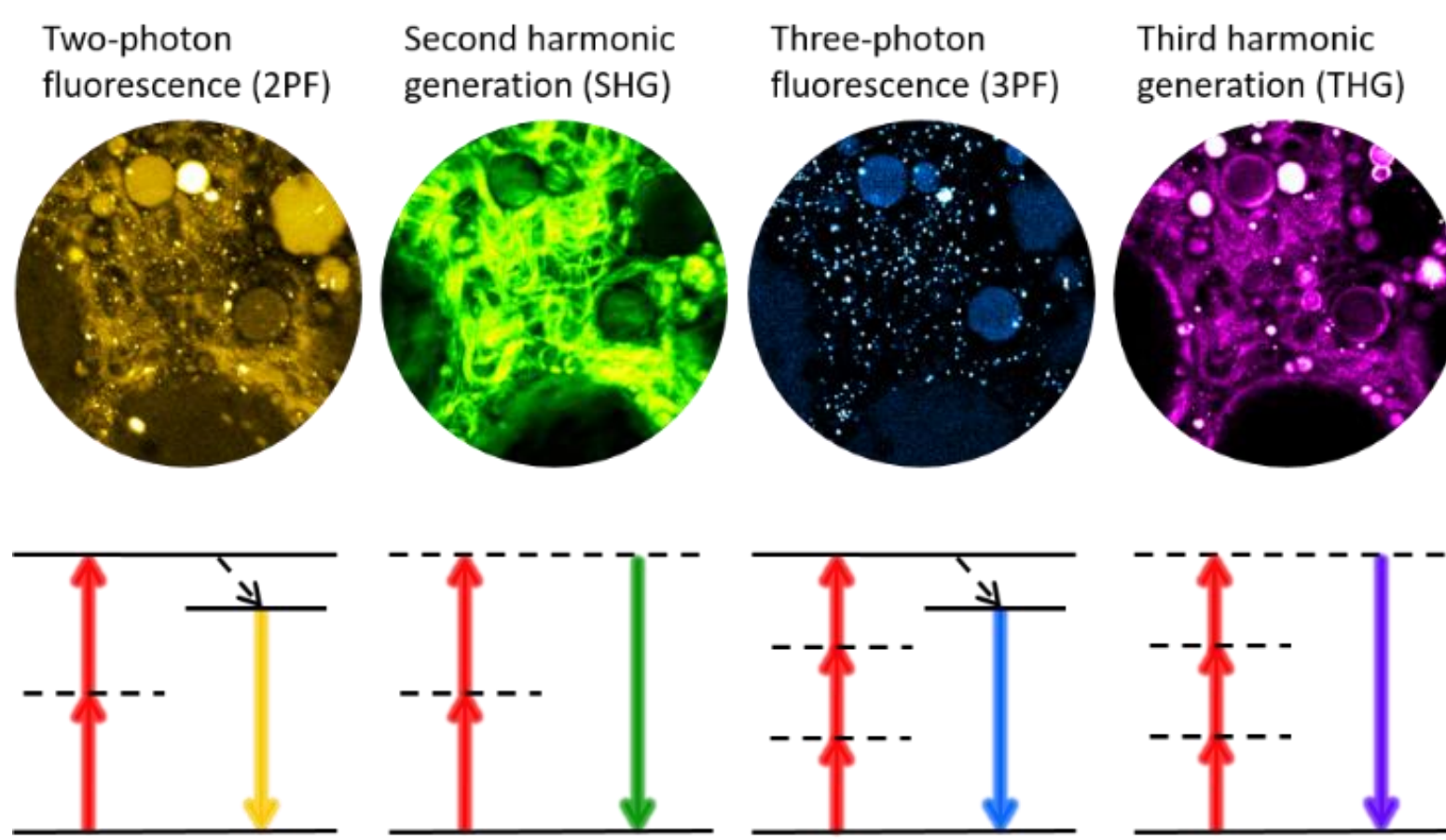


New compact ultrafast lasers for simultaneous 2 and 3 photon microscopy

O. Prochnow¹, J. Ahrens¹, D. Mortag¹, P. König¹, S. Grunert¹

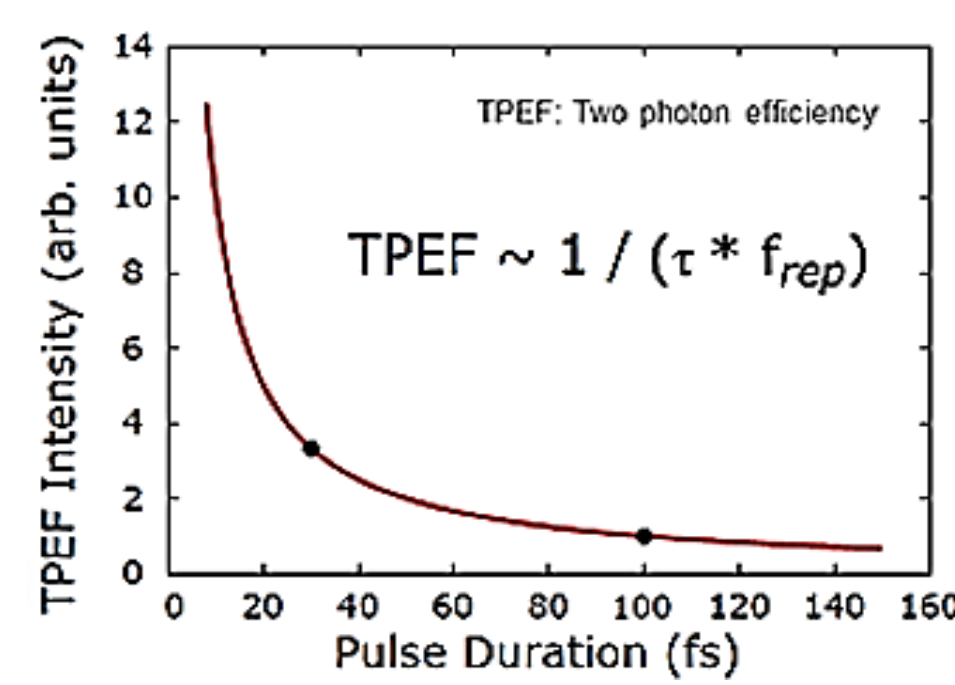
Introduction



