

Label-free super-resolution microsphere-assisted microscopy of biological samples

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Label-free Biomedical Imaging and Sensing

Biological imaging dilemma

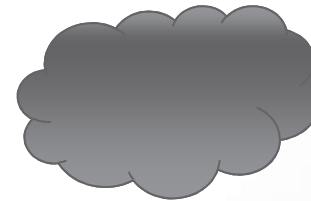
We want:

High resolution
Fast full-field
Large field
Label free
Easy to implement



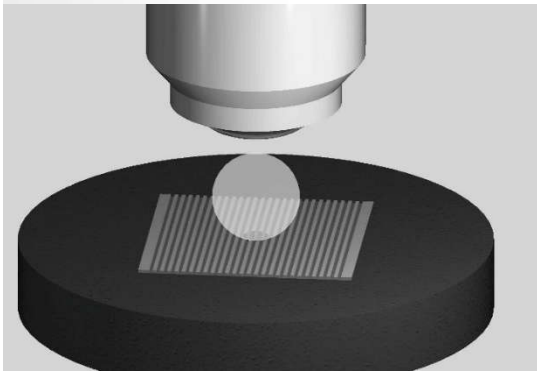
We often have:

Diffraction limit
Long 2D Scanning
X100, 50x50 μm^2 field
Fluorescence
In void & sample preparation

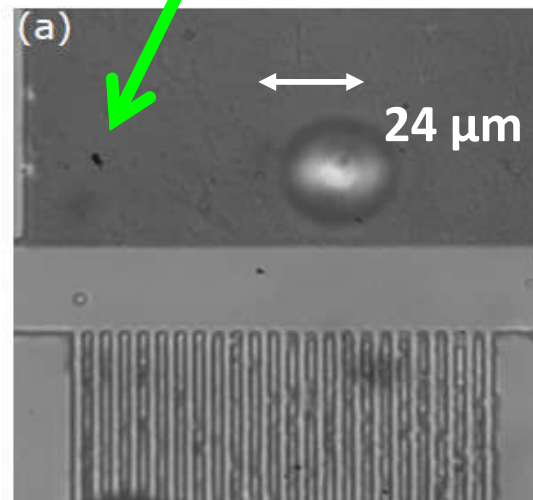


Microsphere-assisted microscopy 3

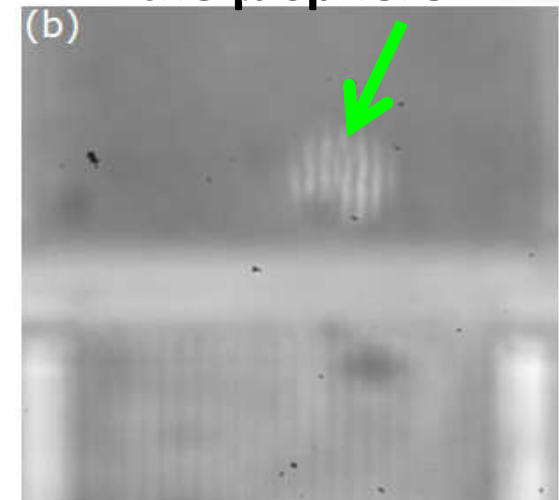
A glass μ -sphere
on a Si grating



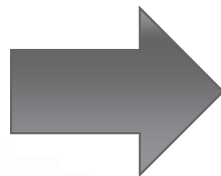
Grating not resolved



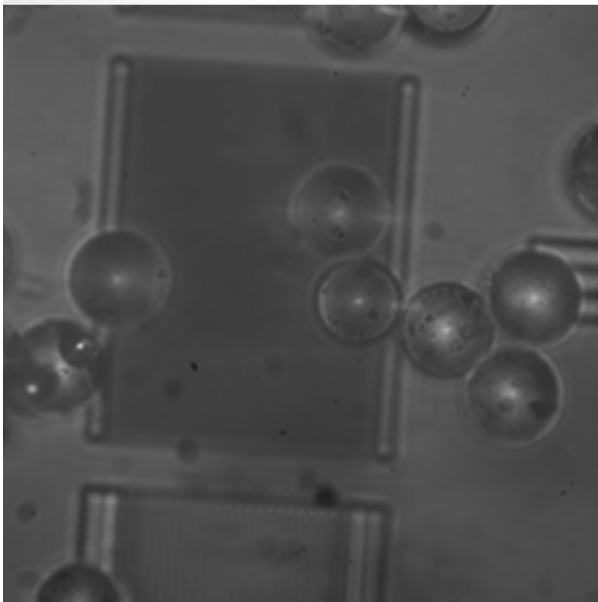
Grating resolved through
the μ -sphere



[Perrin, Label-Free Super-Resol. Micro., Springer, 2019]

