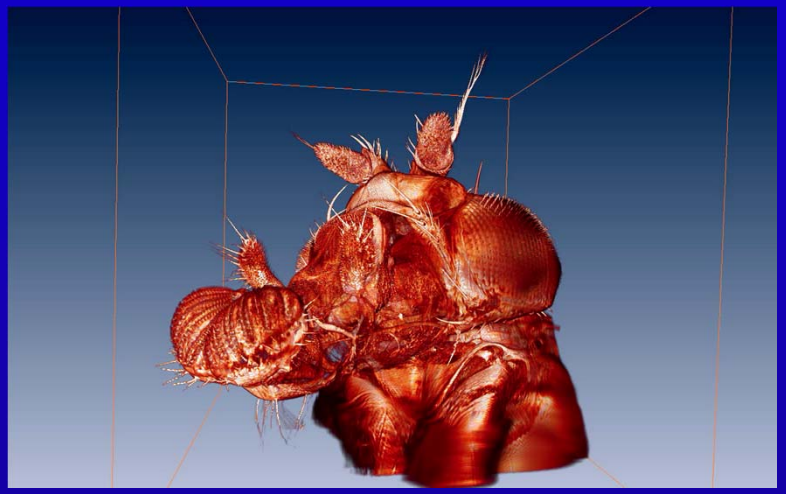
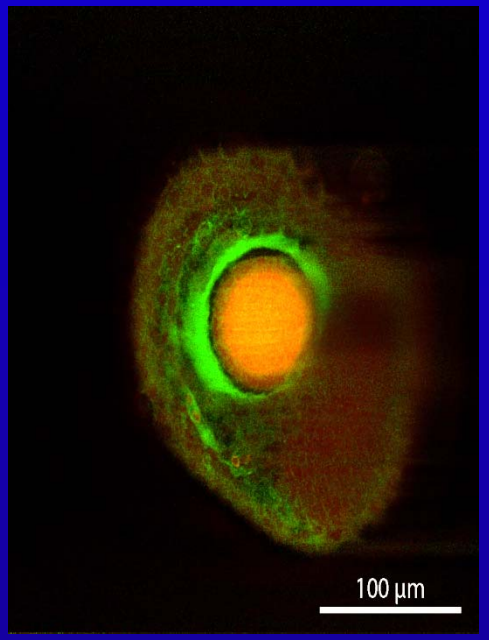
Light Wedge
and
Lightsheet-Raman Microscopy





Walter Mueller, Ulrich Leischner,
Michael Schmitt, Jürgen Popp, Rainer Heintzmann

Leibniz-Institute of Photonic Technology (IPHT),
Friedrich Schiller University of Jena

Trieste, 23.02.2017

Overview

- Introduction: Lightsheet microscopy
- Focusing steeper: The light wedge
- Fast Raman microsopy:
  FT-based Raman lightsheet

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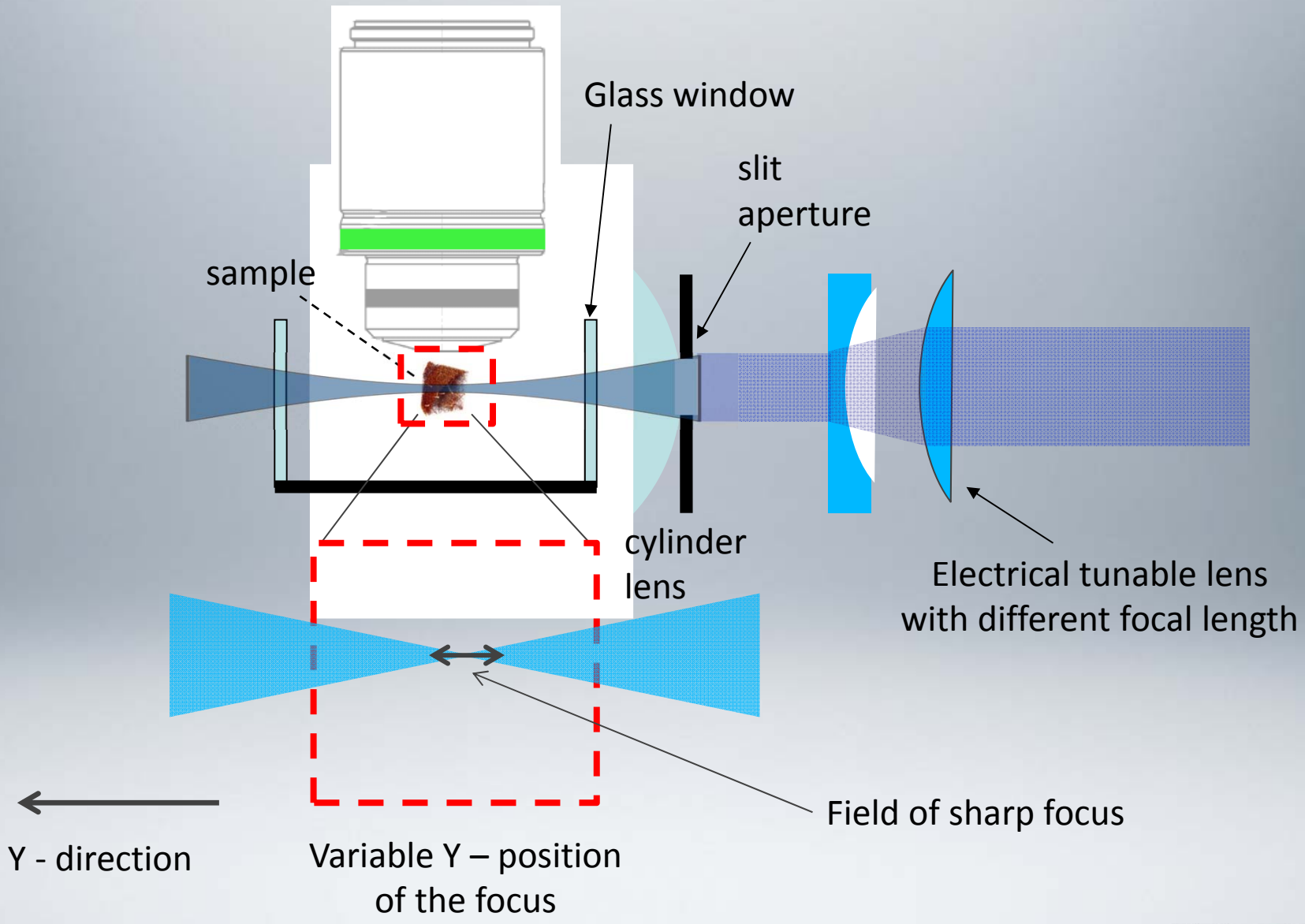
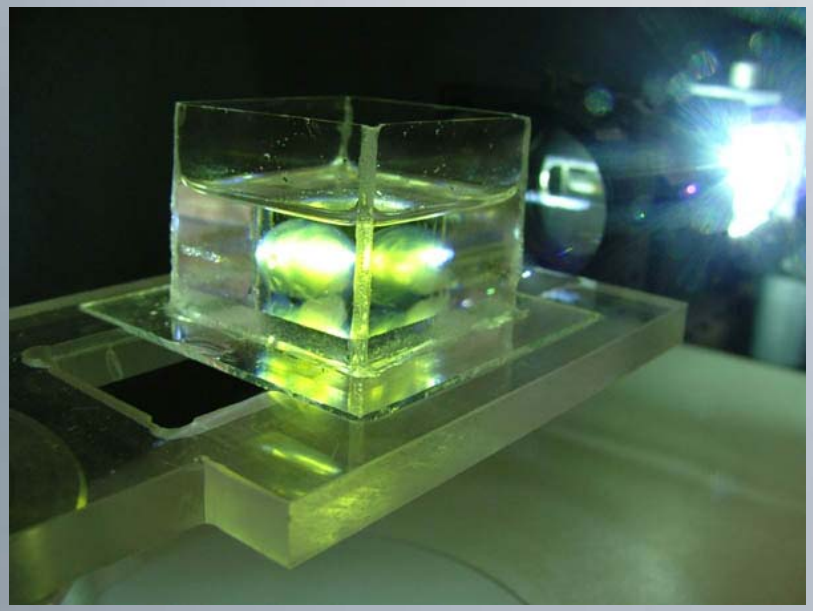
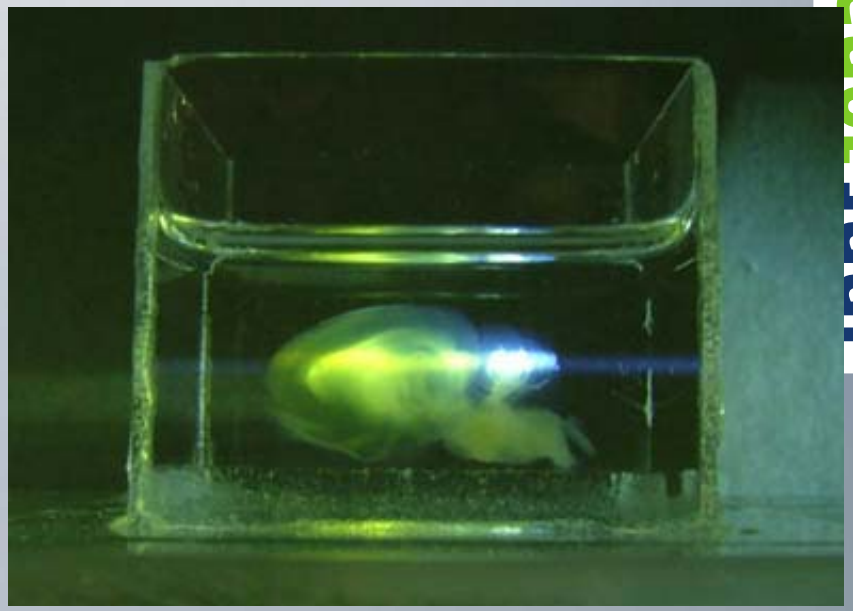
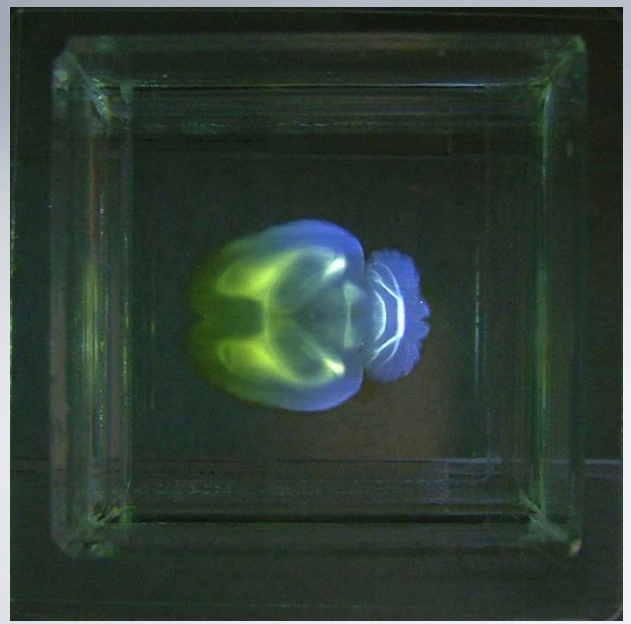


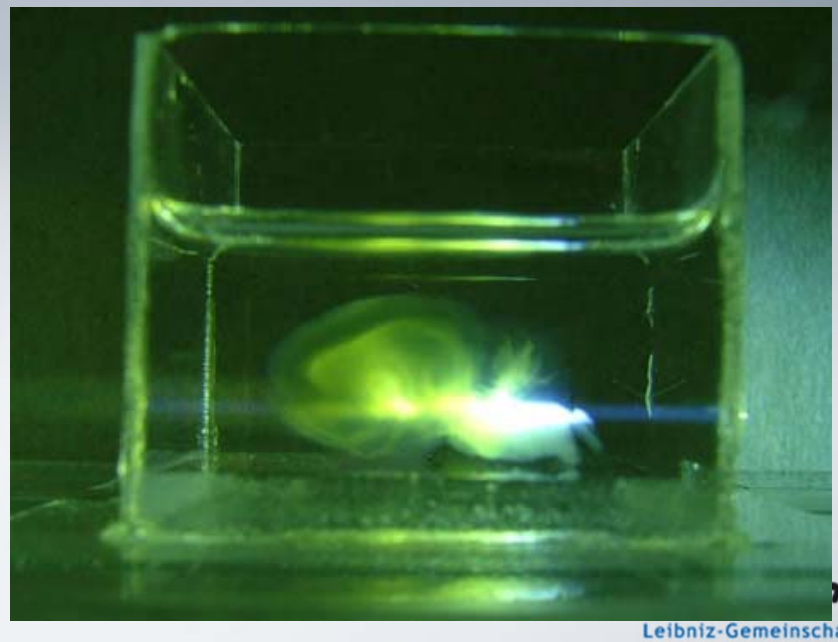
Ulrich Leischner

Optical sectioning of a mouse brain









Ulrich Leischner

IPHT Jena

Leibniz-Gemeinschaft

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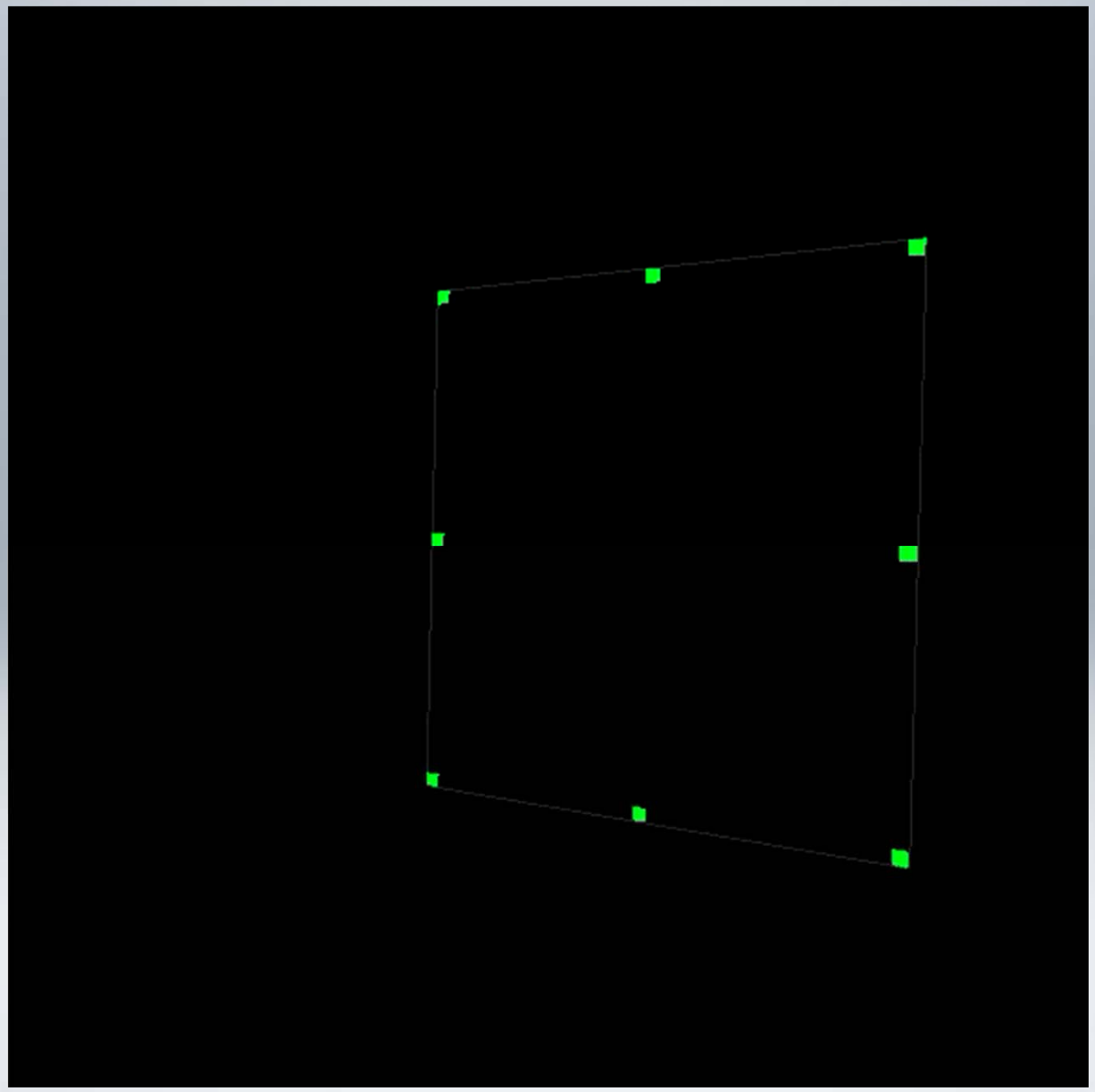

Dodt + Leischner et al., *Nature Methods* 4, 331-336 (2007)

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Mitglied der Leibniz-Gemeinschaft

Overview

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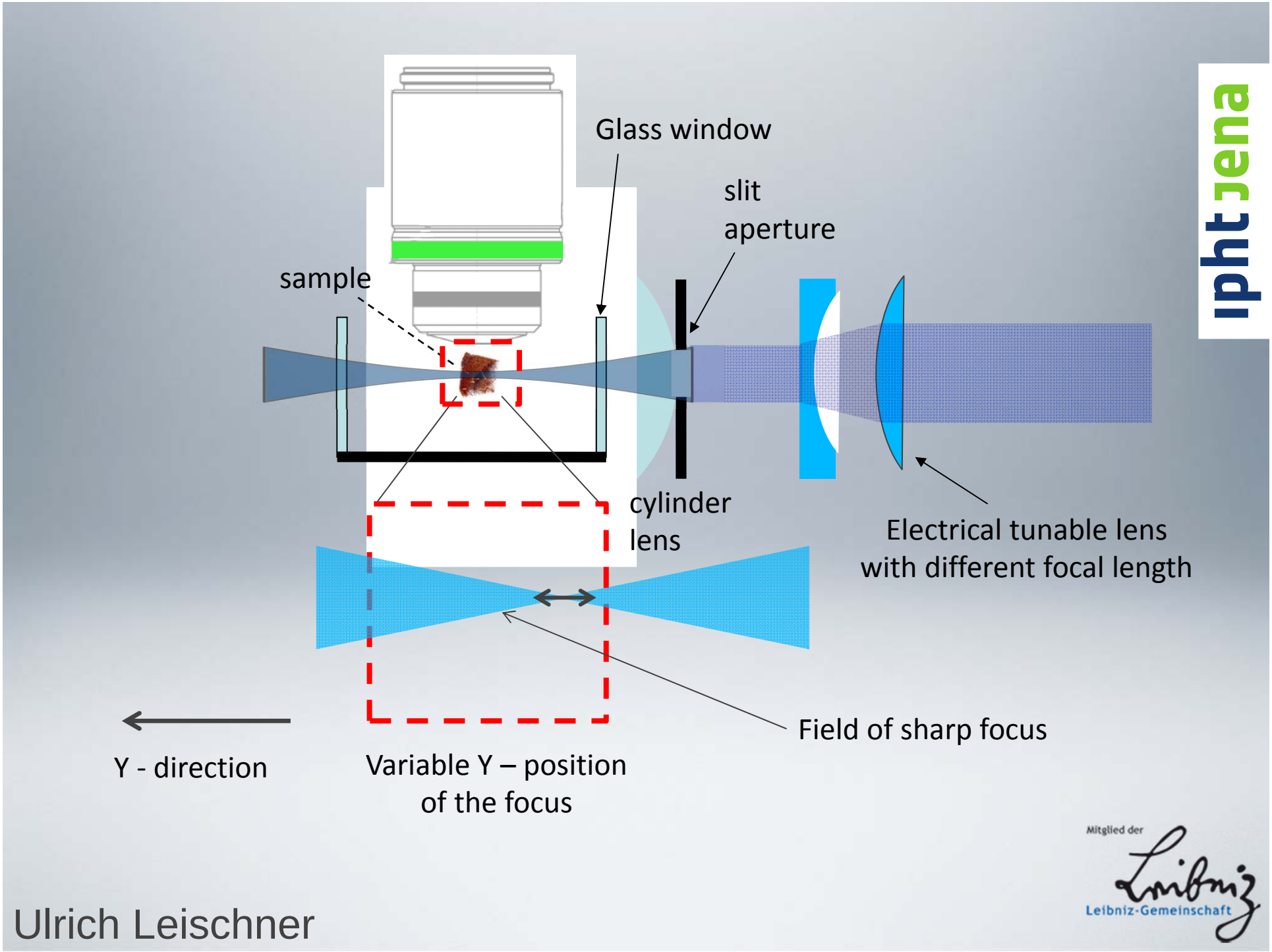
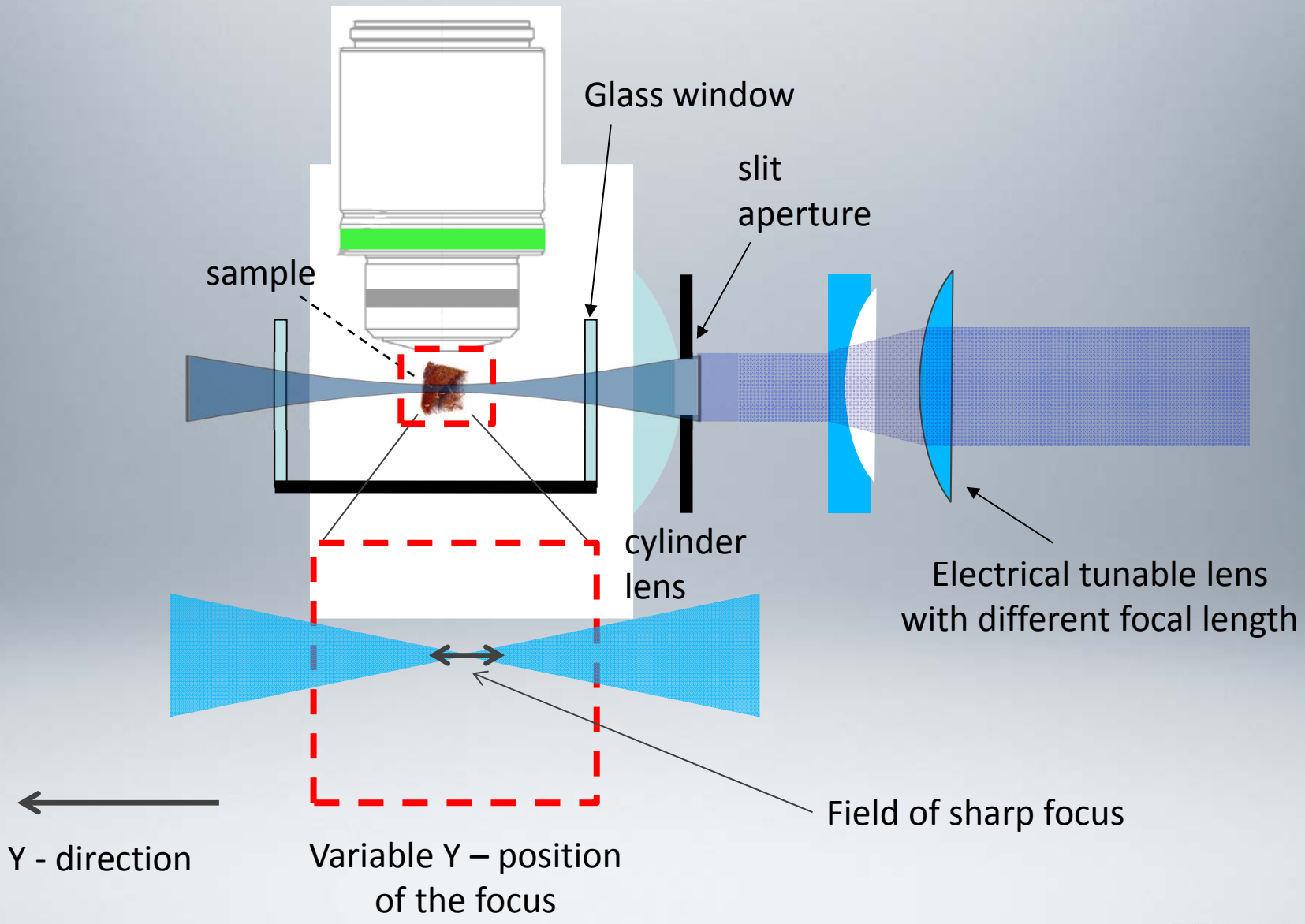
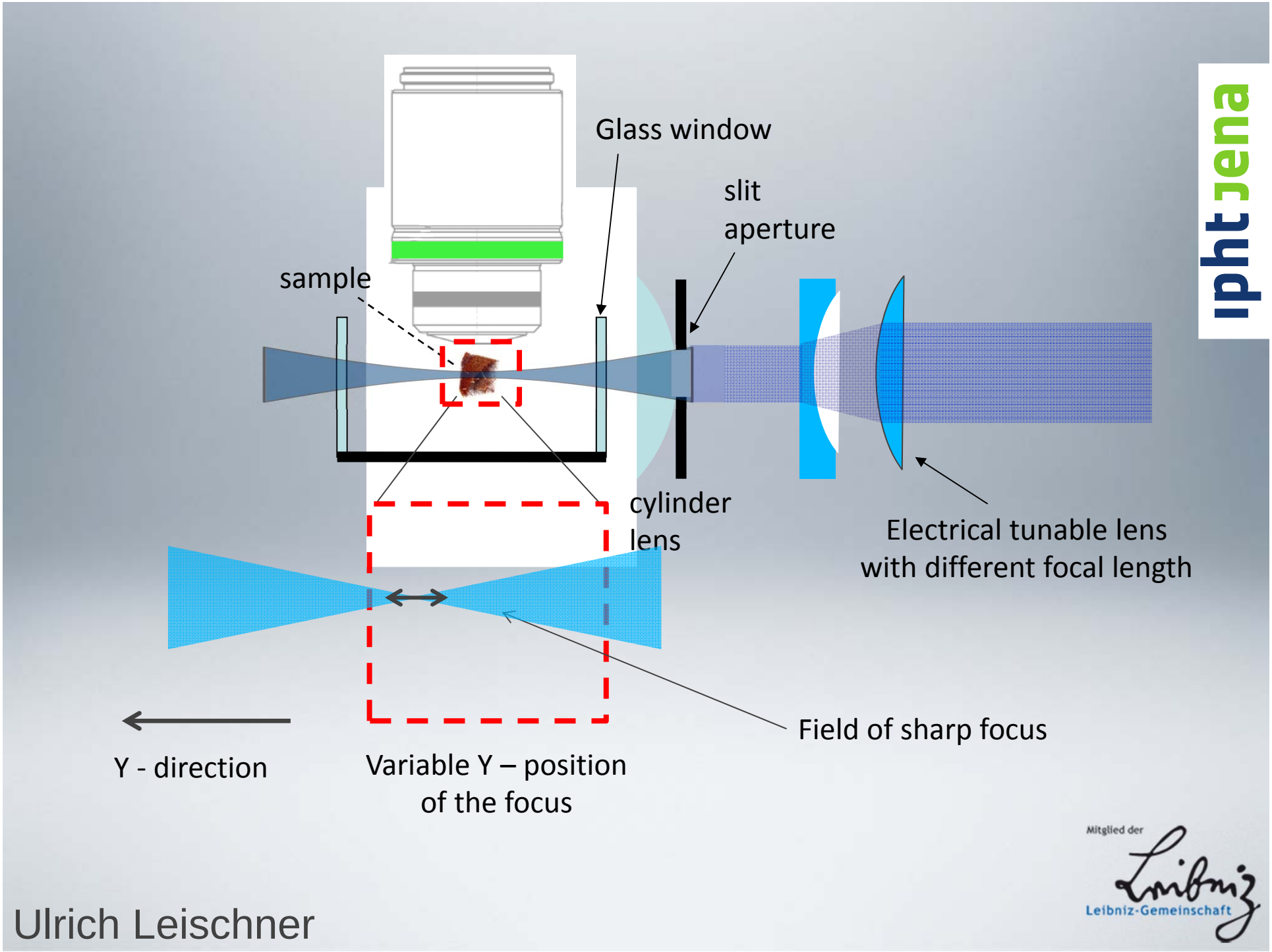


