

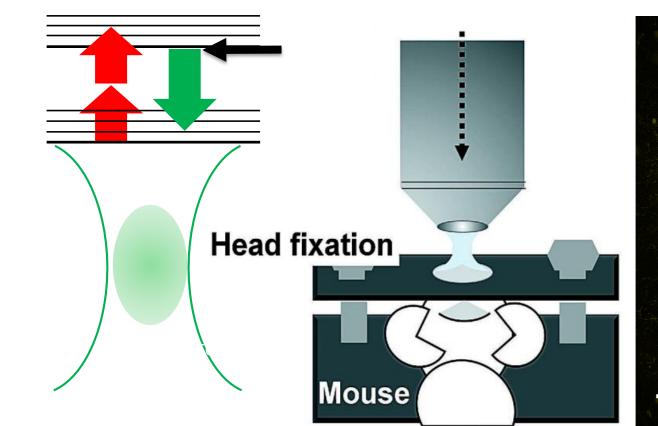


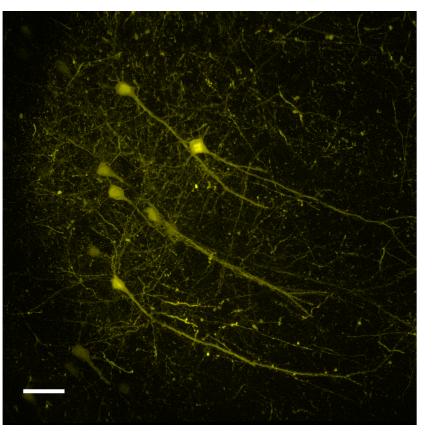


Core Unit Fluorescence Imaging

Rudolf-Virchow-Center for Integrative & Translational Bioimaging, University of Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany

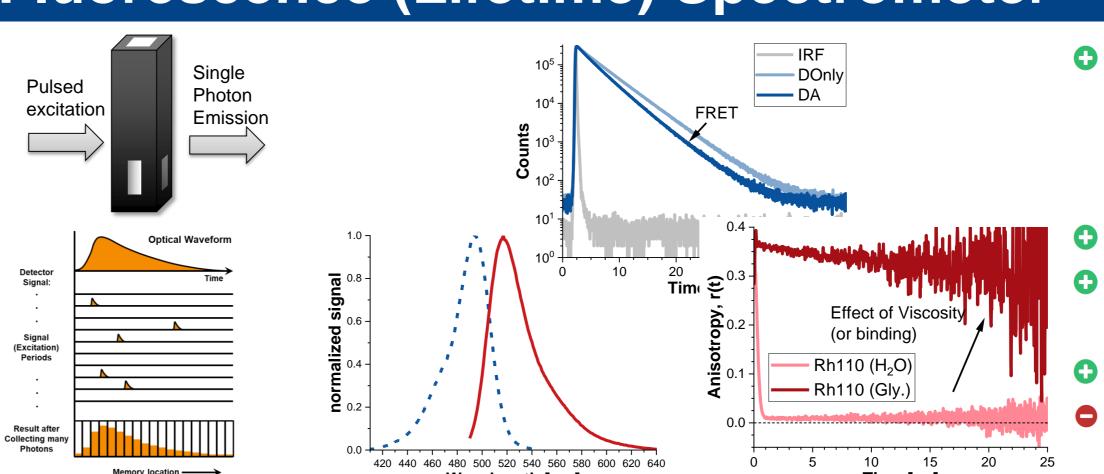
Two-Photon Microscopy (2PM)





