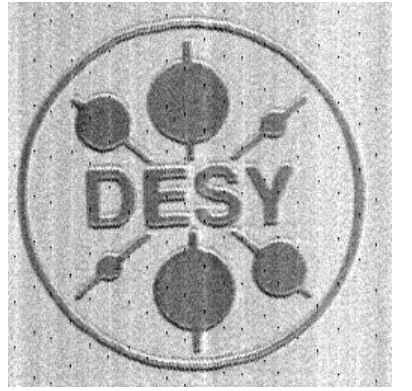


Correlative Bio-Imaging at the GINIX:

Scanning SAXS, Holography, and Optical Fluorescence

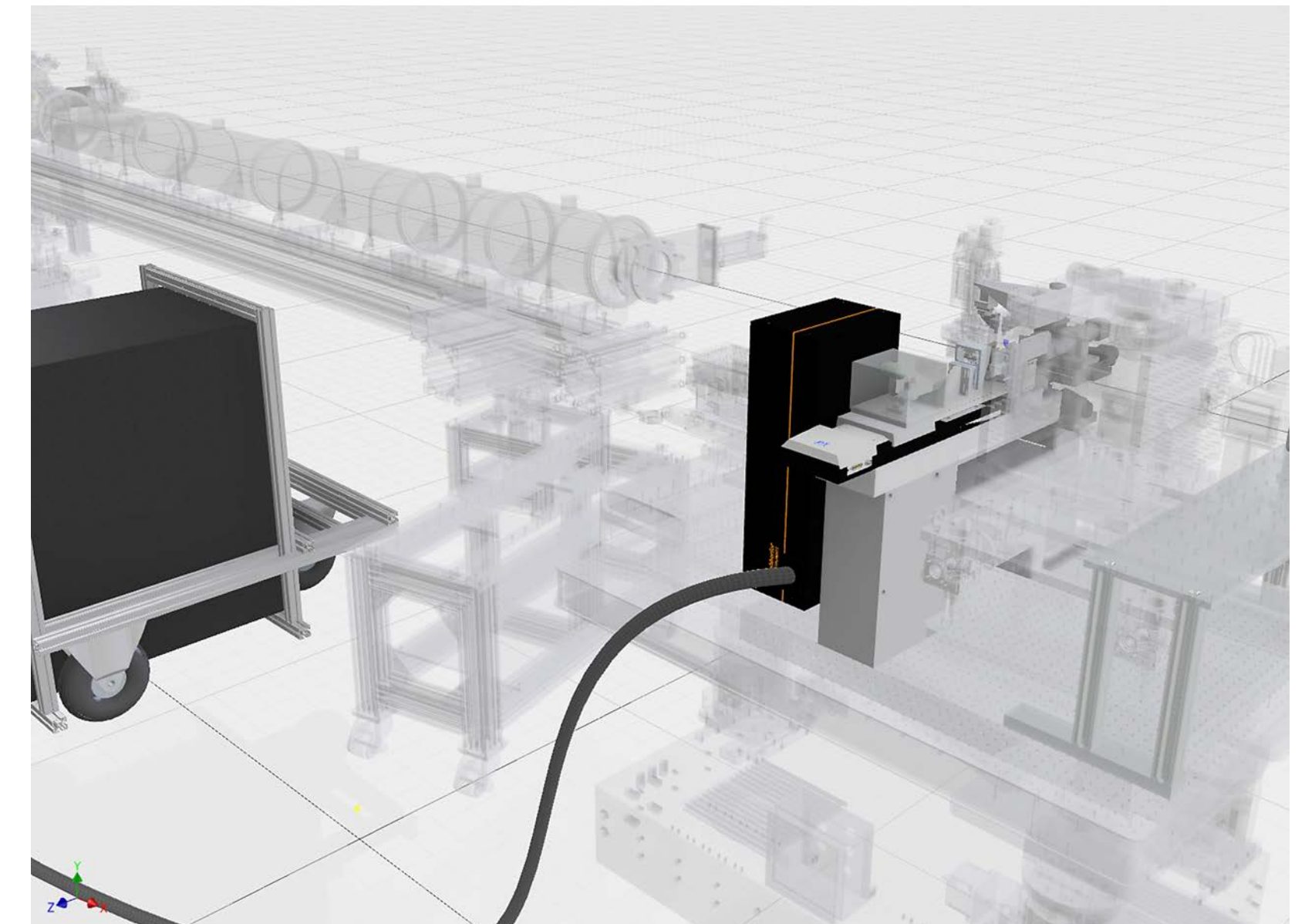
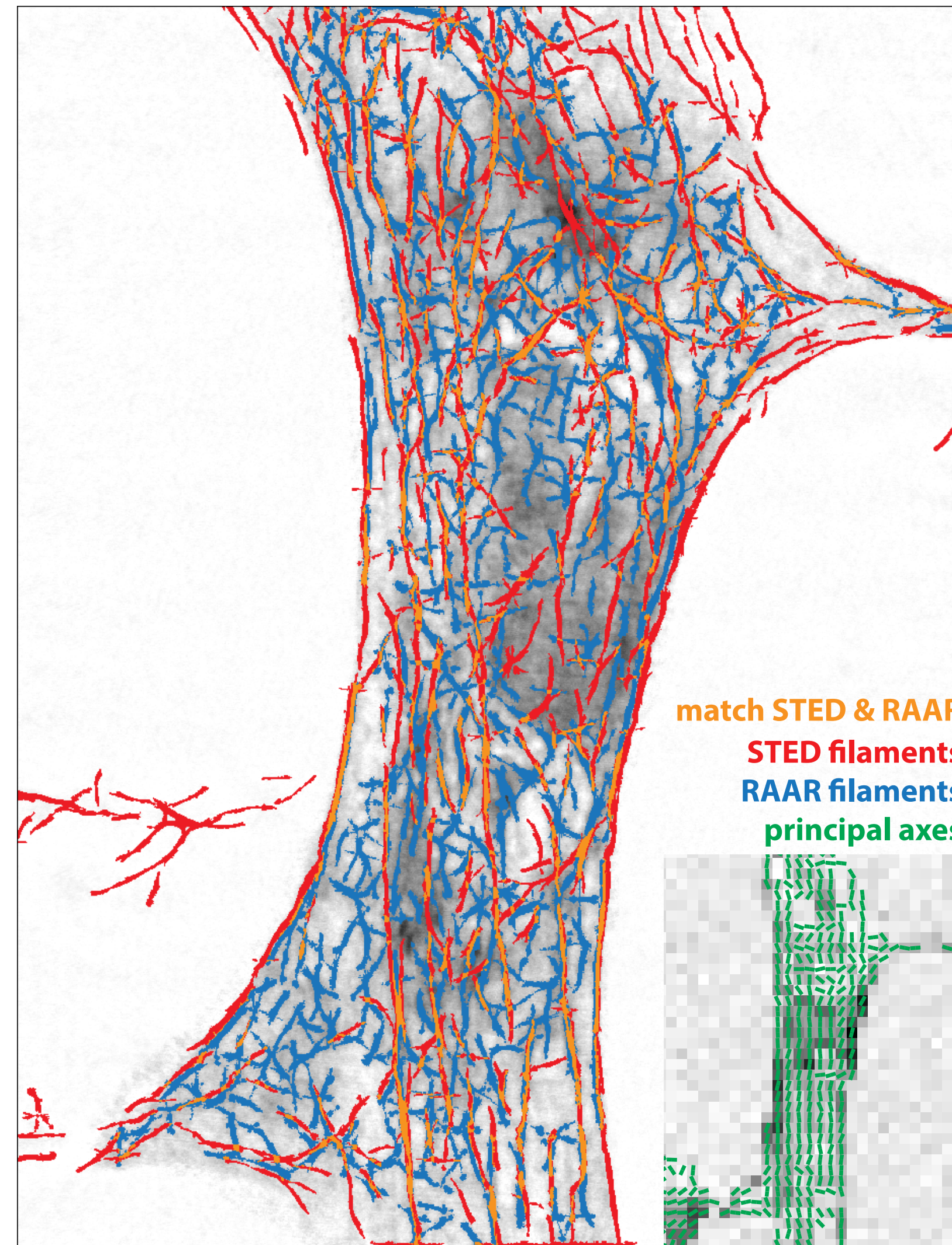


Markus Osterhoff

Marten Bernhardt, J. D. Nicolas,
H. Mittelstädt, M. Reuss, B. Harke,
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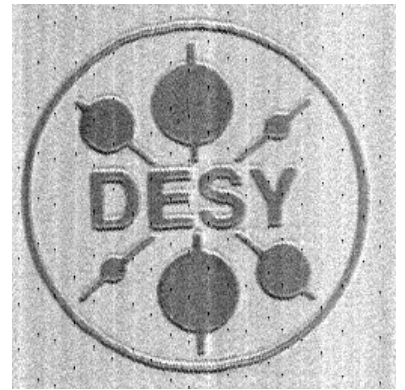
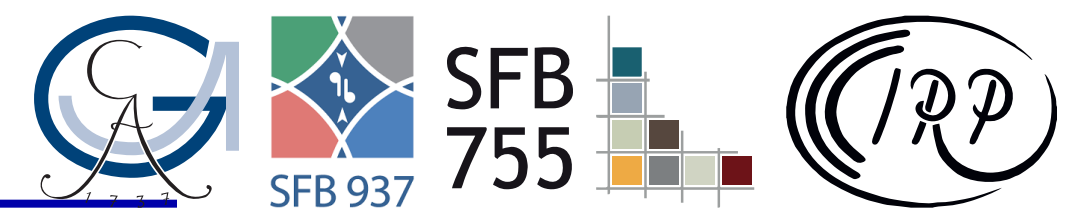
Institut für Röntgenphysik, Uni Göttingen
Abberior Instruments, Göttingen

- ▶ Introduction & Applications
- ▶ Imaging methods:
STED - optical super-resolution;
X-ray holography,
scannig SAXS
- ▶ Instrumentation
- ▶ Results
- ▶ Discussion



Correlative Bio-Imaging at the GINIX:

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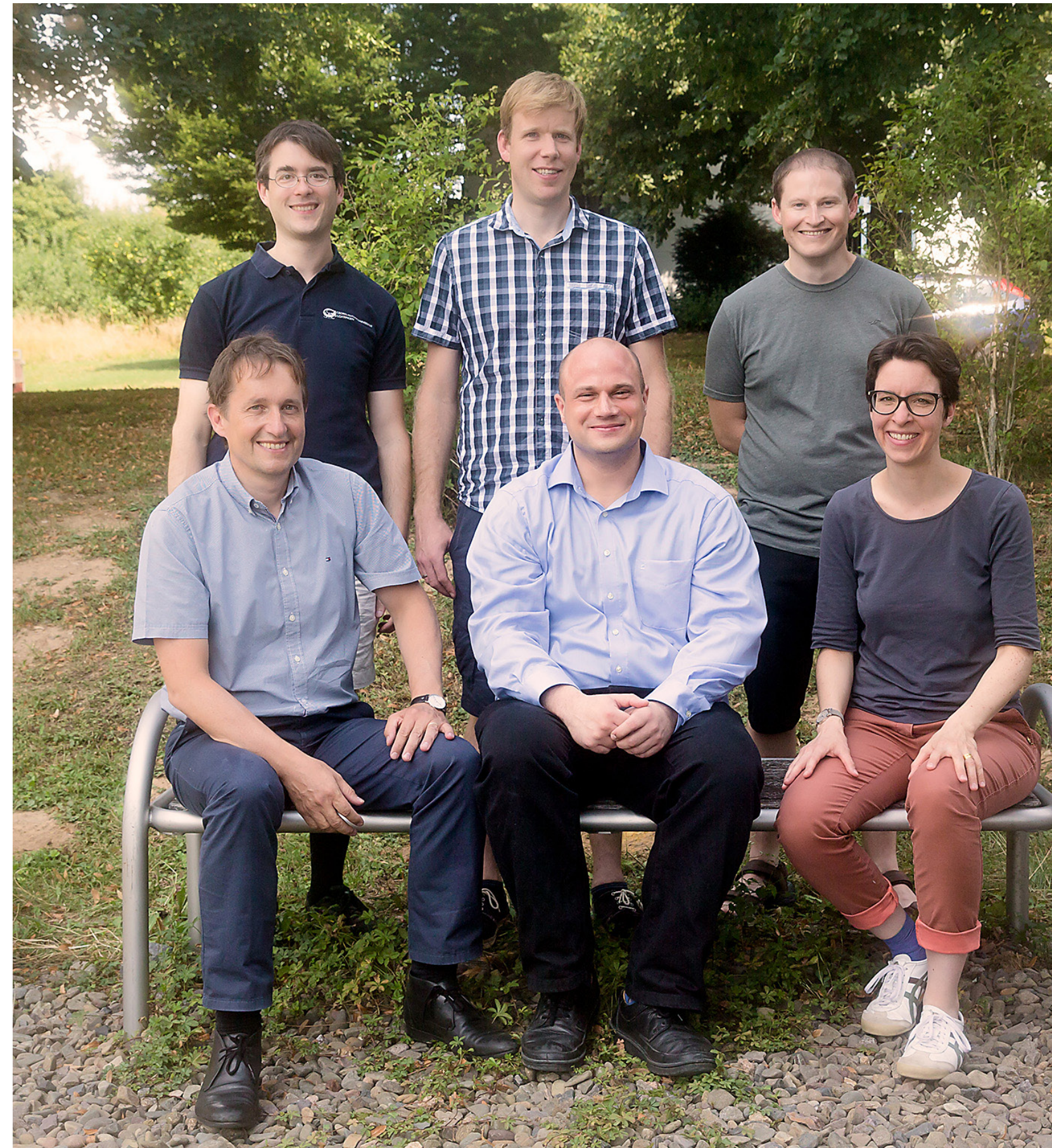


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Thanks to ...

- ▶ Bastian Hartmann,
Peter Luley,
Peter Nieschalk,
Susanne Hengst
- ▶ Stefan Hell
- ▶ Workshops
- ▶ DESY Photon Science
- ▶ funding agencies:
DFG (SFB 755, SFB 937)
BMBF (05K16MG2)

GINIX @ P10 @ PETRA III @ DESY

Göttingen Instrument for
Nano-Imaging with X-rays

- ▶ waveguide-based holography
/ tomography
- ▶ scanning nano-SAXS
- ▶ 300 nm KB, 8.0 / 13.8 keV
- ▶ SPEC controlled :)
flexible setup
- ▶ designed / operated by
group of Tim Salditt / Uni Göttingen



Introduction & Applications

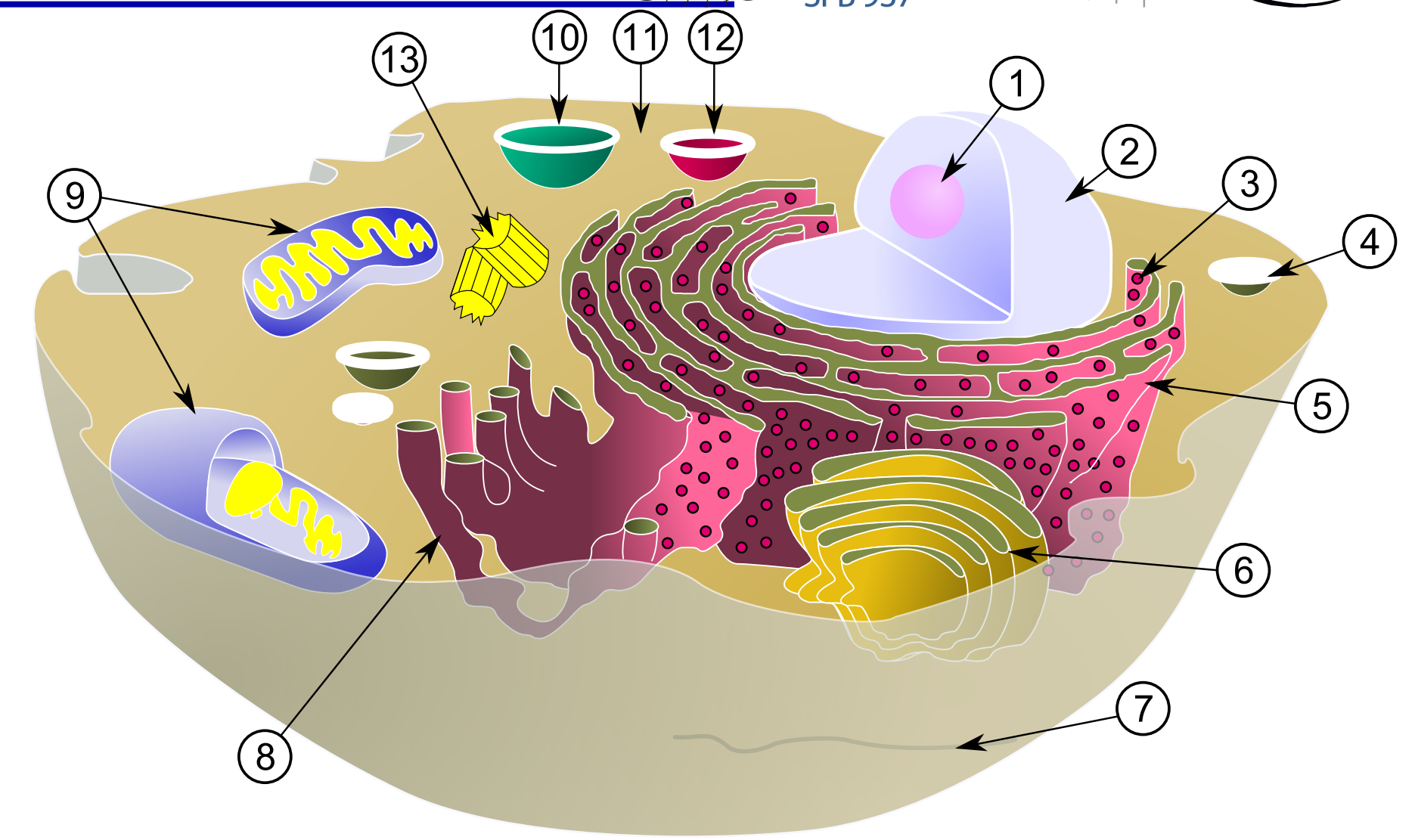
What do we
want to see?

