

# PROTEINOSCOPY

Optical imaging of single protein dynamics

*Marek Piliarik*

*Institute of Photonics and Electronics of the CAS, Praha, ČR*

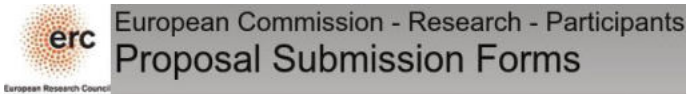


UFE  
NanoOptics

MAREK PILIARIK

*Institute of Photonics and Electronics of the CAS, Praha, ČR  
2011-2016: Max Planck Institute for the science of light, Erlangen*

# PROTEINOSCOPY



To date, it is not possible to monitor conformational changes of a single protein without the use of a label. There exists no means for directly tracking single-protein conformations at time scales characteristic of their transition pathways (e.g. microseconds). This project pursues sensitive optical scattering methods for the monitoring of protein dynamics at a single molecule level. This is done at the temporal resolution of microseconds and faster, and without the need of any fluorescent labels.

- Objectives
  - Develop new methods for fast tracking of dynamics of single proteins.
  - Collect new knowledge about selected biomolecular systems.

# The methodology

- Ultrasensitive microscopy

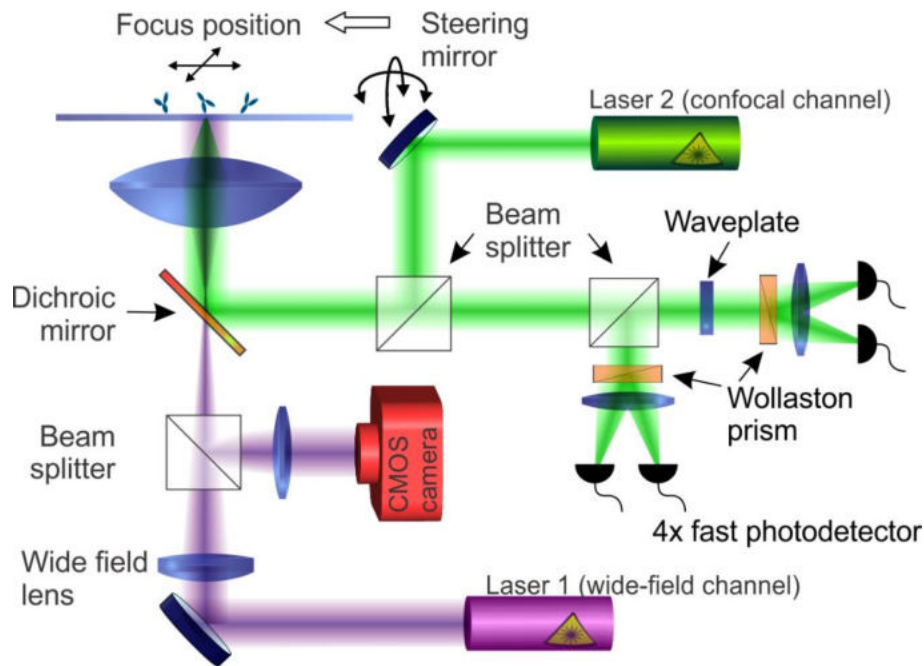


Fig. Microscopy with single-protein sensitivity and interferometric detection of scattering anisotropy.



Fig. Experiment for ultrasensitive monitoring of a single-protein anisotropy.

Further reading:

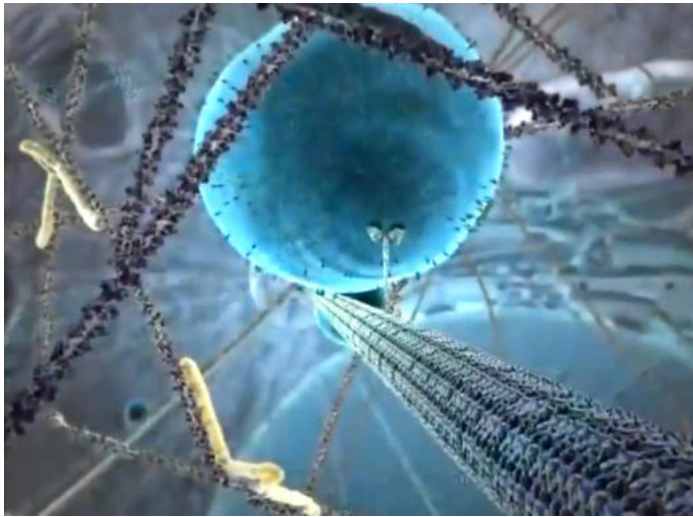
M. Piliarik and V. Sandoghdar, *Nature Communications* **5** (2014) 4495

