PEOPLE MATTER

research
development
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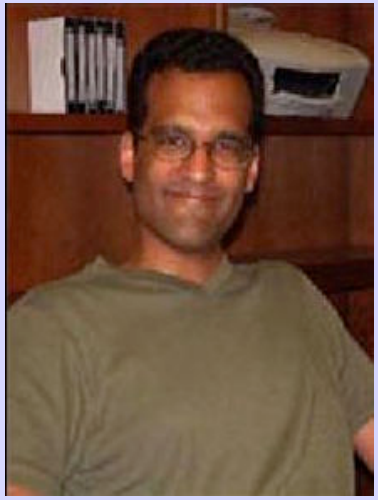
LASER ZENTRUM HANNOVER e.V.

Nanosurgery in live cells using ultrashort laser pulses

Alexander Heisterkamp

Iva Maxwell

Eric Mazur



Sanjay Kumar

Donald Ingber

DEAS, Harvard University
Harvard Medical School

Nanosurgery in live cells using ultrashort laser pulses

- why ablation by fs-pulses?
- differentiation photobleaching – ablation
- live cell applications

- ablation by fs-pulses
- differentiation photobleaching – ablation
- live cell applications

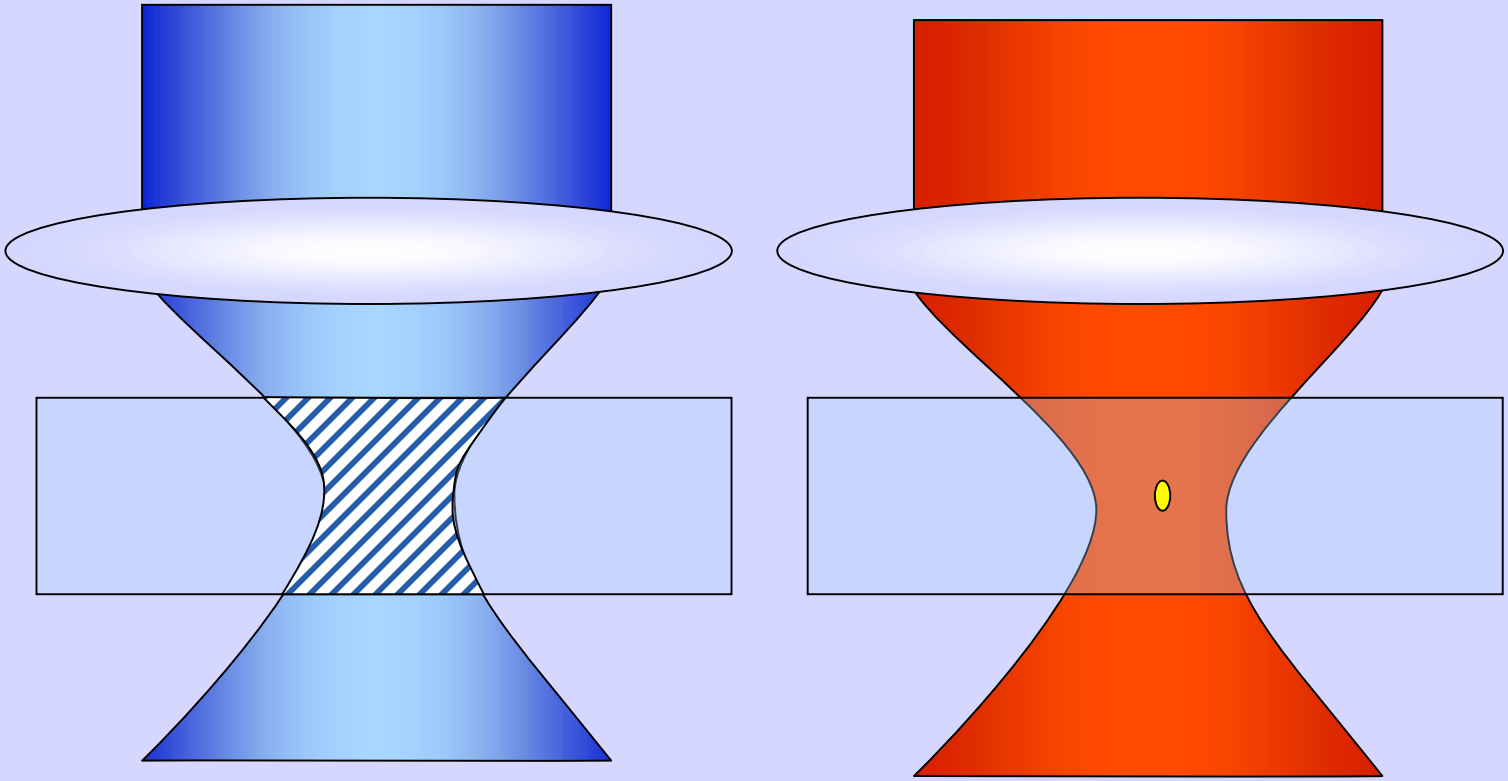
Advantages of fs-pulses

- working inside the bulk of the material
- deep penetration depth
- very low side effects