The living cell

actually, between as dead as a dodo,
and initially alive ...

- ▶ **molecular** transport and dynamics
energy and information
- ▶ (mis-)folding of **proteins**
structure - function
- ▶ filaments and **networks**
mechanical stability, allows for division
- ▶ **organelles**, nucleus, cytoplasm

... from Ångström to 100 μm ...



Von MesserWoland und Szczepan1990 - Eigenes Werk (Inkscape erstellt),
CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=1279365>

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- ... from Ångström to 100 μm ...

Holography / ptychography

- ▶ phase shift / electron density

Tomography

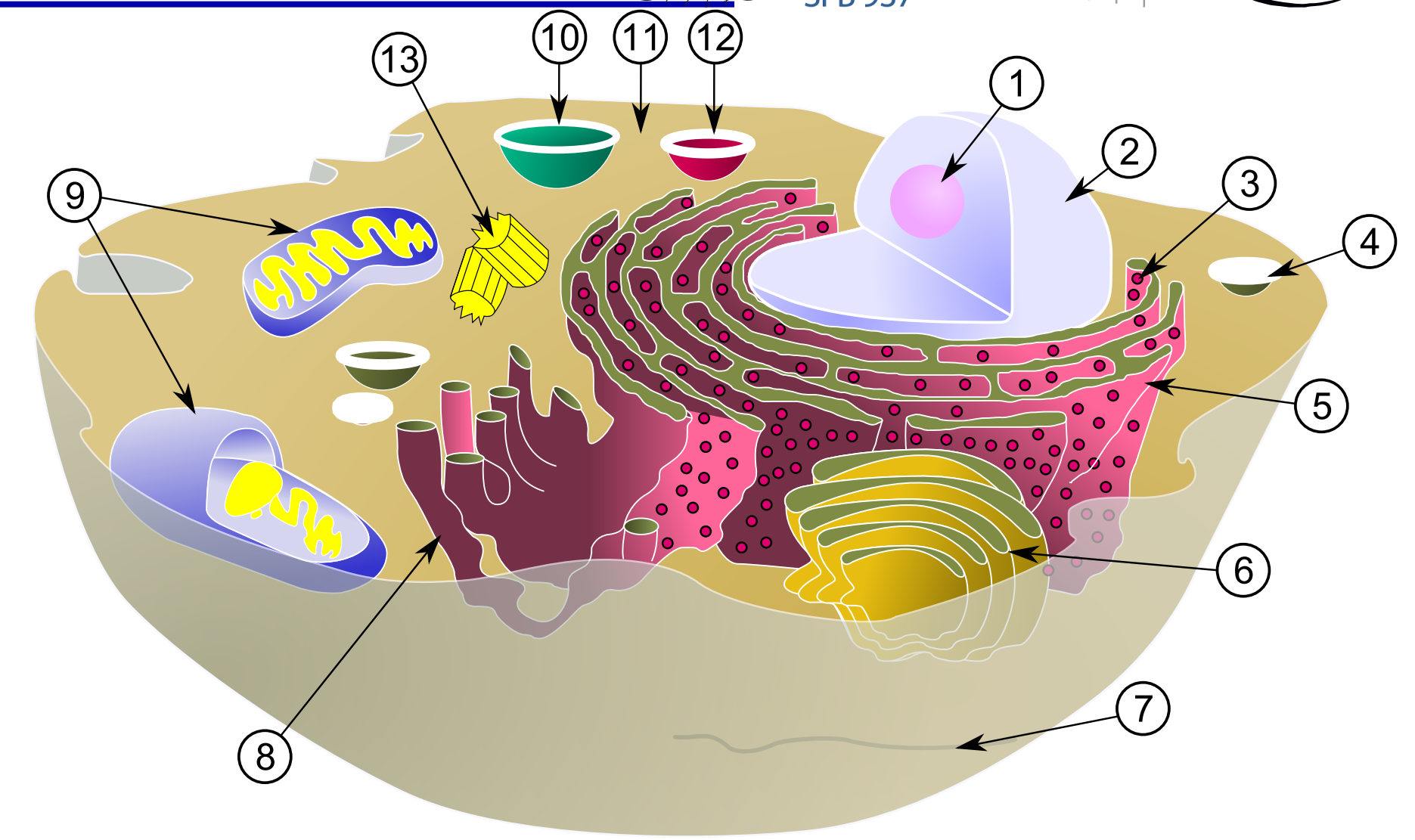
- ▶ electron density in 3D

SAXS / WAXS / crystallography

- ▶ structures, sizes, shapes, ordering

nano-SAXS

- ▶ spatially resolved (≥ 50 nm)



Von MesserWoland und Szczepan1990 - Eigenes Werk (Inkscape erstellt),
CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=1279365>

Soft X-ray microscopy

- ▶ absorption; 2D and 3D

Fluorescence, edges

- ▶ elemental composition,
electronic environment

Molecular movies, structural dynamics

- ▶ fs/ps (XFEL), ns/μs (Synchrotron)

...

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Soft X-ray microscopy

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...

... with optical
fluorescence?

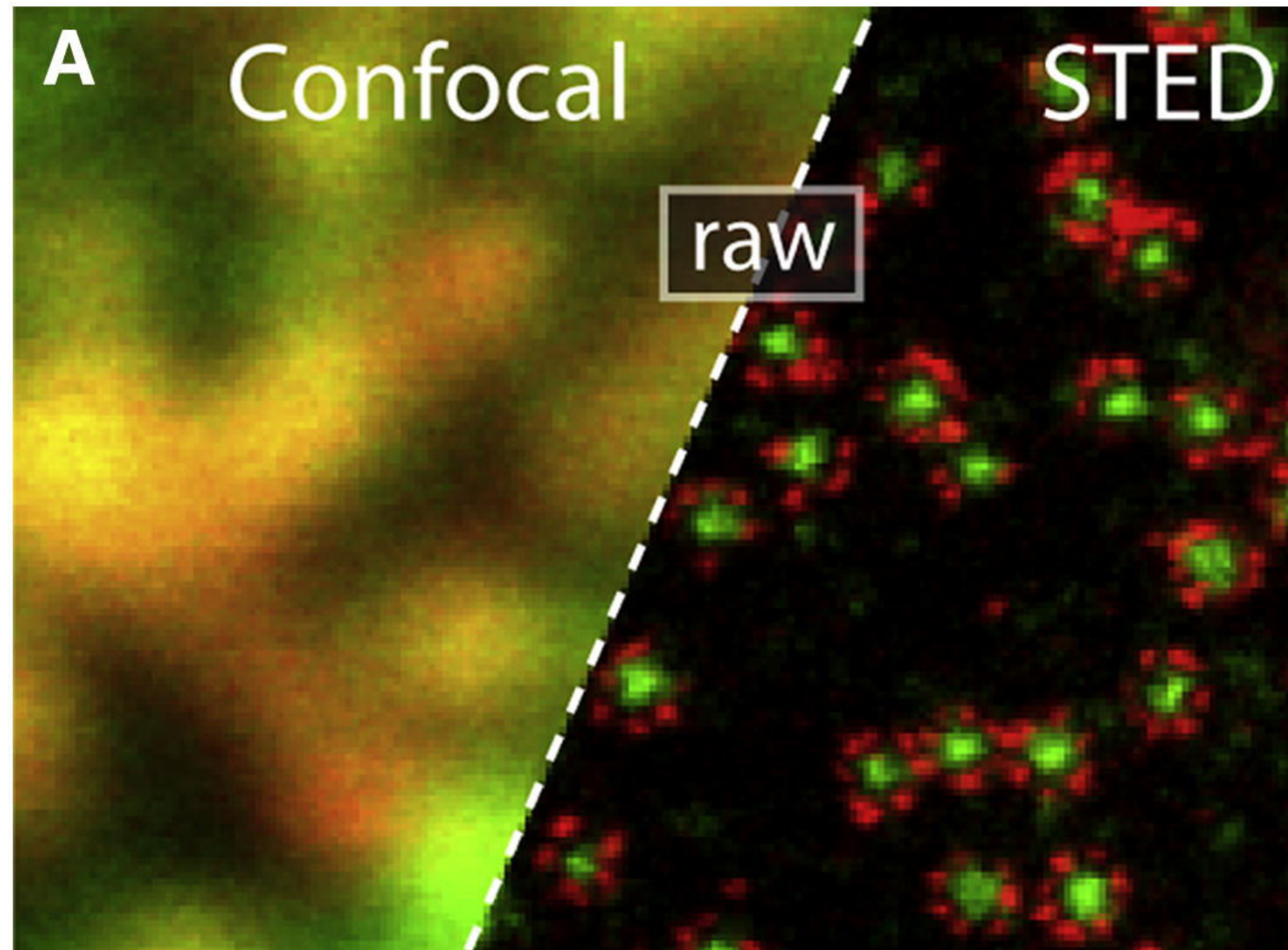
Fluorescent Dyes

- ▶ **molecular markers**,
attached to specific sites
- ▶ **position: high spatial resolution**,
time evolution
- ▶ fluorescent lifetime (FLIM):
electronic environment
- ▶ Förster resonance energy transfer (FRET):
distance calibration
- ▶ total internal reflection (TIRF):
sensitive to the surface

"optical":

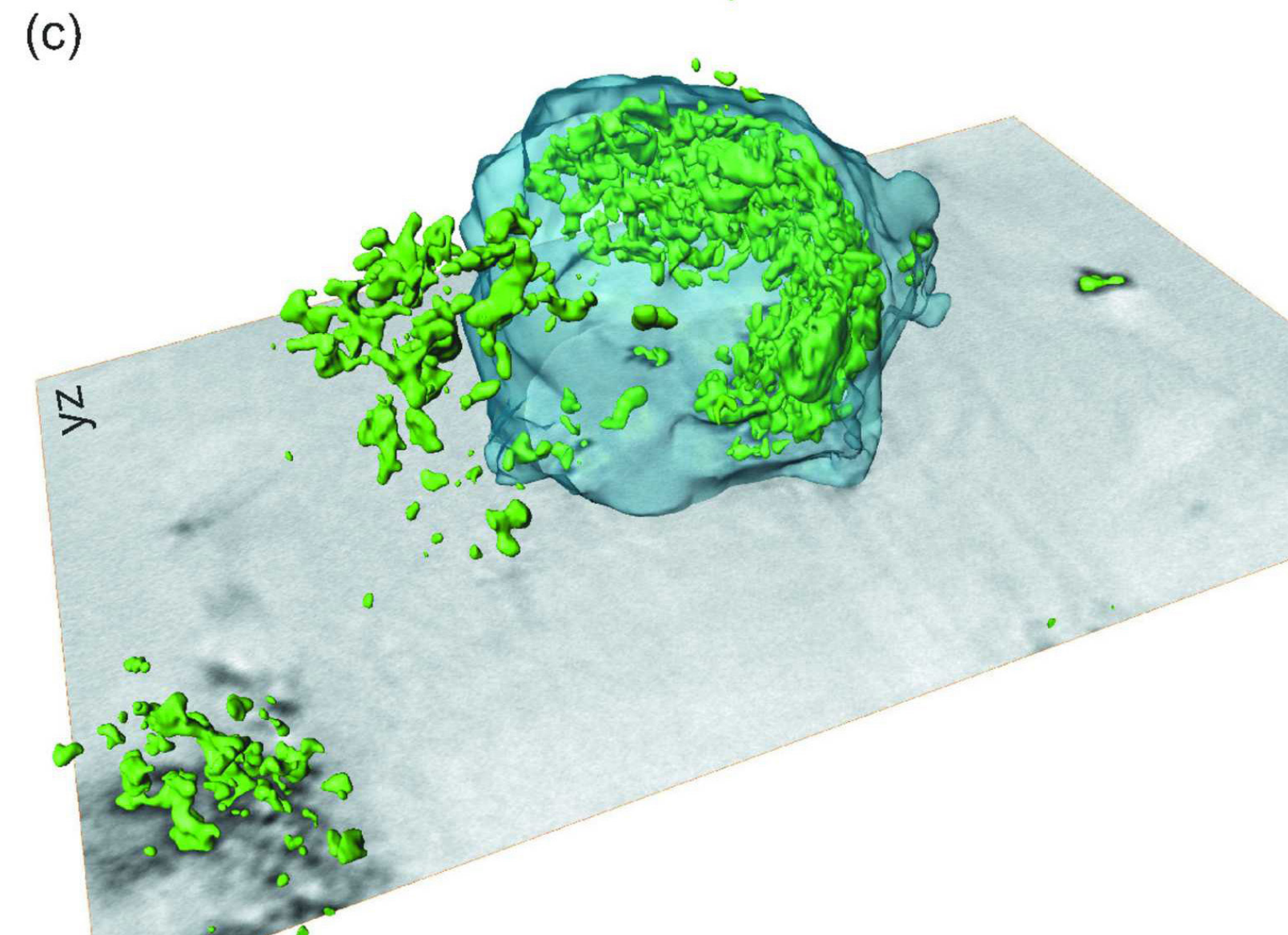
(almost) visible light, e.g. 775 nm
good optics, bad Abbe ...

STED microscopy X-Ray Holography Scanning SAXS



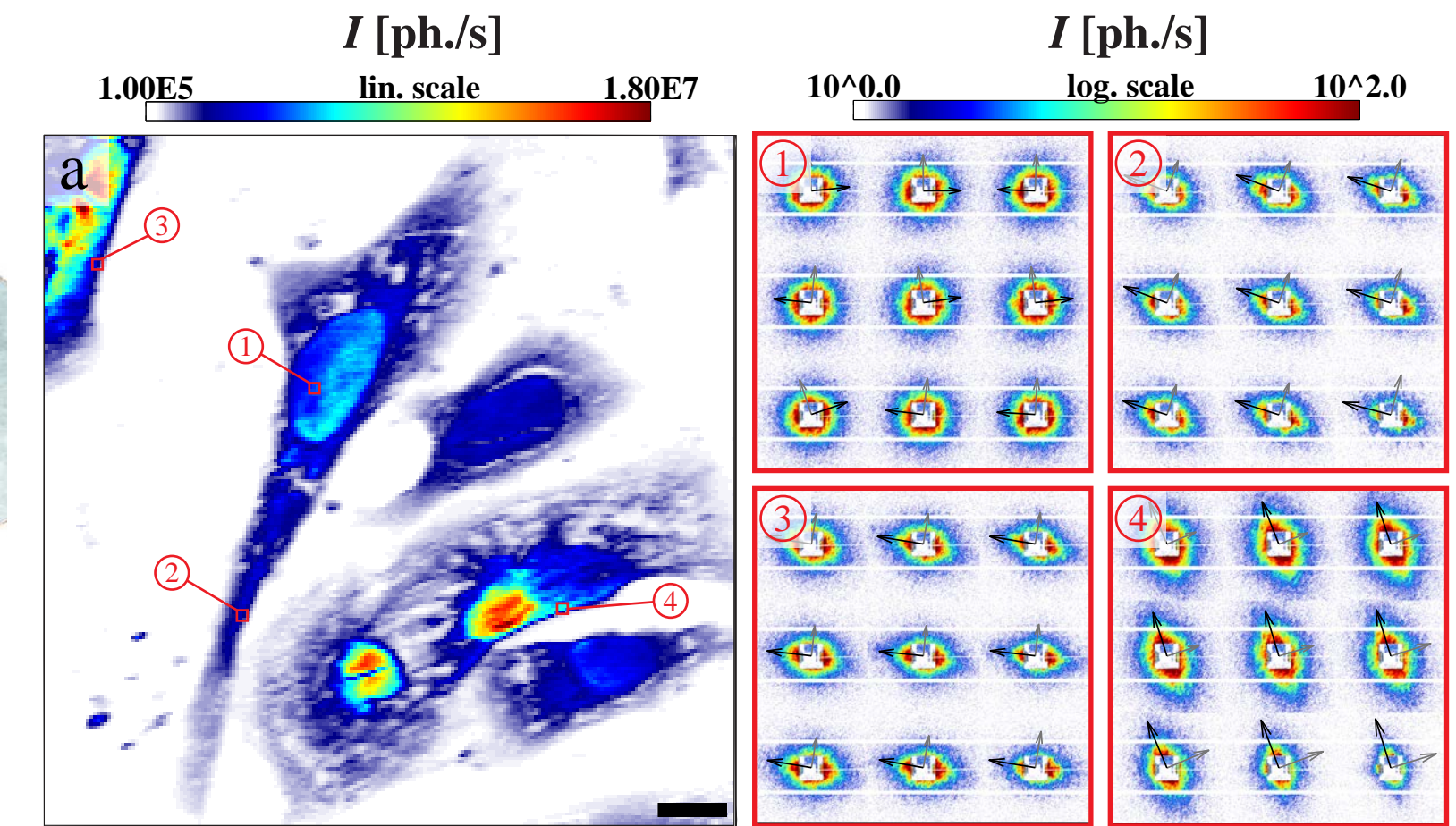
The What (is it?)