M. P. McDonald et al, *Nano Lett.* **18** (2018) 513

K. Holanová, M. Vala, and M. Piliarik, *Opt. Laser Technol.* **109** (2019) 323–327



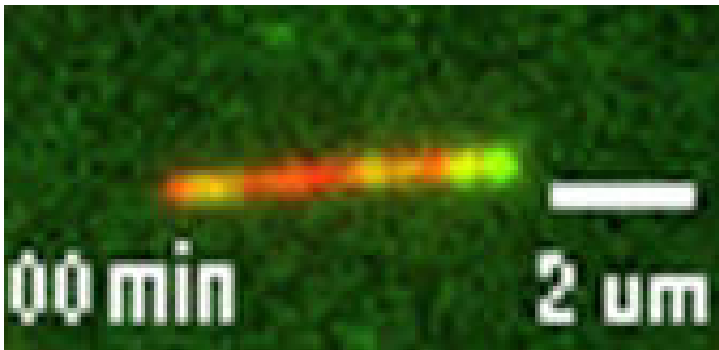
## Biological system under study



Kinesin-1 on a microtubule (cartoon)

Cytoskeleton associated proteins  
and protein motors  
Chemomechanical protein motion

- Directed motion  
e.g. Kinesin-1, Myosin-5
- Diffusive motion  
e.g. Ase1



Ase-1 proteins (green)  
on a microtubule (red).

### Timescales

1h	Mitosis
1 s	Microscopic motion
1 ms	Nanoscope stepping
1 μs	Protein dynamics

**Mitosis** – microtubules (green)  
and chromosomes (red).

# Infrastructure and optics setup







**Nano Optics  
October 2019**



what is  
**nano**  
**optics**



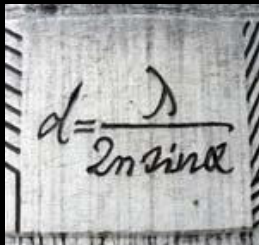




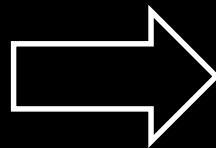
1  $\mu\text{m}$

2 nm

**Fundamental limit**



A photograph of a piece of paper with the handwritten formula  $d = \frac{\lambda}{2n \sin \alpha}$  in black ink. The paper has a grid pattern on the left and right edges.