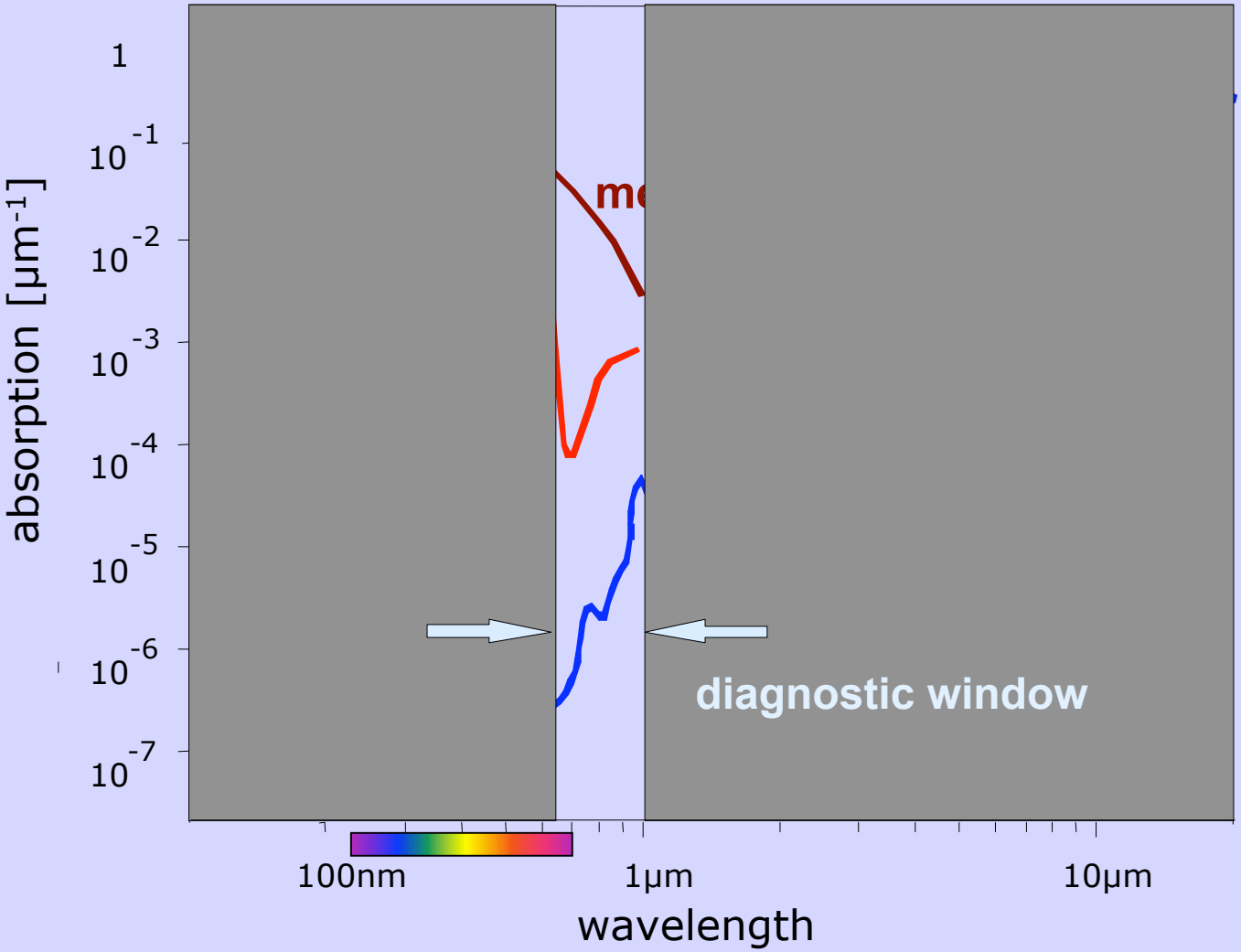
High spatial selectivity

linear absorption

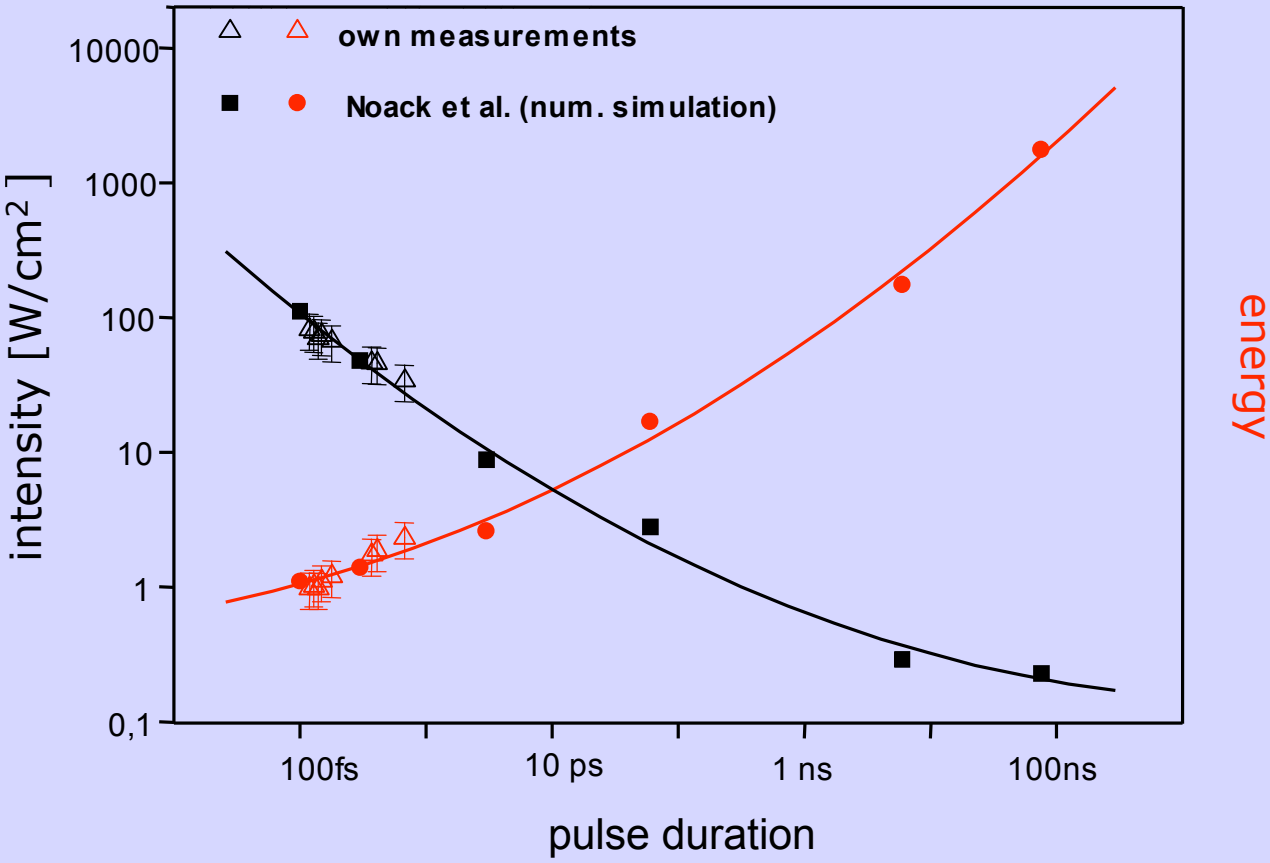
multiphoton absorption



High penetration depth



Very low side effects



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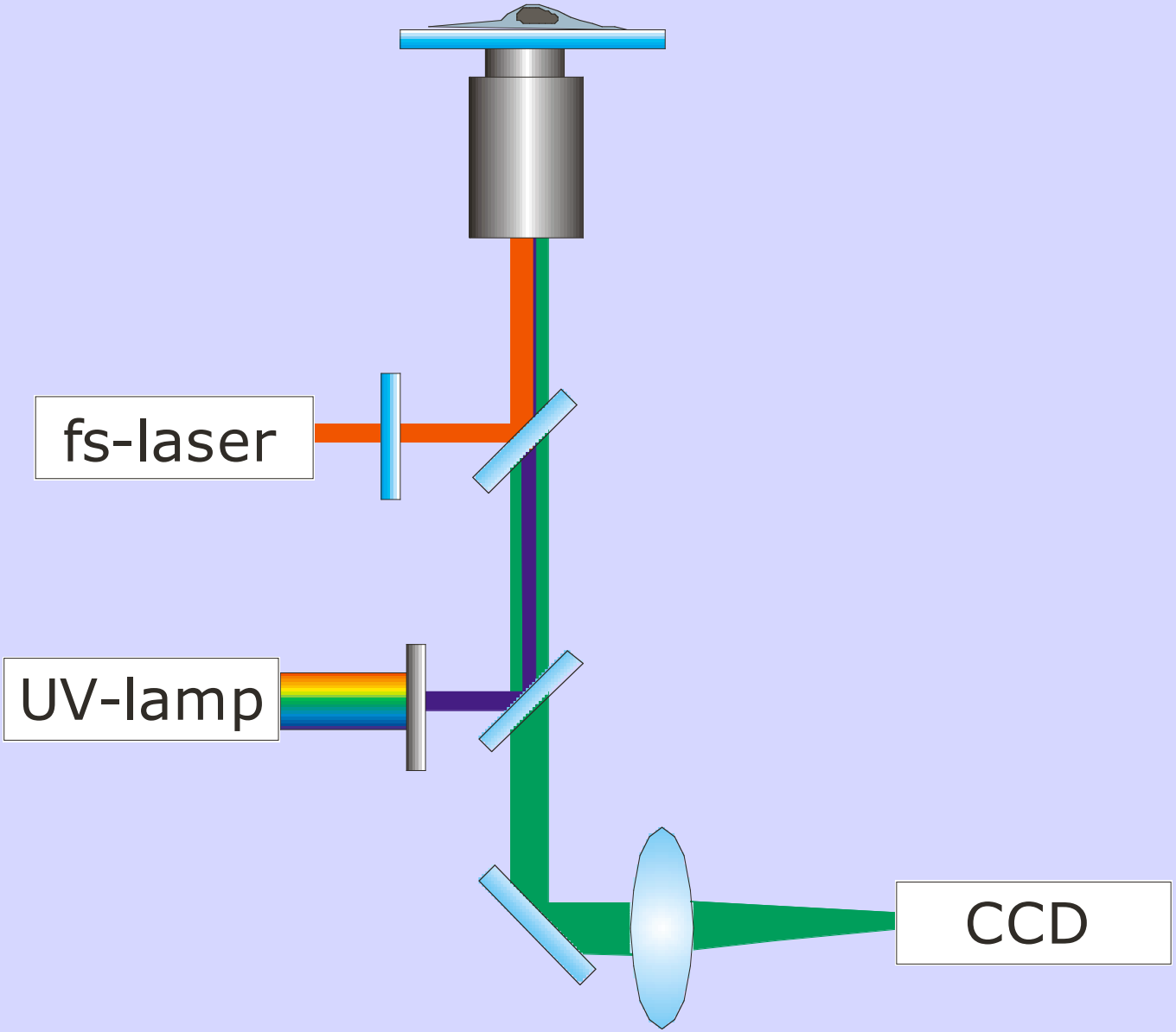
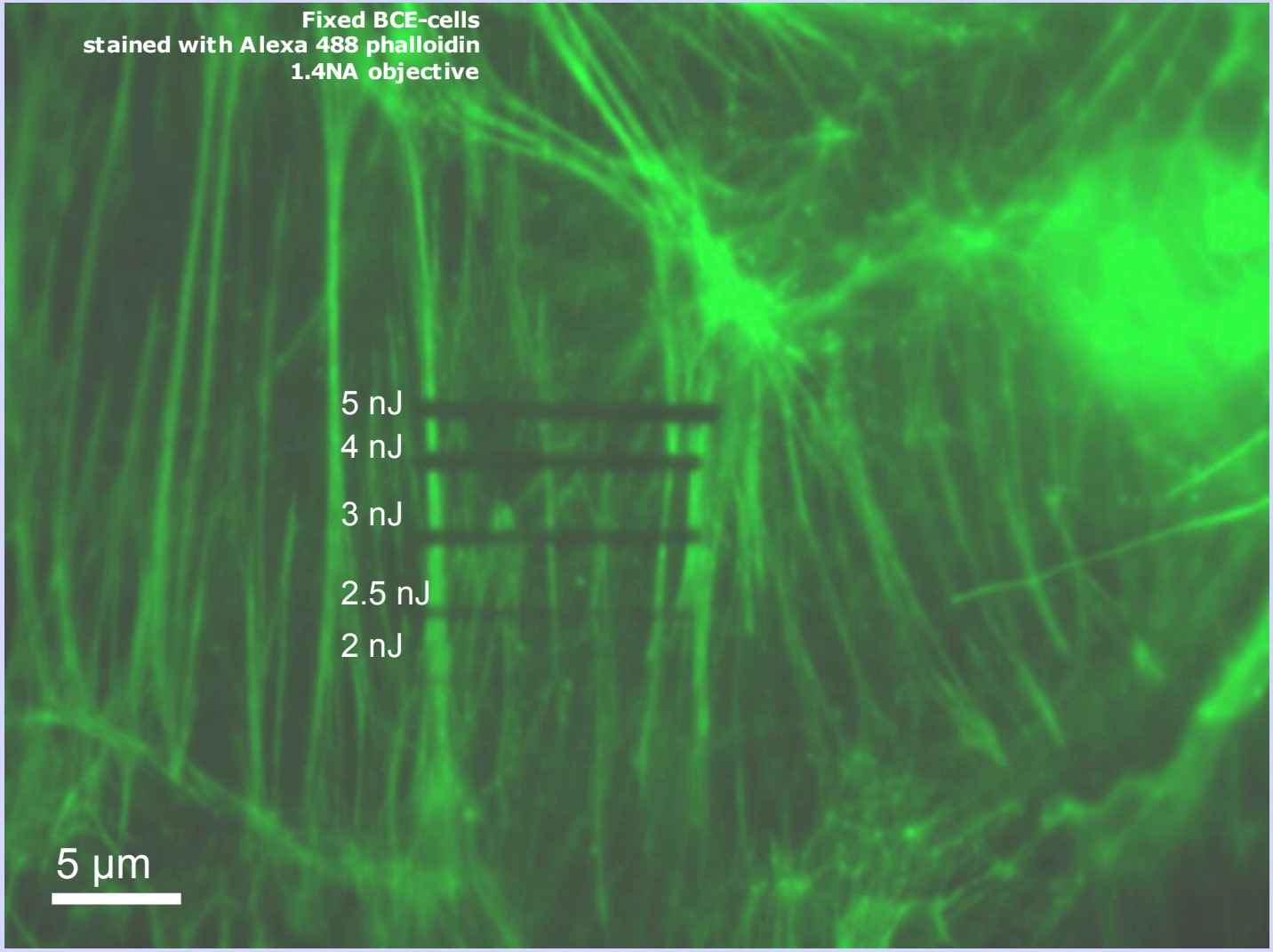
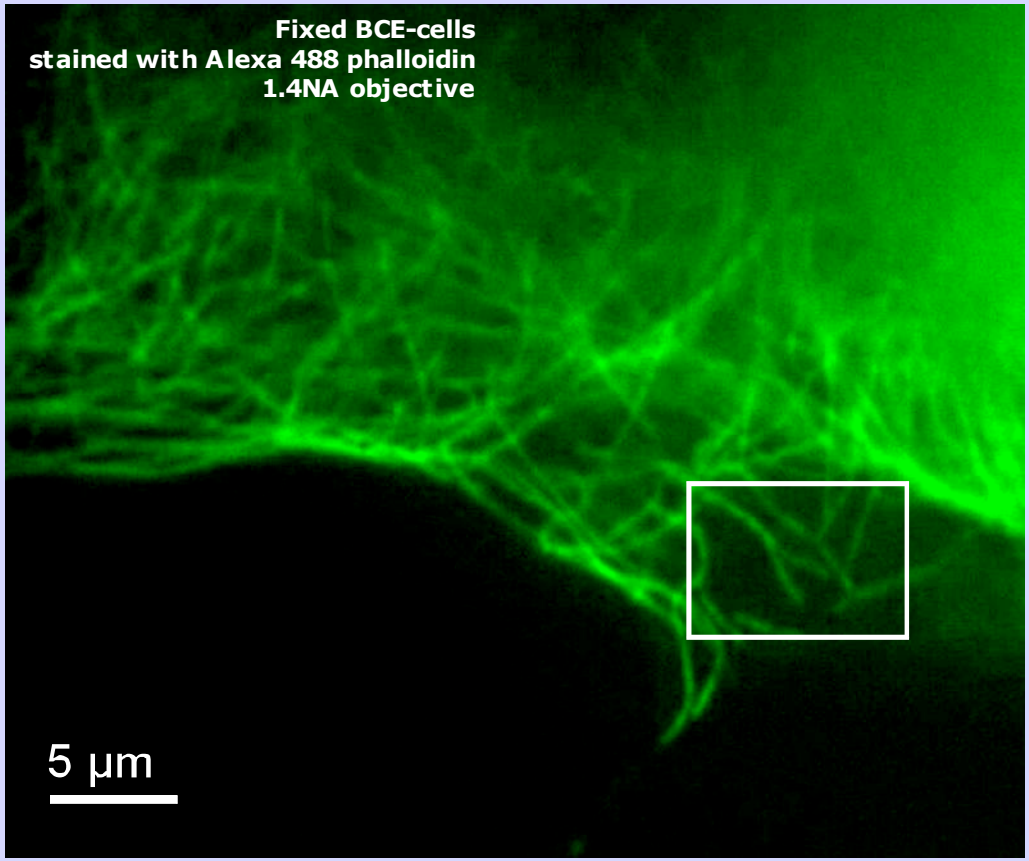