- ▶ molecular markers: specific sites if you see **atto633** (the dye), the Phalloidin ("glue") is attached to **Actin**
- ▶ spatial resolution 10 ... 25 nm at GINIX: ~ 100 nm



The Where (is it?)

- ▶ electron density
- ▶ spatial resolution ≤ 100 nm



The How (does it look like?)

- ▶ local ordering and structures
- ▶ spatial resolution ~ 100 nm, reciprocal resolution ~ 1 nm^{-1}

X-rays: short wavelength

Optics: good optics

always: limited by diffraction

... it's either the λ , or the NA ...

- ▶ **excitation** @ 632 nm
diffraction limited
- ▶ **emission** @ 651 nm
spontaneous, diffraction limited



Imaging methods: **ST**imulated **E**mission **D**epletion

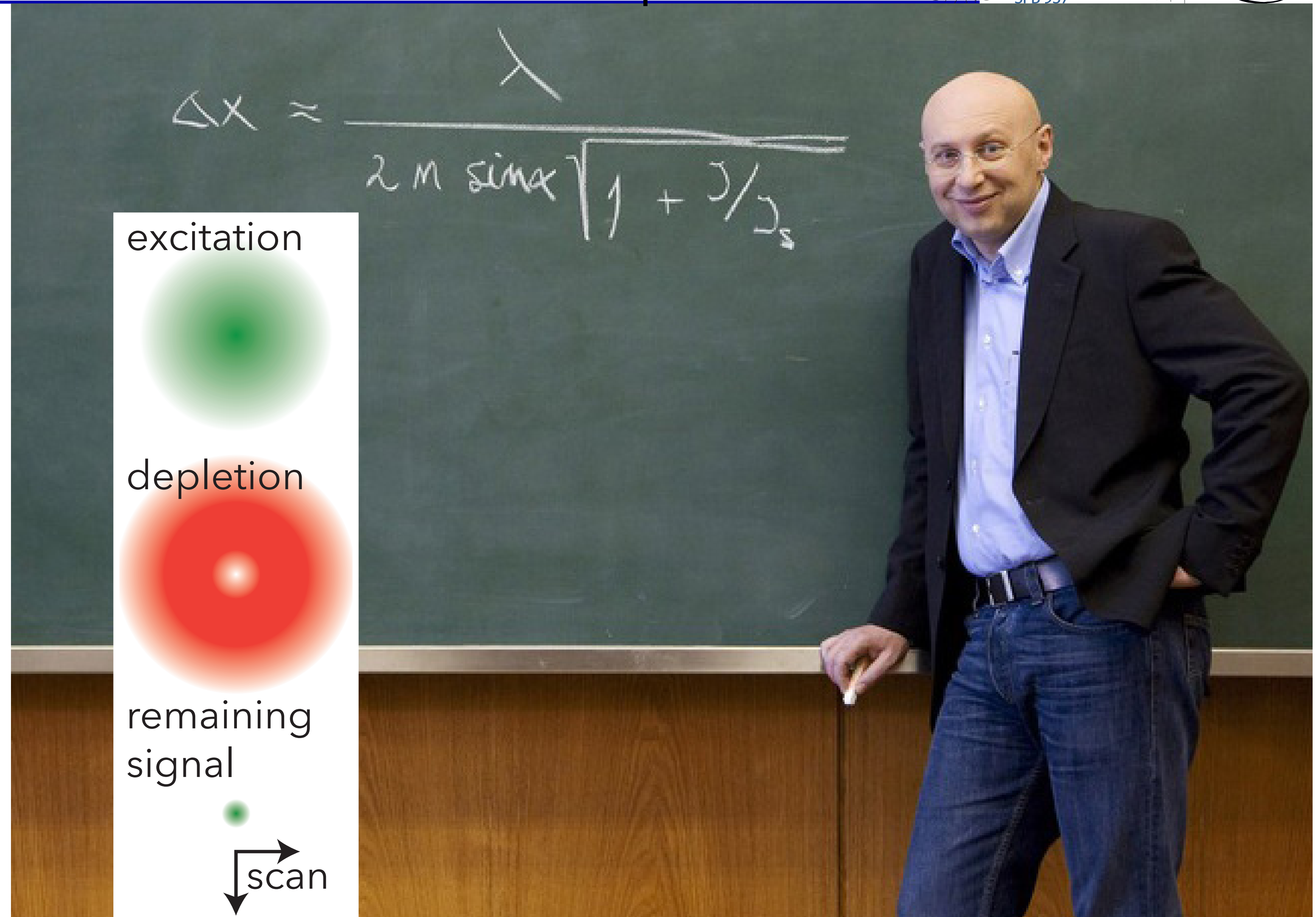
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also diffraction limited,
but non-linear effect:
"effective hole" becomes sharp
- ▶ **emission** @ 651 nm
now limited to ≤ 25 nm region
- ▶ **pulsed and scanned** over full region



Imaging methods: **ST**imulated **E**mission **D**epletion

$$\Delta x \approx \frac{\lambda}{2 n \sin \alpha \sqrt{1 + \frac{3}{2} \frac{\lambda}{\lambda_s}}}$$

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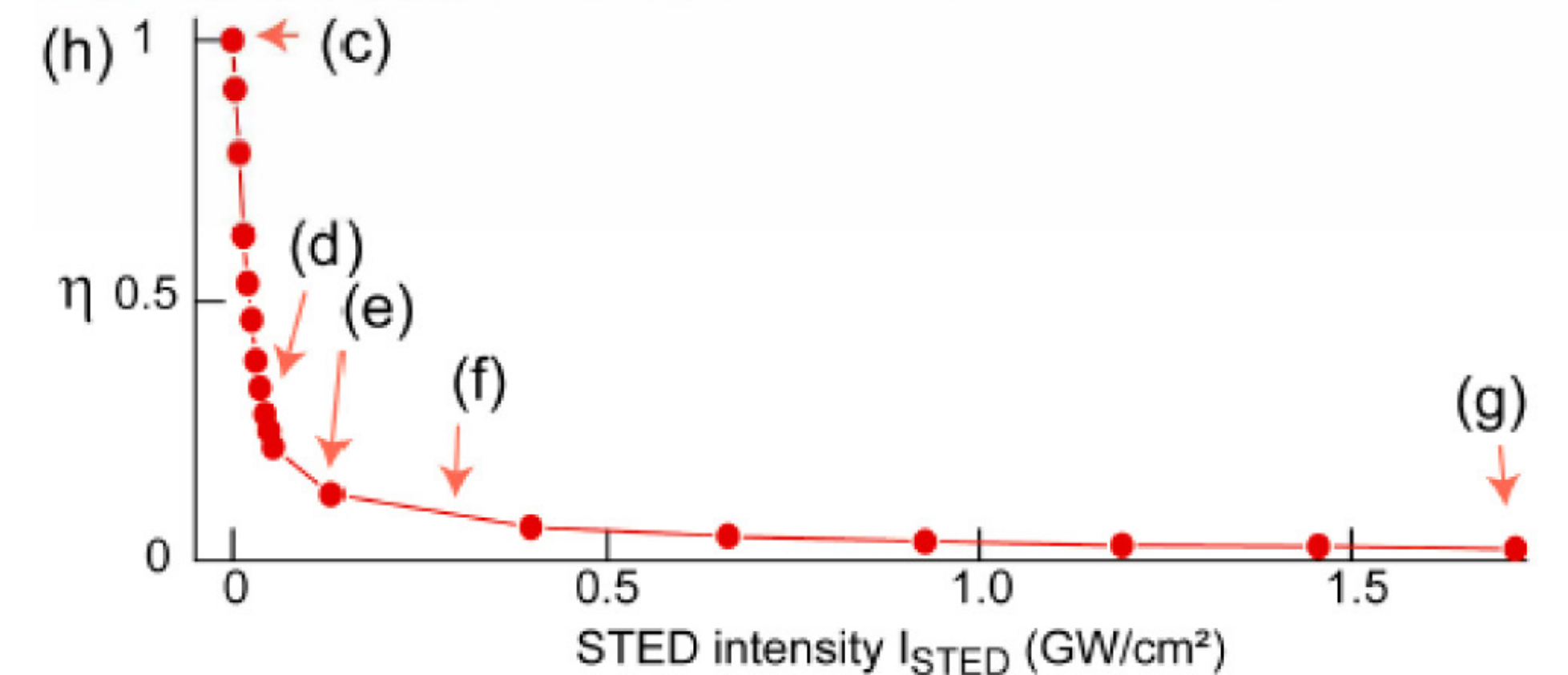
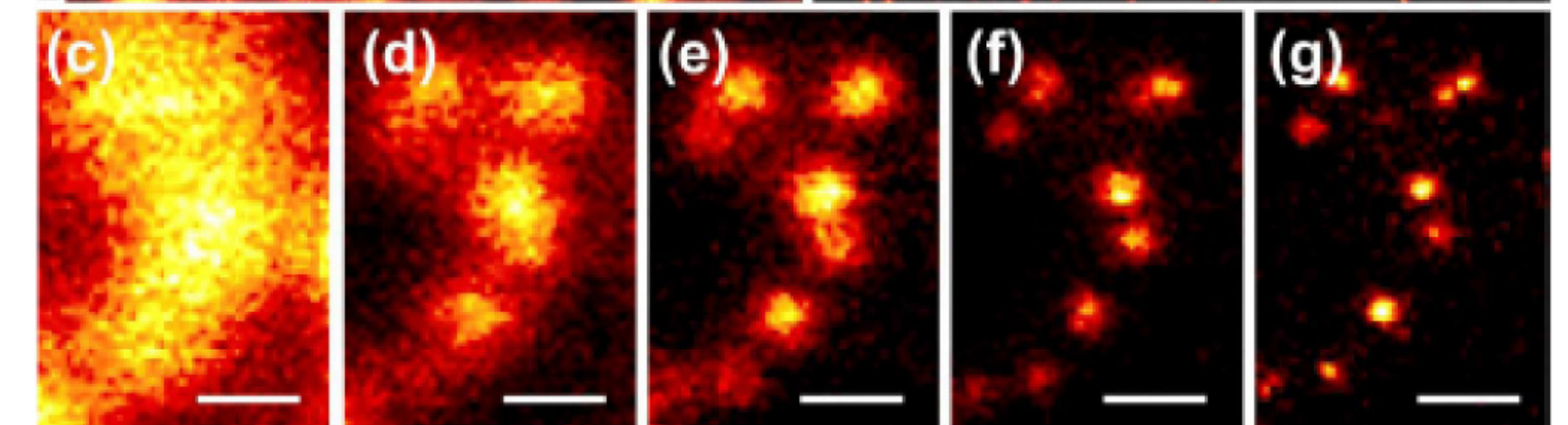
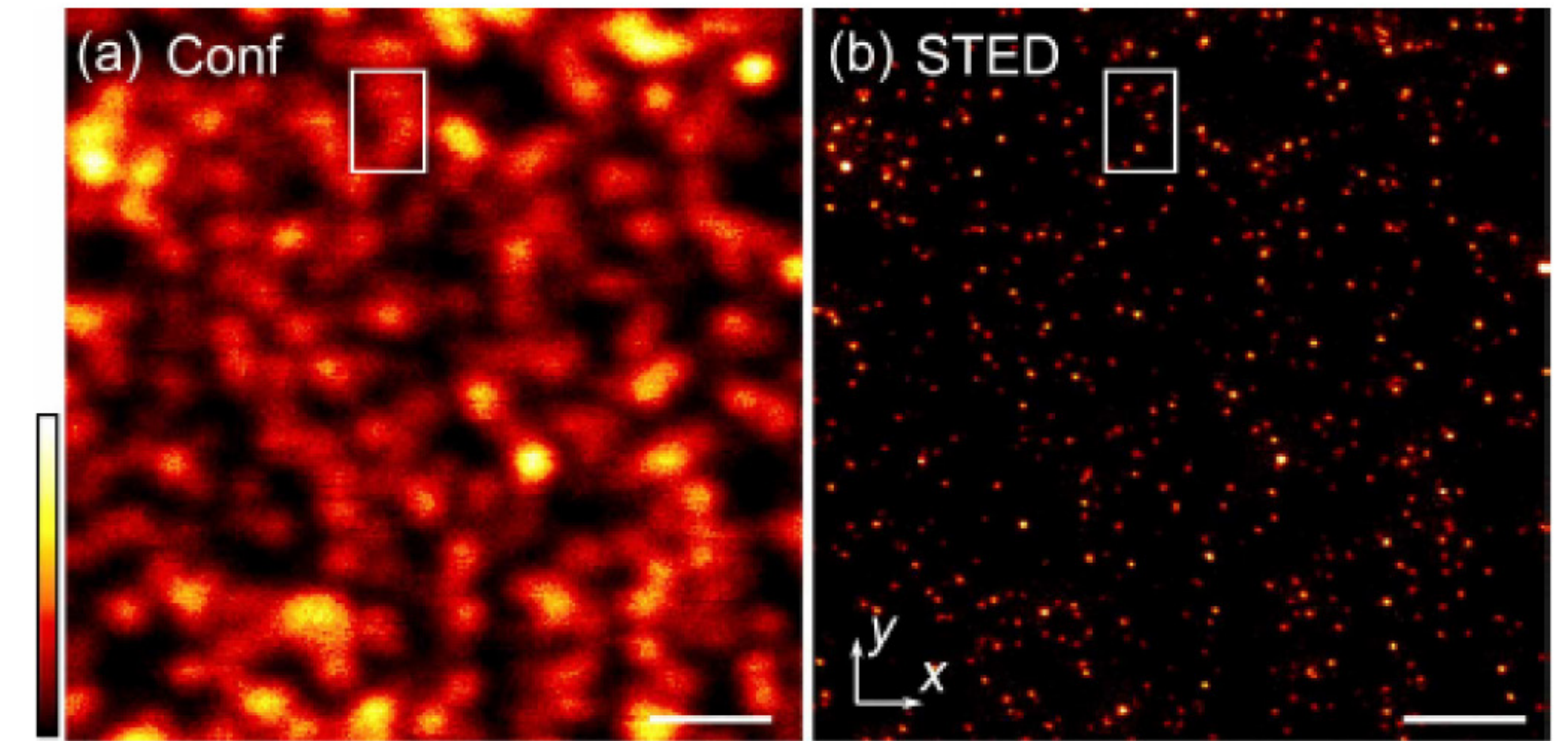
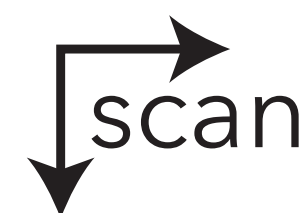
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excitation