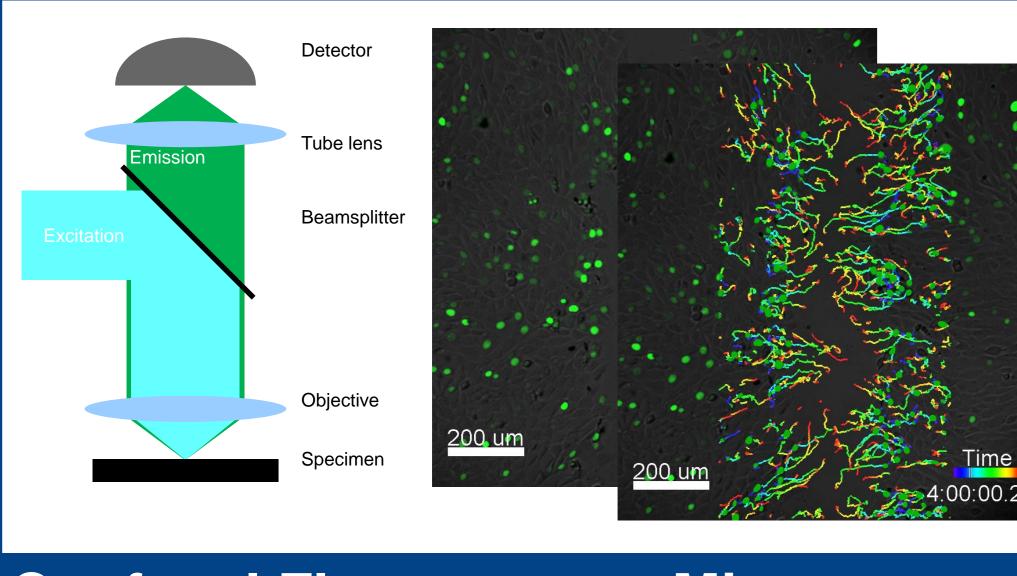
- Optical sectioning
- 3D imaging
- Deep tissue penetration
- ntravital imaging
- _ow autofluorescence
- Limited choice of dyes
- High risk of photobleaching

Fluorescence (Lifetime) Spectrometer



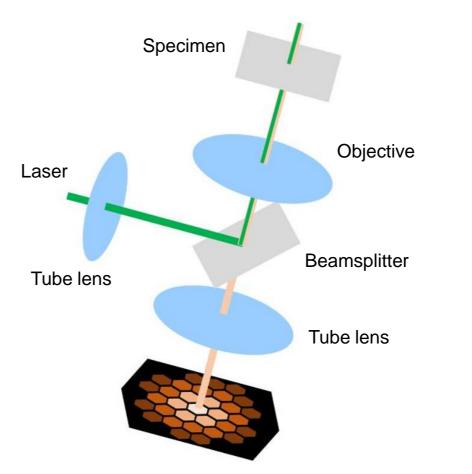
- Fast & automatable
- → up to 4 cuvettes
- → timeseries
- → temperature series
- Anisotropy (binding/rotation)
- FRET (interaction/ protein conformation)
- Environment sensing
- No spatial or mobility information

Fluorescence Video Microscopy

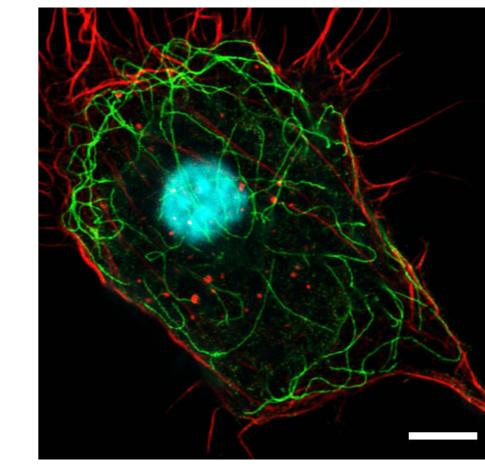


- Fast (camera-based acquisition)
- Multi-color
- Live cell long-term acquisition
- Photon efficient
- No optical sectioning > risk to detect scattered and out-of focus light

AiryScan Super-resolution Microscopy (LSM980)



Airy detector

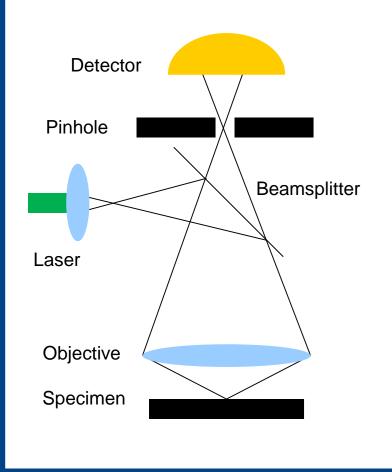


- 3D imaging & Optical sectioning (airy disc elements)
- Up to 1.8x resolution enhancement
- Live cell long-term acquisition