fs-laser
UV-lamp
CCD


Fixed BCE-cells
stained with Alexa 488 phalloidin
1.4NA objective
5 nJ
4 nJ
3 nJ
2.5 nJ
2 nJ
5 µm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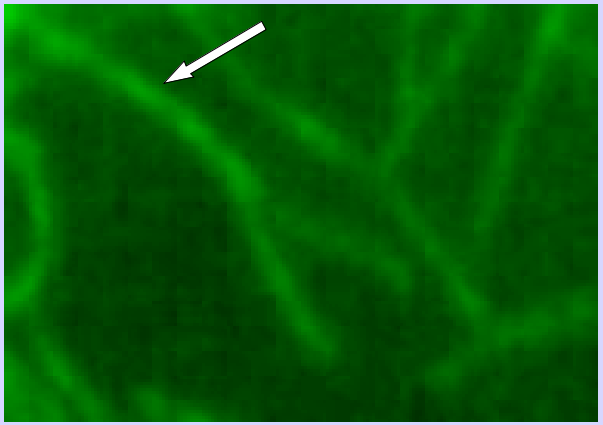
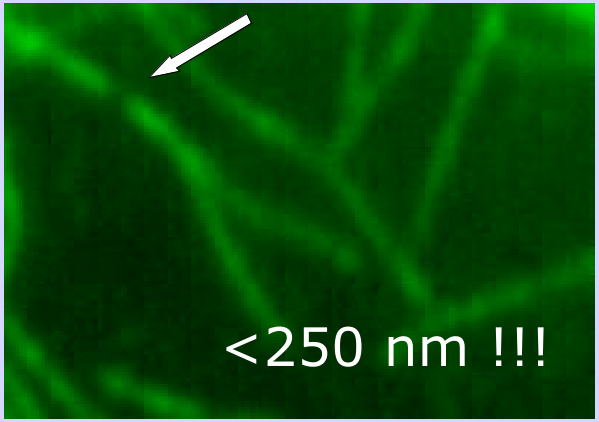
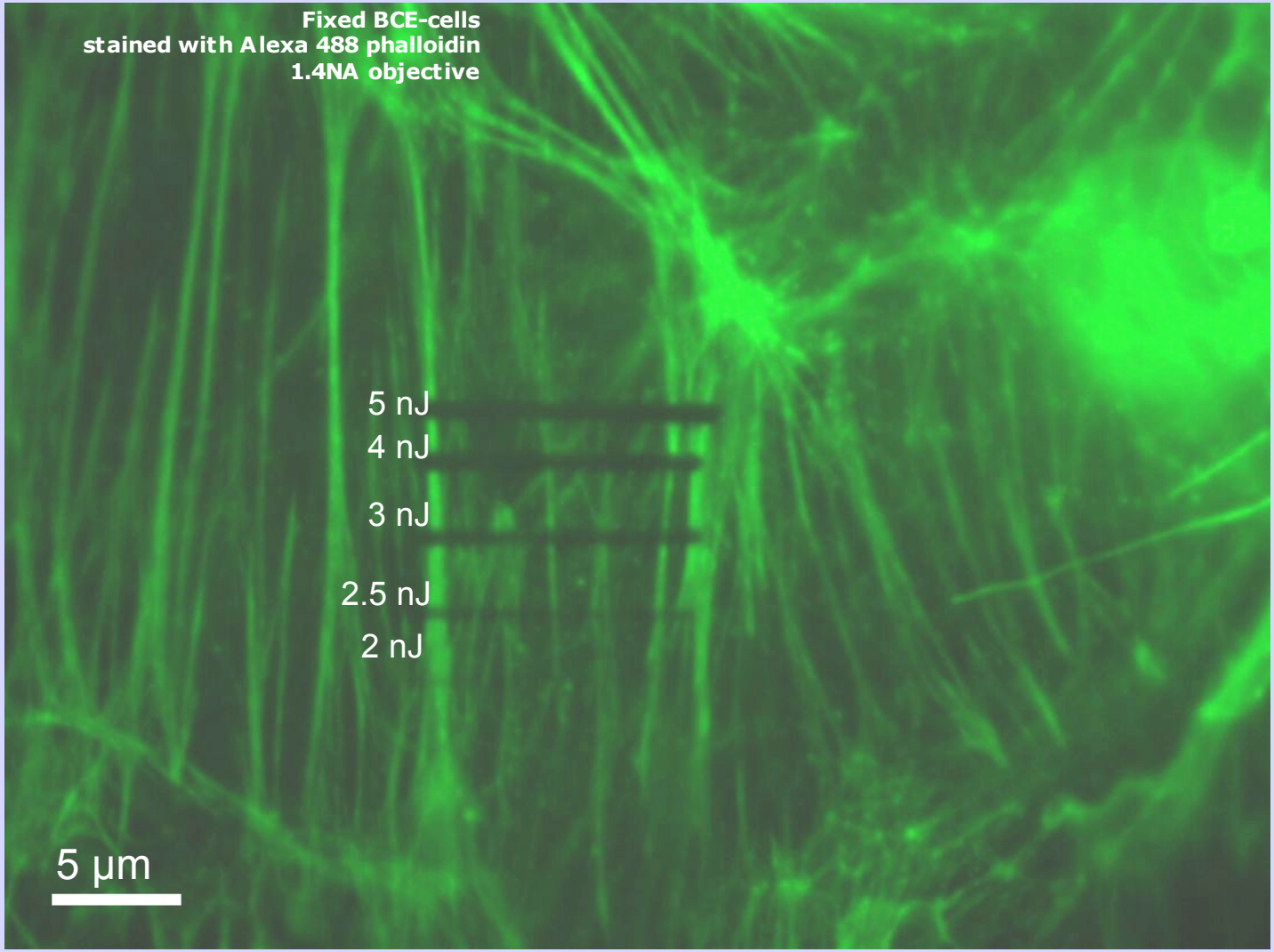


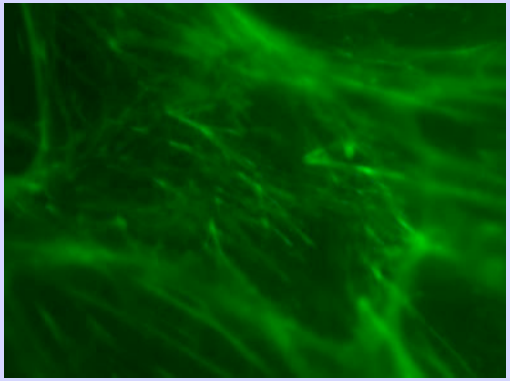

<250 nm !!!


Fixed BCE-cells
stained with Alexa 488 phalloidin
1.4NA objective
5 nJ
4 nJ
3 nJ
2.5 nJ
2 nJ
5 µm

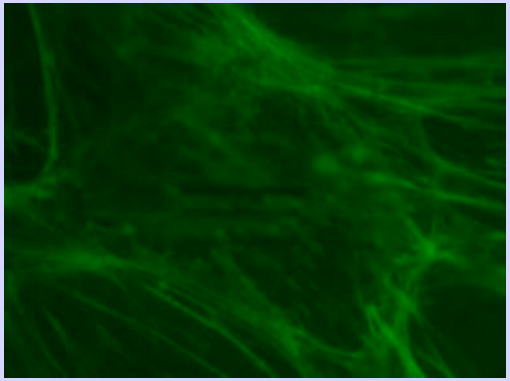
Is this photobleaching or ablation ???