depletion

remaining
signal



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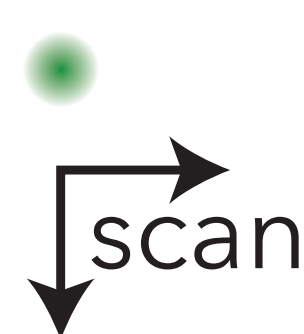
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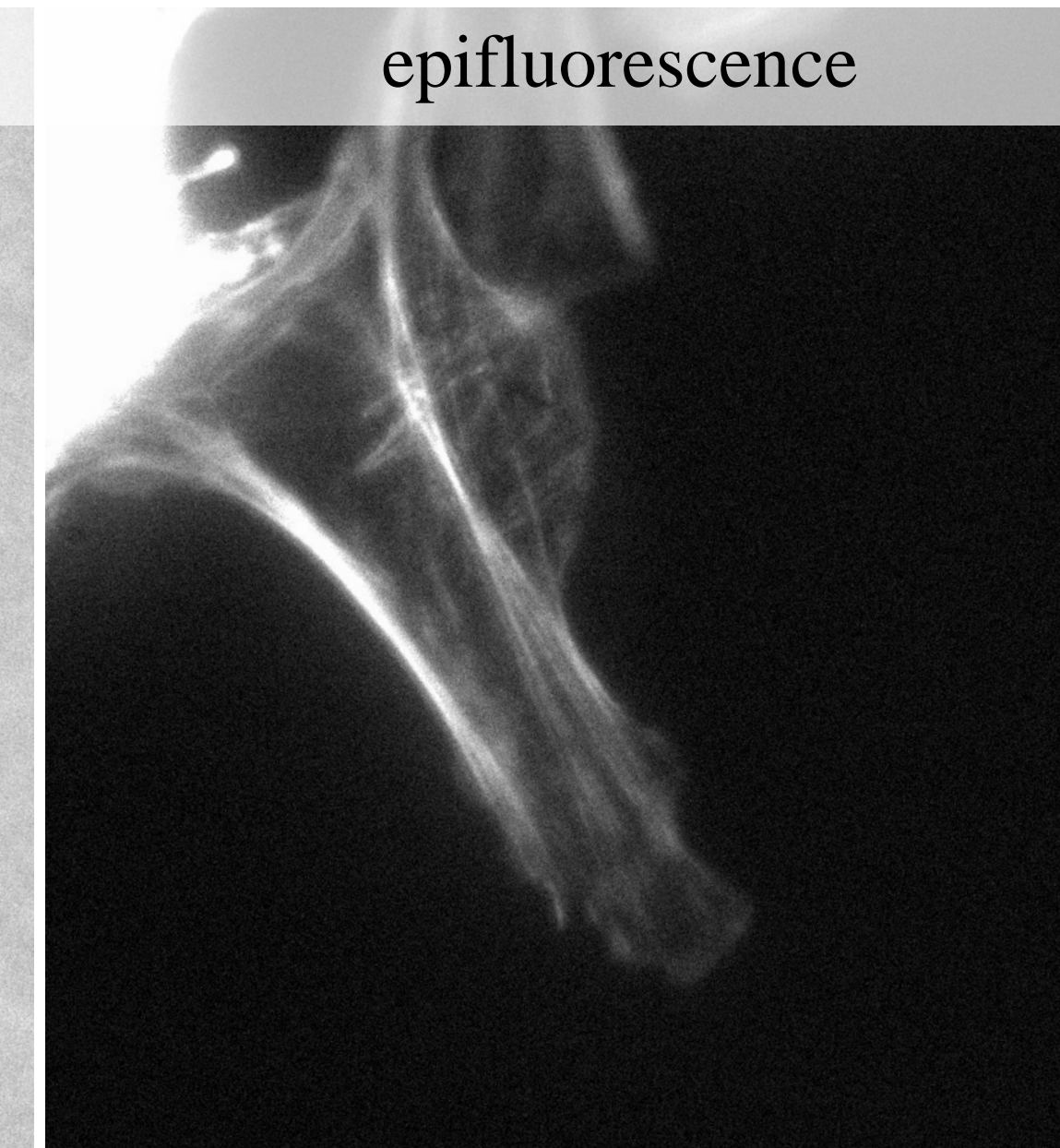
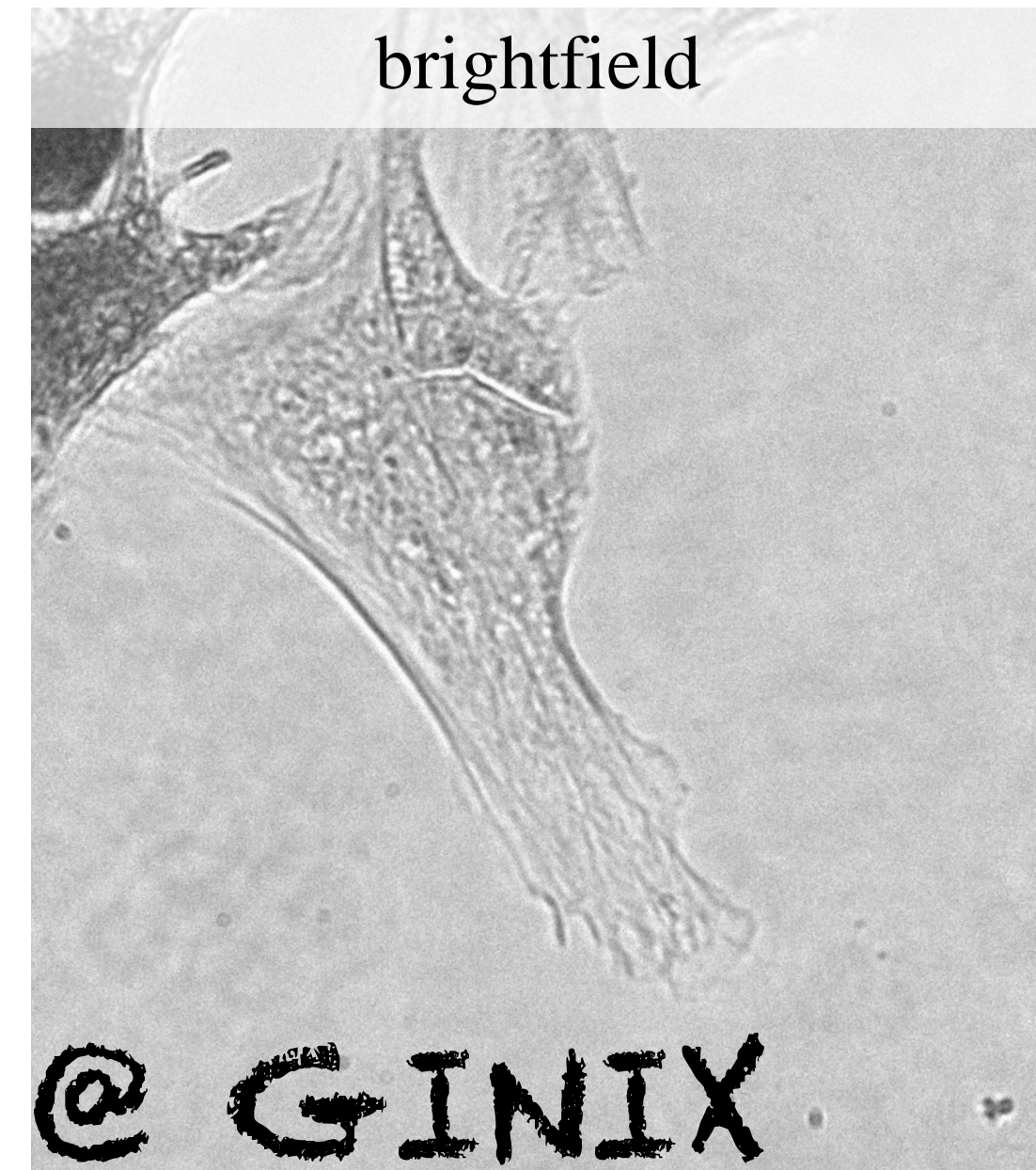
excitation

depletion

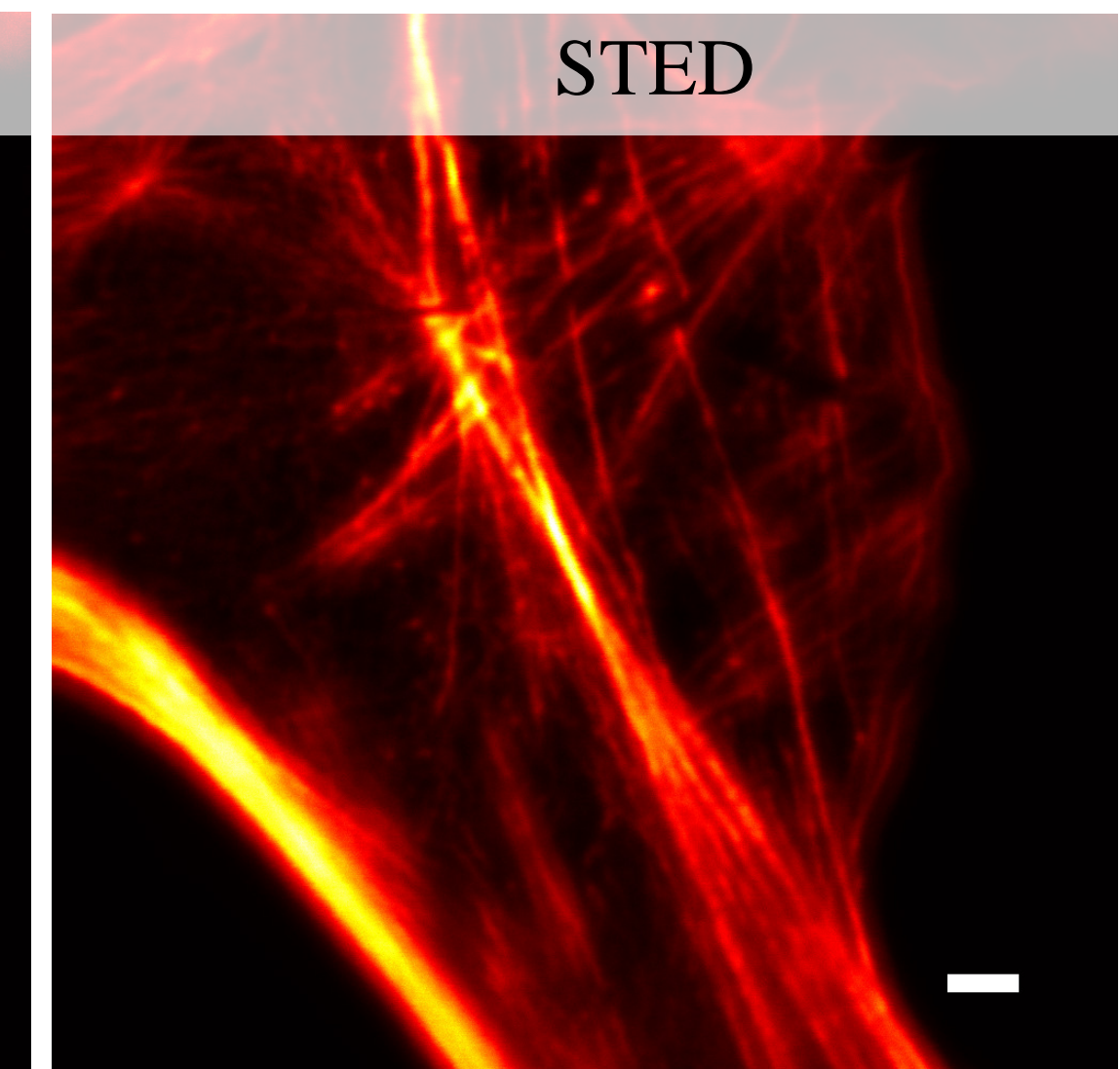
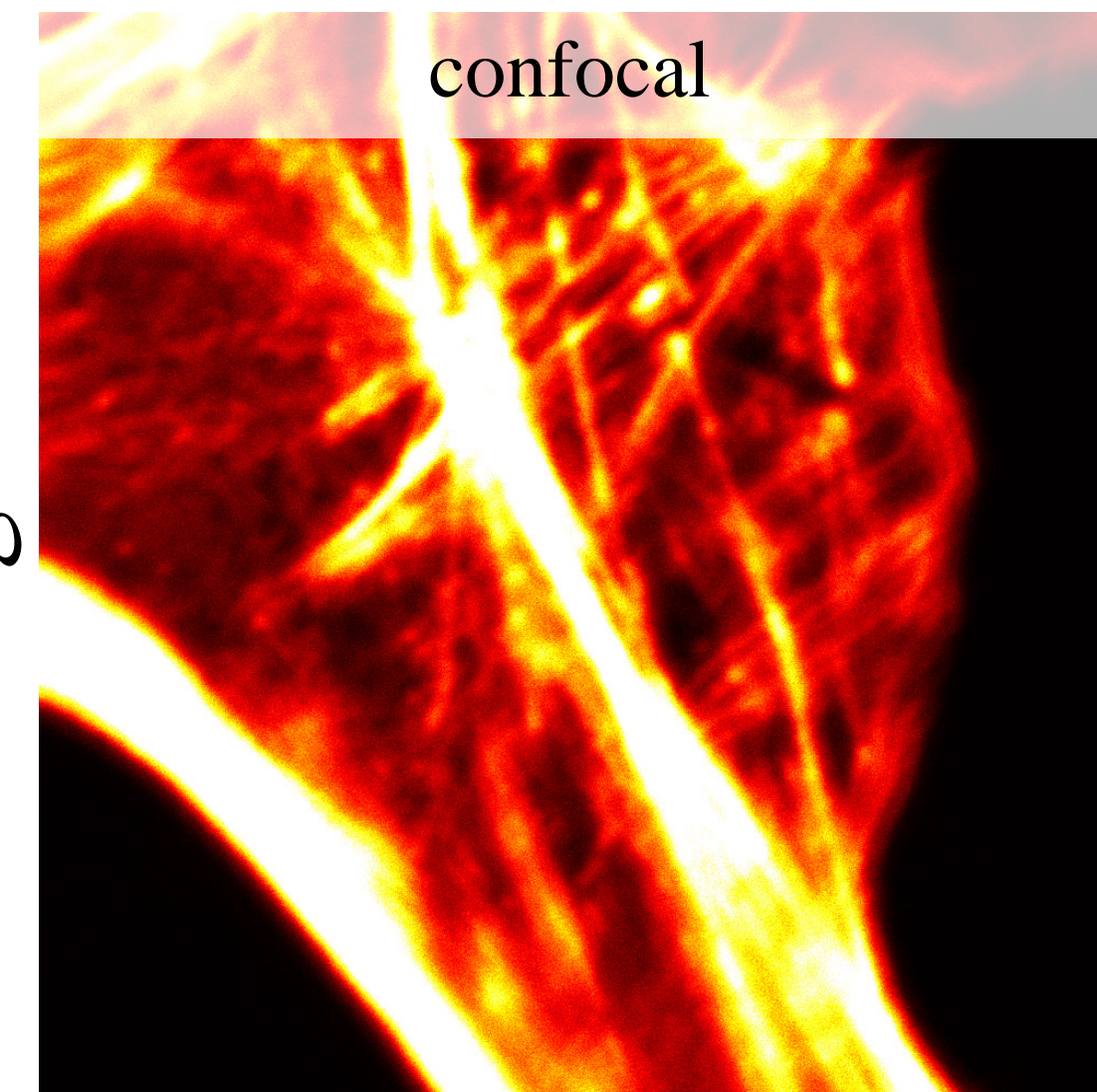
remaining
signal



Full-field mode



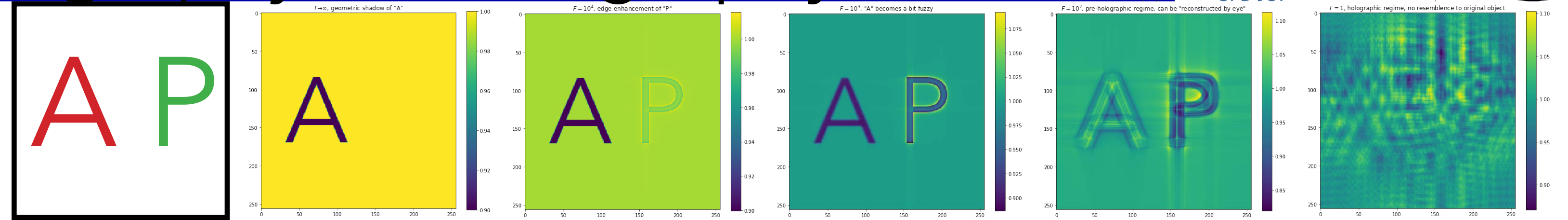
Scanning mode



Imaging methods: Holography & Tomography

$$n = 1 - \delta + i\beta$$

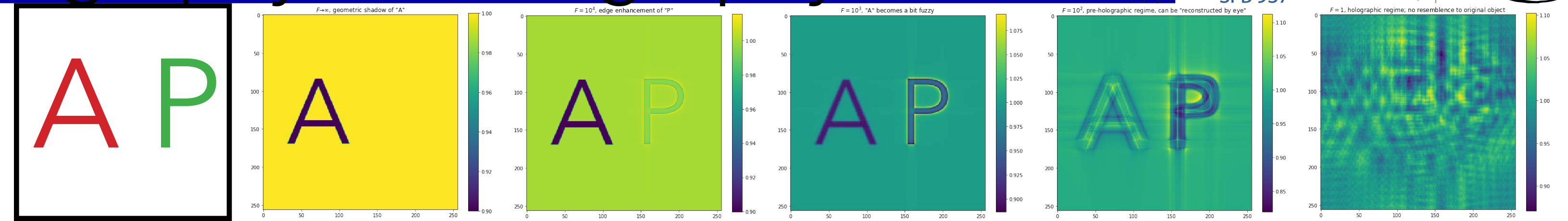
- ▶ complex index of refraction
phase shift + absorption
 $\delta \sim 10^{-5}$, $\beta \sim 10^{-7}$ (soft matter)
- ▶ in-line holography:
scattered wave boosted by
interference with
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- ▶ numerical phase reconstruction
yields quantitative contrast



