Quantitative Phase Imaging and fluorescence imaging

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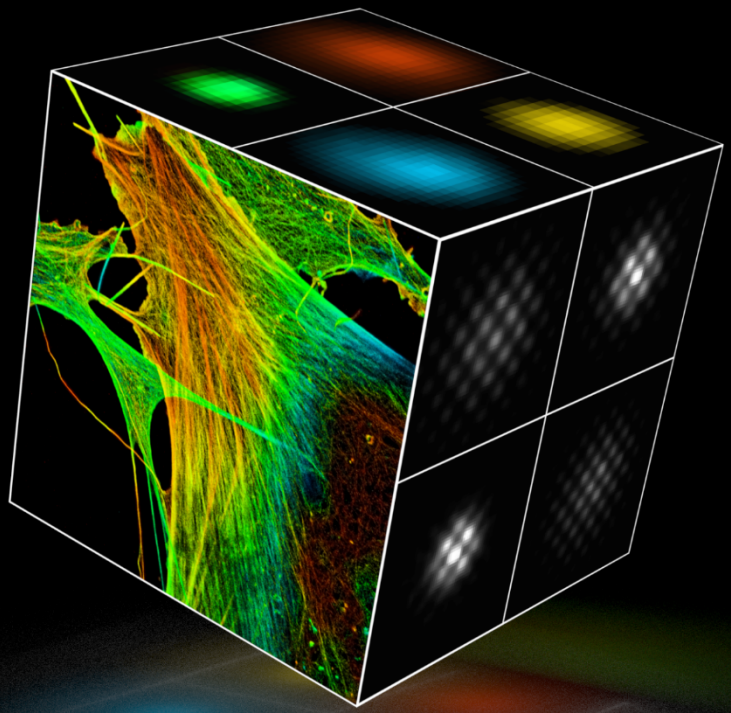
IRIMAS Institut de Recherche en Informatique, Mathématiques, Automatique et Signal (Université de Haute-Alsace, Mulhouse)

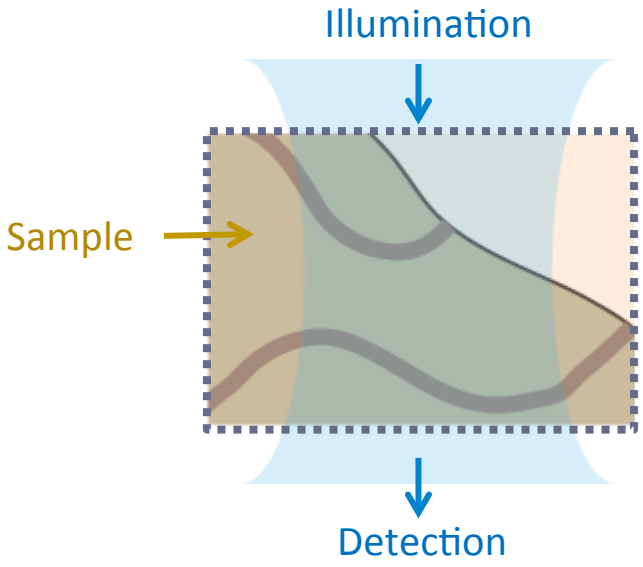


Biological sample imaging

Biological sample

Elastic Light Scattering

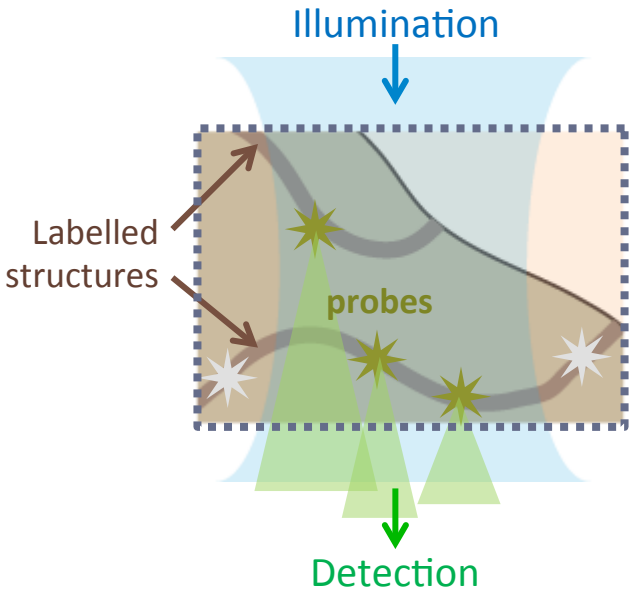
No illumination wavelength change



Dedicated techniques :
Phase contrast, interferometry…

Inelastic Light scattering

With illumination wavelength change



Dedicated techniques :
Fluorescence, Raman spectroscopy…

Biological sample imaging

Biological sample

Elastic Light Scattering

No illumination wavelength change

Illumination

Sample

Detection

Inelastic Light scattering

With illumination wavelength change

Illumination

Labelled

Detection

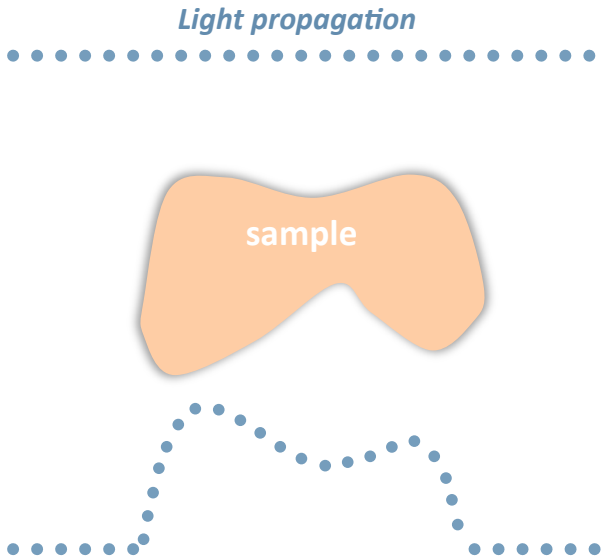
What is the phase of light in both cases ?

How can we interpret it ?

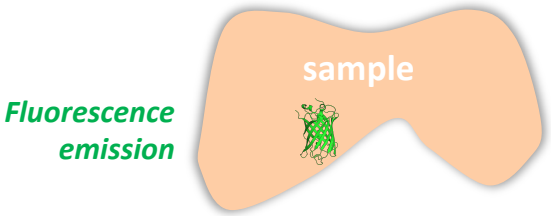
Dedicated techniques :
Phase contrast, interferometry…

Dedicated techniques :
Fluorescence, Raman spectroscopy…

Phase in Label-free VS phase of fluorescent light

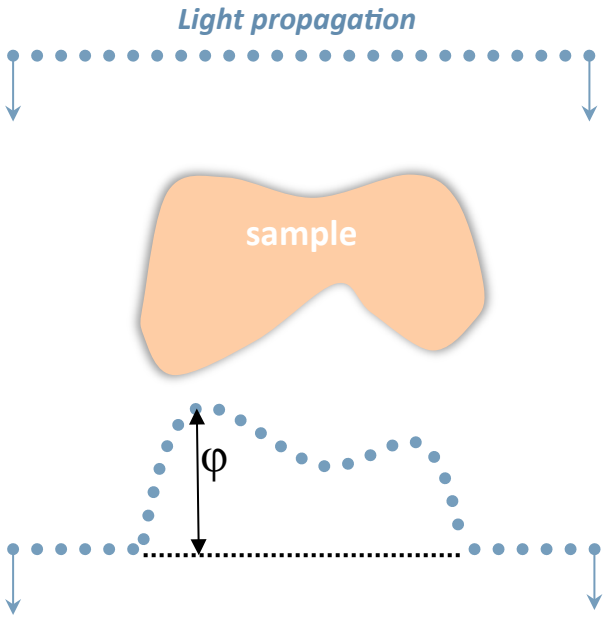


Phase φ in Endogenous contrast



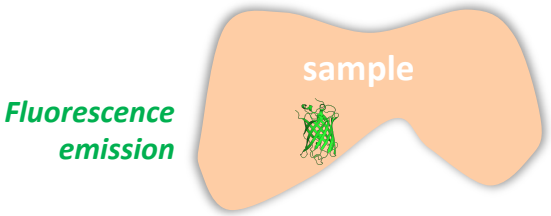
Phase φ in Labelling

Phase in Label-free VS phase of fluorescent light



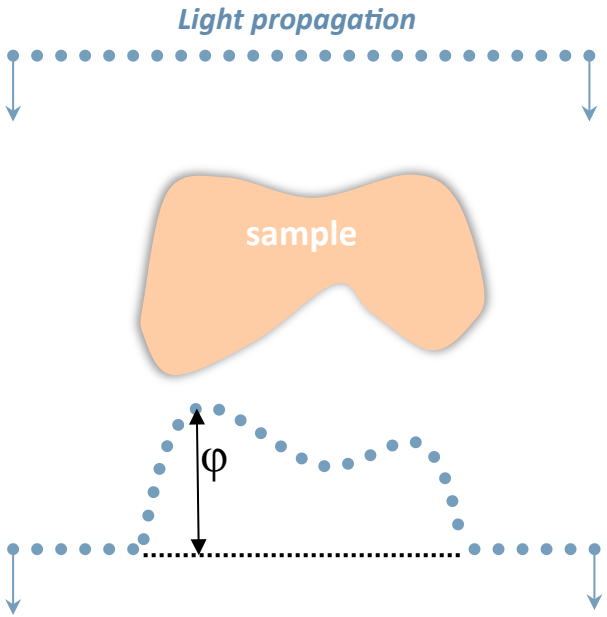
Phase φ in Endogenous contrast

φ <=> sample density of matter



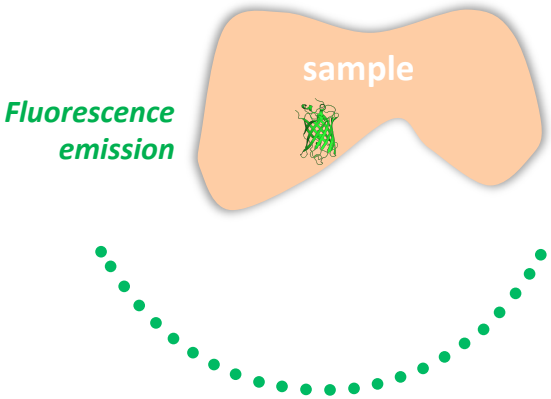
Phase φ in Labelling

Phase in Label-free VS phase of fluorescent light



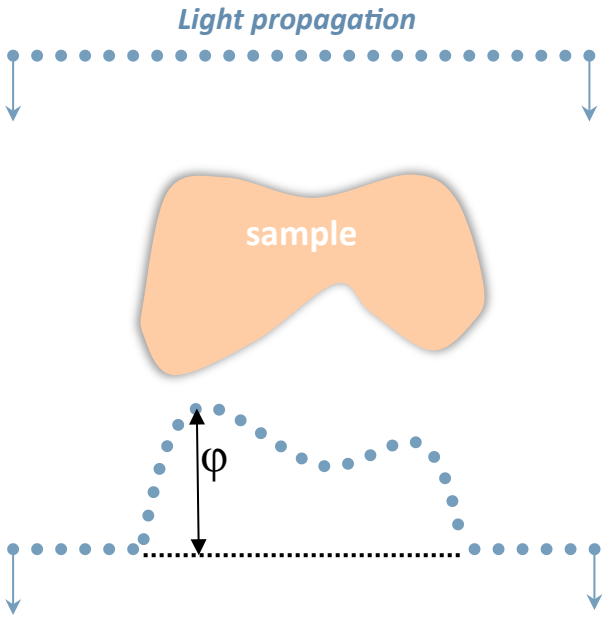
Phase φ in Endogenous contrast

φ ⇔ sample density of matter



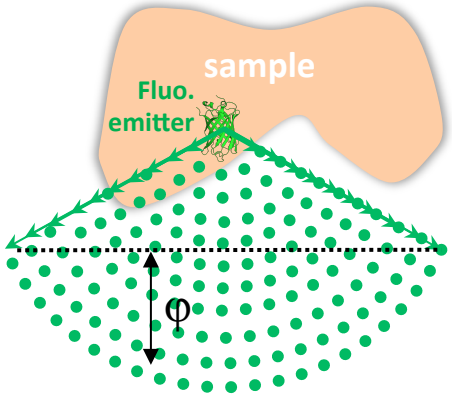
Phase φ in Labelling

Phase in Label-free VS phase of fluorescent light



Phase φ in Endogenous contrast

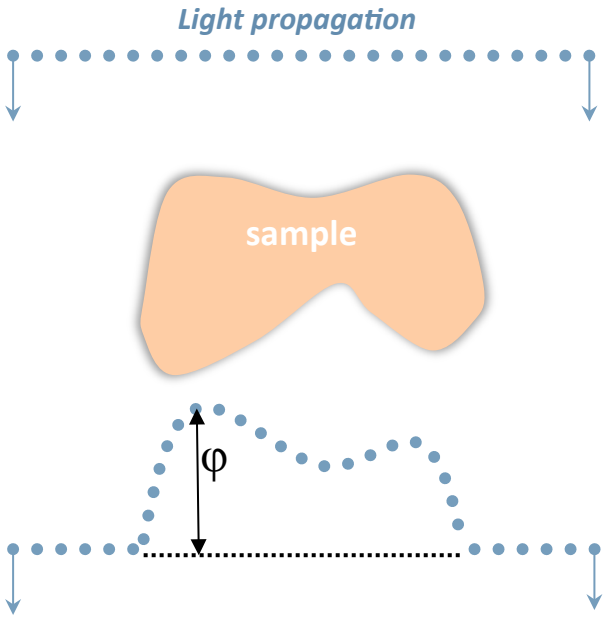
φ ⇔ sample density of matter



Phase φ in Labelling

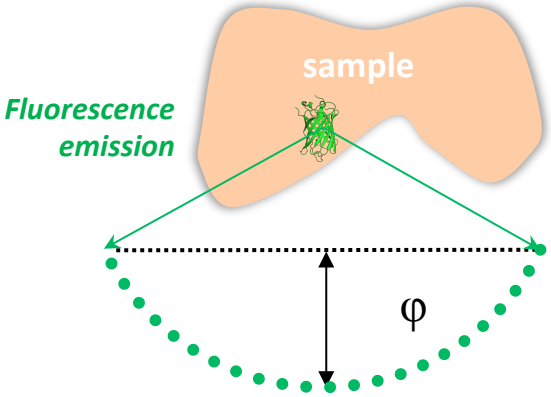
φ ⇔ distance to the light emitter

Phase in Label-free VS phase of fluorescent light



Phase φ in Endogenous contrast

φ ⇔ sample density of matter



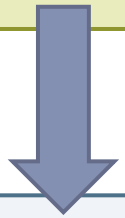
Phase φ in Labelling

φ ⇔ distance to the light emitter

Outlines

- Quantitative Phase Imaging + Fluorescence Imaging
  - Multimodal imaging (with some examples)
- Quantitative Phase Imaging in fluorescence
  - 3D localization of fluorescent emitters: SELFI