- ablation by fs-pulses
- differentiation photobleaching – ablation
- live cell applications

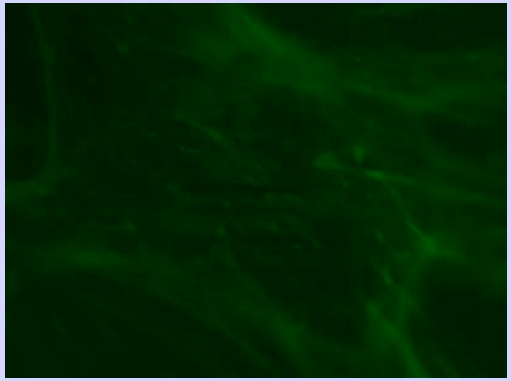
Photobleaching



0 seconds



30 seconds



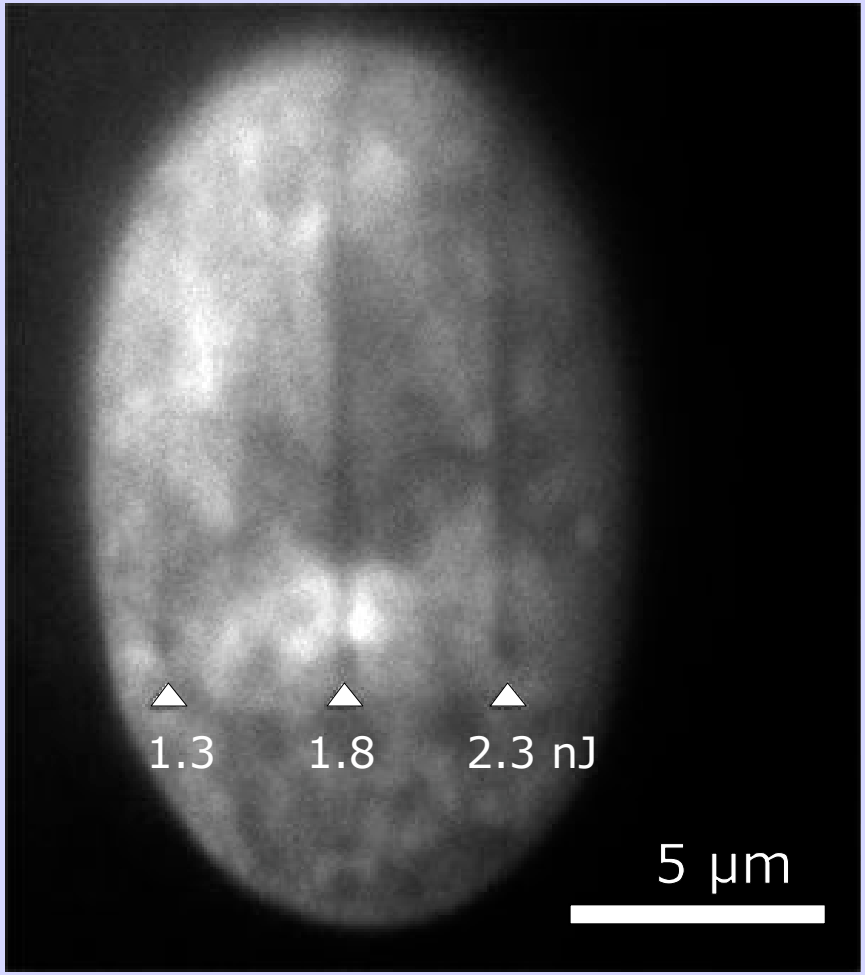
1 minute

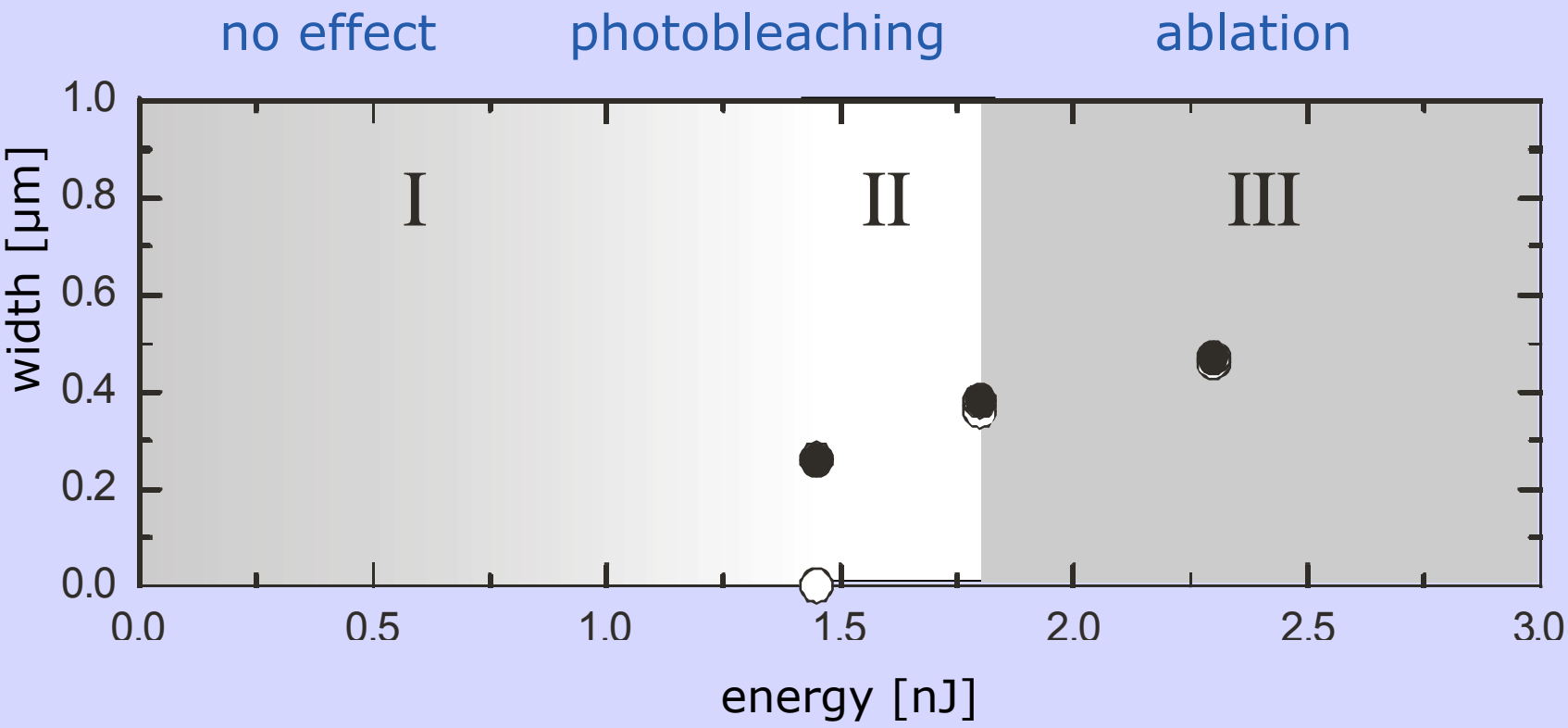
Effects during fs-illumination

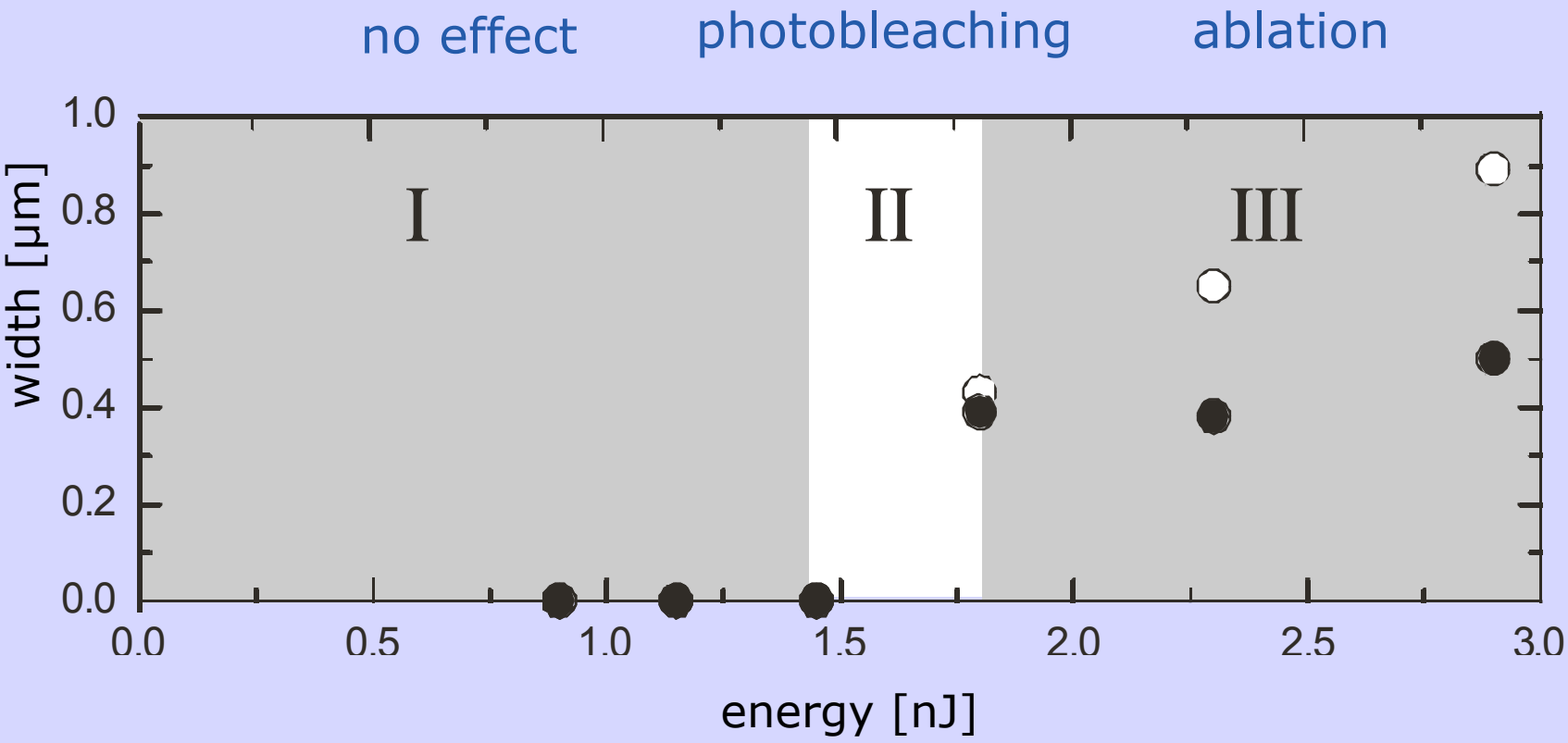
- 1-10 mW — typical imaging power
- 5-15 mW — unwanted chemical side effects "Photobleaching"
- >80 mW — Ablation!!!