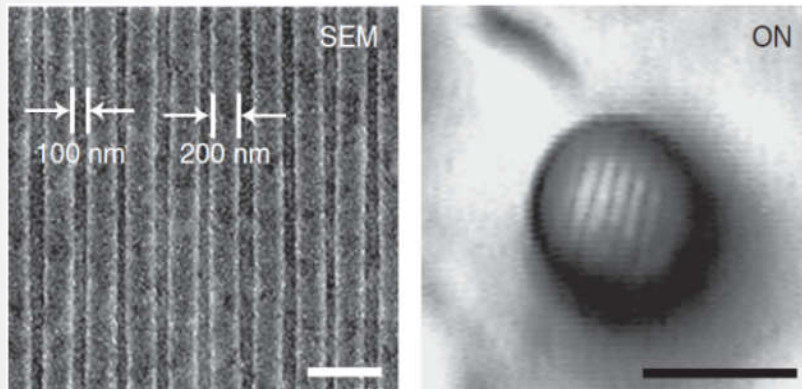


The microsphere behaves as a lens



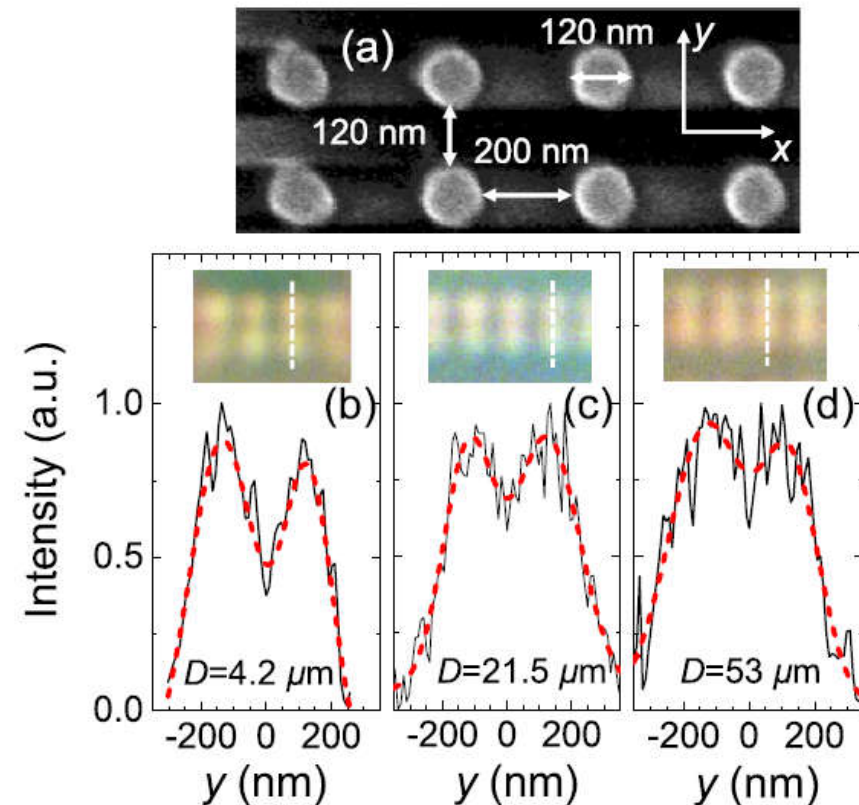
2D super-resolution through sphere

Blu-ray DVD through
a $4.7\ \mu\text{m}$ glass μ -sphere in air



[Wang ZB. Nat. Comm. (2)218, 2011]

Nano-dots in immersion $\lambda = 800\ \text{nm}$



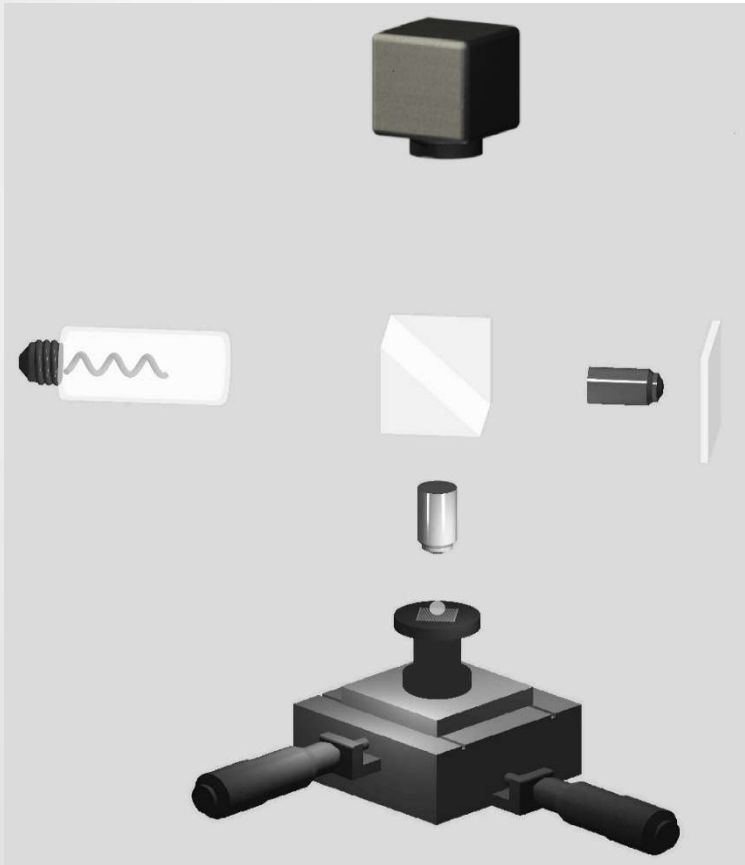
[Darafsheh, Appl. Phys. Lett. 101, 141128 (2012)]



(air) $\lambda/4 > \text{resolution} > \lambda/7$ (immersion)

3D measurement through μ -sphere

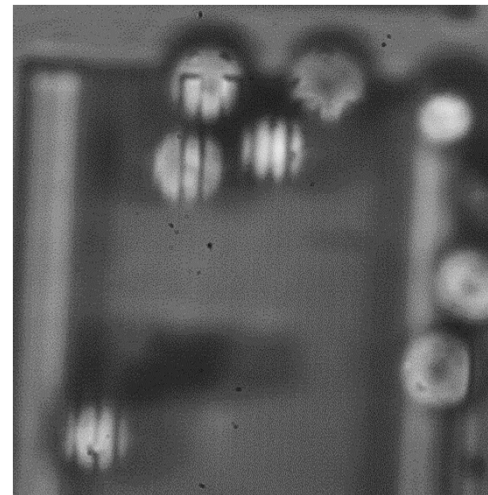
Linnik Interferometric setup



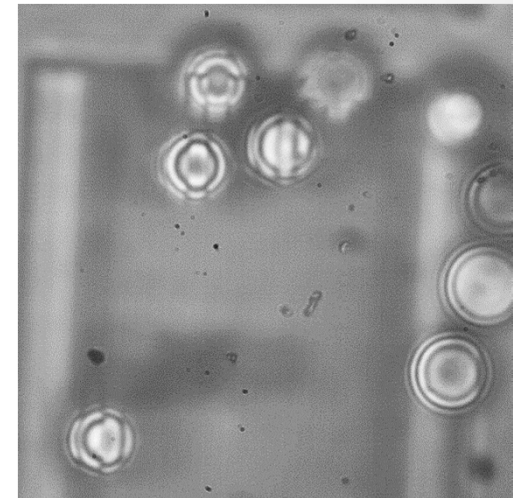
Linnik configuration in white light
with 50x objectives (ON = 0.85)

[S. Perrin et al., Applied Optics 56 (2017)]

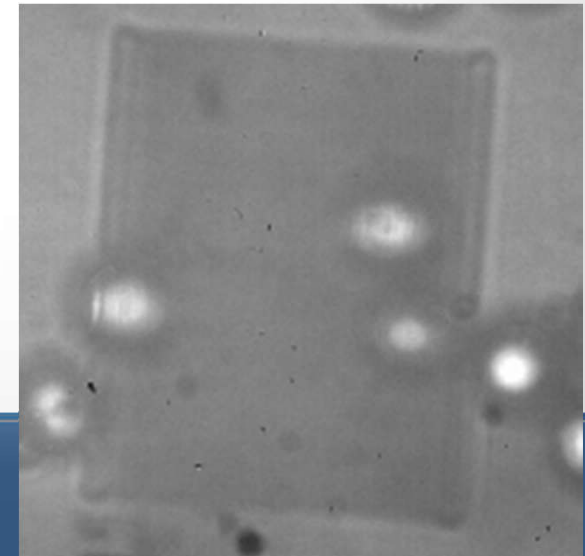
Linnik without
fringes



Linnik with
fringes



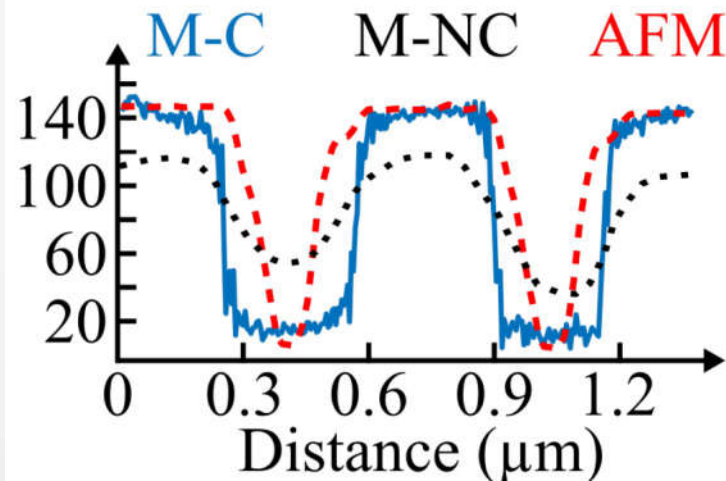
Resolution standard:
Si gratings
Period = 3 μm



