

LIGHTOPTICAL
NANOSCOPIE

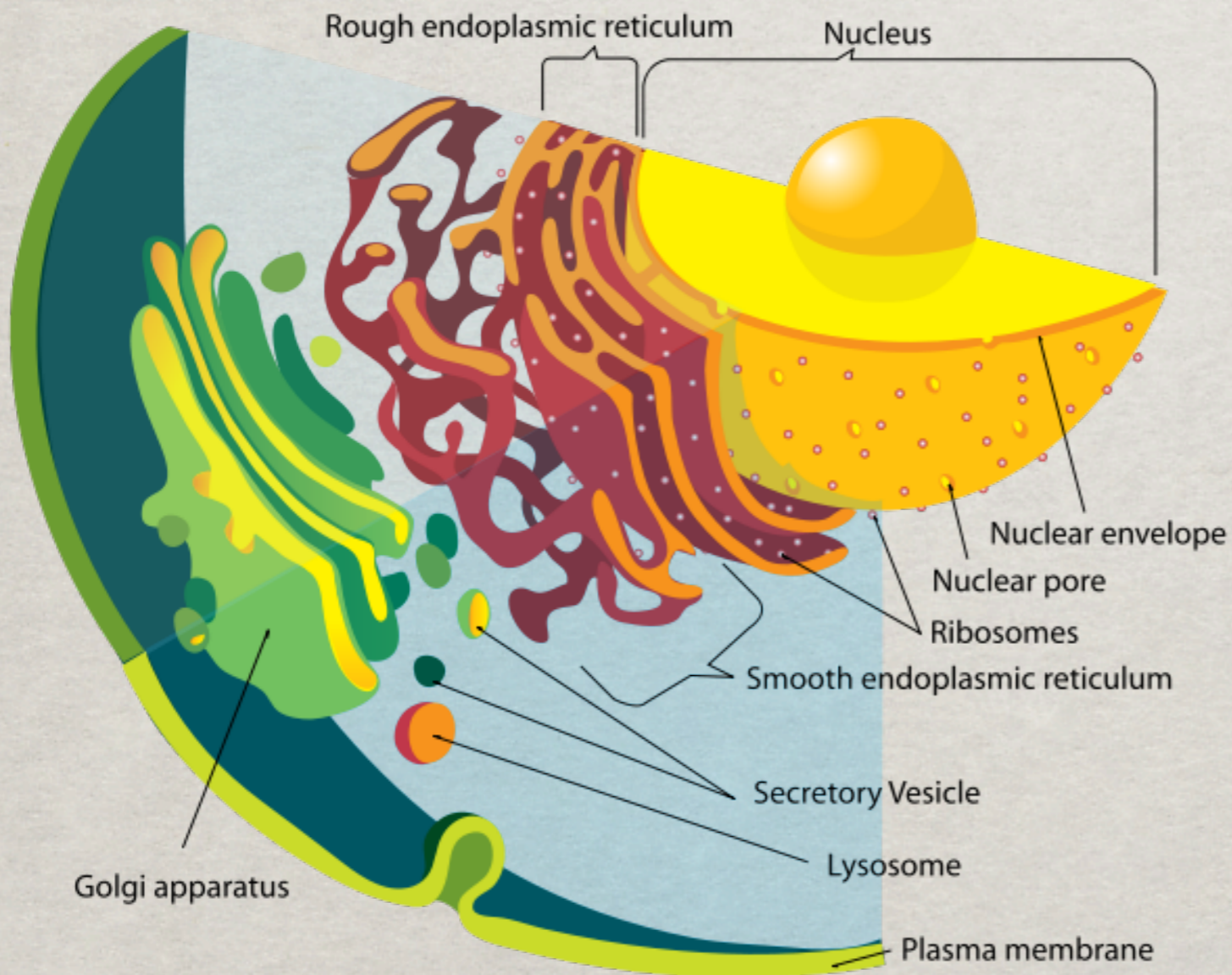
MARCH 12TH, 2013

NICK KEPPER

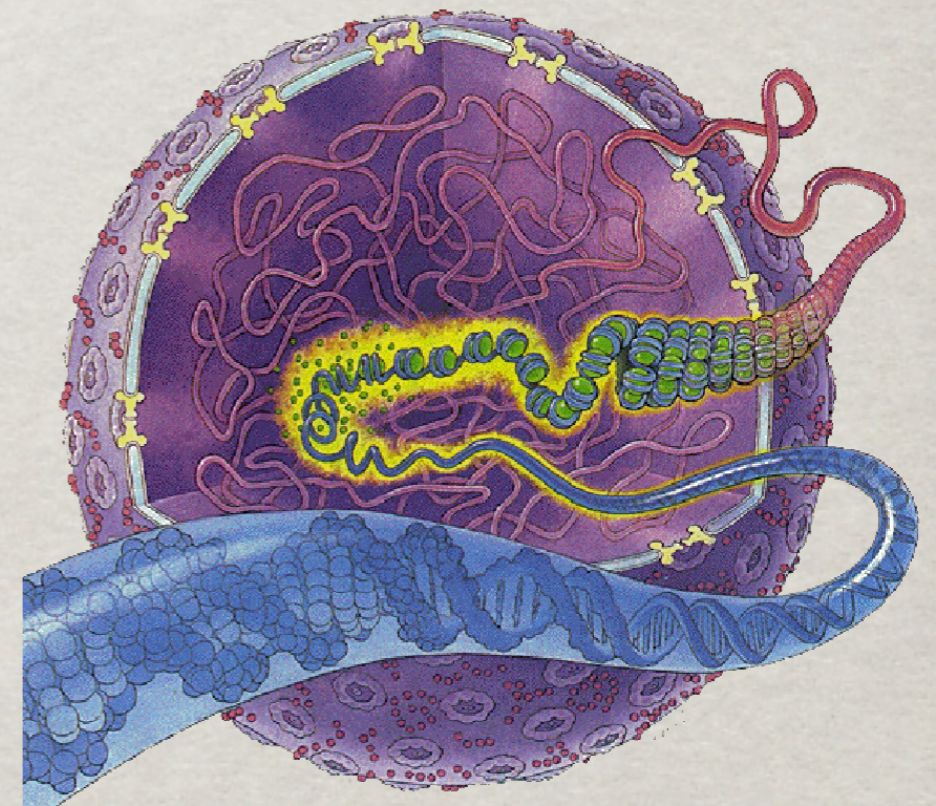
MICHAEL HAUSMANN

JÜRGEN HESSER

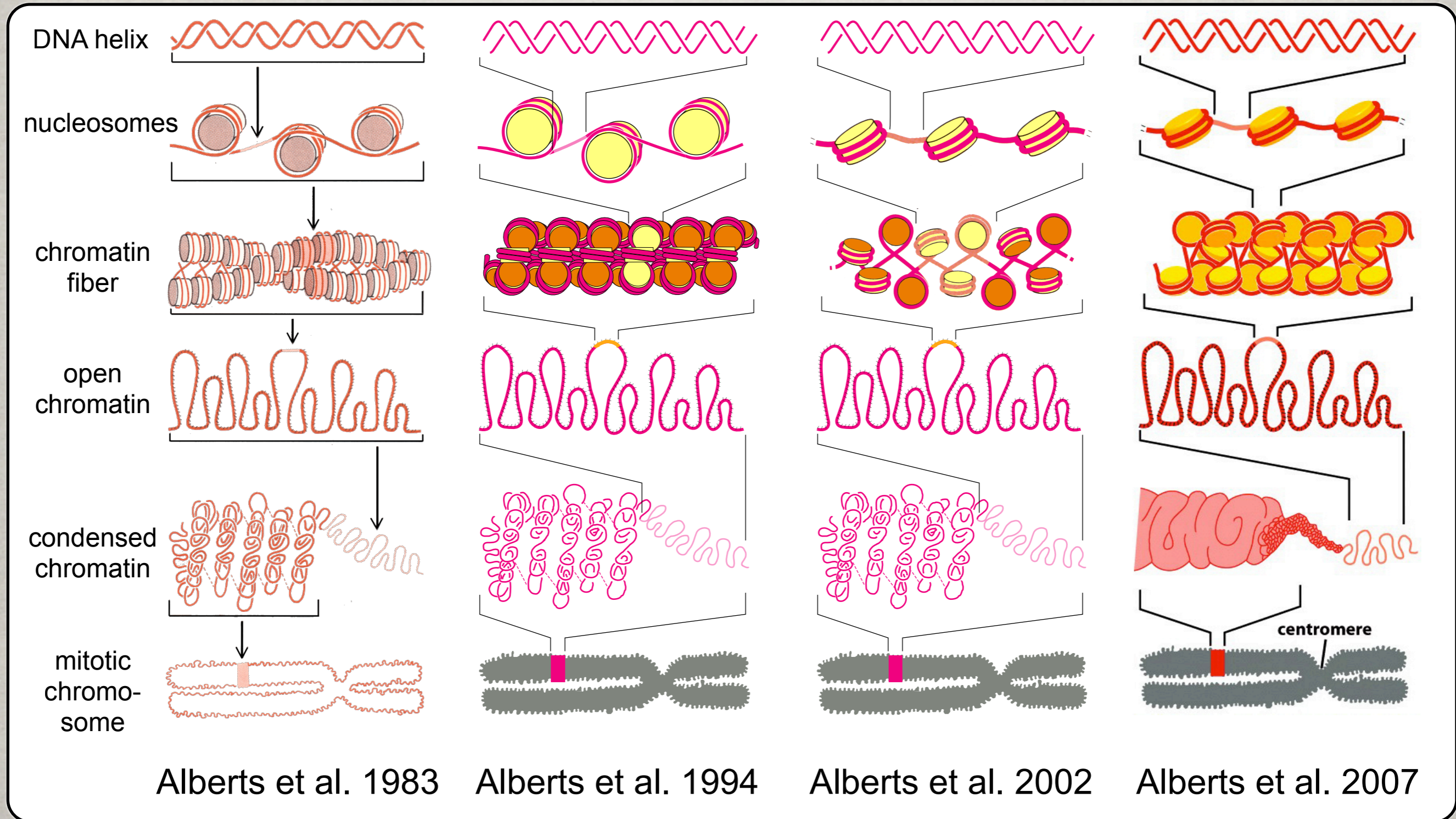
WALK THROUGH THE NANO-COSMOS OF THE CELL



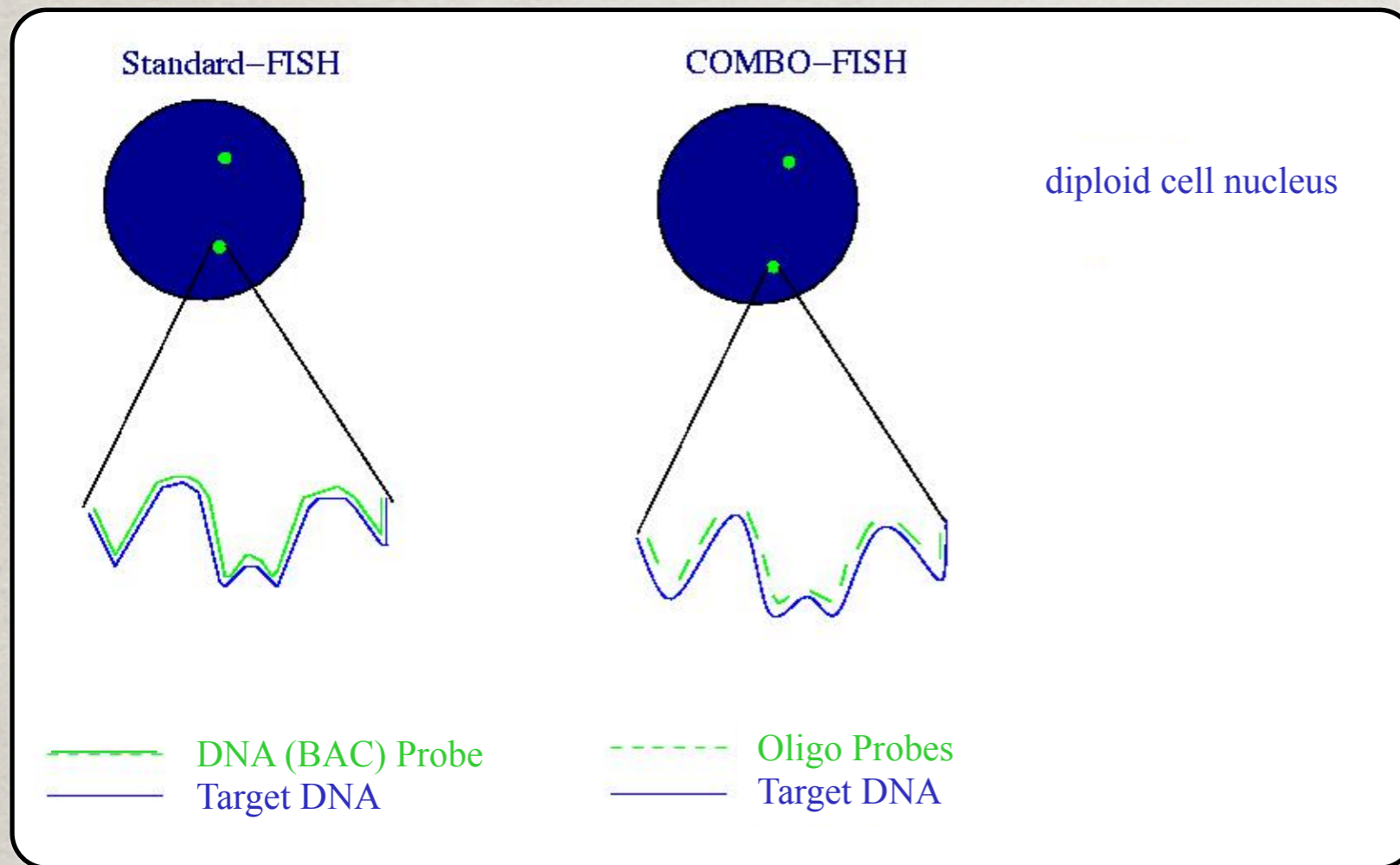
cell nucleus



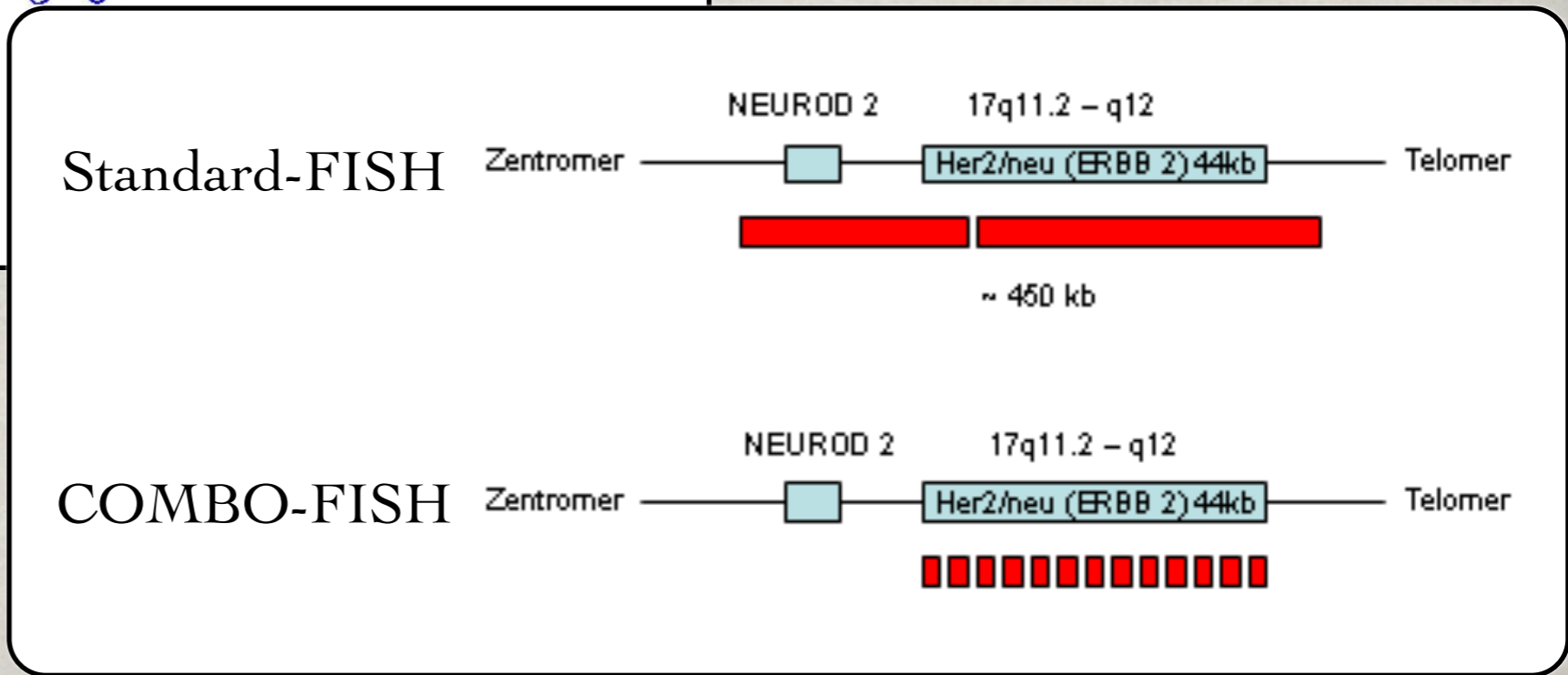
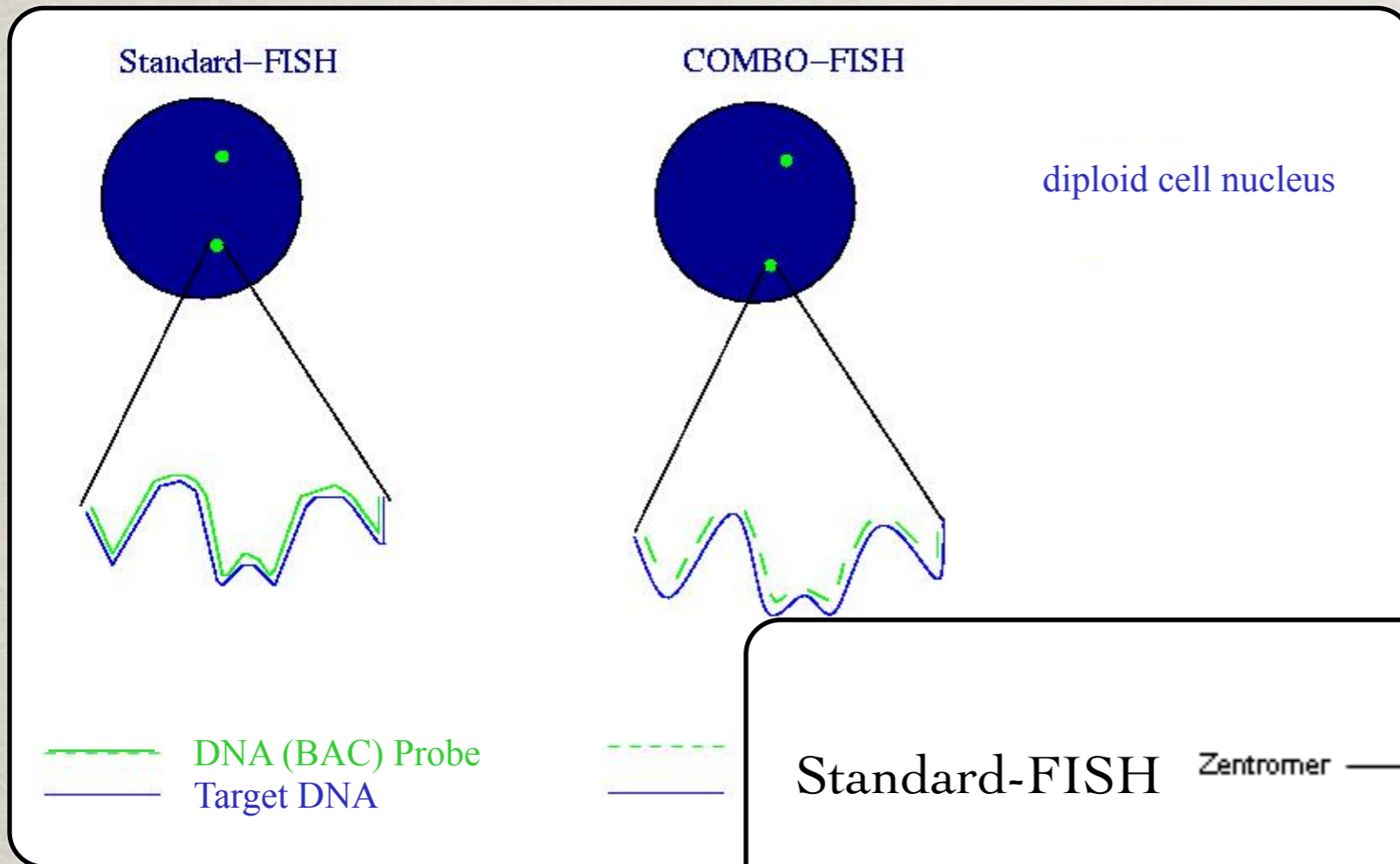
THE LAST 25 YEARS OF CHROMATIN STRUCTURE RESEARCH



STANDARD FISH VS. COMBO-FISH



STANDARD FISH VS. COMBO-FISH



FROM COMBO TO FISH

5'-ccccctctcttttccc-3'

5'-tttctctctctctt-3'

5'-tccttccctttccccc-3'

5'-ttctctctctcttcccc-3'

5'-cttctctccctcttcc-3'

5'-ccccctctctcccc-3'

5'-cttctctctctctctc-3'

5'-tcctctctctcttcc-3'

5'-ctctctctcttctcc-3'

5'-tctccccctcccttct-3'

5'-cttccctcccttcccc-3'

5'-ccttctcttctctttctct-3'

5'-ccctccctctctctttctc-3'

5'-cctctcttccctcttcc-3'

5'-tcctctctcttccccc-3'

5'-tctccccctctctctcc-3'

5'-tcttcccttccctctcc-3'

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5'-ccccctcttctctctc-3'

5'-tcttccctctcttctccc-3'

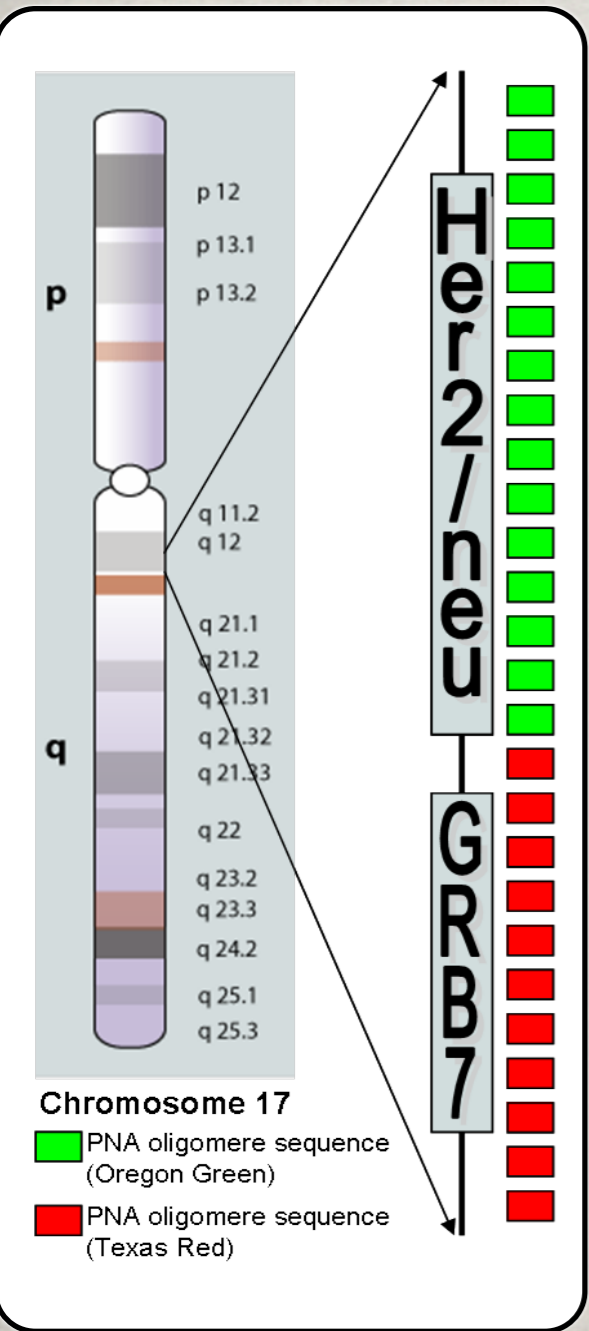
5'-tctcttctcttcccc-3'

5'-ccctctcttcttccctcc-3'

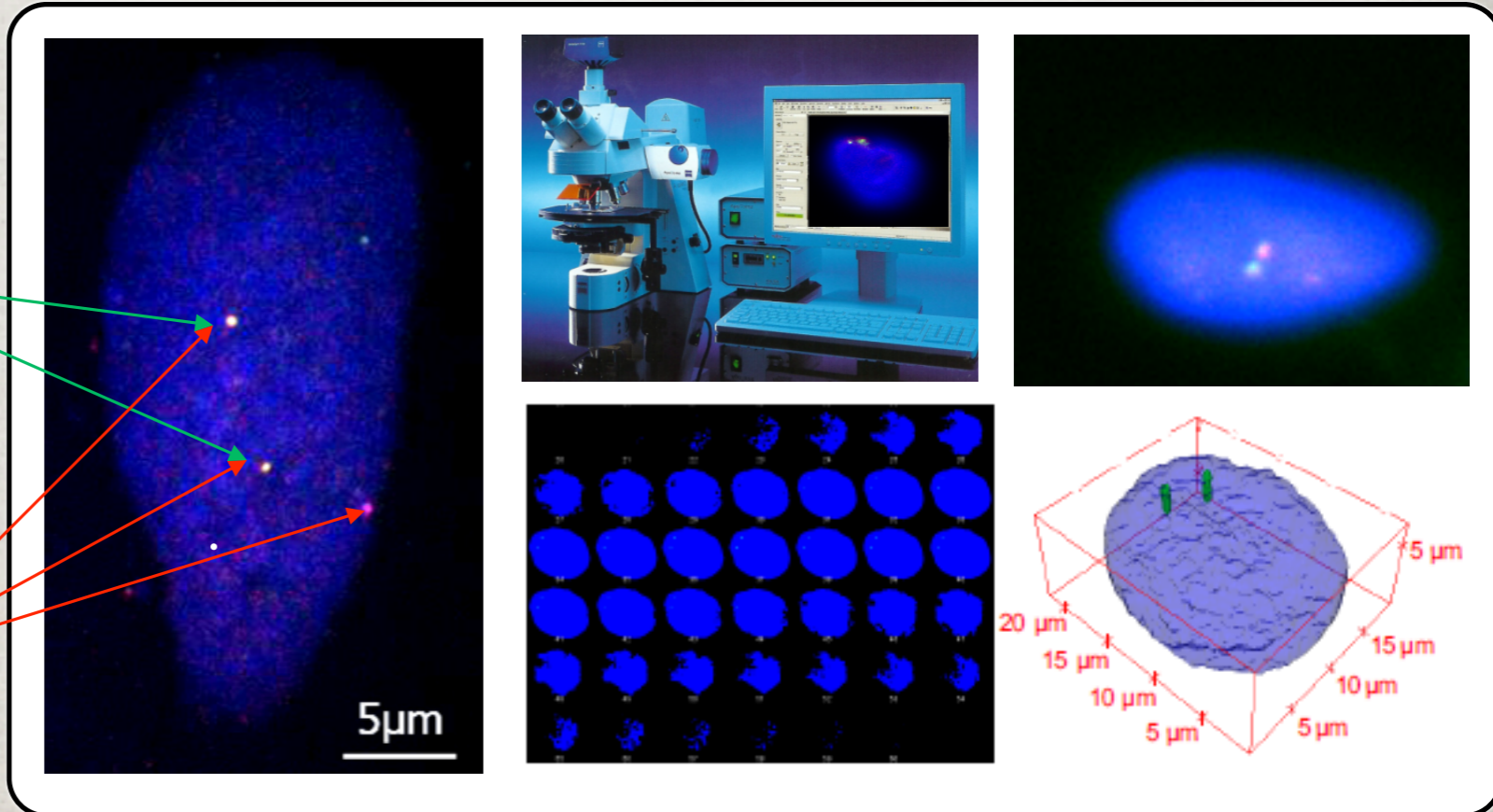
5'-ctctctctcttctctt-3'