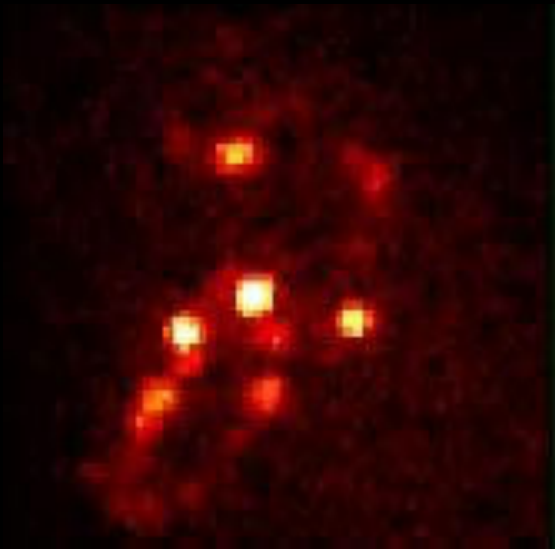


**Technical limit**

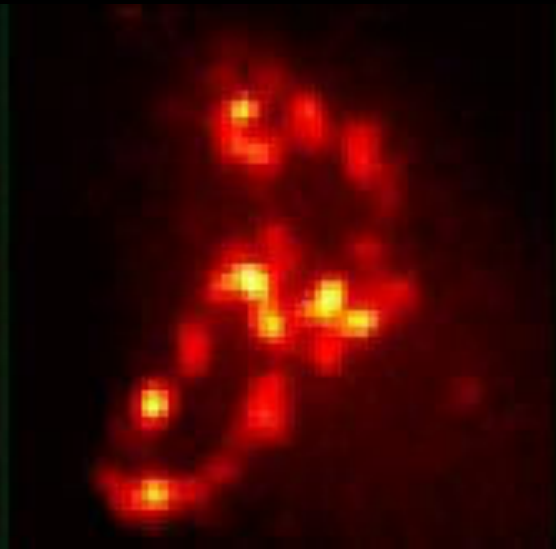
**SNR**

# from the “dot” back to an image

recorded frames



diffraction-limited image



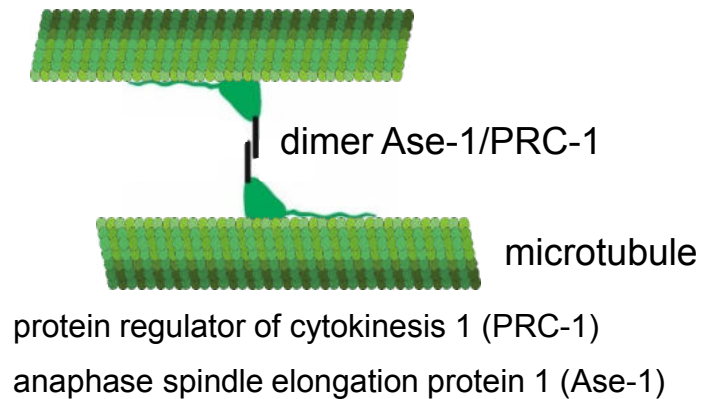
live reconstruction



E. Betzig, G. Patterson, R. Sougrat, O. Lindwasser, S. Olenych, J. Bonifacino, M. Davidson, J. Lippincott-Schwartz, and H. Hess, *Science* **313**, 1642 (2006).