With sphere compensation in the ref. arm

24 μm glass sphere

Si Grating period: 600 nm

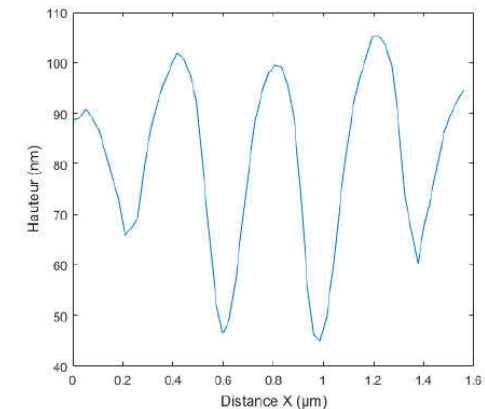
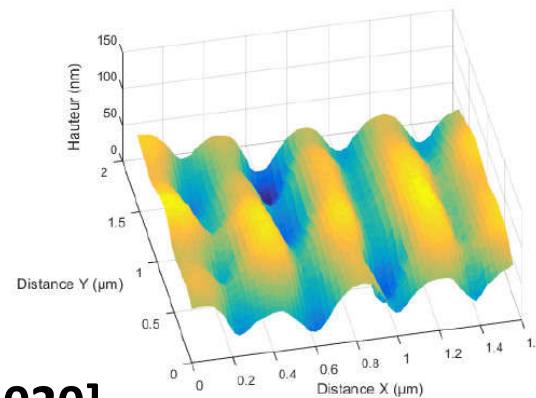


M-C: Microsphere - Compensated

M-NC: Microsphere – Non Compensated

AFM : Atomic Force Microscope

Si Grating period: 400 nm



[Perrin, Physical Review Applied 2020]

What about biological samples?

Difficulties :