5'-ttcttctcttcttccctc-3'

5'-tttttctctcccttcttcc-3'



Probe selection

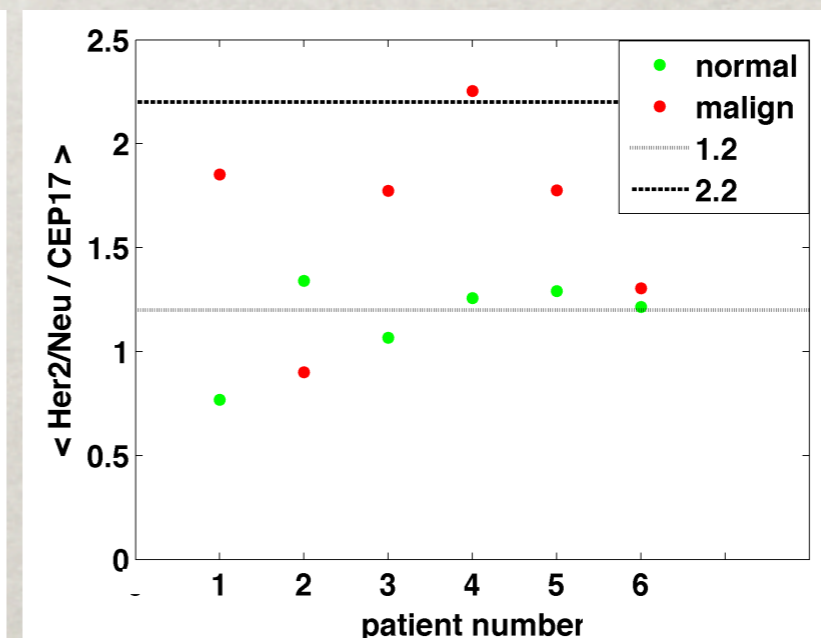
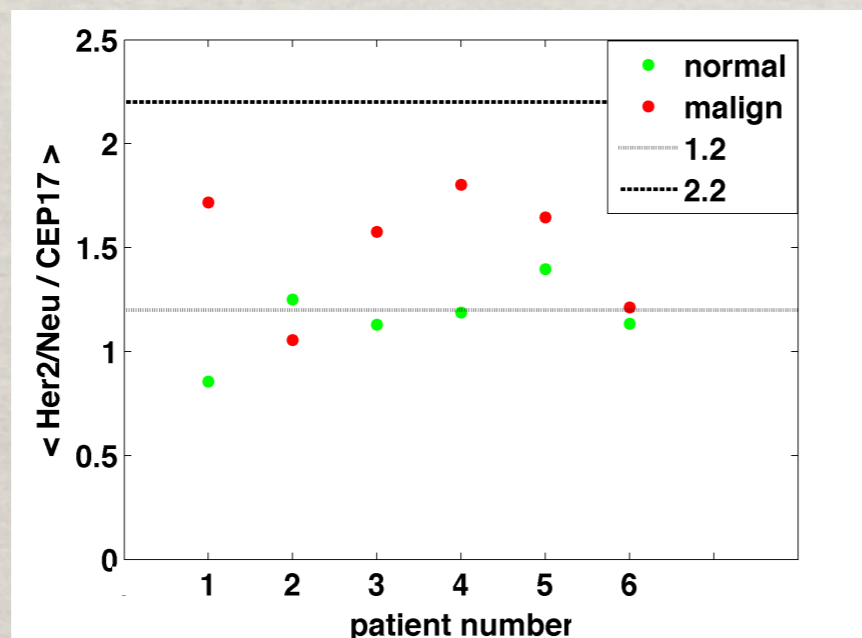
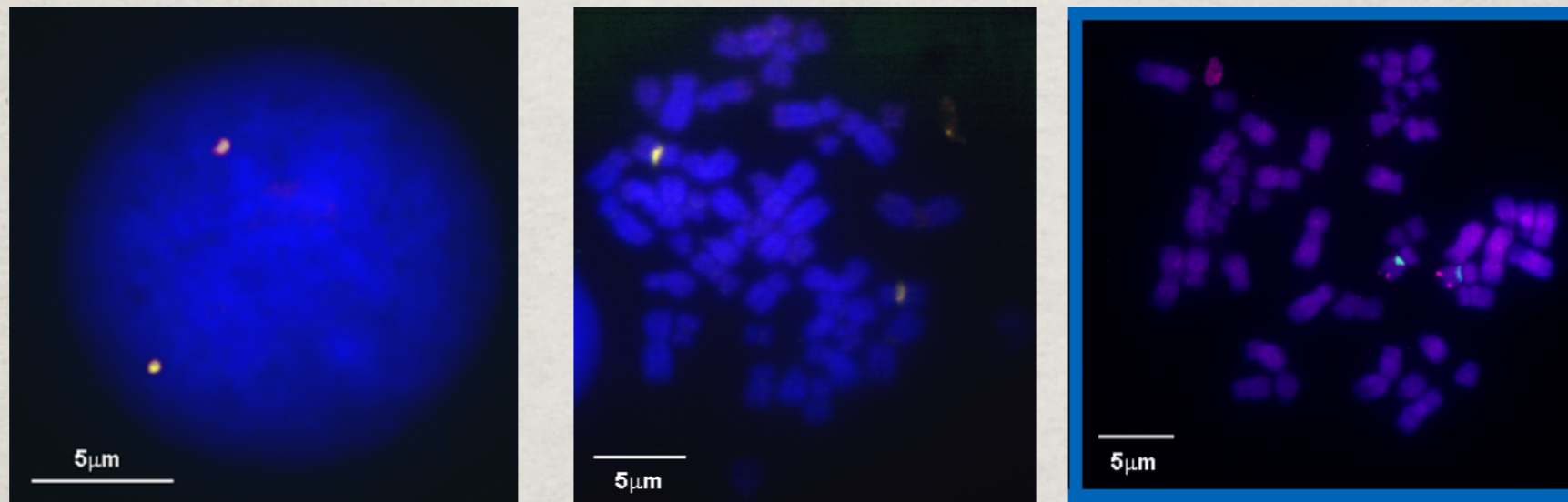


Imaging

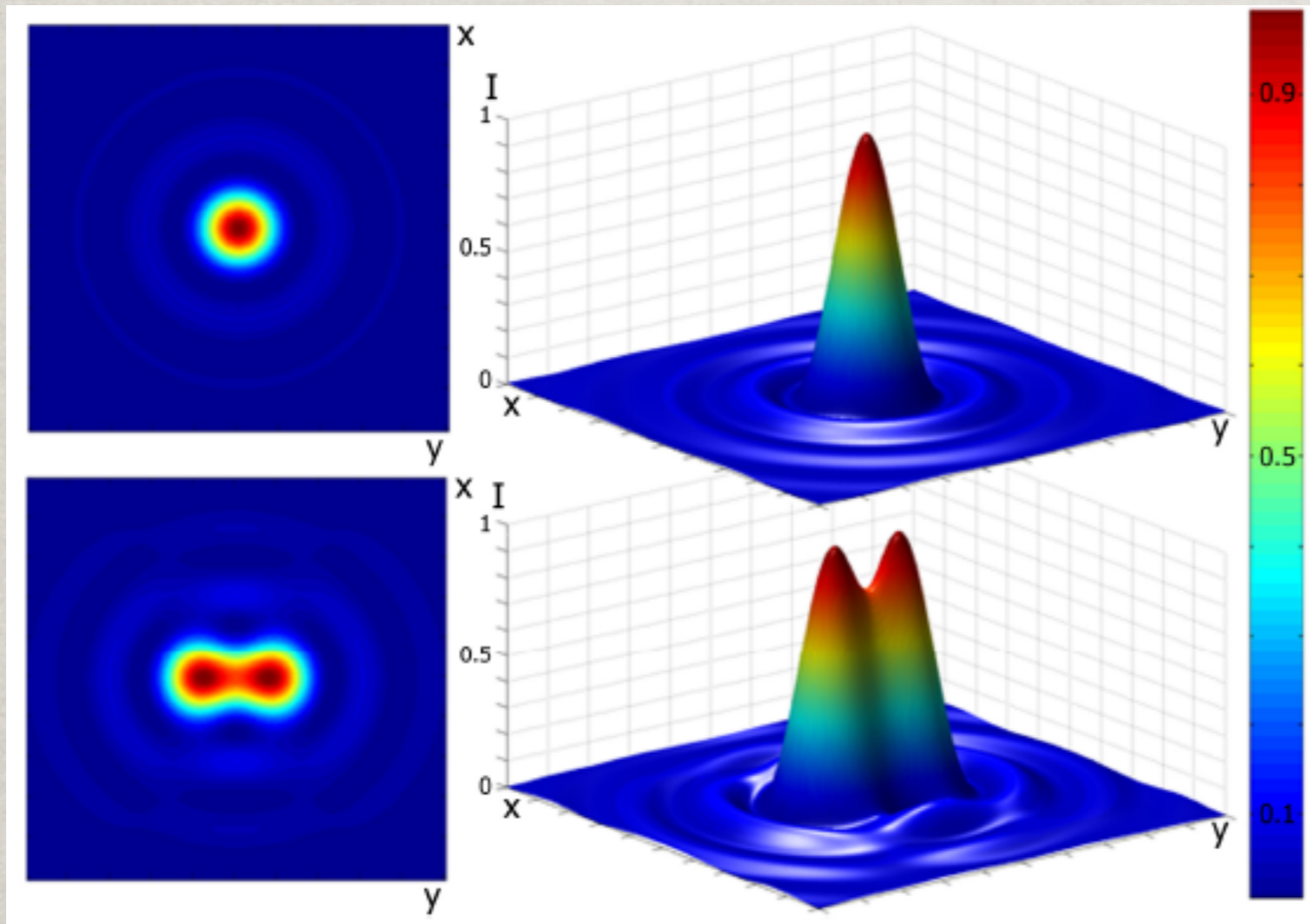
Diagnosis

From laboratory to clinical application

RELATION HER2/NEU TO CEP17 CAN BE CONNECTED TO BREAST CANCER



DIFFRACTION LIMIT IN CONVENTIONAL LIGHT MICROSCOPY

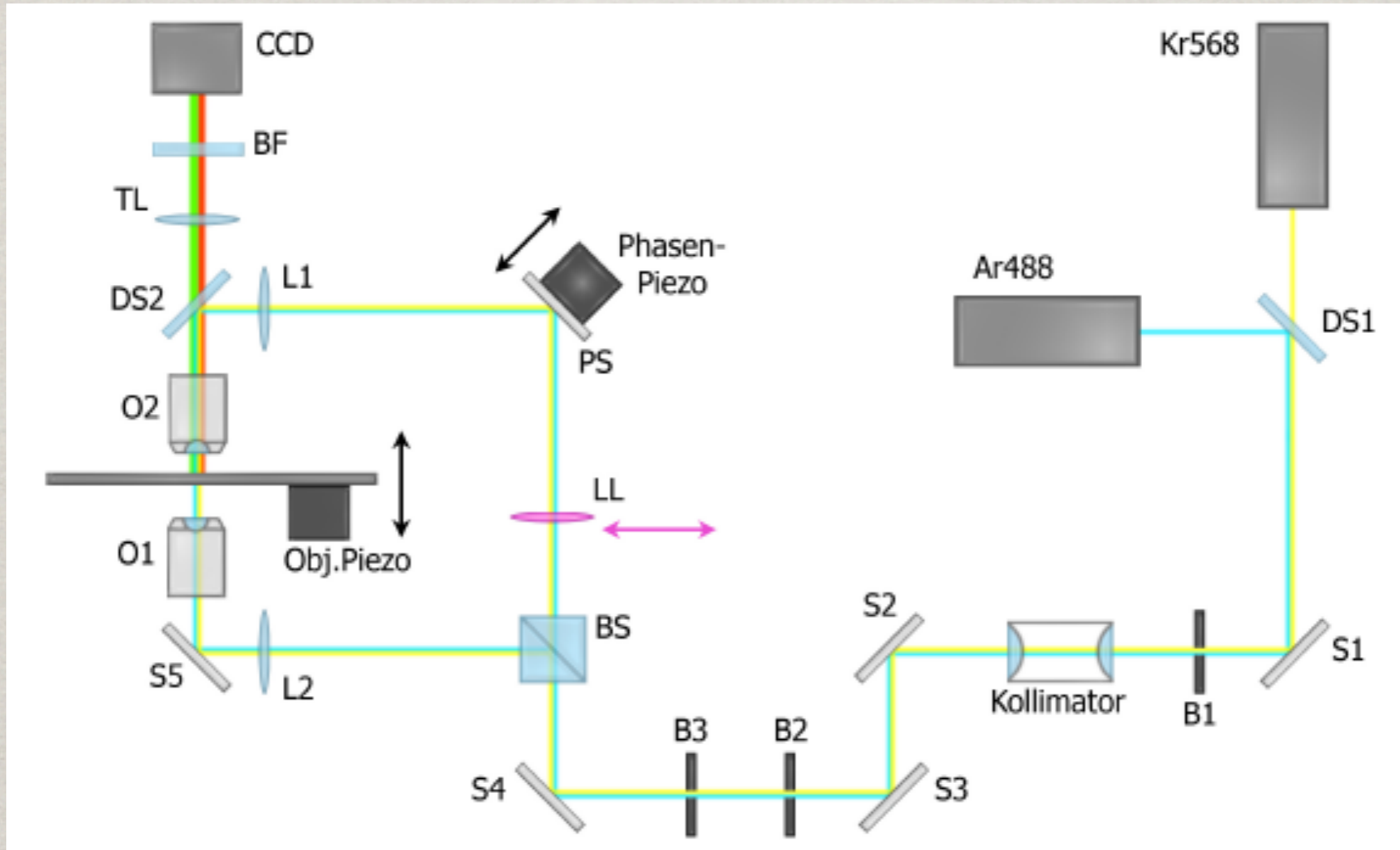


Resolution limit (diffraction limit):

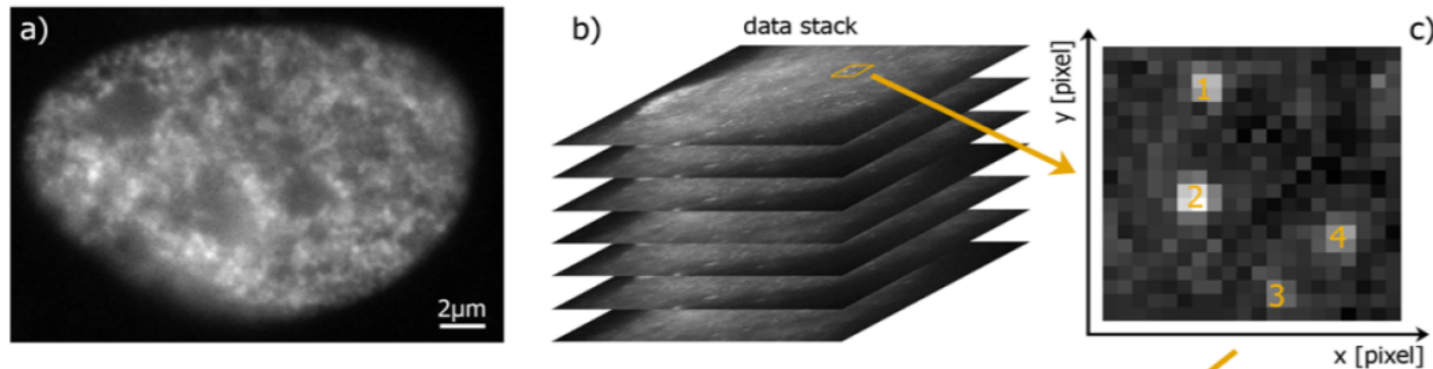
Full Width at Half Maximum of the Point Spread Function (FWHM of PSF)

not considering: optical separation by different colours or dye effects

SPECTRAL POSITION DETERMINATION MICROSCOPY



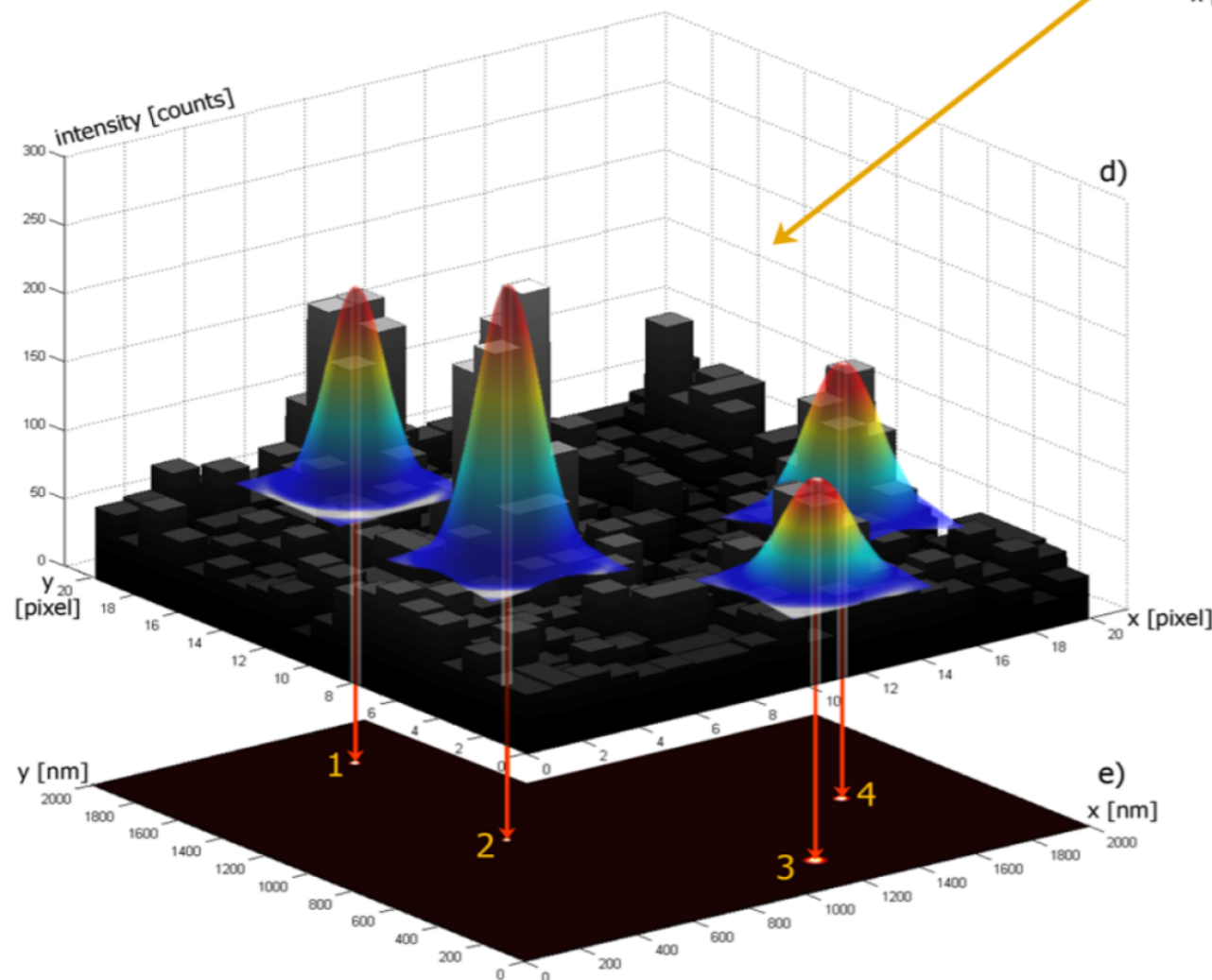
SPDM: LOCALIZATION NANOSCOPY USING DYES THAT UNDERGO REVERSIBLE PHOTOBLEACHING



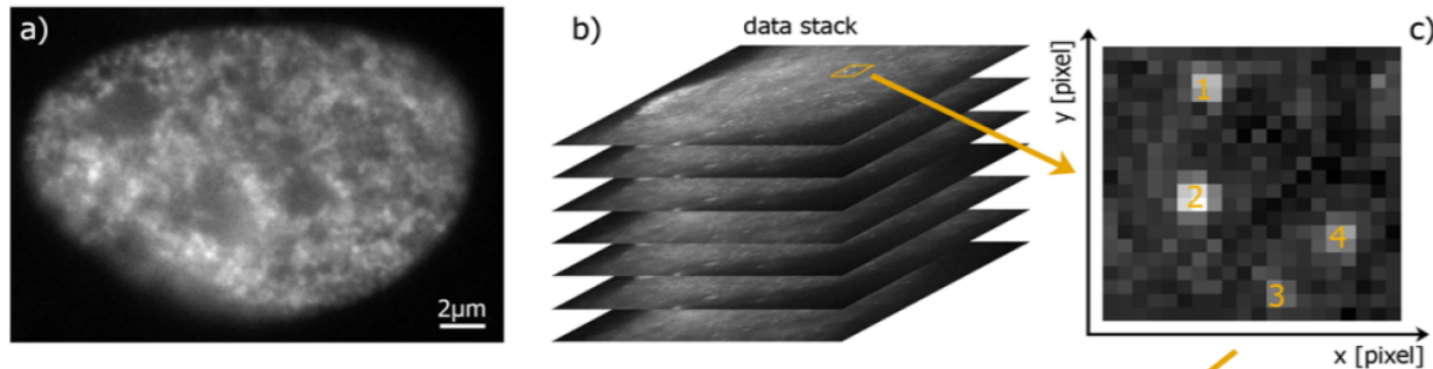
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