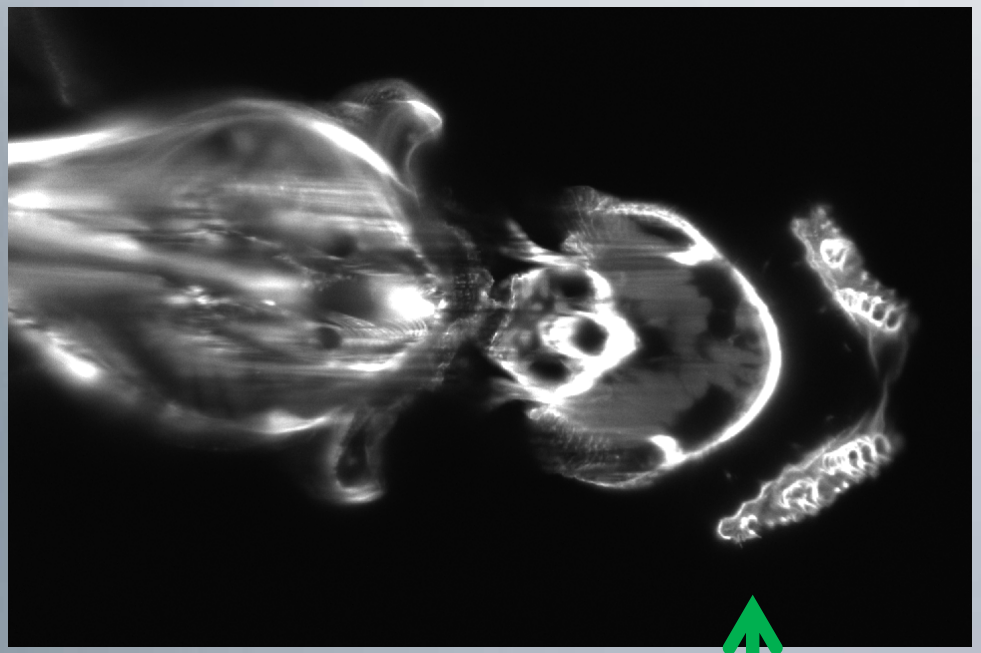
Ulrich Leischner

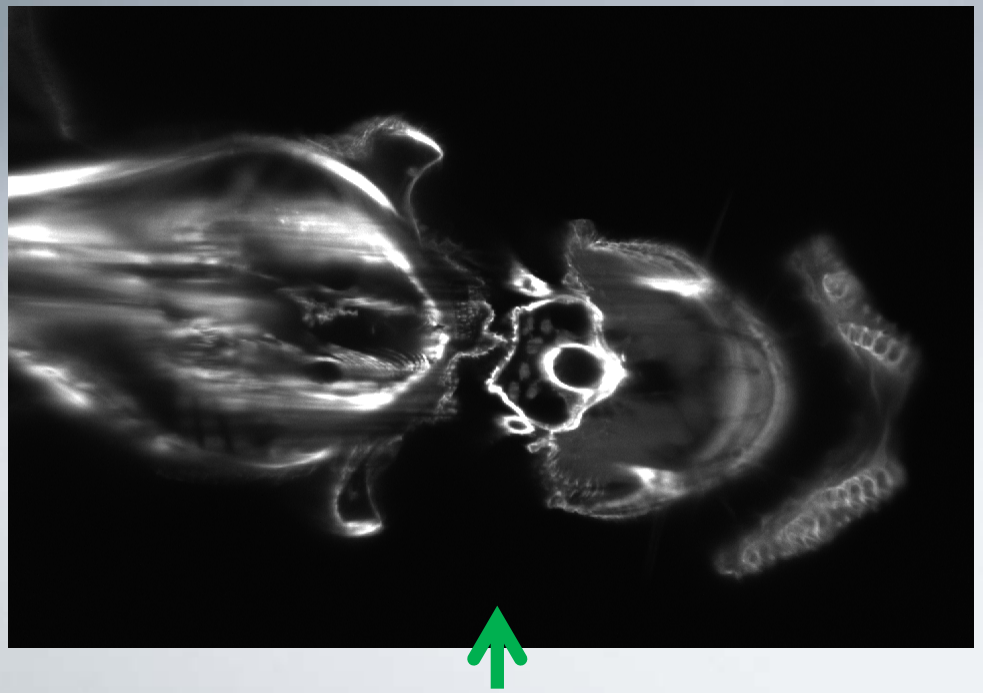


Ulrich Leischner



Ulrich Leischner



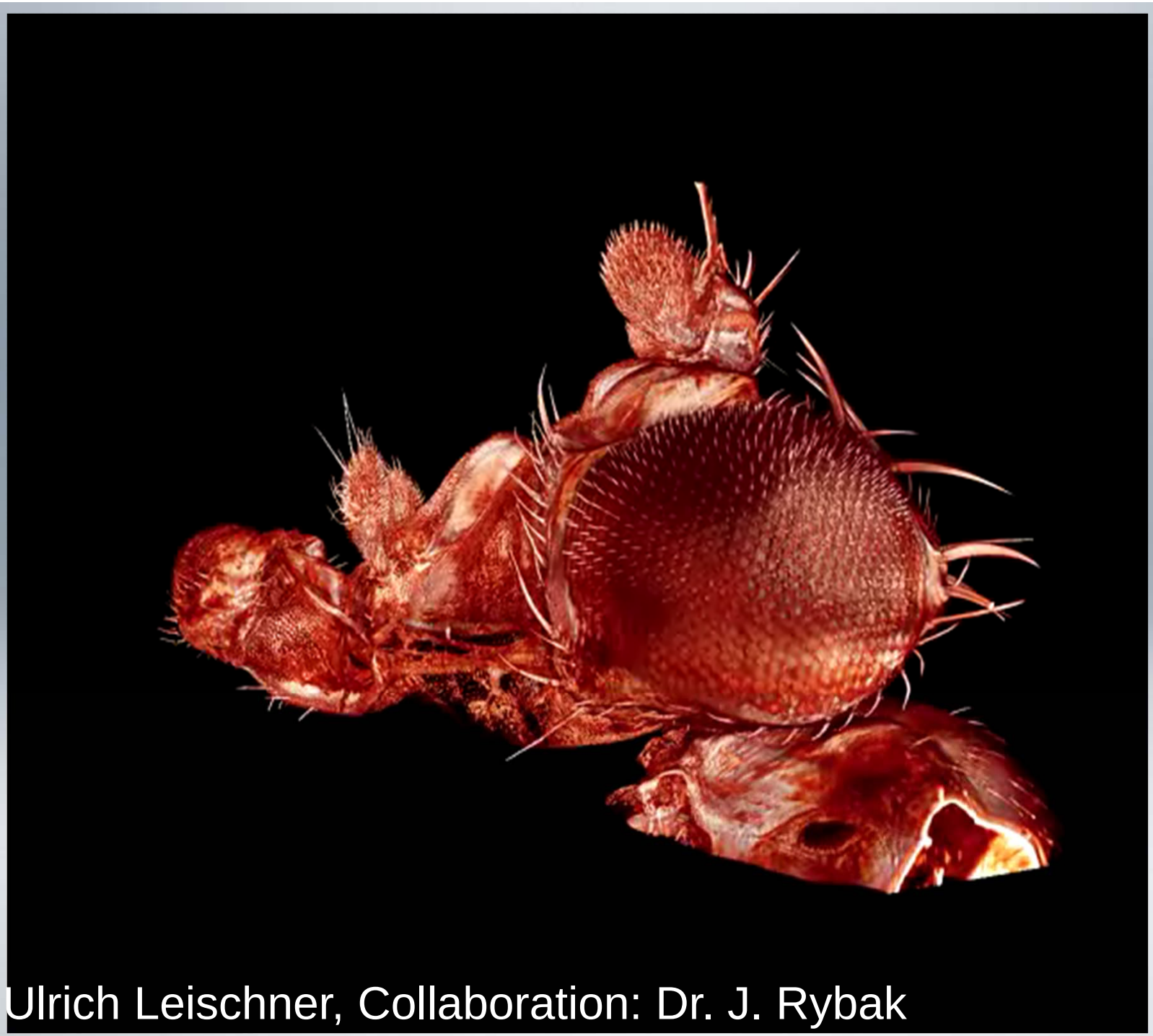


Detection of the sharp parts of the image

join images

Raw Data:
300 GB (one stack only)

Ulrich Leischner



Ulrich Leischner, Collaboration: Dr. J. Rybak

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Mitglied der Leibniz-Gemeinschaft

Ulrich Leischner, Kooperation: Dr. J. Rybak

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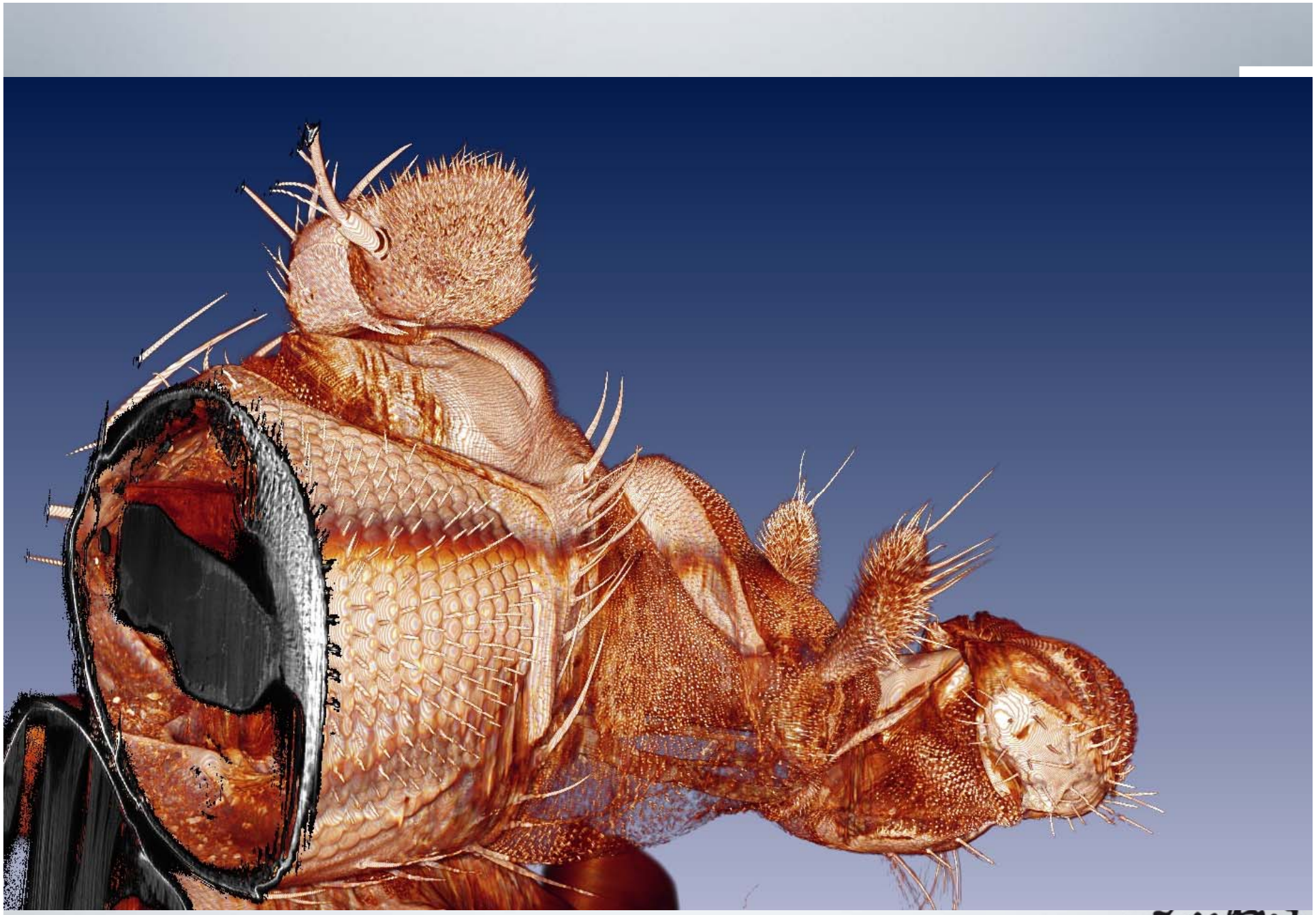


Ulrich Leischner, Kooperation: Dr. J. Rybak

Leibniz-Gemeinschaft

Ulrich Leischner, Kooperation: Dr. J. Rybak

Leibniz-Gemeinschaft

Volumetric datasets

Brain and muscle segmented in *Drosophila melanogaster*

Ulrich Leischner, Jürgen Rybak, Rolf Beutel,

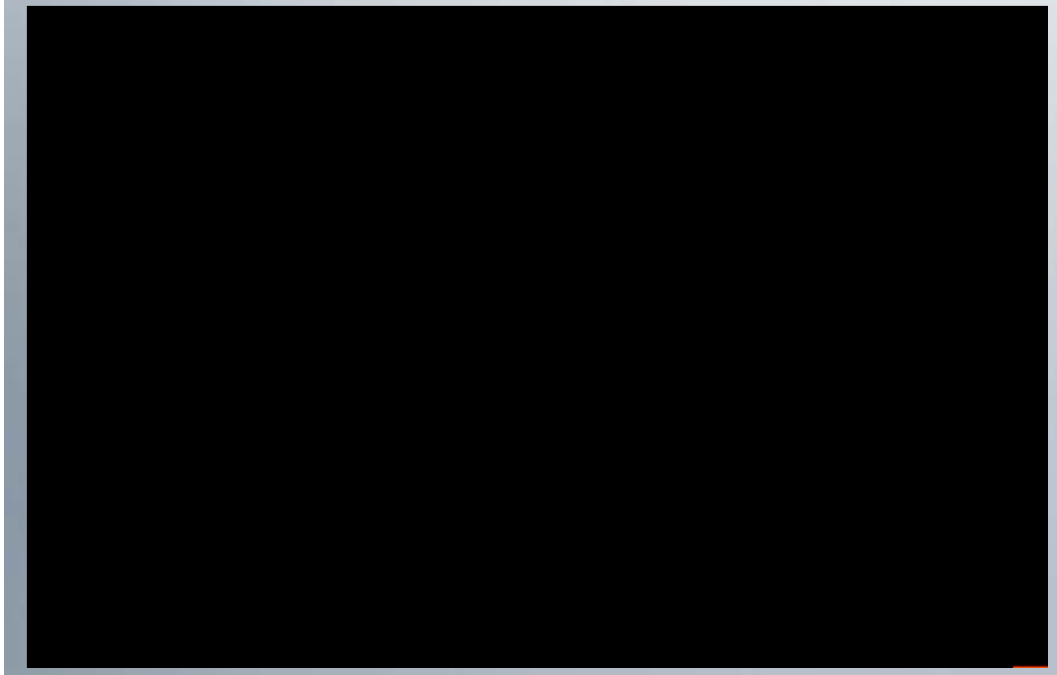
Ulrich Leischner:
Drosophila Melanogaster

Sample from Jürgen Rybak

Open File: D:\Users\pi96doc\Documents\Vorträge\Powerpoint\Material\DrosoDeconvVersusNonDeconv.avi