Software

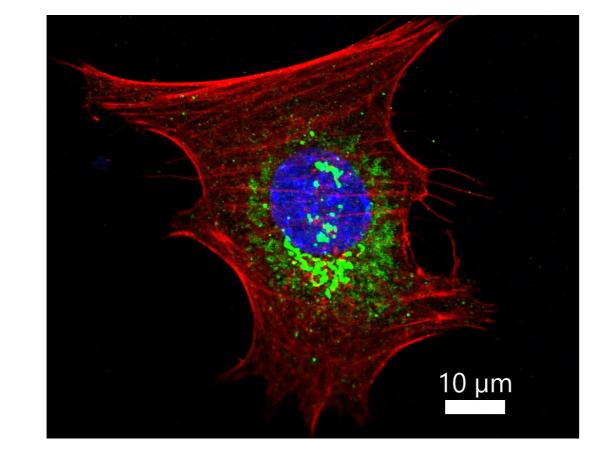
- Multicolor
- Risk of photobleaching
- Low sample penetration
- Slow

Confocal Fluorescence Microscopy



Excitation

Light source



Fluorescence Lifetime Imaging Microscopy (FLIM)

- Optical sectioning (pinhole)
- 3D imaging
- Live cell long-term acquisition
- Multi-color
- High risk of photobleaching
- Low sample penetration

Time-resolved information

→ Interactions (homoFRET)

→ protein-protein interaction

→ structural information

Confocal sectioning

→ Binding/Rotation

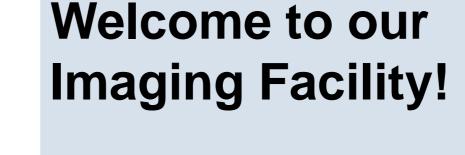
Anisotropy

FRET

Slow

Slow

Katrin

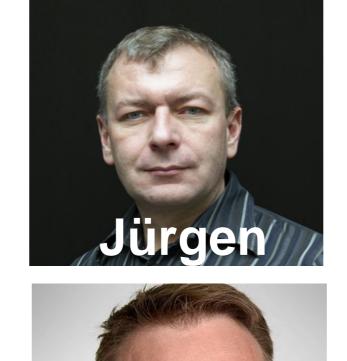




PLAN and familiarize yourself with all facility rules.



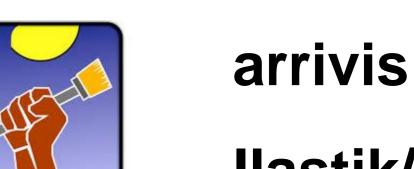
BOOK off-peak times if possible, or schedule long experiments overnight.



LOG OUT after closing all windows on the computer to secure and protect your data.



Huygens



Imaris

llastik/Labkit

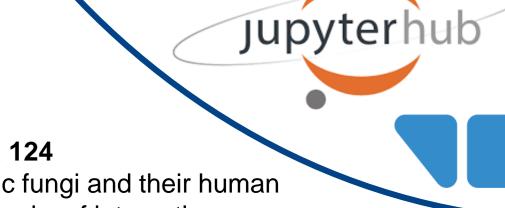
SymPhoTime



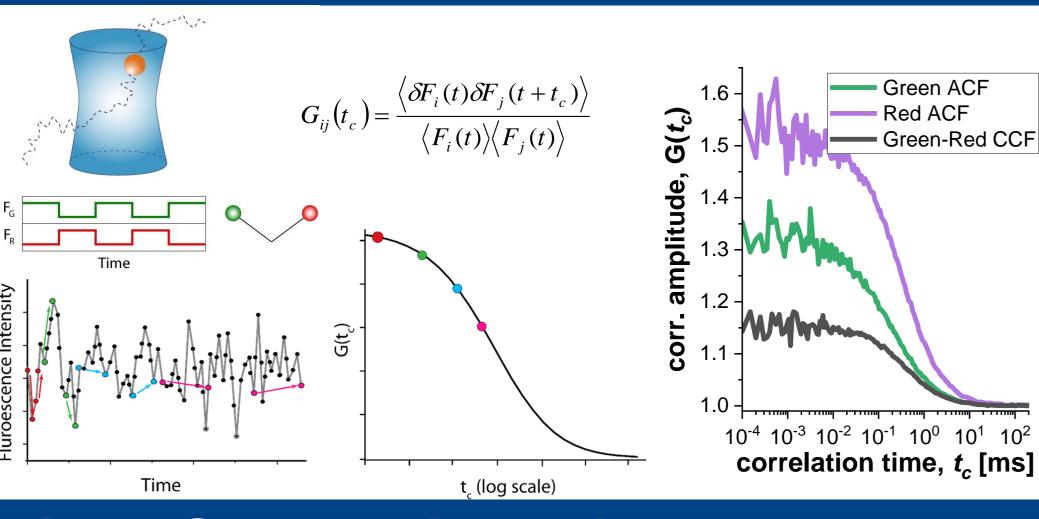
JupyterHub



MATLAB[®]