Multiphoton microscopy allows for

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

*Shorter pulses lead to increased two photon efficiency



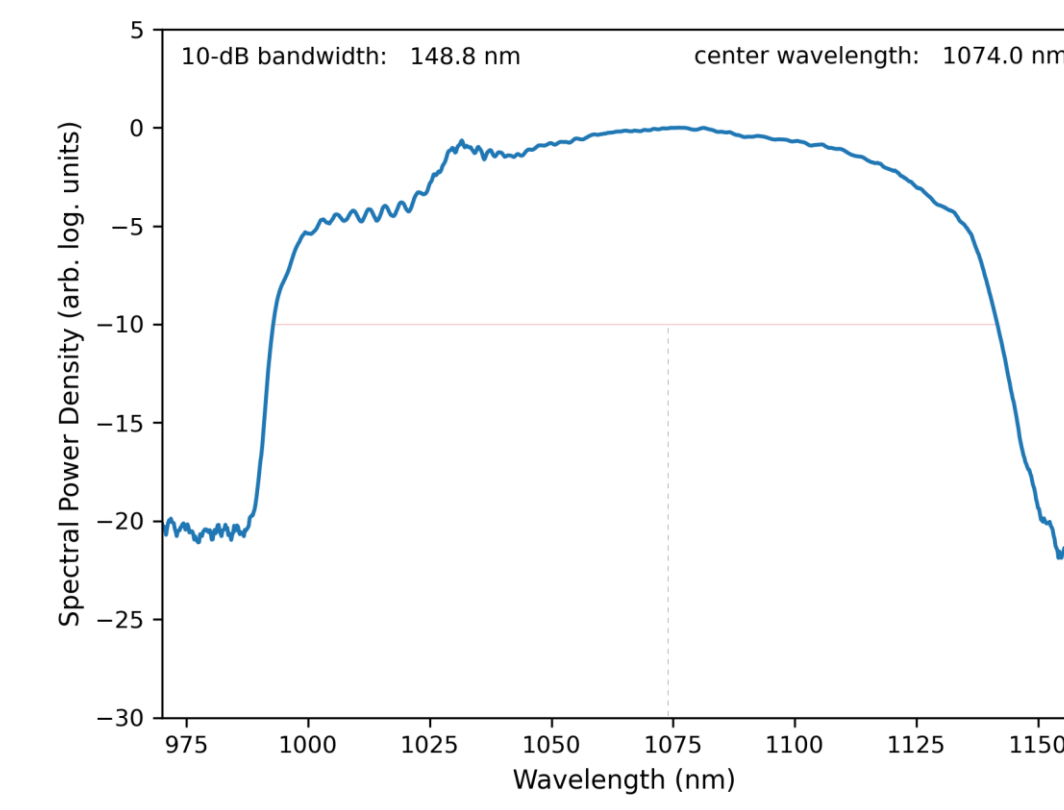
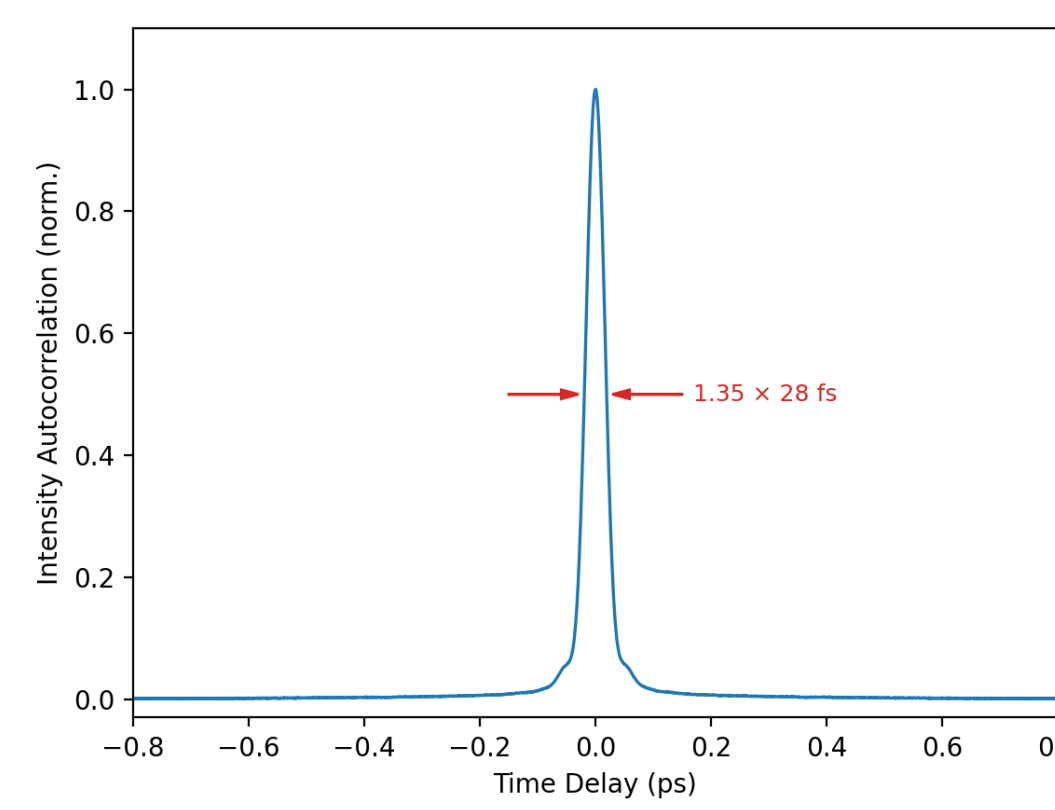
New ultra broadband femtosecond lasers

Specifications

- Spectral bandwidth: 990 nm -1140 nm (@-10dB)
- Pulse duration: typ. 30 fs
- Repetition rate: 30 MHz
- Output power: 3 W
- Nearly perfect Gaussian beam profile ($M^2 < 1.25$)