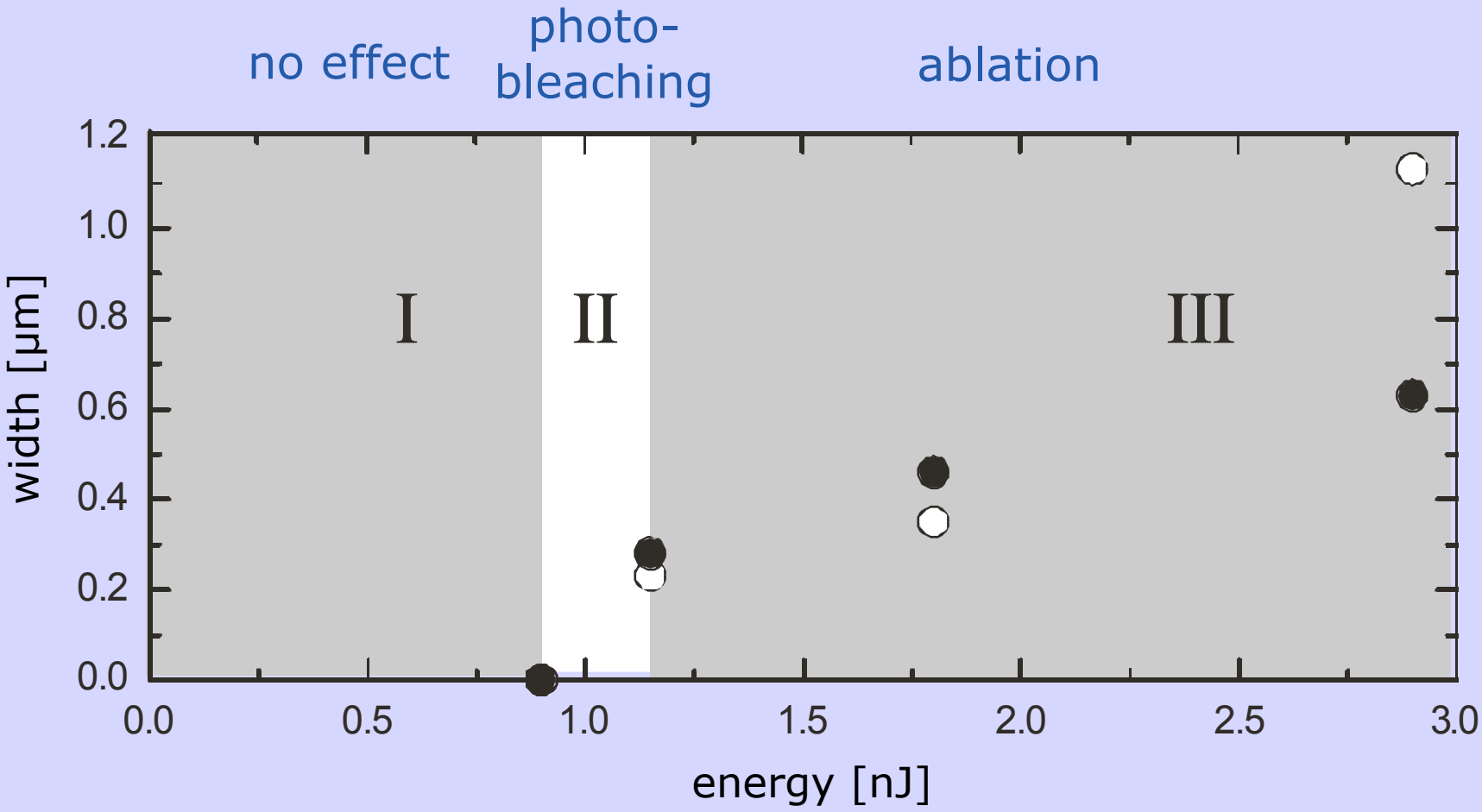
fluorescence

TEM



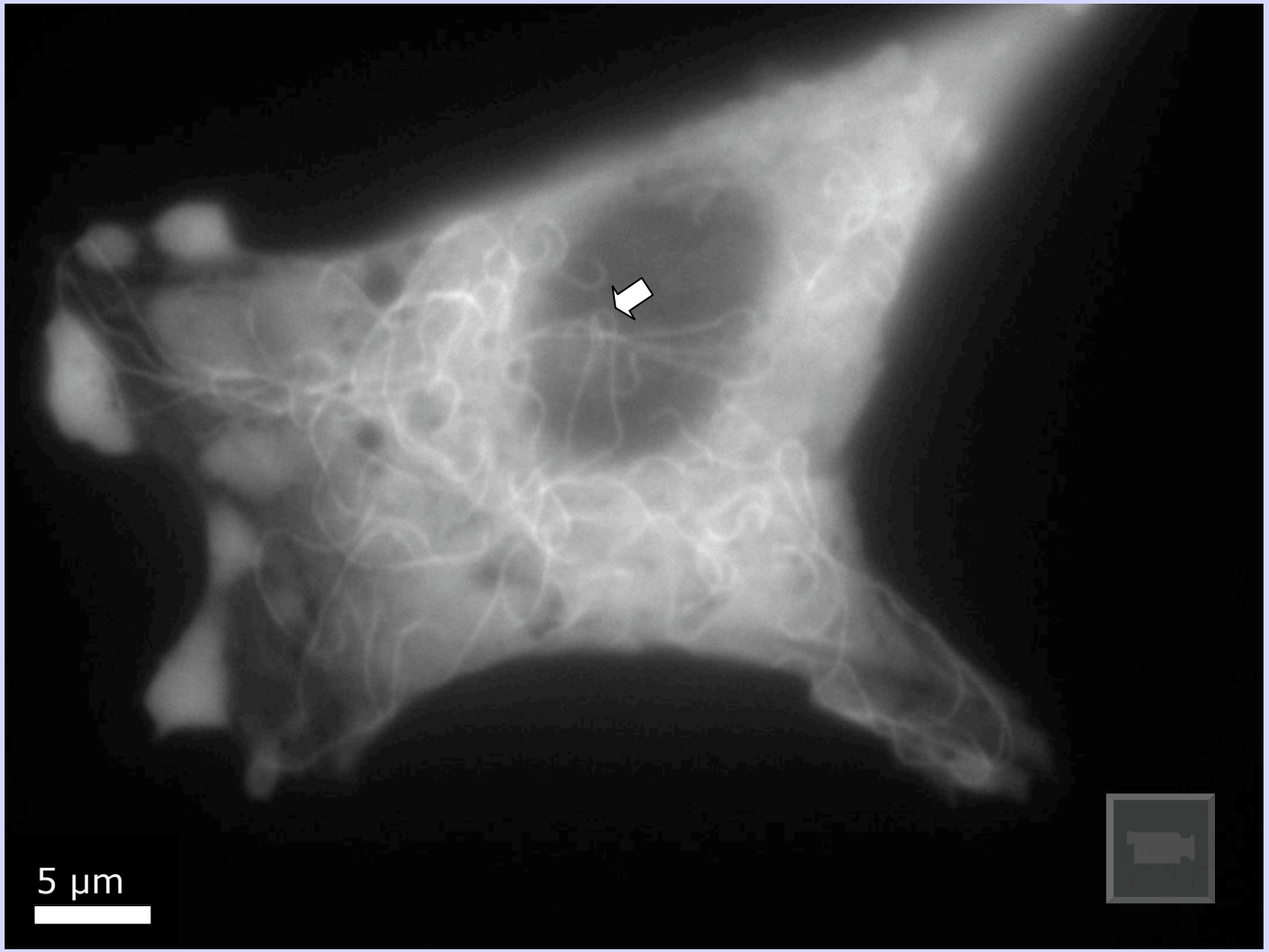

no effect
photobleaching
ablation
I
II
III
width [µm]
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
energy [nJ]