Low reflectivity

Low contrast

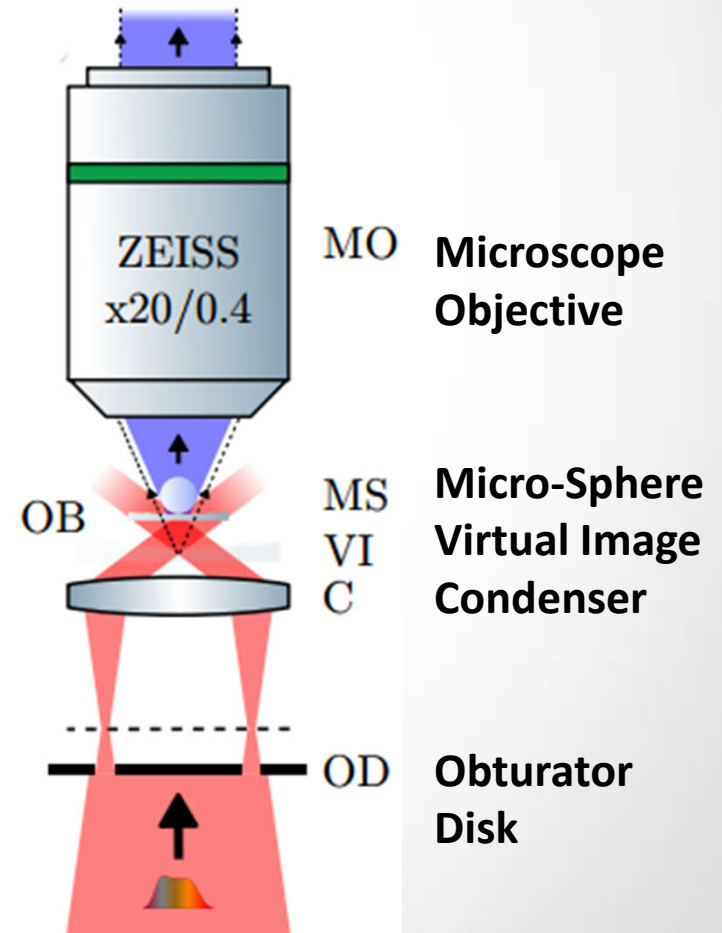


Measure in transmission
using dark-field illumination

25 μm sphere

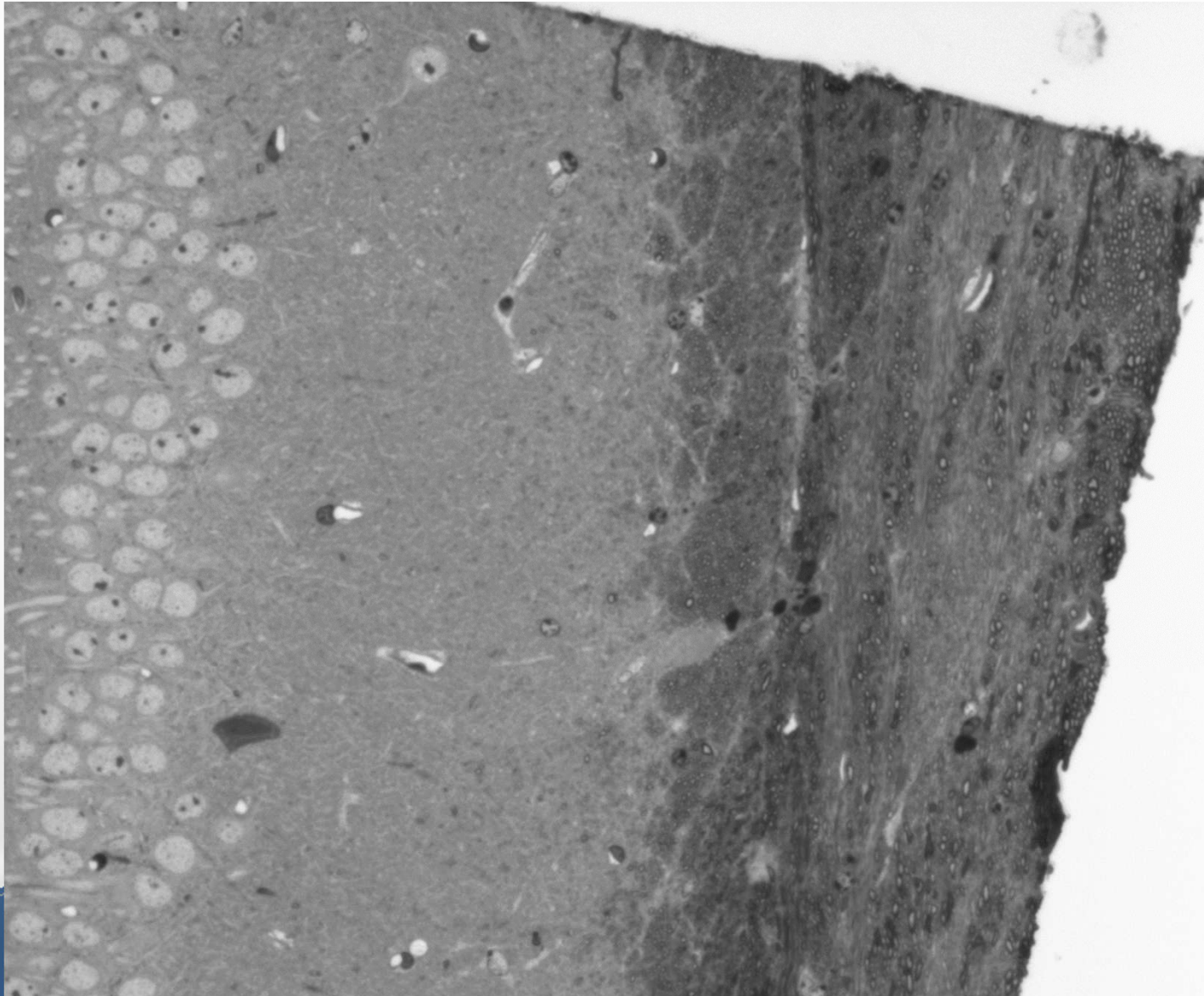
Soda lime

[Perrin, Pss-Rapid research let. p.1800445, 2018]



Fixed mouse brain (x20)

nerve sections in the hippocampus

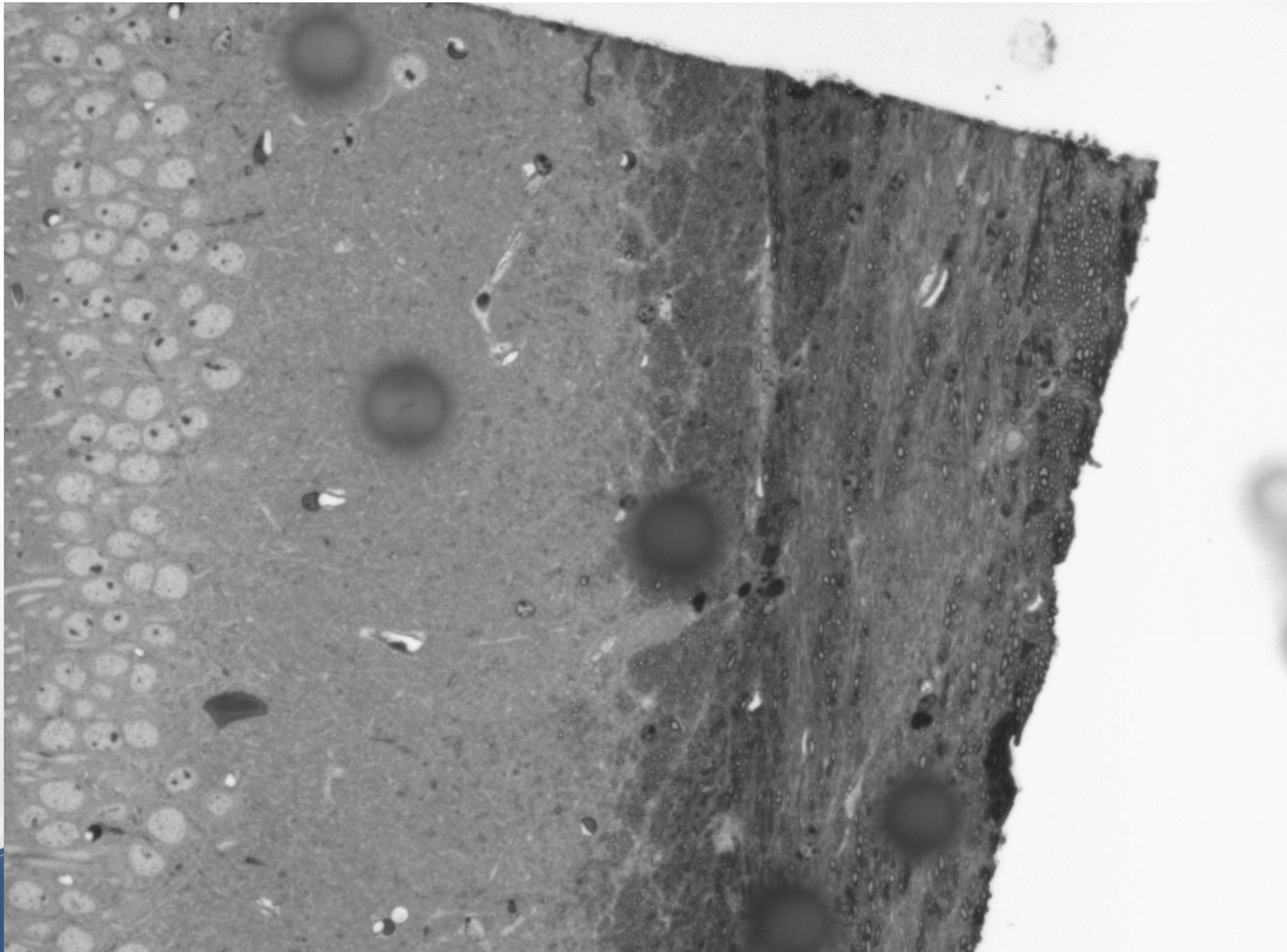


With sphere (x20 obj.)