Resolution
Distance: ~ 10 nm



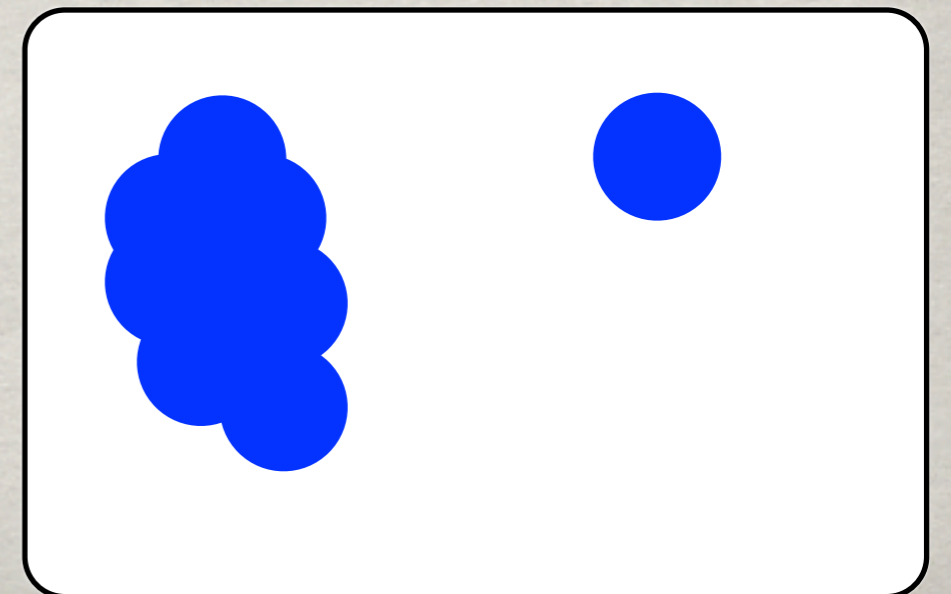
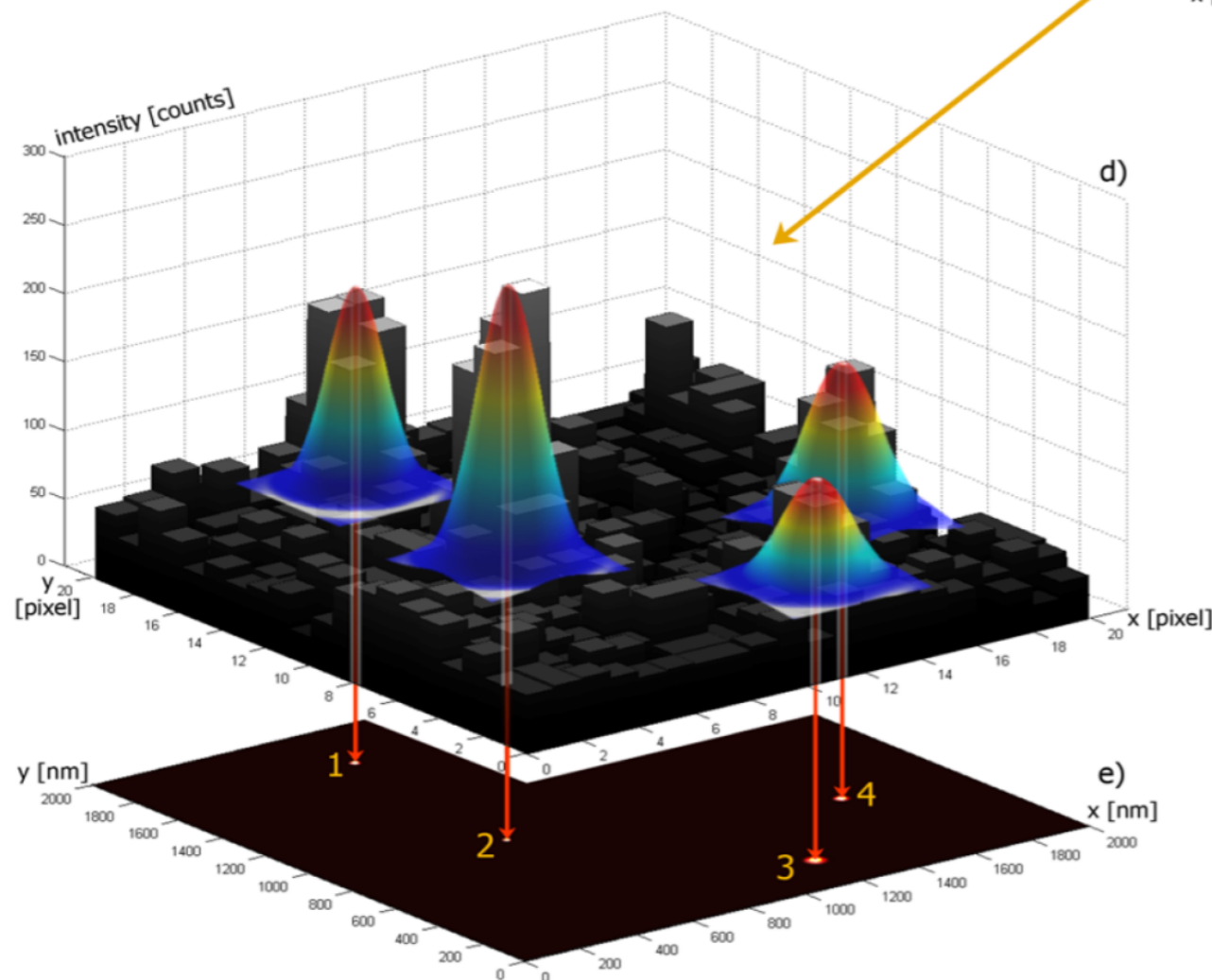
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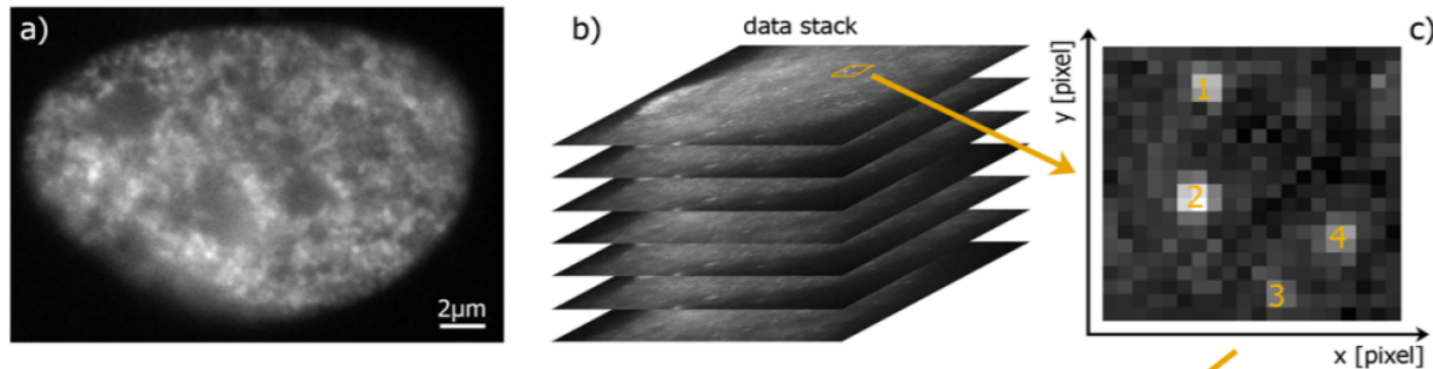
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

Resolution
Distance: ~ 10 nm



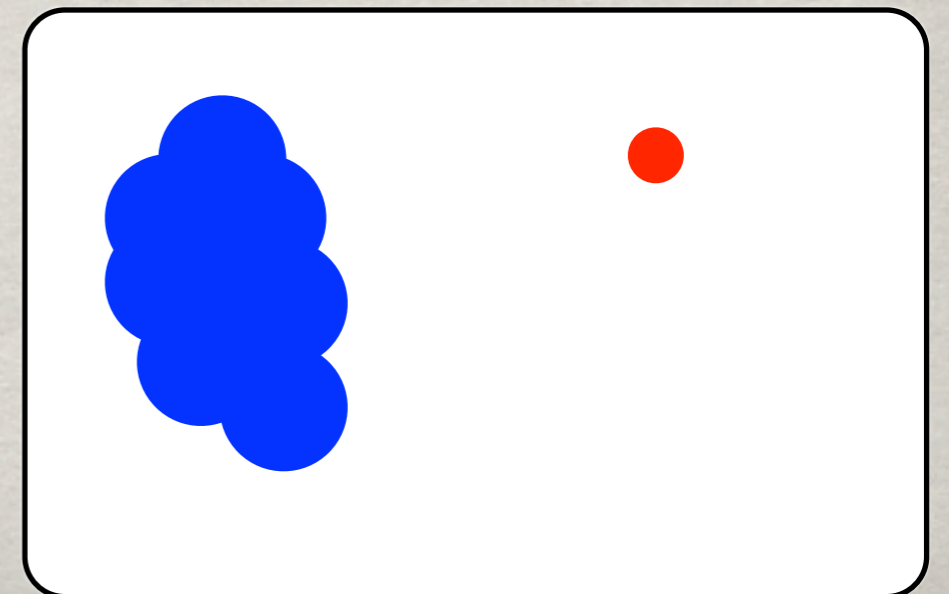
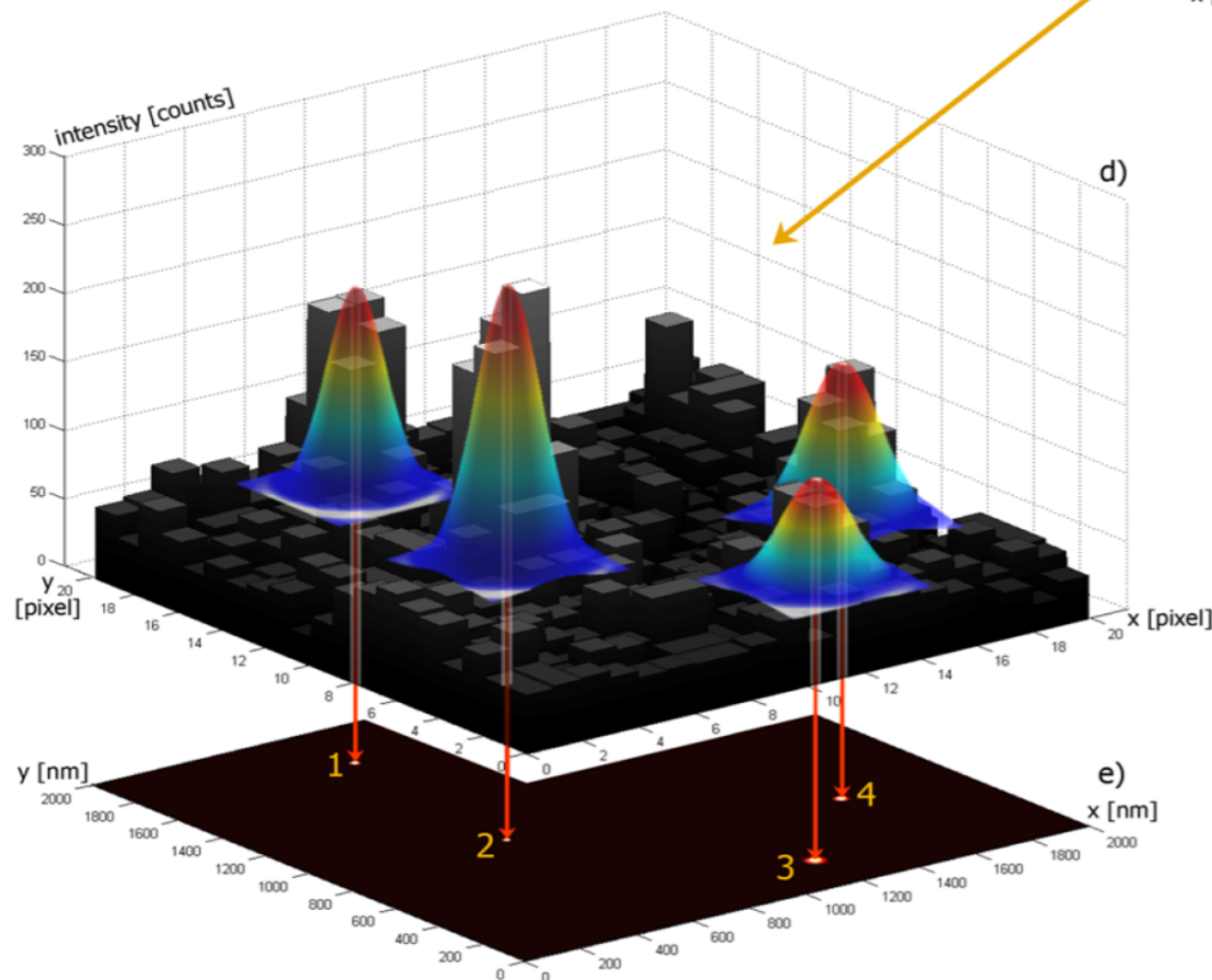
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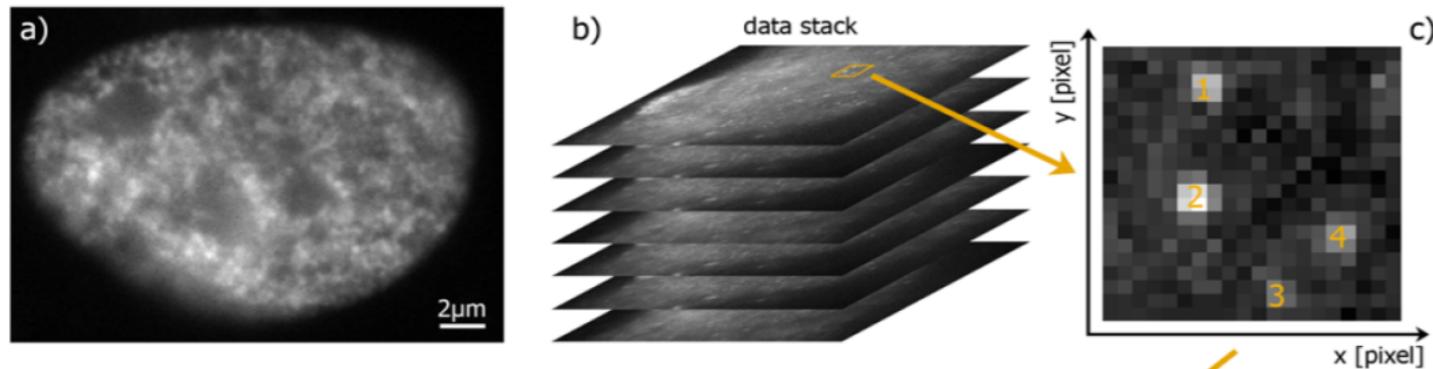
Time stack of images:
400 – 2000 frames

Precision
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Resolution
Distance: ~ 10 nm