Features

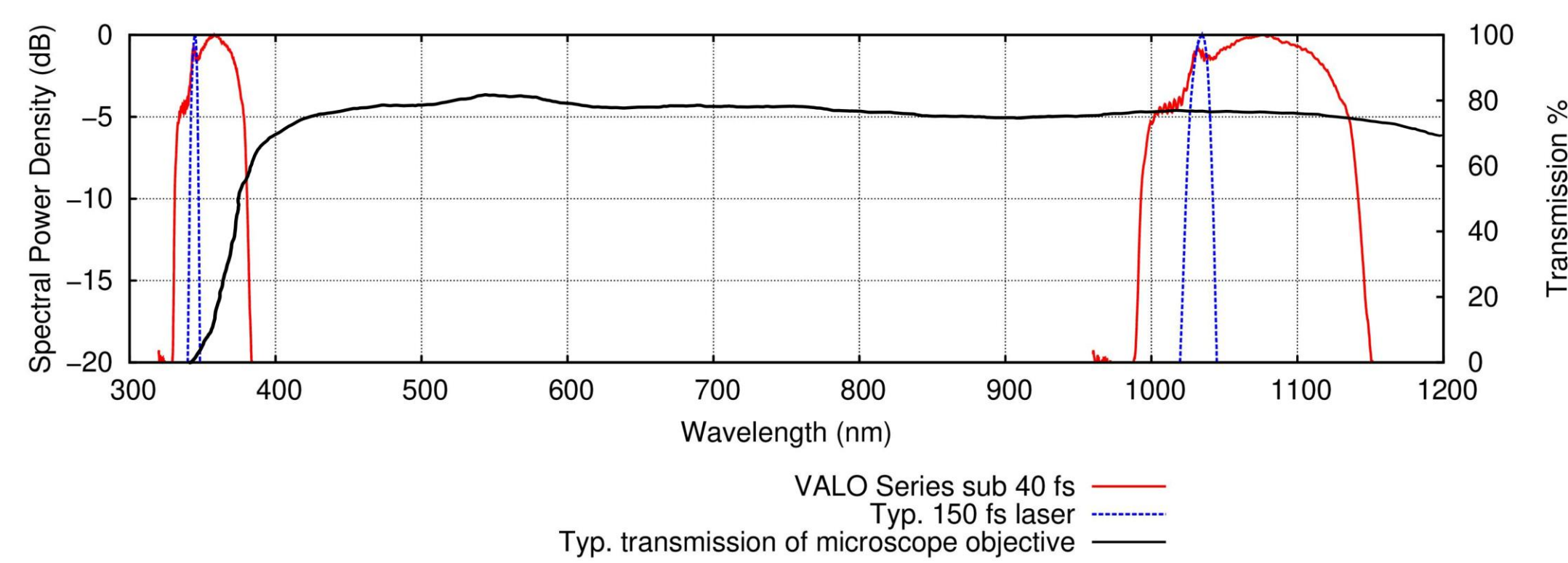
- No water cooling, no fan
- Dispersion precompensation module integrated
- Remote controllable
- Active power stabilization - always at optimum
- No training / no installation required