Fixed mouse brain cells: nerve sections in the hippocampus

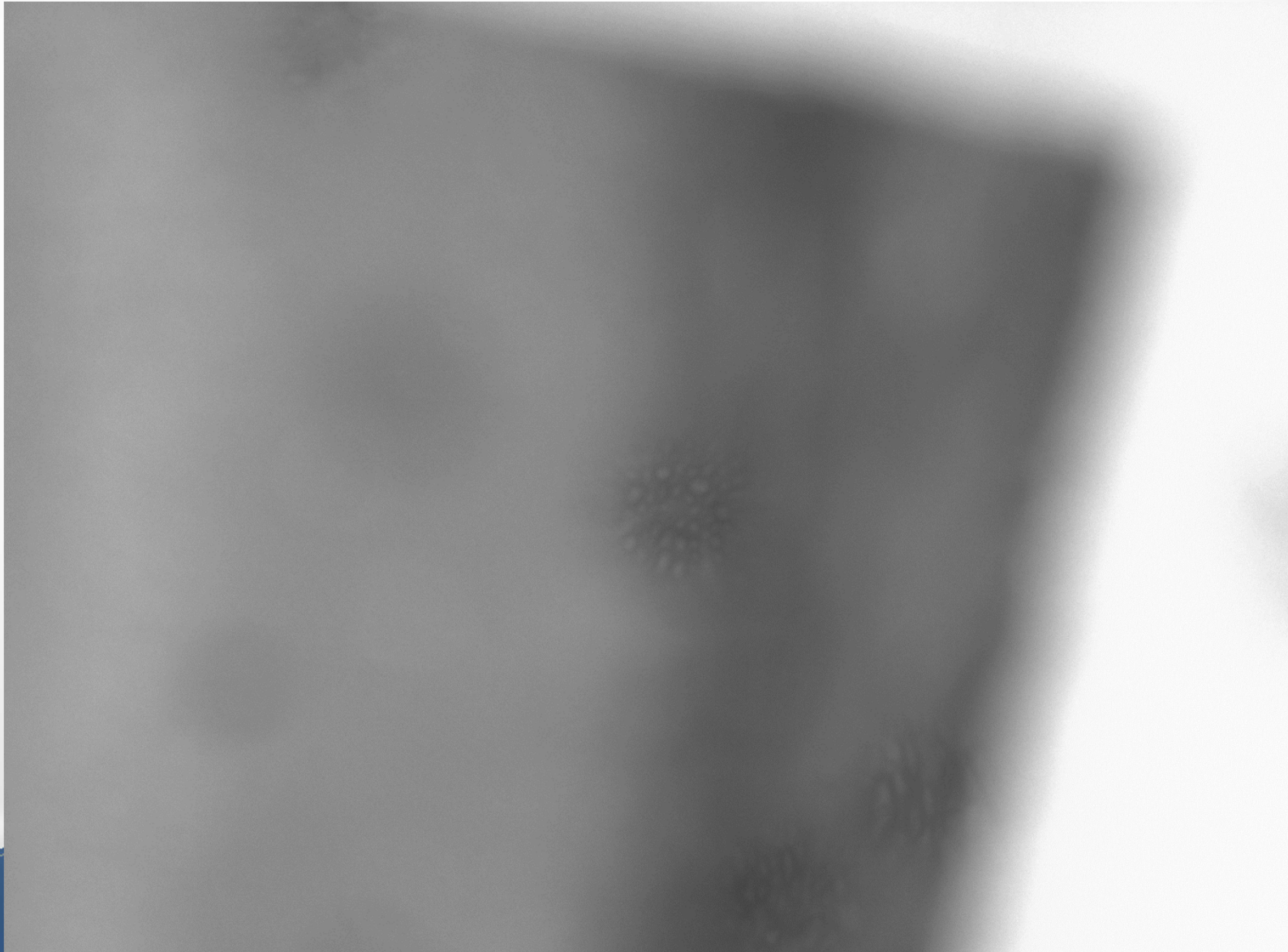
25 μm
sphere

Soda
lime



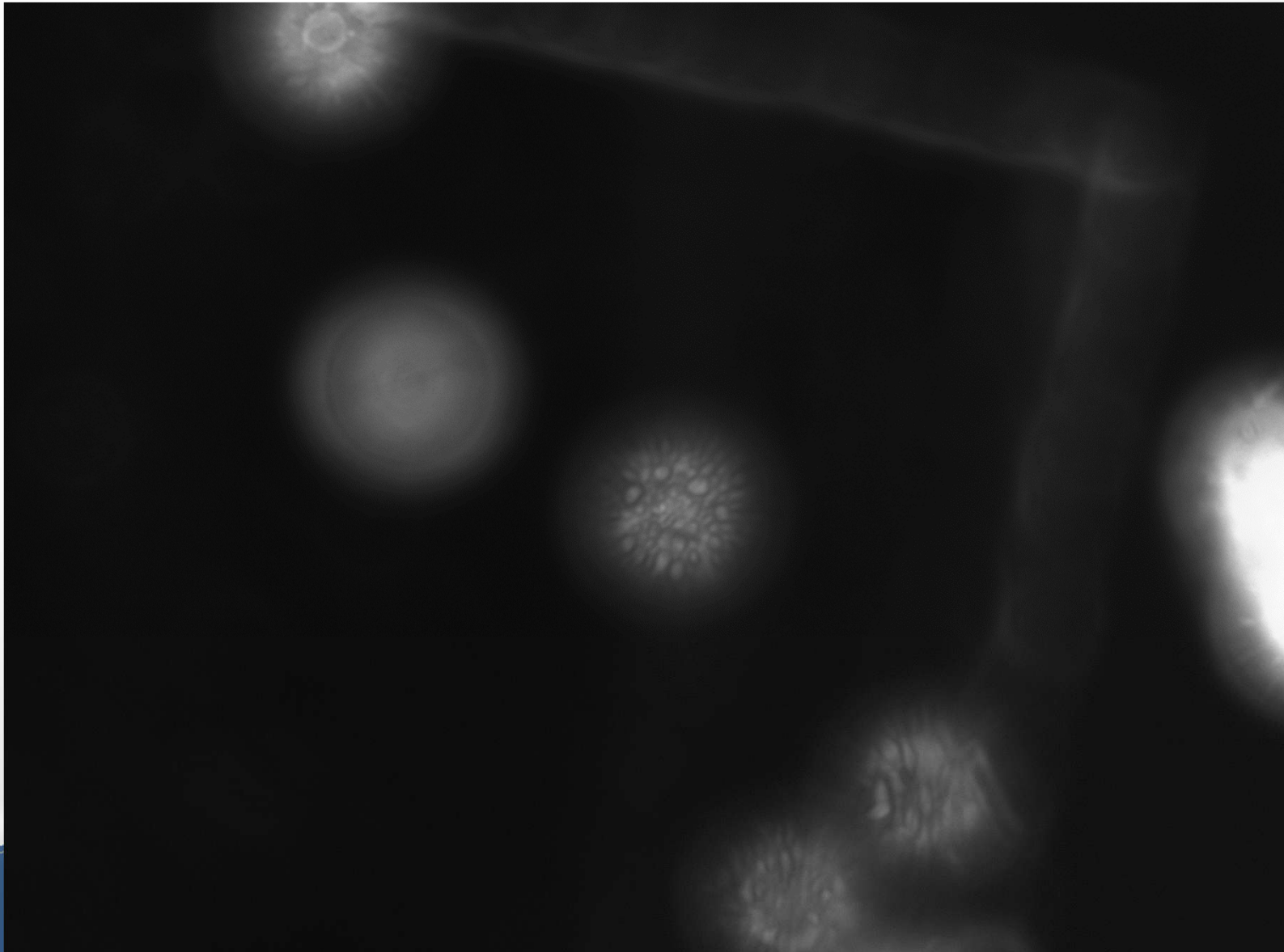
Through sphere in bright field