electron density

- ▶ δ proportional to ρ
- ▶ assuming negligible β ,
or single material

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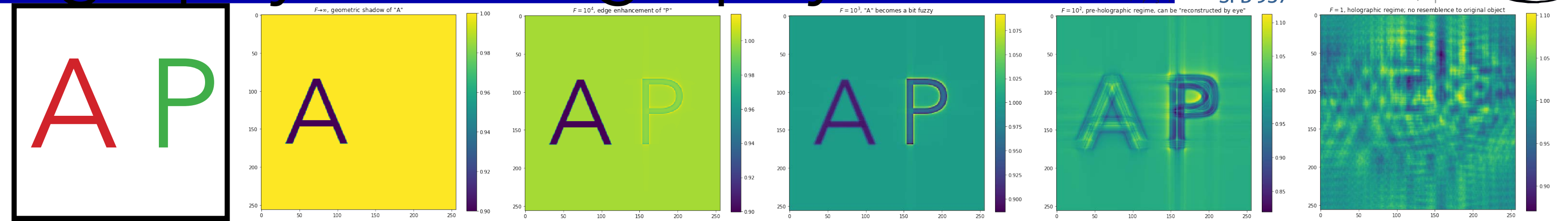
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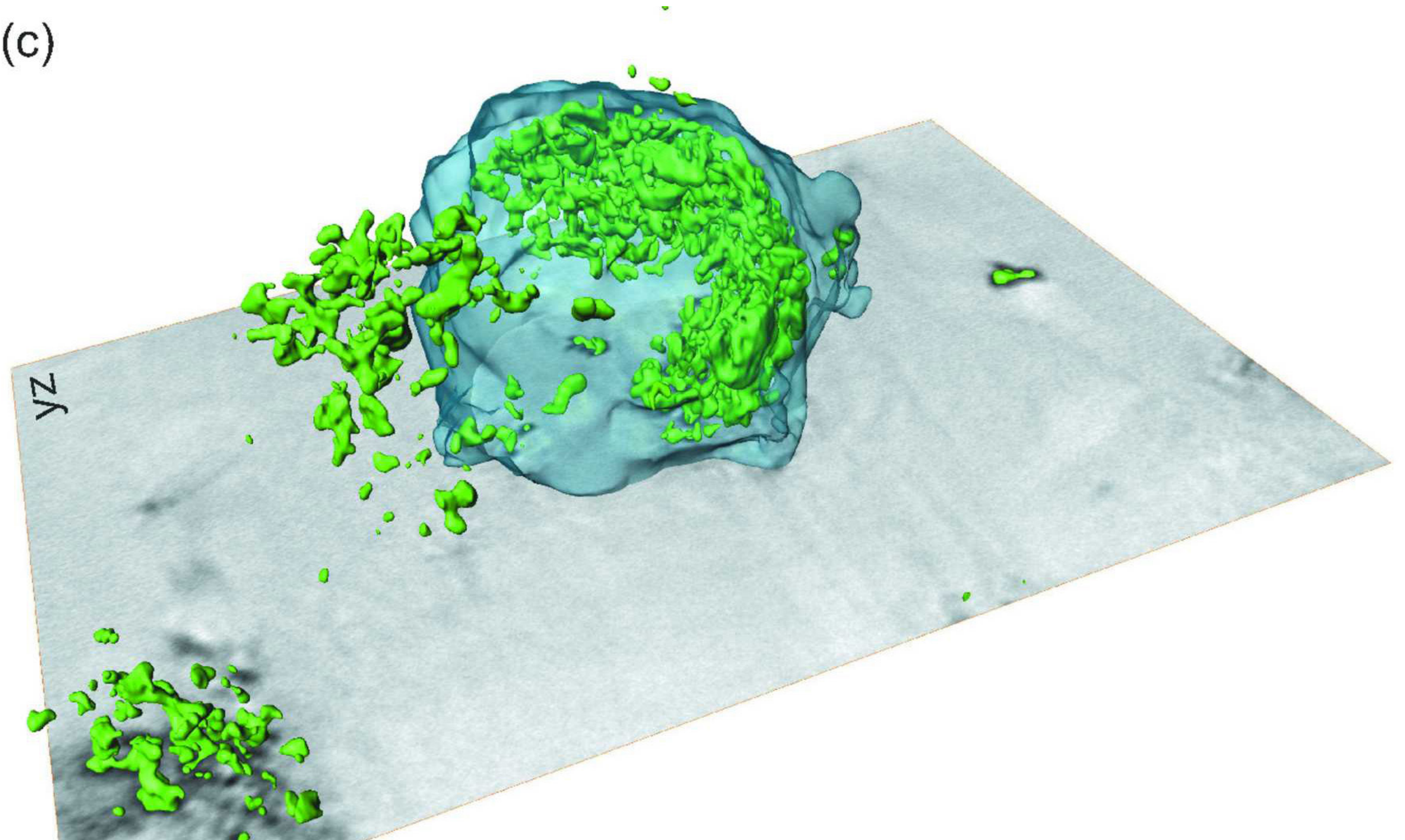
- ▶ δ proportional to ρ
- ▶ assuming negligible β ,
or single material

from 2D to 3D

- ▶ projections over 180°
- ▶ inverse Radon transformation



(c)



Imaging methods: scanning nano-SAXS

Small Angle X-ray Scattering

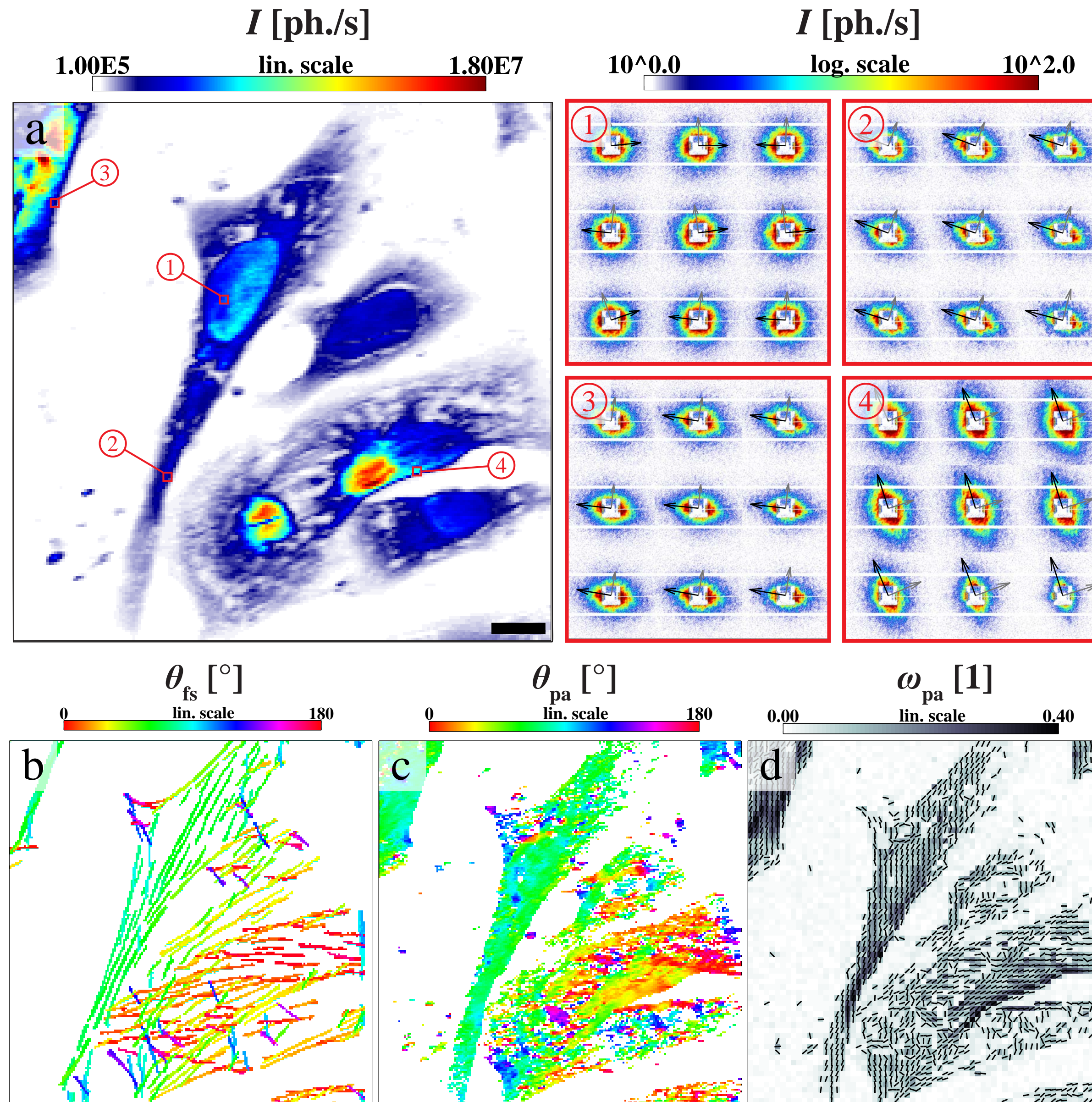
- ▶ access to shapes and sizes
- ▶ volume average over beam size

nano-SAXS

- ▶ volume average over ~ 100 nm
- ▶ reciprocal resolution $\sim 1 \text{ nm}^{-1}$

scanning nano-SAXS

- ▶ spatially resolved scattering map



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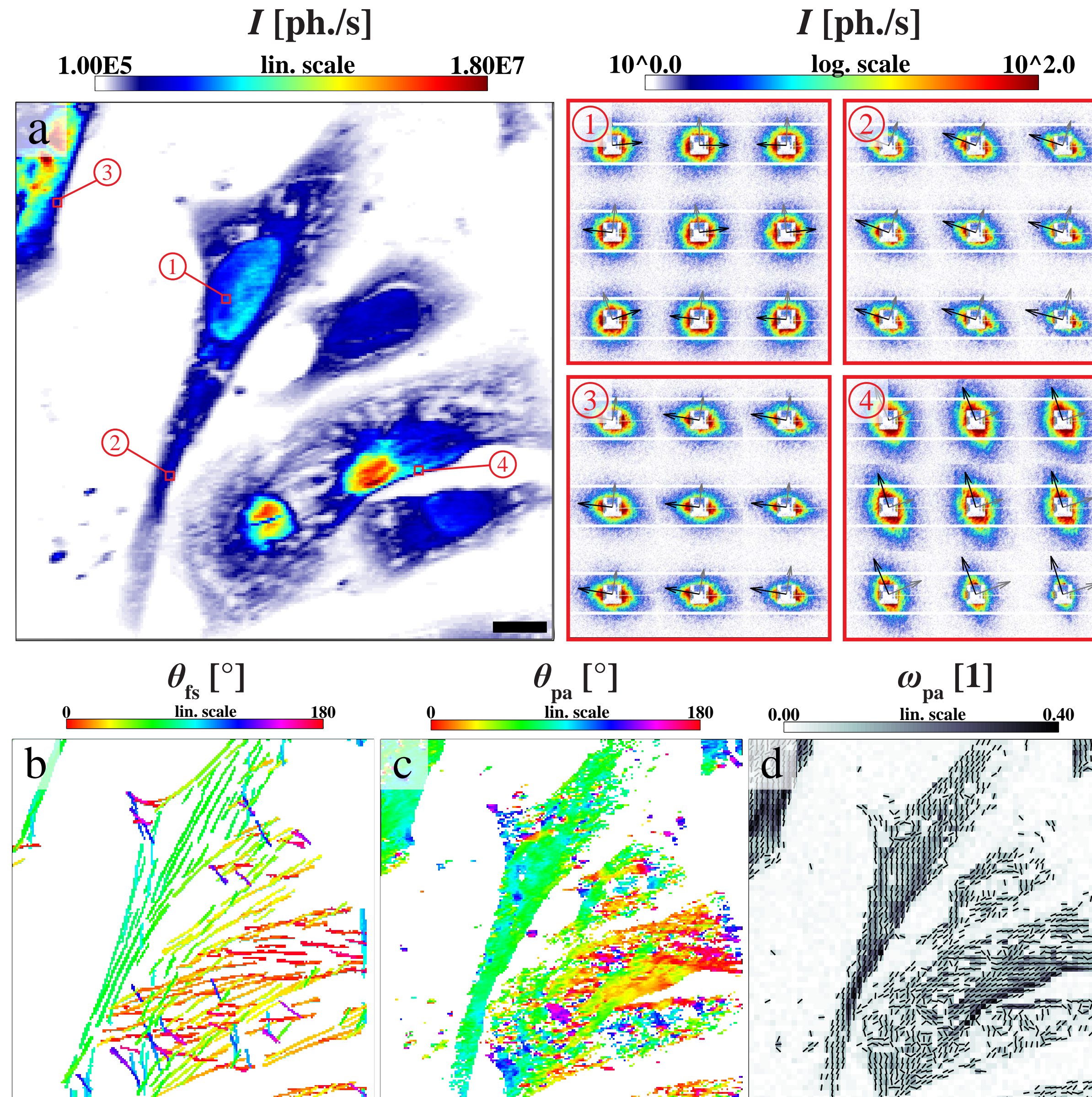
- ▶ spatially resolved scattering map

ptychography

- ▶ diffraction \rightarrow phase map

actin network

- ▶ where are the filaments bundles?
what size? how many?
cross-connections?
- ▶ mechanical stability
- ▶ (dis-)assembly during cell division



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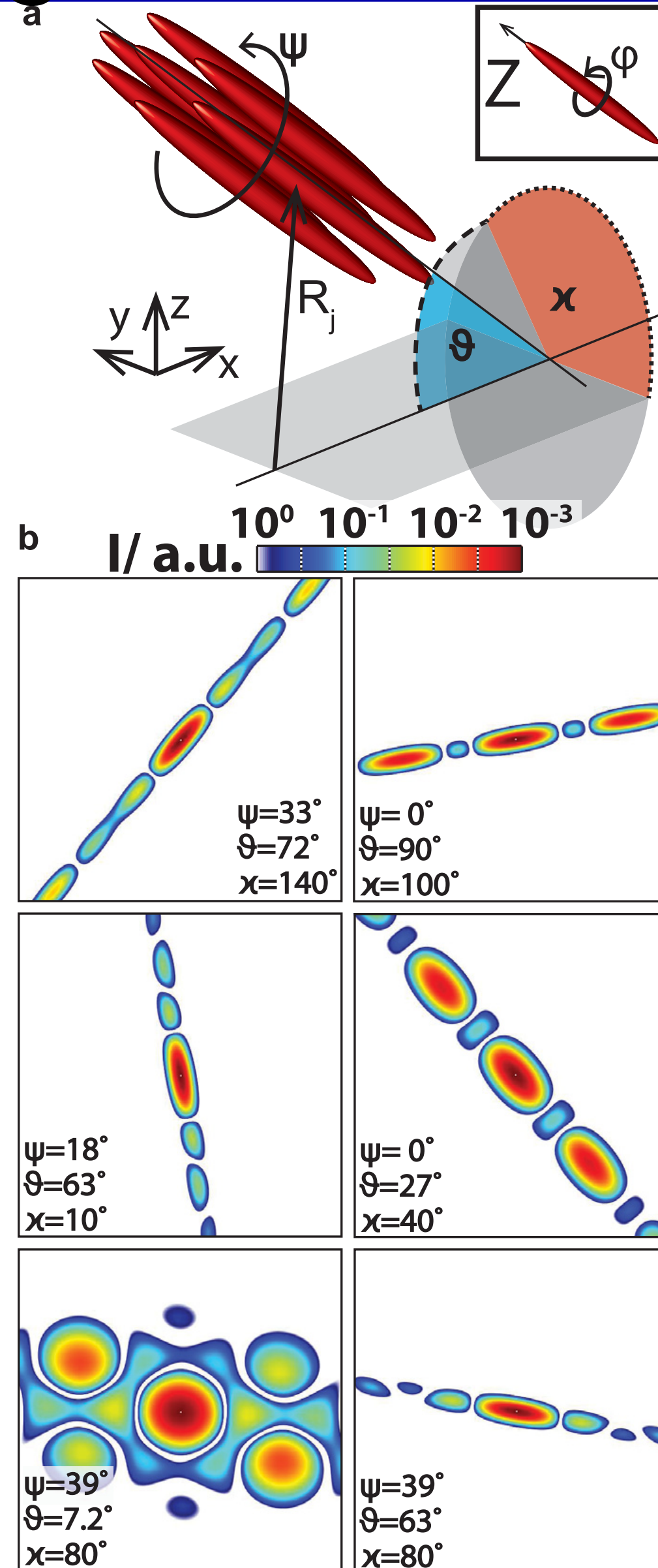
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actomyosin filament network
in *Dictyostelium discoideum*

model: Gaussian cigars

$$F(Q_z, Q_{\parallel}) = \frac{1}{\sqrt{2\pi}L_p} \exp(-0.5Q_z^2 L_p^2) \times 2J_0(R Q_{\parallel}) / (R Q_{\parallel}),$$

- ▶ what size? – persistence length L_p
- ▶ how many? – filament radius R

Table 2. Comparison of the Results for Fitting the Data Shown in Figure 4a with a 7 versus 19 Filaments Model, Including the Error Estimate of Each Parameter^a

parameter	7 filaments	19 filaments	EM
r (nm)	6.8 ± 0.5	5.1 ± 0.6	4–5
a (nm)	23.5 ± 0.5	14.3 ± 0.4	11–14
\emptyset (nm)	60.6 ± 2.1	67.4 ± 2.9	81 ± 22
ψ (deg)	139.8 ± 0.6	155.7 ± 0.6	–
resnorm	3.6	2.9	–

^aAdditionally the expected values from EM experiments are given.^{38,59} The EM values of the bundle diameters are given as mean and standard deviation.³⁸ The norm of residuals is defined as the sum of the square of the difference between the estimated value and the data: $\text{resnorm} = \sum (I_{\text{estimated}} - I_{\text{data}})^2$.