Deconvolved

Original



Ulrich Leischner:
Drosophila Melanogaster
larval stage

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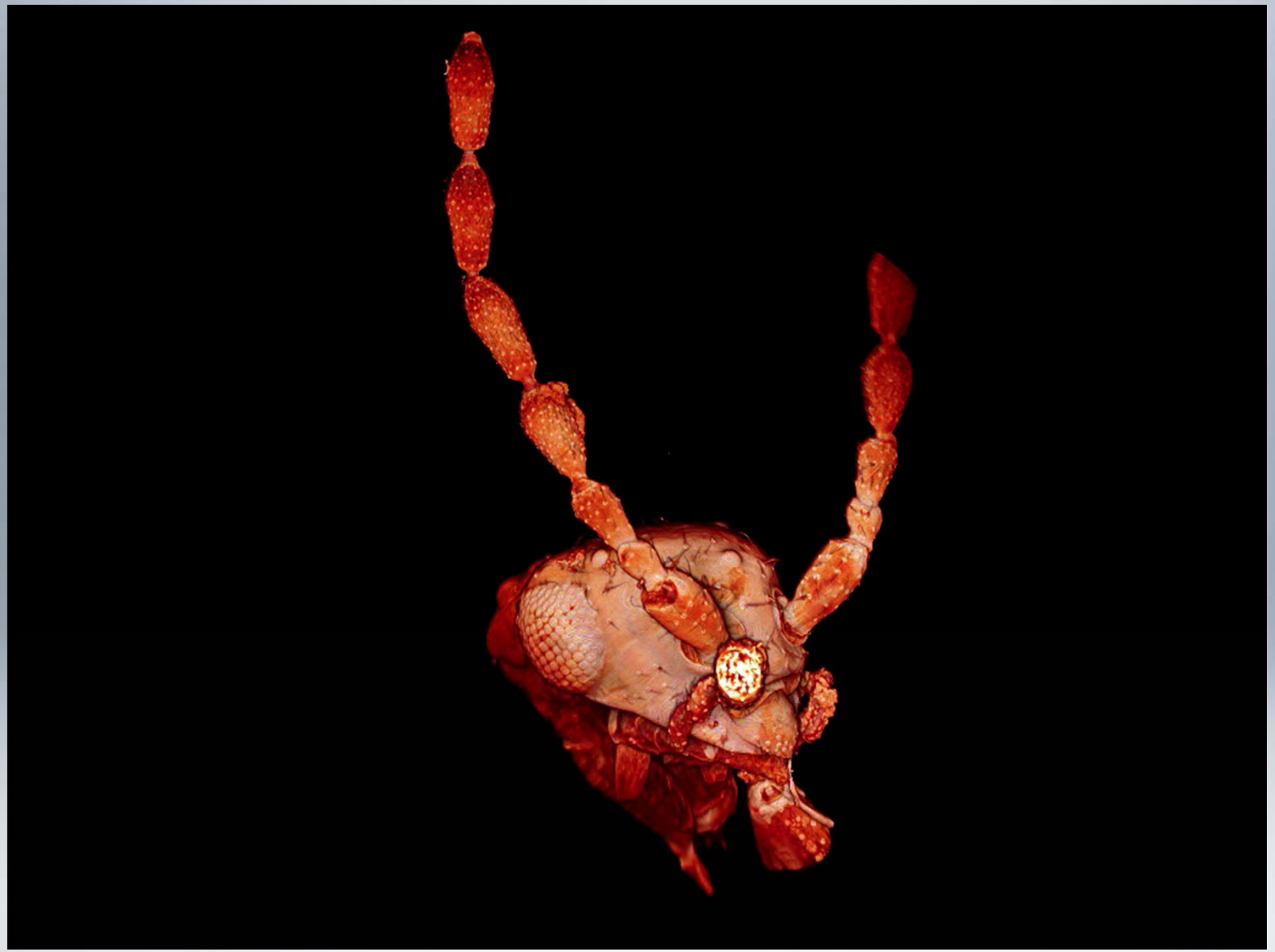
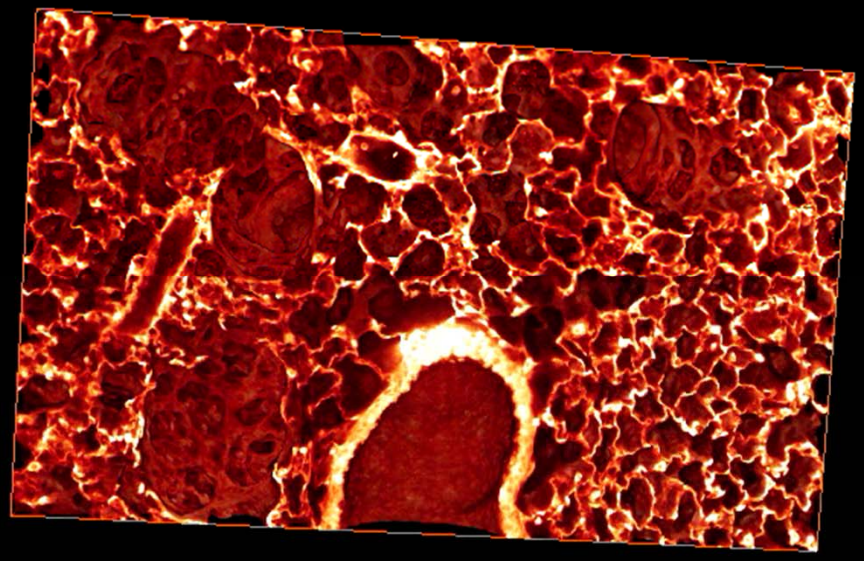


tropical zoraptera, zorotypus weidneri

Ulrich Leischner, Kooperation: Y. Matsumura, Prof. R. Beutel



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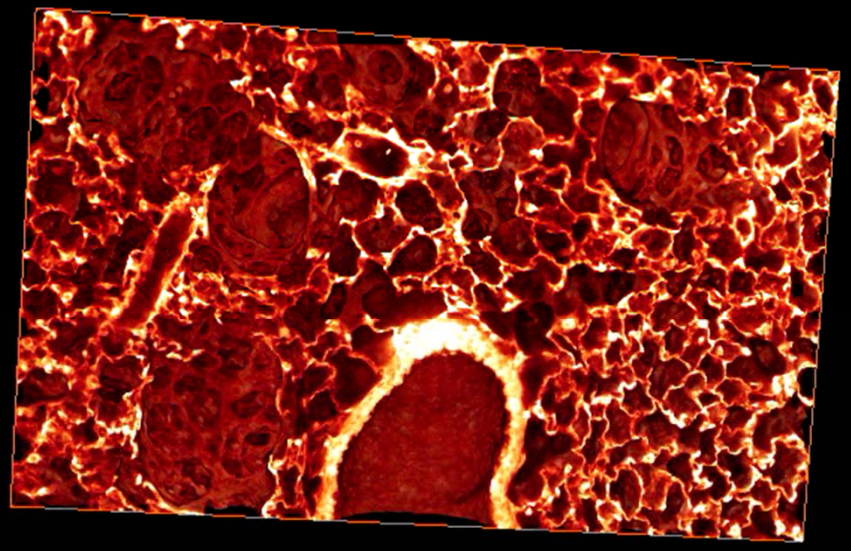
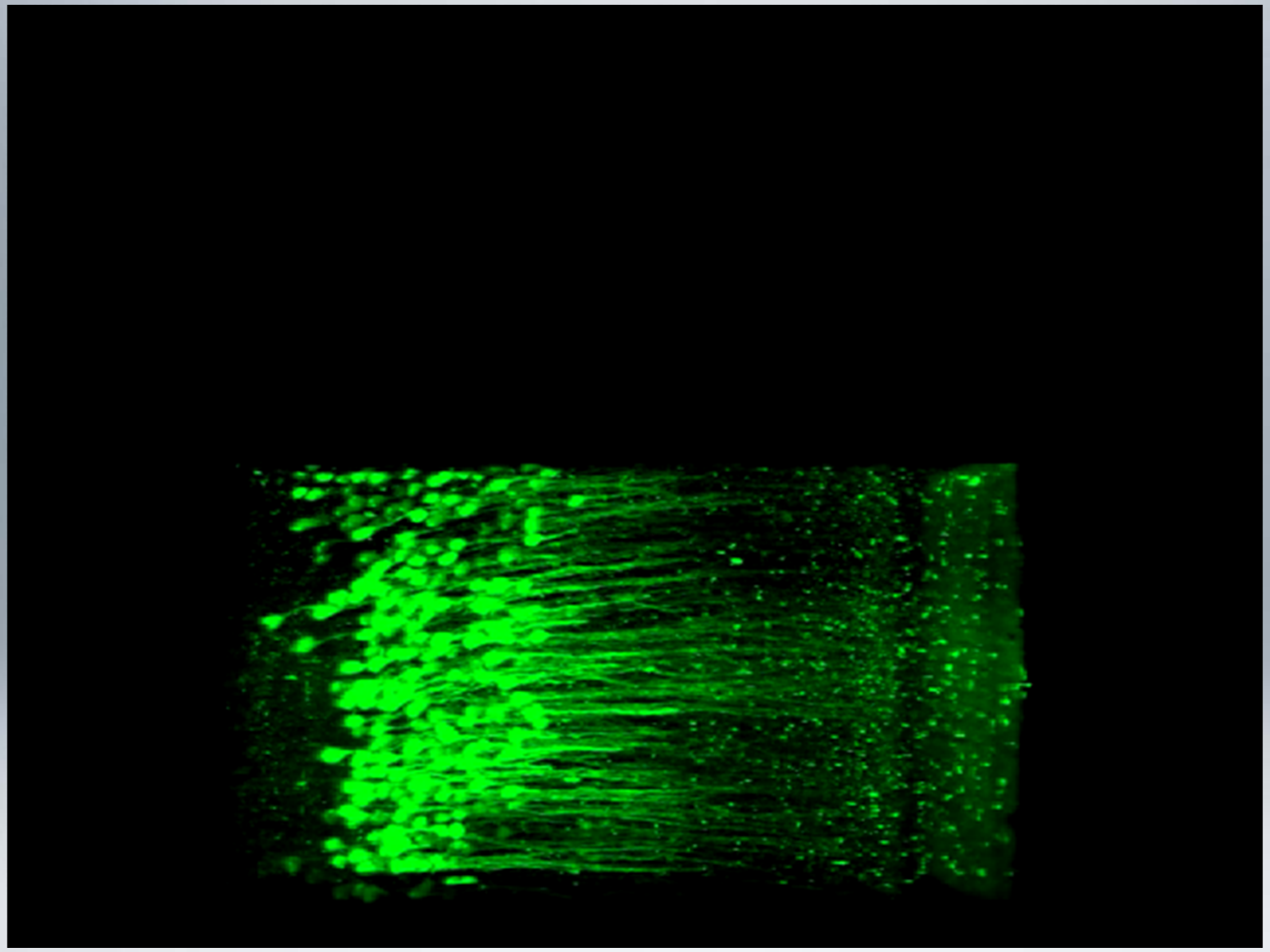


preserving clearing of
mouse lung tissue
Sample: Hortense Slevogt

Ulrich Leischner



GFP-labelled mouse brain neurons and autofluorescence

Ulrich Leischner

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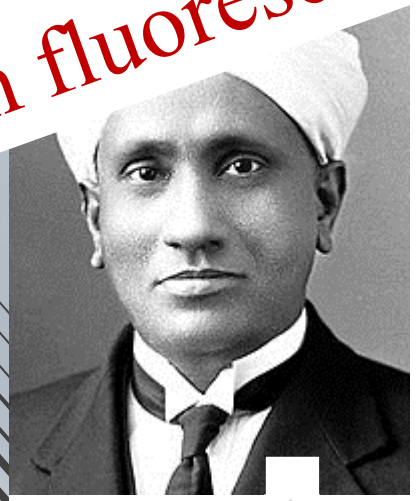
Raman Scattering

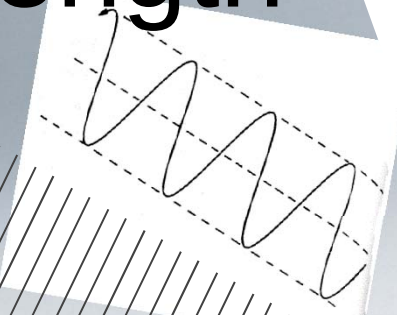
nuclei positions modify scattering strength

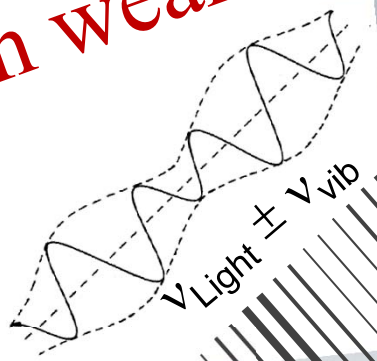
Sir C.V. Ra
(11.07.18

Signal is EXTREMELY weak
(much weaker than fluorescence)

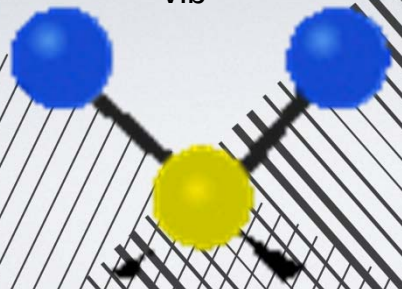


http://www.rtoz.org/2013/11/07/
c-v-ramans-125th-birthday-google-doodl

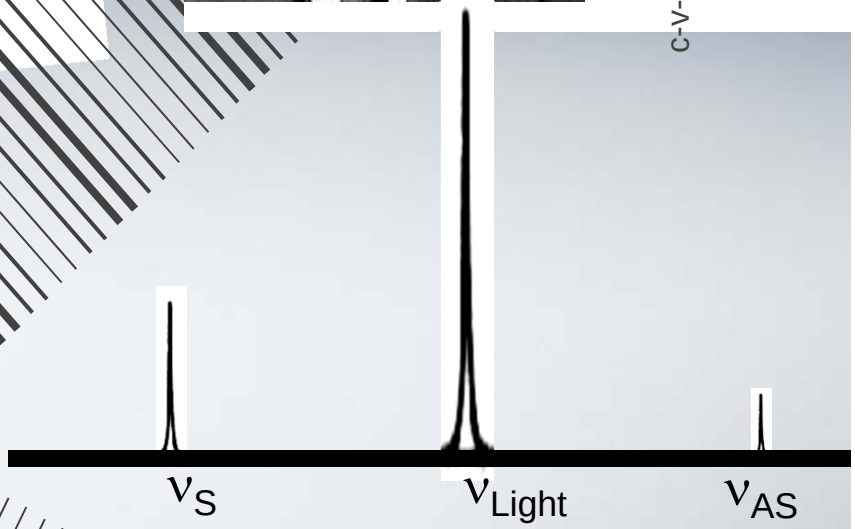




ν_{vib}



ν_{Light}



The Idea

Light sheet properties:

→ tissue tolerates more intensity

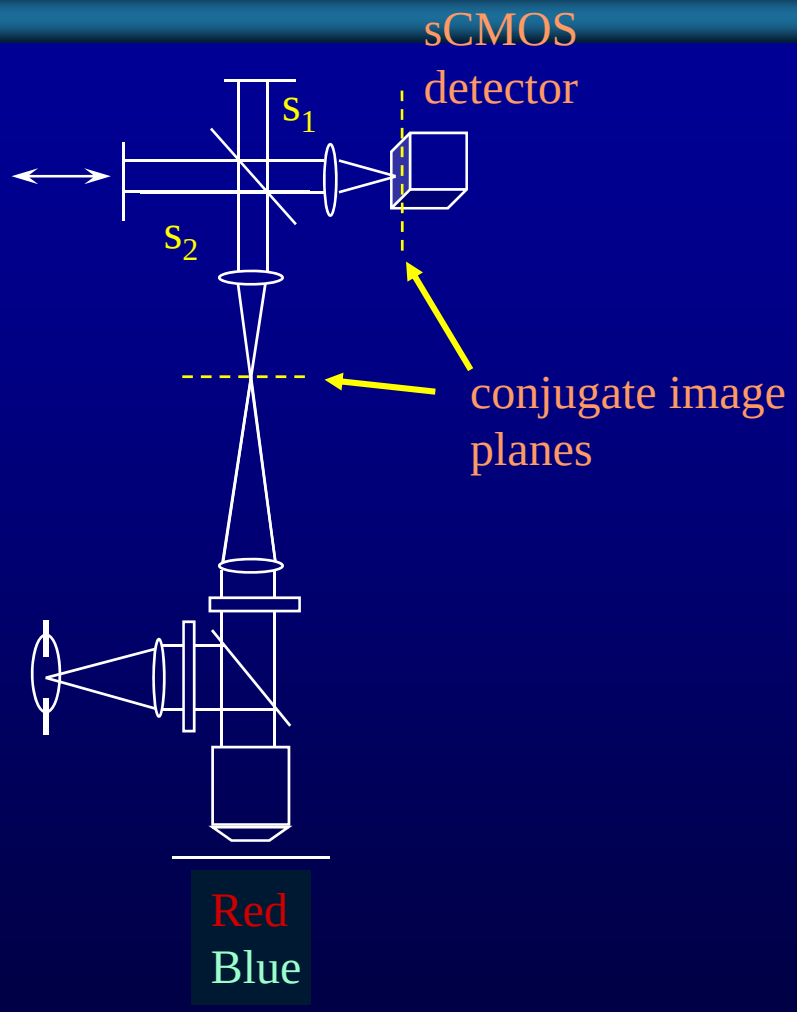
→ “recycles” light

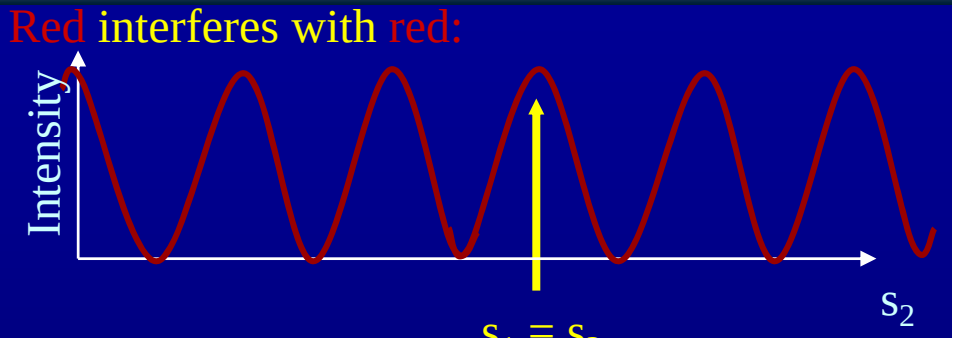
→ The multiplex advantage

→ Use it for Raman imaging,
which is inherently slow

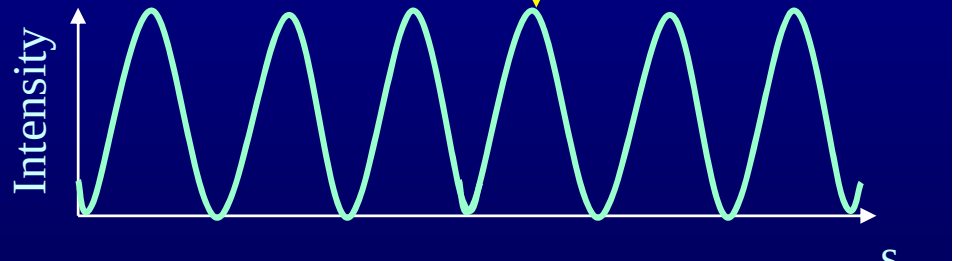
But: How do we measure the hyperspectral information?

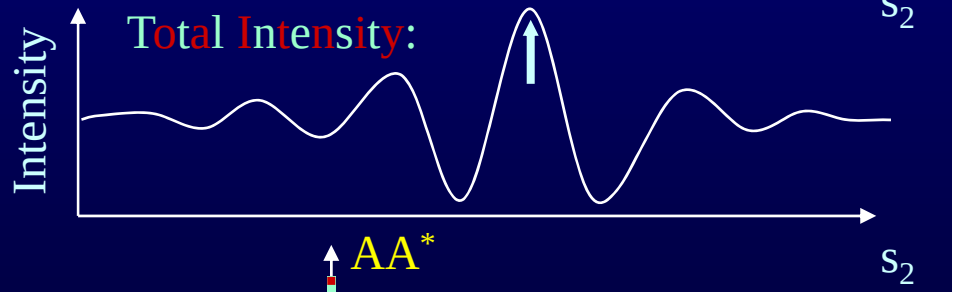
Spectral (Fourier) Encoding



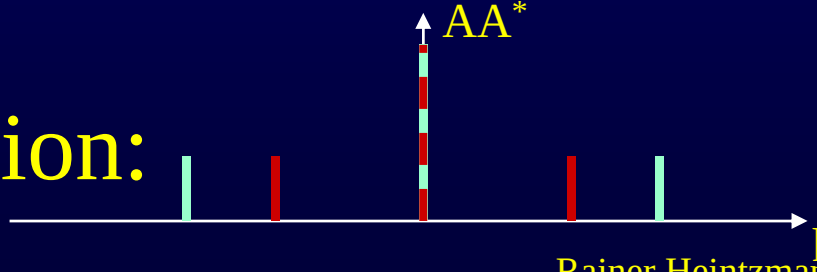


$s_1 = s_2$

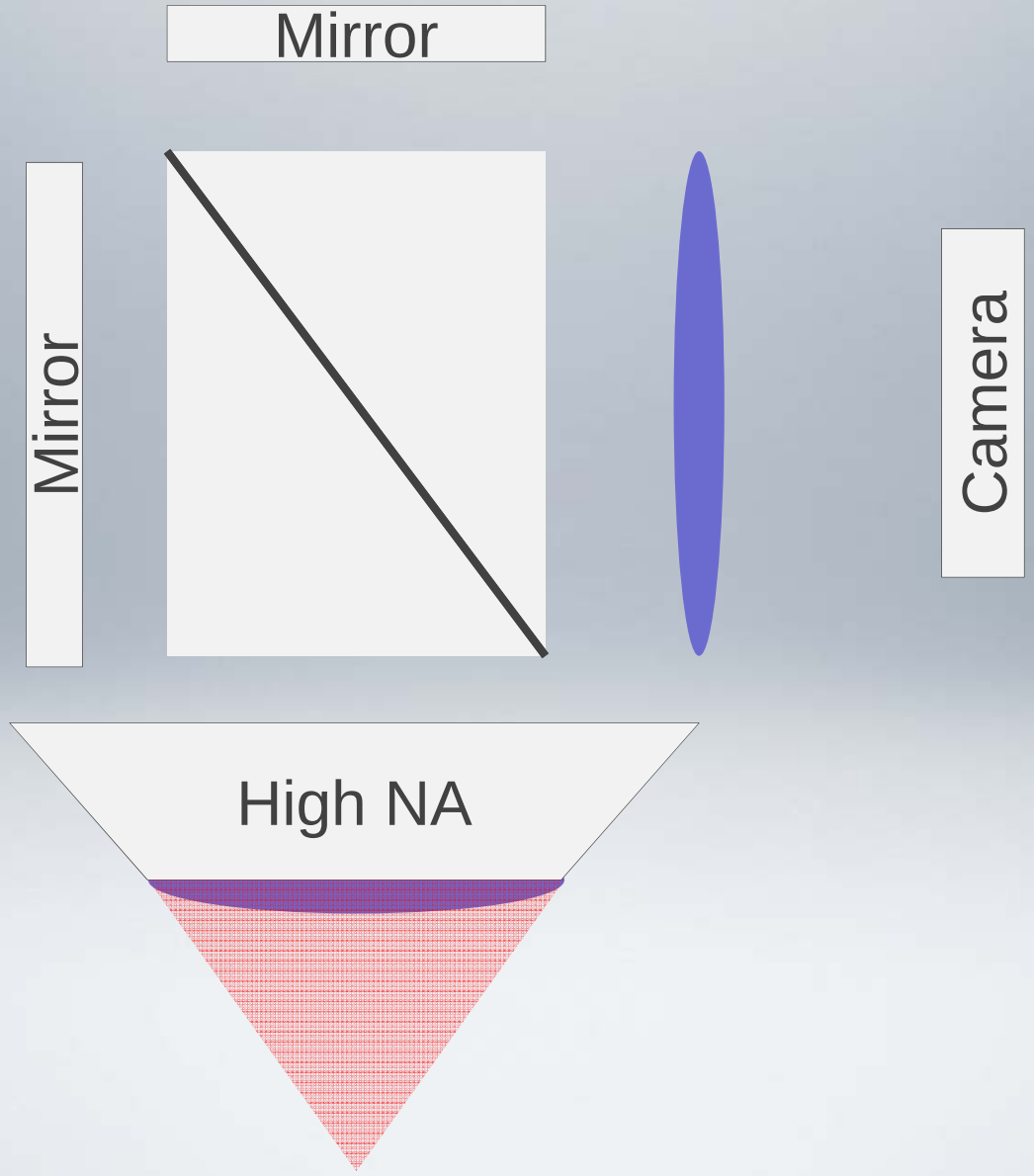




Fourier-Transformation:



The idea - parallel spectroscope - FT approach



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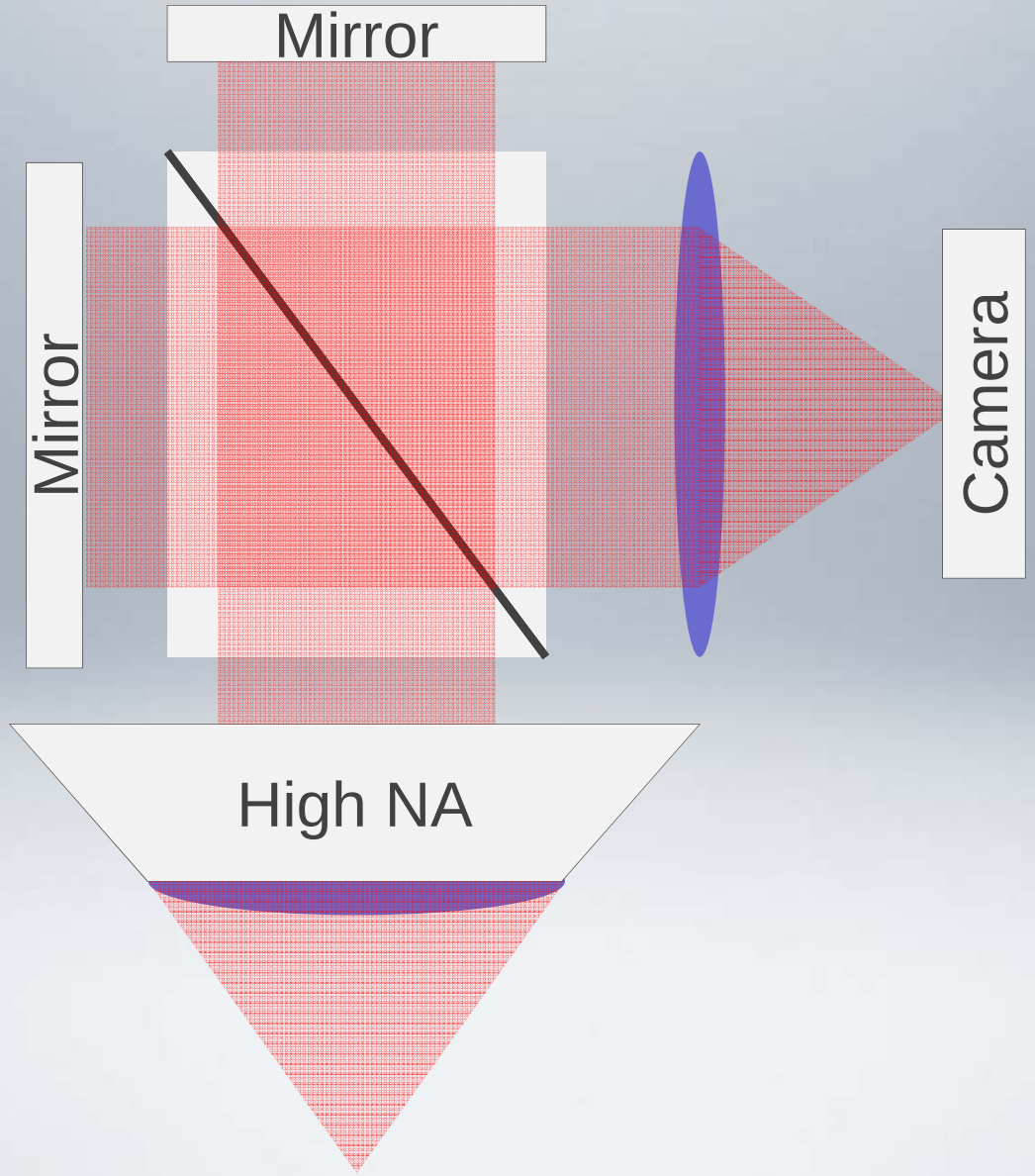

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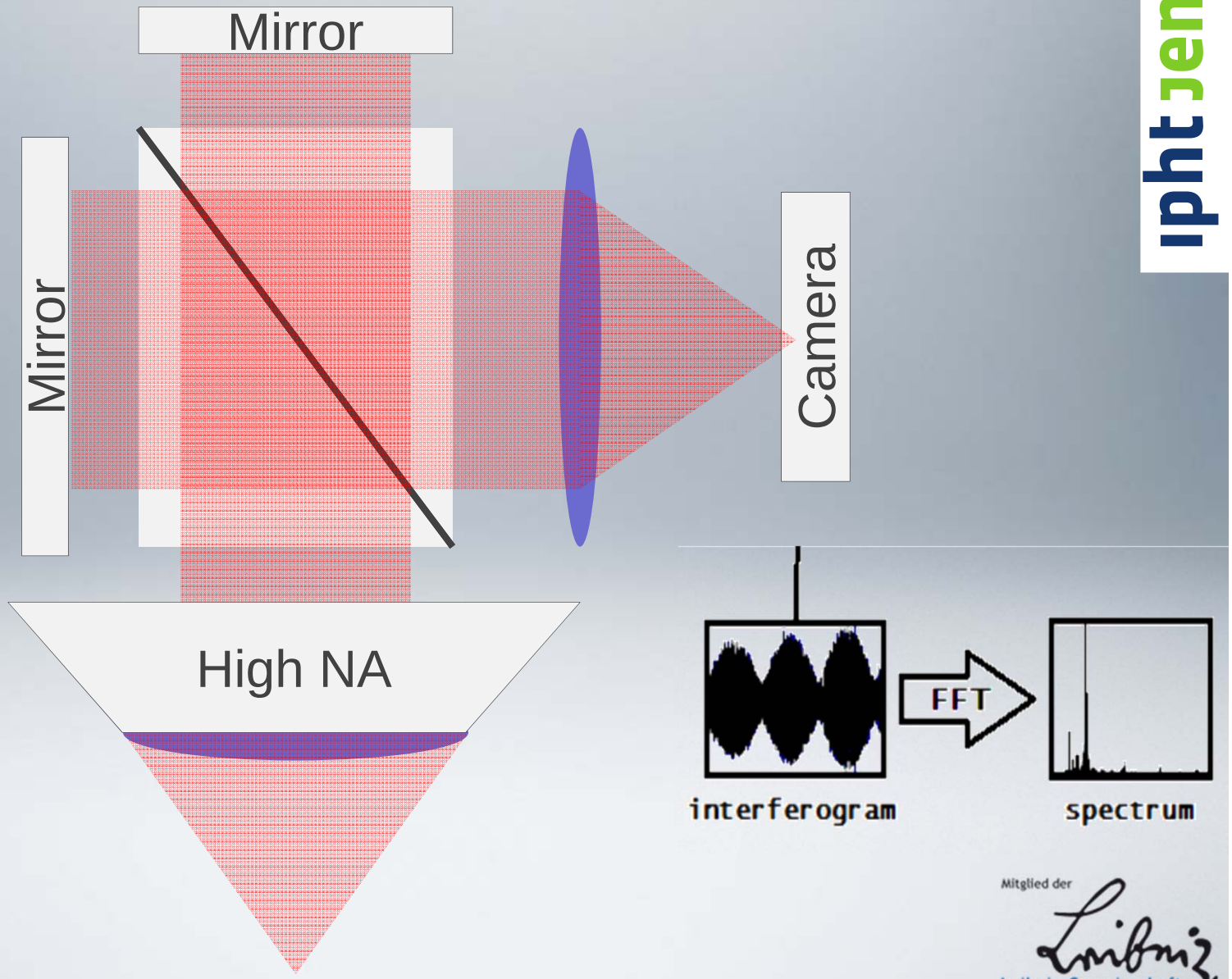
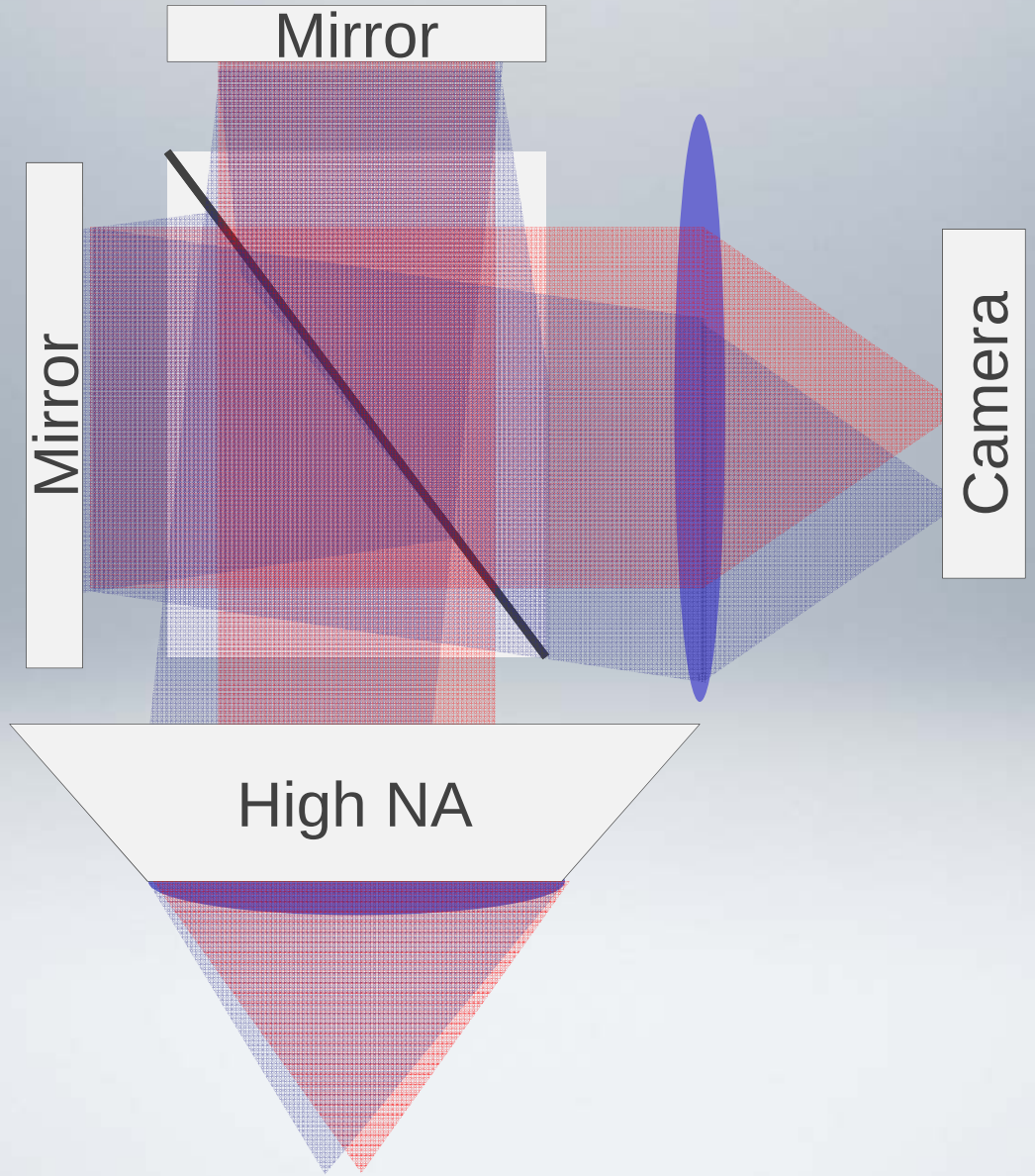
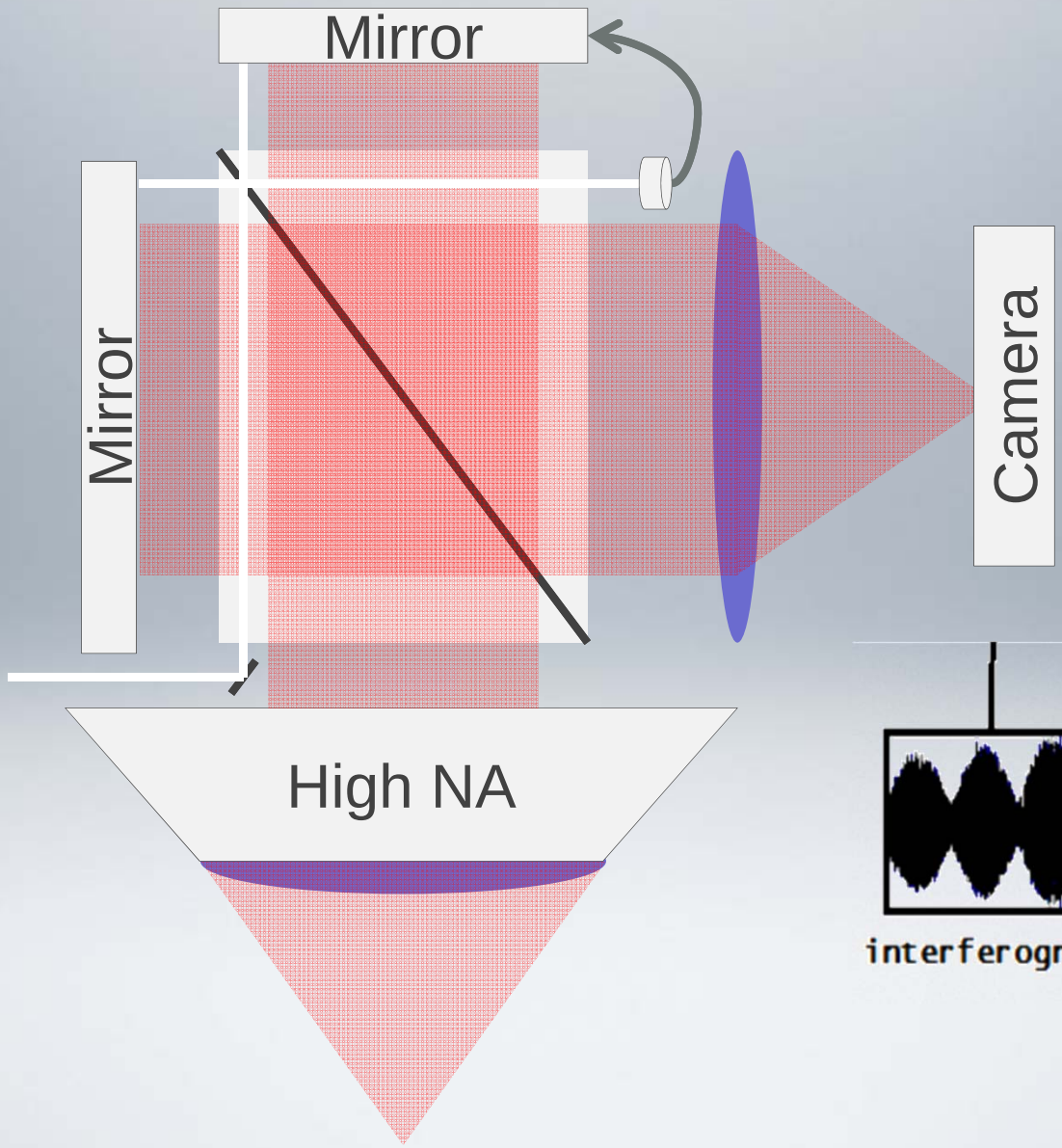
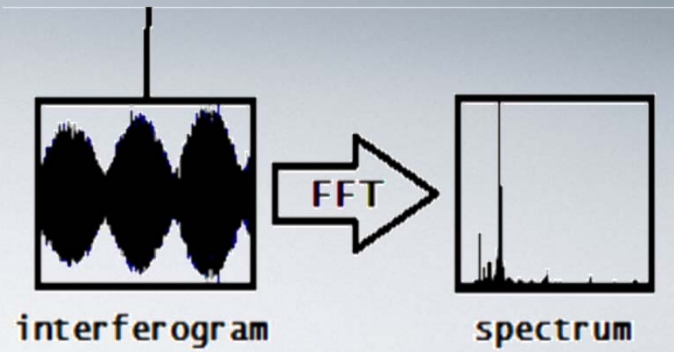


Works in parallel for all pixels (tiny corrections needed)

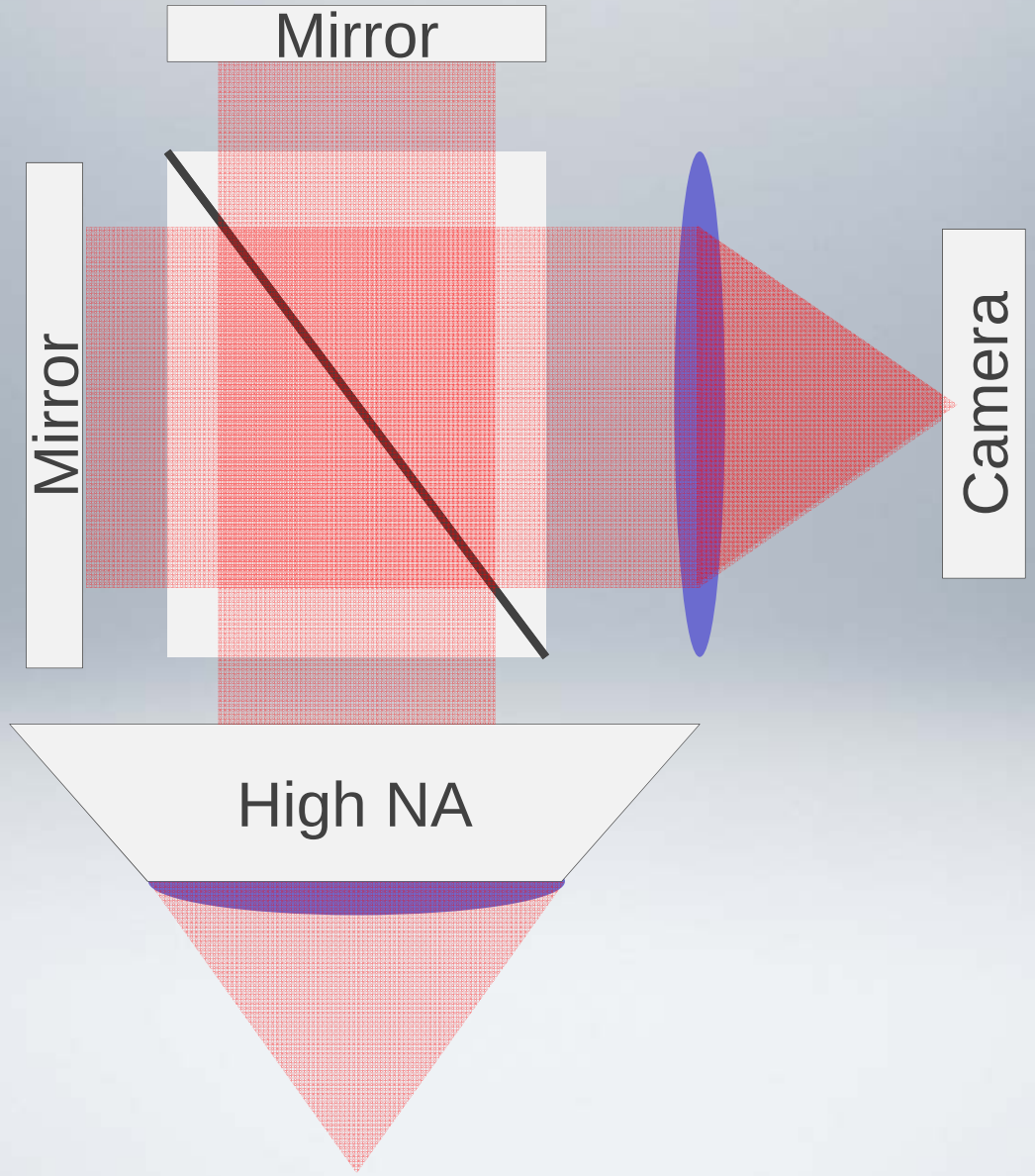


Make it precise over large distances:





The Problem: Mirror Stability



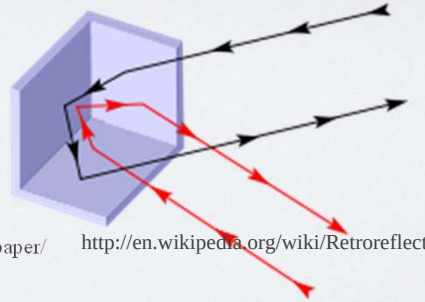
Solutions to stability problem

- Three piezos and active feedback
  → expensive, complicated

- Common path interferometer (e.g. Sagnac)
  → reduced optical throughput
  (FOV vs. spectral resolution)