Fixed mouse brain cells: nerve sections in the hippocampus



Through sphere in dark field

Fixed mouse brain cells: nerve sections in the hippocampus

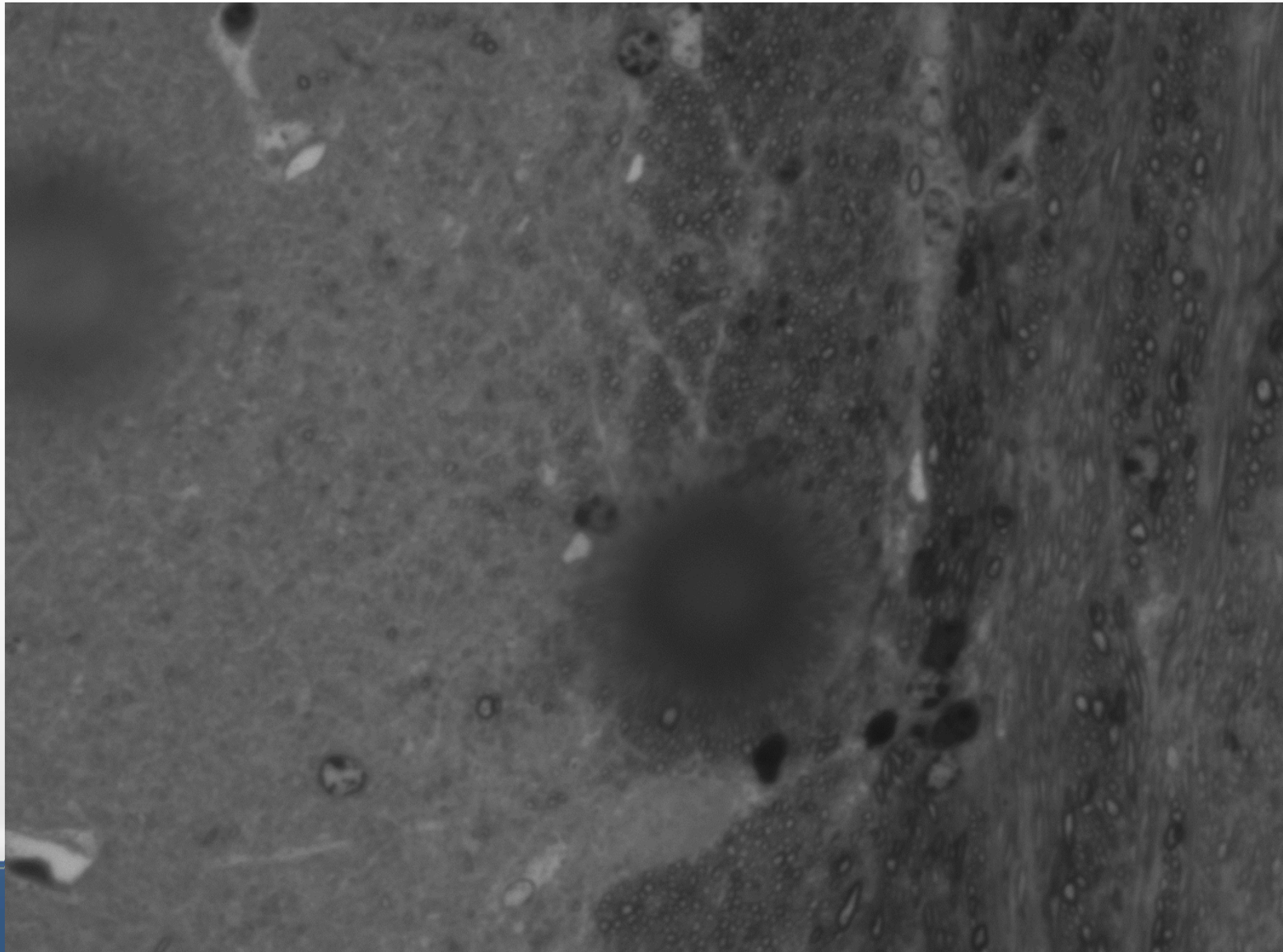


Fixed mouse brain (obj. X50)