Fluorescence Correlation Spectroscopy (FCS)



Confocal sectioning

→ Biosensors

- Time-resolved information Single molecule sensitive
- Determining
 - → binding affinities
 - → concentrations
 - → molecular interaction
- → protein dynamics
- No spatial information (image)
- From the fundamentals of biofabrication towards functional tissue models

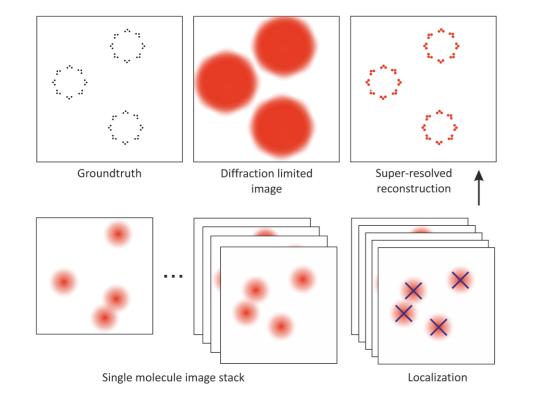
Joachim

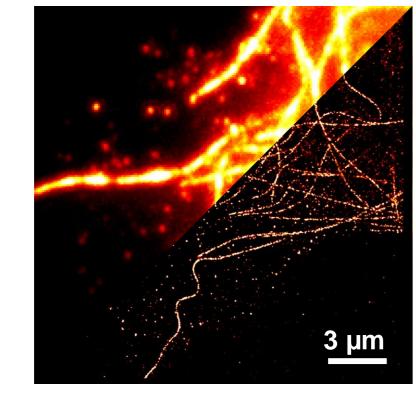




CRC/TRR 124 Pathogenic fungi and their human host: Networks of interaction

Direct Stochastic Optical Reconstruction Microscopy (dSTORM), mirror enhanced (me)STORM





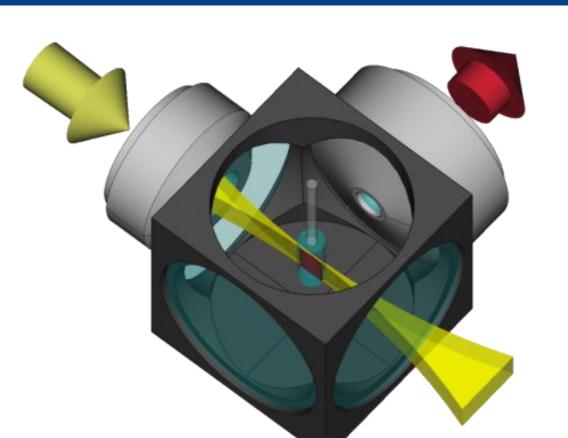
- Camera-based acquisition
- Single molecule detection
- Super-resolution
- Very limited choice of dyes Limited live cell compatibility
- Slow

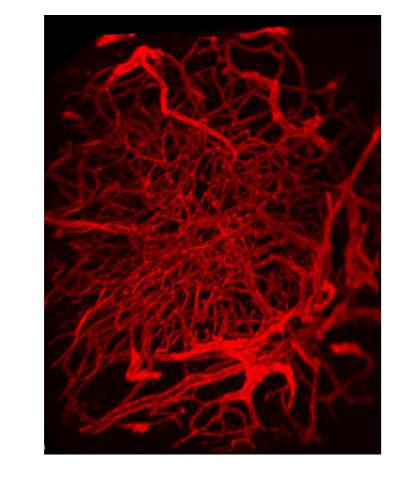
Micro-Patterning System (PRIMO)



- Property of Prof. Dr. Markus Bender (JMU) Hosted by the CU Imaging Attached to widefield microscope
 - Protein micropatterns in cell culture substrates
 - Cell migration & adhesion
 - 3D confinement
 - microfabrication
 - Slow

Light-Sheet Fluorescence Microscopy (LSFM)





- 3D imaging of large specimen with cellular resolution
- Fast camera-based acquisition
- Multi-color
- _imited choice of dyes
- Optical clearing required
- Long sample preparation → low throughput

Computational & Data Analysis Resources