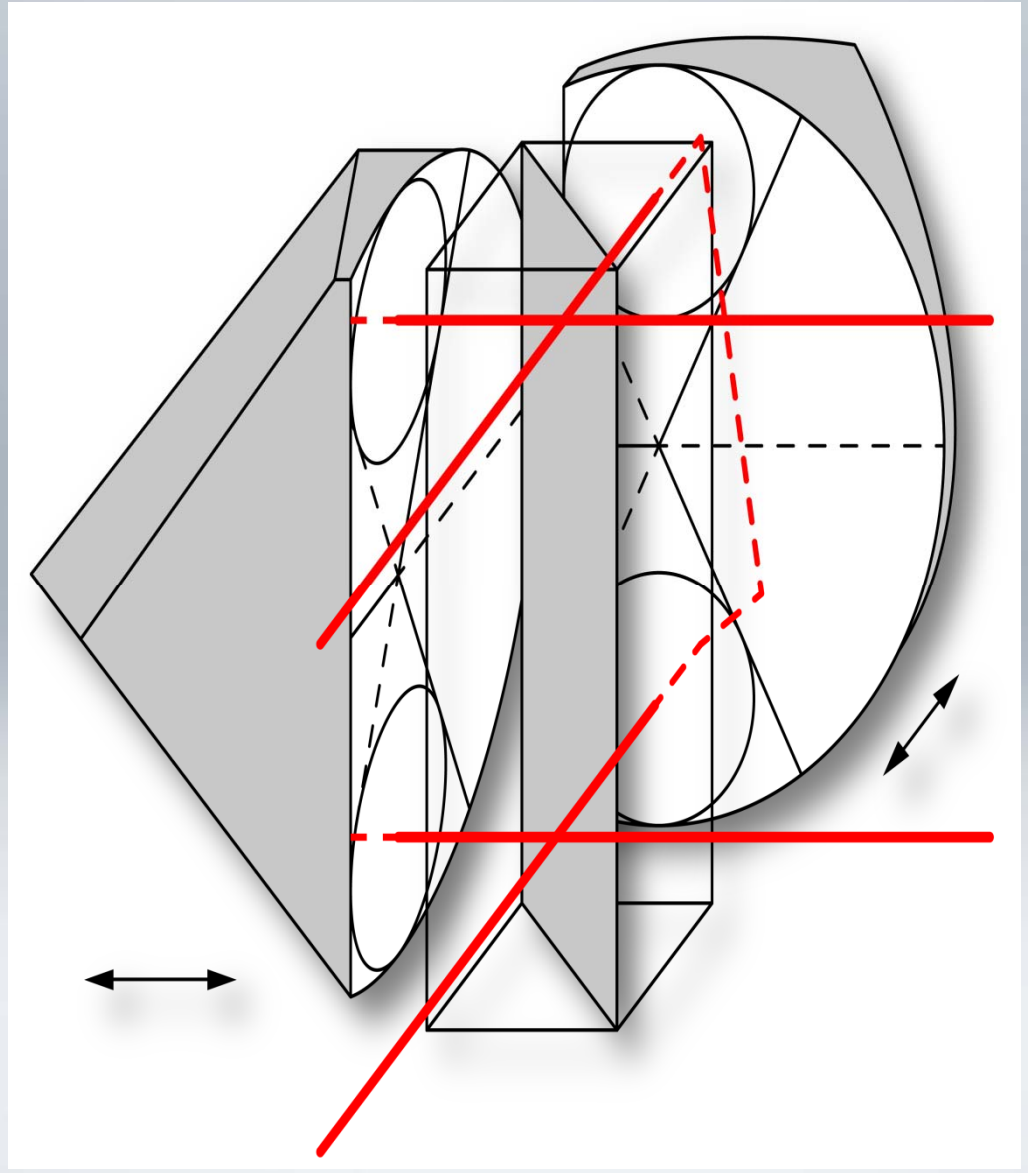
- Avoid the Tip/Tilt problem optically:
  The cat-eye trick!

http://stuffpoint.com/cats/image/39175/cats-eye-wallpaper/



http://en.wikipedia.org/wiki/Retroreflector

The FT-Raman setup



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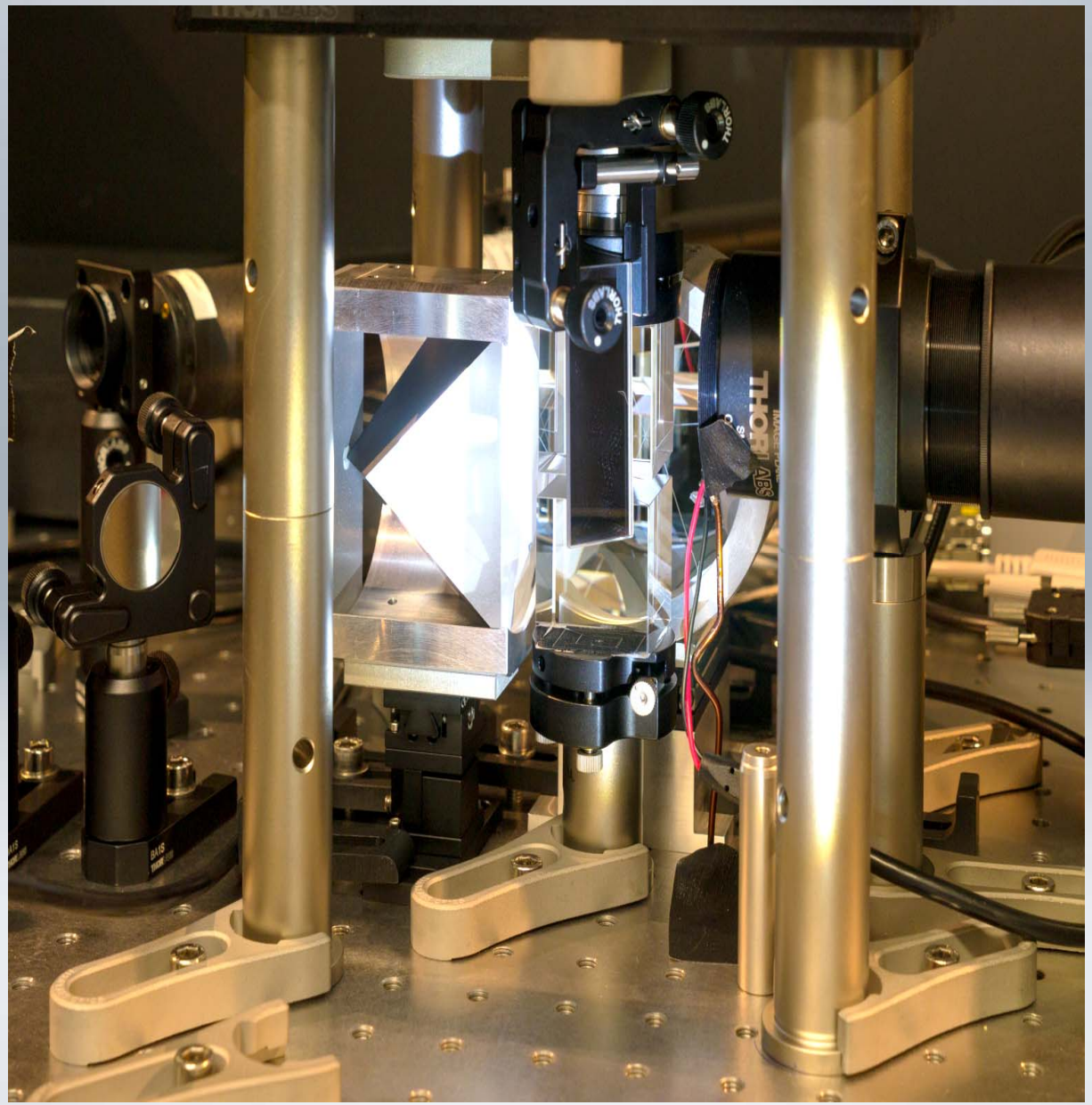

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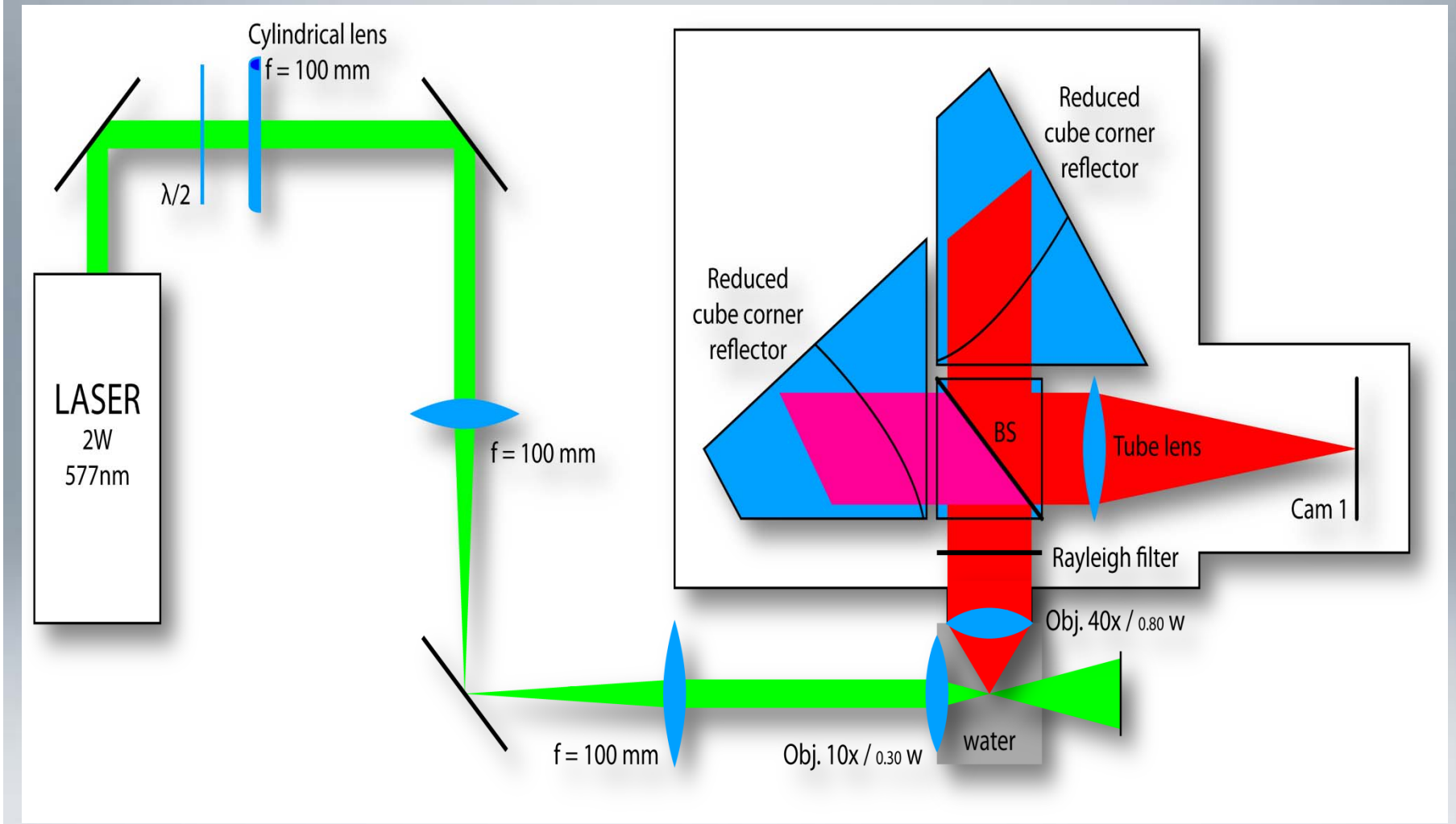
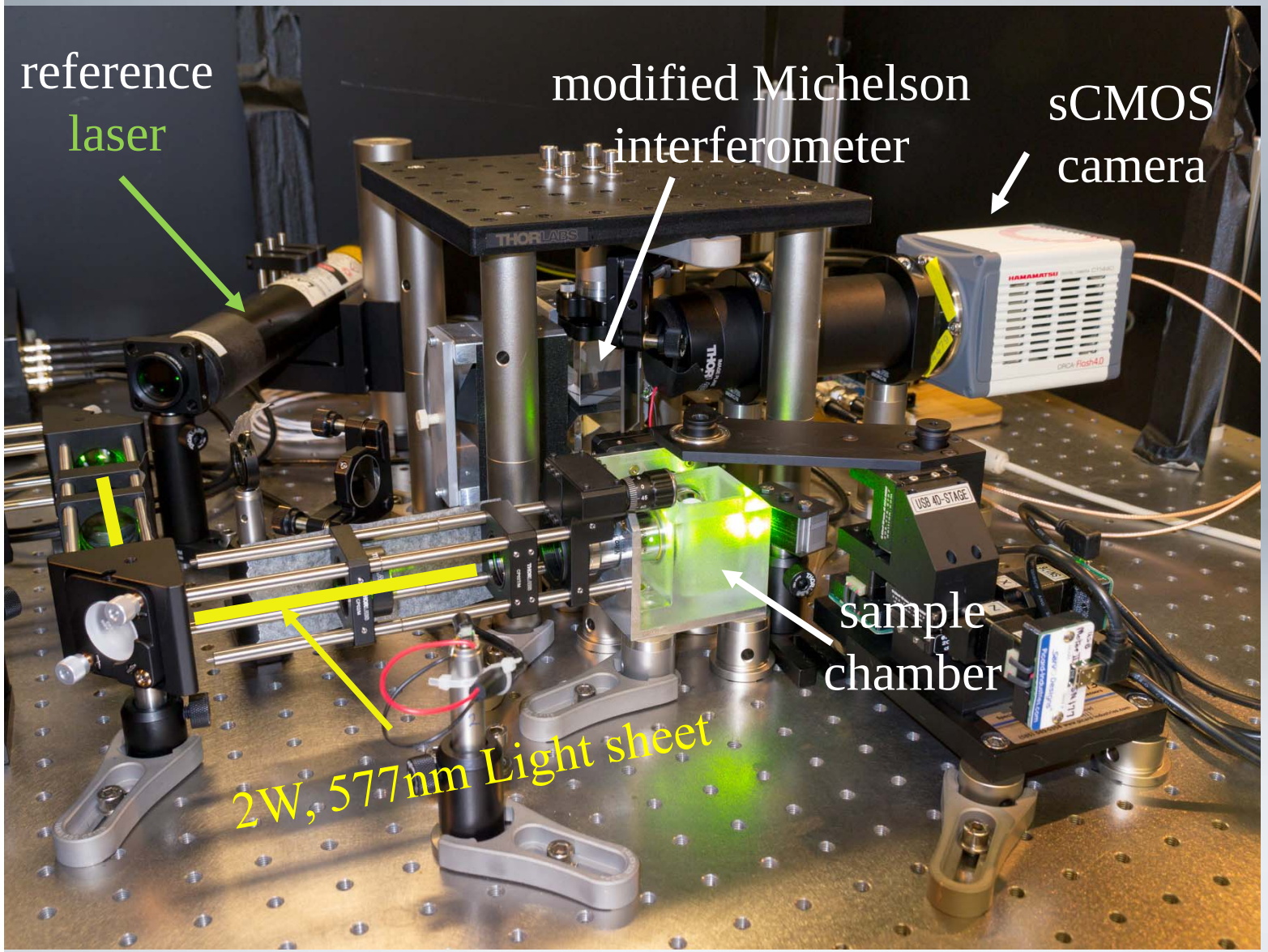


Objective: Water dipping 0.8 NA 40× Nikon
XY-resolution: 440 nm
FWHM Slice thickness: 3.1 µm in the center

FT - Raman Microscopy



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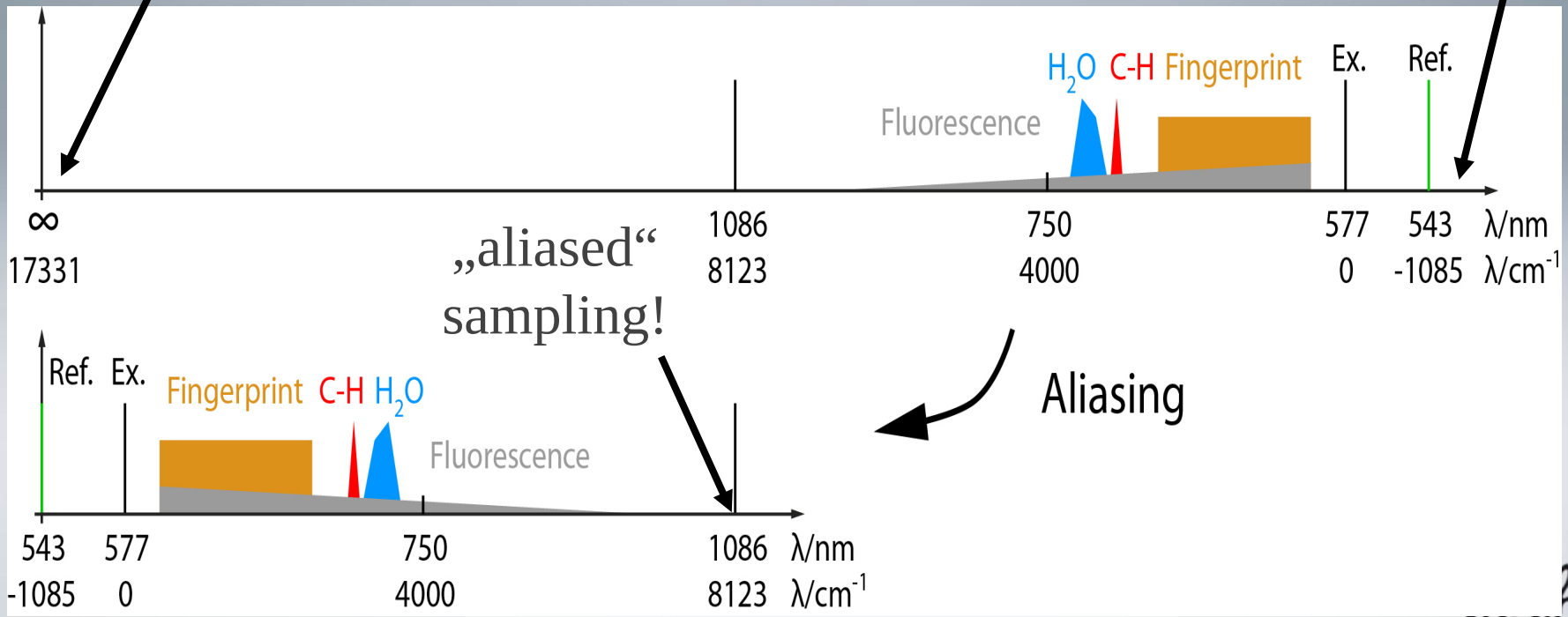
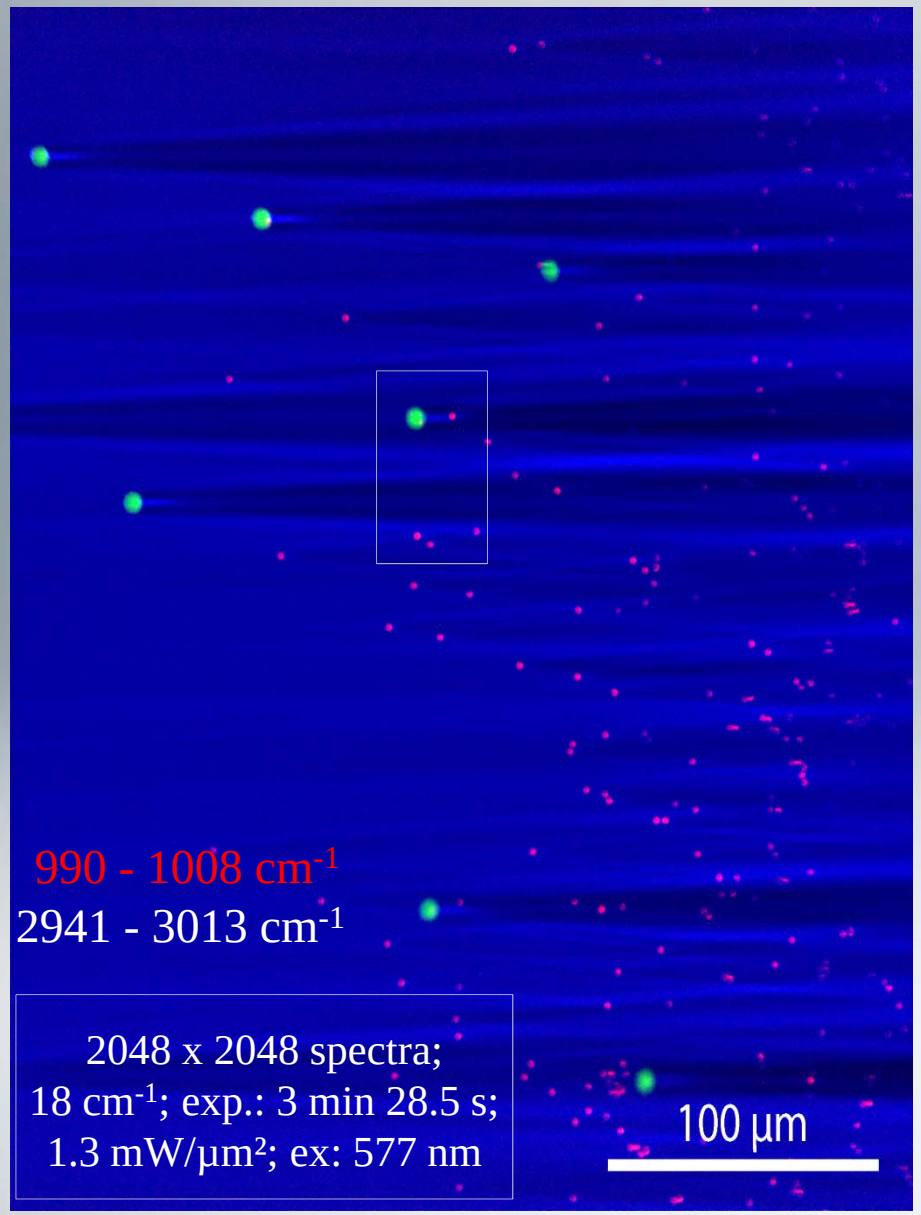
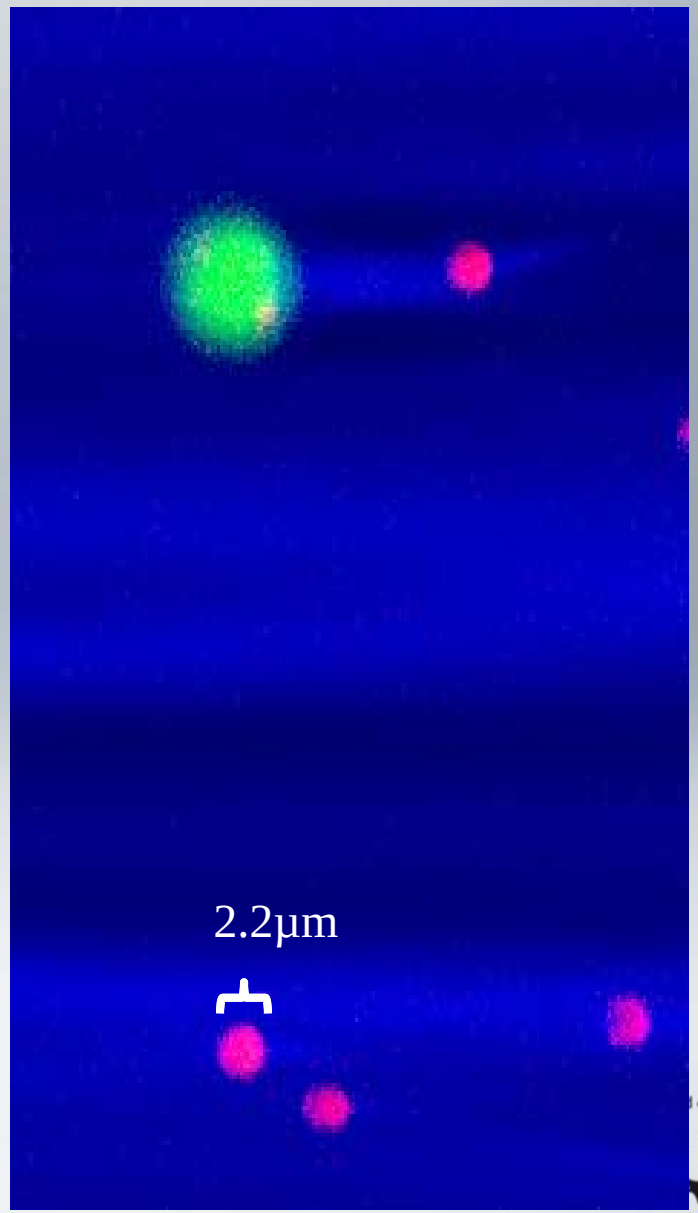
Sampling the interferogramm