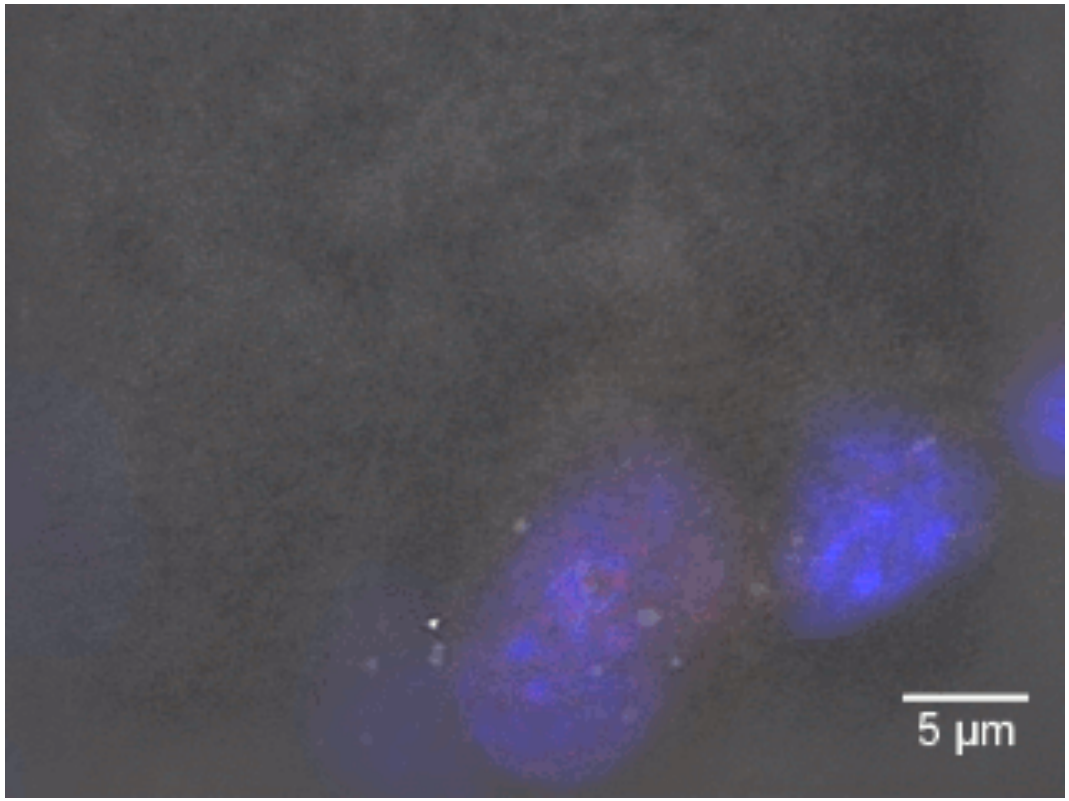
Fluorescence for features identification in QPI



Ex: label-free Organelles & cytoskeleton imaging

Diffraction Tomography & Fluorescence

***Z-stack of A549 cells* (63x, NA=1.32)**



Red fluorescence = viral nucleoprotein (human influenza H3N2 virus)

Blue = DAPI (DNA)

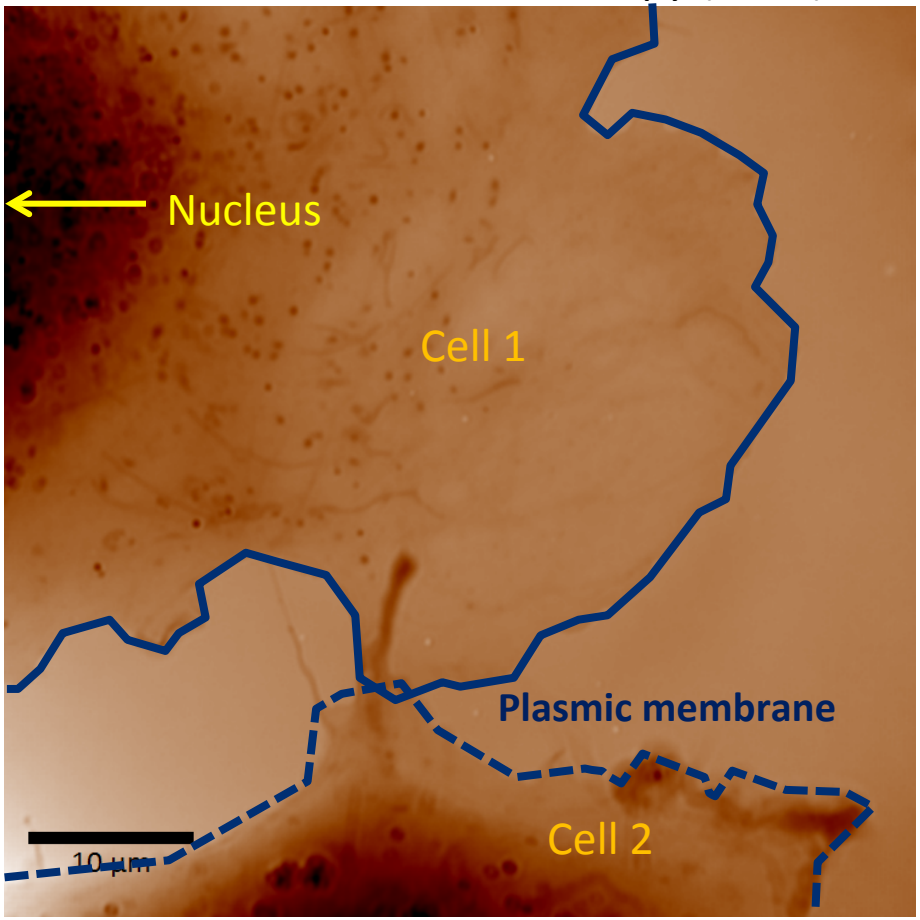
Grey level = refraction (QPI)

"High resolution tomographic diffractive microscopy of biological samples"
B. Simon, et al., J. Biophotonics **3**, p. 462 (2010)

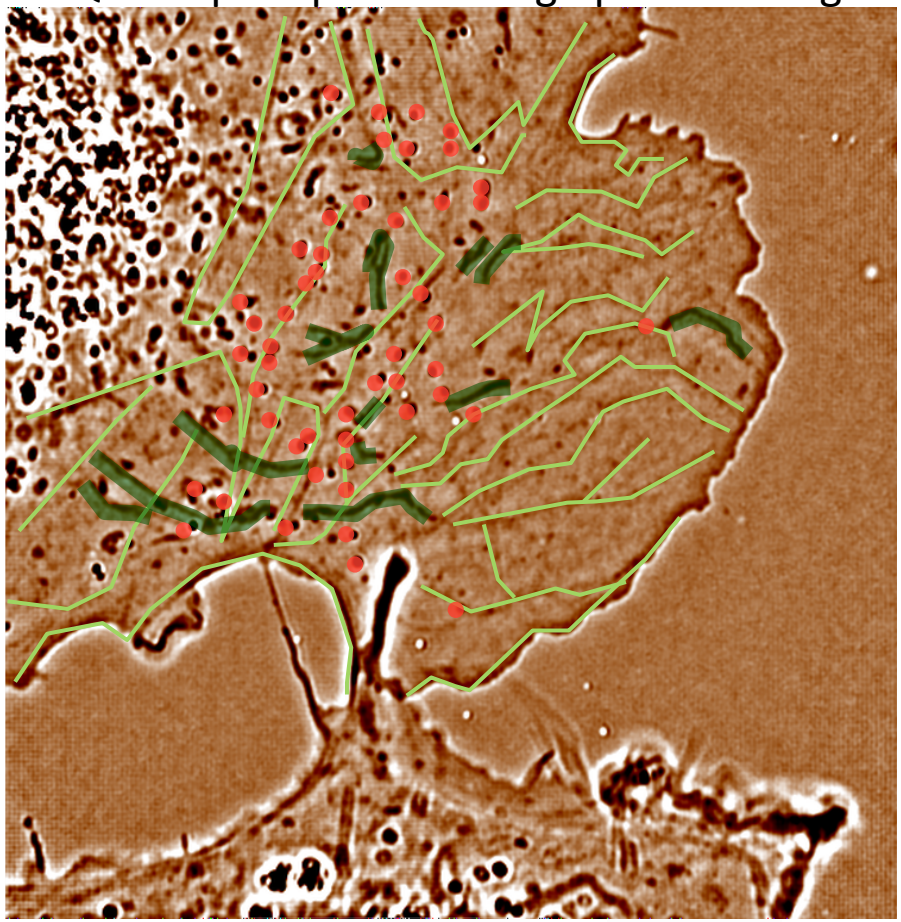
Label-free cytoskeleton imaging

- *Living CHO cells* (60x NA=1.49)

Quantitative Phase Microscopy (QPM)



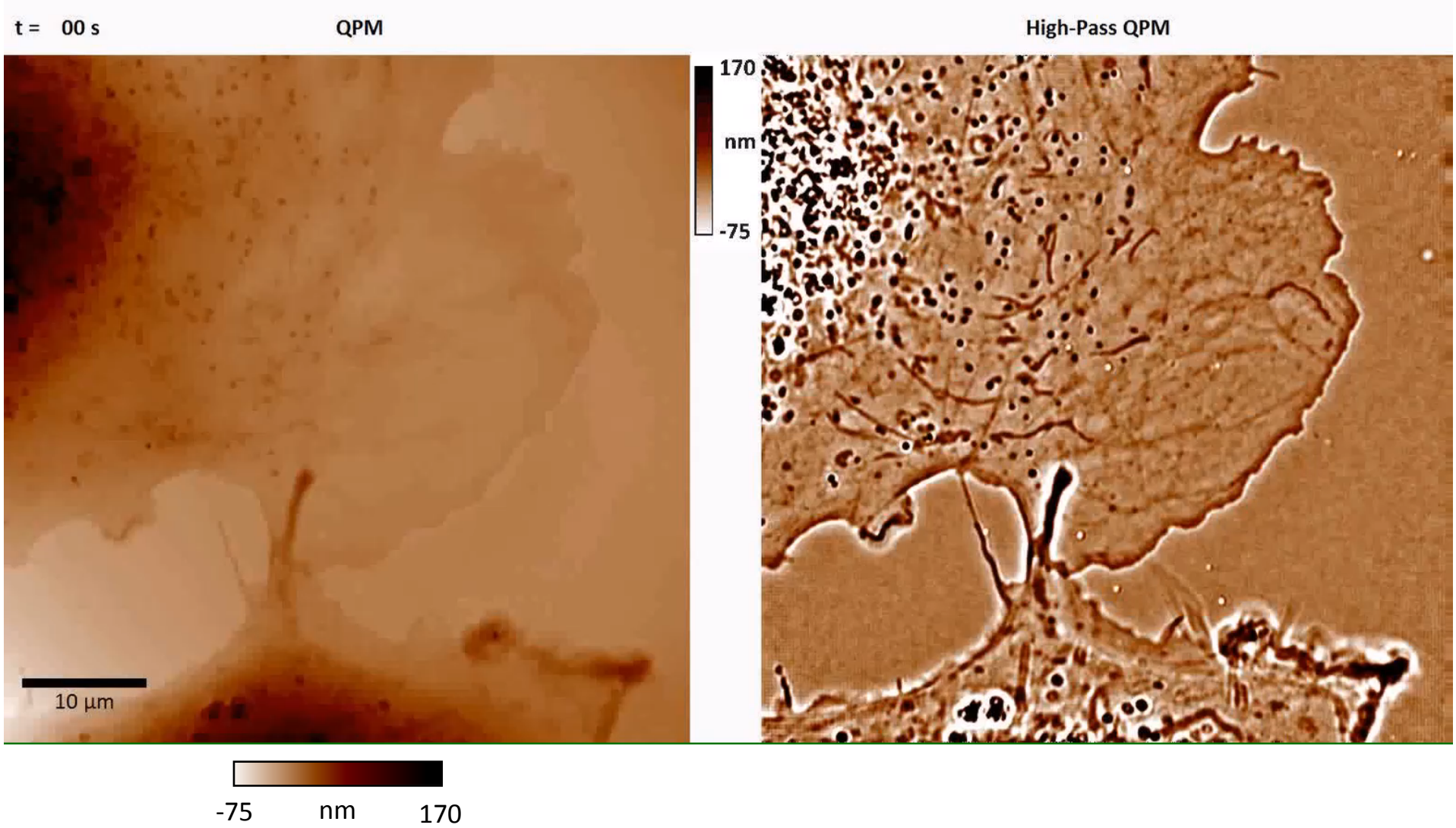
QPM + post-process : High pass filtering



Bon *et al.*, Biophys. J. 2014

Label-free cytoskeleton imaging

- *Living CHO cells* (60x NA=1.49)

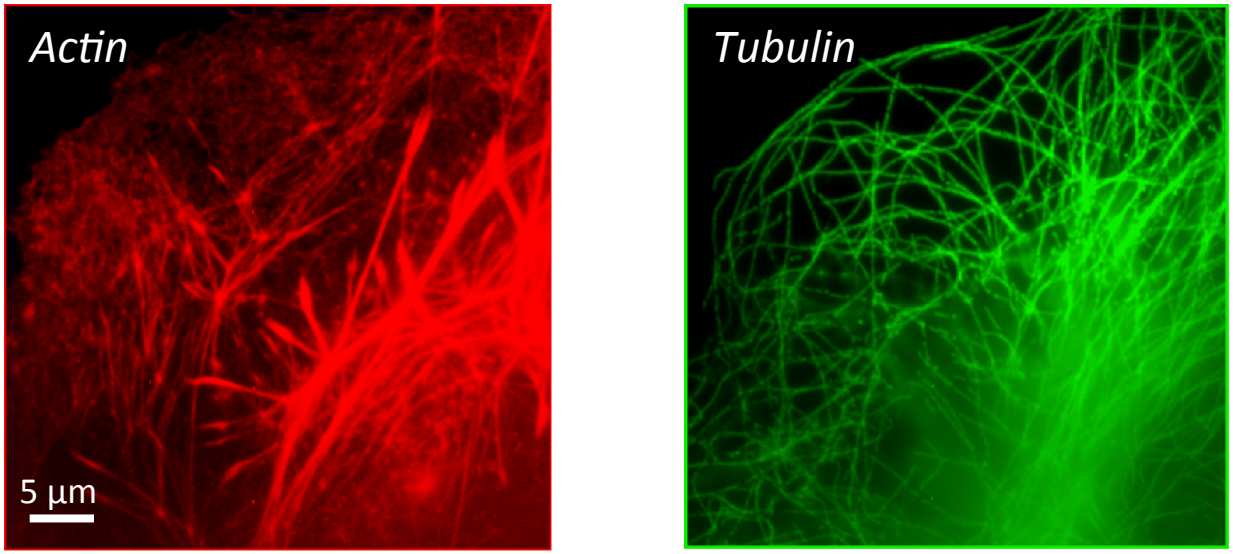


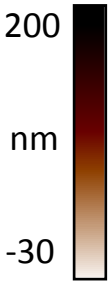
Bon *et al.*, Biophys. J. 2014

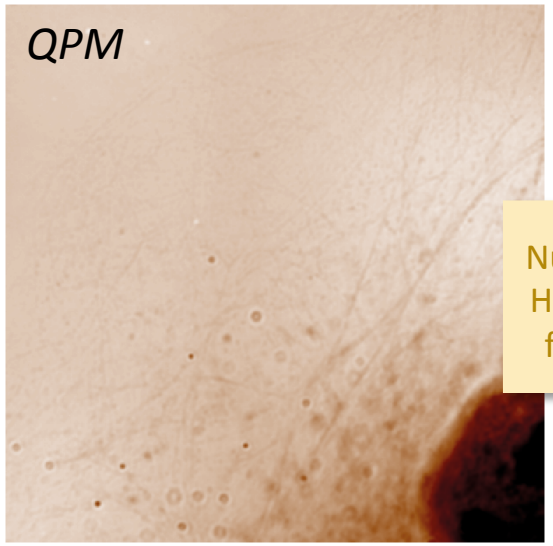
Fluorescence colocalization for demonstration

- Multimodality phase / fluorescence[1,2]
- Fixed CHO cells, double immunofluo. label. F-actin + α-tubulin

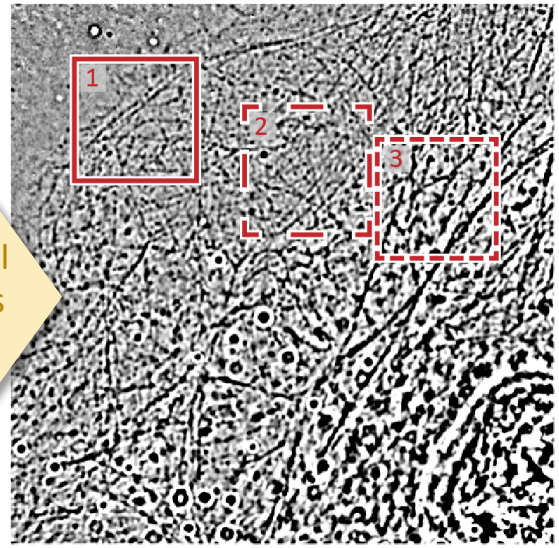
1 : Bon *et al.*, JBO 2012
2 : Bon *et al.*, Biophys. J. 2014