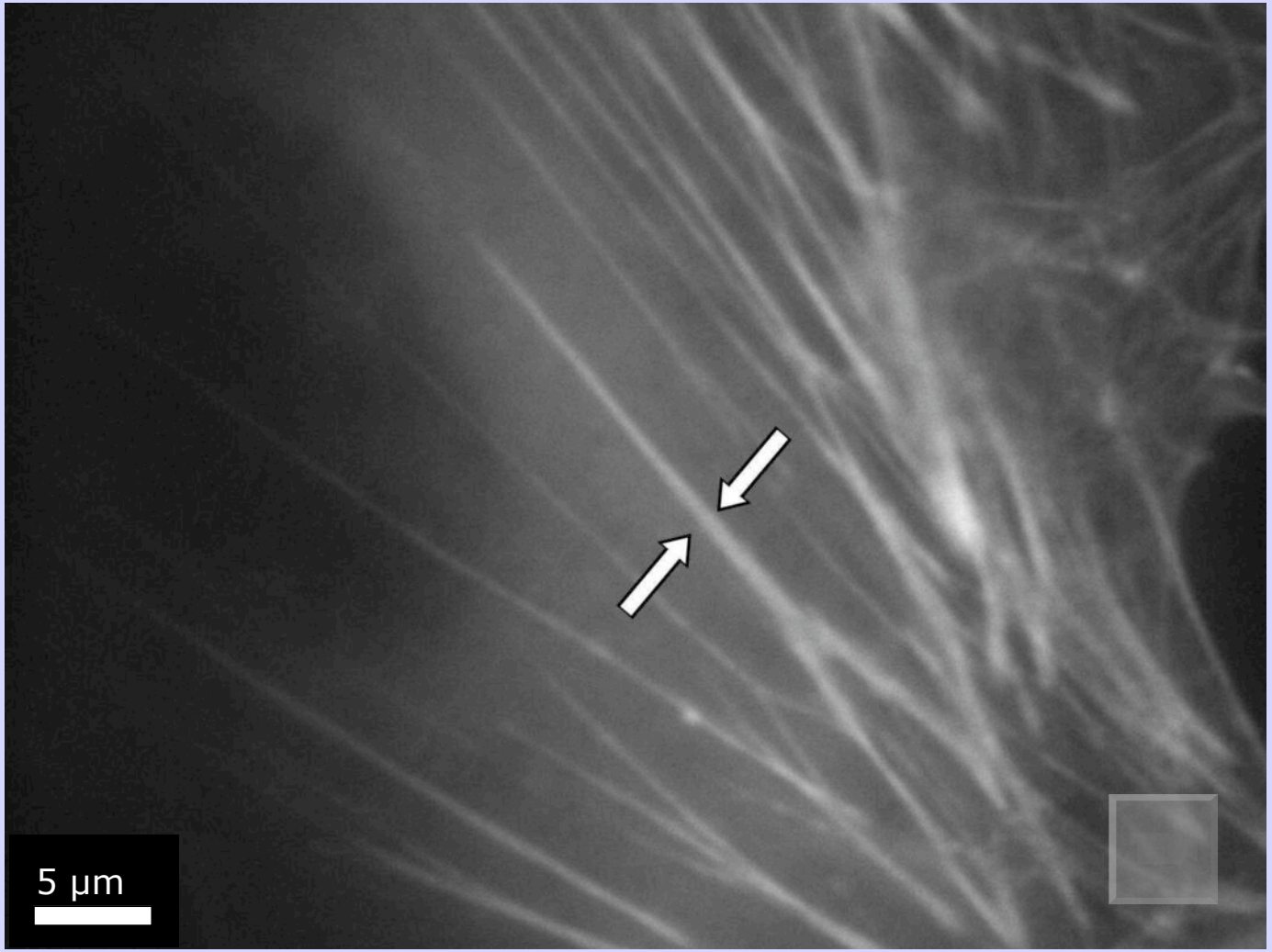

no effect
photobleaching
ablation
I
II
III
width [µm]
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
energy [nJ]

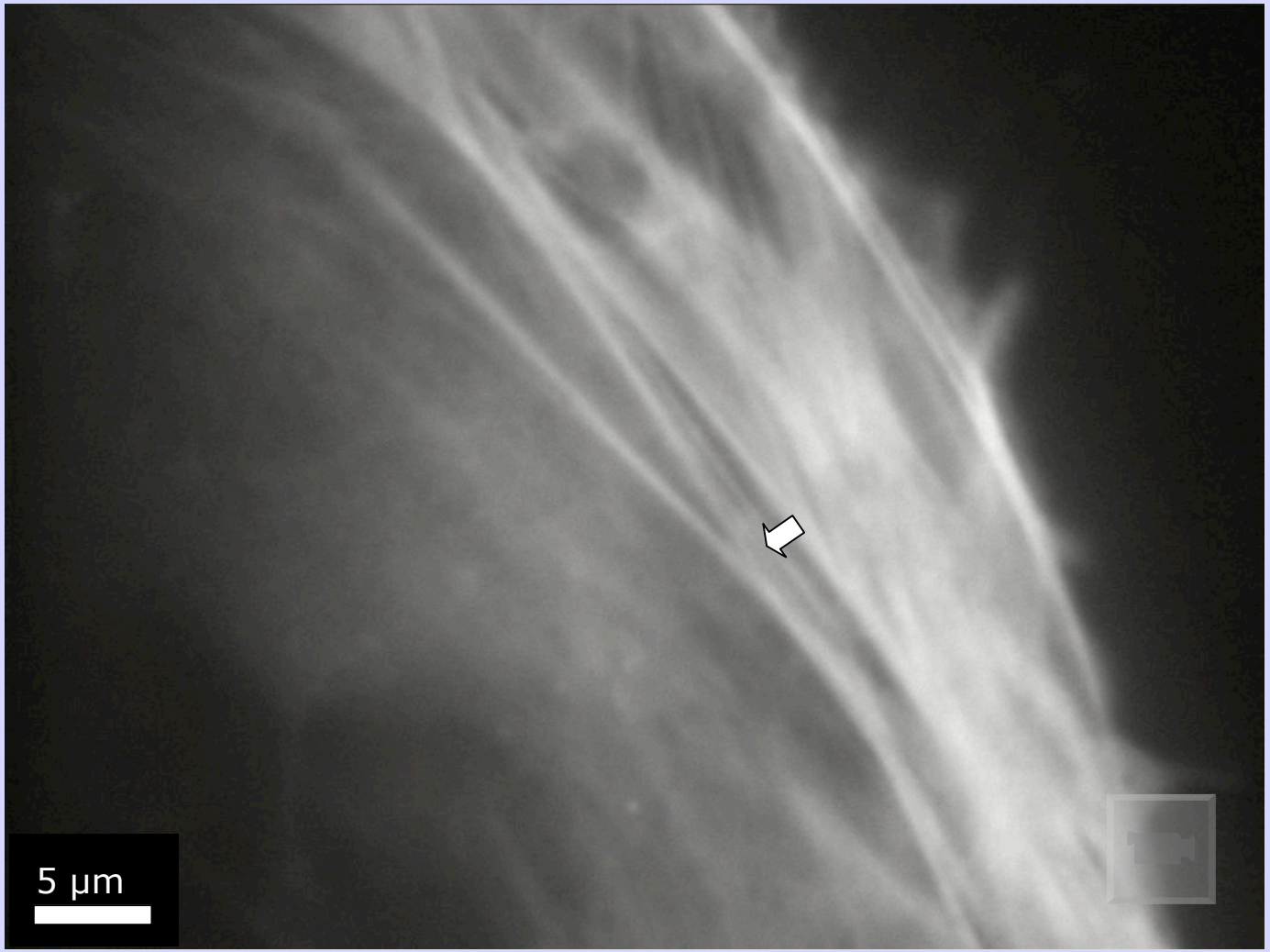

no effect
photo-
bleaching
ablation
I
II
III
width [µm]
energy [nJ]
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.0
0.5
1.0
1.5
2.0
2.5
3.0