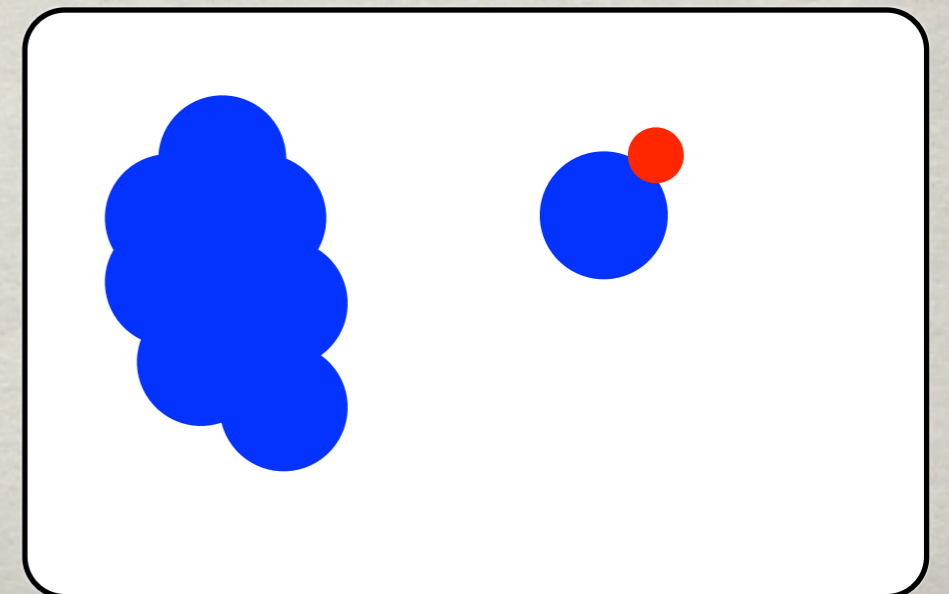
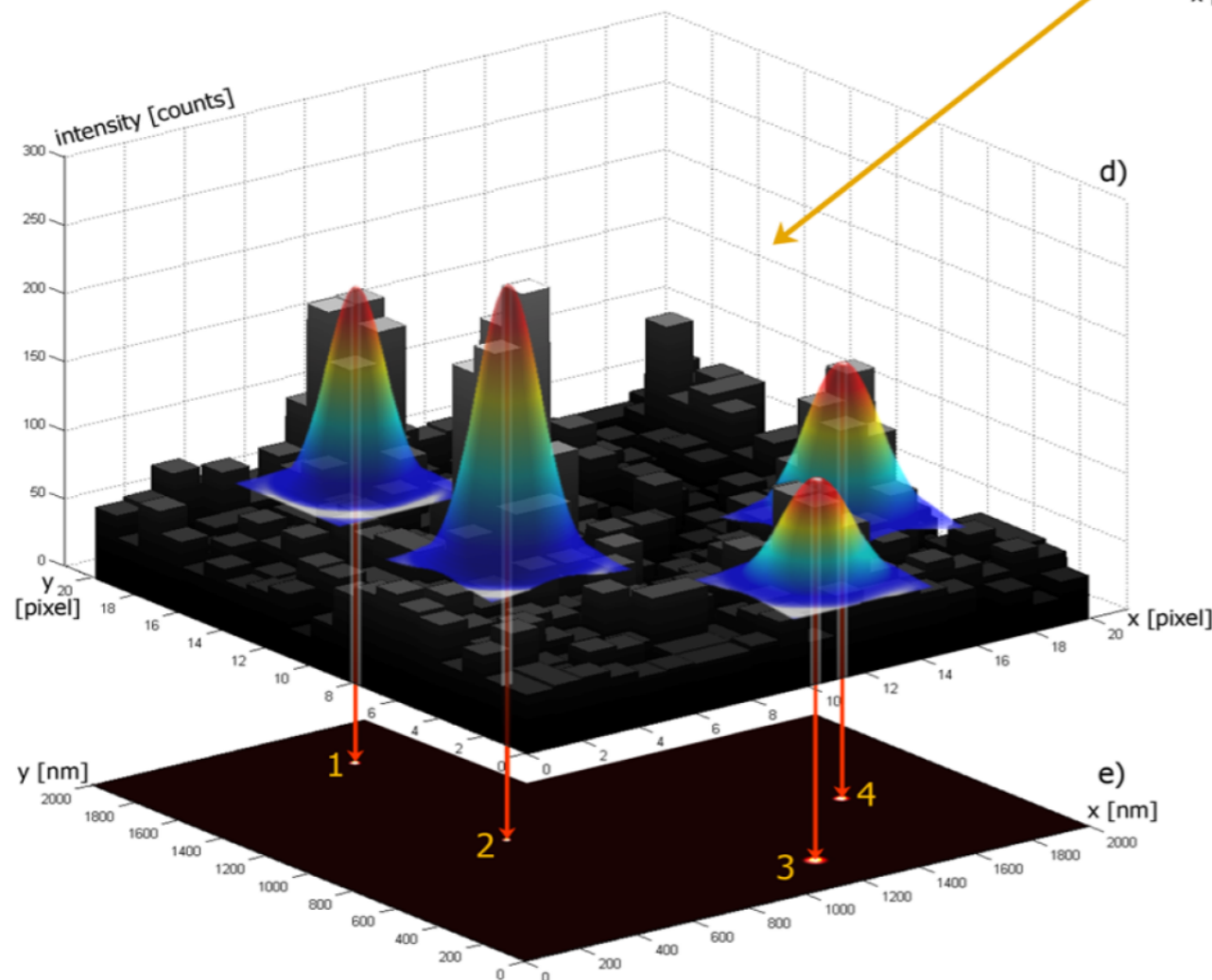
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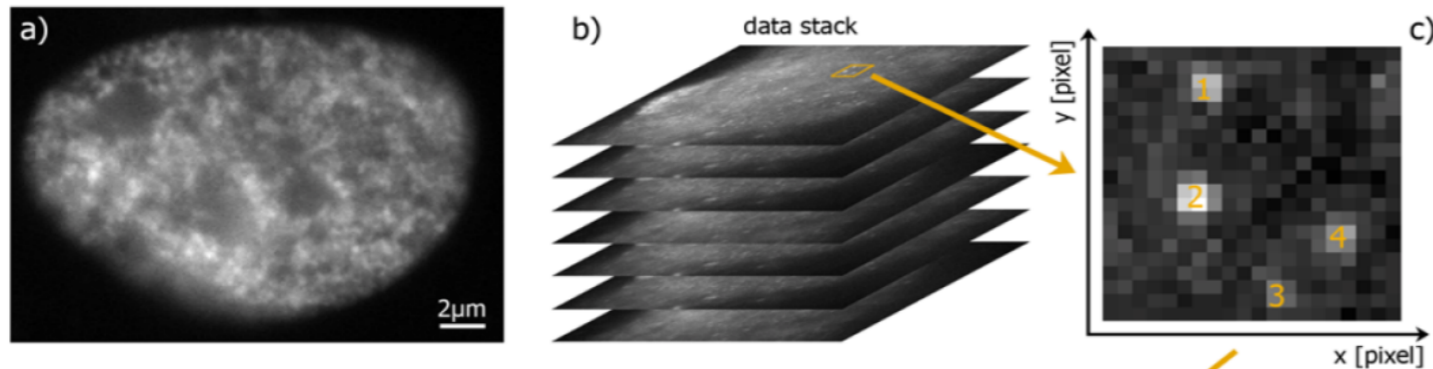
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Precision
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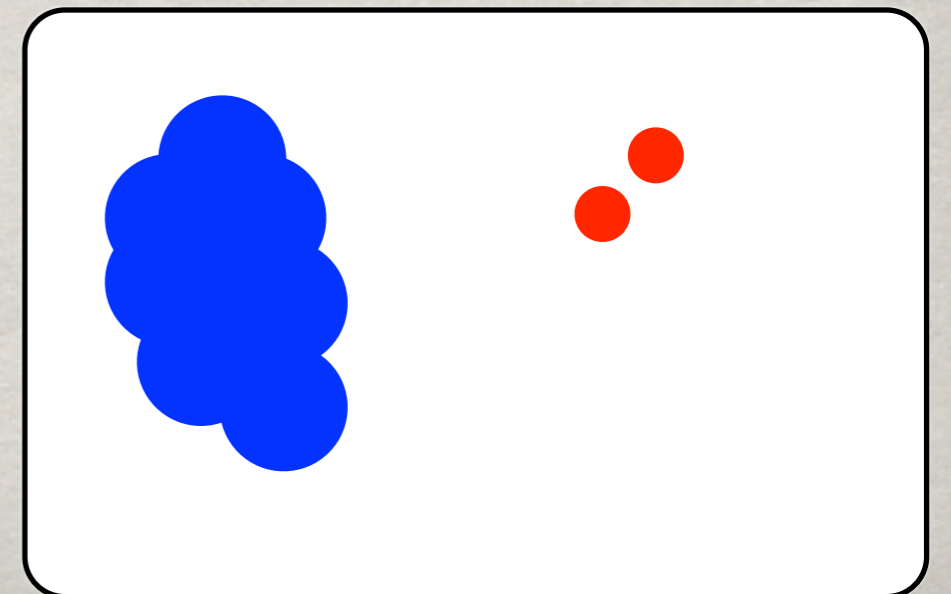
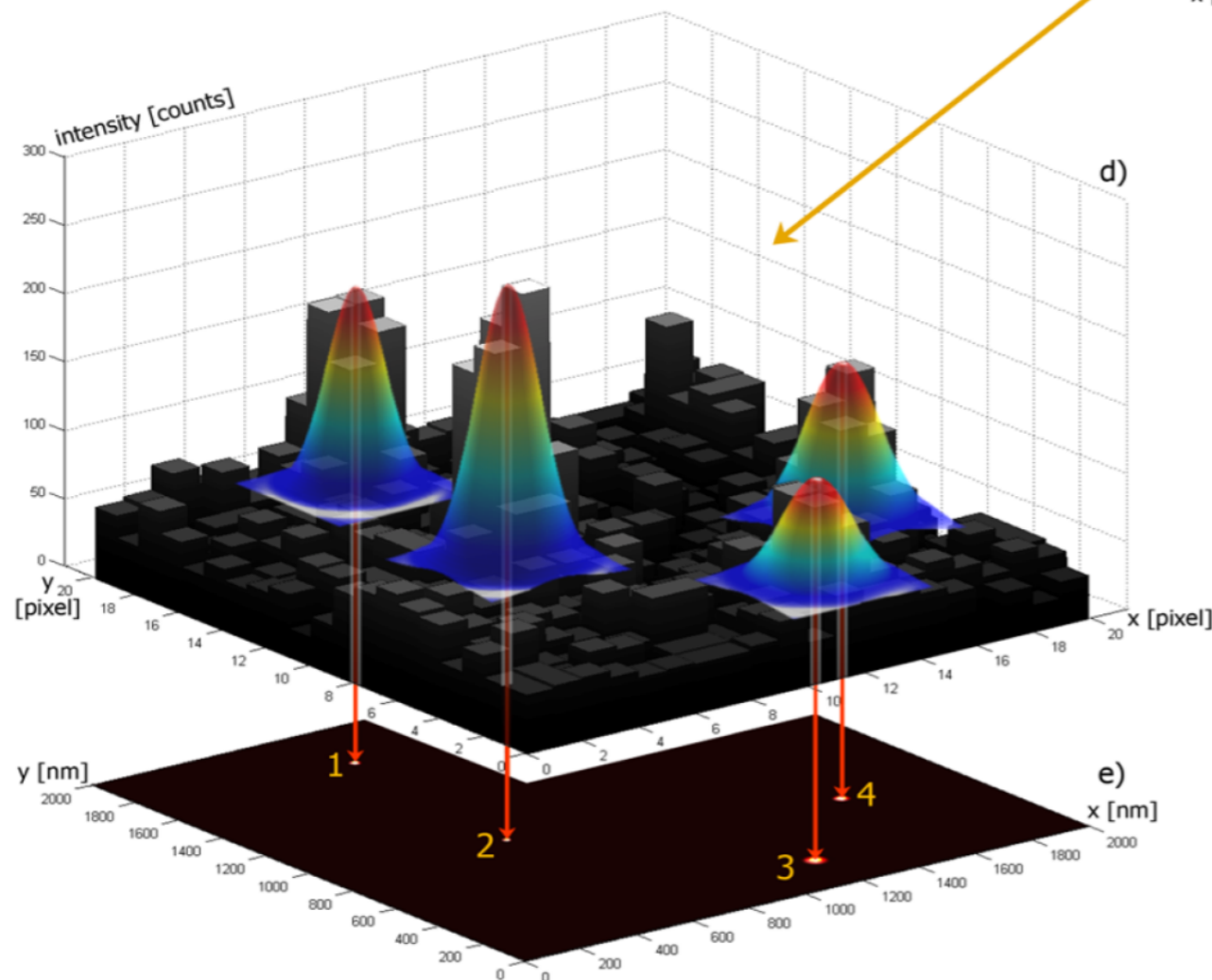
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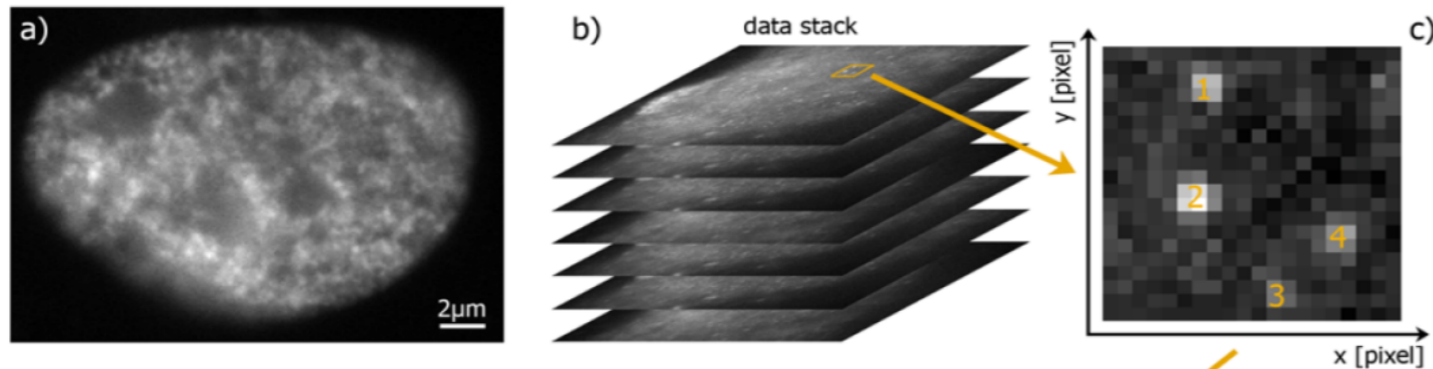
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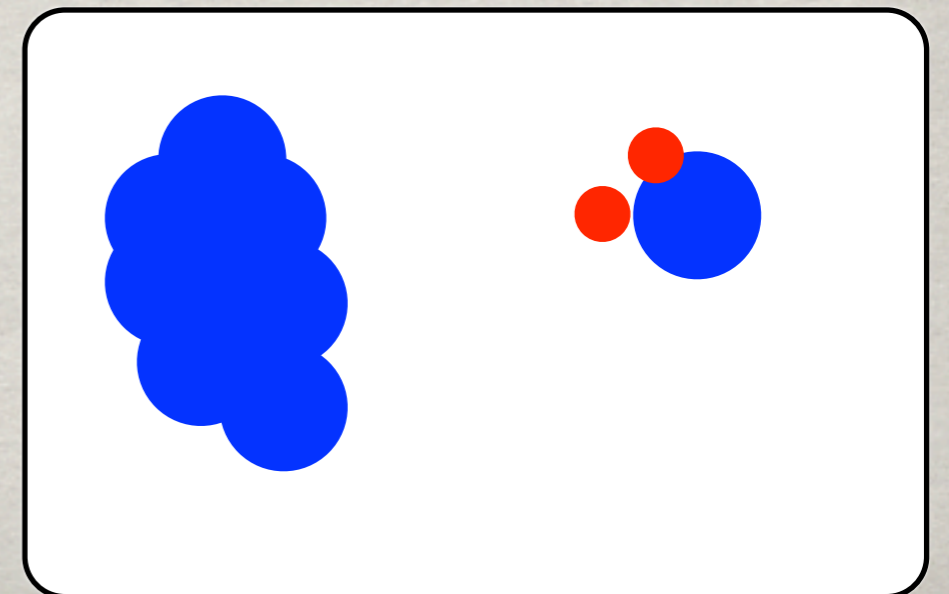
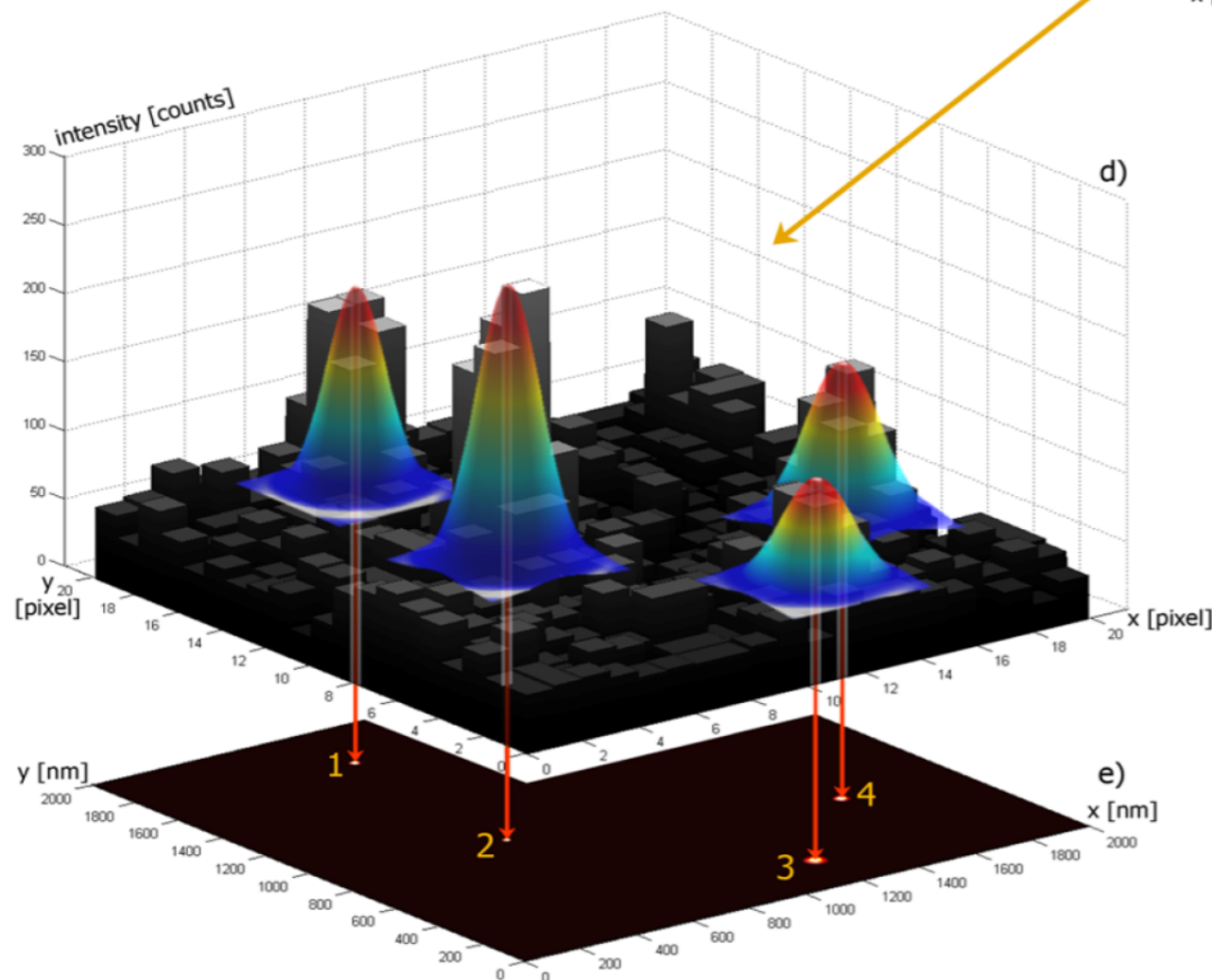
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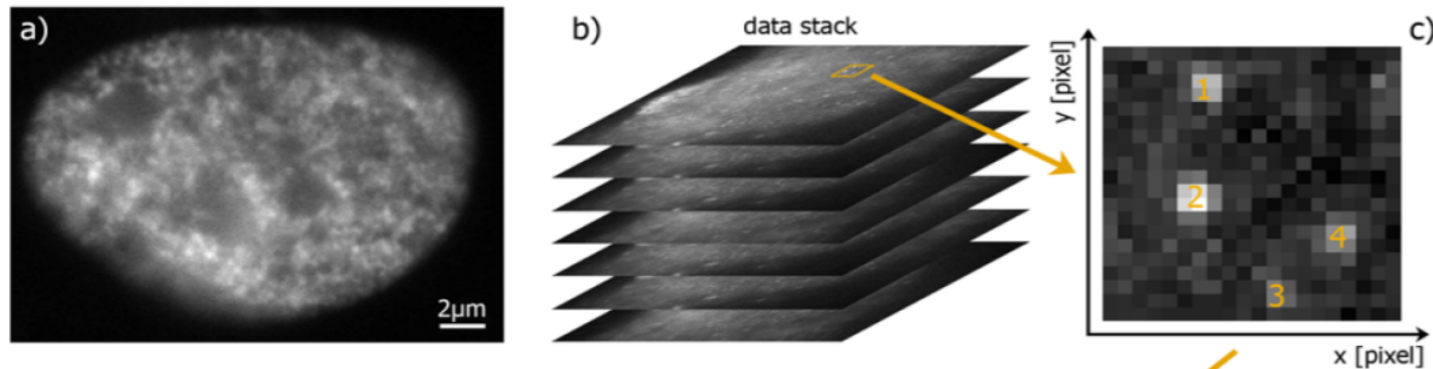
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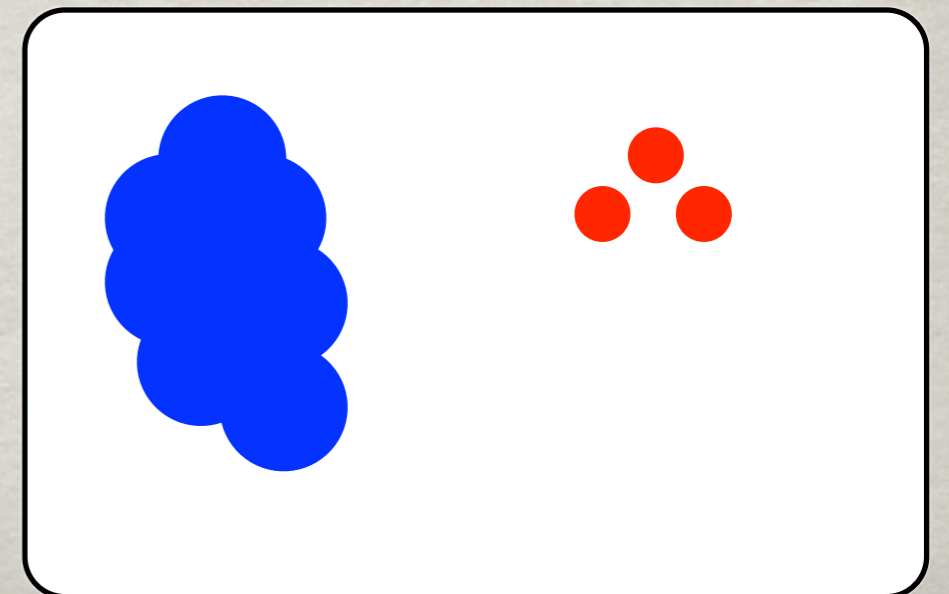
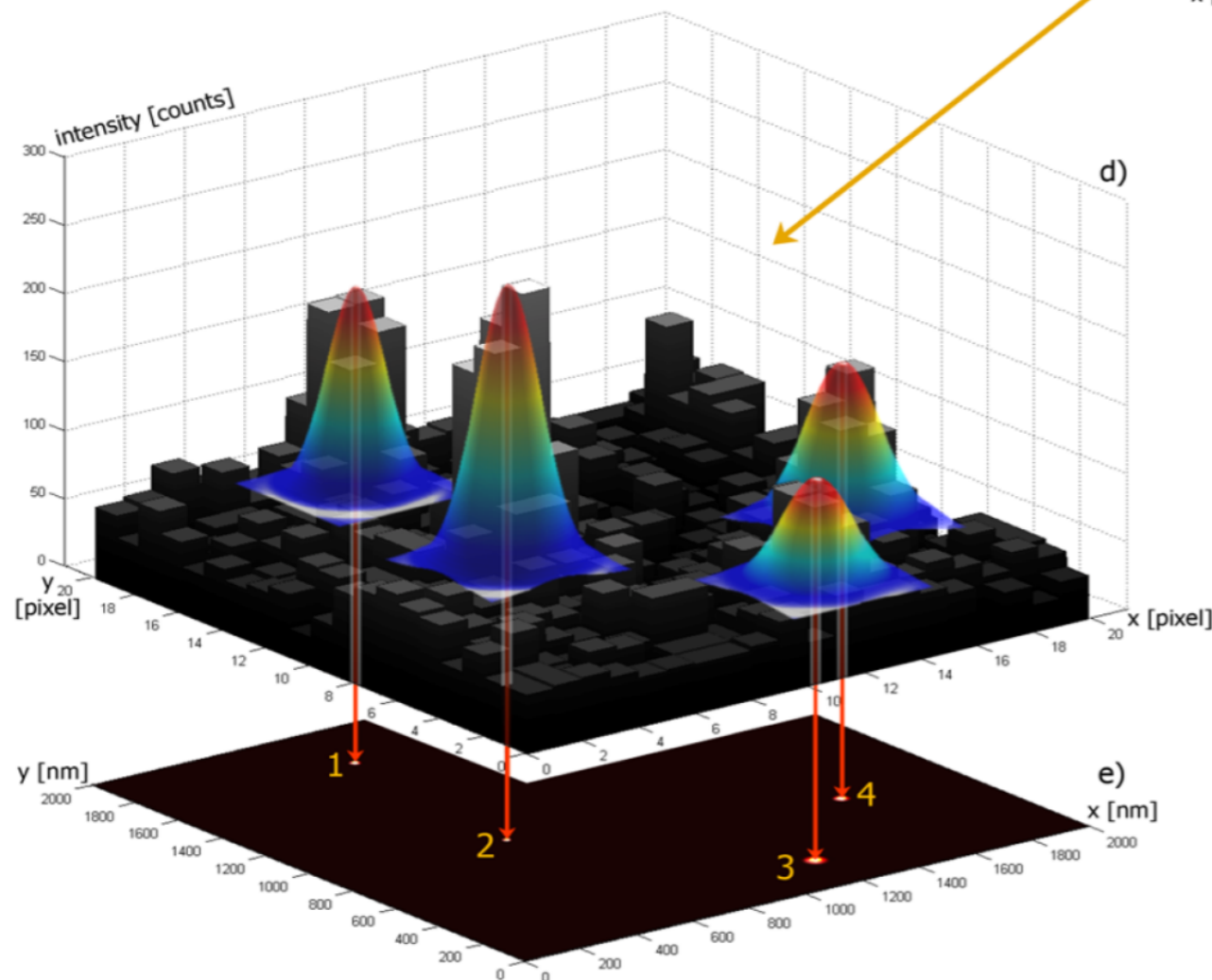
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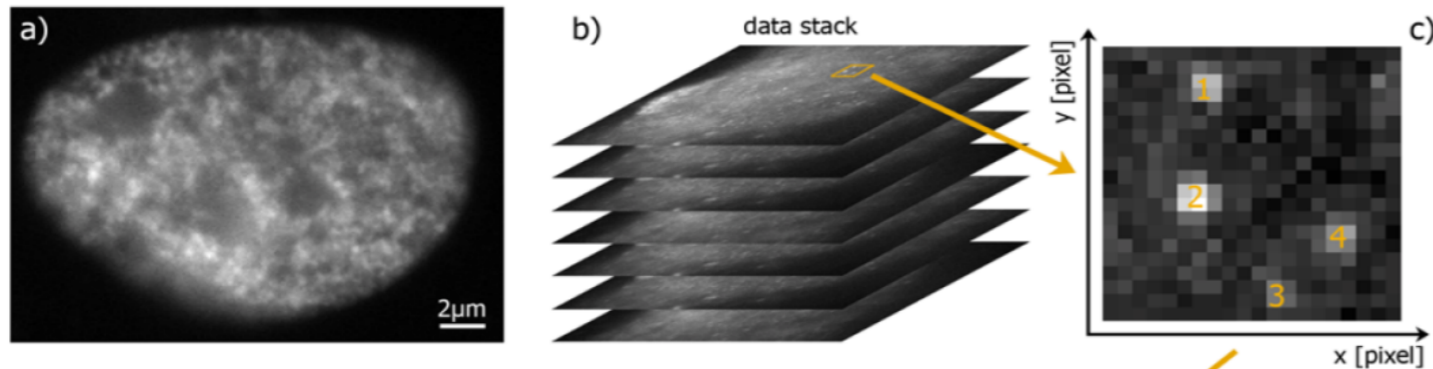
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Precision
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Resolution
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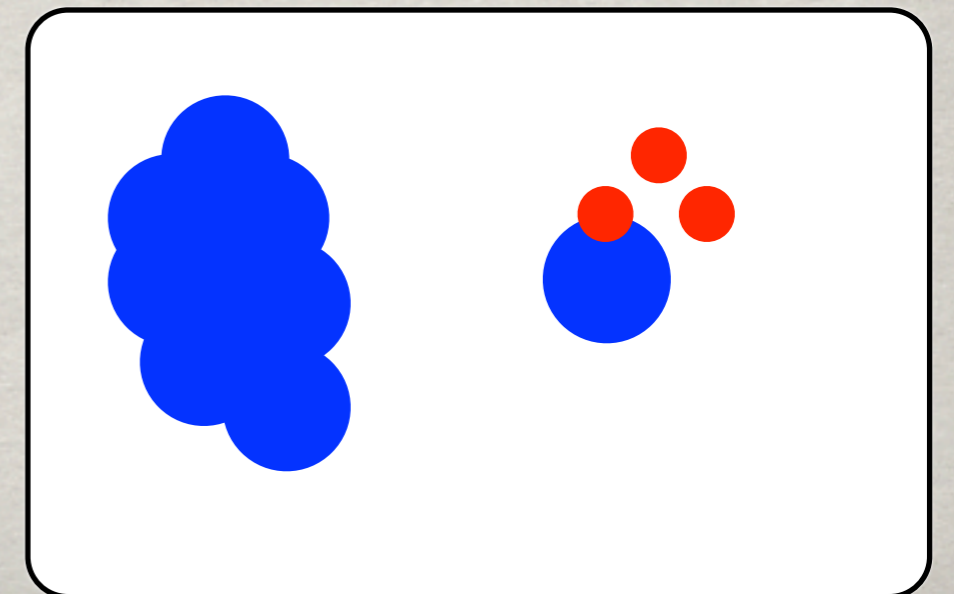
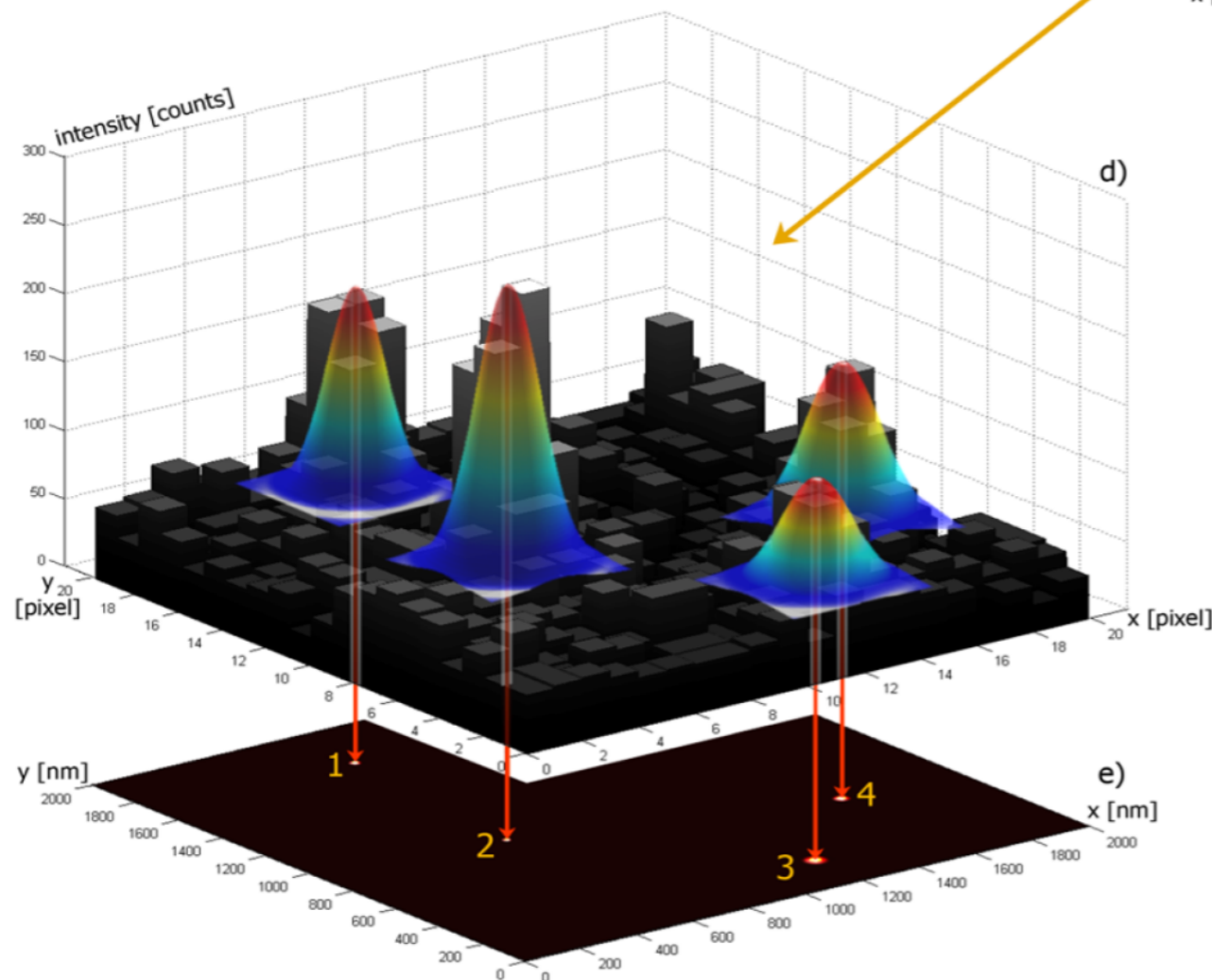
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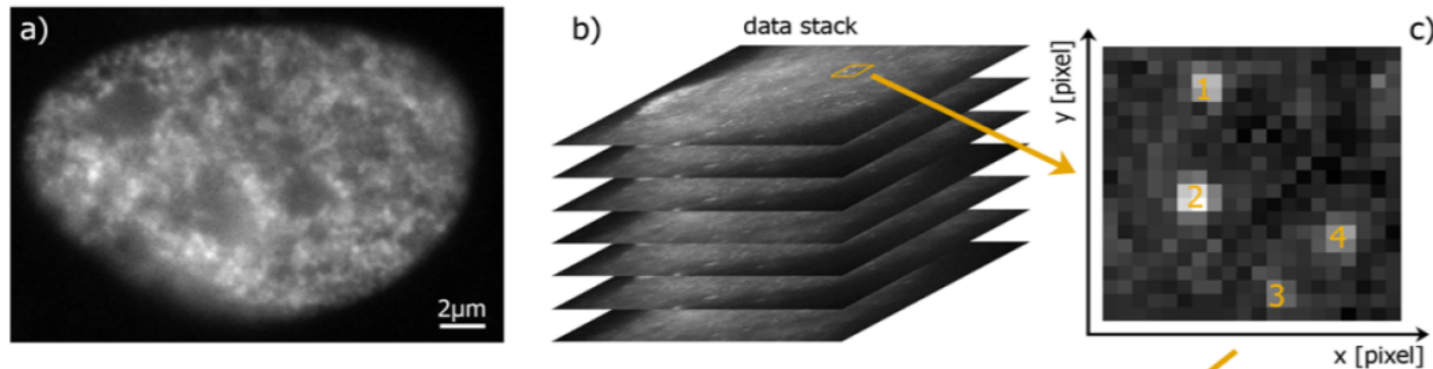
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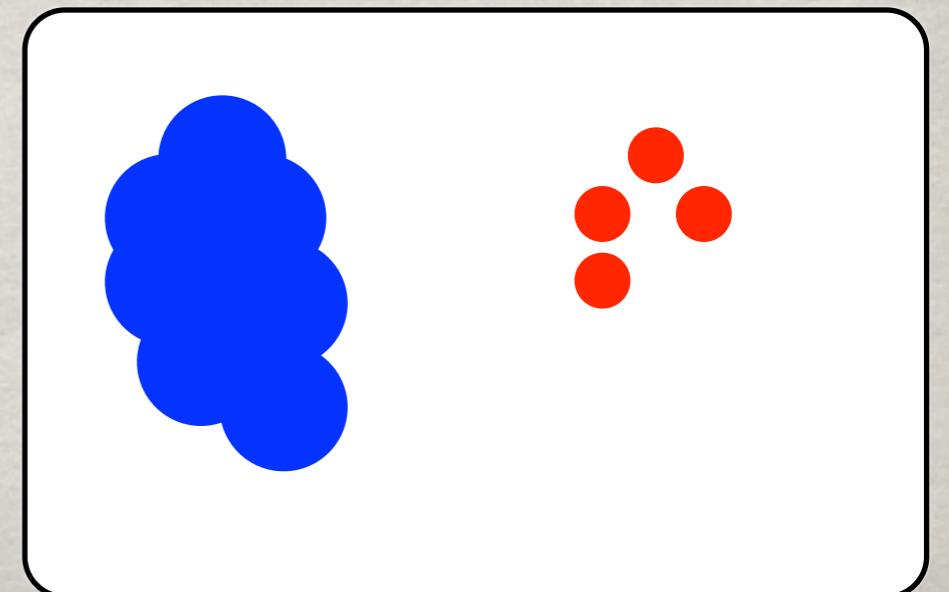
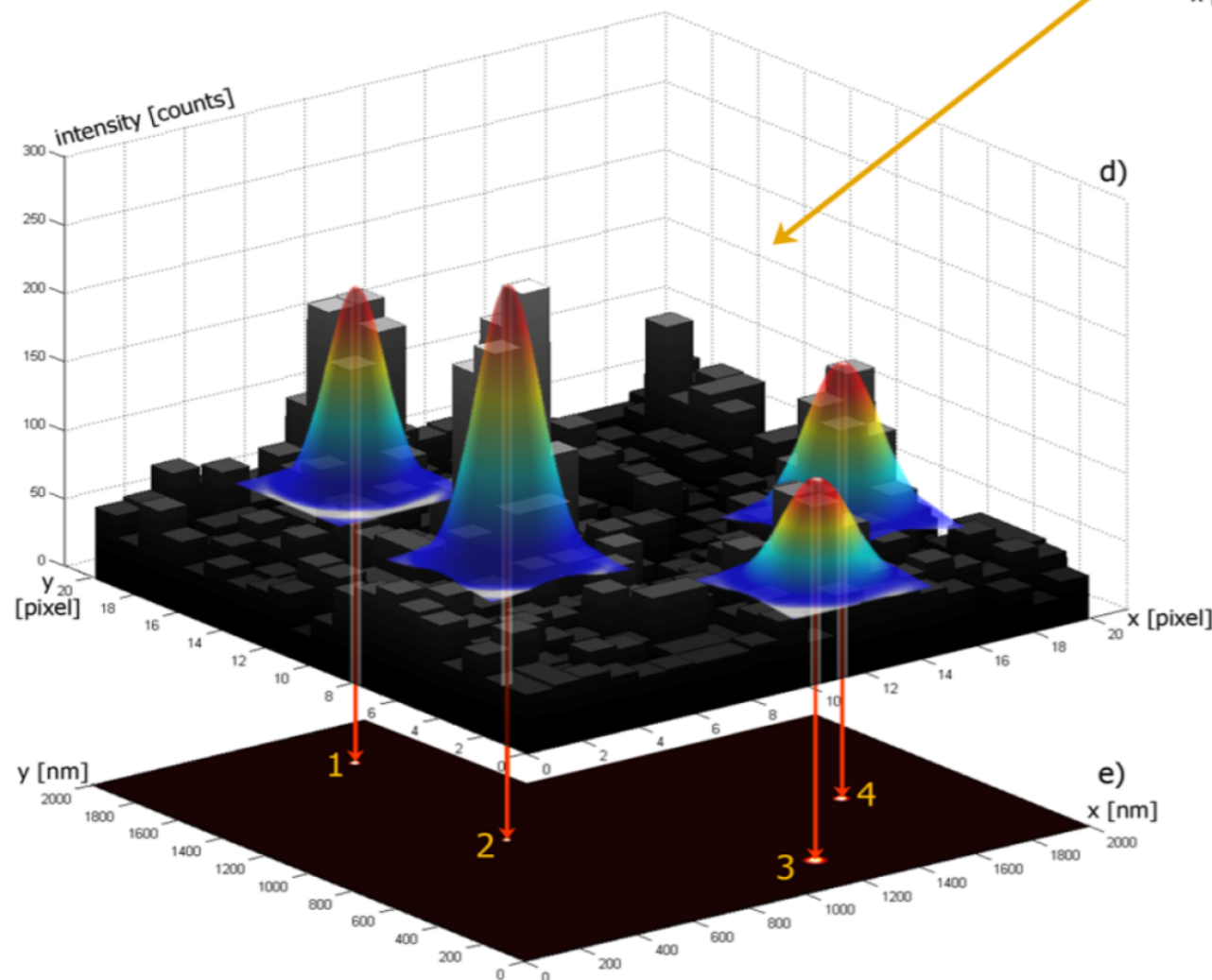
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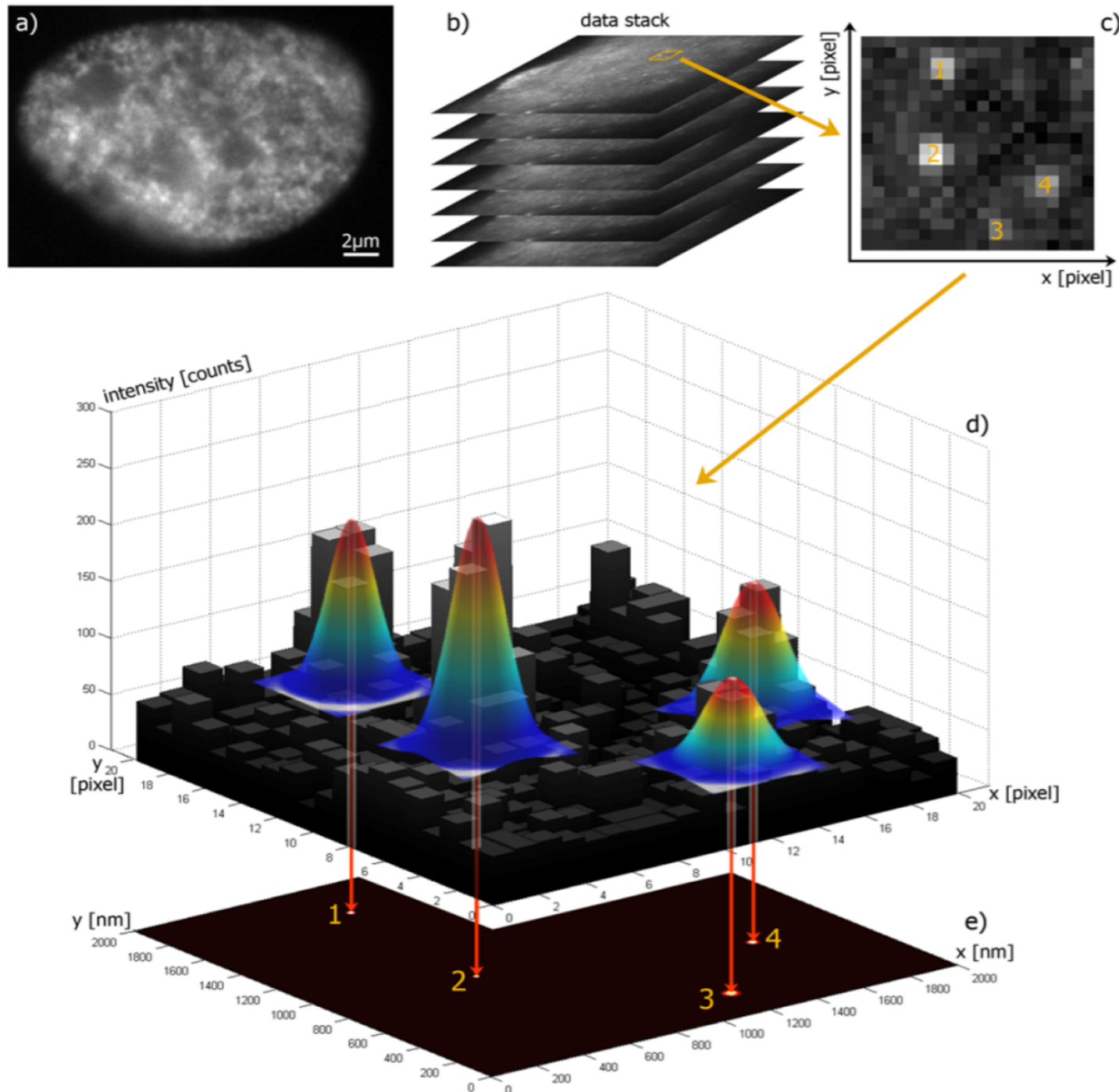
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