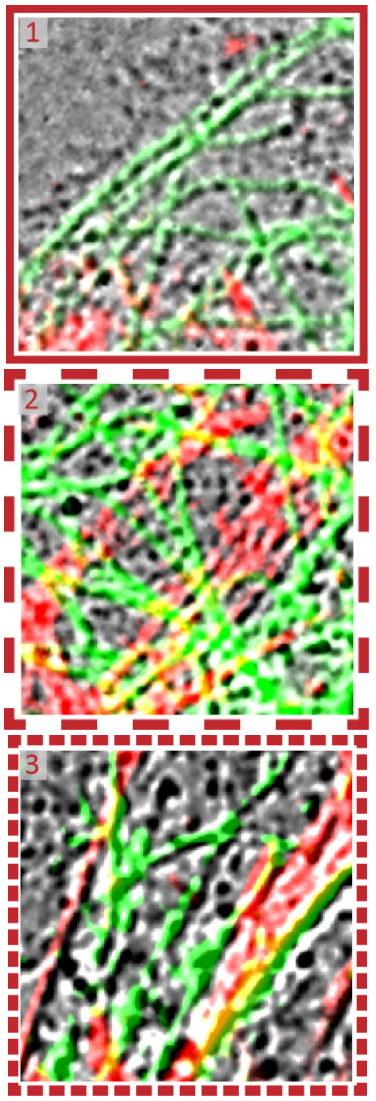






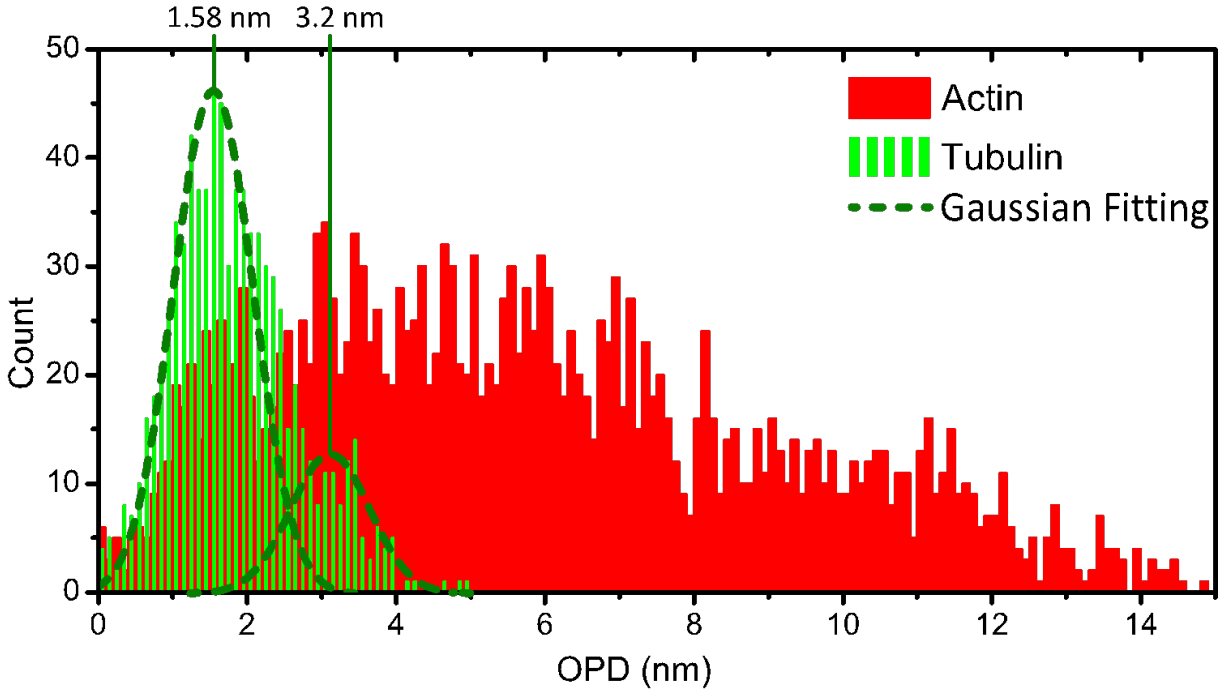
Numerical High-pass filtering



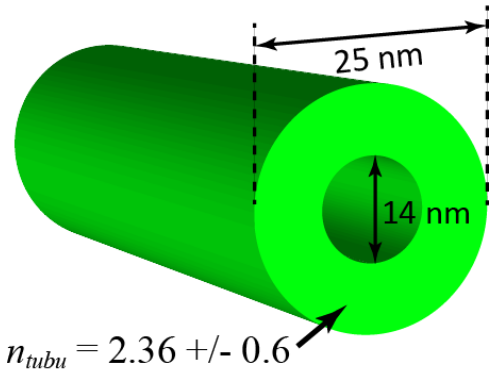


Quantitative difference between Actin and tubulin fibers

OPD histograms (using fluo. for identification)



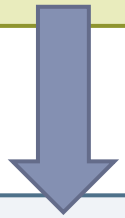
Tubulin microtubule



- ✓ **Microtubules can be differenciated from Actin** using the OPD value
- ✓ Refractive index has been determined[1] for microtubules to be $n_{tubu} = 2.36 \pm 0.6$

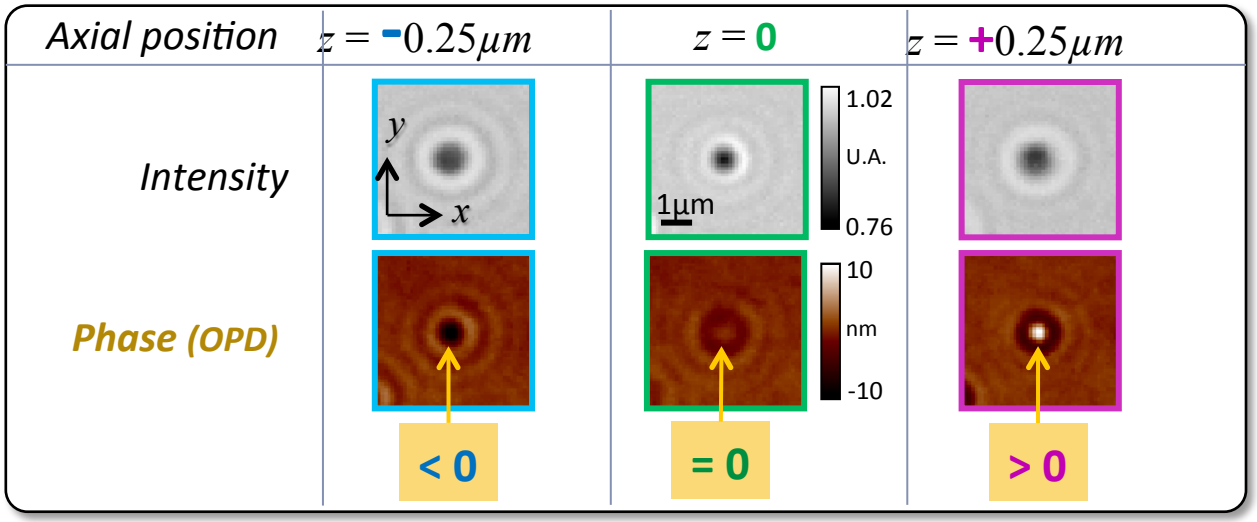
Bon *et al.*, Biophys. J. 2014

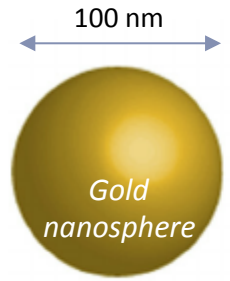
QPI to enhance fluorescence imaging

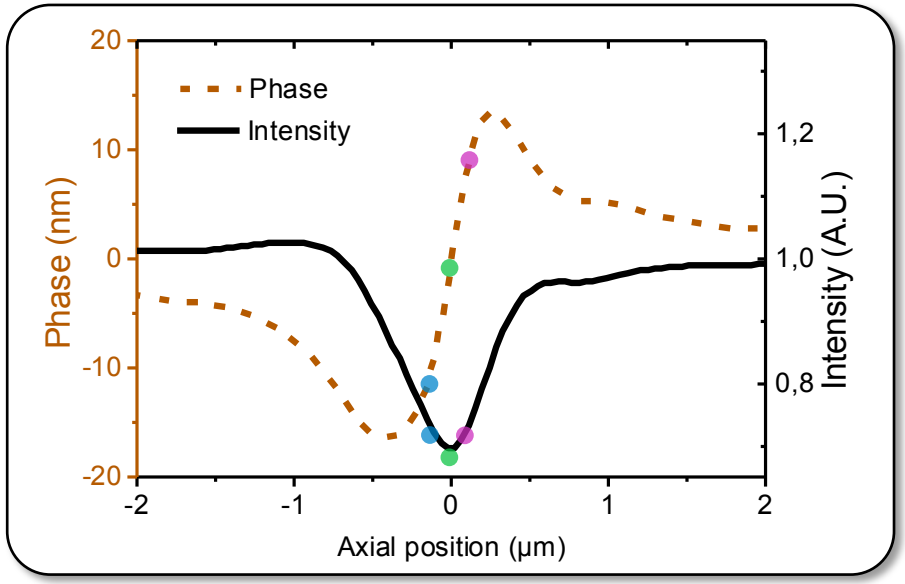


Ex: 3D autofocus with QPI for microscope stabilization

Intensity and phase measurements of absorbing particles







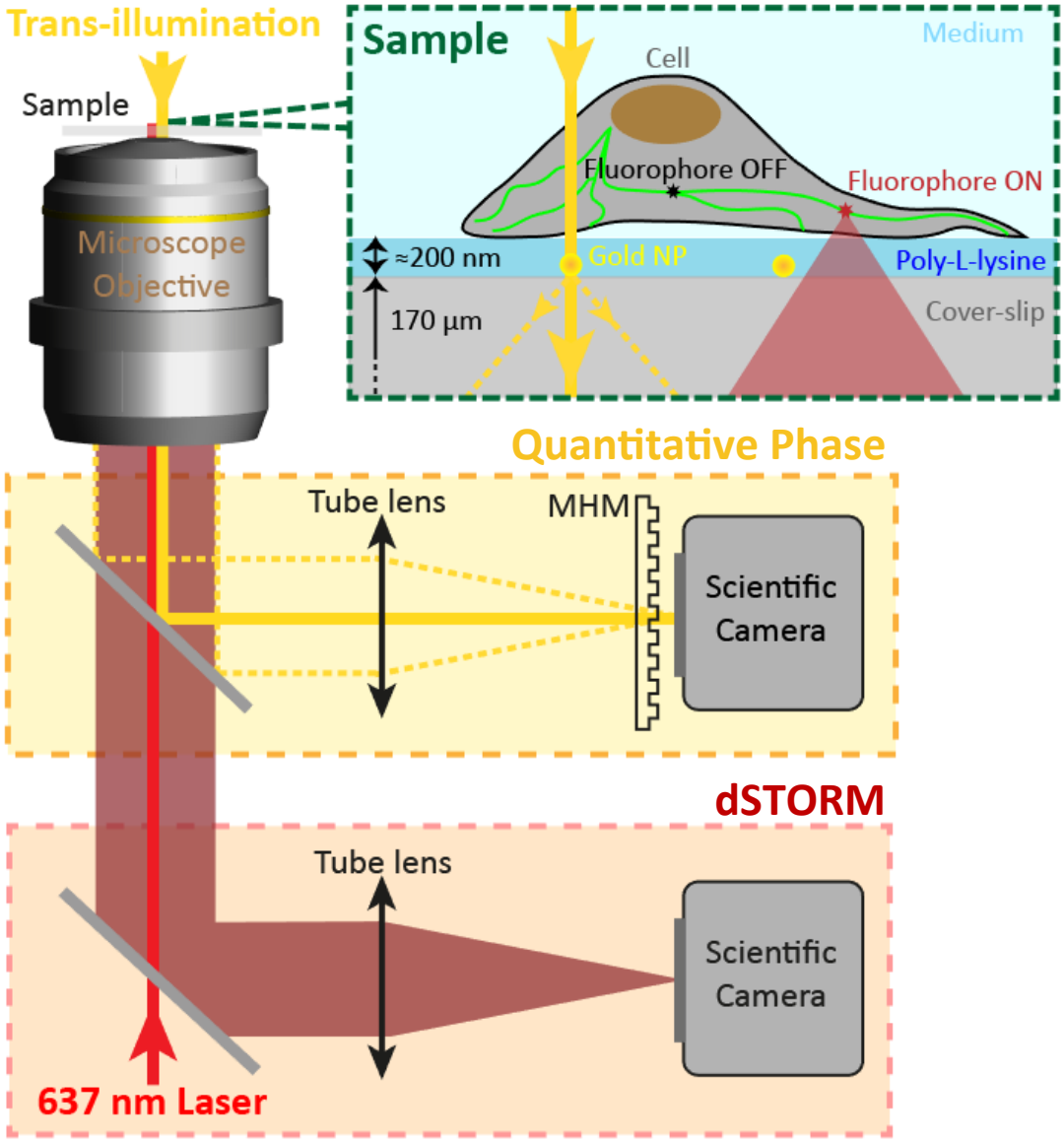
3D Localization

- ✓ **Intensity** = lateral (xy)
- ✓ **Phase** = axial (z)

Collab. J. Wenger (Institut Fresnel), S. Lévêque-Fort (ISMO)

Bon *et al.*, Nature Communication, 2015

Application: 3D drift correction for super-resolution



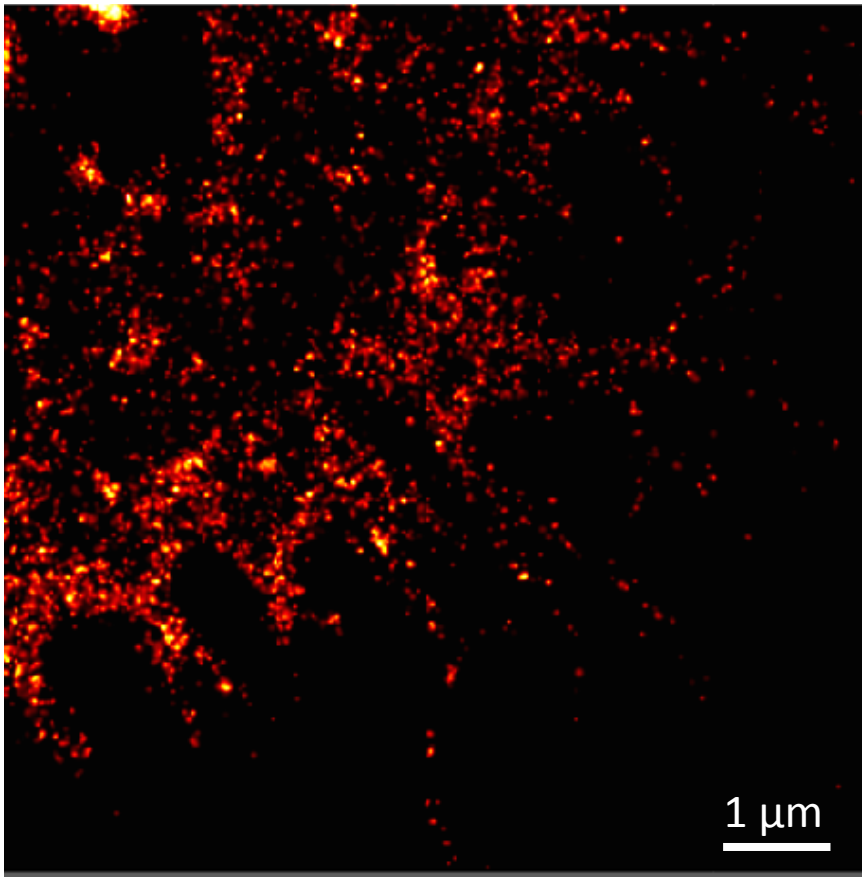
Collab. J. Wenger (Institut Fresnel), S. Lévêque-Fort (ISMO)