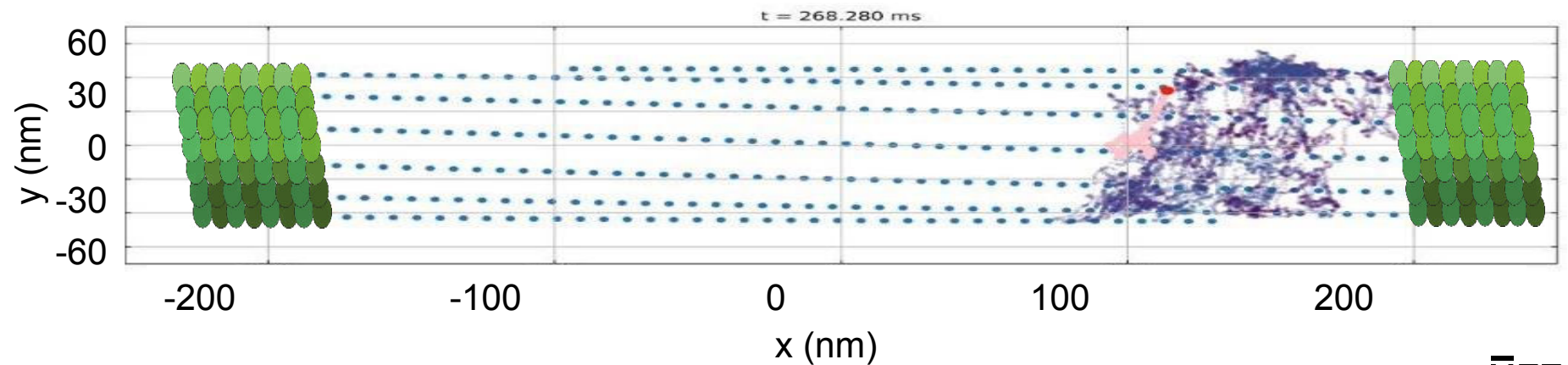


# Ultrafast tracking of single-protein motion

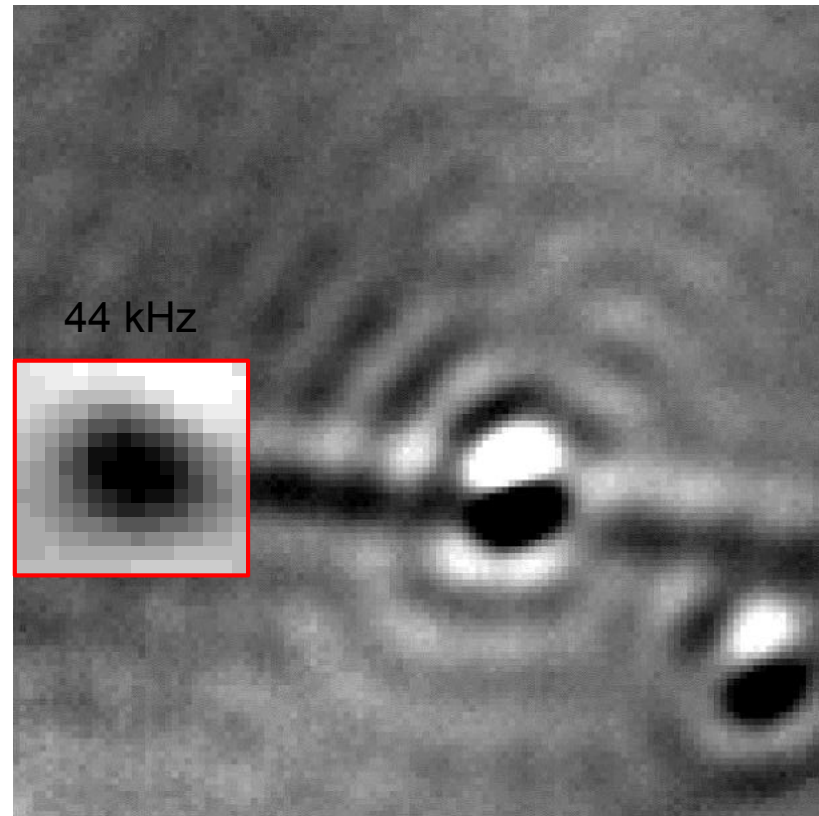
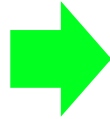
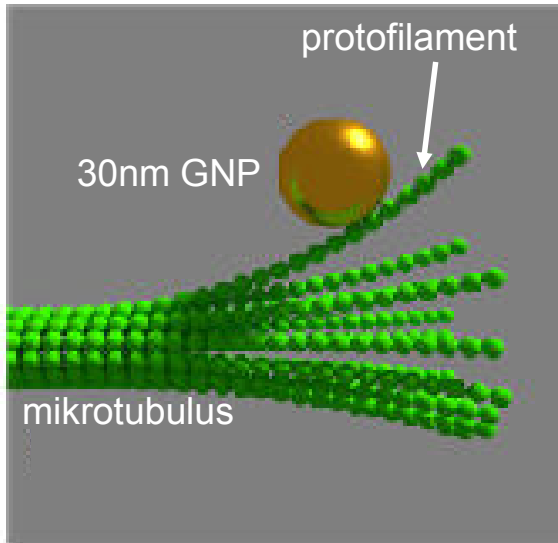


Tracking single proteins on a microtubule  
<20  $\mu$ s temporal resolution  
<2 nm spatial (3D) resolution