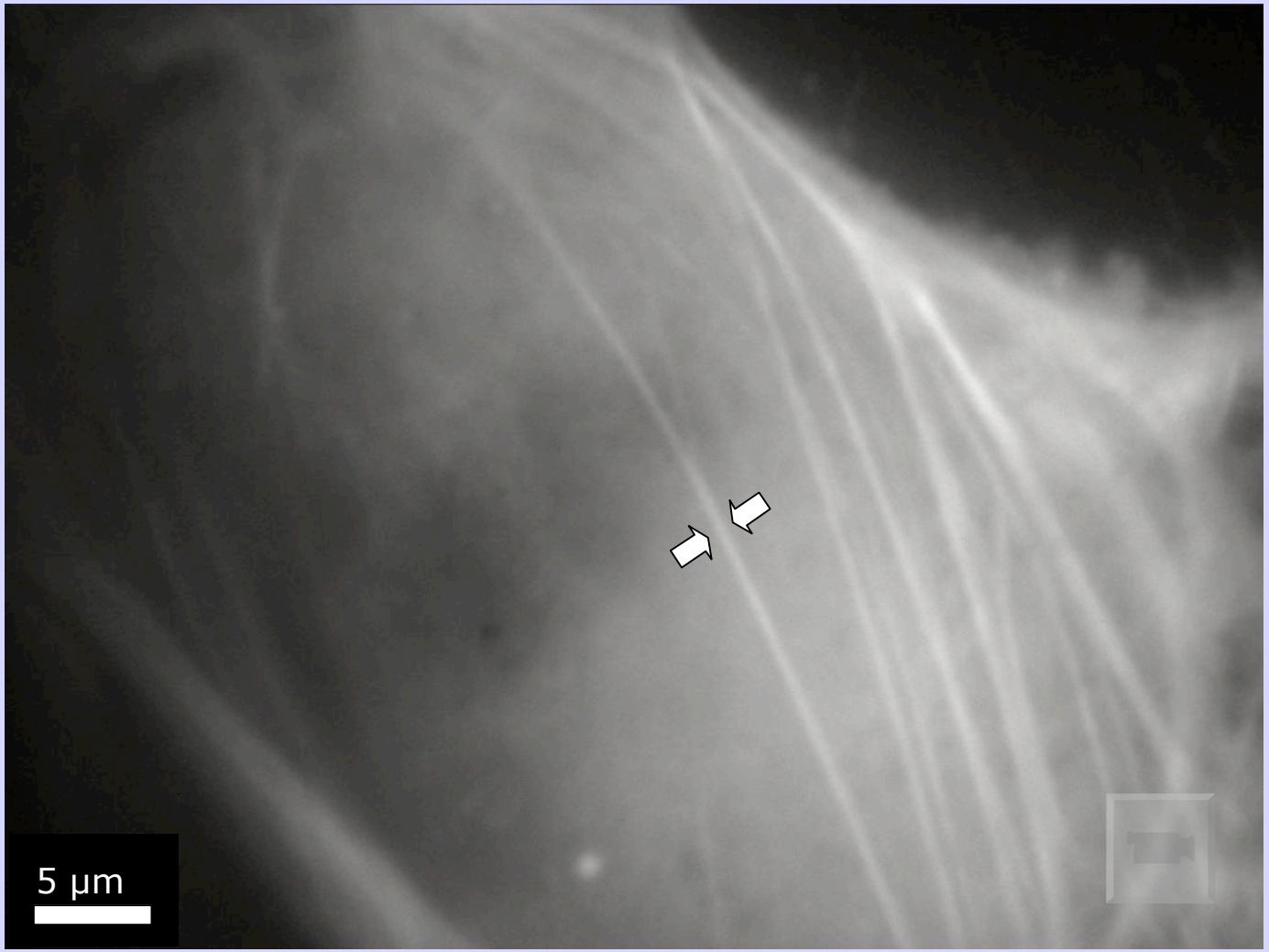
20% above threshold leads to ablation

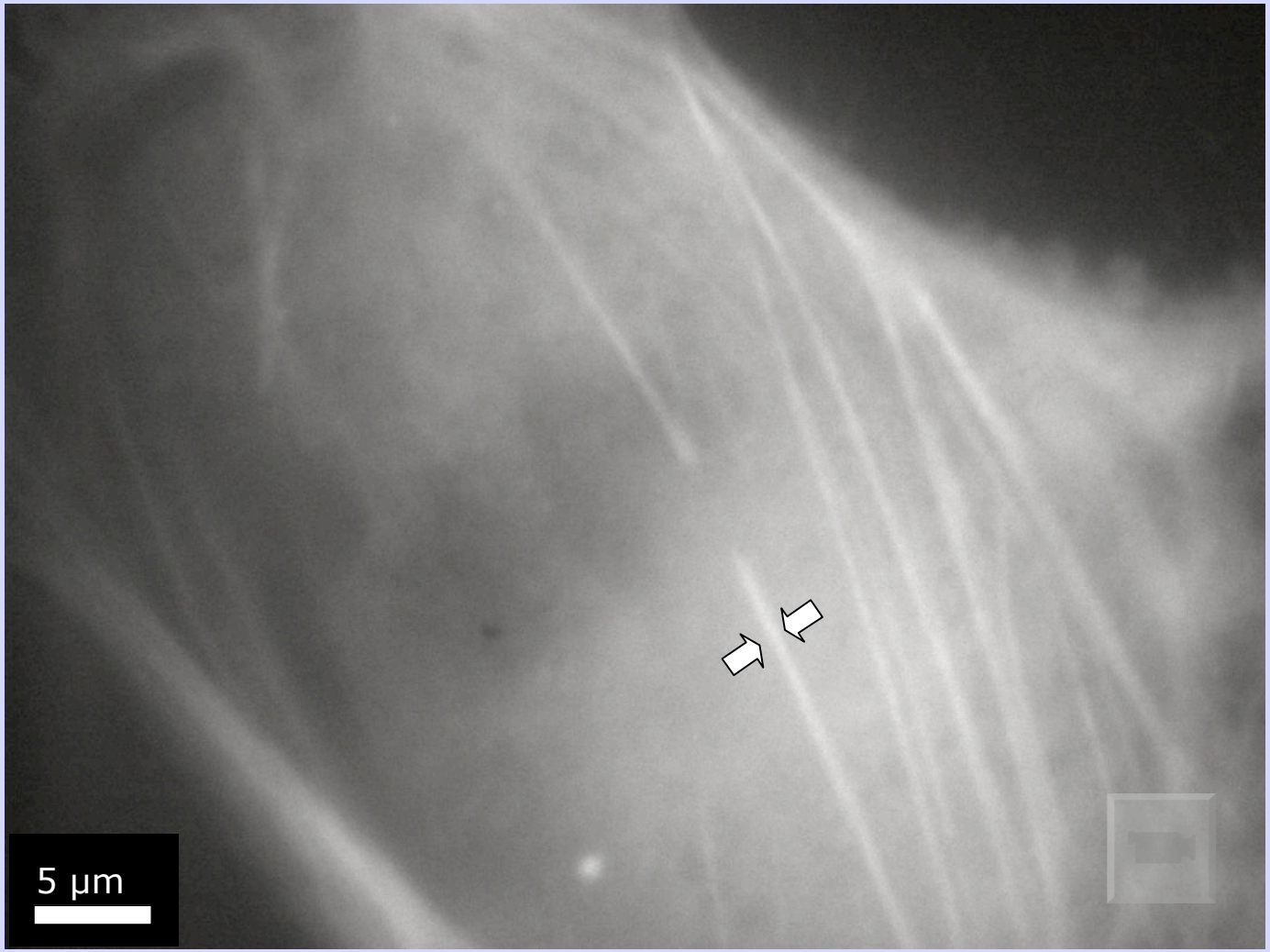
- ablation by fs-pulses
- differentiation photobleaching – ablation
- live cell applications


5 µm


5 µm

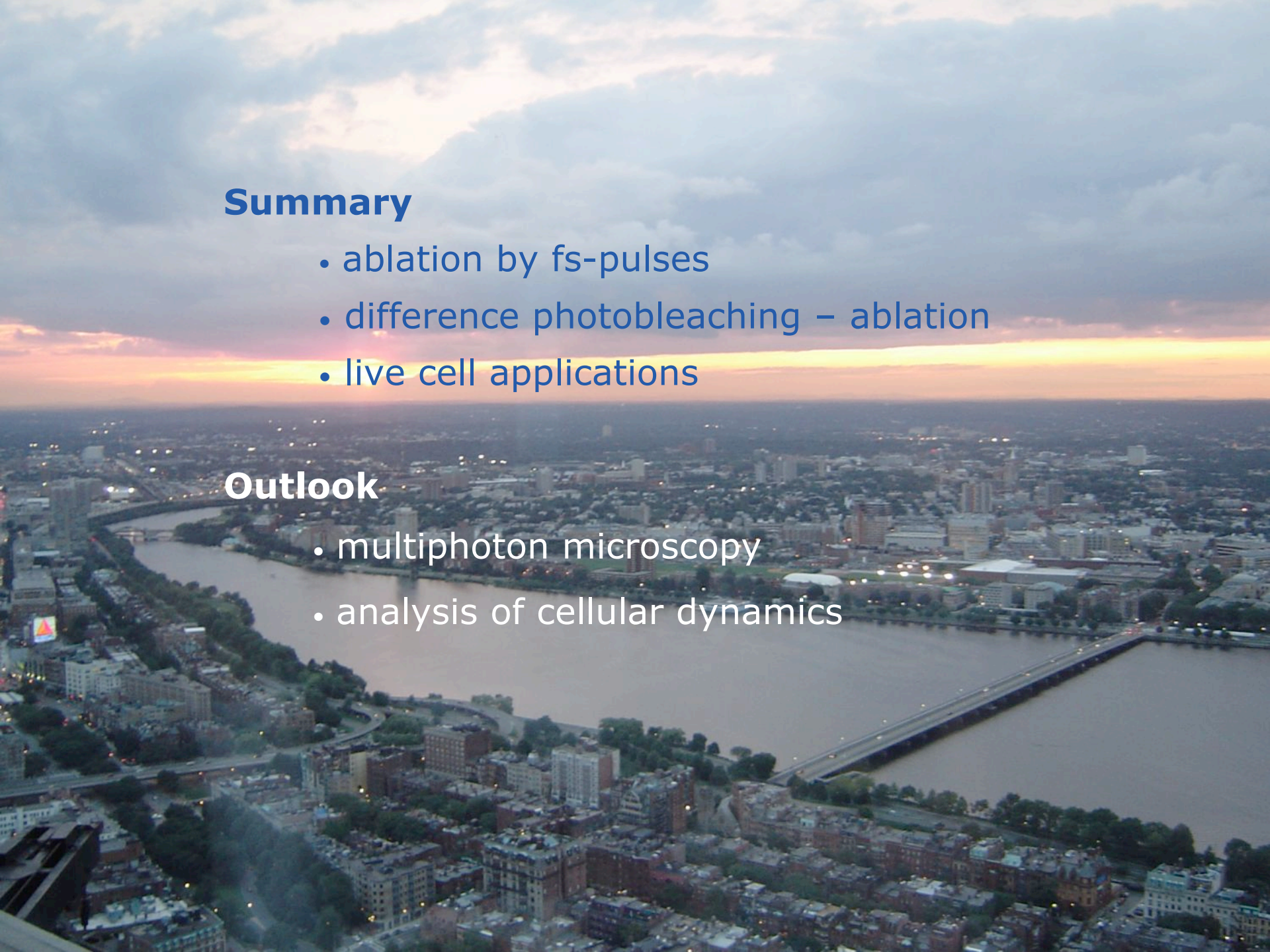

5 µm


5 µm


5 µm

What can we learn from this?