Bon *et al.*, Nature Communication, 2015

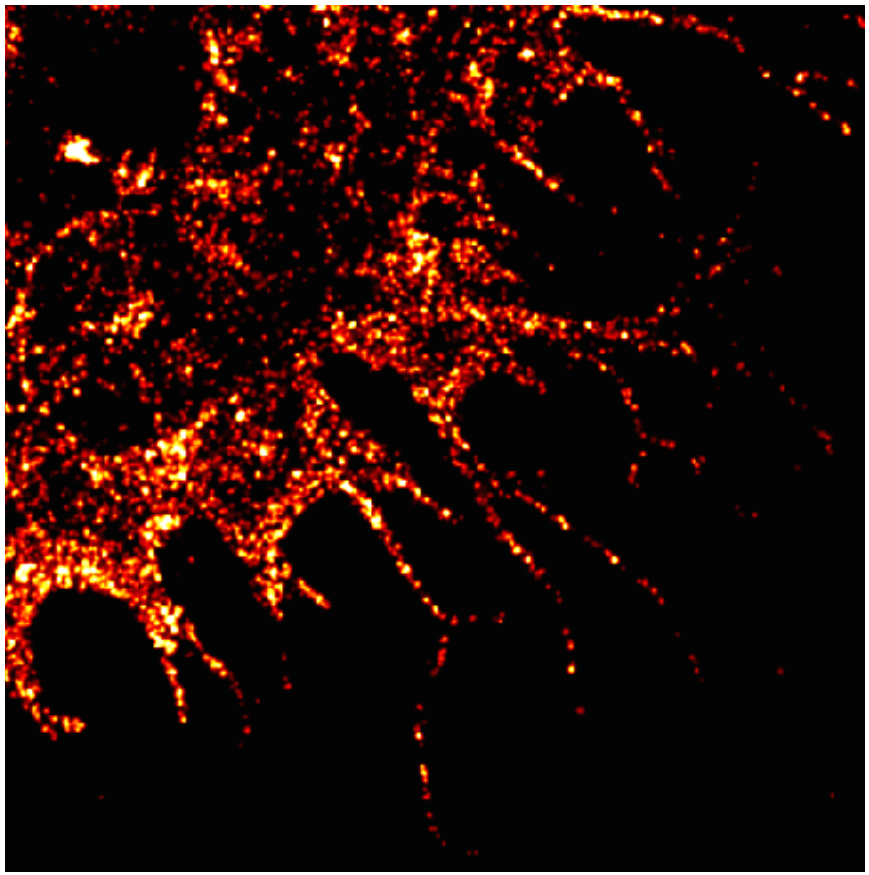
Application: 3D drift correction for dSTORM

dSTORM F-actin imaging (CHO cells)

No drift compensation



3D drift compensation



Collab. J. Wenger (Institut Fresnel), S. Lévêque-Fort (ISMO)

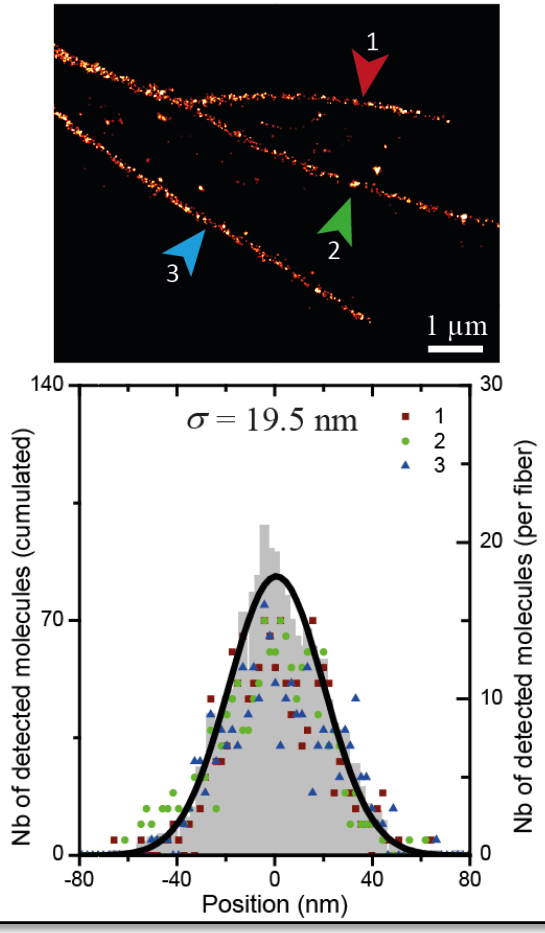
Bon *et al.*, Nature Communication, 2015

Comparison with standard drift-compensation approach

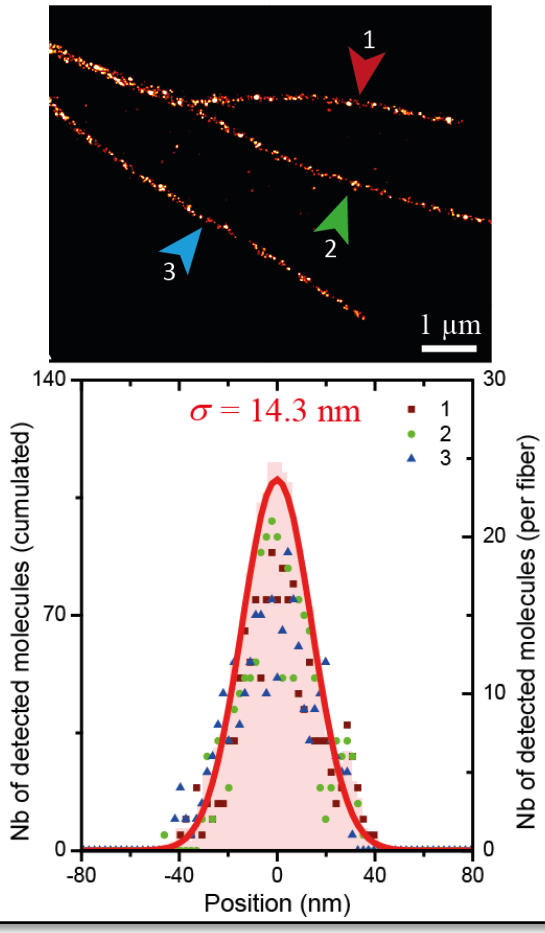
dSTORM F-actin imaging (CHO cells)

Bon *et al.*, Nature Communication, 2015

Fluorescent bead (*xy* drift)
+ Nikon Perfect focus (*z* drift)



Our 3D approach

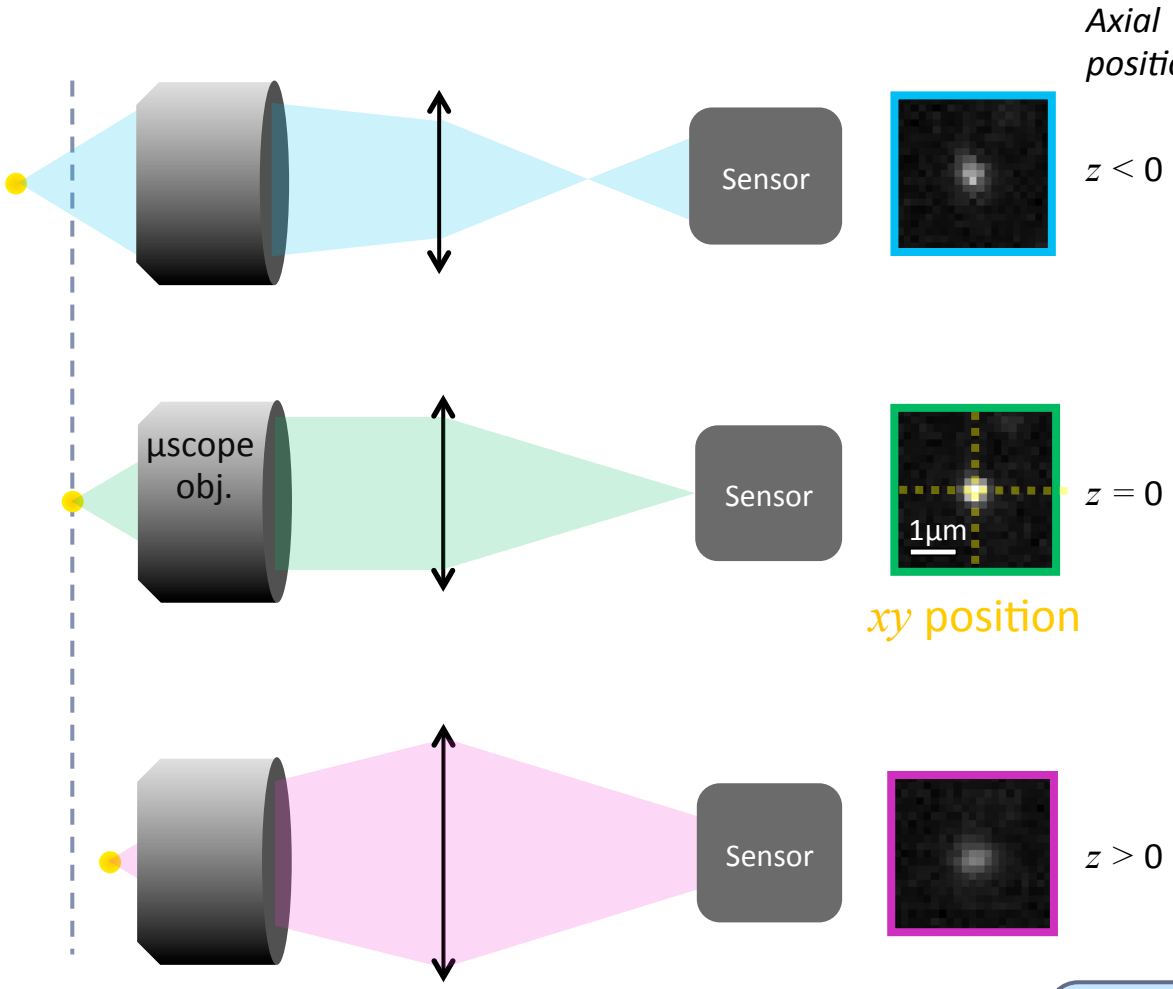


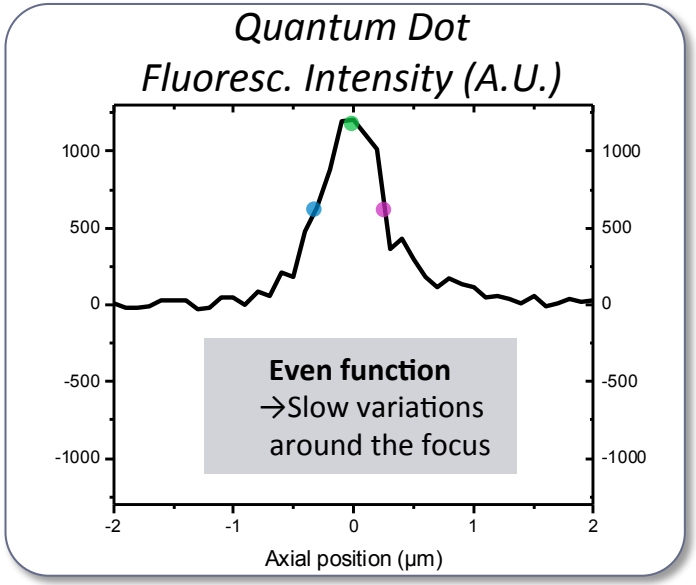
26 % resolution improvement

Outlines

- Quantitative Phase Imaging + Fluorescence Imaging
  - Multimodal imaging (with some examples)
- Quantitative Phase Imaging in fluorescence
  - 3D localization of fluorescent emitters: SELFI

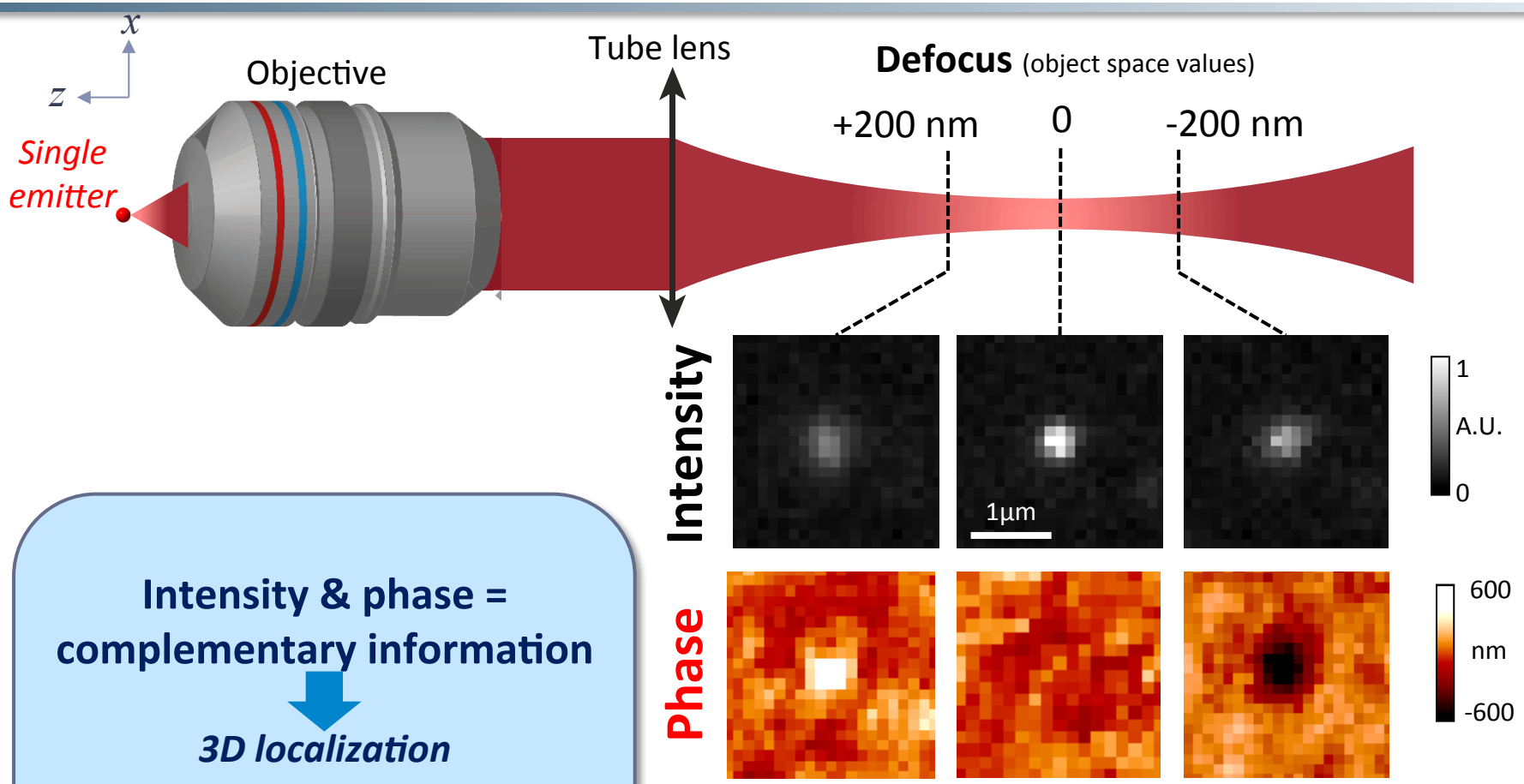
3D super-localization ?





How to obtain axial superlocalization ?