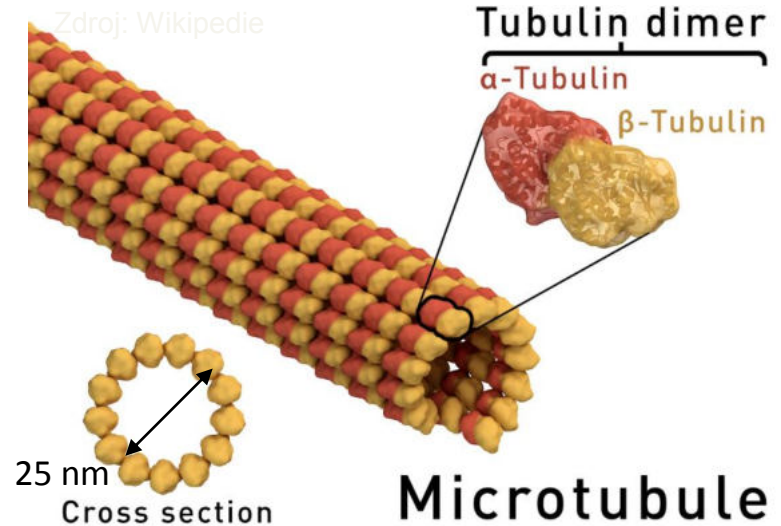
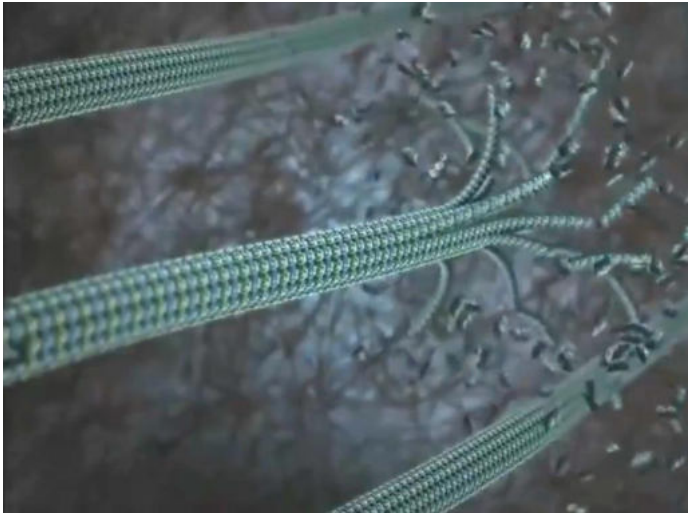
MAP proteins Ase-1/PRC-1 family