A Need for Correlative Imaging

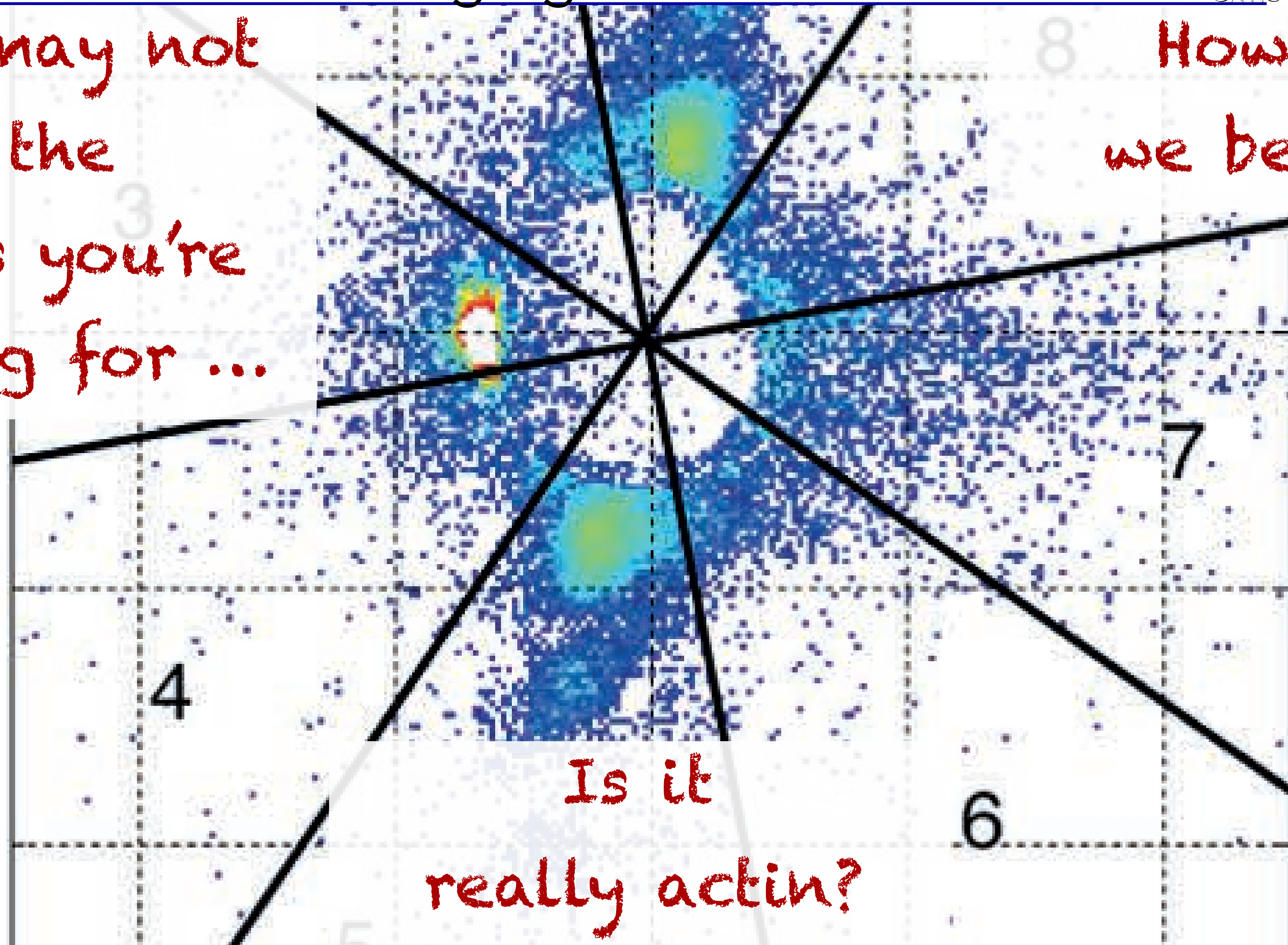
These may not
be the
droids you're
looking for ...

How can
we be sure?

Is it
really active?

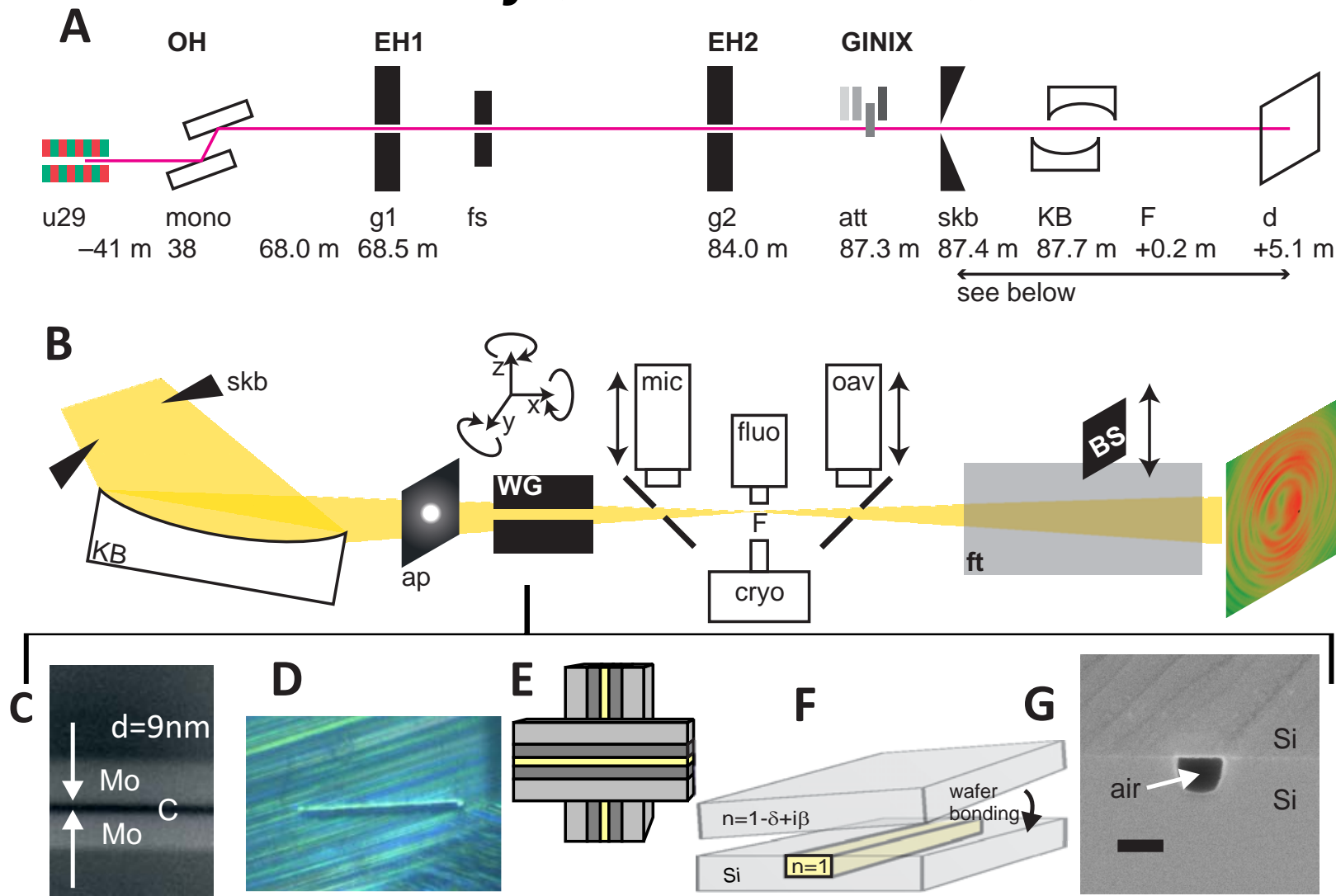
q_z ()
0.2
0.4

log(number of photons)

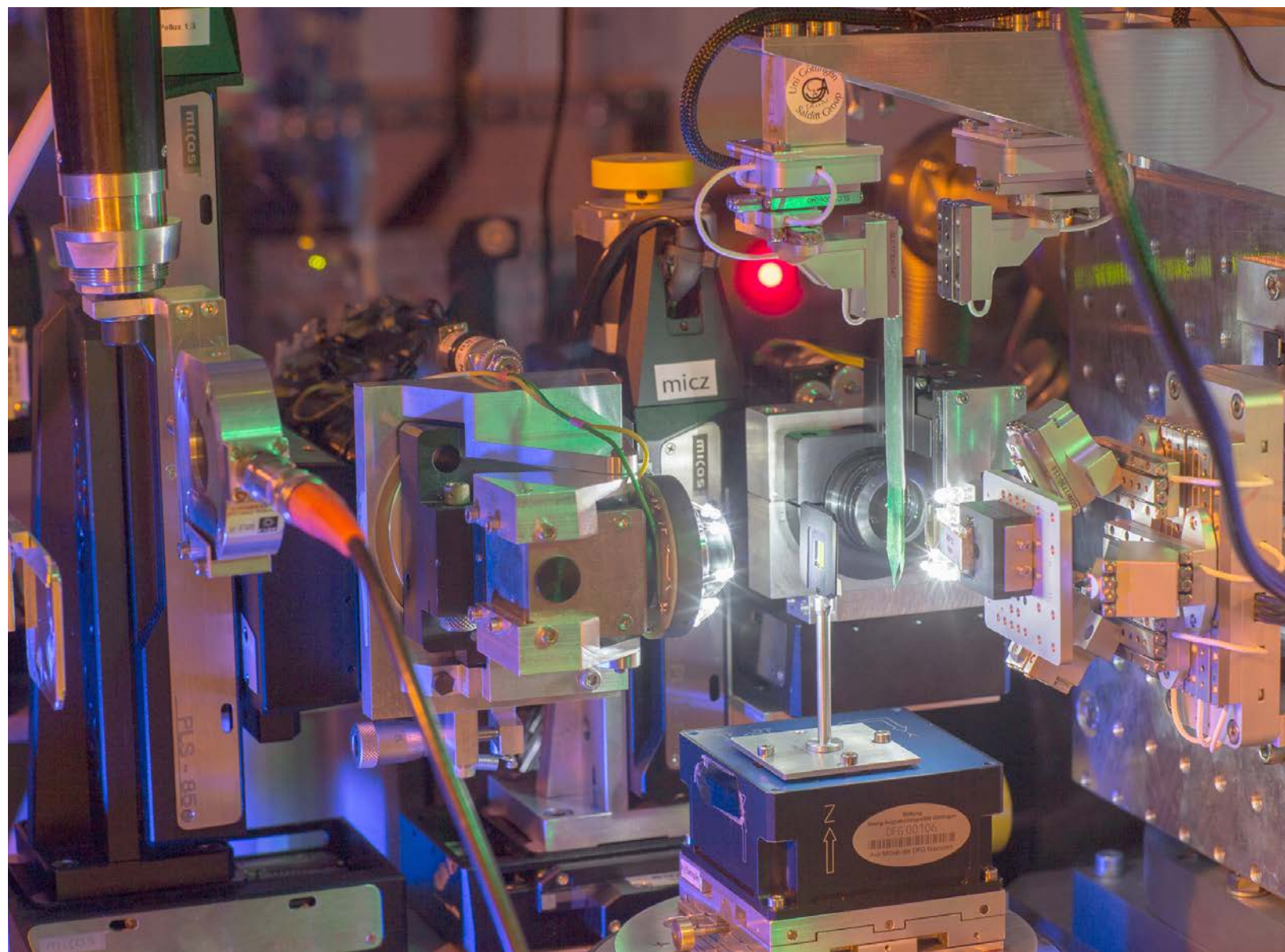
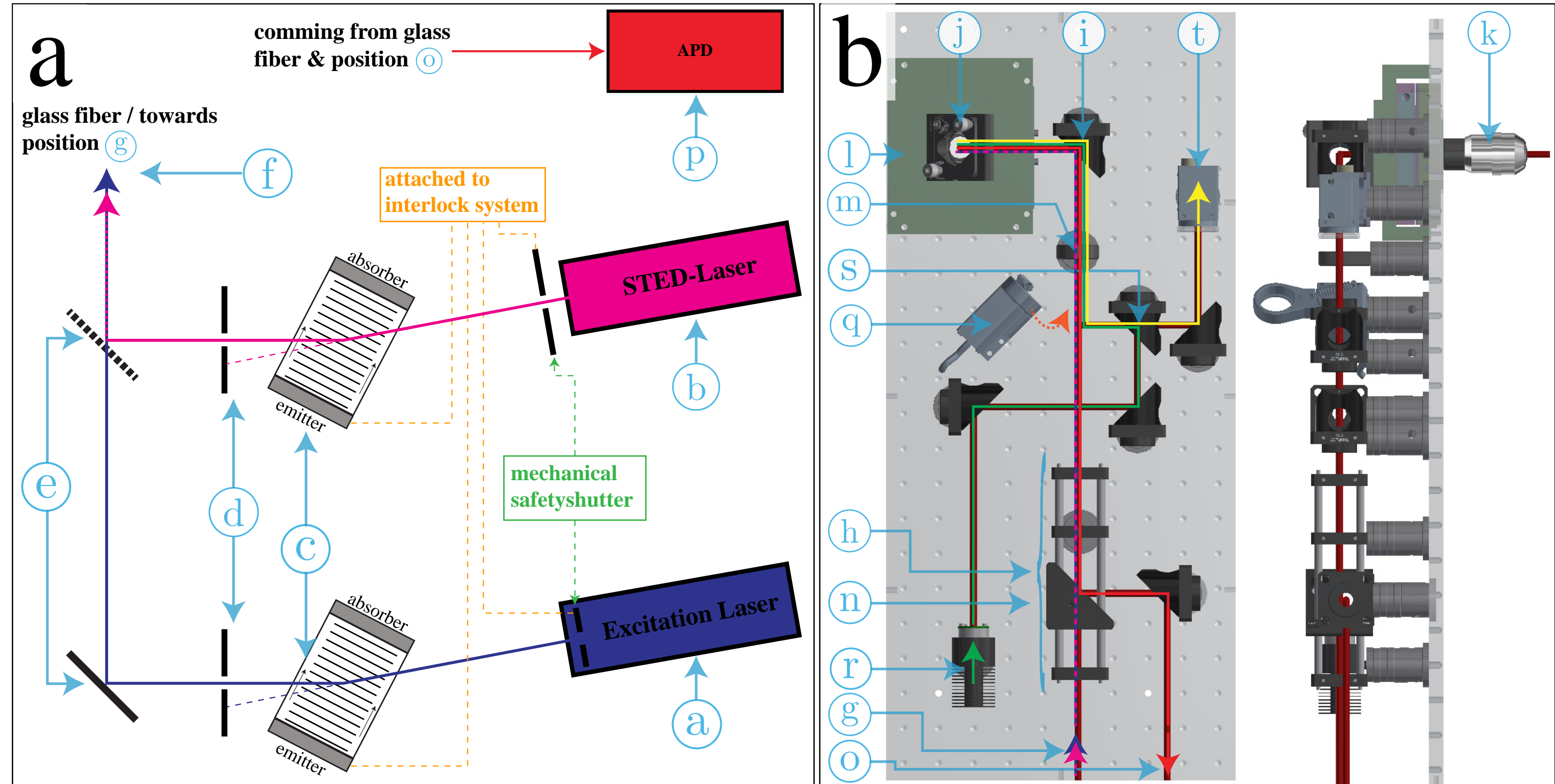


How to get a STED microscope into the X-ray station

X-Ray (GINIX @ P10)