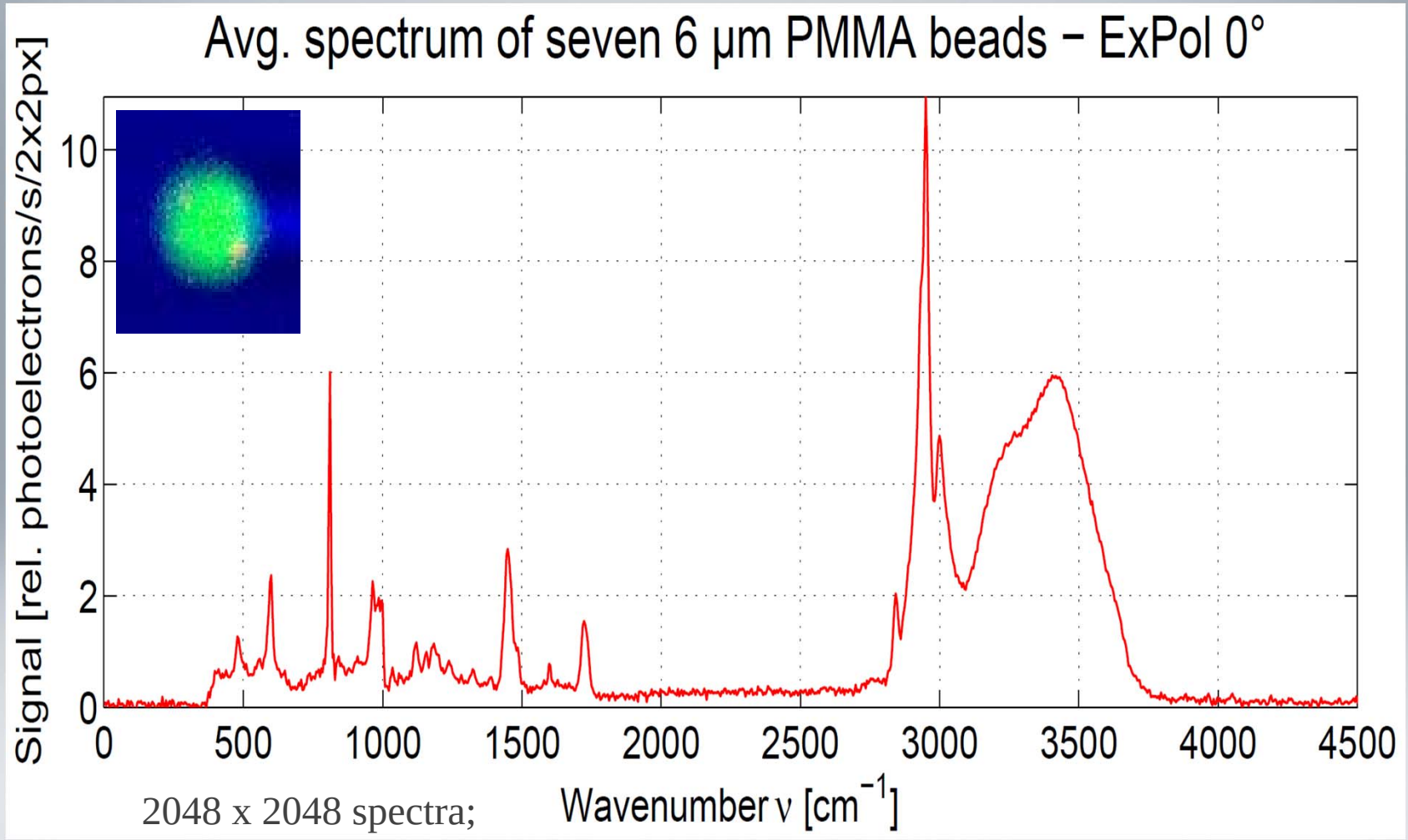


2,2 µm Polystyrene and 6 µm PMMA beads



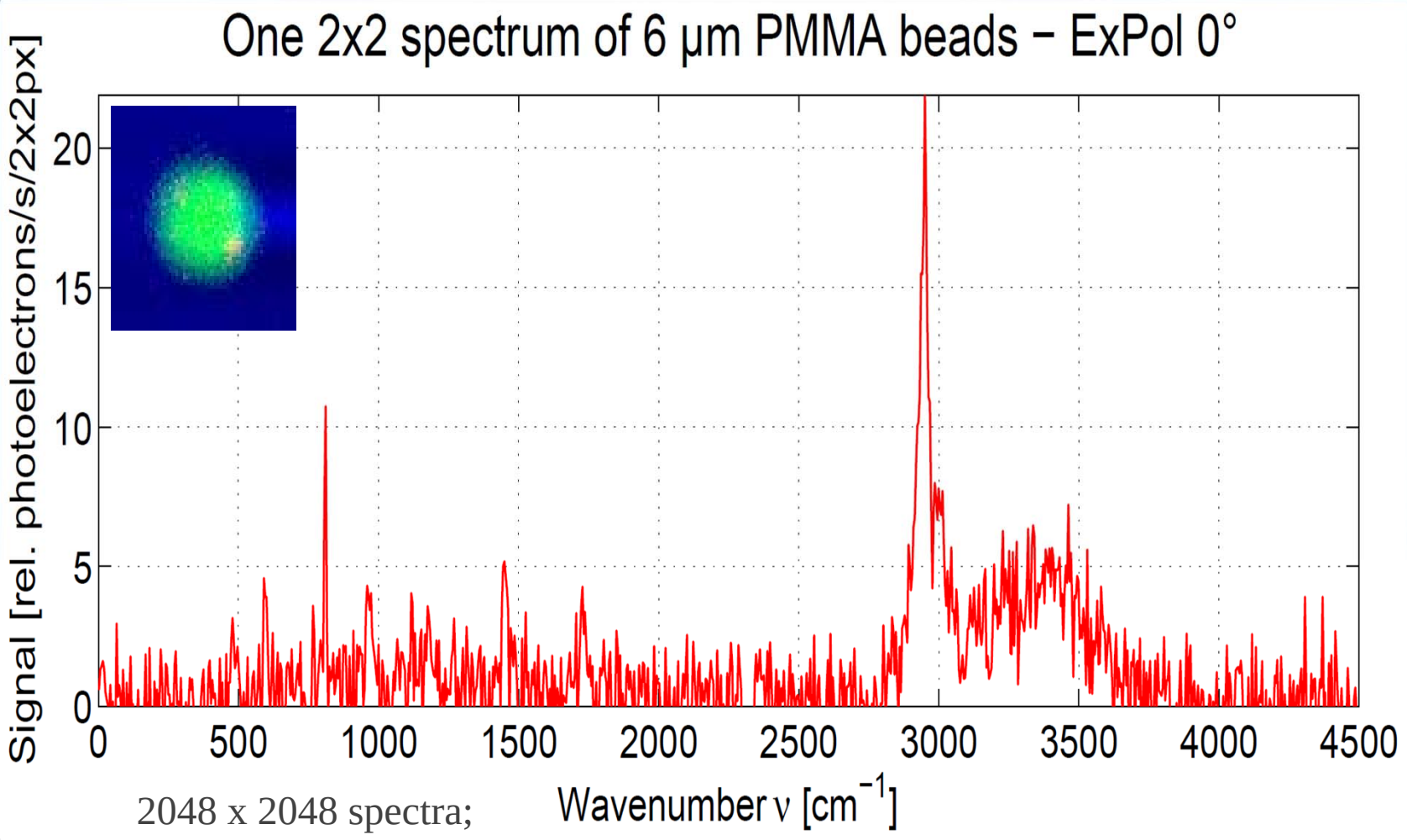


6 µm PMMA beads



2048 x 2048 spectra;
4.4 cm^{-1}; exp.: 13 min 39 s;
1.3 mW/µm²; ex: 577 nm

6 µm PMMA beads



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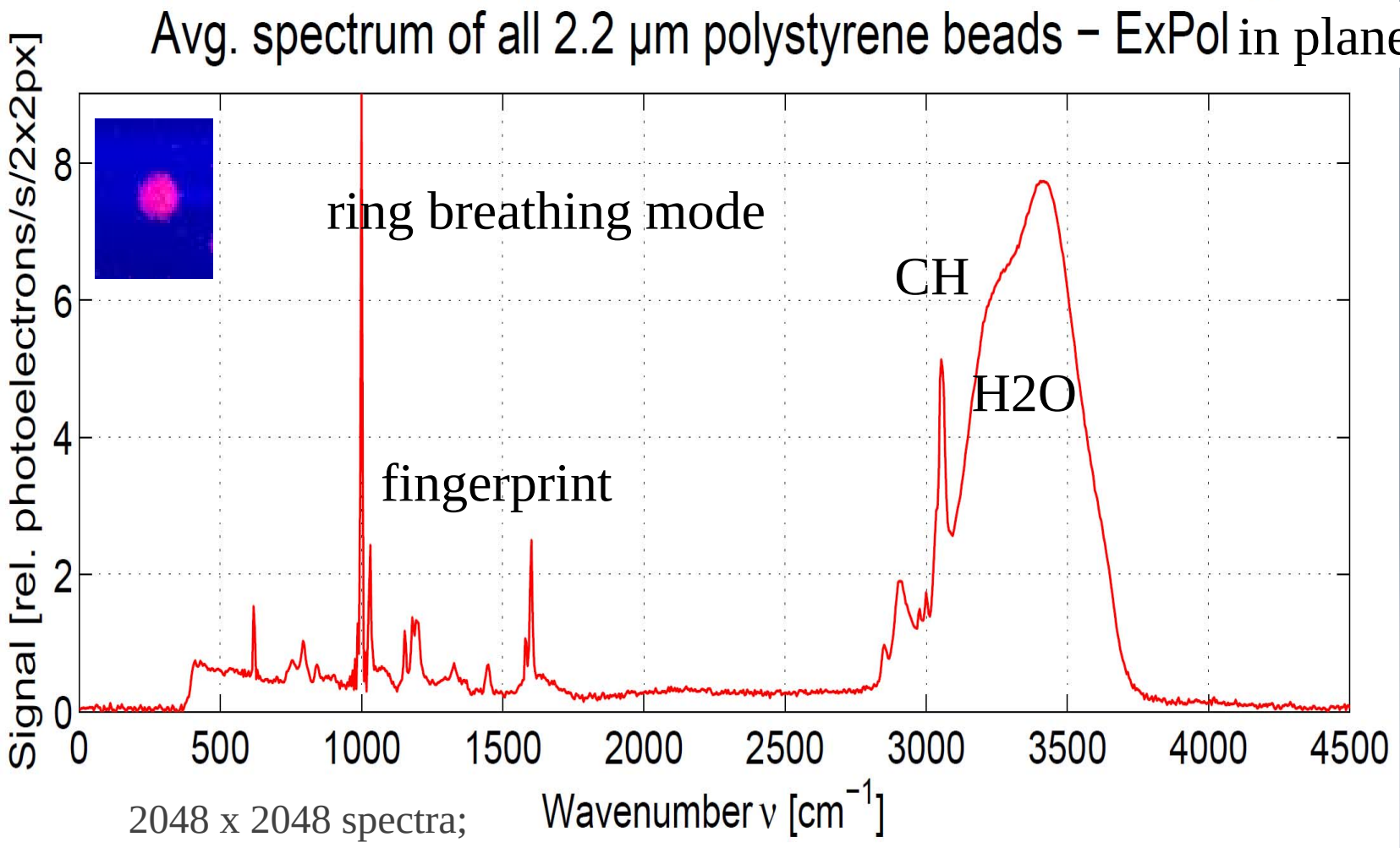
2.2 µm Polystyrene beads



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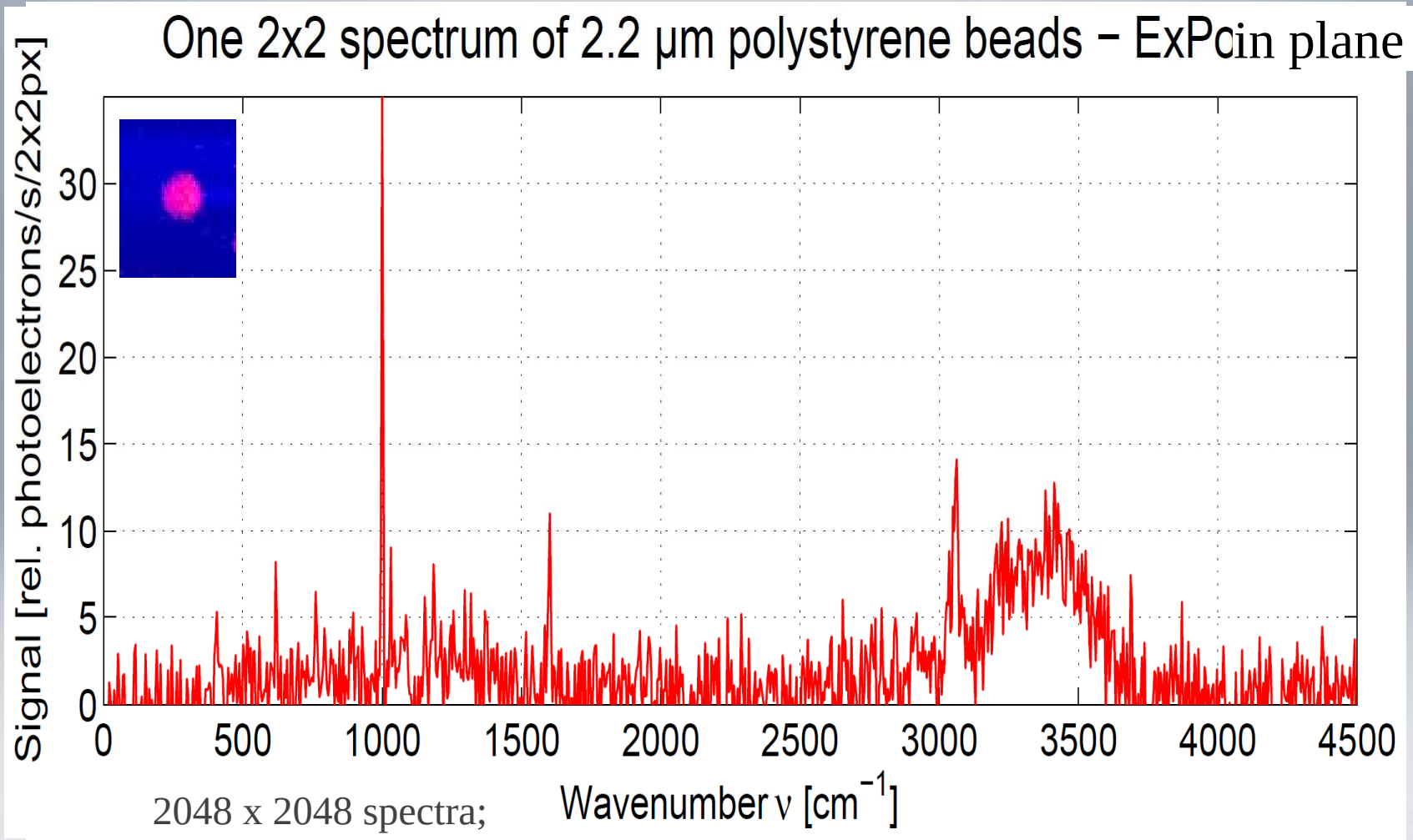

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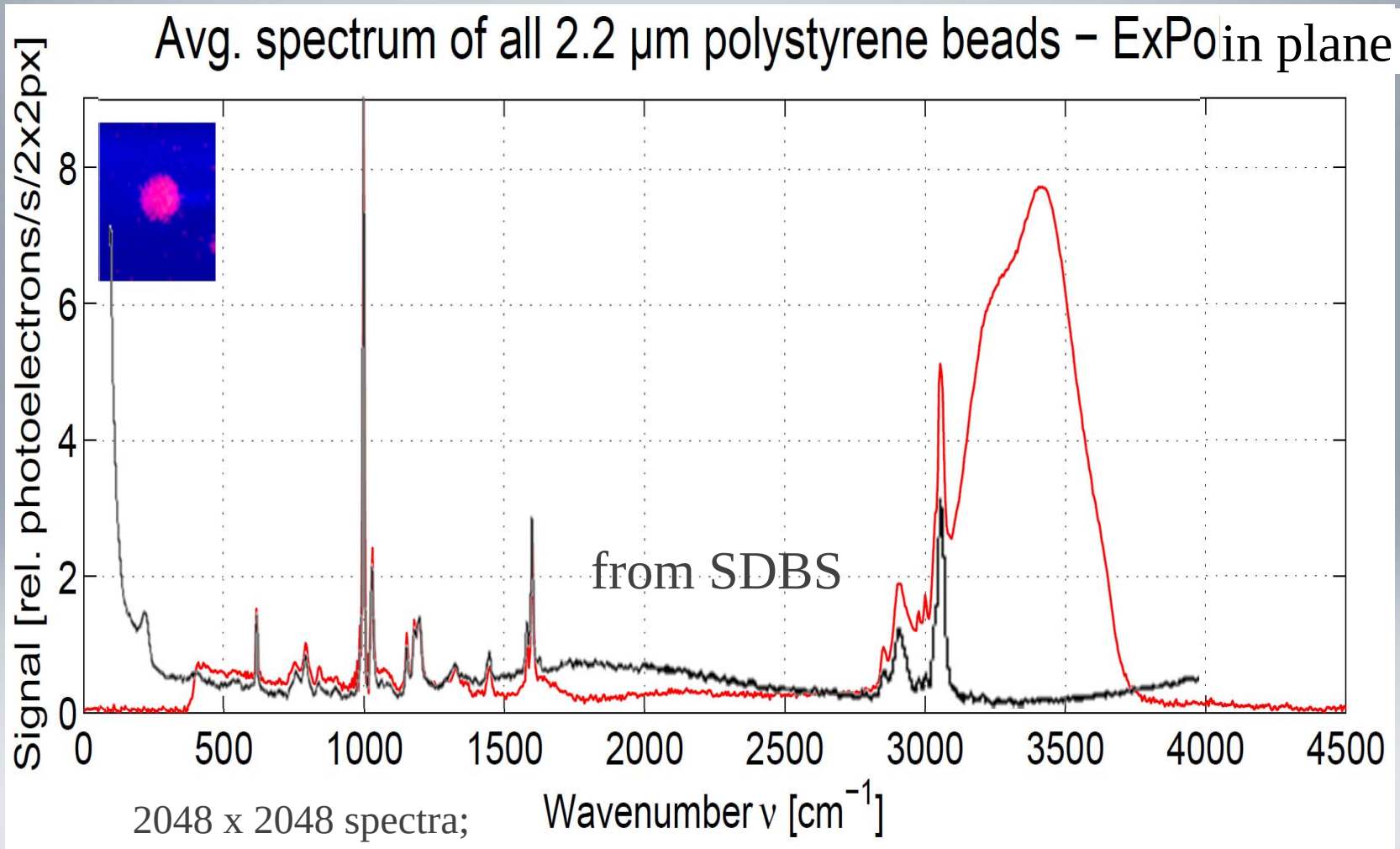
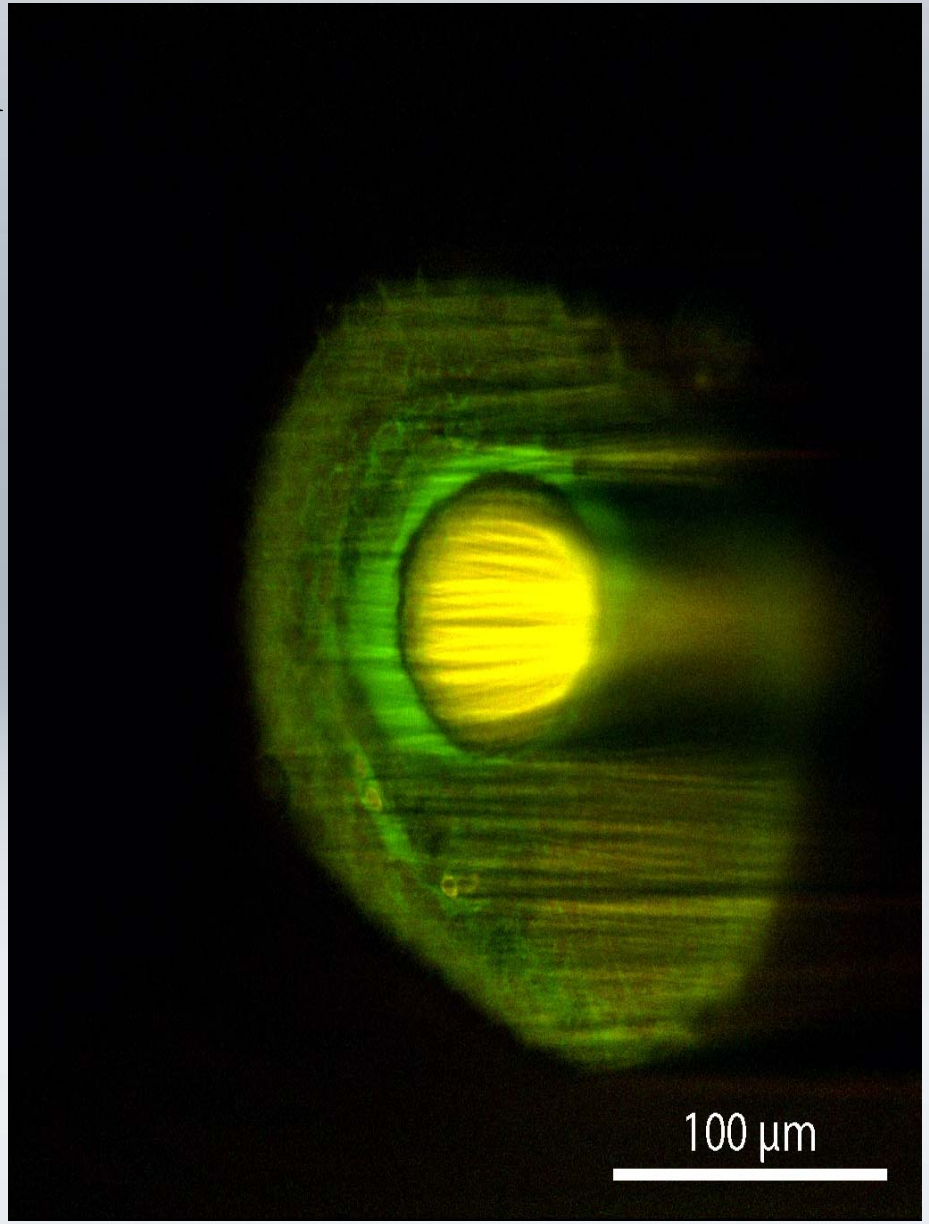