# Tracking single depolymerizing tubulin

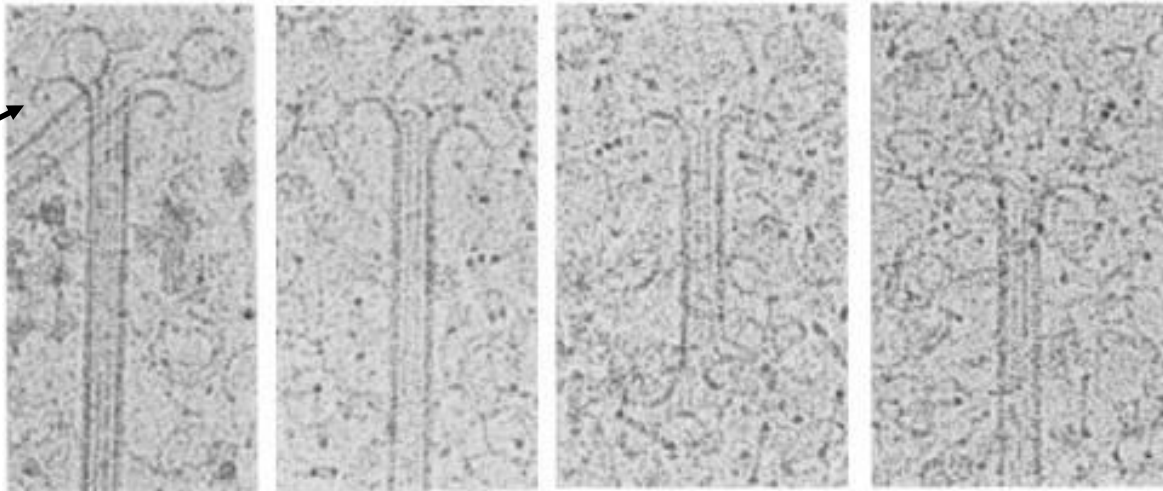


# Depolymerization of microtubules



Ram's horn

**Diameter  
30-50 nm**

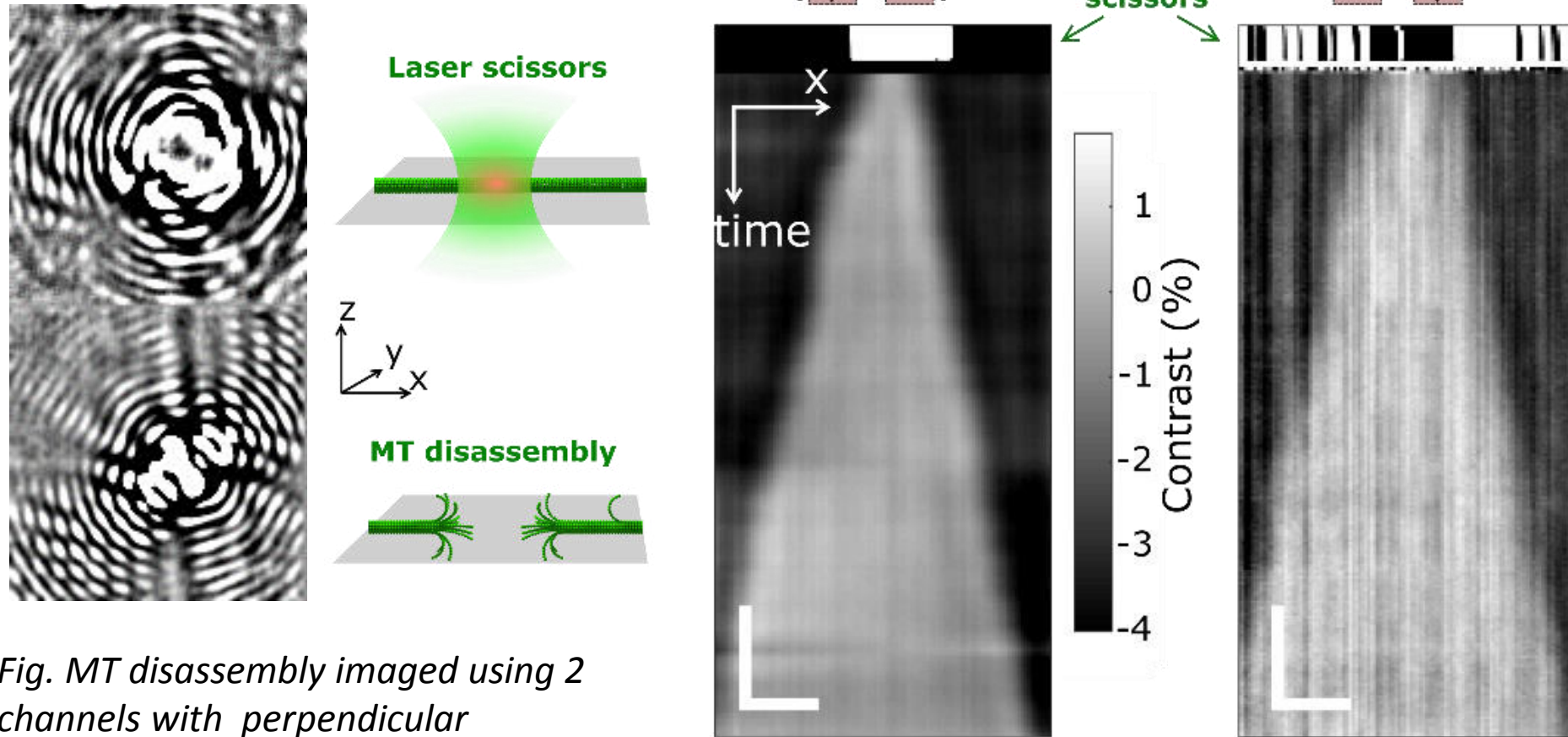


SEM  
static images

E.-M. Mandelkow, E. Mandelkow, and R. A. Milligan, "Microtubule dynamics and microtubule caps: a time-resolved cryo-electron microscopy study," *J. Cell Biol.*, vol. 114, no. 5, pp. 977– 991, 1991.