3D: phase and intensity of a single fluorescent emitter



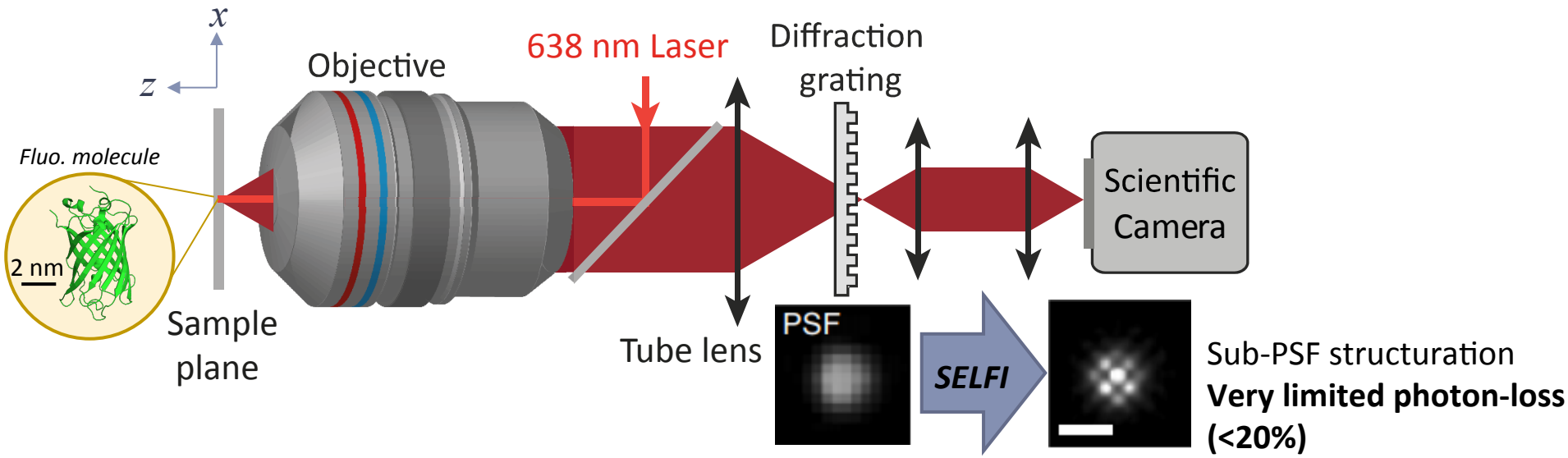
Intensity & phase = complementary information

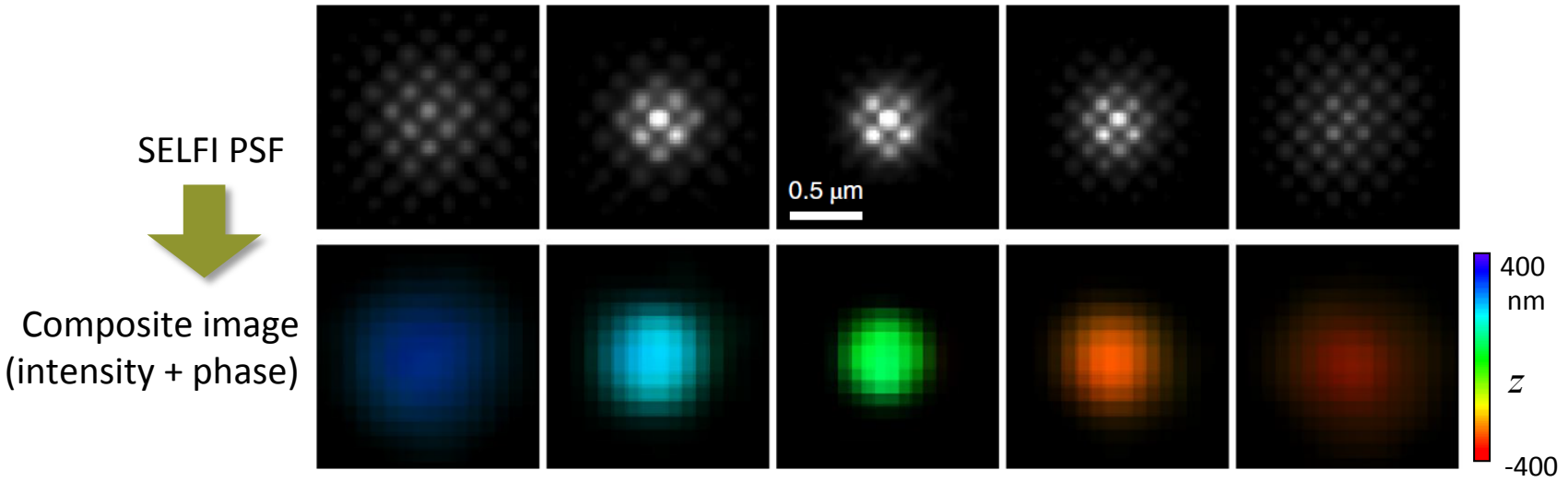
3D localization

✓ **Intensity** = lateral (xy)
✓ **Phase** = axial (z)

Bon *et al.* Nat. Methods 2018

« Self-interferences »: SELFI





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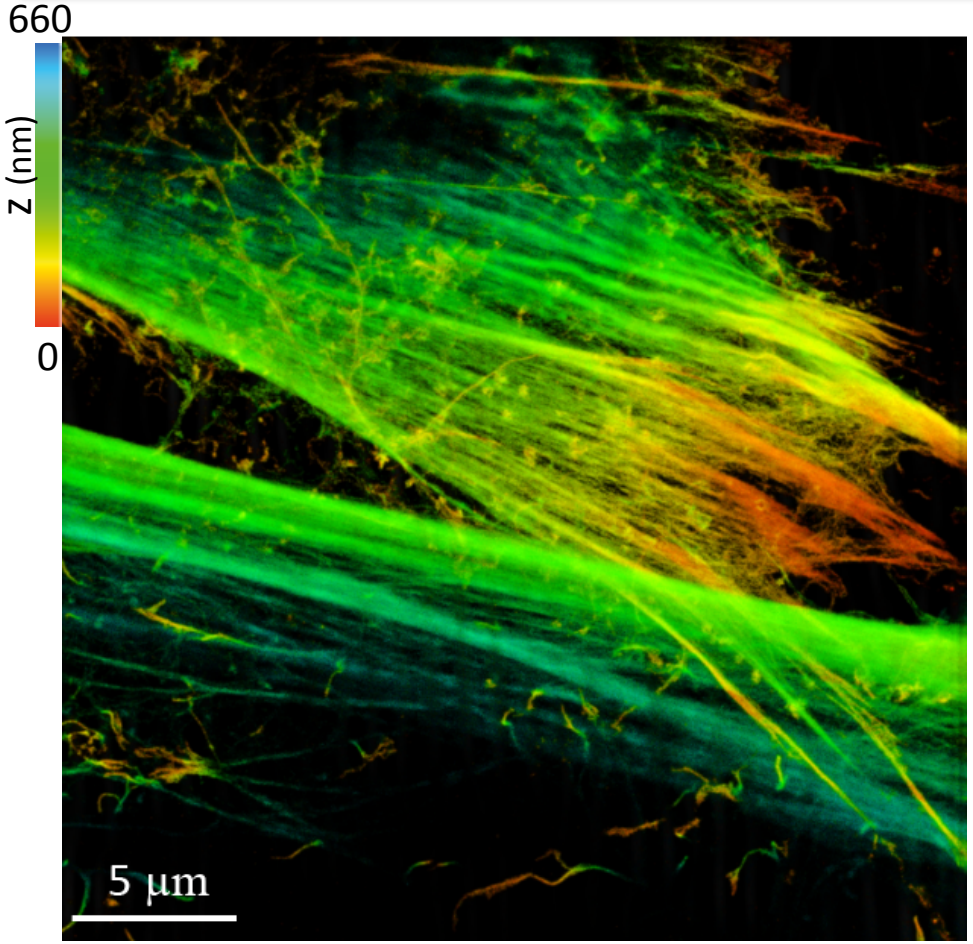
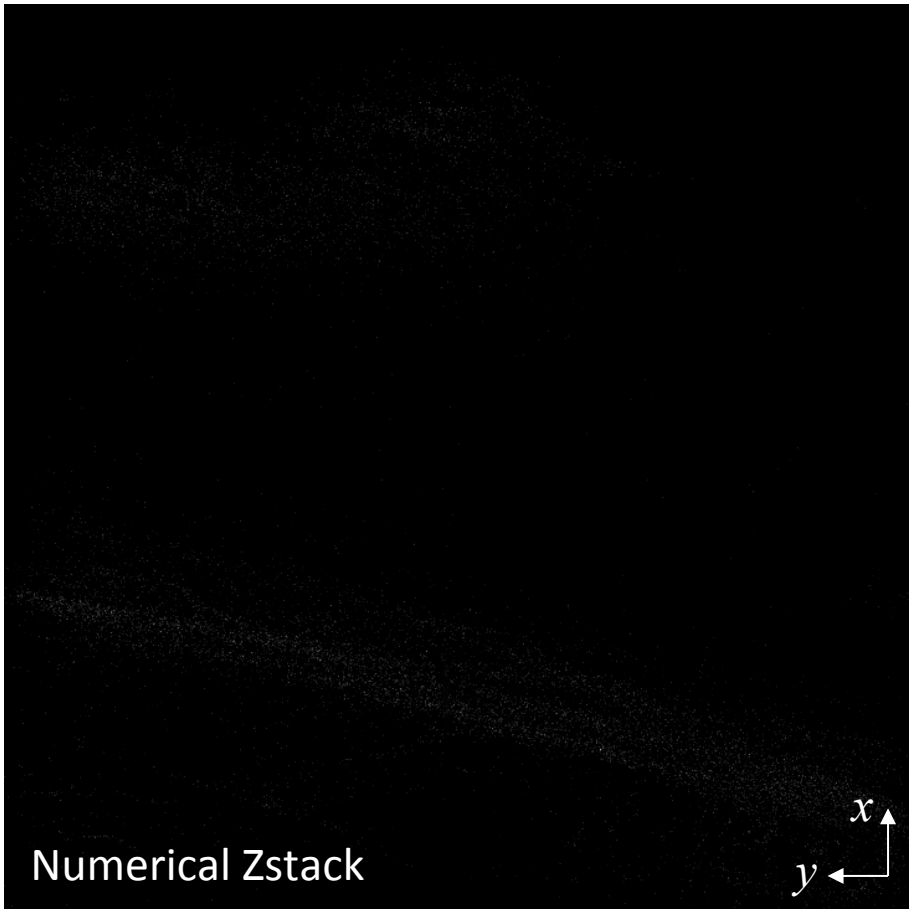
3D dSTORM reconstruction near a coverslip





Human fibroblast, f-actin labelled with A647/Phalloidin
60x NA=1.3, 3D dSTORM imaging ***during 8 hours (24M molecules)***

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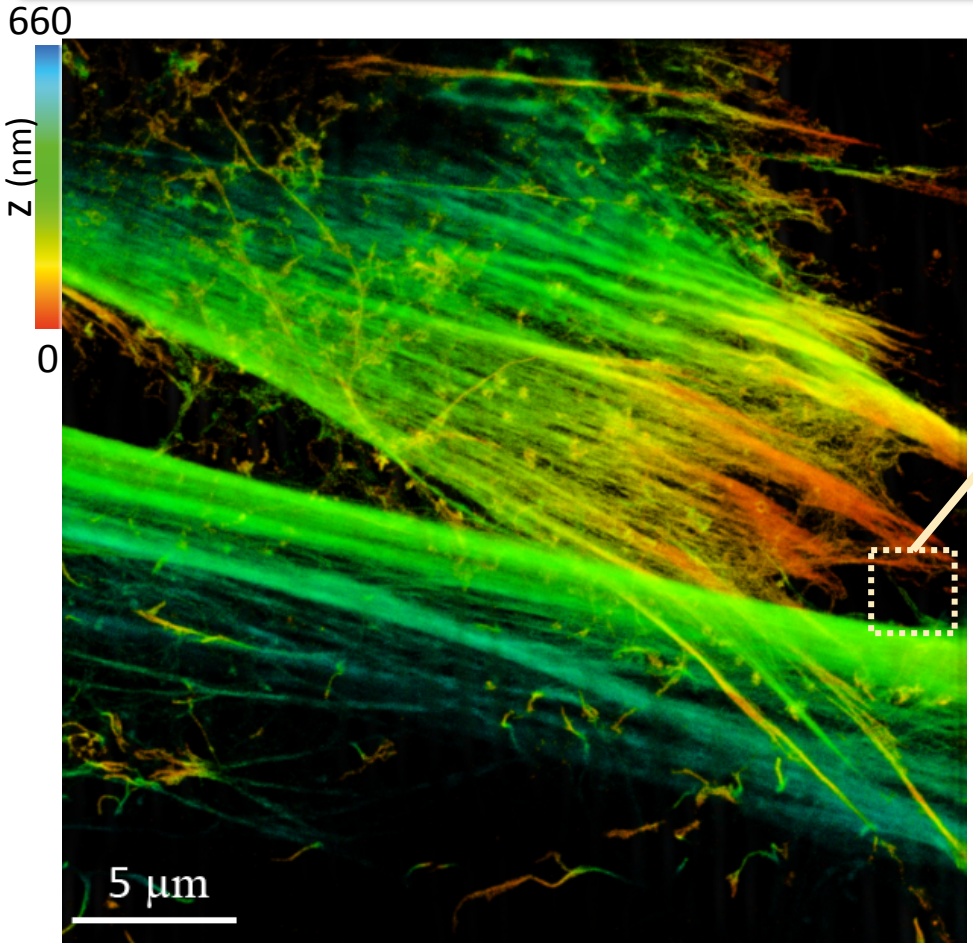
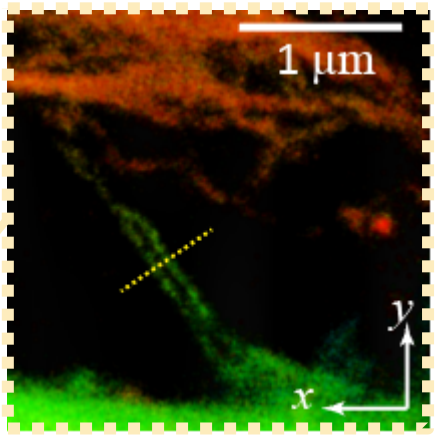
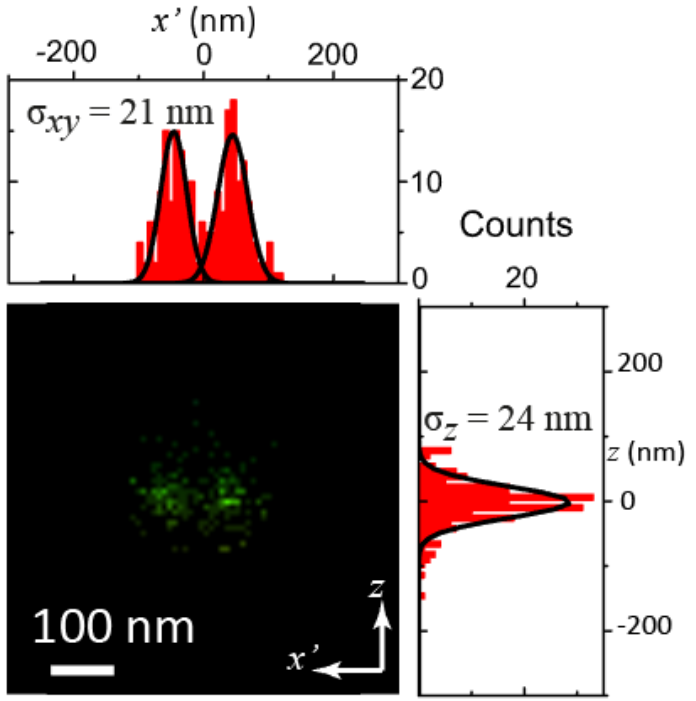
3D dSTORM reconstruction near a coverslip







≈ **isotropic 3D resolution**

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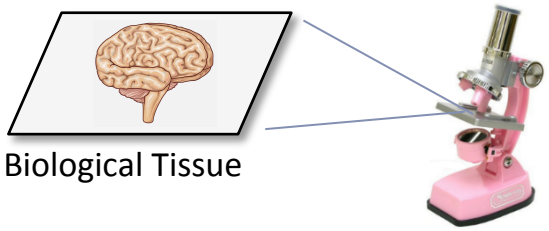
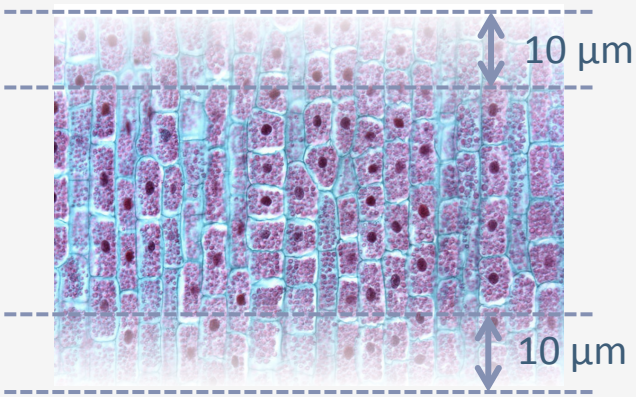
Biological tissue imaging challenges