Nerve sections in the hippocampus

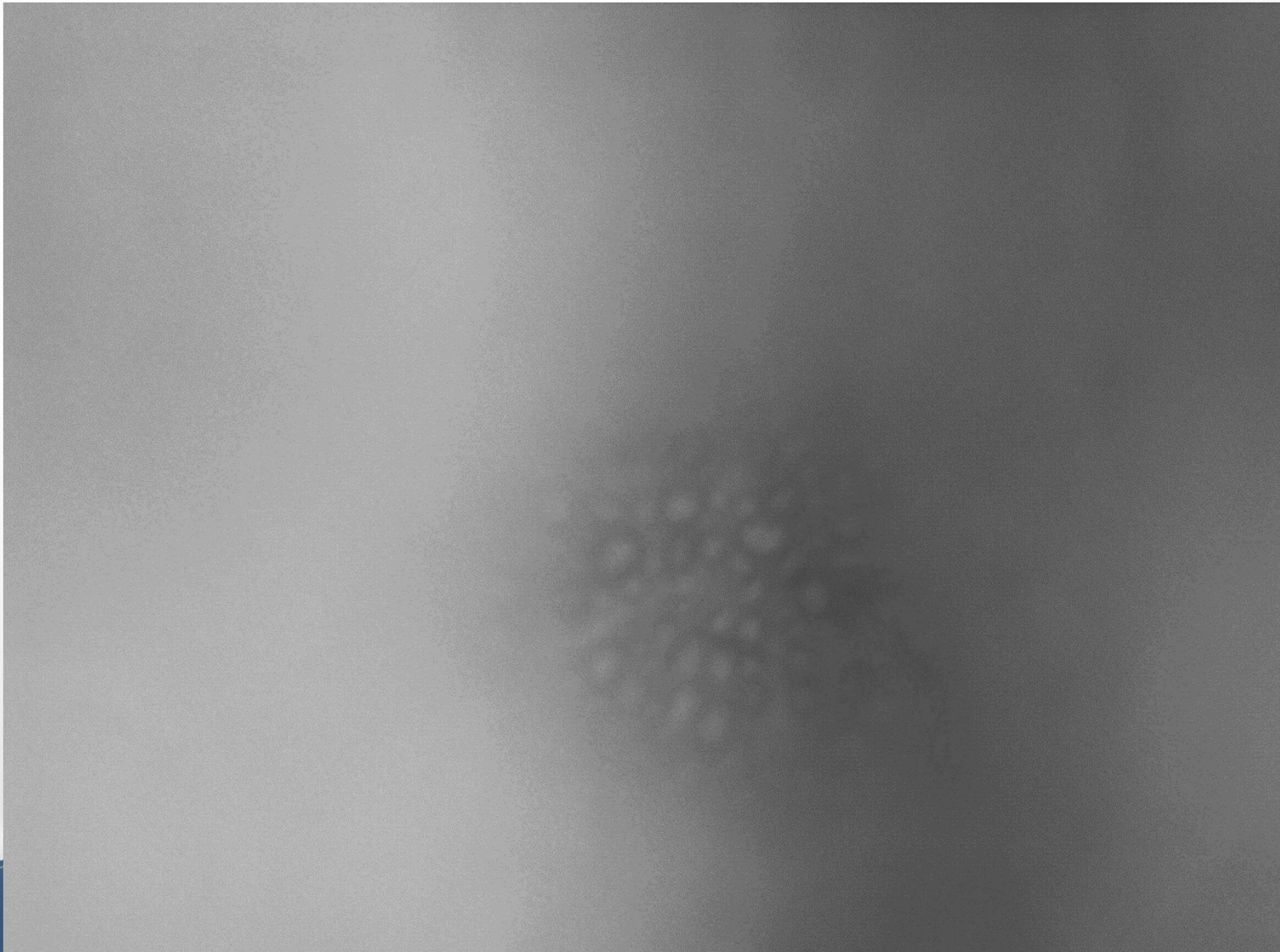
12

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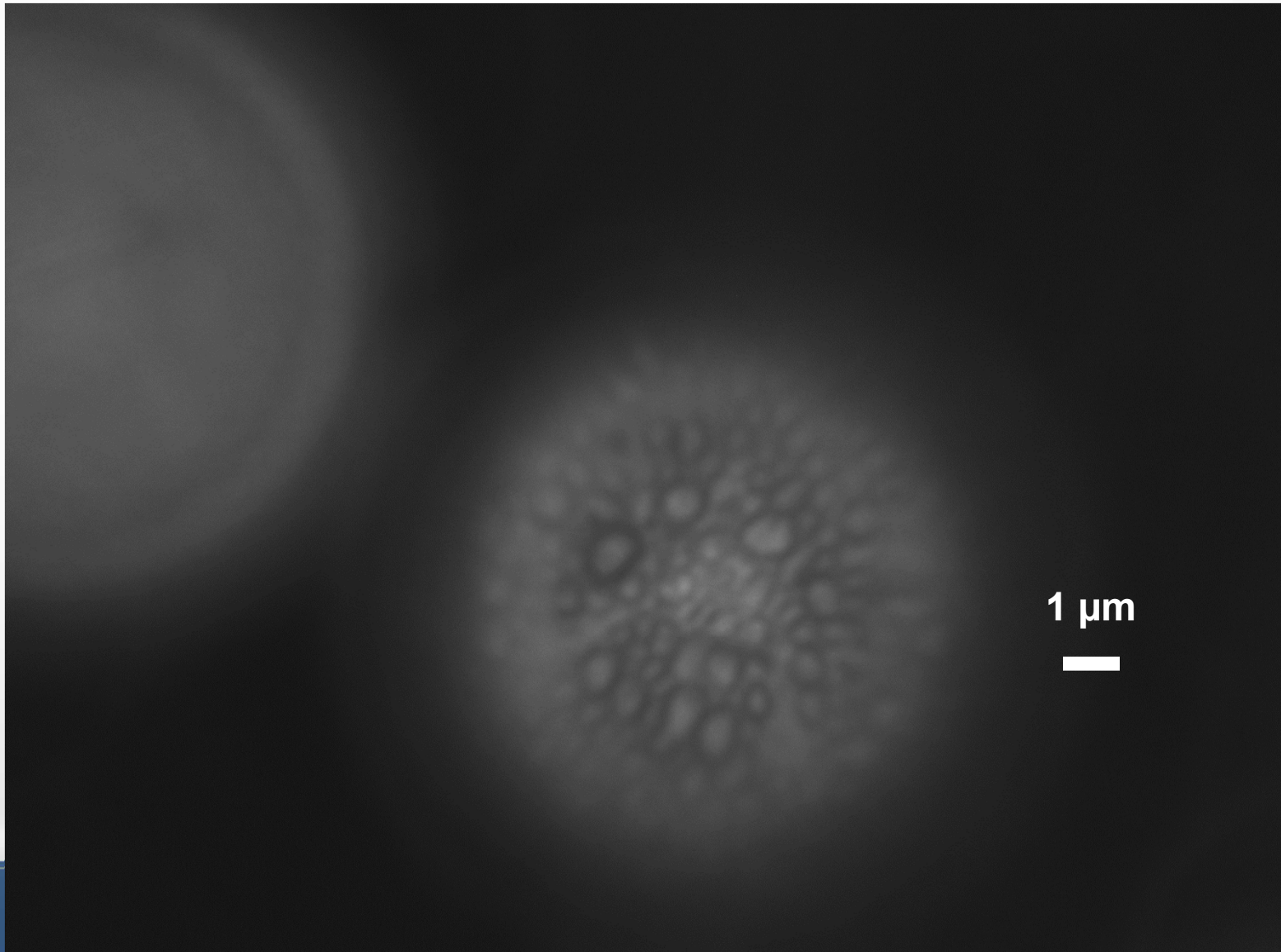
Through sphere in bright field