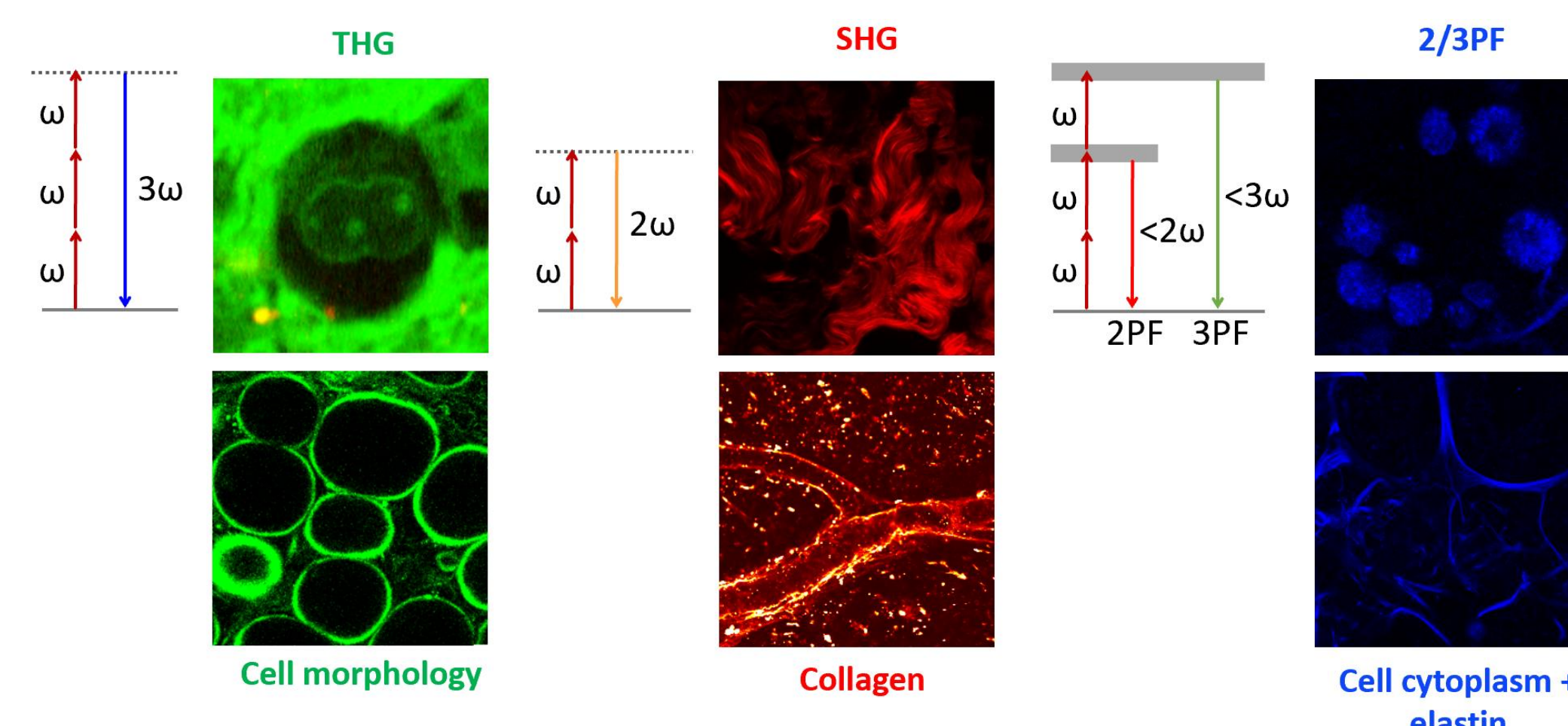
Objective transmission

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!



Label free 2 and 3 photon excitation



SHG:

- visualizing connective tissues, collagen, cytoskeletal structures and fibrillar proteins