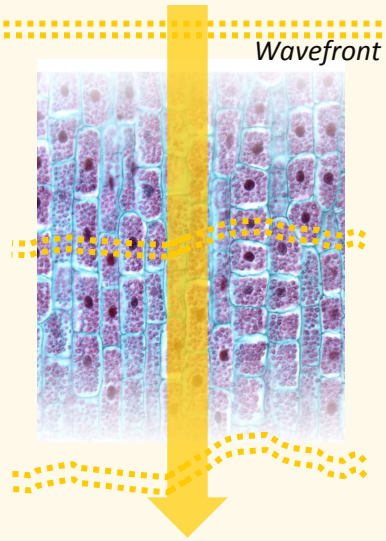


Sample limitations

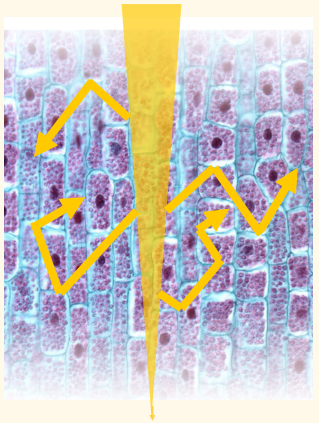


Surface structures are damaged

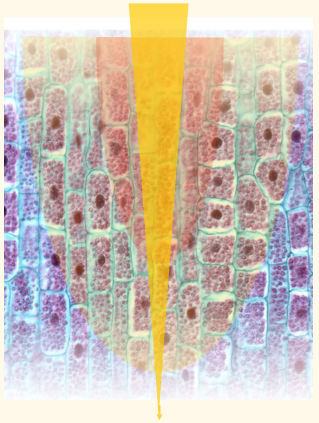
Optical limitations



Aberrations



Scattering



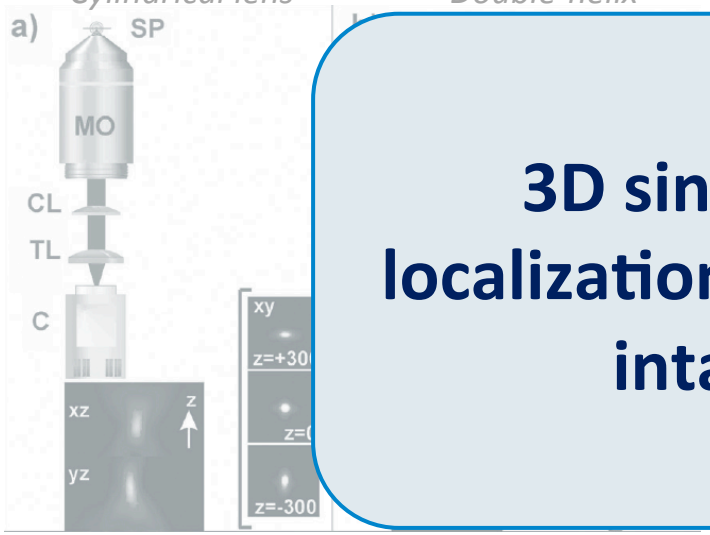
Absorption

Photon loss

Main strategies to detect in 3D… Challenging in tissue

PSF shaping

Cylindrical lens



Kao and Verkman, Biophys. J., 1994

Double-helix

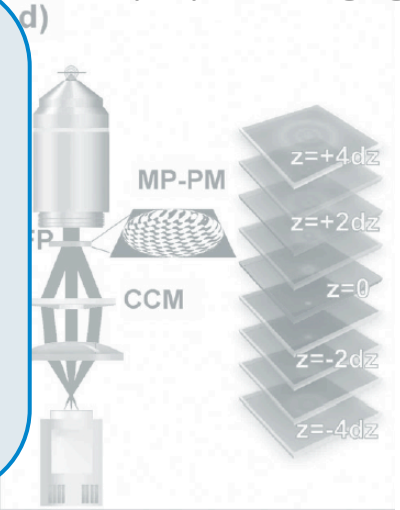
Pavani and Piestun, Optics Express, 2008

Multiple plane imaging

Bi-plane Imaging

Prabhat et al., *IEEE Trans Nanobiosci., 2004*

Grating assisted multiple-plane Imaging



Abrahamsson et al., *Nat. Meth., 2013*

3D single molecule localization is challenging in intact tissue!

Limitations:

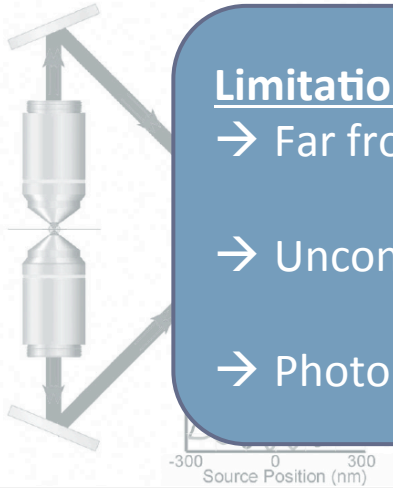
→ Far from the interface

→ Uncontrolled optical aberrations

→ Photon-loss

iPALM

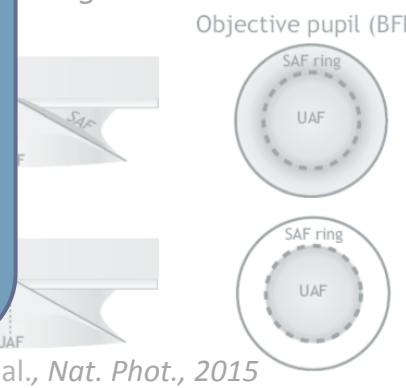
Double-obj. +3 detectors



Shtengel et al., *PNAS, 2009*

…ssion properties

…l Angle Fluorescence

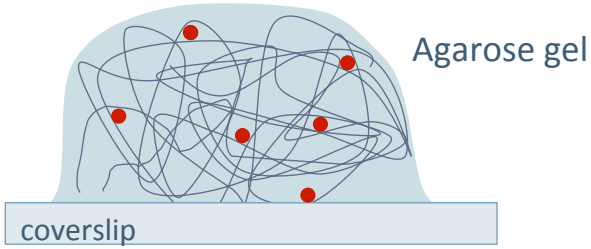


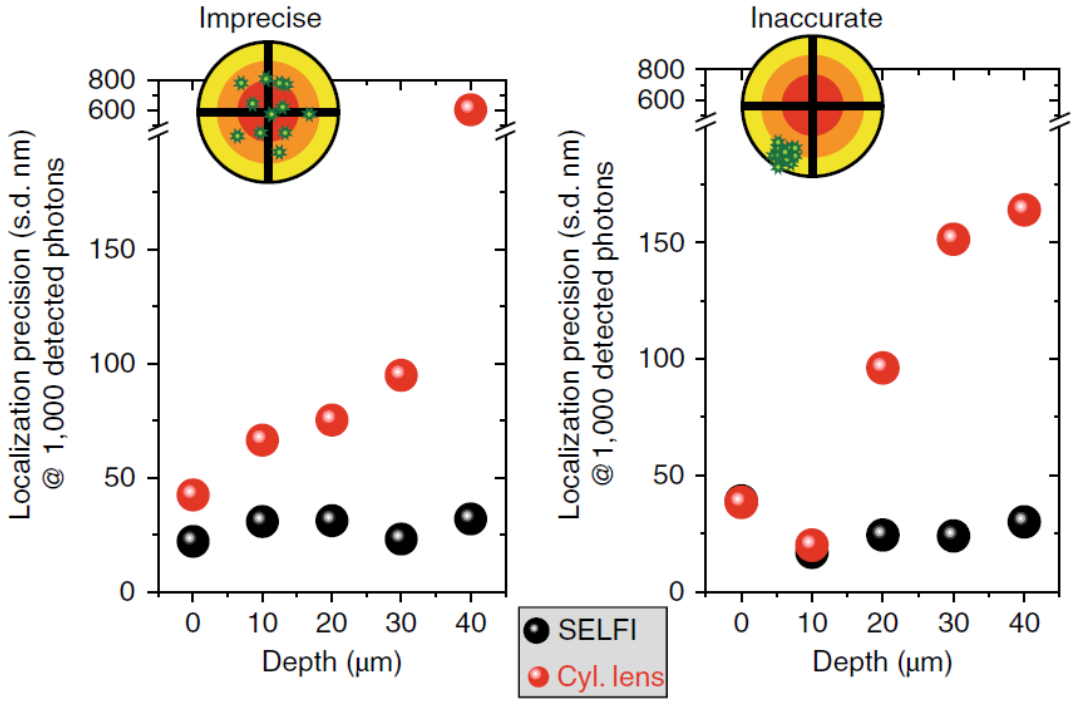
Bourg et al., *Nat. Phot., 2015*

SELFI is weakly sensitive to aberrations

3D sample phantom

100 nm fluo. beads

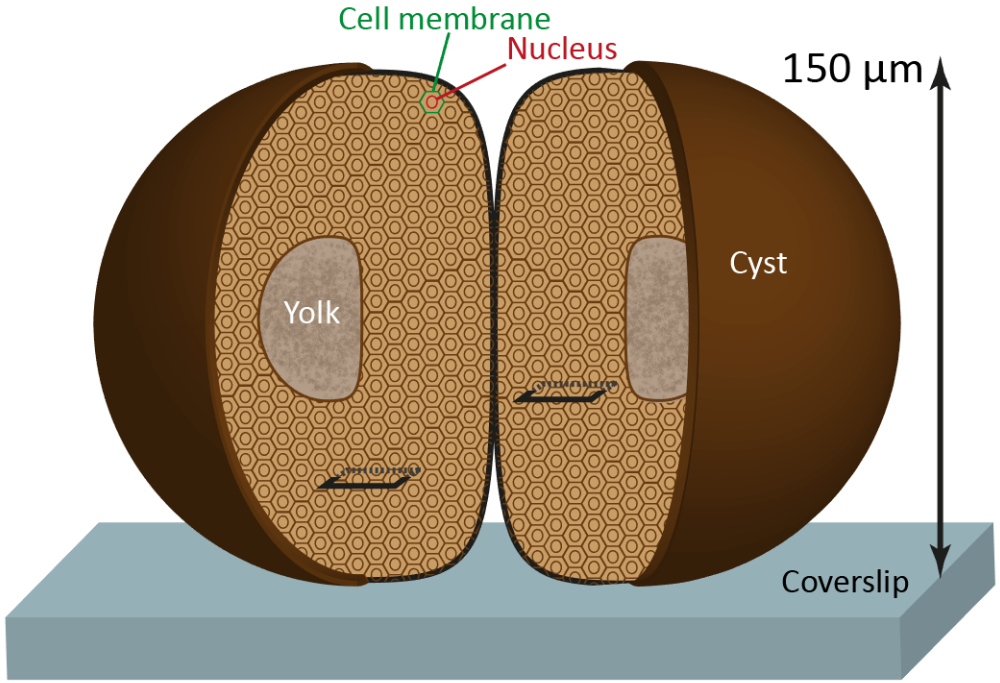




For each position :
1000 images of 100 nm fluorescent bead

Tissue 3D super-resolution is unlocked with SELFI!

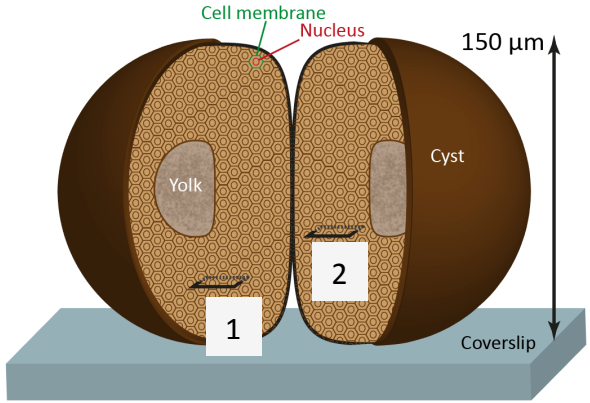
Non-cleared Tissue super-resolution



Human stem cells, f-actin labelled with A647/Phalloidin
60x NA=1.3, 3D dSTORM imaging

Collaboration with P. Nassoy
(LP2N, Bordeaux)

Non-cleared Tissue super-resolution



Human stem cells, f-actin labelled with A647/ Phalloidin
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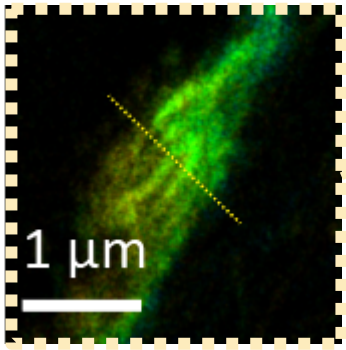
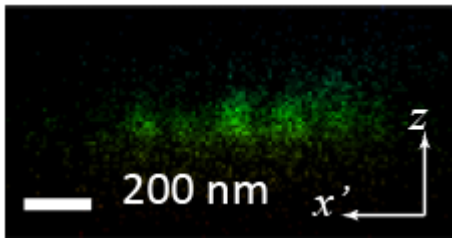
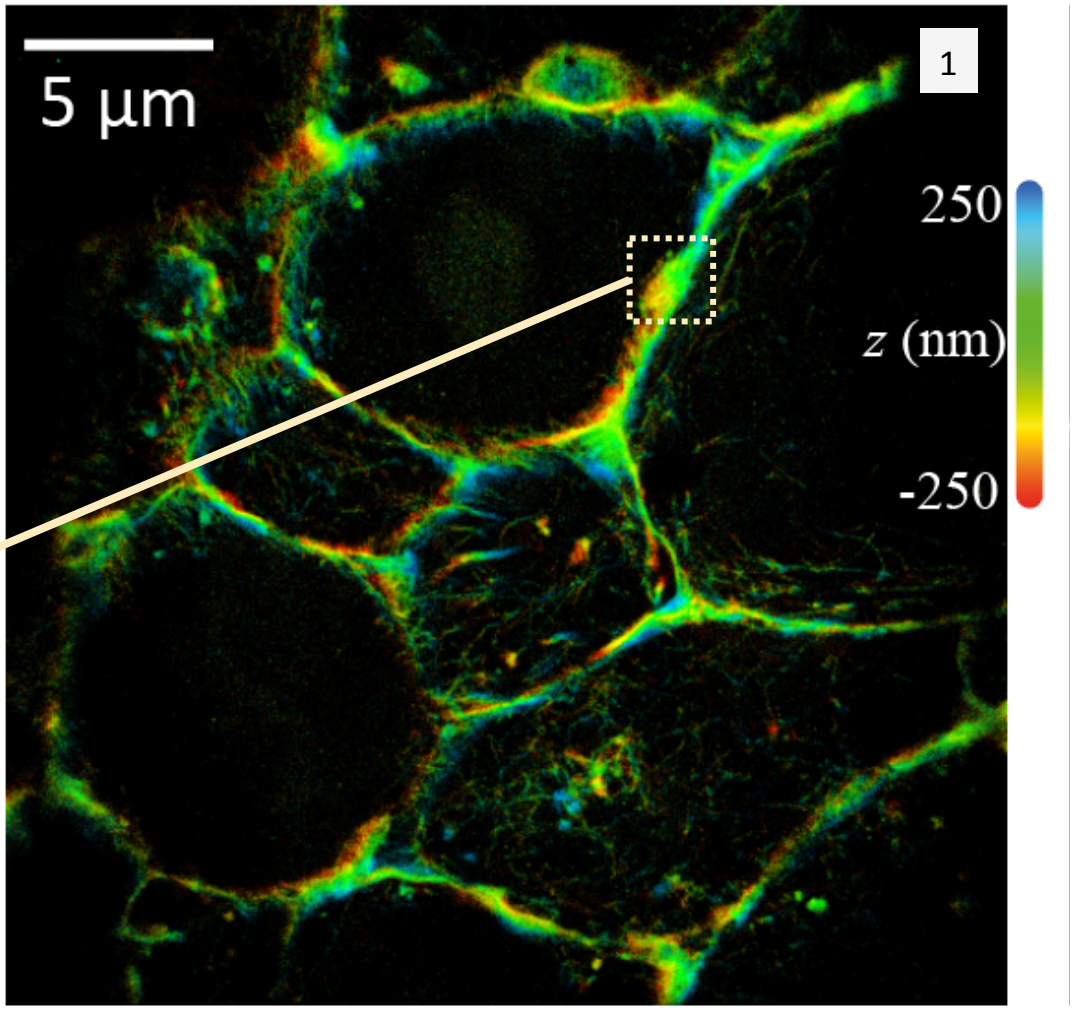