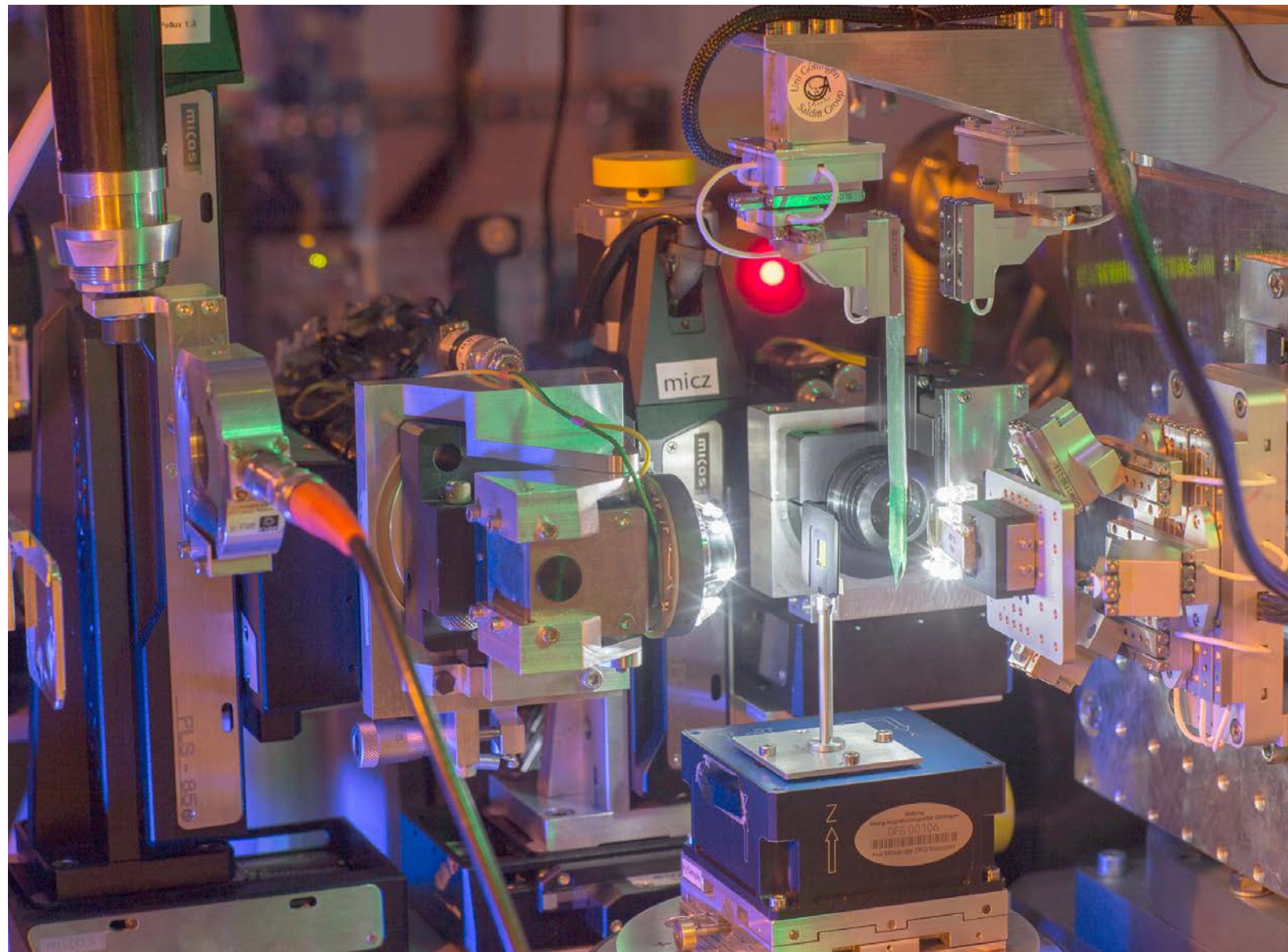
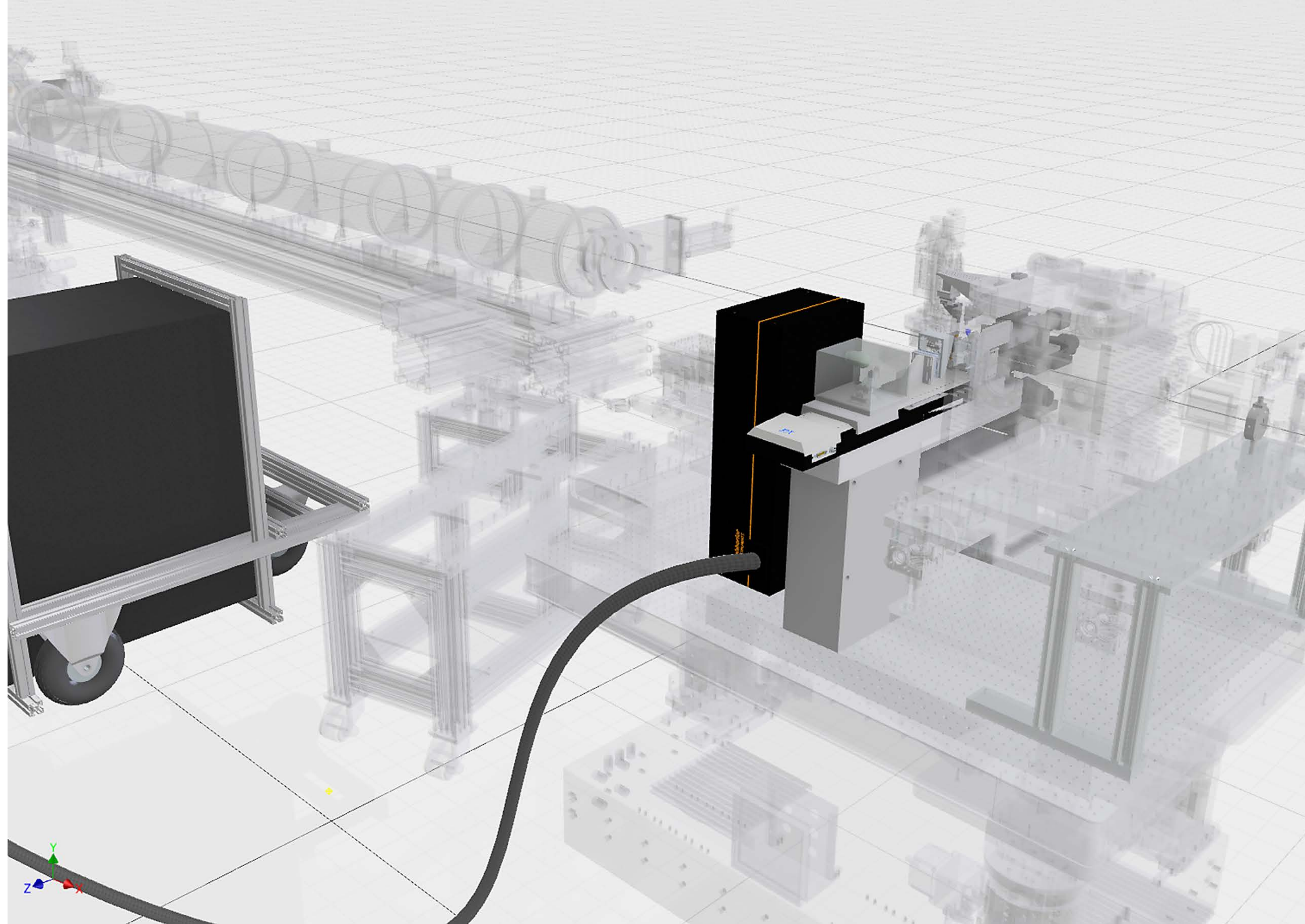
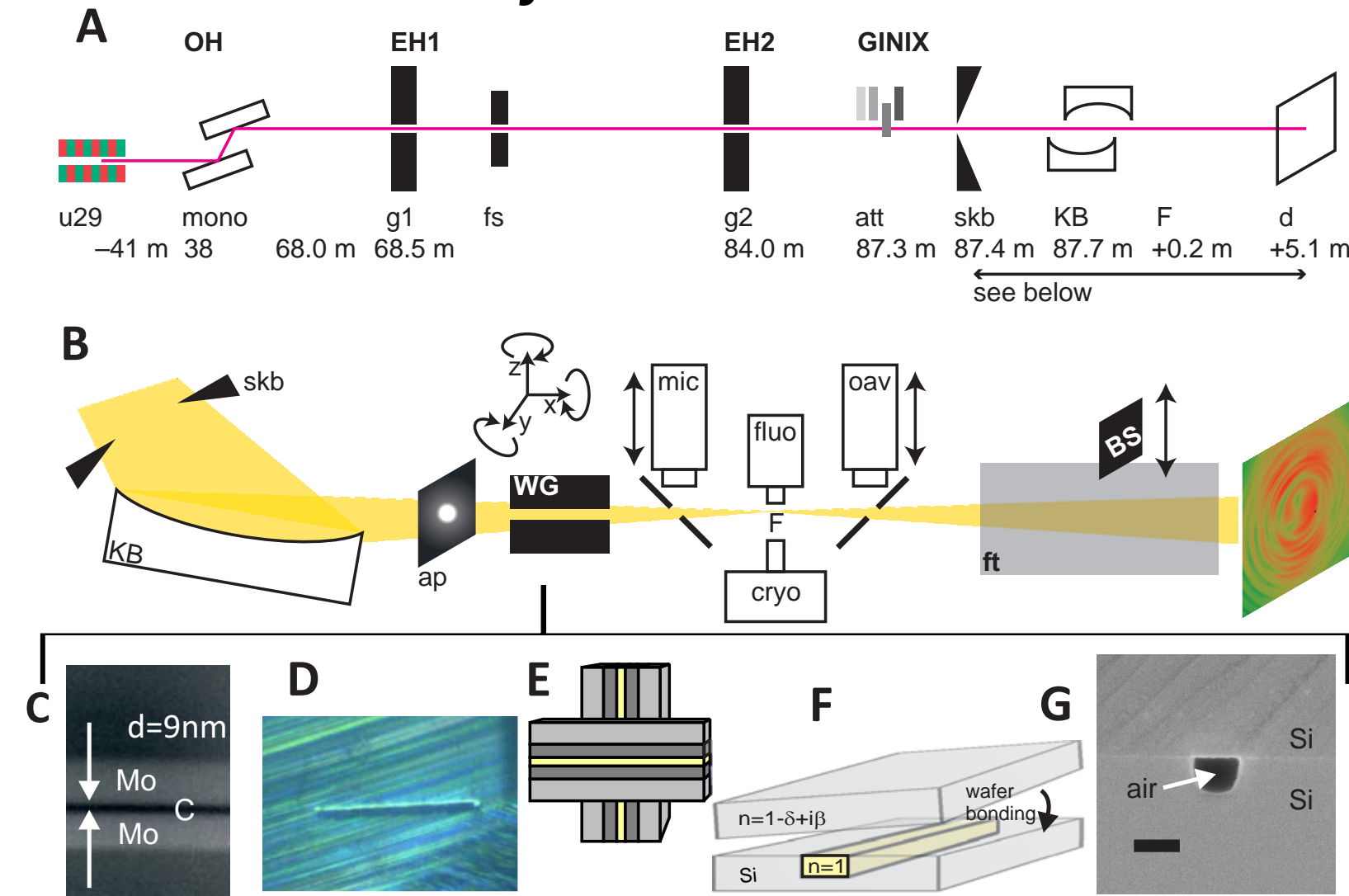
STED



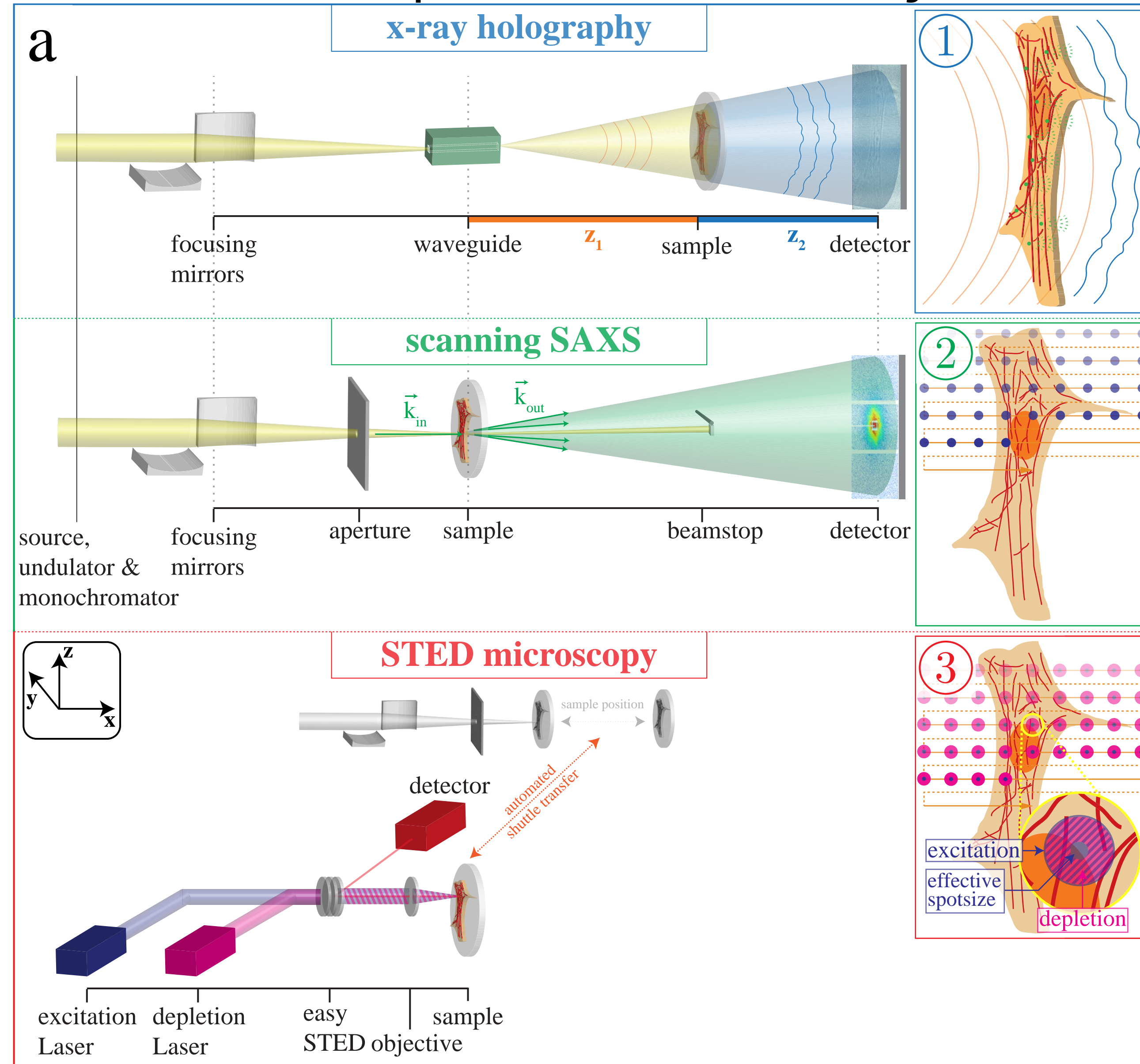
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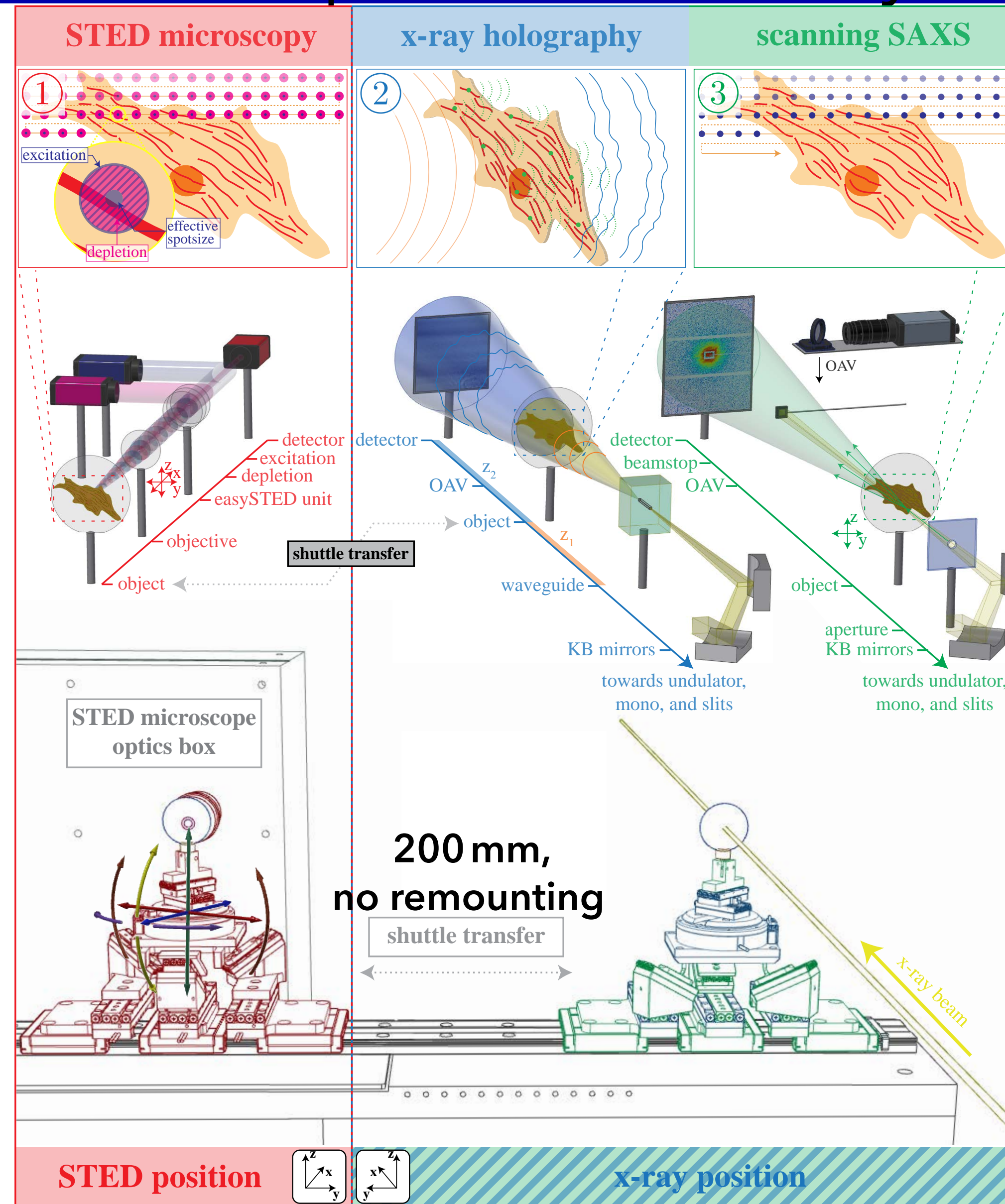
STED @ GINIX @ P10 @ PETRA III @ DESY