Tension-dependent retraction of severed stress fibers
Control
Y27632
Nocodazole
Distance between severed ends (µm)
Time (sec)
0
1
2
3
4
5
6
7
0
5
10
15
20
25
30
35
40

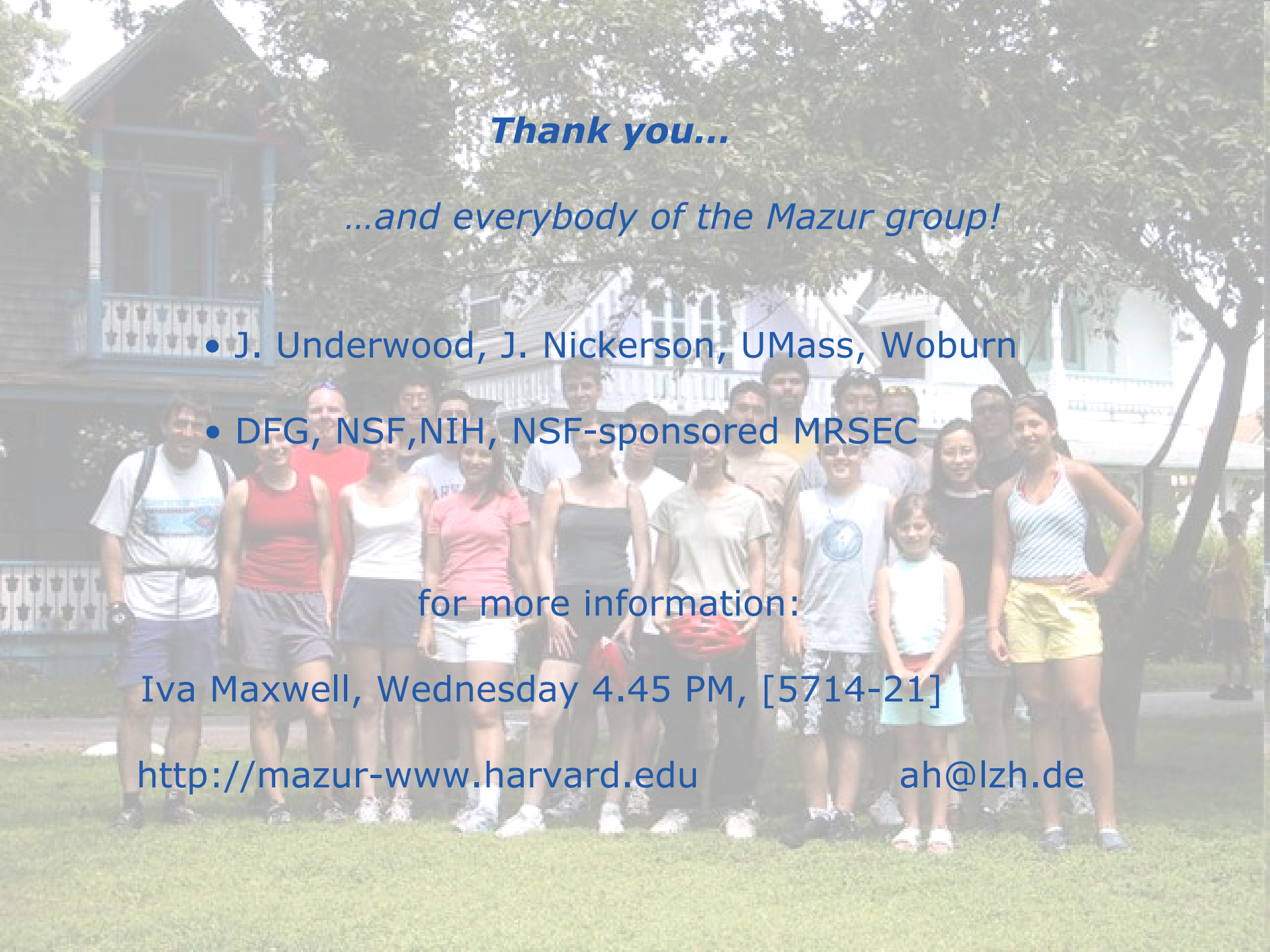


Summary

- ablation by fs-pulses
- difference photobleaching – ablation
- live cell applications

Outlook

- multiphoton microscopy
- analysis of cellular dynamics



Thank you…

…and everybody of the Mazur group!

- J. Underwood, J. Nickerson, UMass, Woburn
- DFG, NSF,NIH, NSF-sponsored MRSEC

for more information:

Iva Maxwell, Wednesday 4.45 PM, [5714-21]

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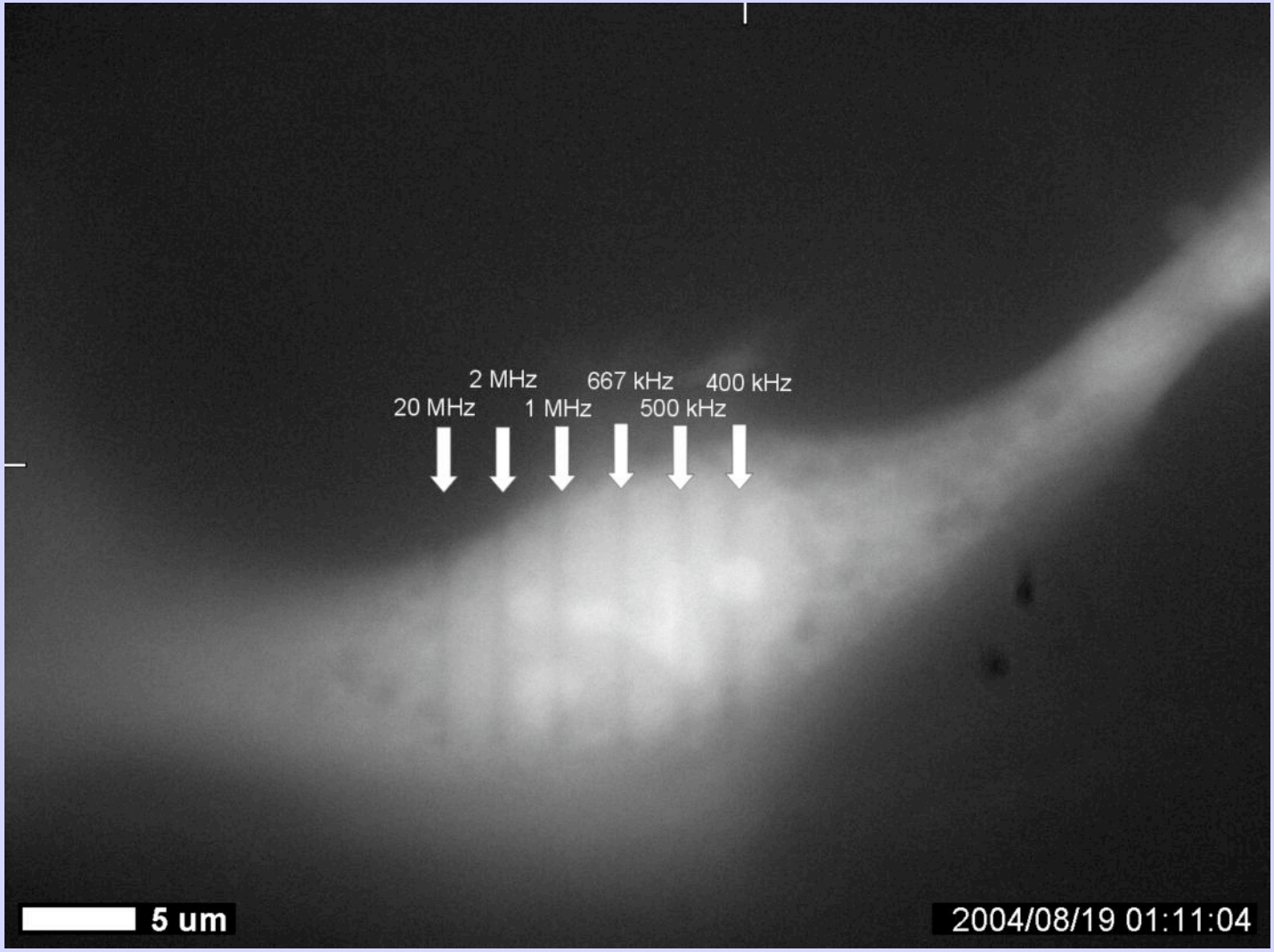
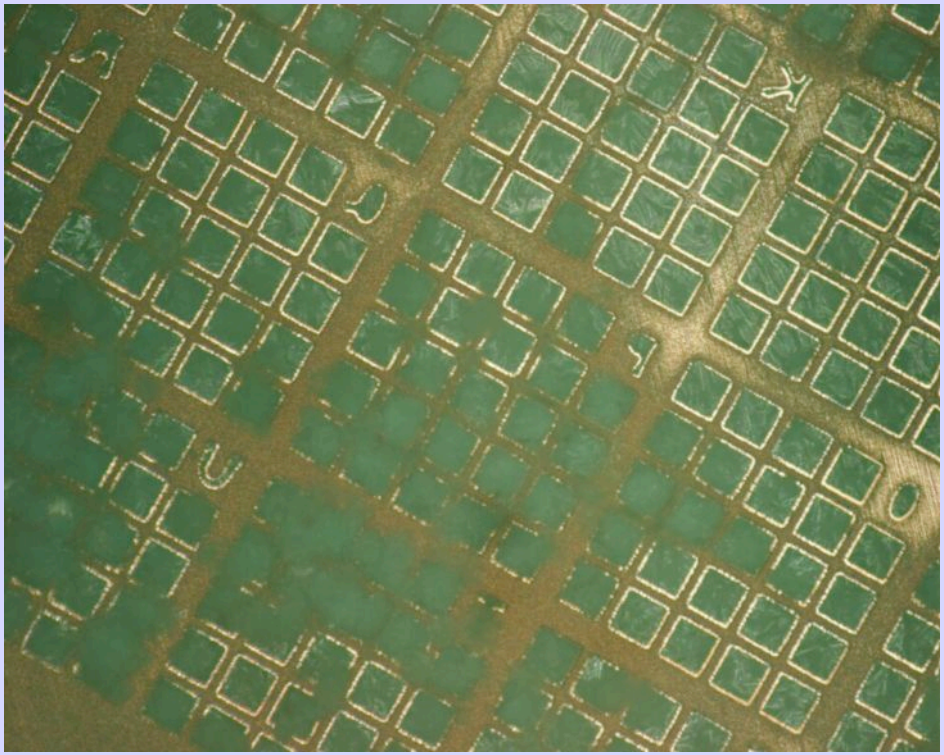
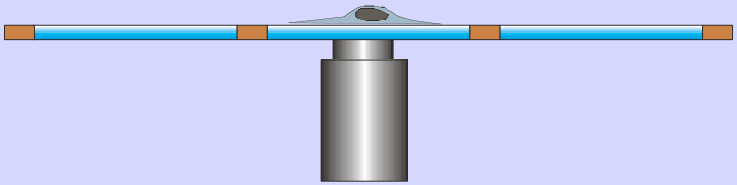
Rep-rate dependence





Transmission-Electron Microscopy (TEM)



Comparison of fluorescence images to TEM