Resolution
Distance: ~ 10 nm



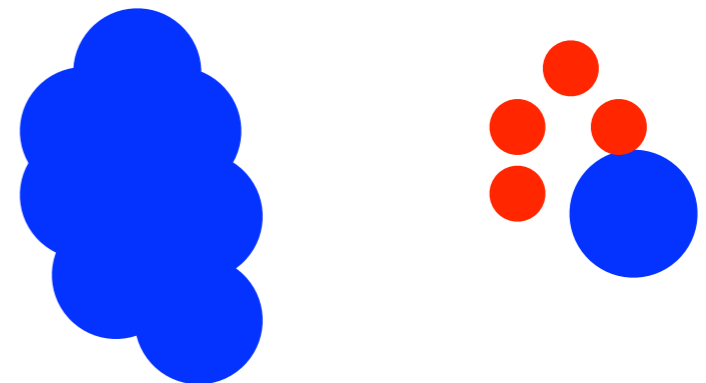
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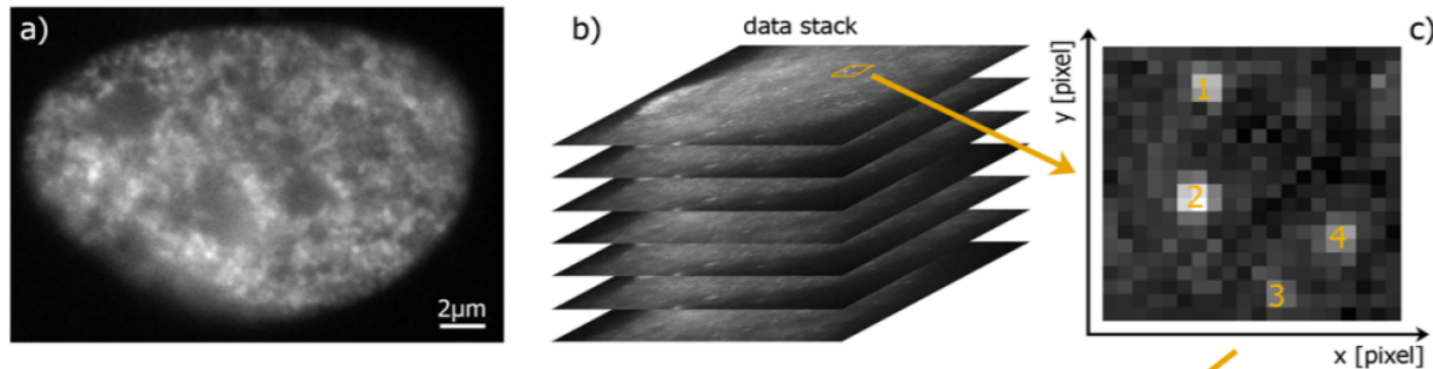
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5 \text{ nm}$

Resolution
Distance: $\sim 10 \text{ nm}$



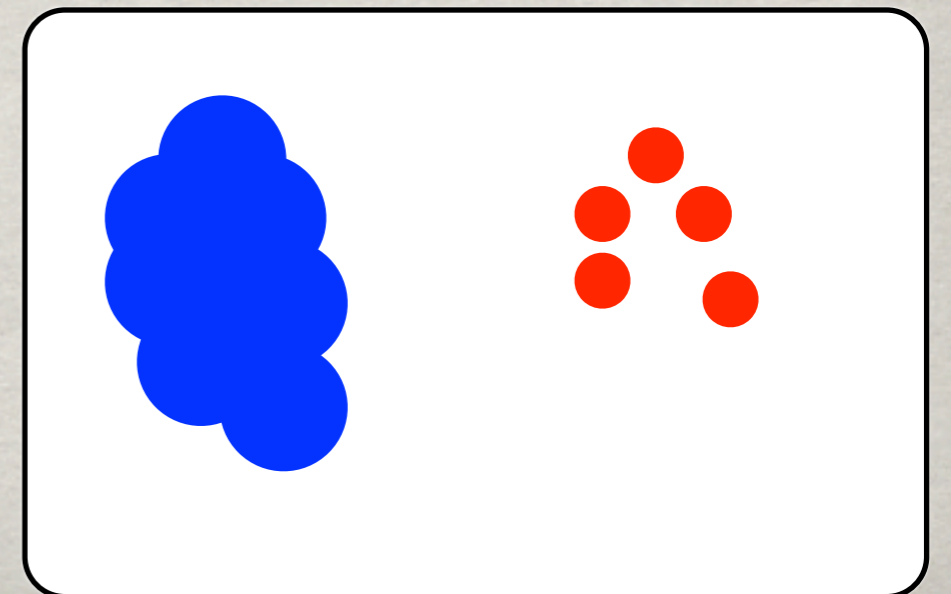
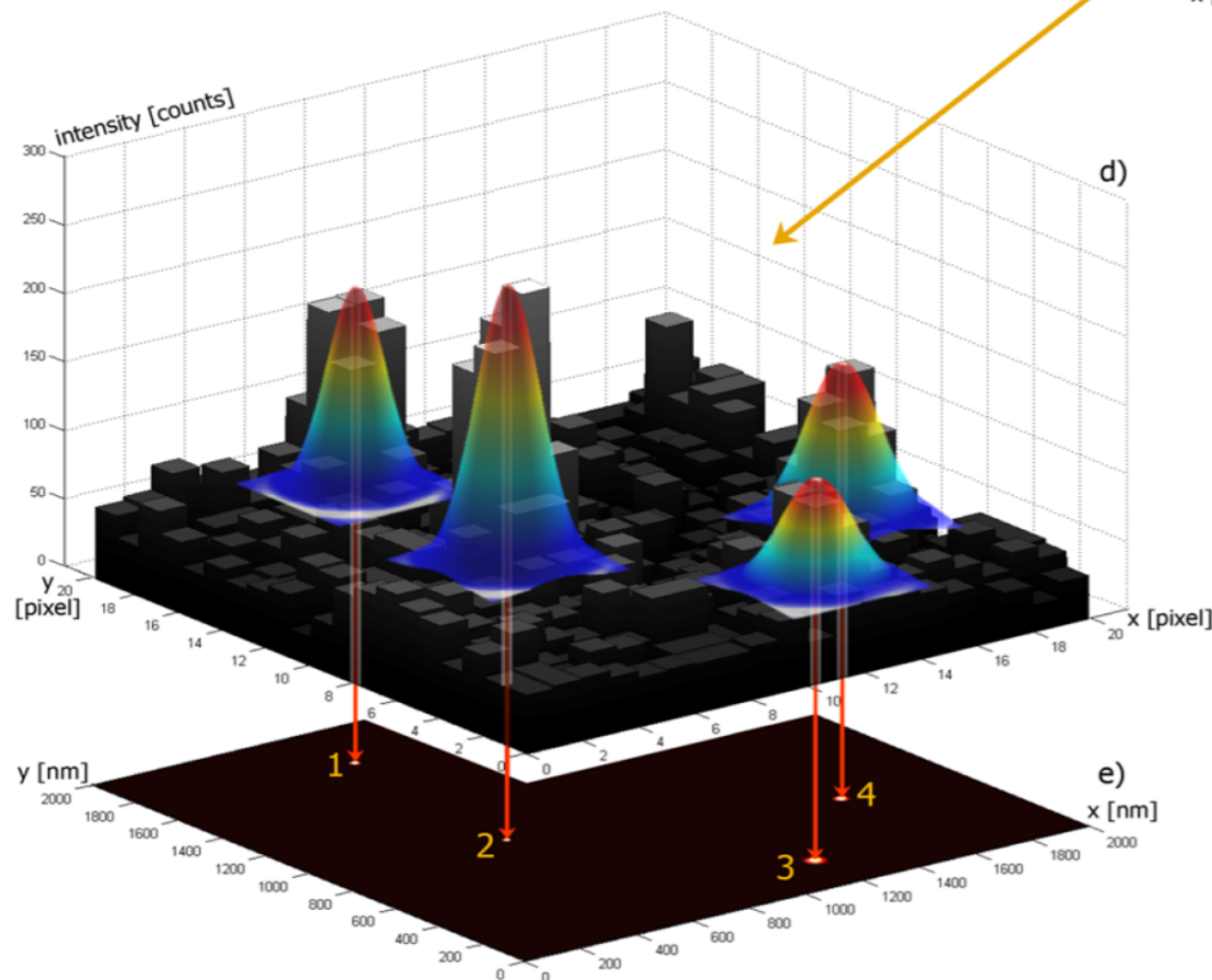
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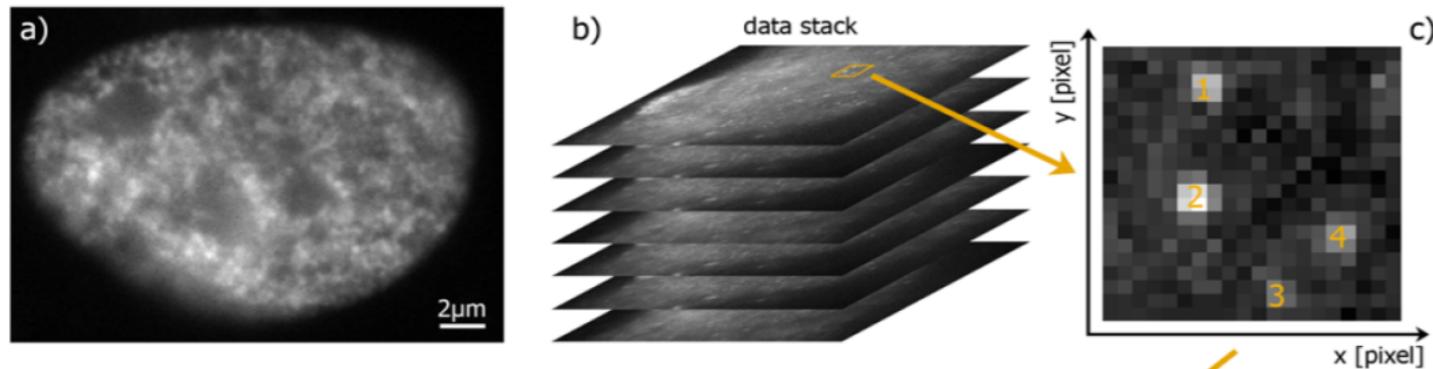
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

Resolution
Distance: ~ 10 nm



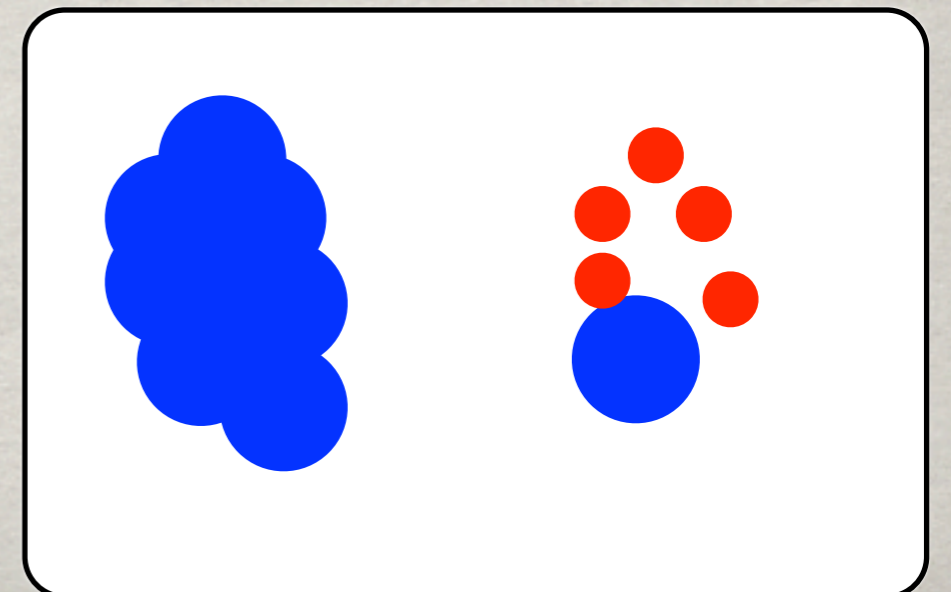
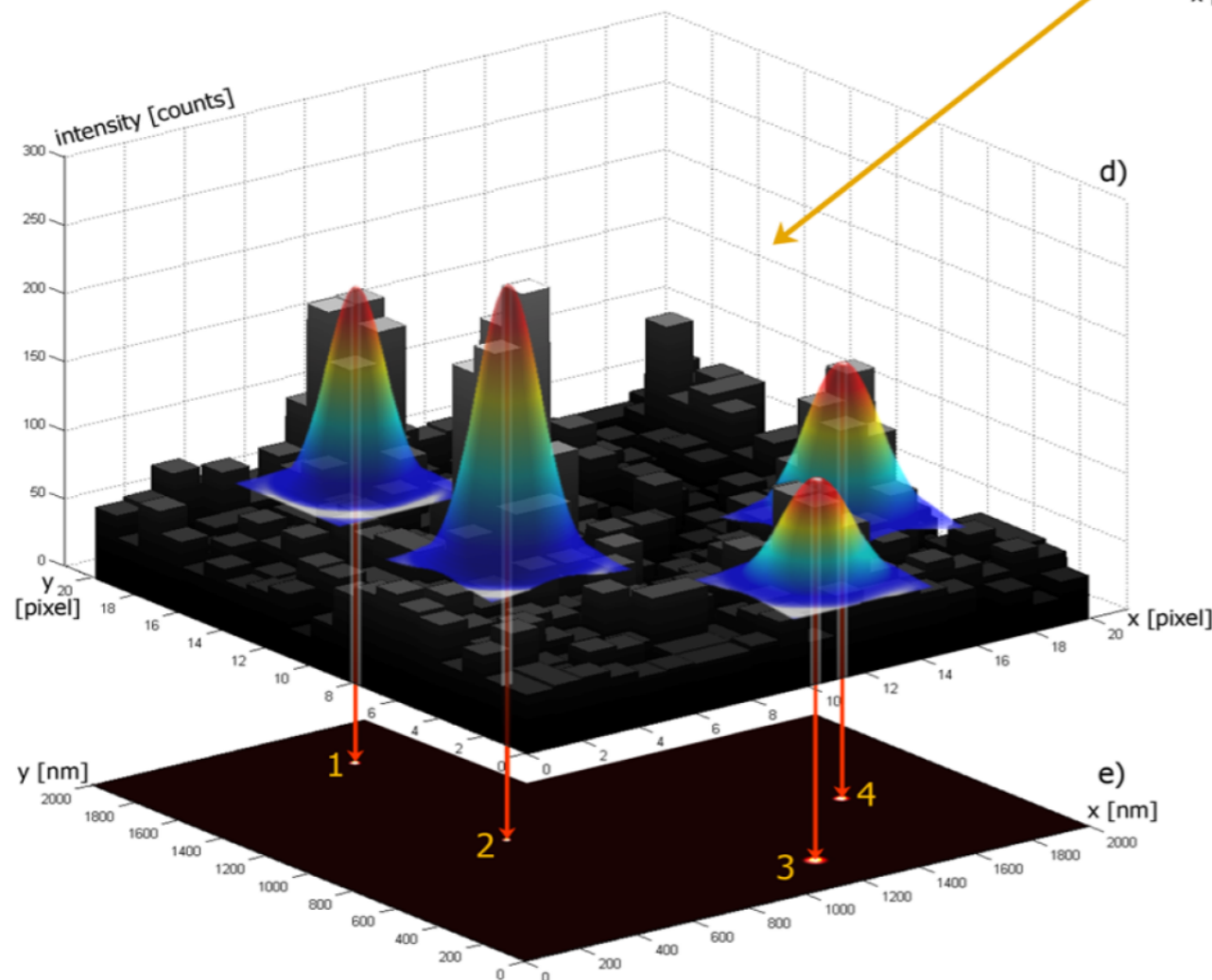
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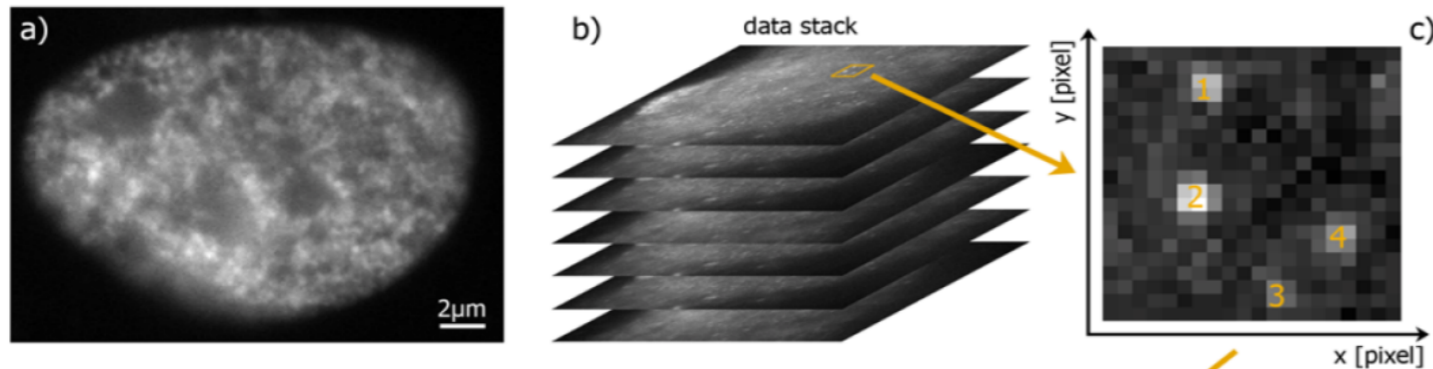
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

Resolution
Distance: ~ 10 nm



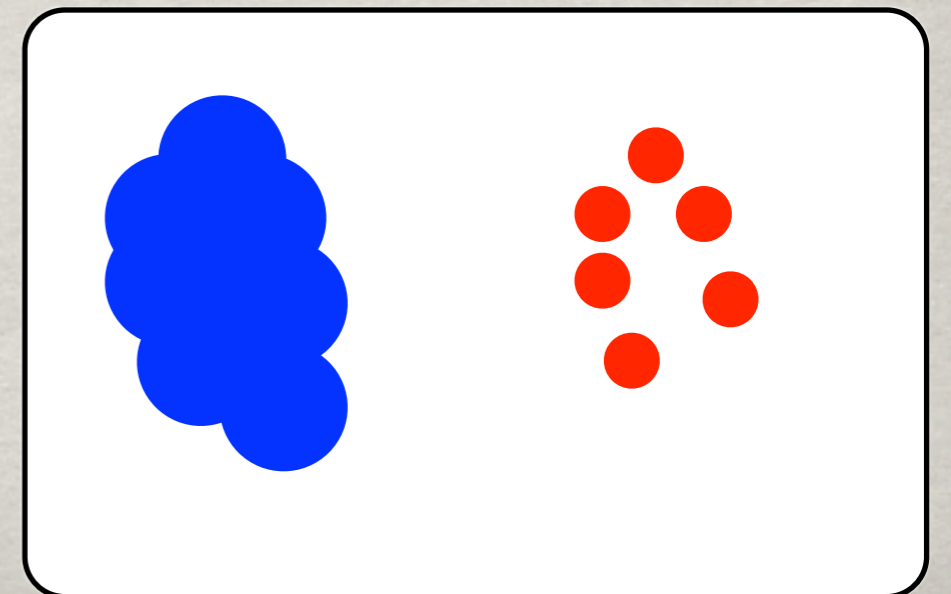
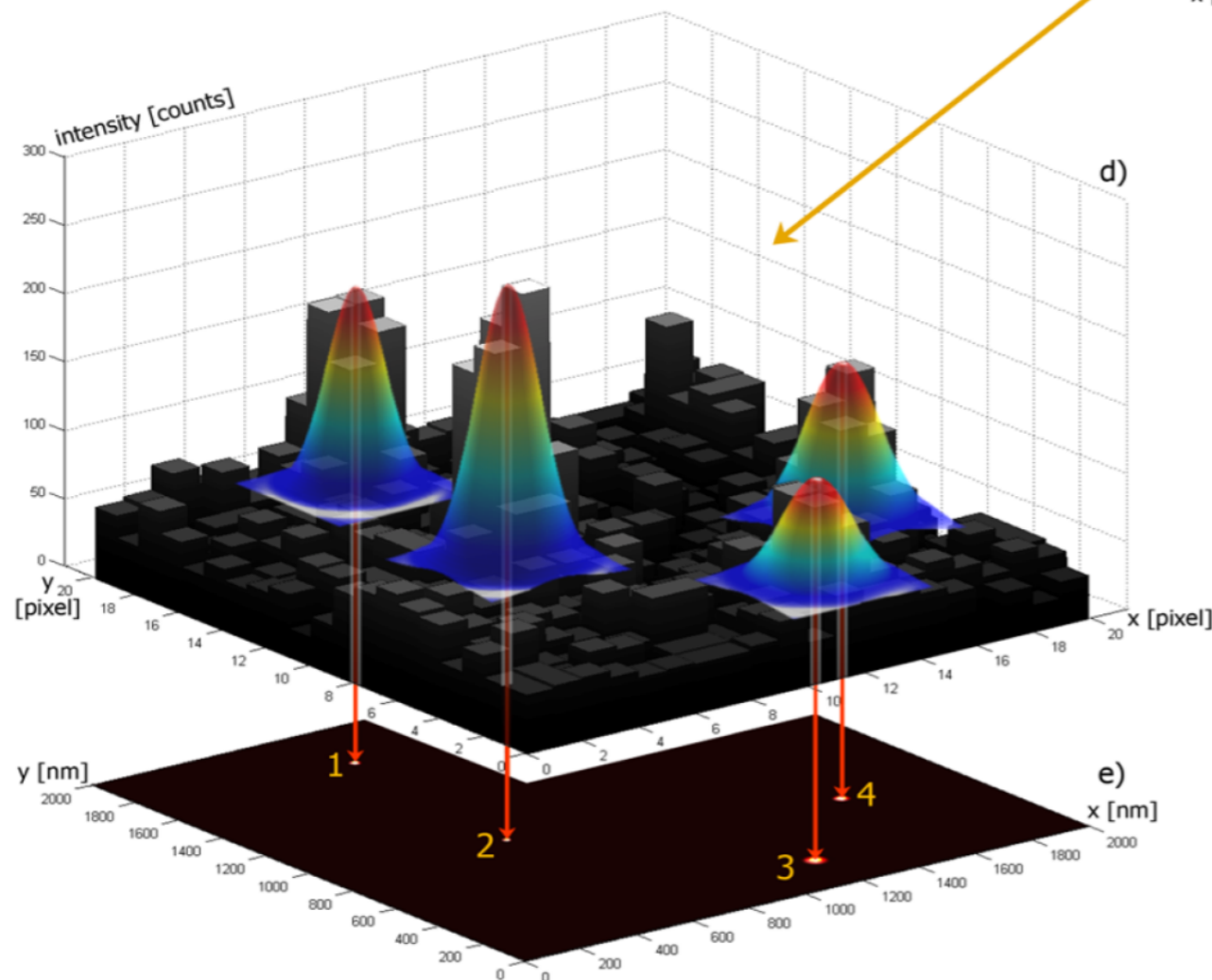
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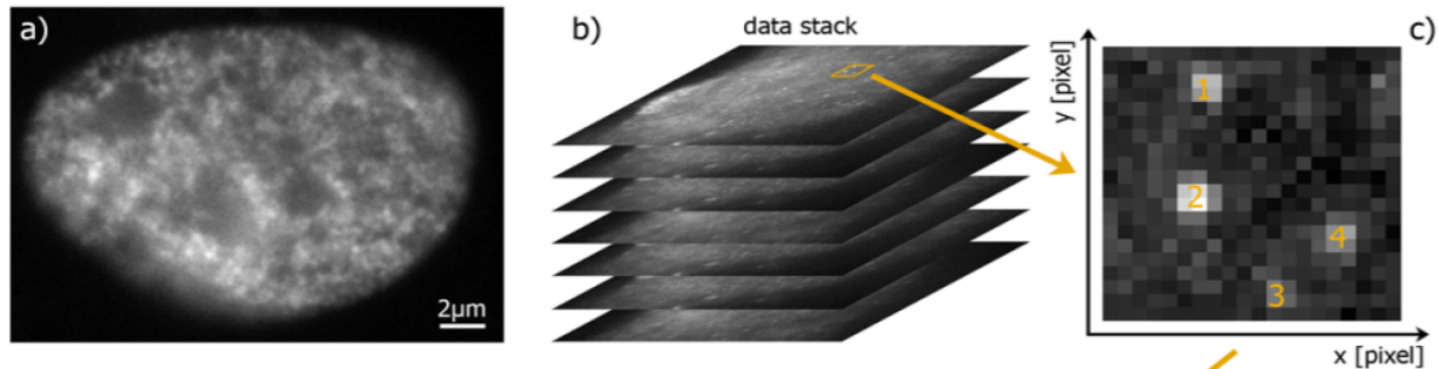
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