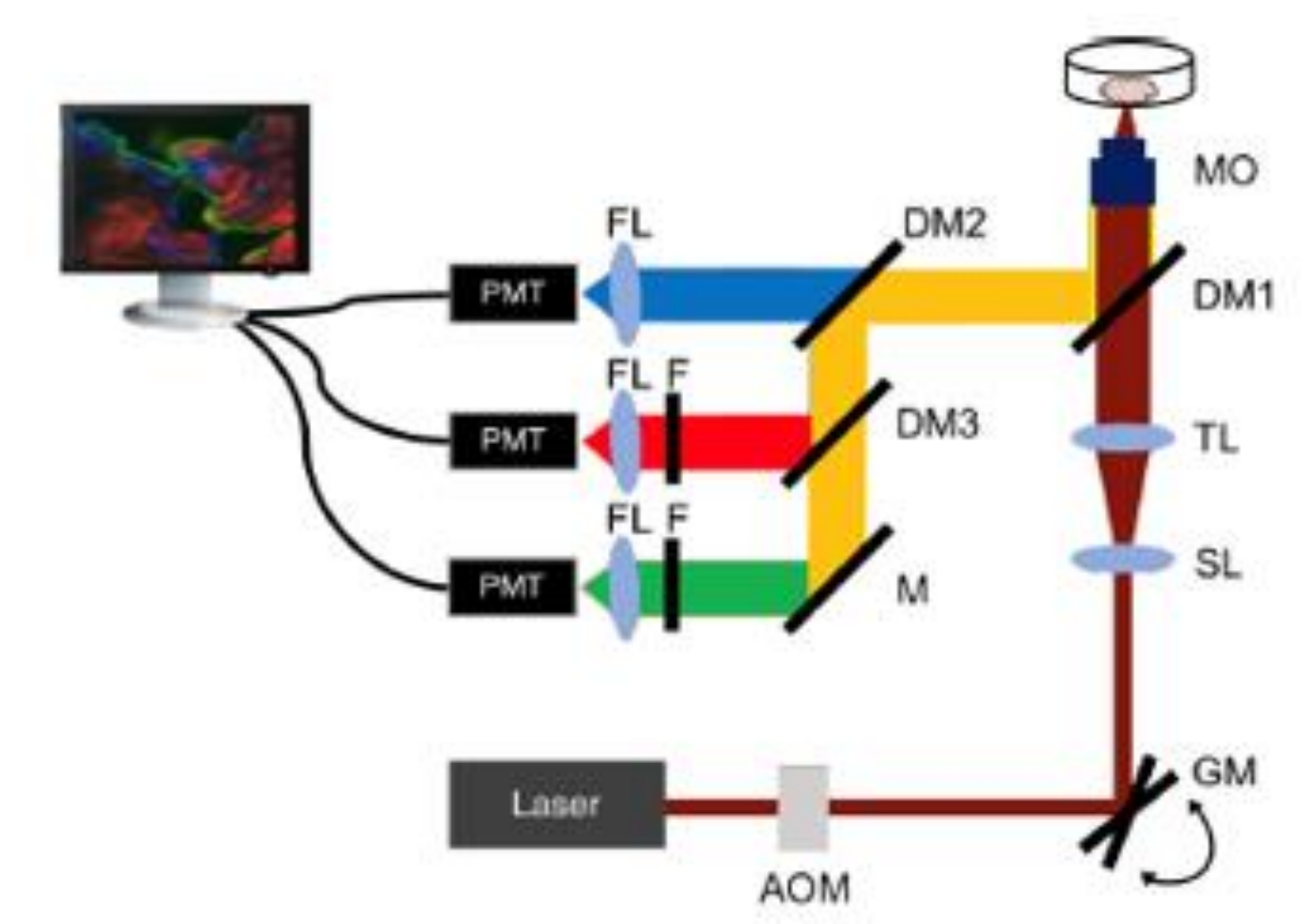
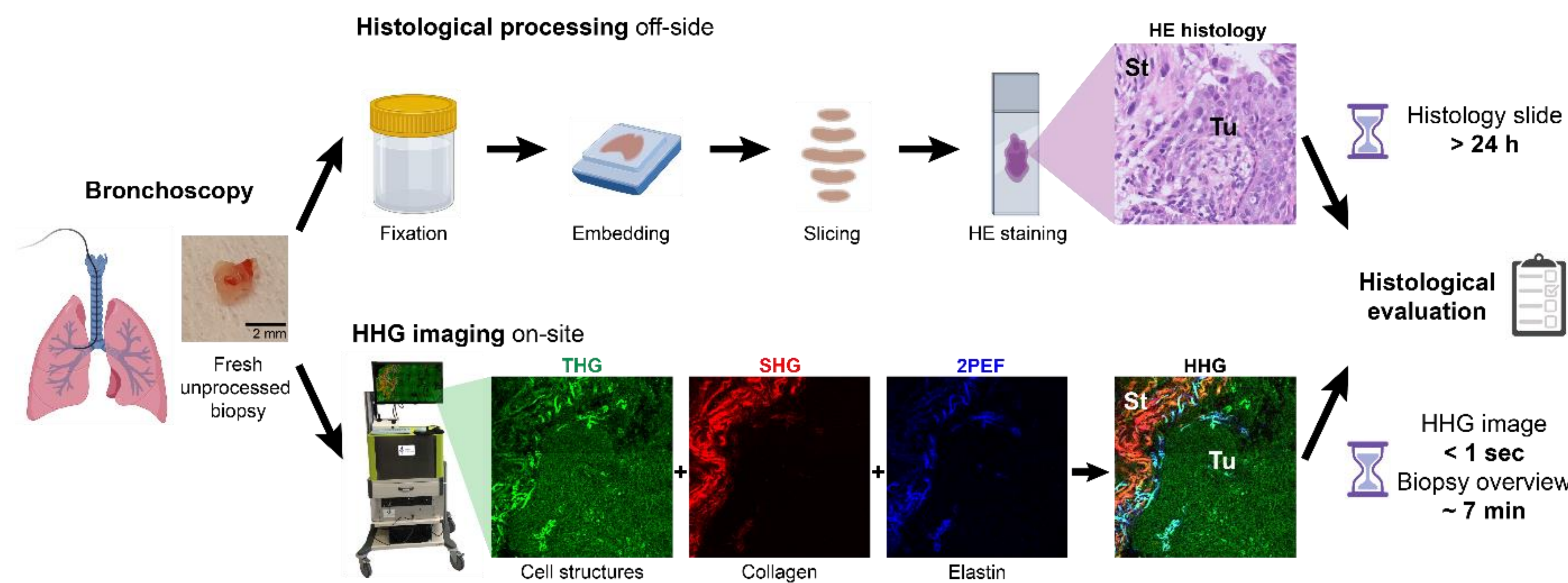
THG:

- imaging of interfaces, lipid rich structures, cell membranes and organelle boundaries

2PE/3PE:

- Imaging of cell cytoplasm and elastin

Real life application: Instant pathology



- Simultaneous excitation of 2 and 3 photon processes
- Separation of signals by dichroic mirrors
- Very short pulses – highest peak power – low average power at the probe – no heating
- Highest contrast measurements

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

The authors gratefully acknowledge the support of the group of Prof. Marloes Groot, Biophotonics and Medical imaging, Faculty of Science, Vrije Universiteit Amsterdam

Summary

New class of ultrabroadband femtosecond lasers allow for simultaneous excitation of 2 and 3 photon processes

- Spectral bandwidth of the laser is extended for higher 3 photon signals
- Very high peak power allows for very low average power
- Long term imaging - no heating of the tissue
- Dynamics of cells in acute tissue can be followed over hours and even days



Read More