2048 x 2048 spectra;
4.4 cm^{-1}; exp.: 13 min 39 s;
1.3 mW/µm²; ex: 577 nm

Zebrafish eye

- 1024 × 1024 spectra (2 × 2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 17 min
- 0.8 $mW/\mu m^2$
- ex: 577 nm

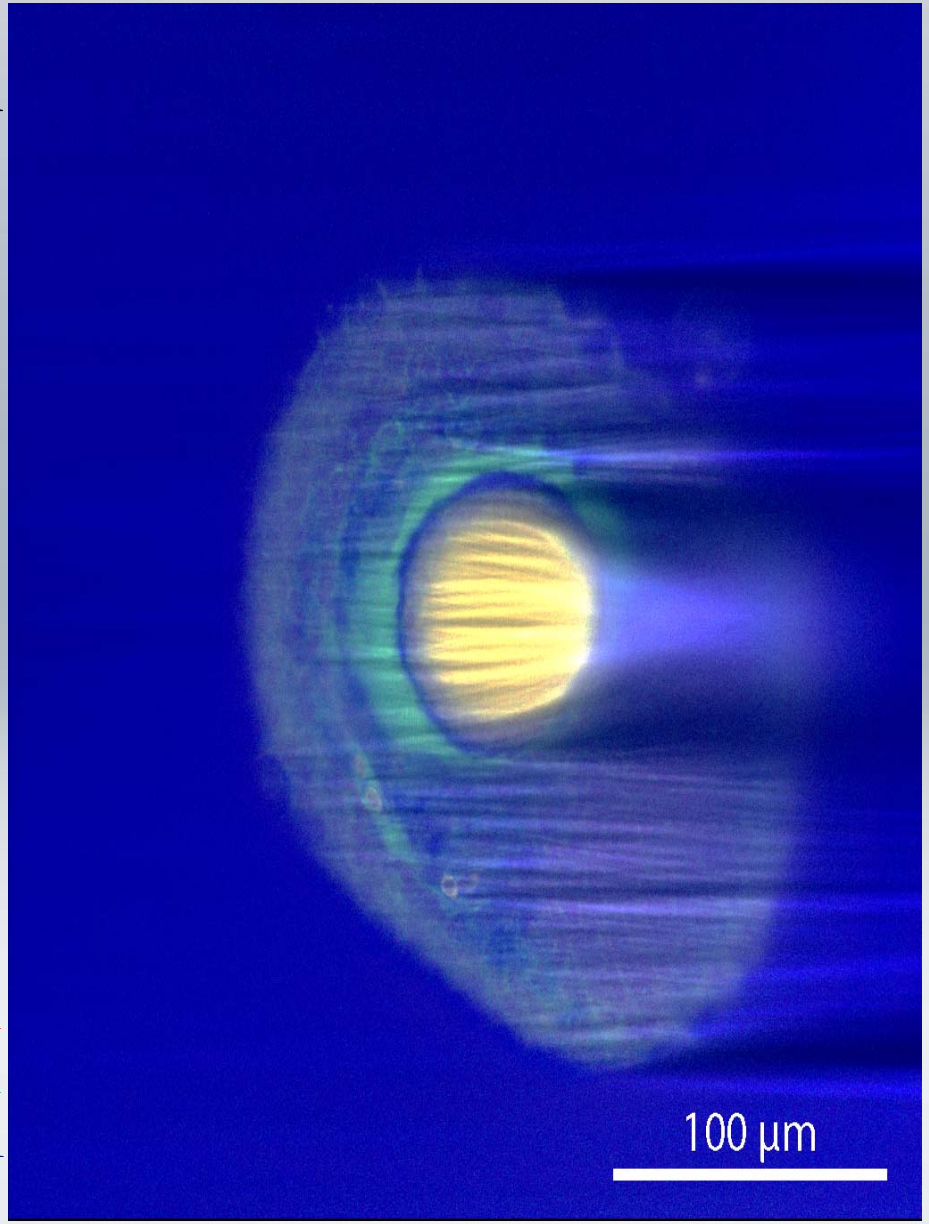


C-H: 2941 - 3017 cm^{-1}

C-H: 2851 - 2910 cm^{-1}

Zebrafish eye

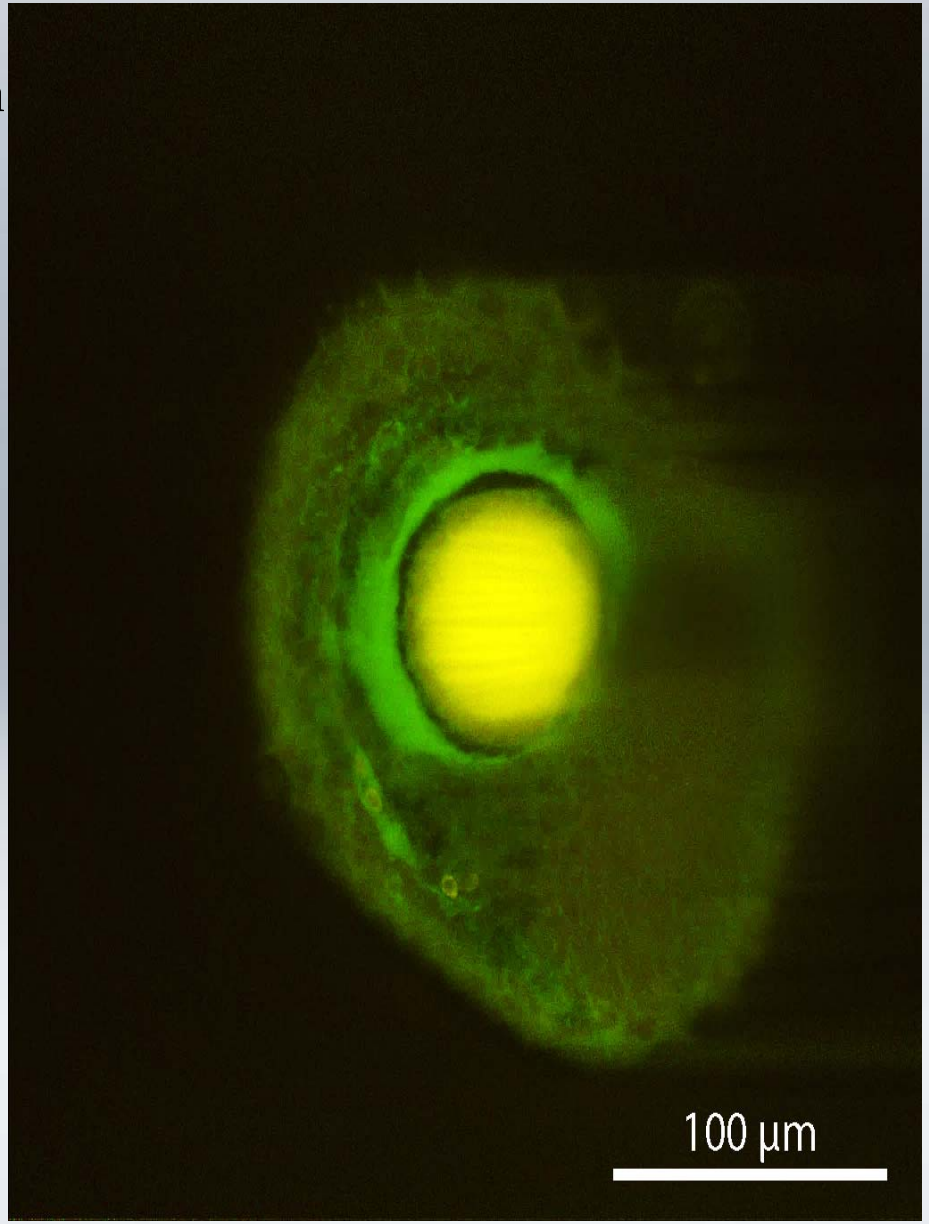
- 1024 × 1024 spectra (2 × 2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 17 min
- 0.8 mW/µm²
- ex: 577 nm



C-H: 2941 - 3017 cm^{-1}
C-H: 2851 - 2910 cm^{-1}
H_2O: 3103 - 3646 cm^{-1}

Zebrafish: Stripes removed

- 1024 × 1024 spectra (2 × 2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 17 min
- 0.8 mW/µm²
- ex: 577 nm

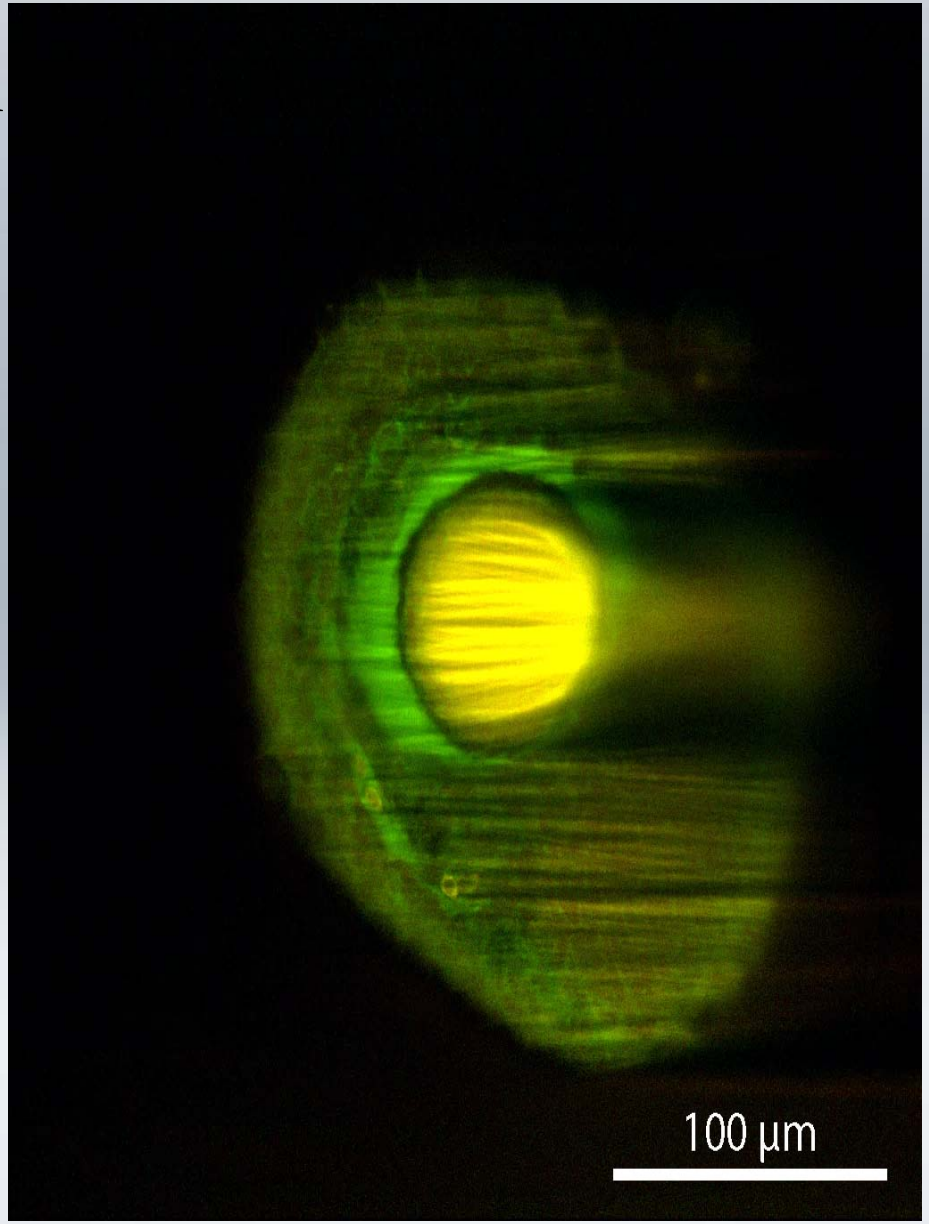


C-H: 2941 - 3017 cm^{-1}
C-H: 2851 - 2910 cm^{-1}

corrected

Zebrafish with Stripes

- 1024 × 1024 spectra (2 × 2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 17 min
- 0.8 mW/µm²
- ex: 577 nm

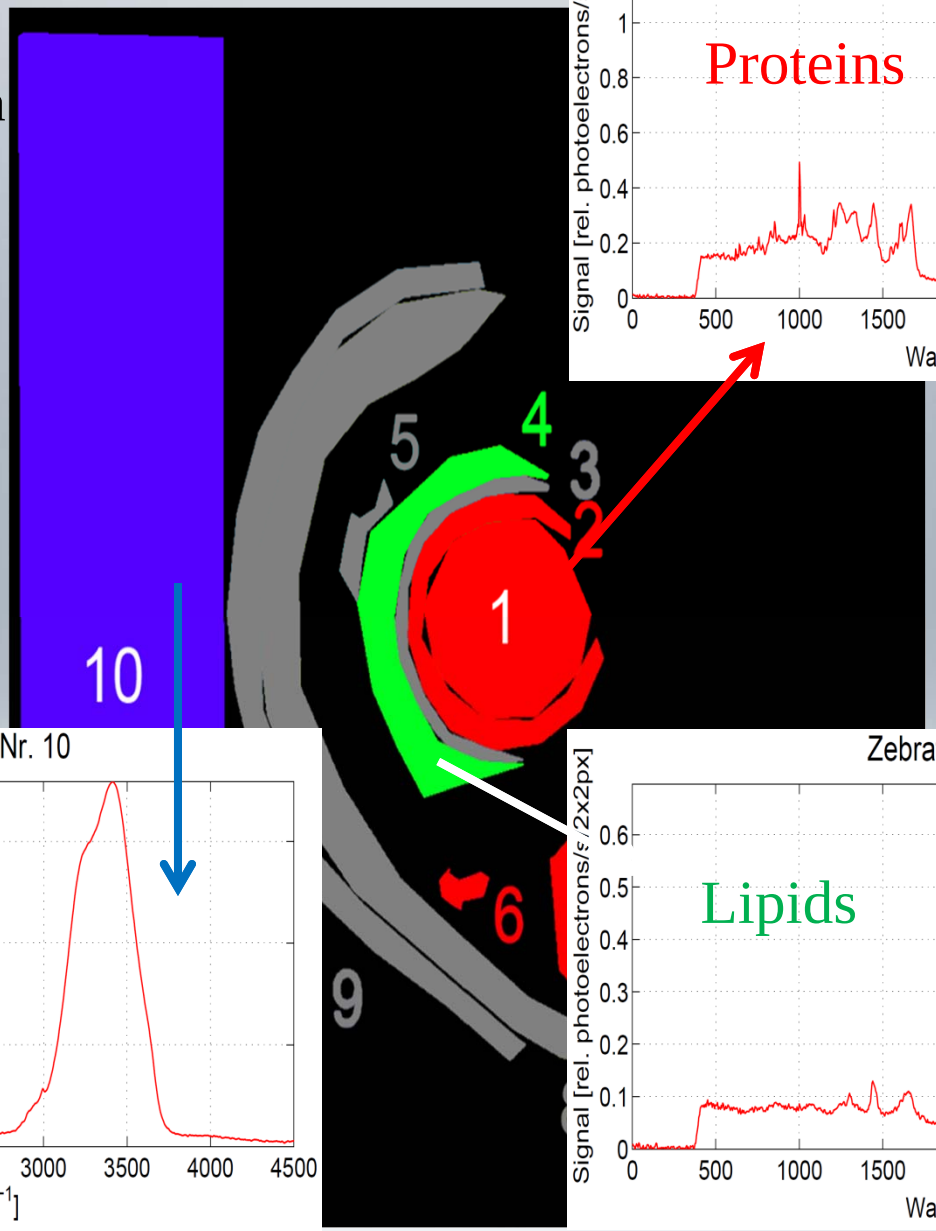


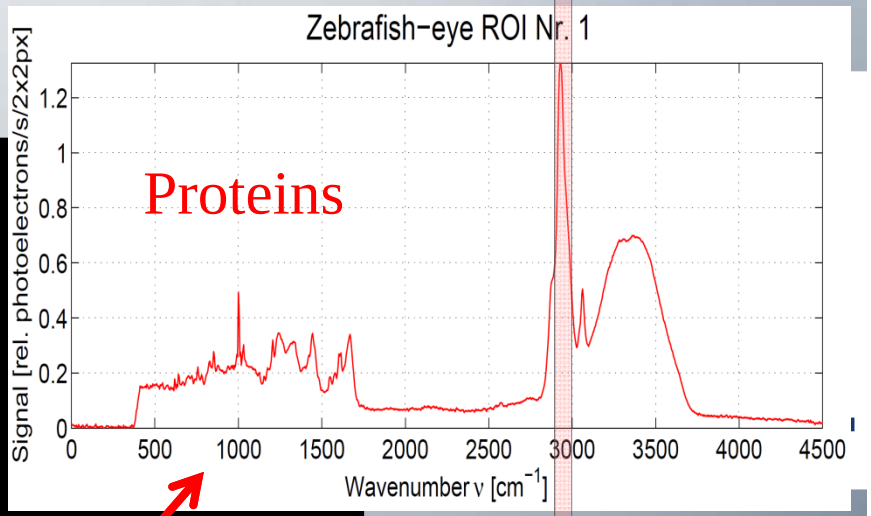
C-H: 2941 - 3017 cm^{-1}

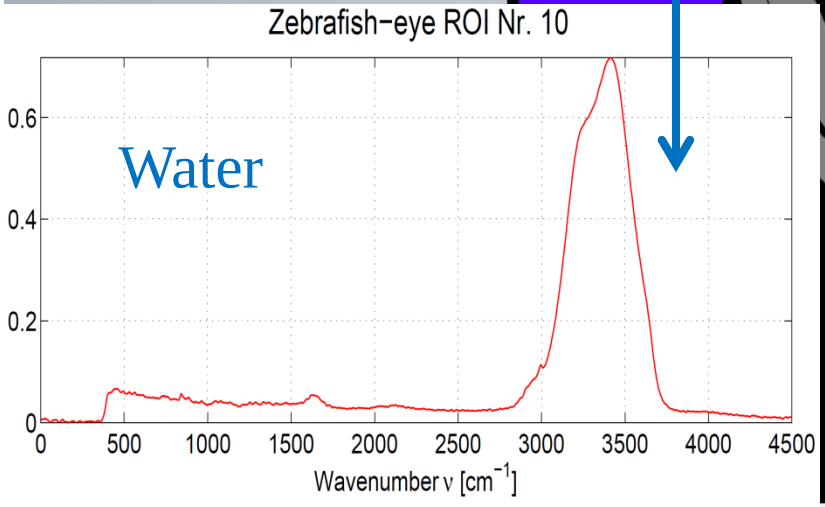
C-H: 2851 - 2910 cm^{-1}

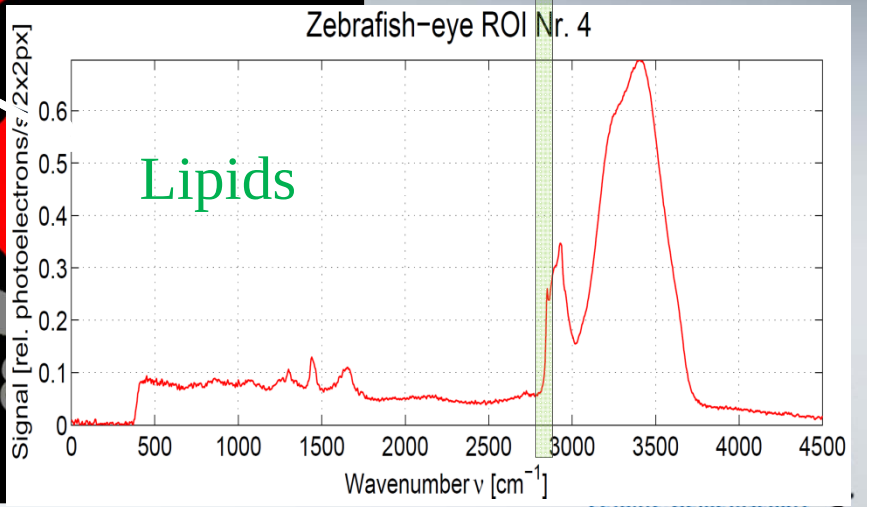
Zebrafish eye

- 1024 × 1024 spectra (2 × 2 binned)
- Resolution: 4 cm^{-1}
- Tot. exp.: 68 min
- 0.8 mW/µm²
- ex: 577 nm