Resolution
Distance: ~ 10 nm



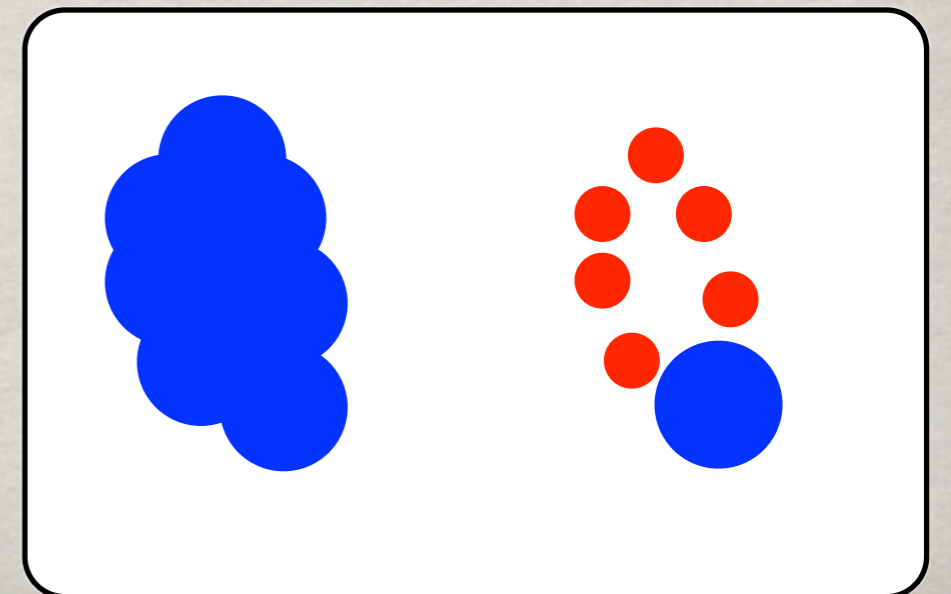
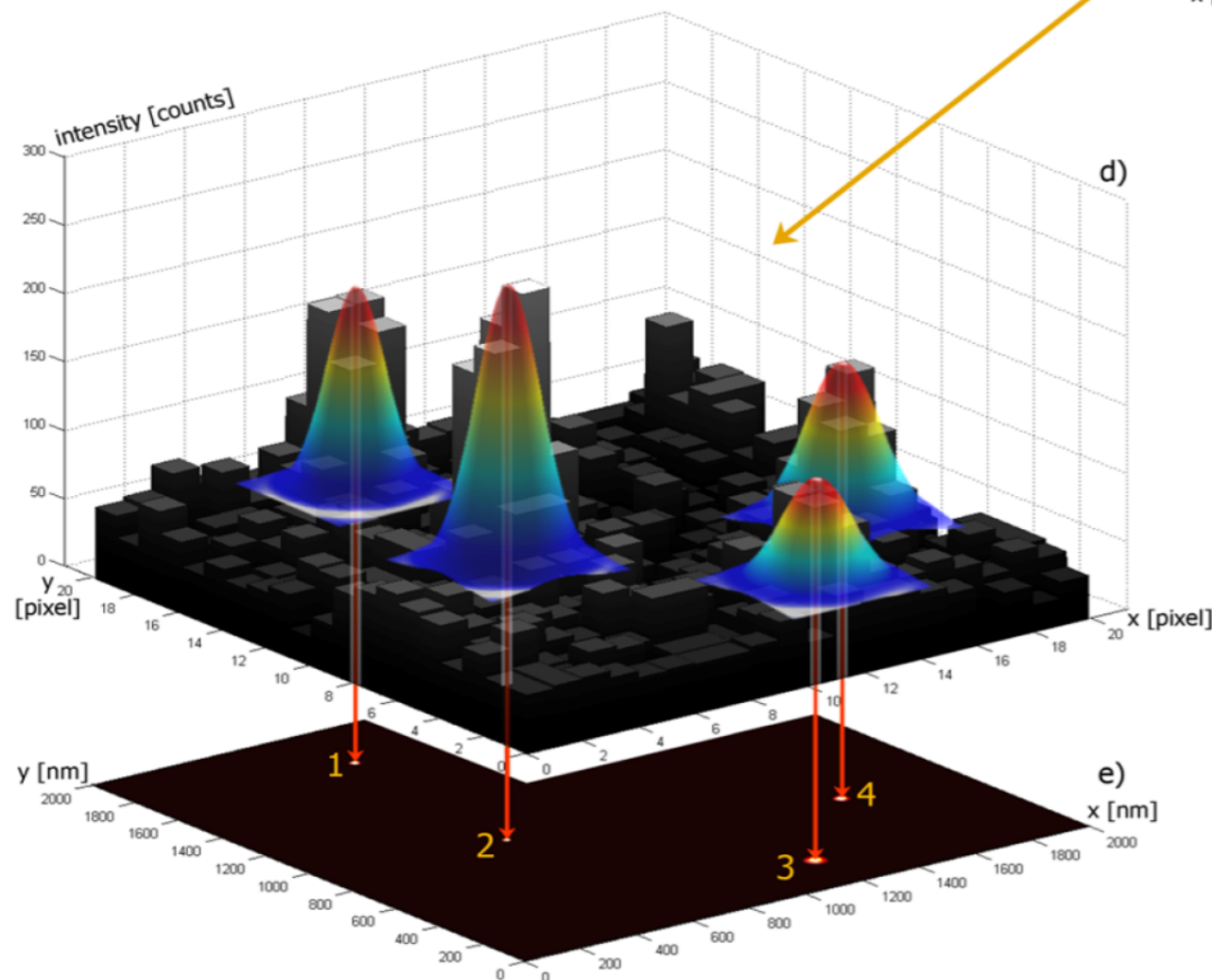
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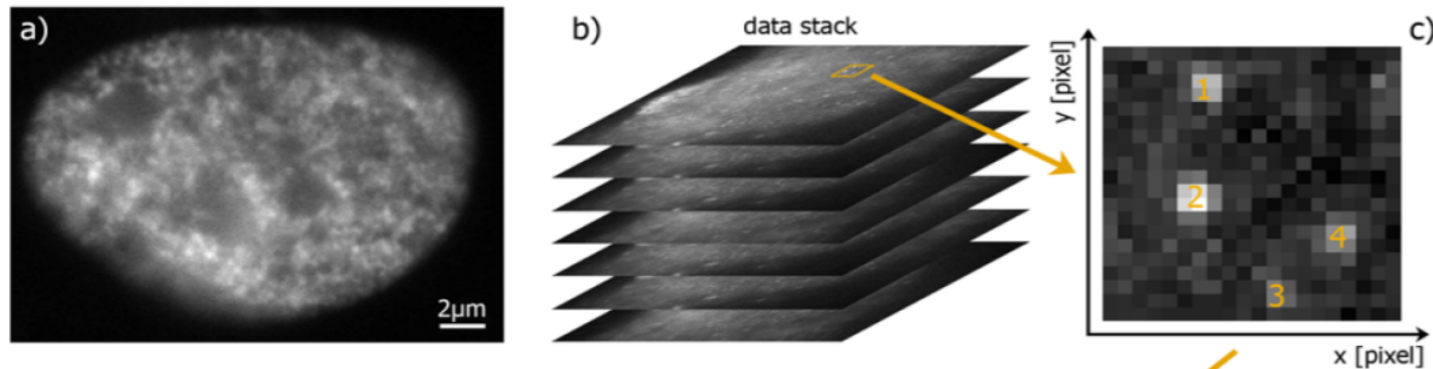
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

Resolution
Distance: ~ 10 nm



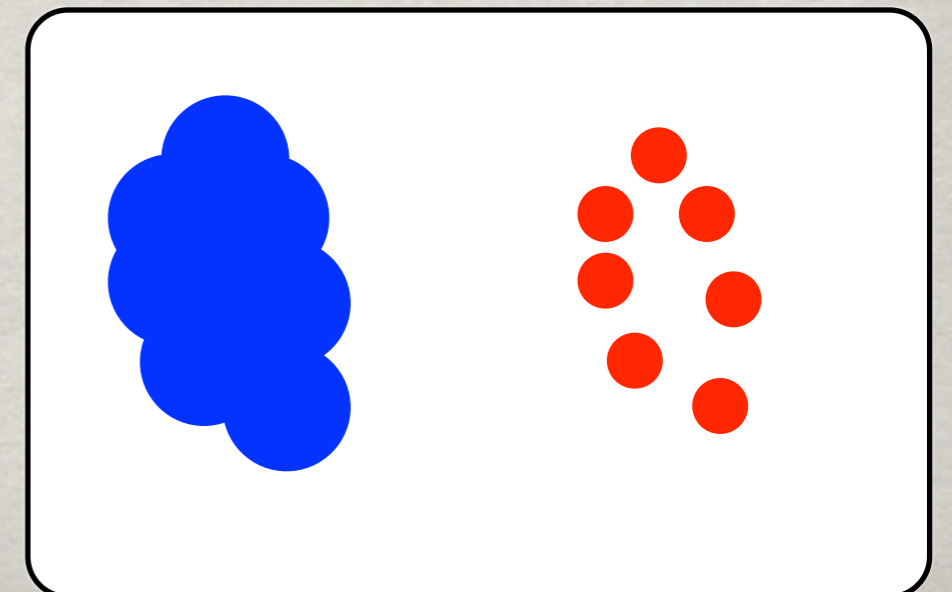
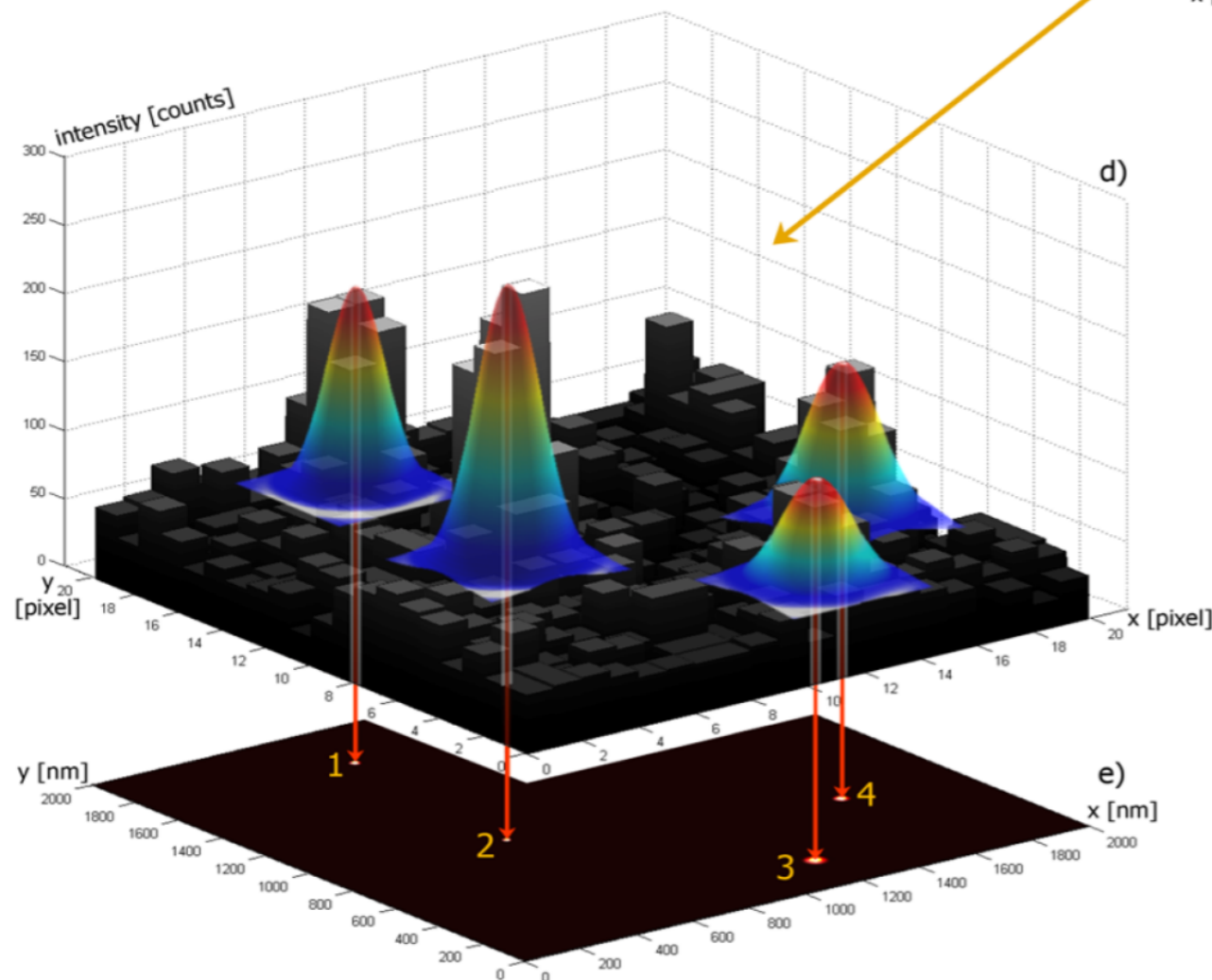
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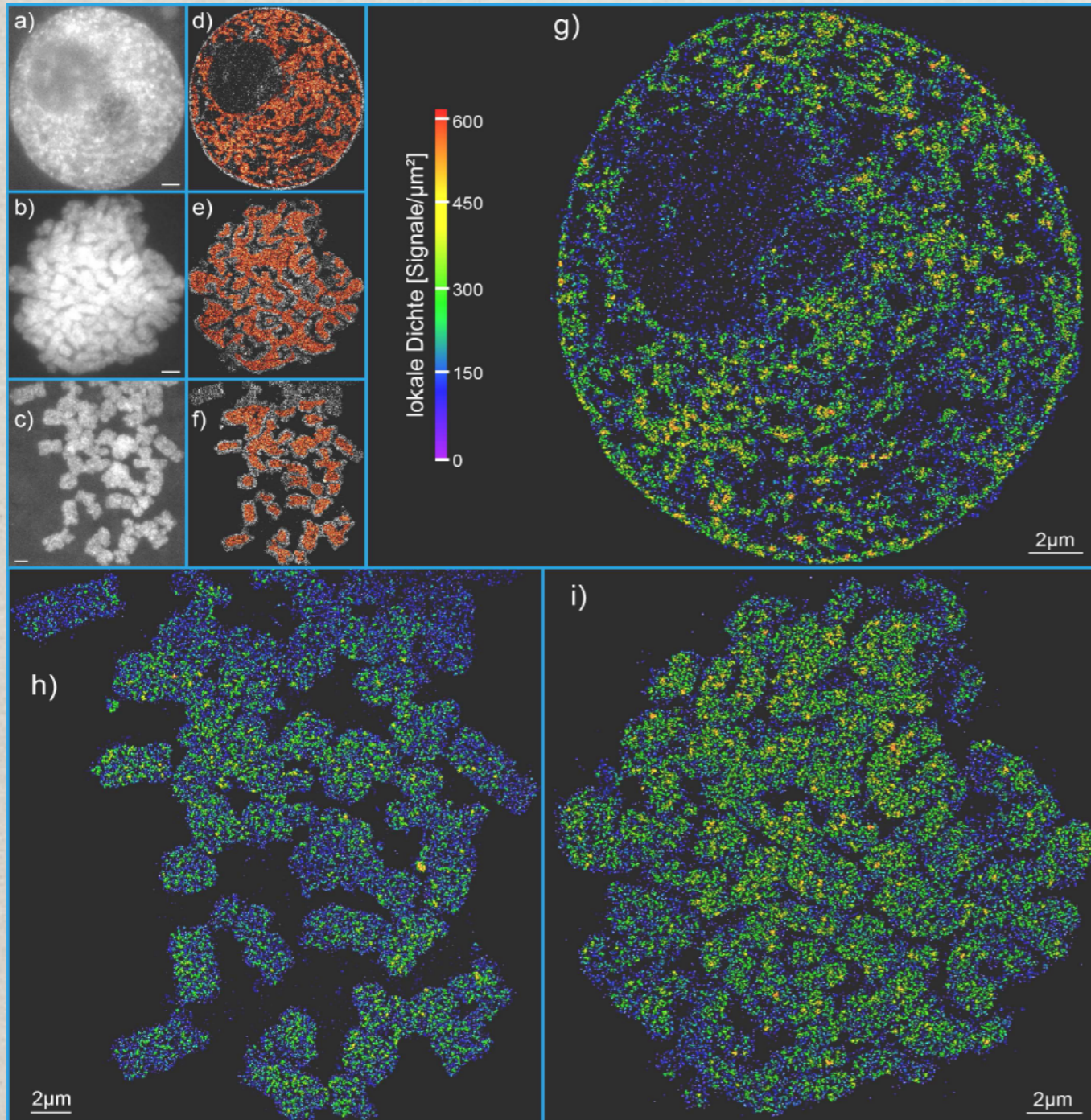
Time stack of images:
400 – 2000 frames

Precision
Localization: $< + 5$ nm

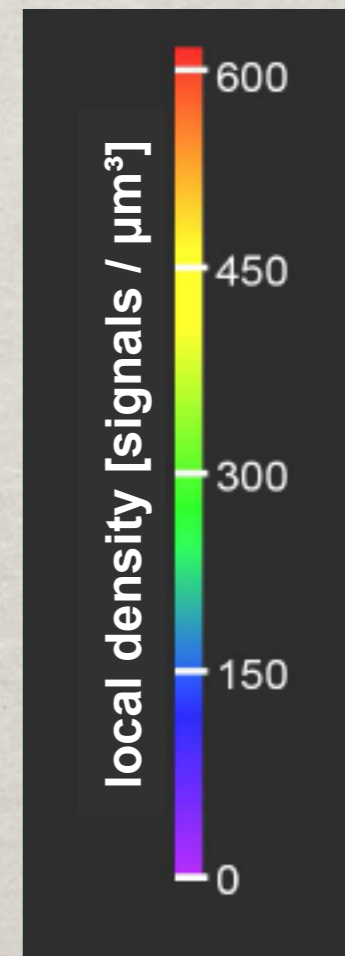
Resolution
Distance: ~ 10 nm



SPDM OF NUCLEOSOMES HELA CELL LINE WITH H2B - GFP



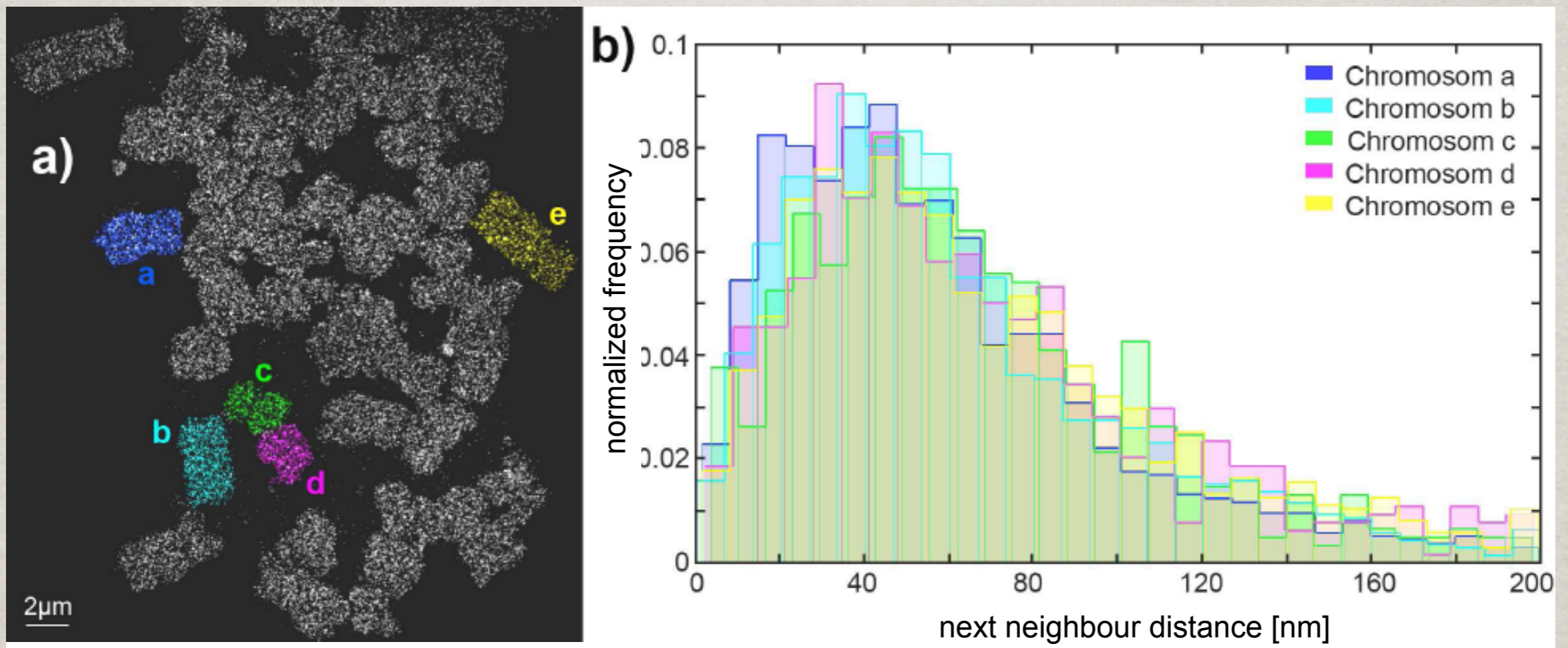
interphase



prometaphase

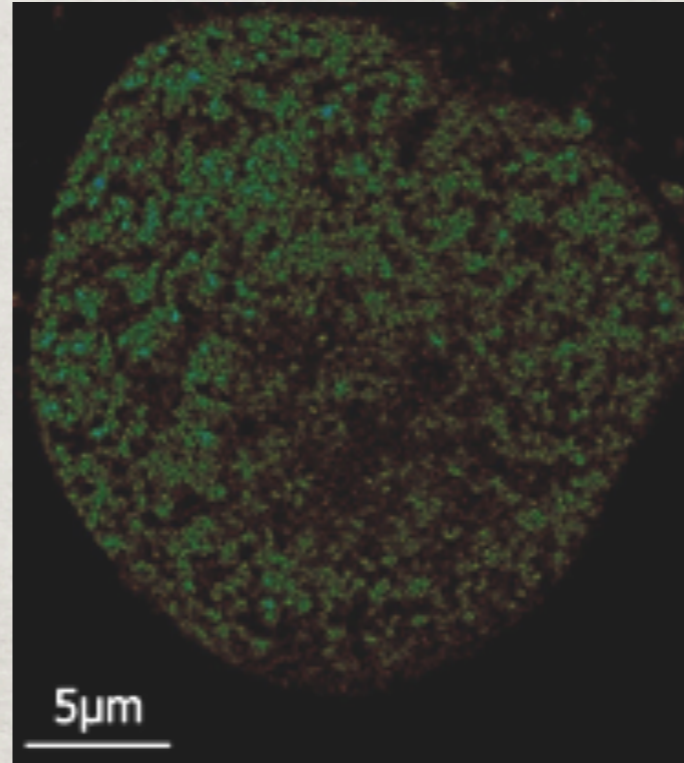
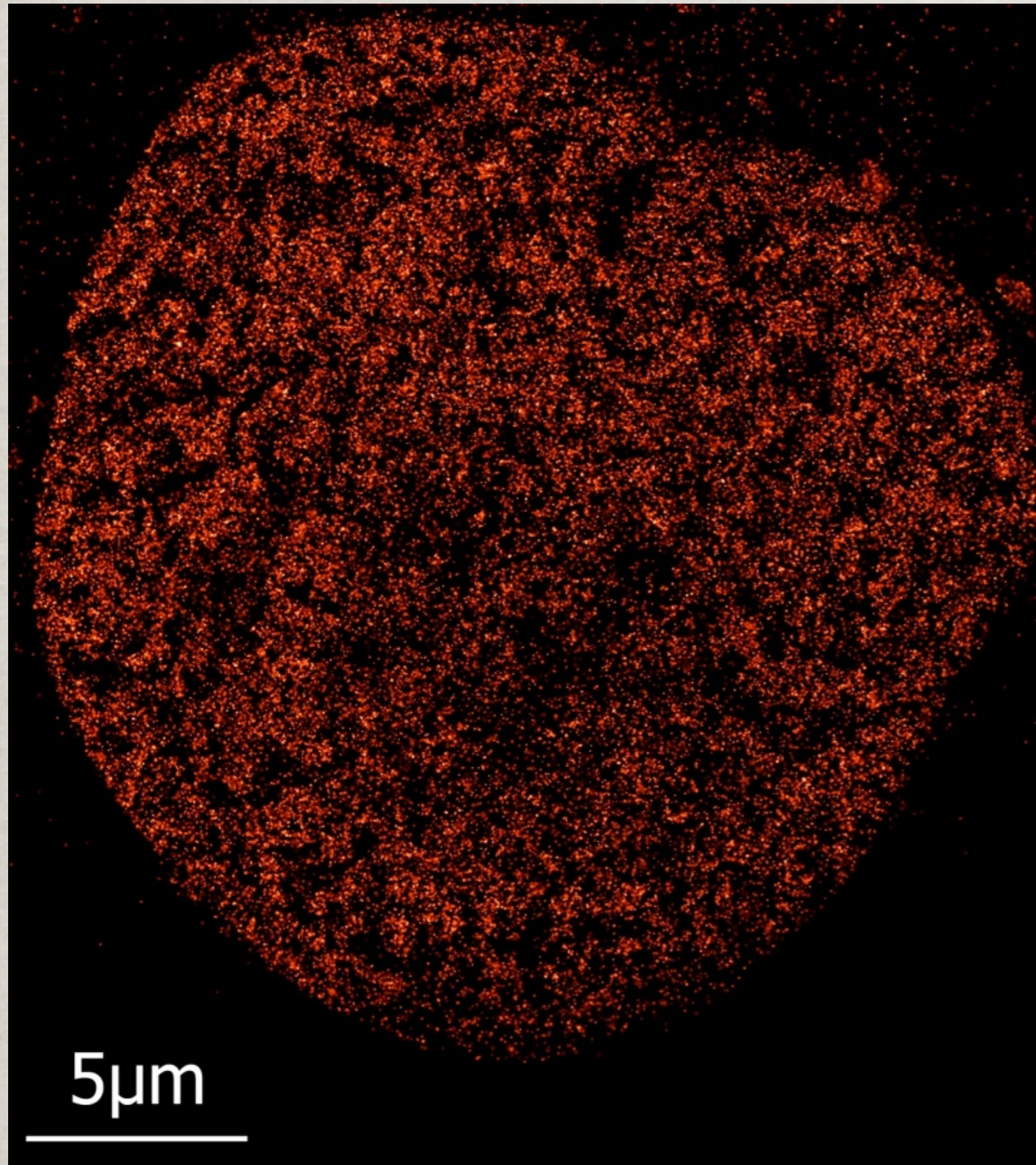
metaphase