Depth = 25 µm



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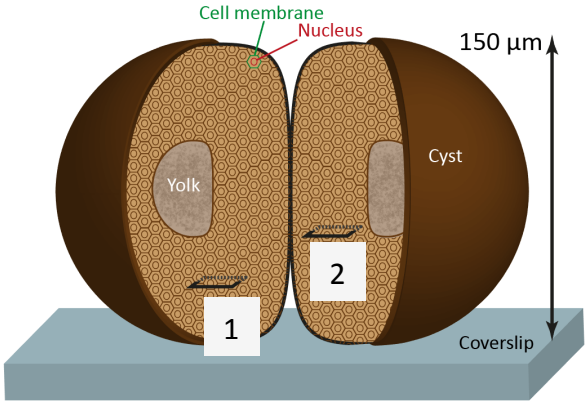
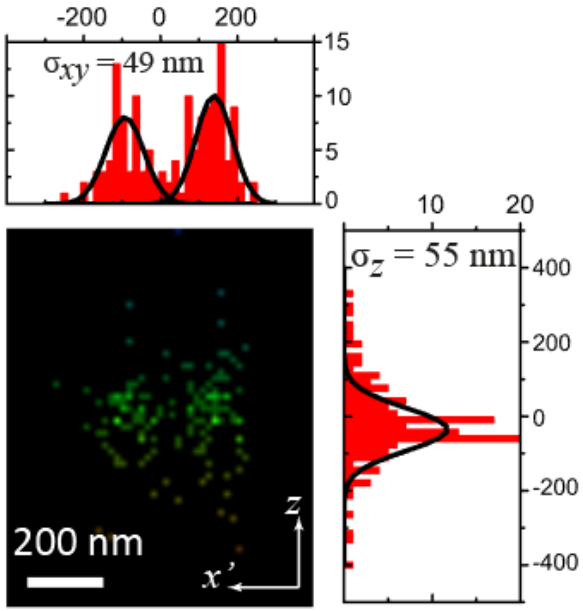
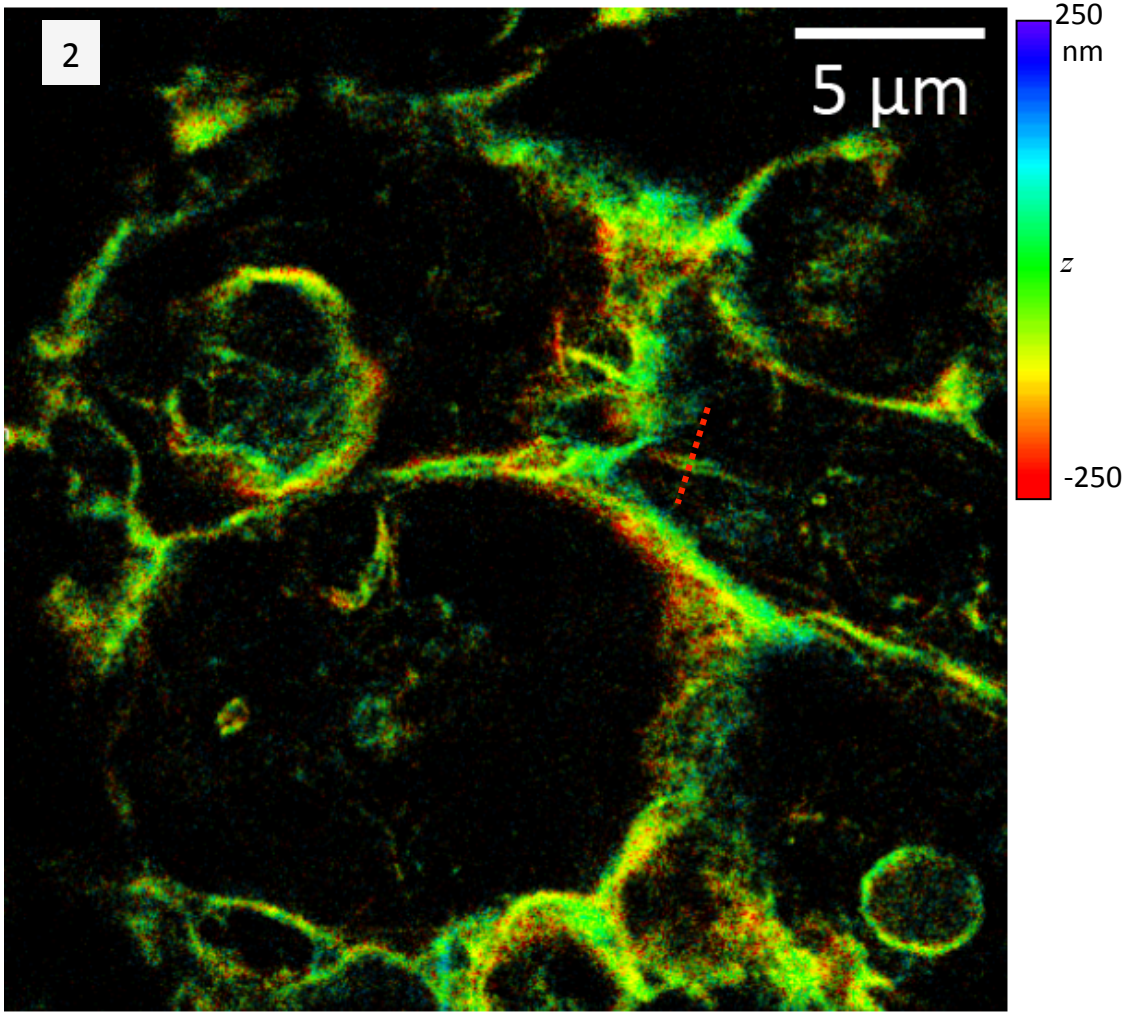
Non-cleared Tissue super-resolution



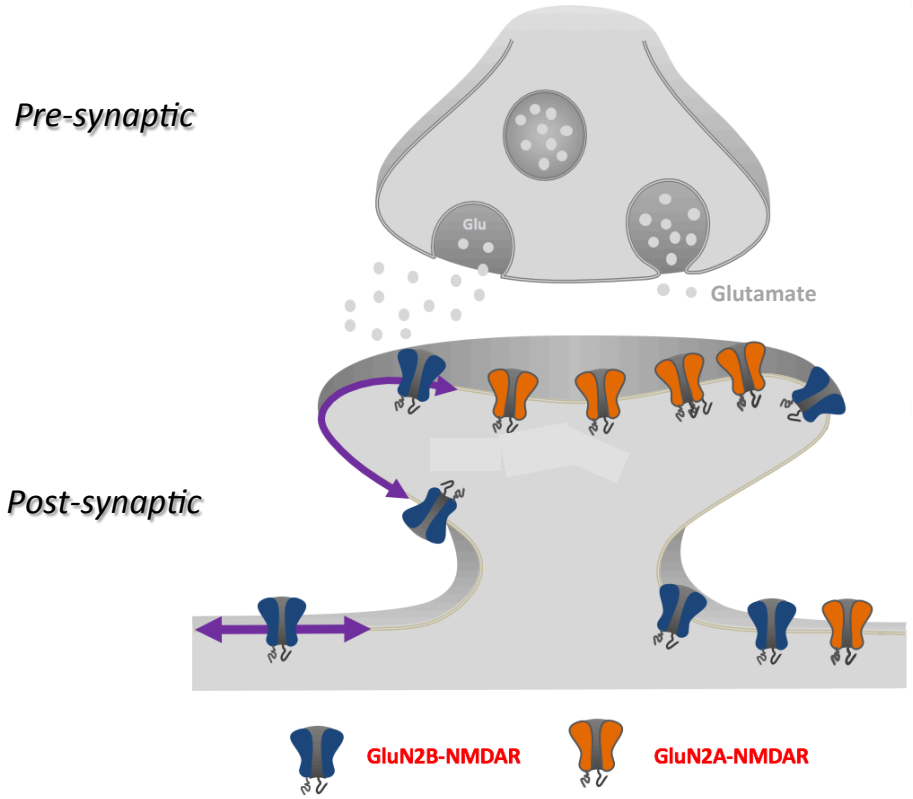
Human stem cells, f-actin labelled with A647/ Phalloidin
60x NA=1.3, 3D dSTORM imaging



Depth = 54 µm



NMDA Receptor clustering in brain



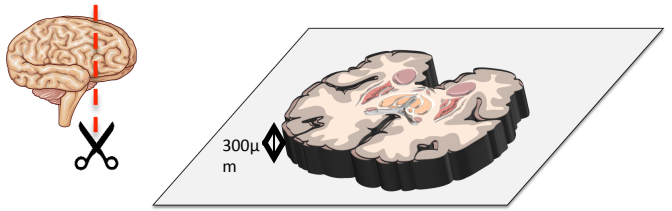
Glutamate receptor distribution in synapses

⇔

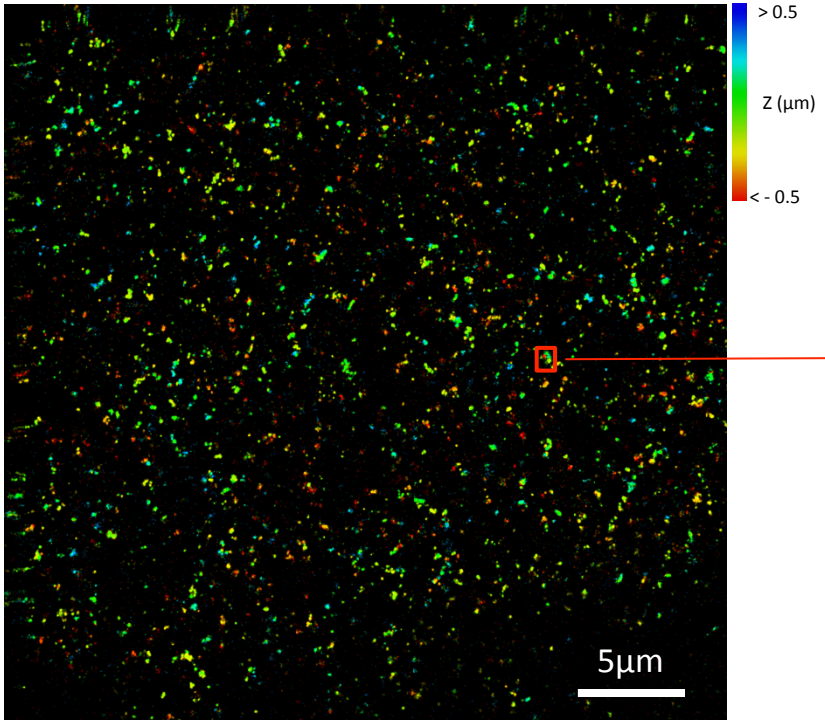
Info. on strength & reliability of synaptic communication

Collaboration with J.Ferreira, B.Kellermayer, L.Groc
(*Interdisciplinary Institute for NeuroScience at Bordeaux*)

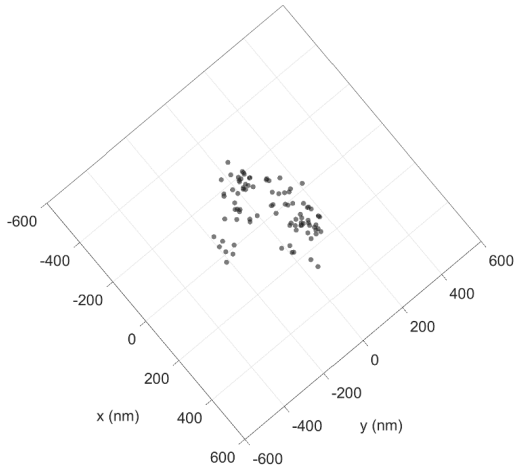
NMDA Receptor (GluN2B) clustering in brain



Brain tissue



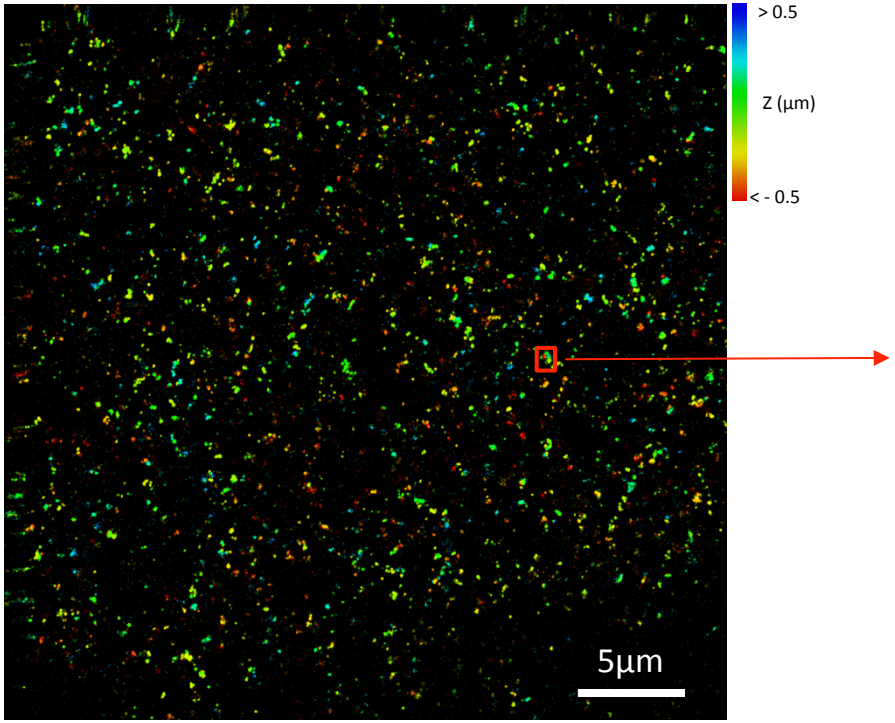
3D super resolved image
@ 20µm deep



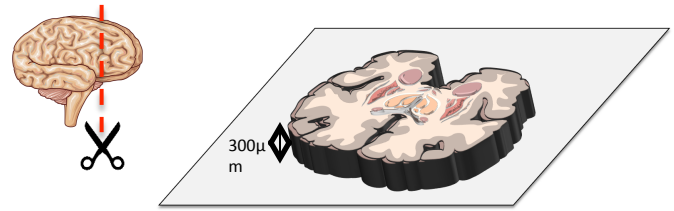
Super resolved image

Collaboration with J.Ferreira, B.Kellermayer, L.Groc
(*Interdisciplinary Institute for NeuroScience*)

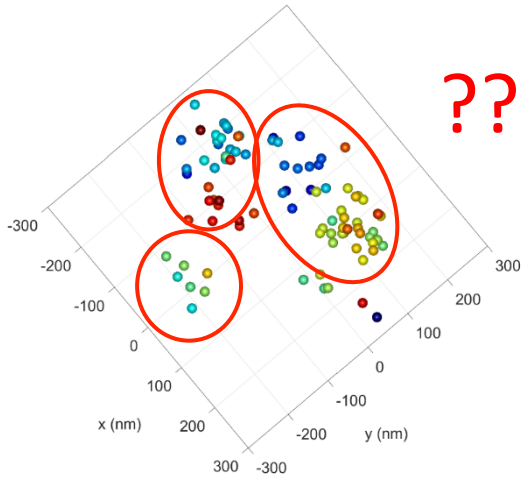
NMDA Receptor (GluN2B) clustering in brain



3D super resolved image
@ 20µm deep



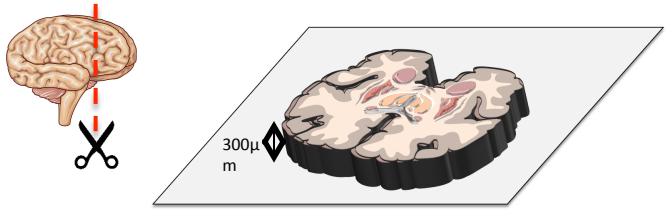
Brain tissue



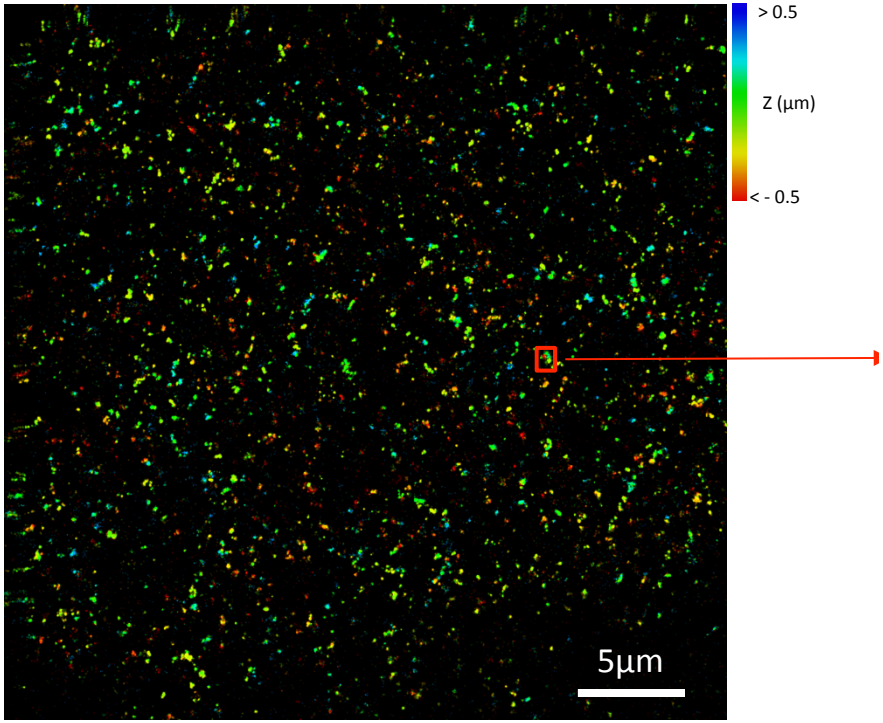
How many clusters ?