Fixed mouse brain cells: nerve sections in the hippocampus



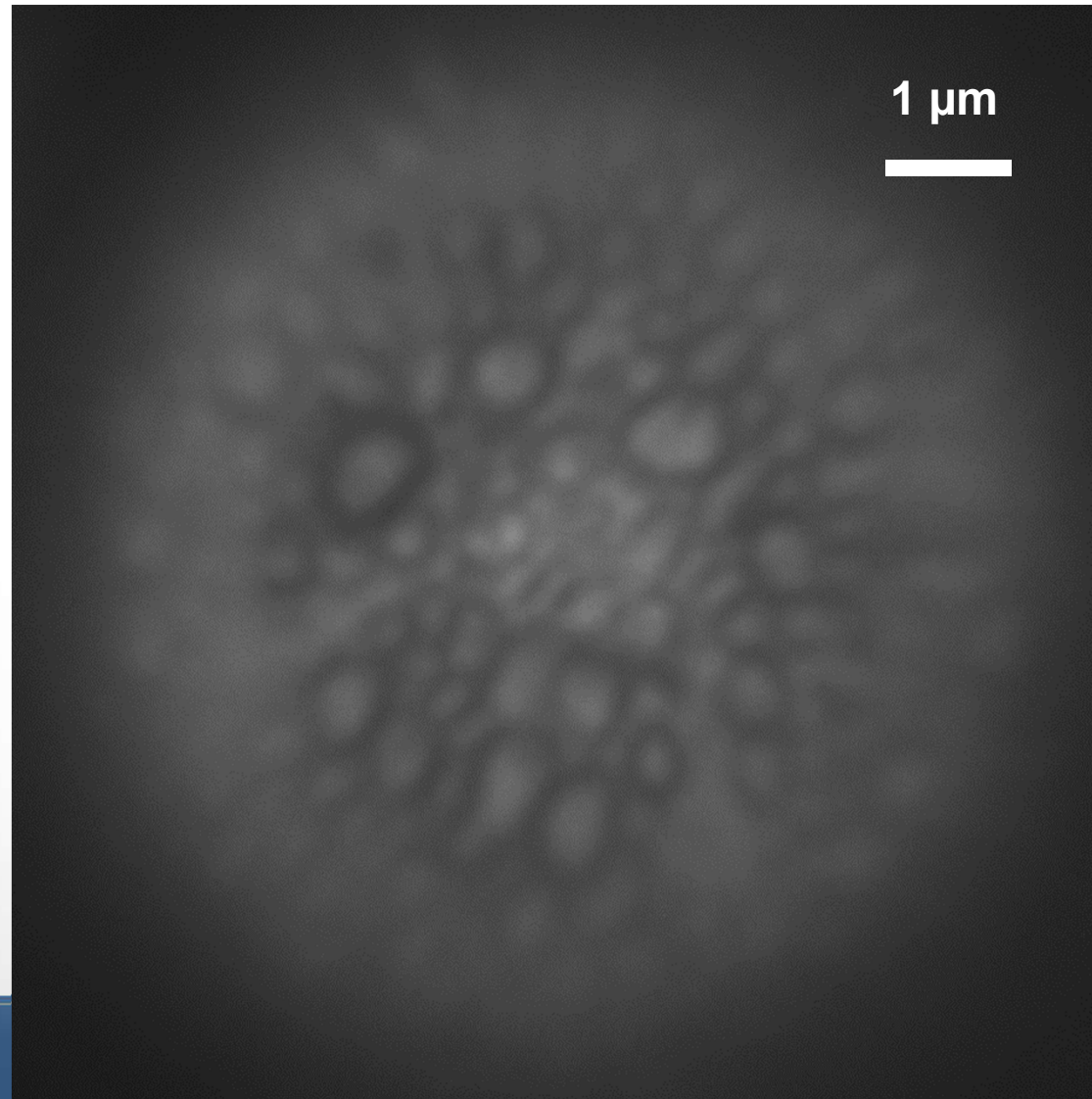
Through sphere in dark field

Fixed mouse brain cells: nerve sections in the hippocampus



Through sphere in dark field

Fixed mouse brain cells: nerve sections in the hippocampus



Resolution estimation

16

Sample:

Transparent 250-nm
of side squares
(EPFL – CSEM)

Sphere:

Diameter = 25 μm
Soda lime

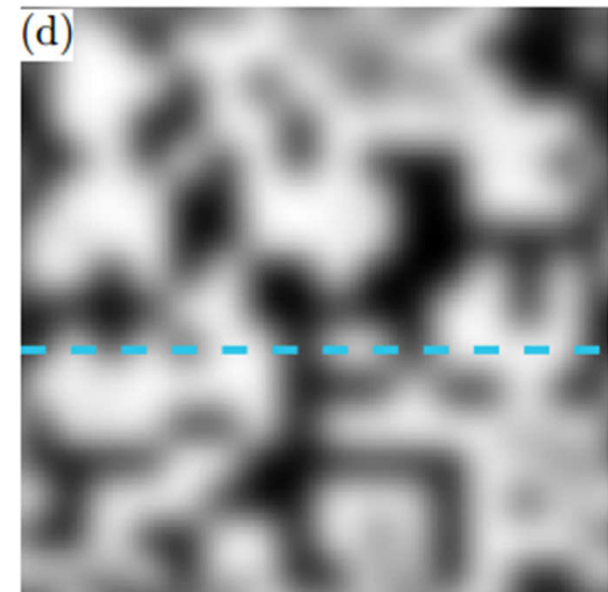
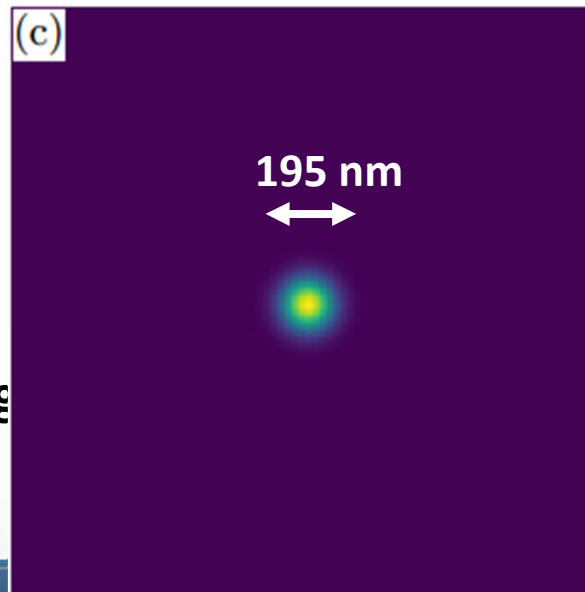
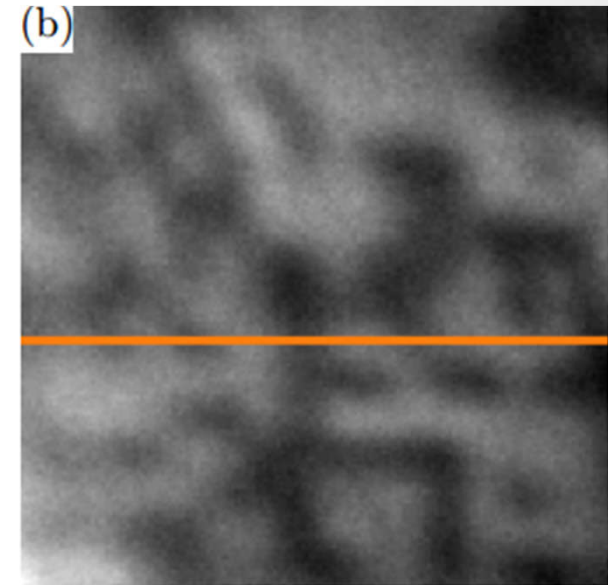
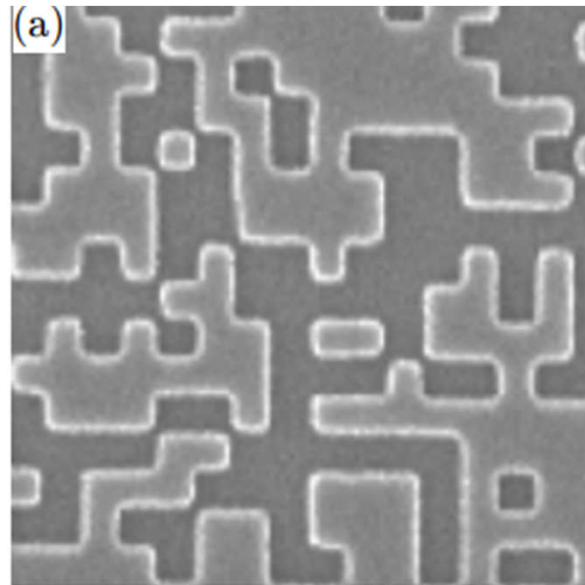
Figures:

(a) SEM image

(b) Through μ -sphere

(c) Estimated PSF

(d) Computed image taking
into account the PSF



Conclusion

Micro-sphere assisted microscopy allow:

- label free,
- full field,
- easy to implement,

2D super-resolved ($\lambda/5 \sim 100$ nm) measurements

3D reconstructions

The technique can be adapted to bio-sample

- Working in transmission
- Using dark-field illumination

Thanks for your attention



And thanks to :

Giorgio Quaranta (EPFL)

Nadia Messaddeq, Nicolas Lemercier, Jean-Luc Vonesch (IGBMC)