How to get a STED microscope into the X-ray station



How to get a STED microscope into the X-ray station



Results: three contrasts, same mounted cell

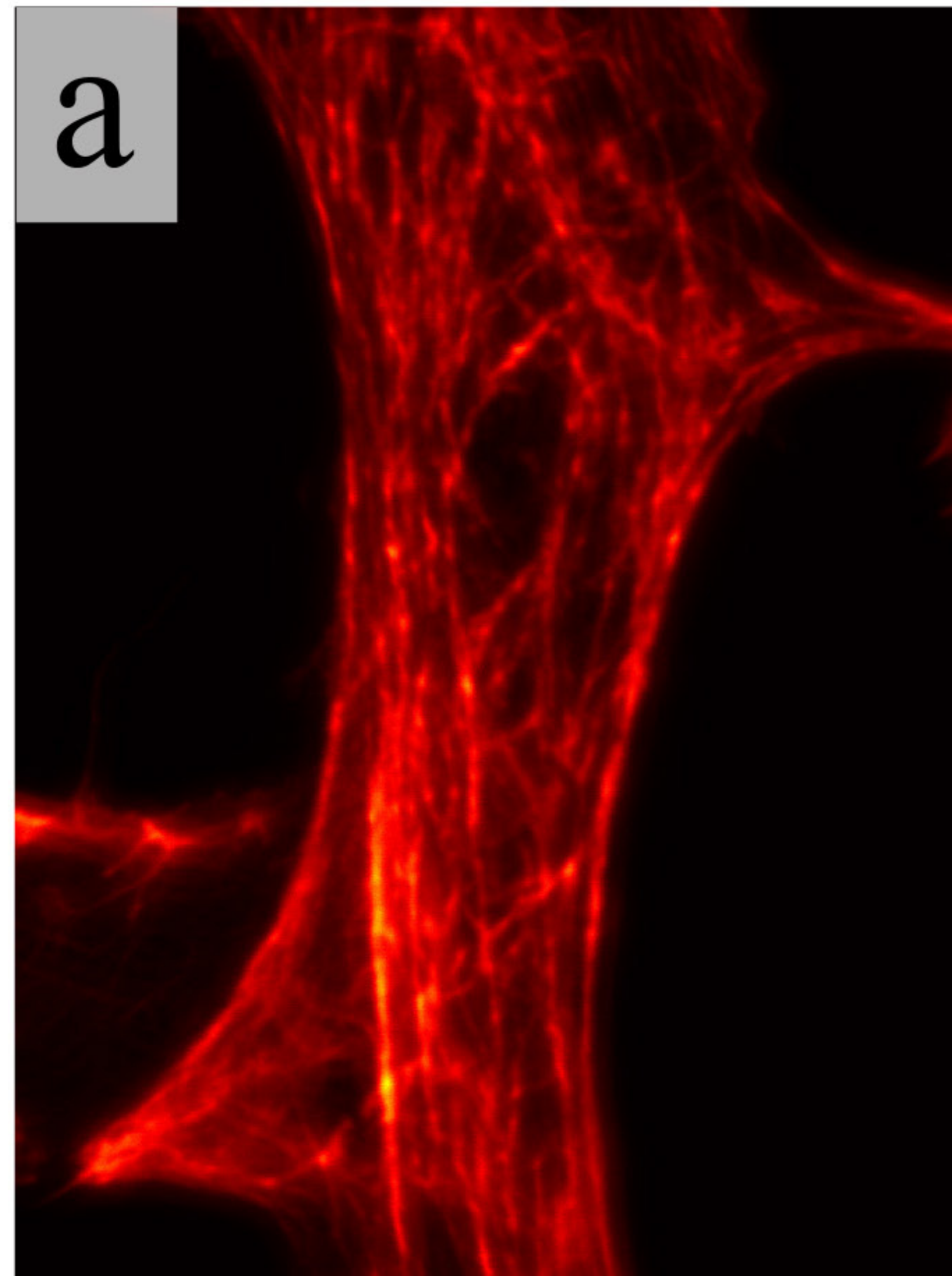
STED

Holography

scanning nano-SAXS

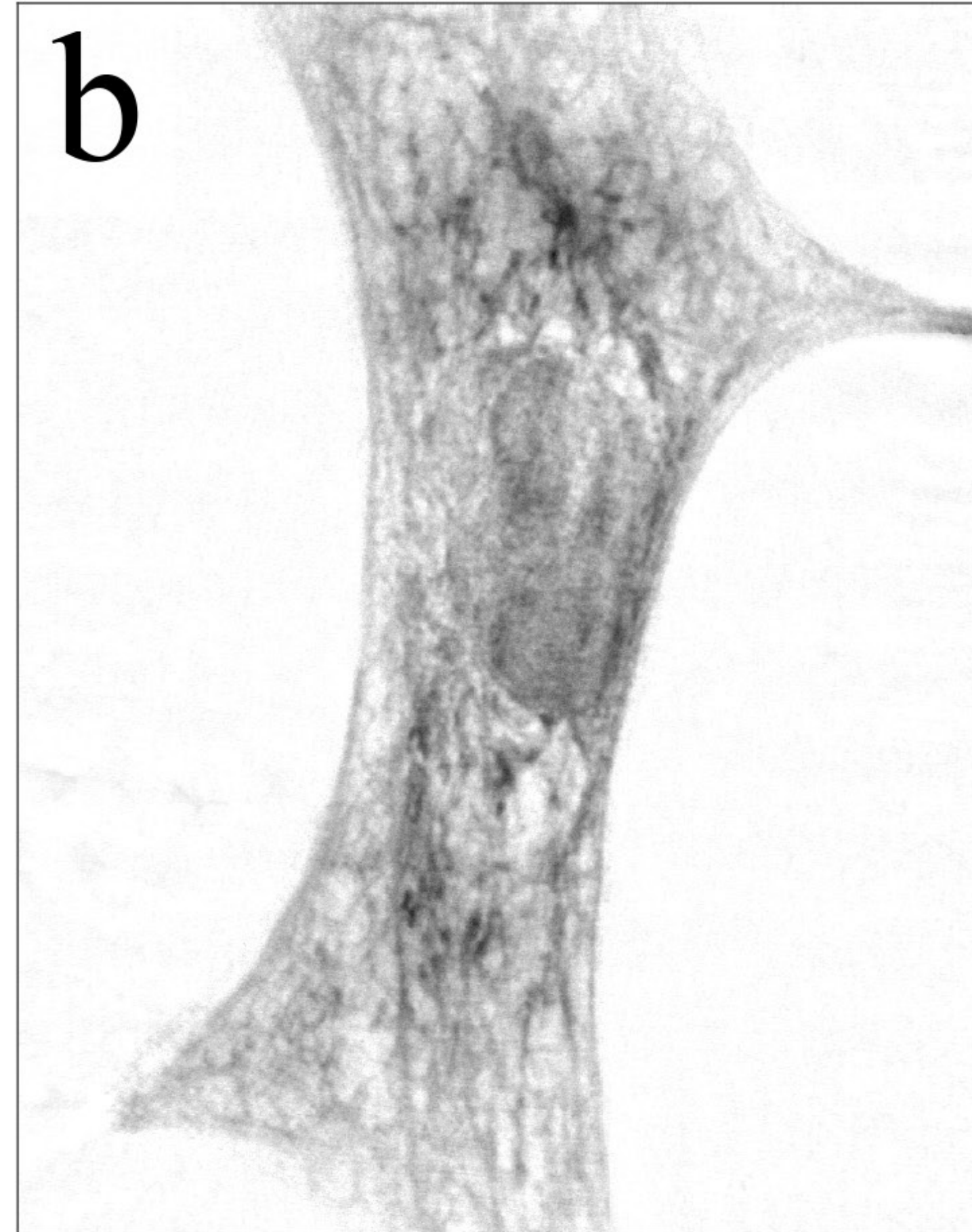
I [ph./s]

0.00E0 lin. scale 1.50E7



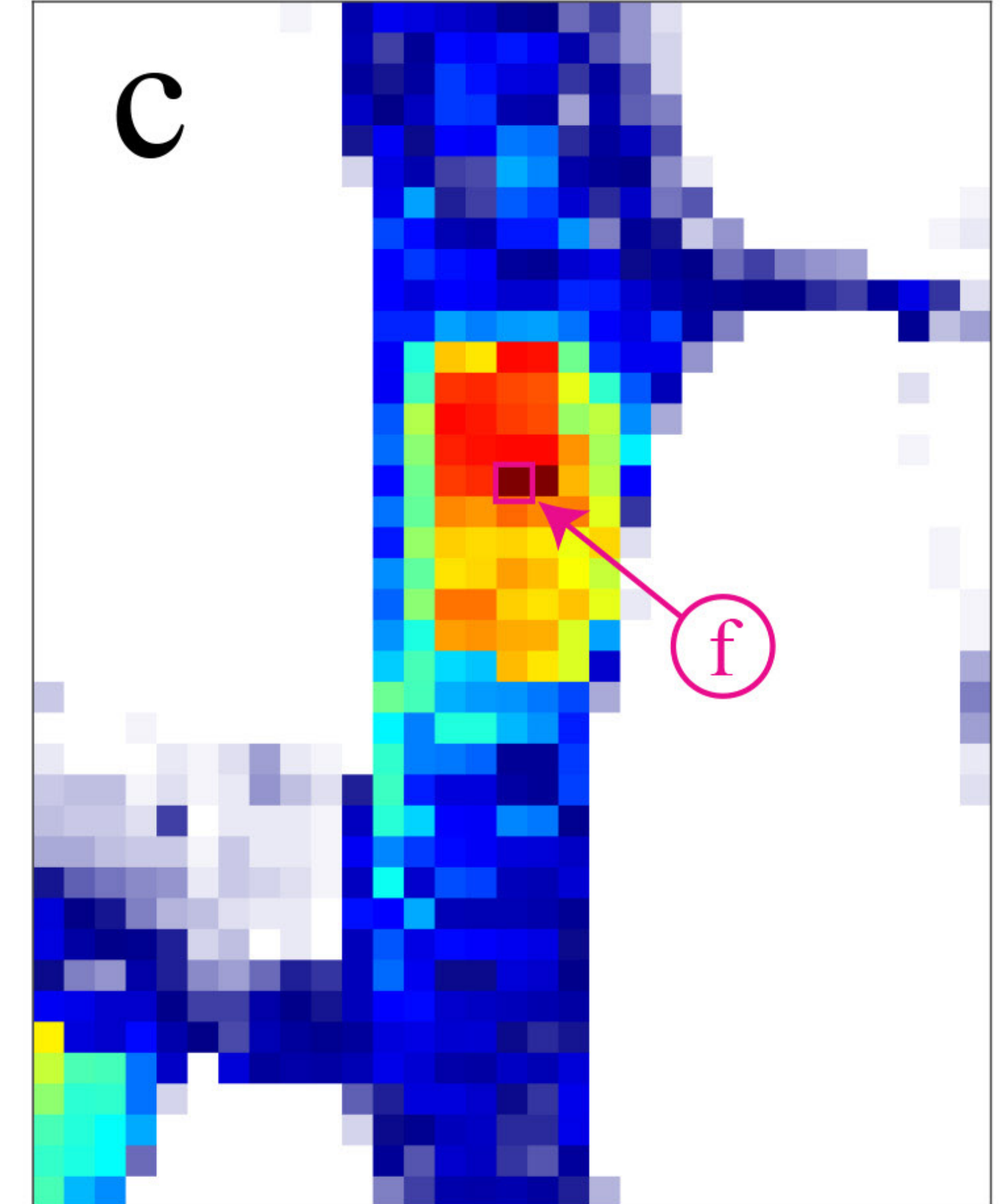
φ [°]

-0.07 lin. scale 0.00



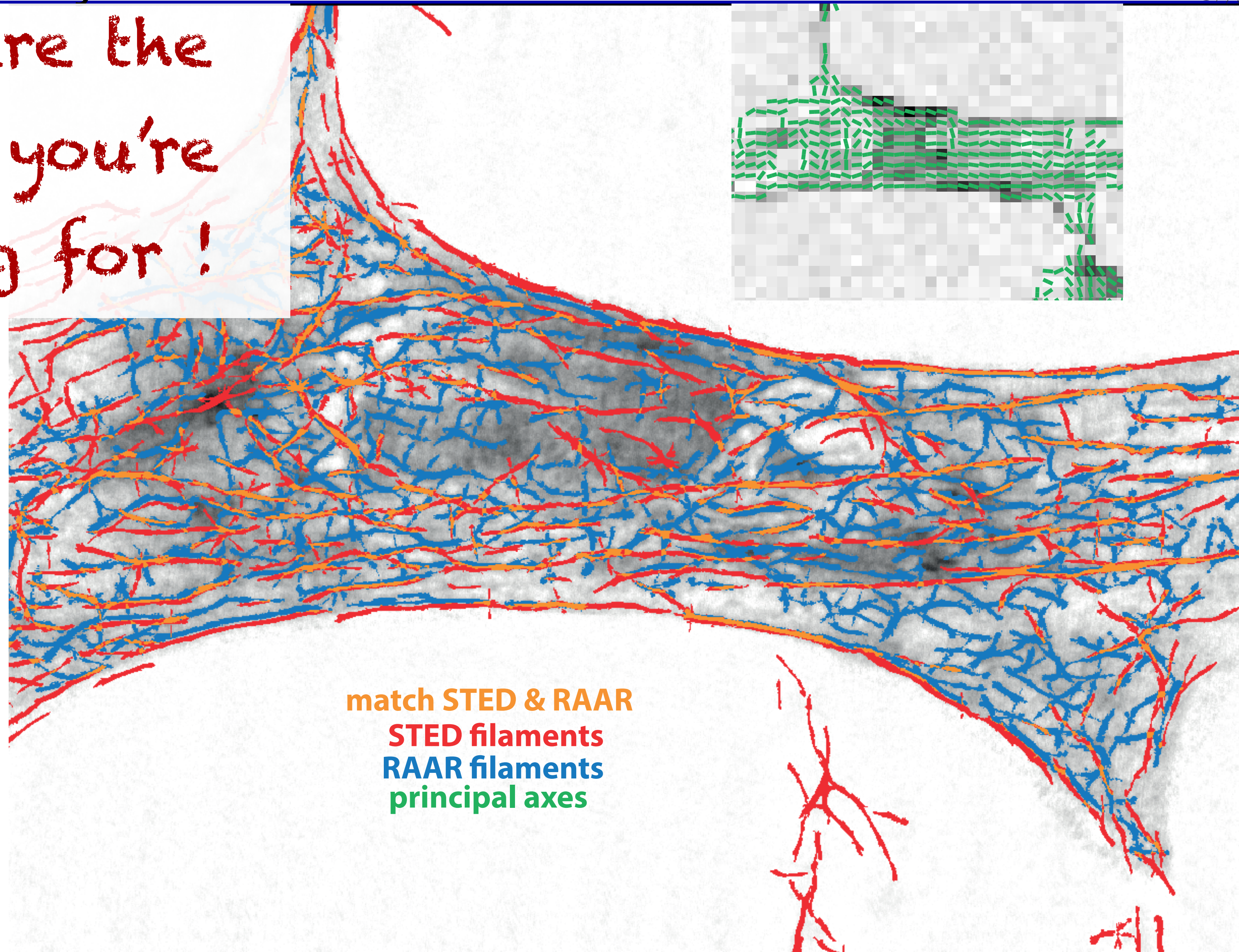
I [ph./s]

6.50E5 lin. scale 2.90E6



Results: overlay of fibres

These are the droids you're looking for!



Summary / Outlook / Discussion

Three modalities shown here:

- ▶ Optical Fluorescence
"name tags" for specific molecules
- ▶ X-ray Holography
electron density, "the surroundings"
- ▶ nano SAXS
structures and sizes

Future prospects

- ▶ higher resolution, sub-100 nm
- ▶ fluorescence mapping
- ▶ tomography
- ▶ throughput