# Imaging of geometrical changes during disassembly



*Fig. MT disassembly imaged using 2 channels with perpendicular polarizations*

# So how evil is the European Research Council?





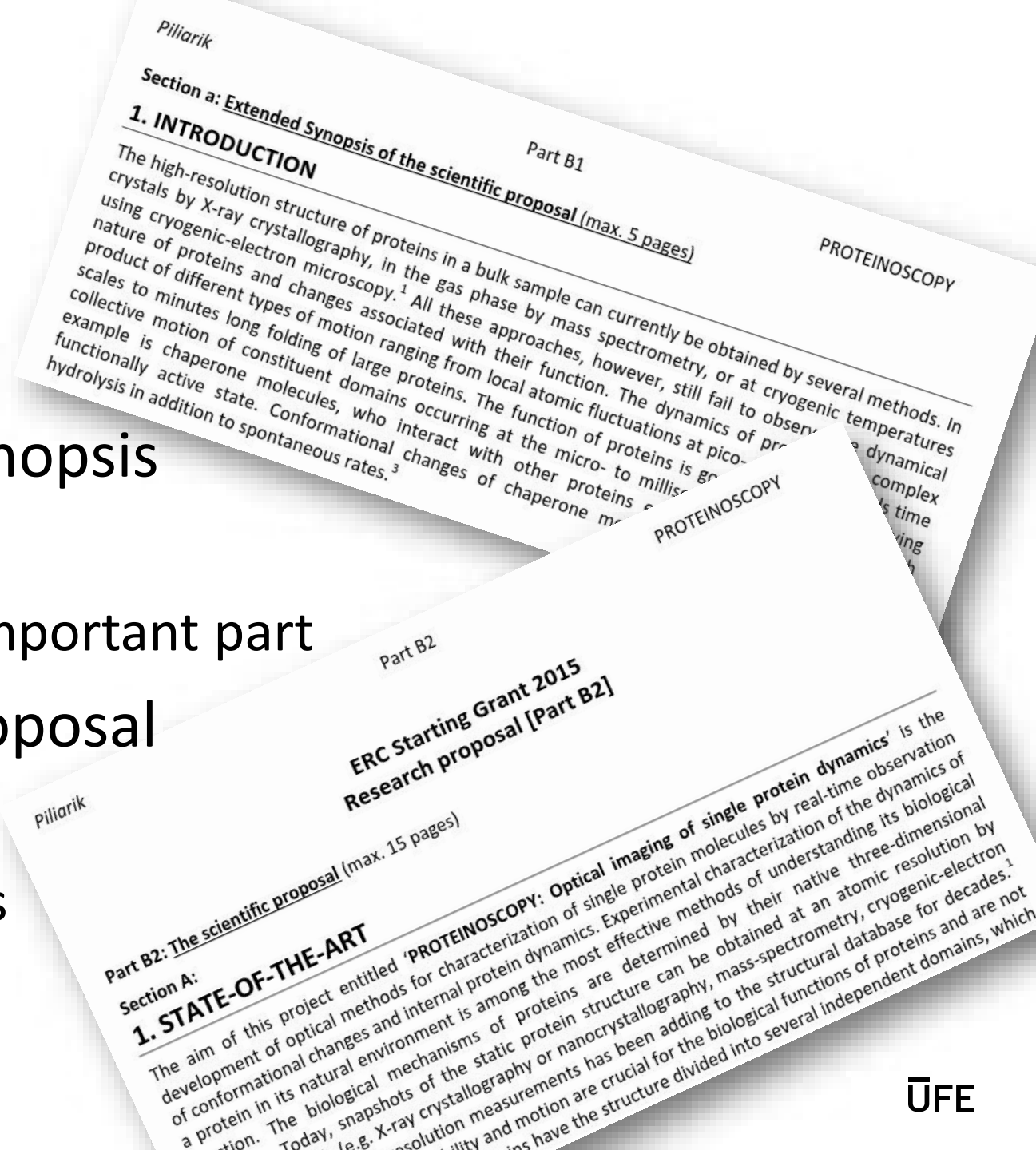
## Lessons learned

- Panel members are not there to appreciate your wisdom
  - Do not try to trick them with high level of technical details.
- Referees will find out if you know your story
  - Do not lure the referees into discussion you are not 100% sure of.



# Proposal

- Abstract
- Extended synopsis
  - 5 pages
  - The most important part
- Research proposal
  - 15 pages
  - For referees





## One more thing...

- Seek help in revising the project proposal
- Ask for more feedback from your colleagues
- Ask for an opinion a stranger you run into in the corridor
- Ask a native speaker for revision
- In case of a positive feedback ask someone else

# NanoOptics



WHERE HETEROGENEITY IS AN ADVANTAGE



Acknowledgements:



ERC-CZ Starting grant



Lab tours at any time, visitors welcome!

Thank you



This Monday is happy  
because new opportunities  
for exciting science opened  
in ERC call.

#ERCrocksChelseaSucks