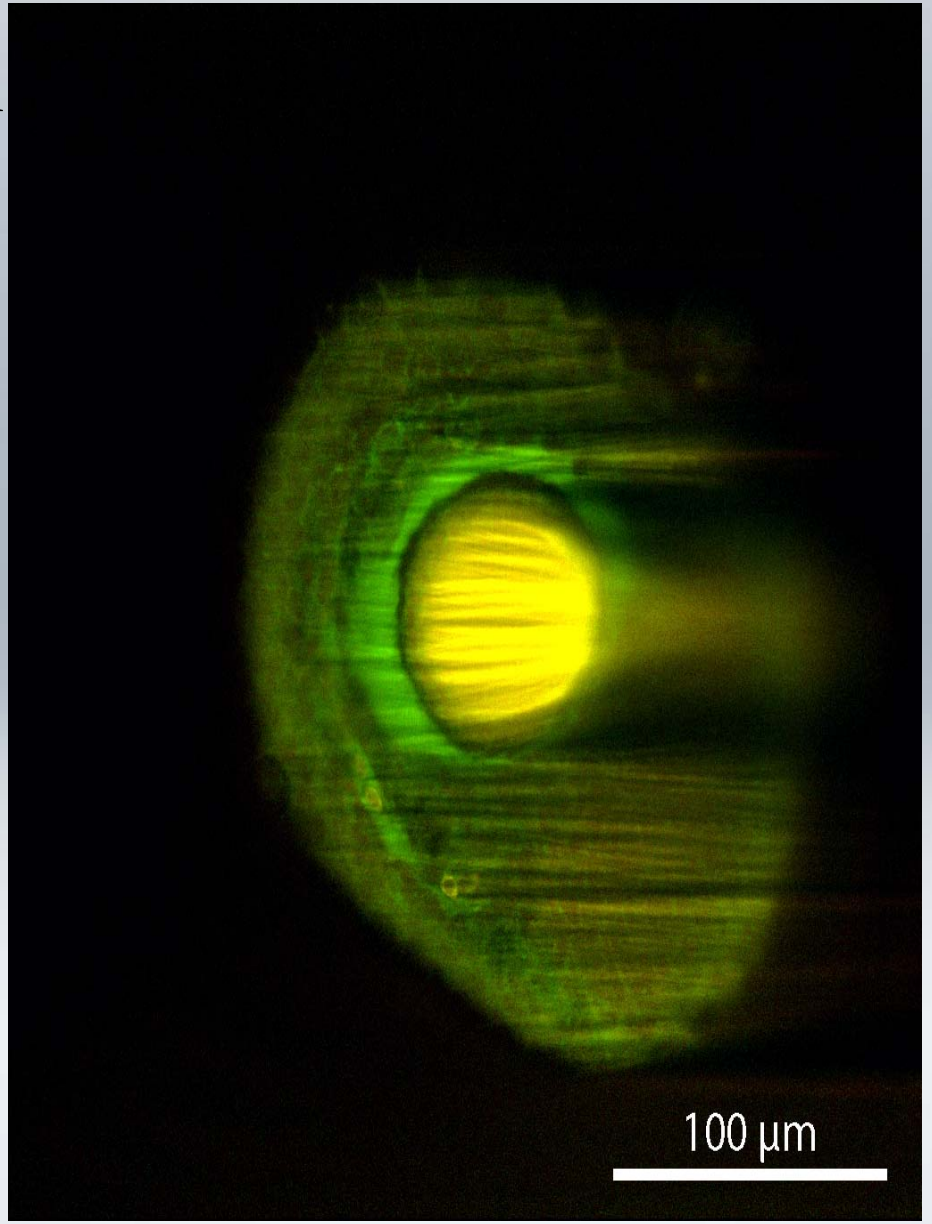






Zebrafish eye

- 1024 × 1024 spectra (2 × 2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 17 min
- 0.8 $mW/\mu m^2$
- ex: 577 nm

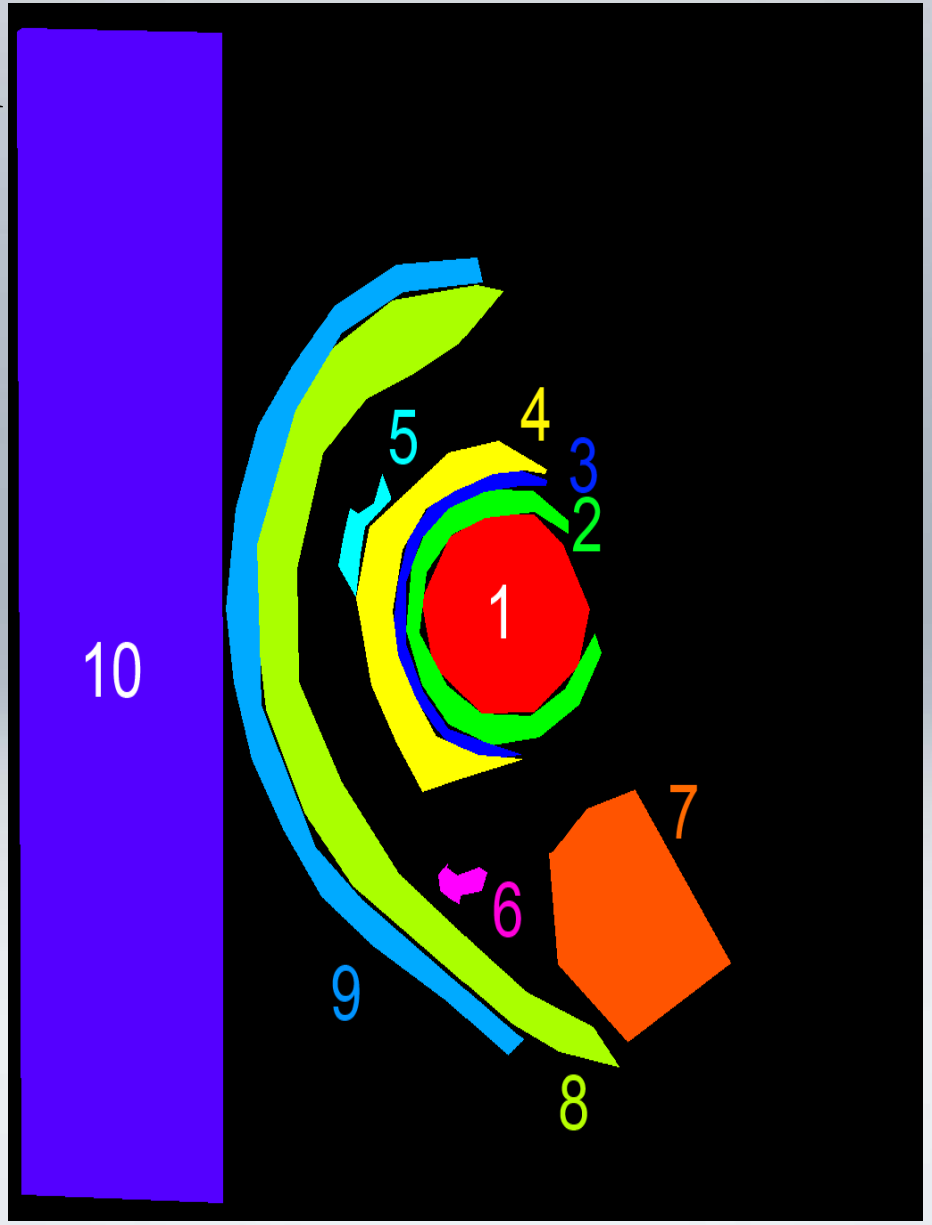


C-H: 2941 - 3017 cm^{-1}

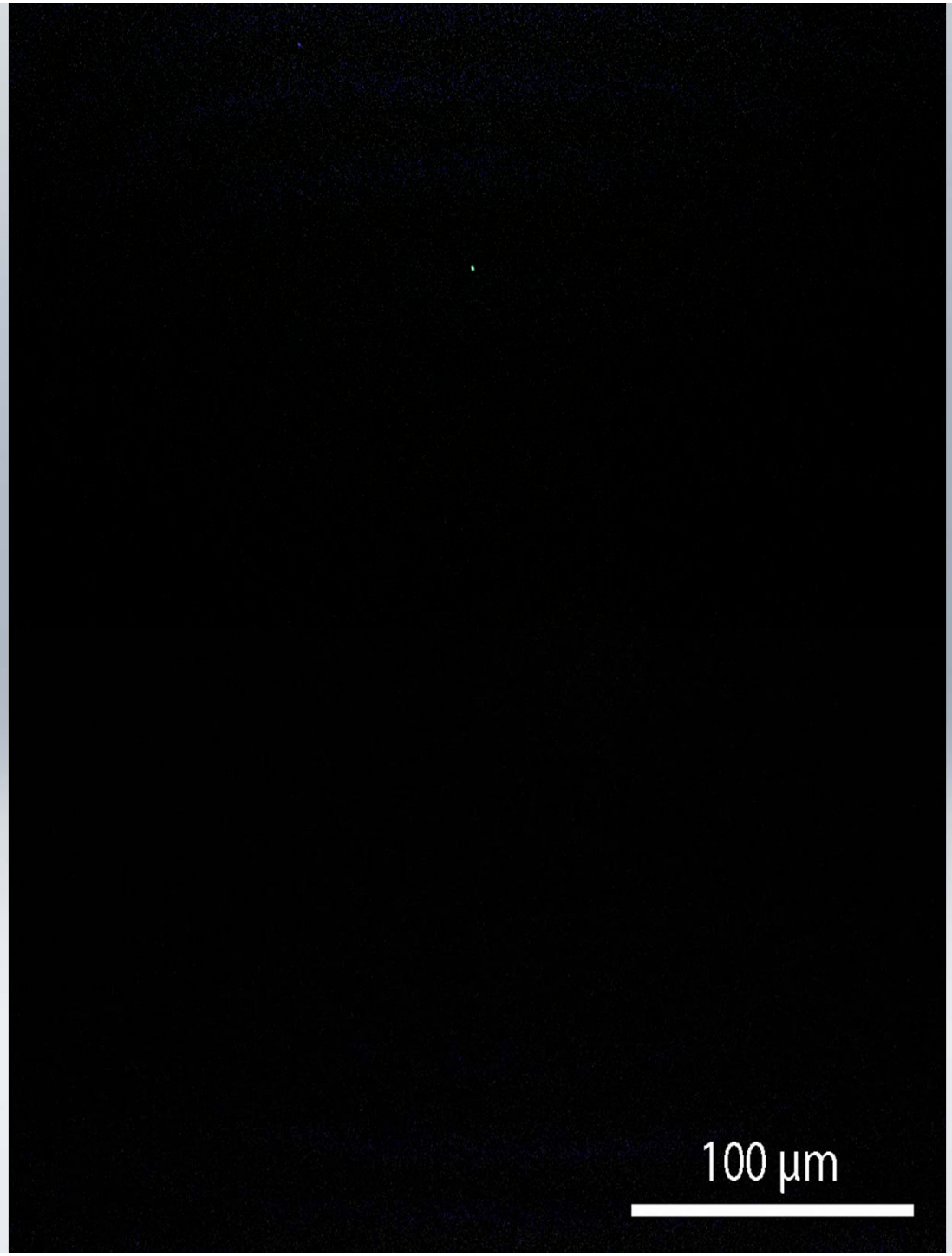
C-H: 2851 - 2910 cm^{-1}

Zebrafish eye

- 1024 × 1024 spectra (2 × 2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 17 min
- 0.8 mW/µm²
- ex: 577 nm



- 50 Slices
- 1024 × 1024 spectra (2×2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 50 · 17 min
- 0.8 mW/µm²
- ex: 577 nm

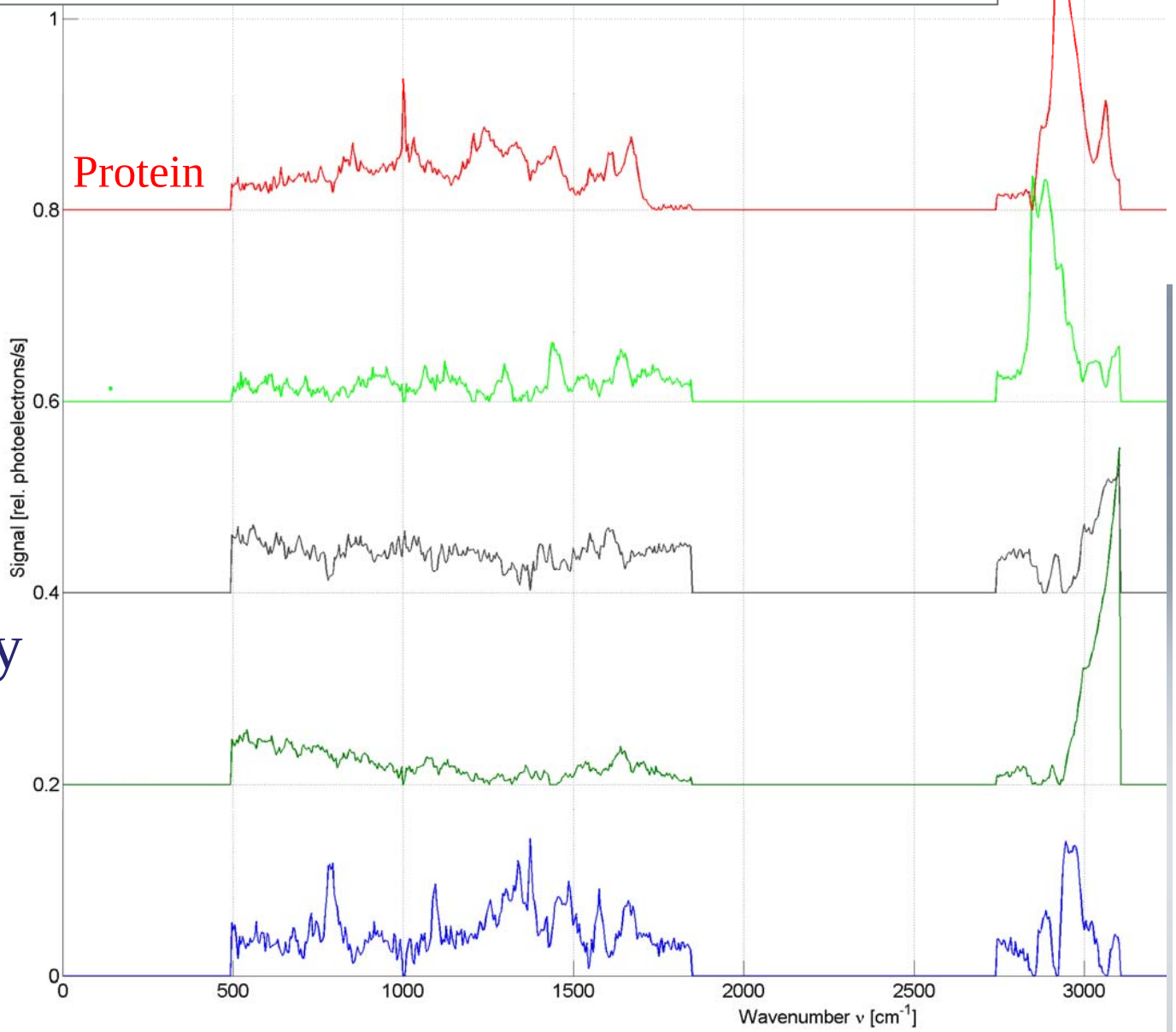


How to find “components”

Can we find the molecular constituents (components) automatically?

→Non-negative matrix factorization (NMF)

Non-negative Matrix Factorization (NMF) : Finding constituents automatically

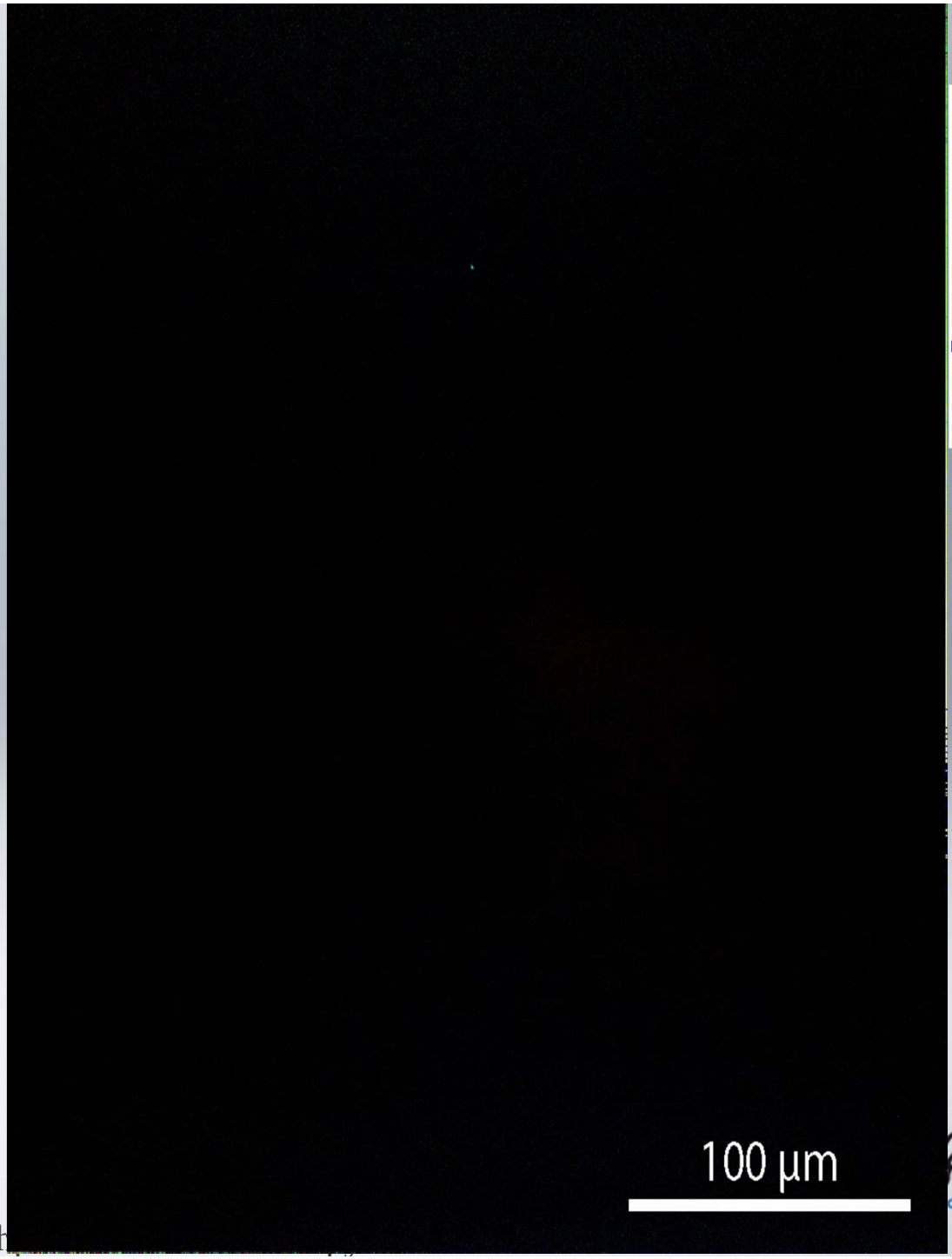


5 components only on the two regions

- 50 Slices
- 1024 × 1024 × 50 spectra (2 × 2 binned)
- Resolution: 18 cm^{-1}
- Tot. exp.: 50 · 17 min
- 0.8 mW/µm²
- ex: 577 nm

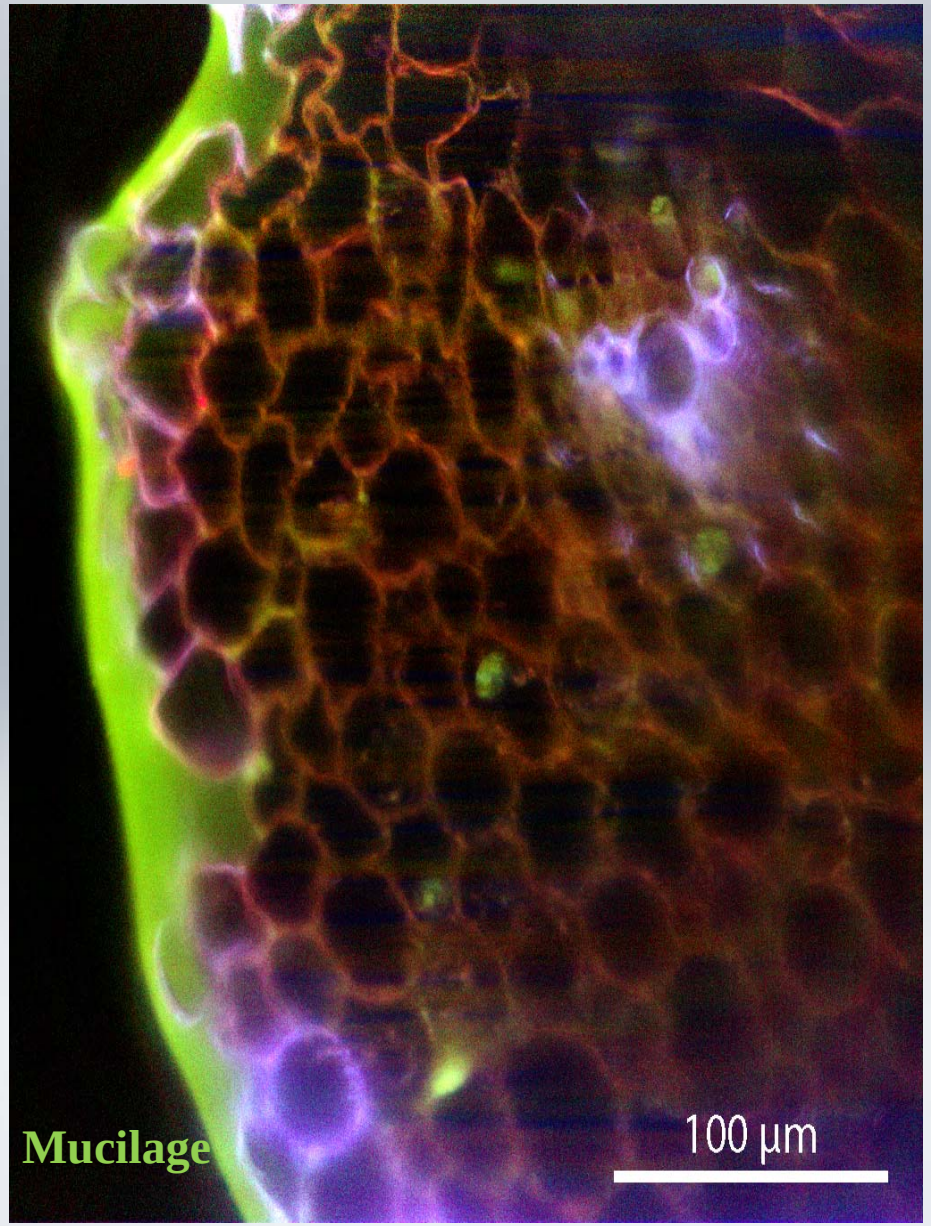
- 4% paraformadehyde
  2% agarose
  14h 13min total exposure

red: Protein/Collagen
green: Lipid
blue: DNA



Galanthus nivalis L. (snowdrop root)

- 1024 × 1024 spectra (2×2 binned)
- 18 cm^{-1}
- Tot. exp.: 17 min
- 1.7 mW/µm²
- ex: 577 nm



red: C-H band
green: C-H band
blue: residual fluroescence

Future directions

- **Build more machines**
- **Applications: Biofilms, Sepsis, living material**
- **Software Autofocus**
- **Heat control**
- **Wavelength: 785nm**
- **Use second interferometer output**
- **Combine with Expansion Microscopy**

Properties/Summary

- **Low out-of-focus light or system-induced Raman**
- **> 300 times lower local intensity compared with confocal Raman system**
- **Exposure time independent of the number of spectra**
- **> 4 000 000 spectra simultaneously**
- **Spectral resolution of < 4 cm^{-1} possible**
- **Shadows can be reduced by water normalisation**
- **Narrow band sampling possible (intentional aliasing)**
- **3D possible**

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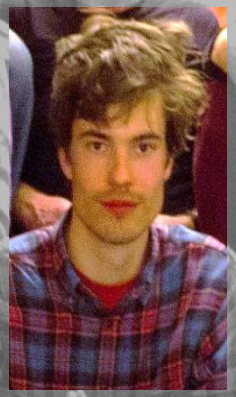

Ulrich Leischner