Collaboration with J.Ferreira, B.Kellermayer, L.Groc
(*Interdisciplinary Institute for NeuroScience*)

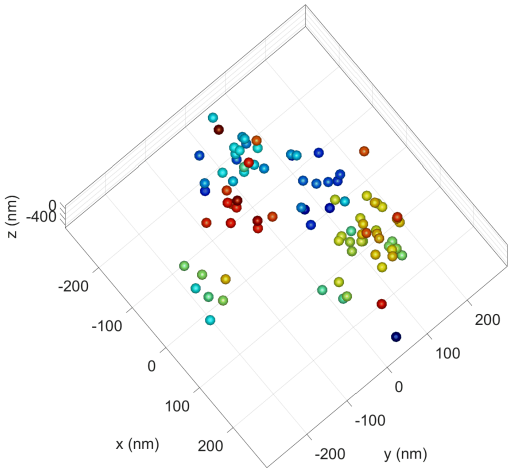
NMDA Receptor (GluN2B) clustering in brain



Brain tissue

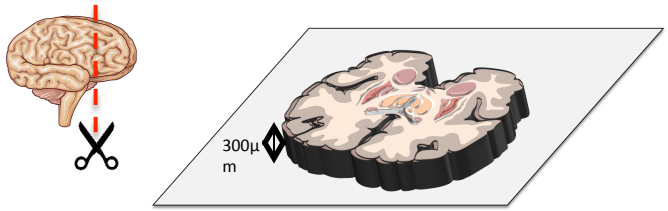


3D super resolved image
@ 20µm deep

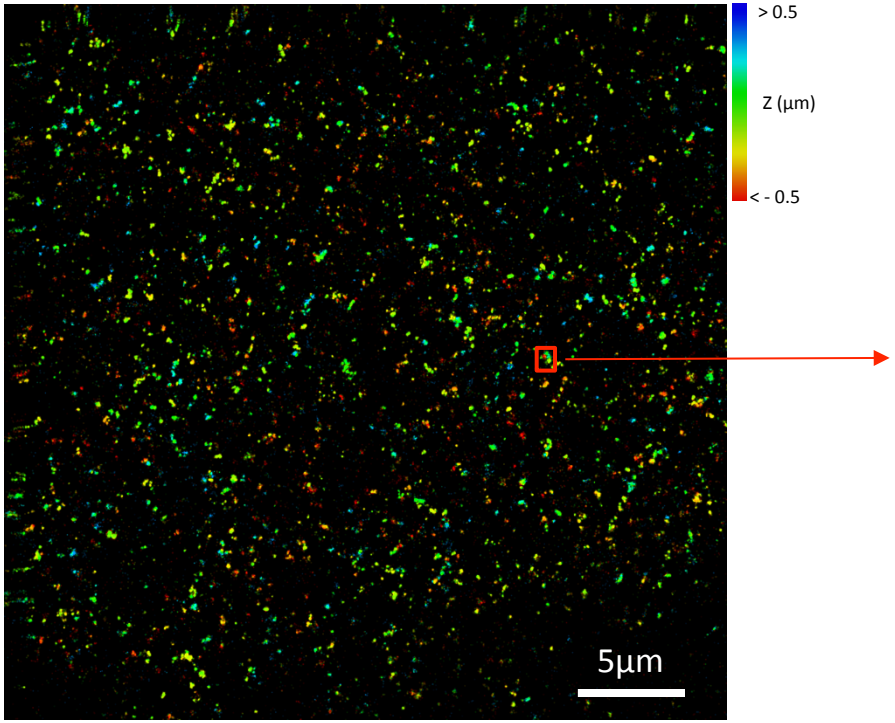


Collaboration with J.Ferreira, B.Kellermayer, L.Groc
(*Interdisciplinary Institute for NeuroScience*)

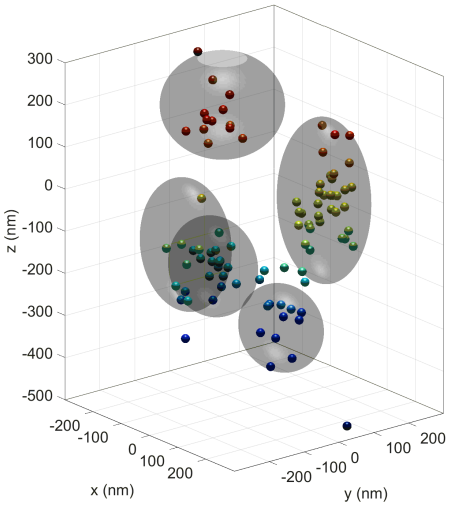
NMDA Receptor (GluN2B) clustering in brain



Brain tissue

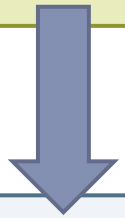


3D super resolved image
@ 20µm deep



Collaboration with J.Ferreira, B.Kellermayer, L.Groc
(*Interdisciplinary Institute for NeuroScience*)

QPI and fluorescence imaging for complementary information

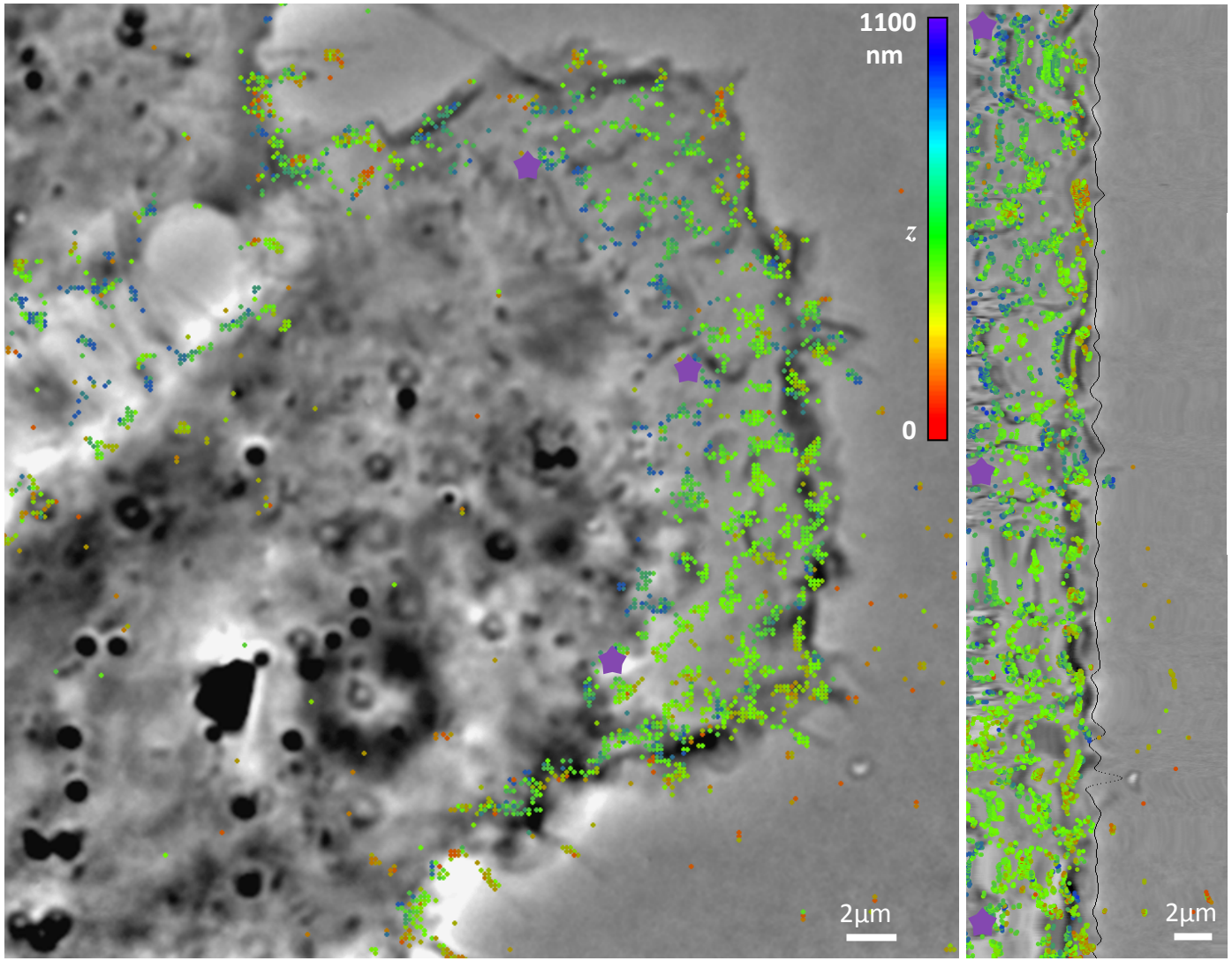


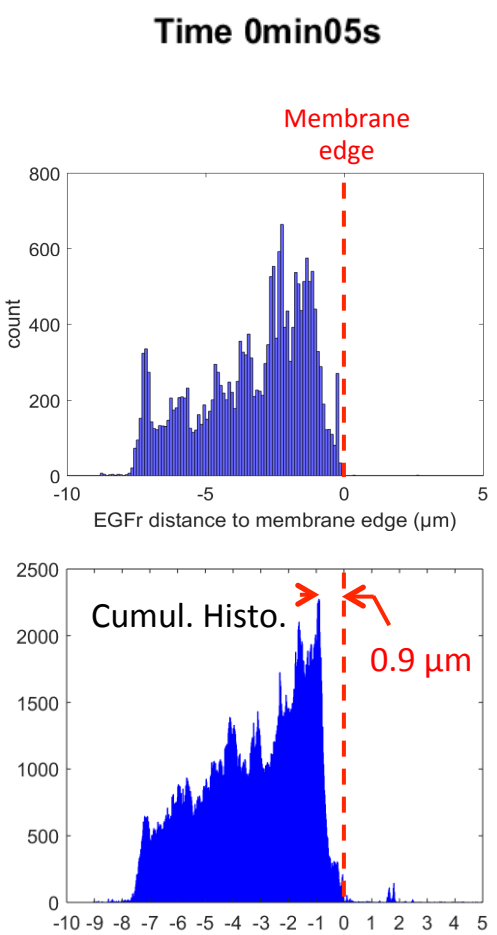
Ex: 3D QPI single molecule detection + label-free QPI

Live super-resolution in fluorescence (uPAINT) + QPI

Cell A431,60X, NA=1.3

Linearized membrane





Gray = Quantitative phase image (High-pass filtered)

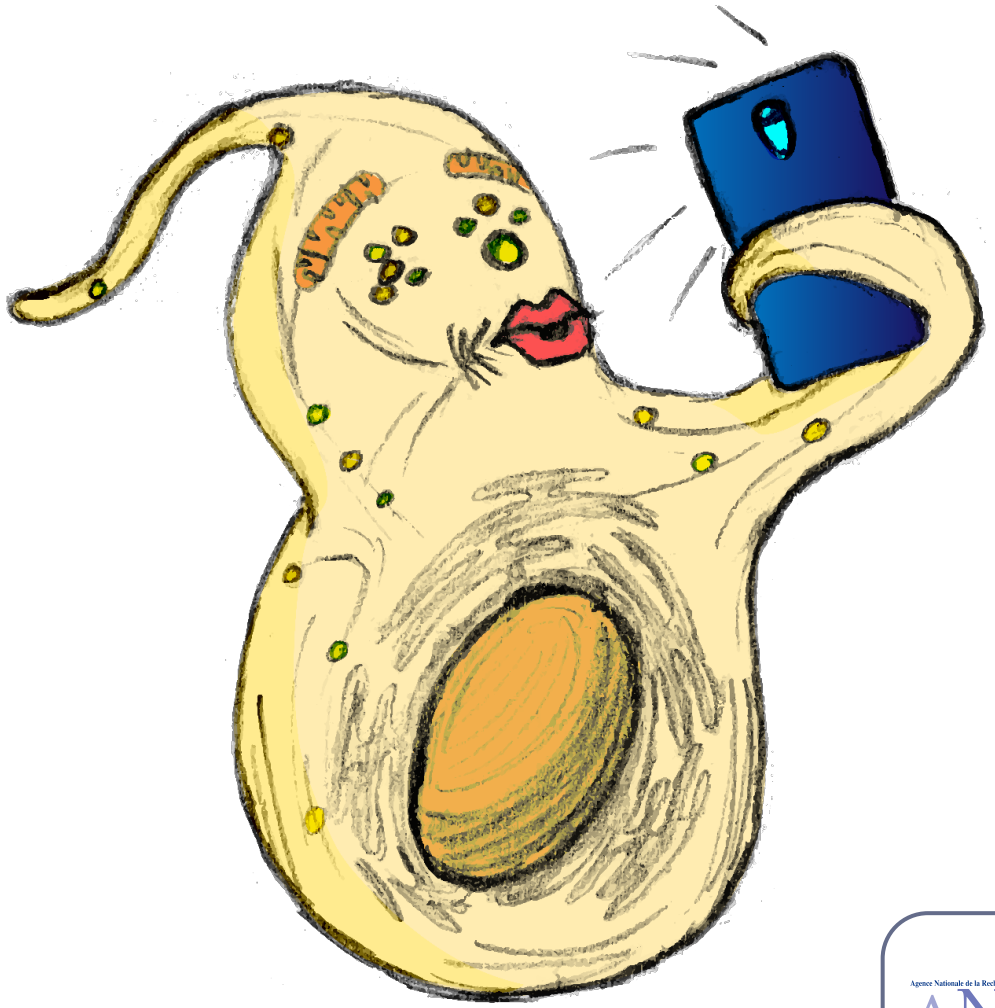
Color = EGF receptors in single molecule detection regime

Conclusions

- Fluorescence and QPI are roomate!
- Each modality can help the other and work in parallel for complementary information
- QPI can even be applied in the fluorescence signal

Thank you for your attention

No financial interest



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