SPDM OF NUCLEOSOMES METAPHASE CHROMOSOMES



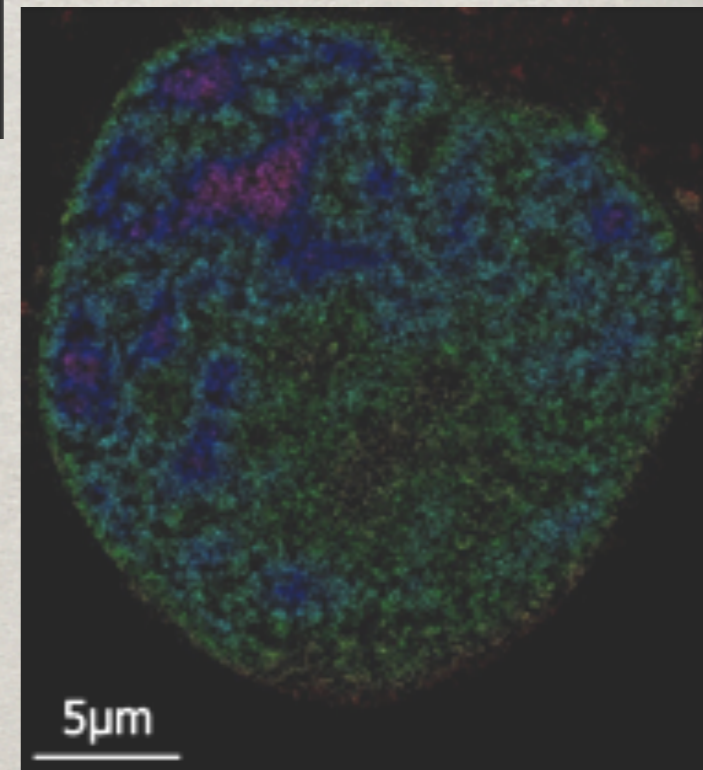
no significant compaction difference

3D – NANOARCHITECTURE OF NUCLEOSOMES

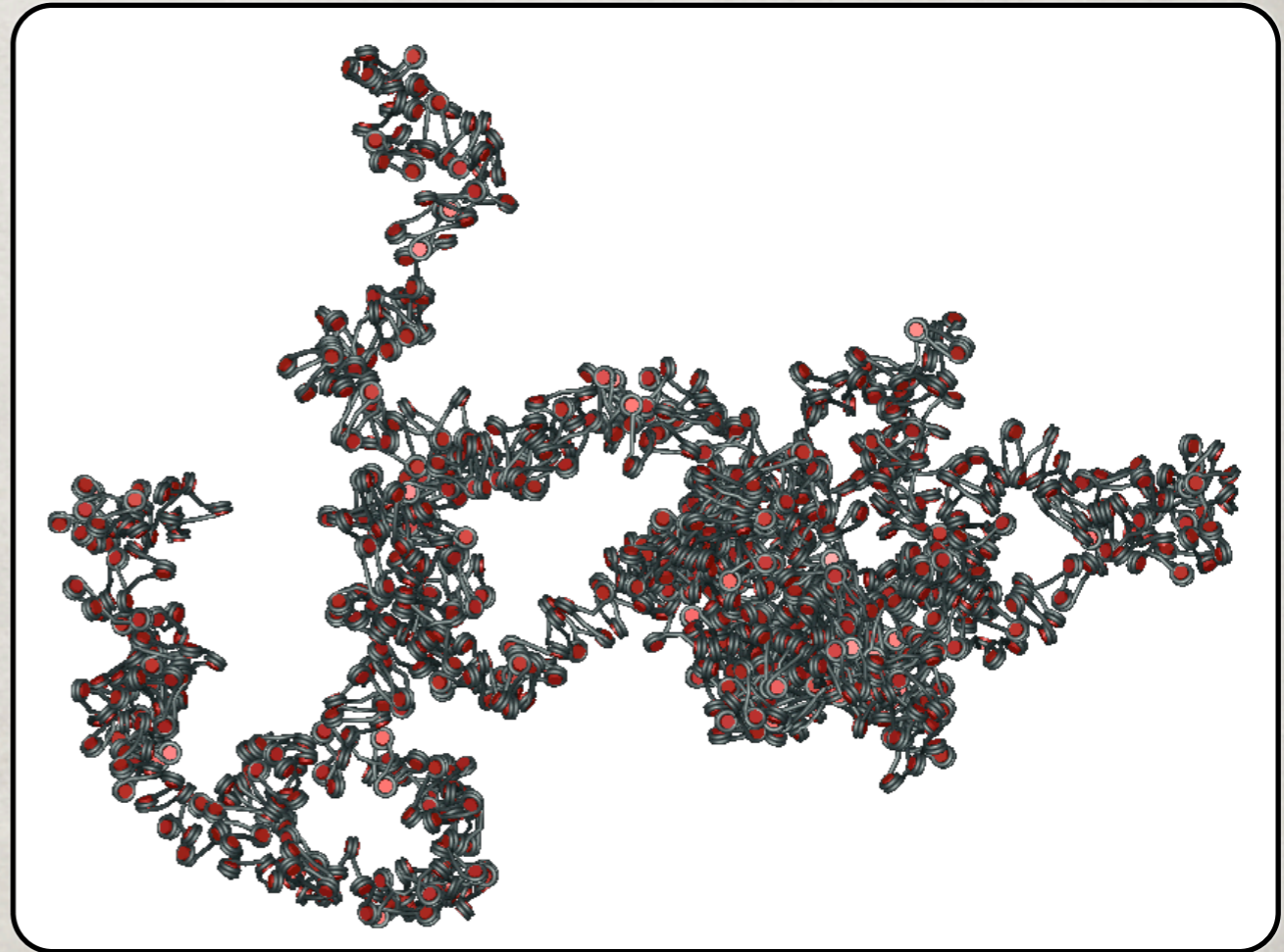
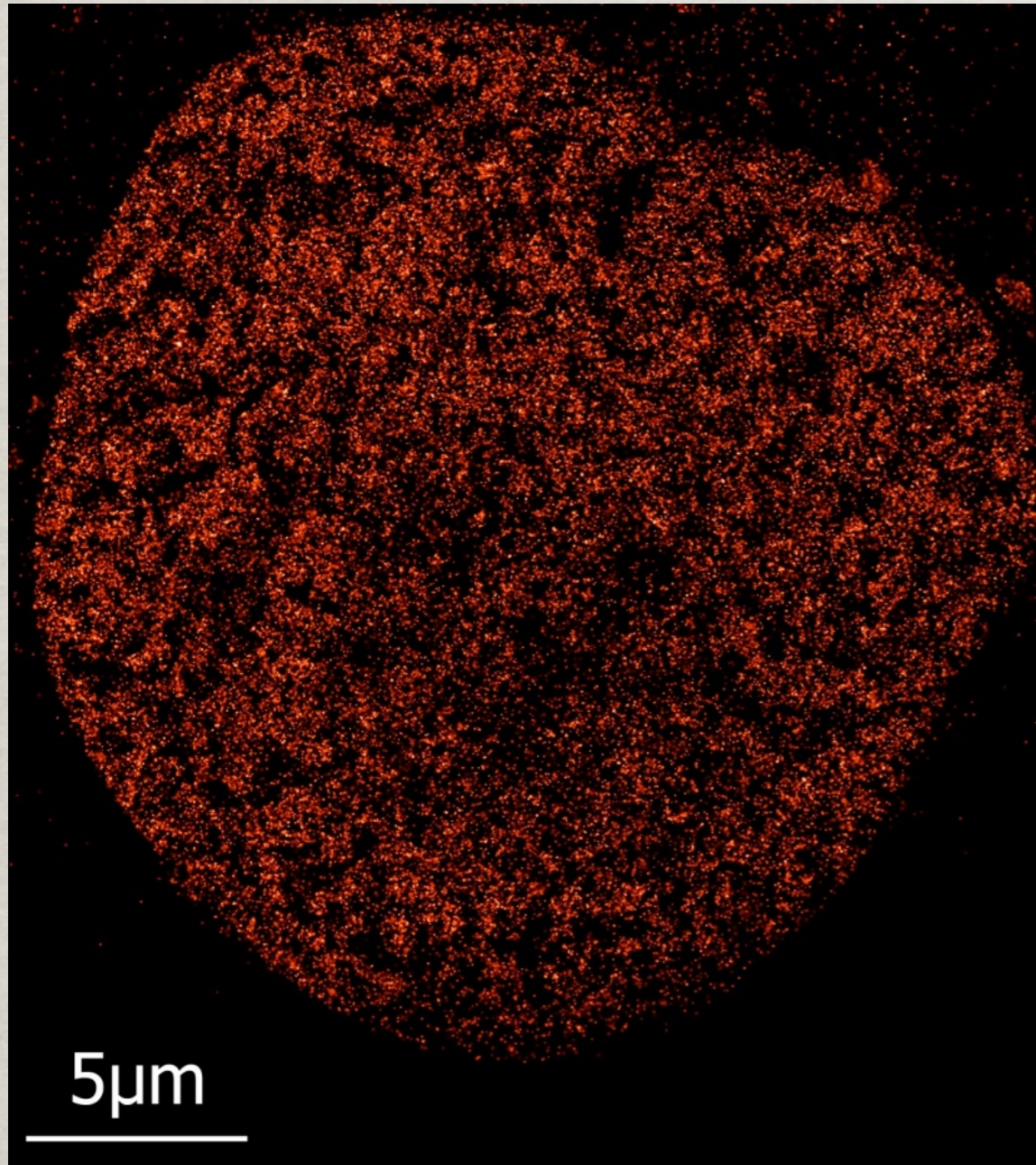


Neighbors
200 nm / 1000 nm

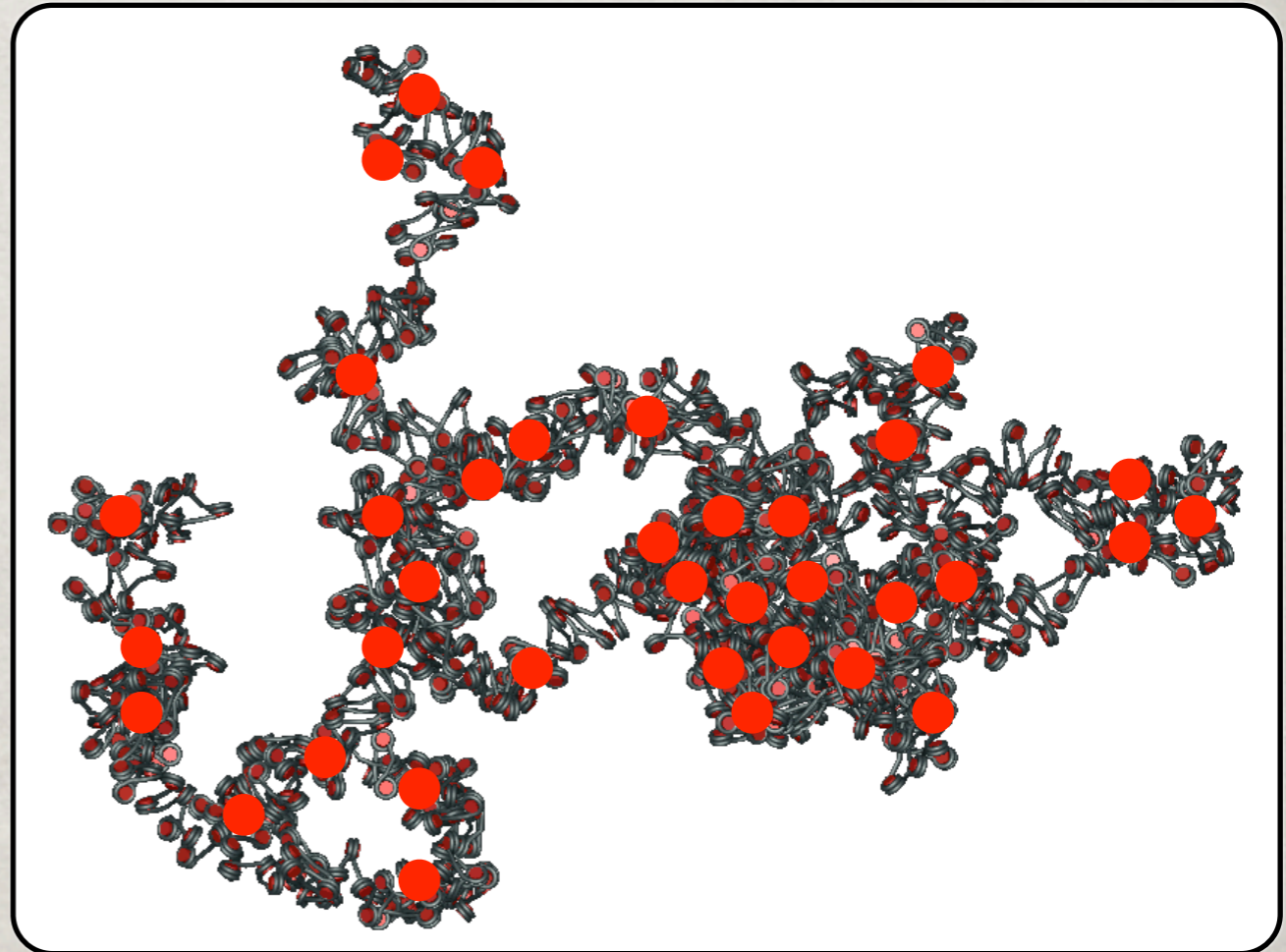
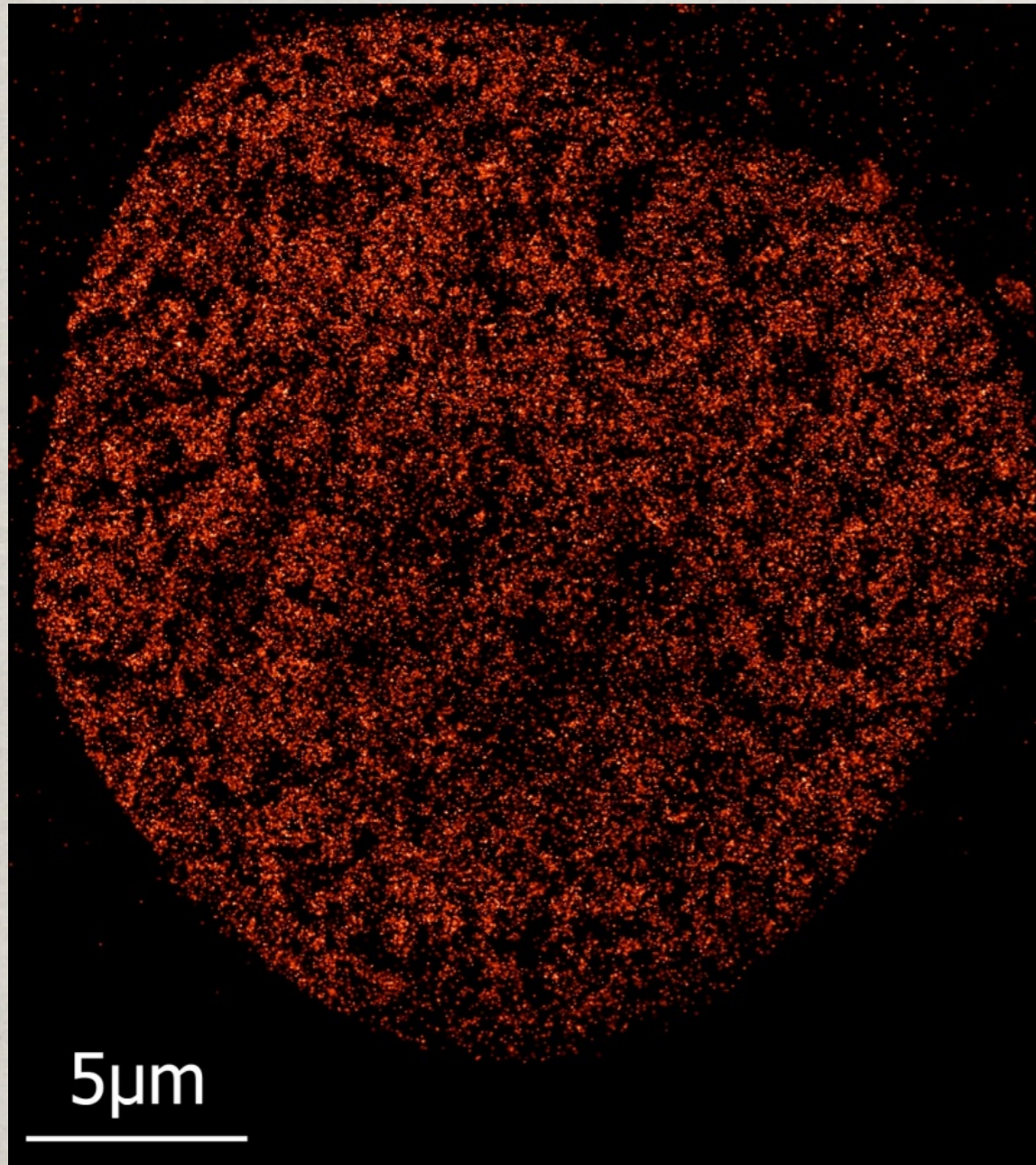
Localization image of
nucleosomes:
Detected fluorochromes:
~ 70000 /image section



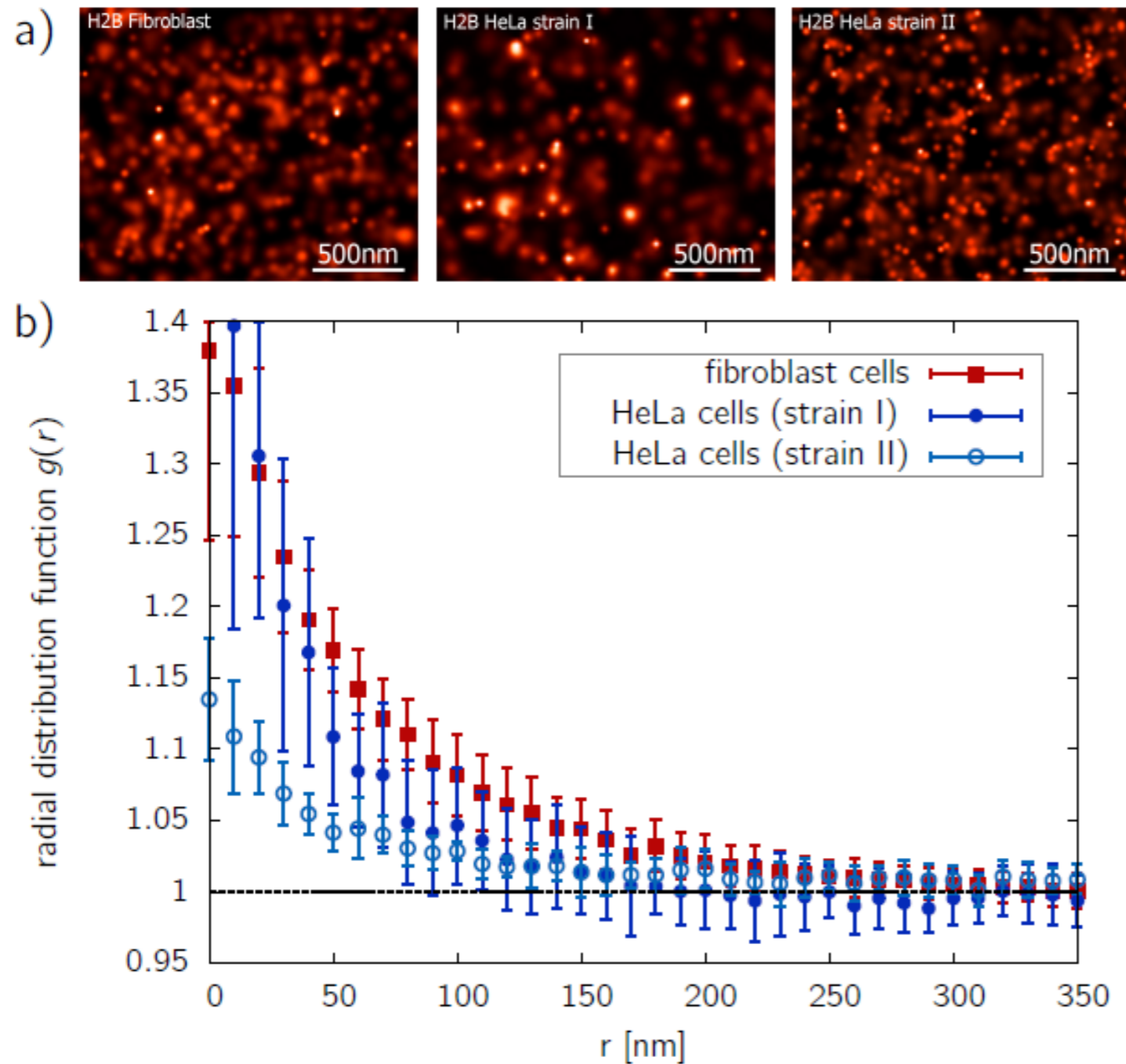
3D – NANOARCHITECTURE OF NUCLEOSOMES



3D – NANOARCHITECTURE OF NUCLEOSOMES



SPDM OF NUCLEOSOMES: FIBROBLASTS HELA CELL LINE



Nanoscale results:

Distinct structures below
dimensions of 100 nm

Different expression
mechanisms lead to
different structures

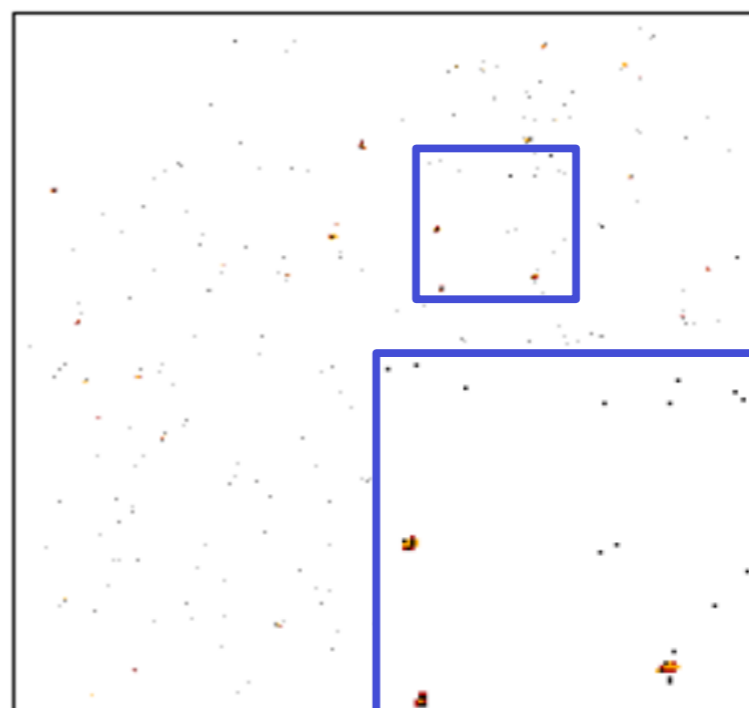
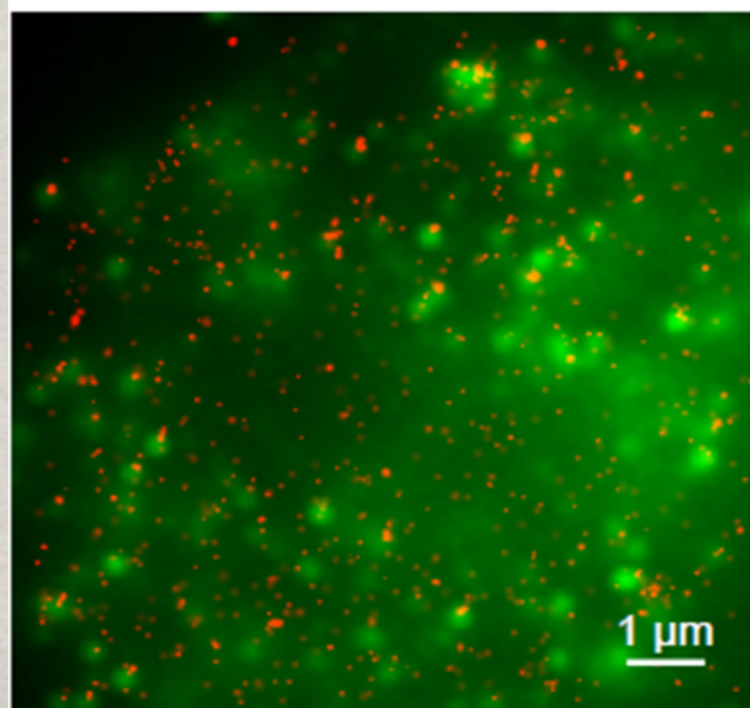
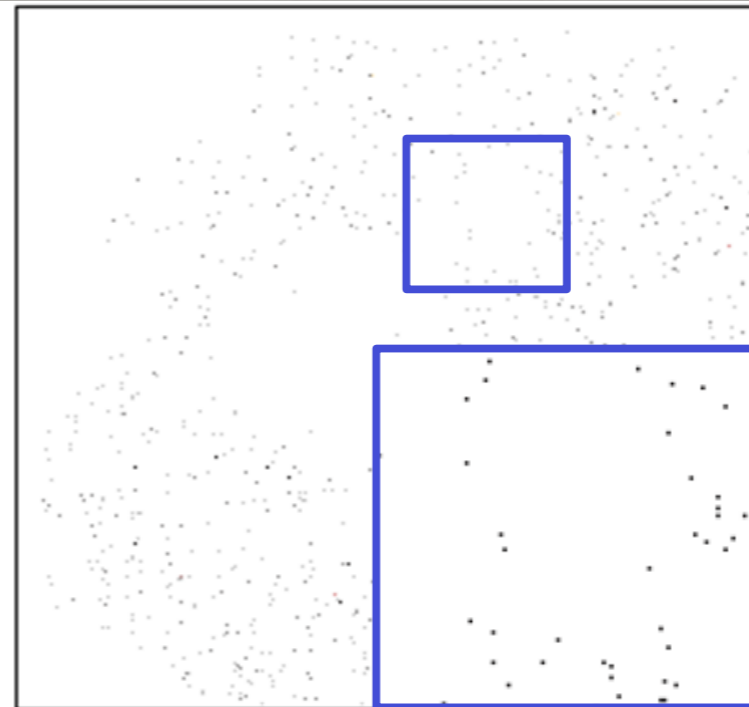
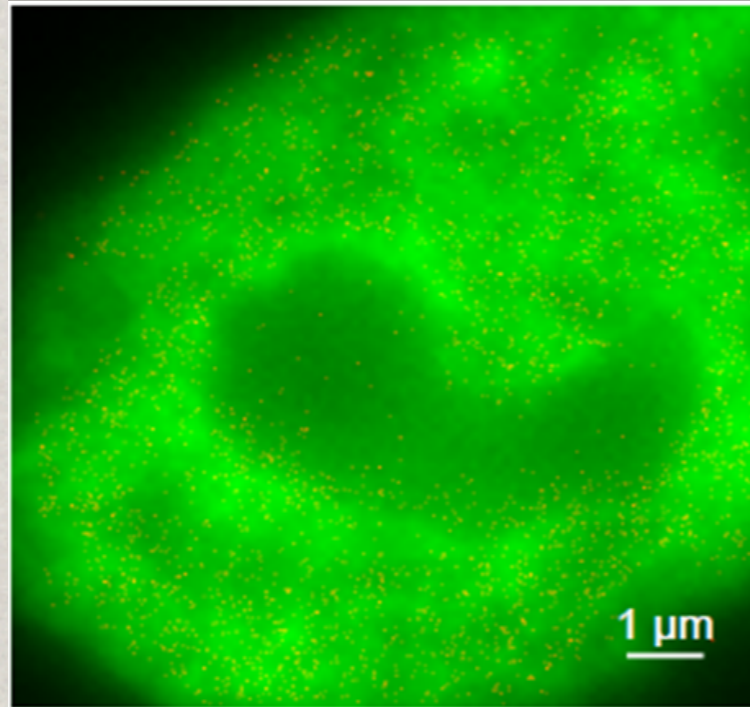
No cell type dependence

SPDM OF HETERO- AND OF EU- CHROMATIN AFTER γ - IRRADIATION

Nucleosomes:
H2A-YFP
H2B-GFP

Alexa 568:
antibodies against
heterochromatic
regions:
H4K20me3

antibodies against
euchromatic
regions:
H3K4me3

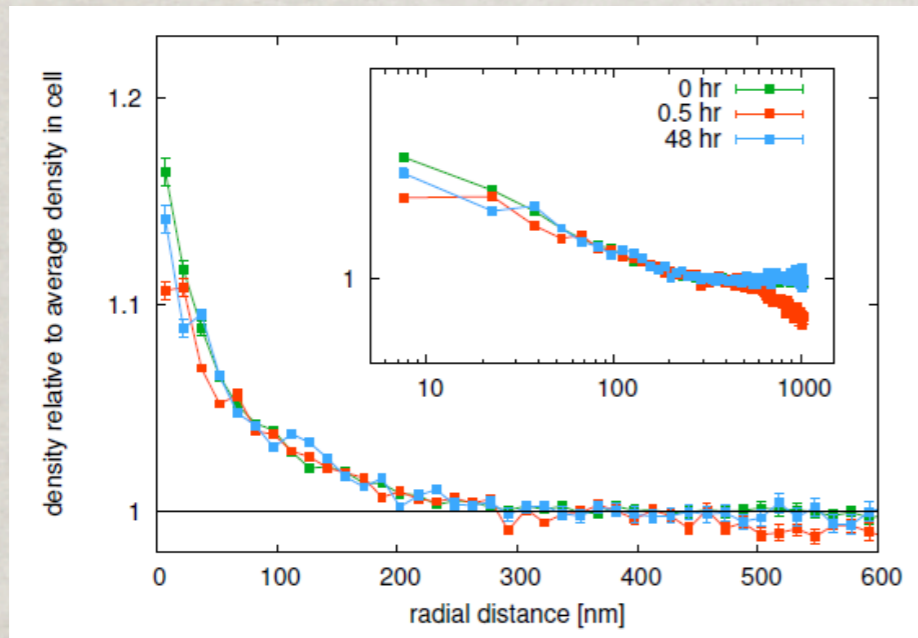


Cluster
determination:
Within 40 nm
minimum
3 next
neighbours

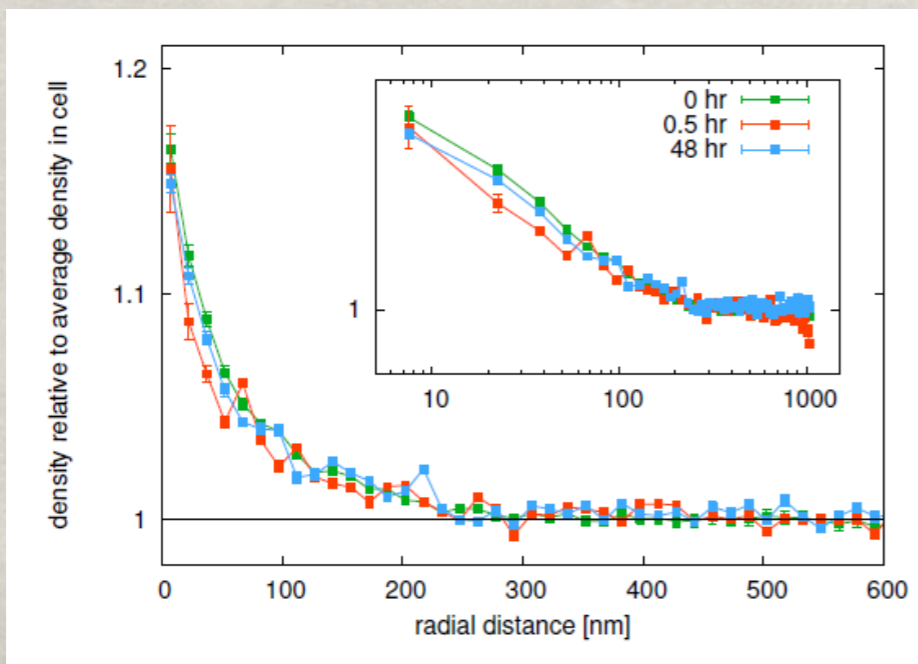
CHANGES OF NANOARCHITECTURE DURING REPAIR

all nucleosomes

0.5 Gy



4 Gy

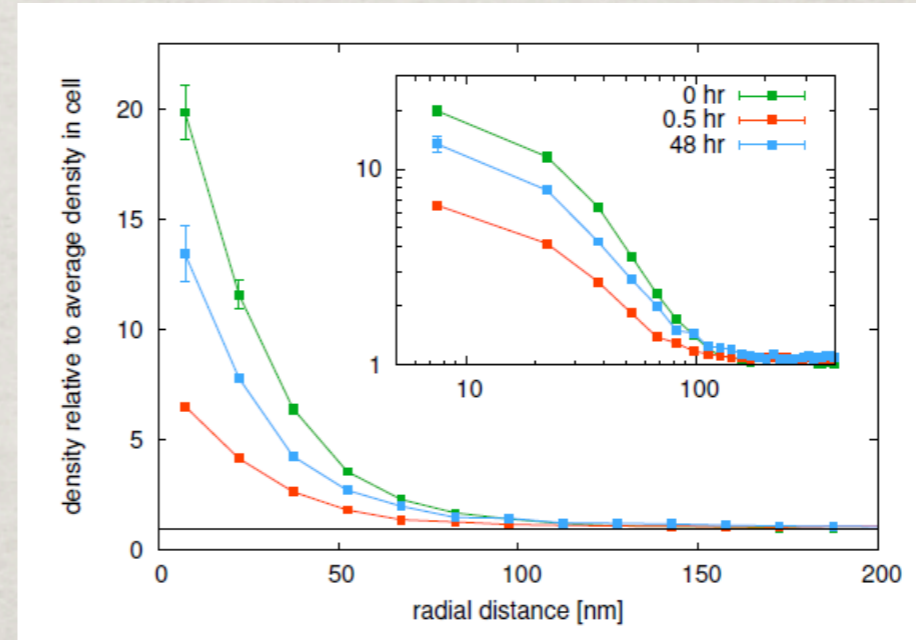
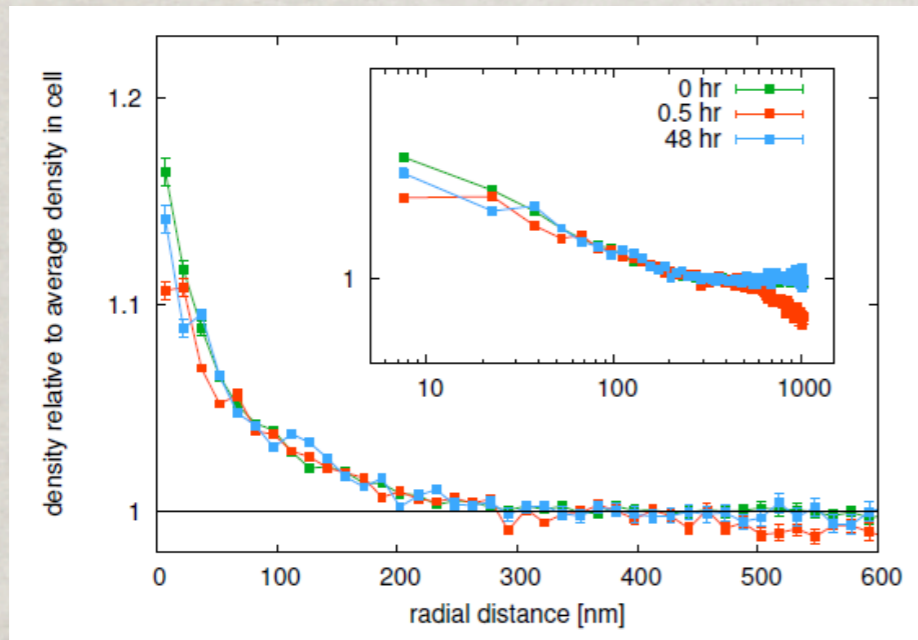


CHANGES OF NANOARCHITECTURE DURING REPAIR

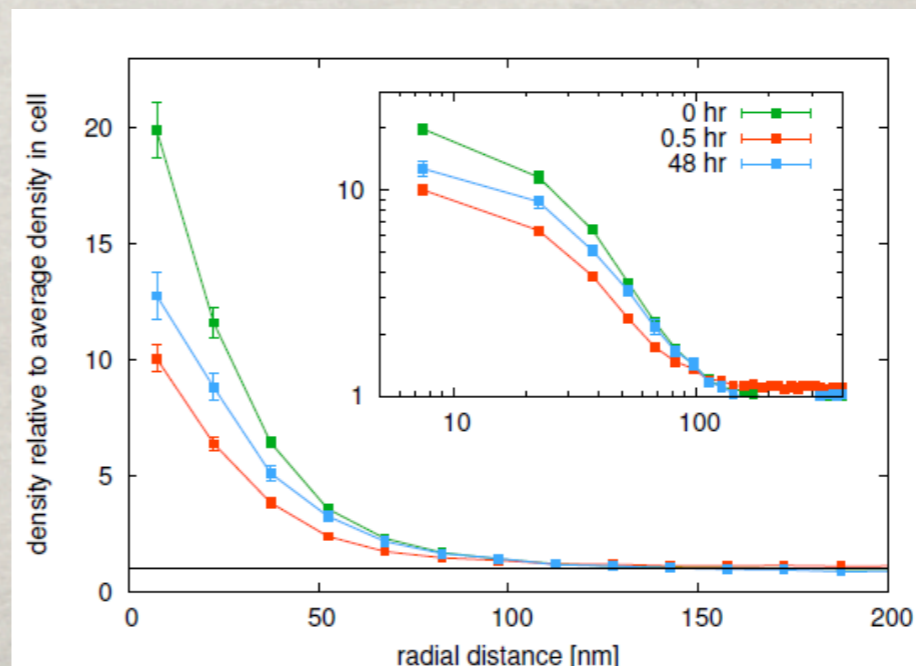
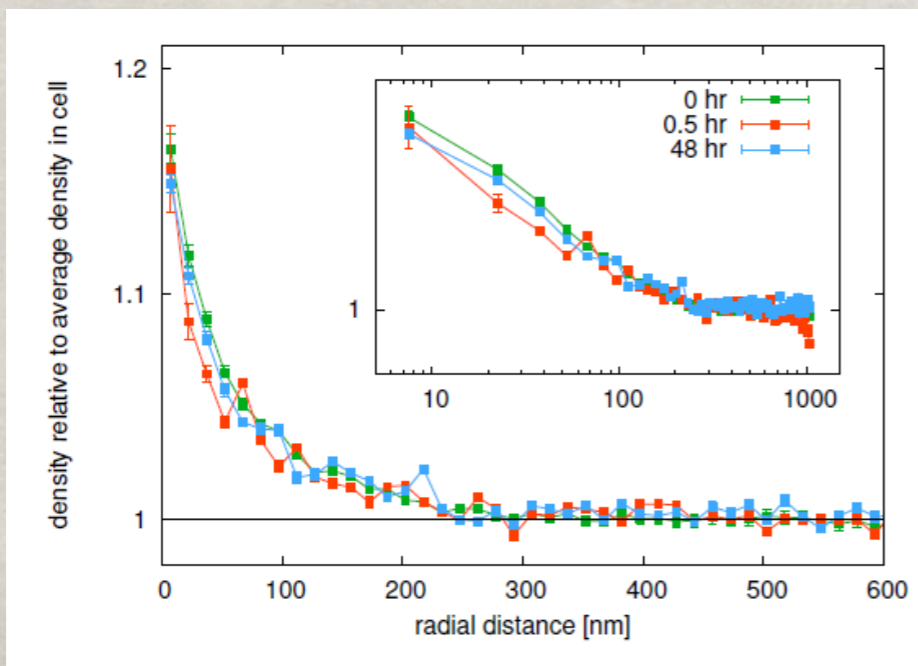
all nucleosomes

heterochromatin

0.5 Gy



4 Gy



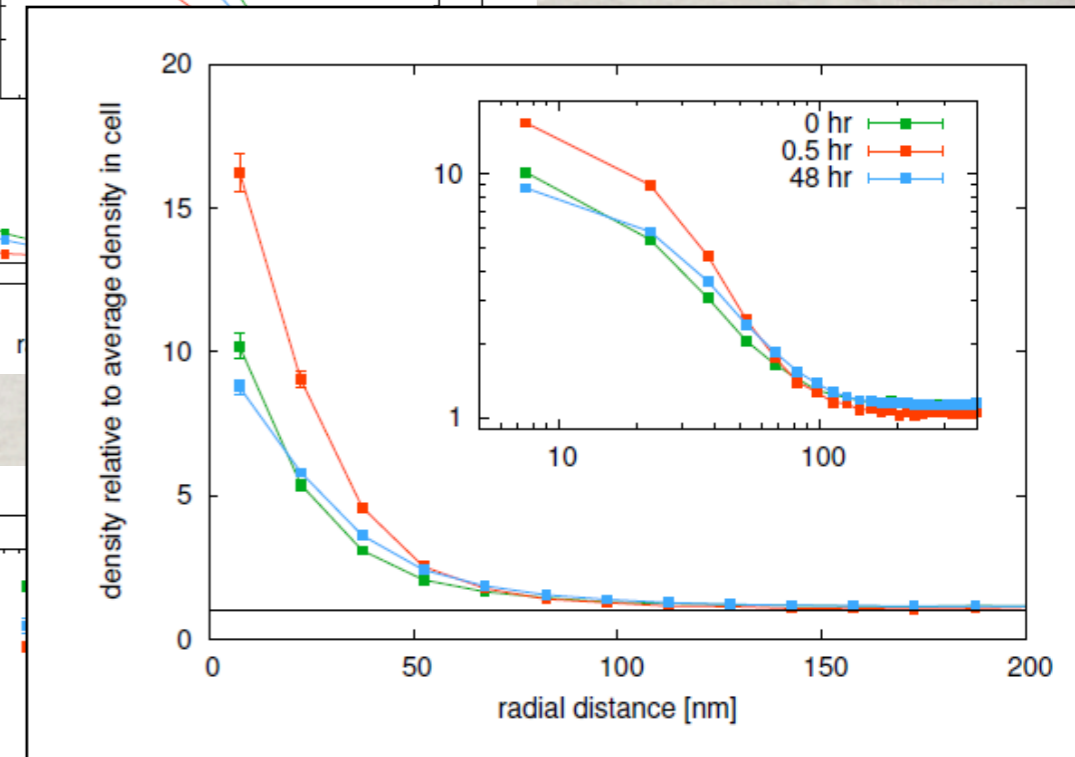
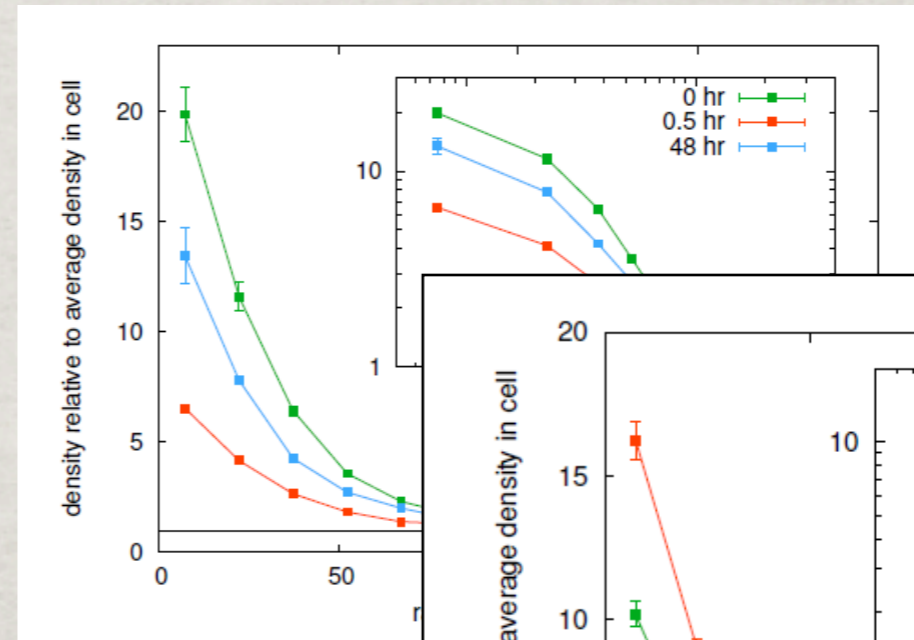
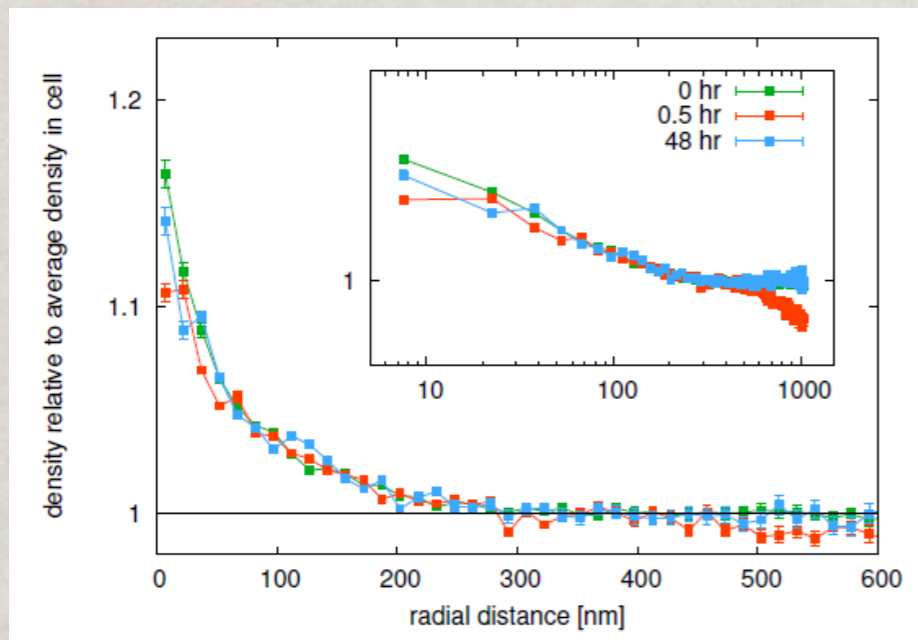
CHANGES OF NANOARCHITECTURE DURING REPAIR

all nucleosomes

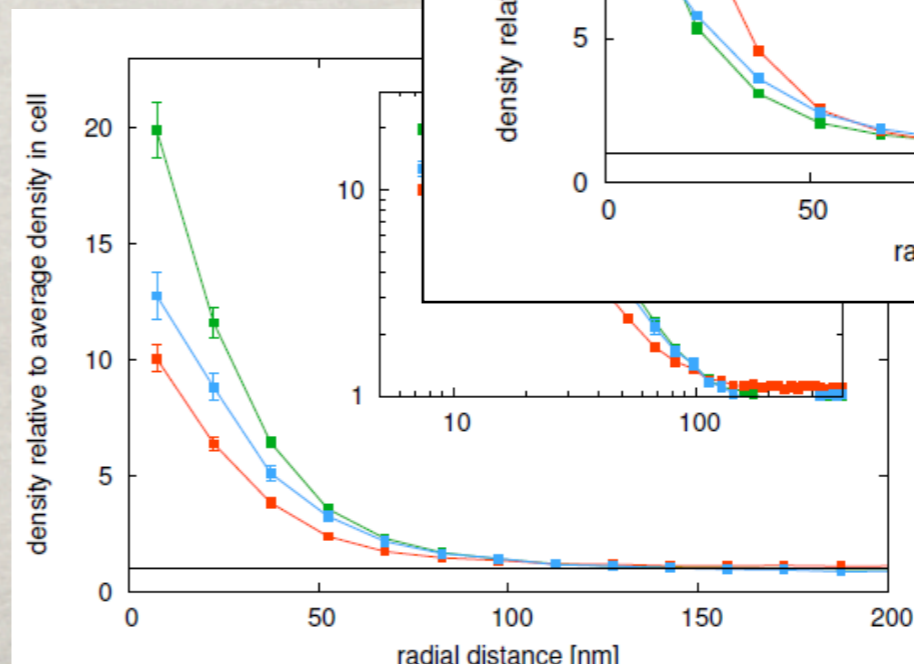
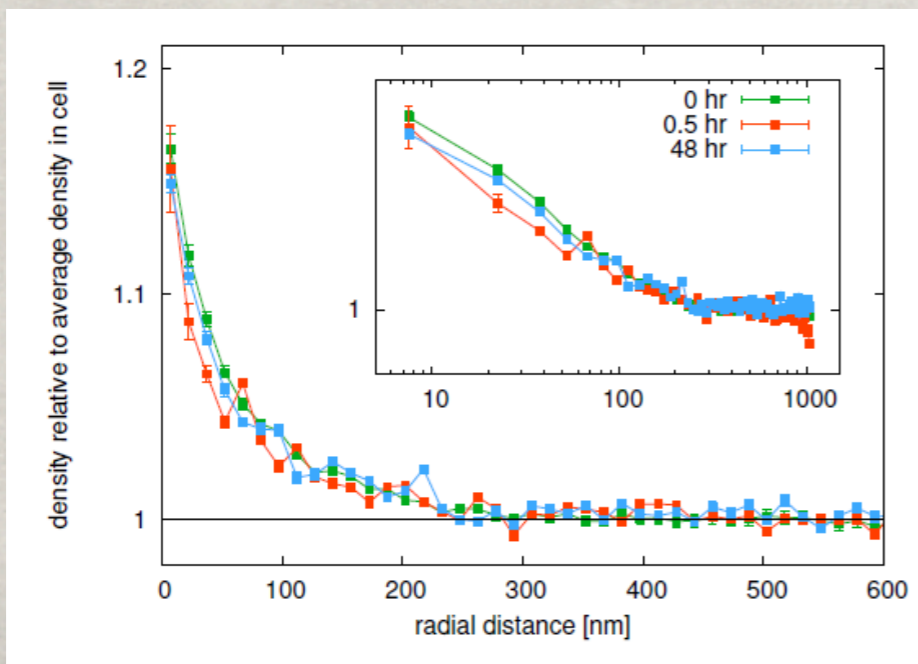
heterochromatin

euchromatin

0.5 Gy



4 Gy



CONCLUSION

- Distance measurements in the 10 nm range (= 1/50 wavelength of green light) with a precision of < 5 nm
- Tool to analyse 2D/3D nano-structures in intact nuclei and cells
- Fast image acquisition (~ 2 min / 2D; ~ 2 h / 3D)
- Approx. 2 GB per 2D SPDM image / 100 GB per 3D SPDM image

ACKNOWLEDGEMENT

- The Labs of M. Hausmann and J. Hesser at Heidelberg University, P. Müller, J. Wagner
- C. Cremer, IMB Mainz
- D. Heermann, Heidelberg University



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