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Faculty of Fisheries
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of Waters

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v Českých Budějovicích
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in České Budějovice
Czech Republic

Superresolution Using Simple Microscopes

Renata Štysová-Rychtáriková et al.

Laboratory of Experimental Complex Systems
Institute of Complex Systems
Faculty of Fisheries and Protection of Waters
University of South Bohemia in České Budejovice

Palacký University in Olomouc, 23 May 2018

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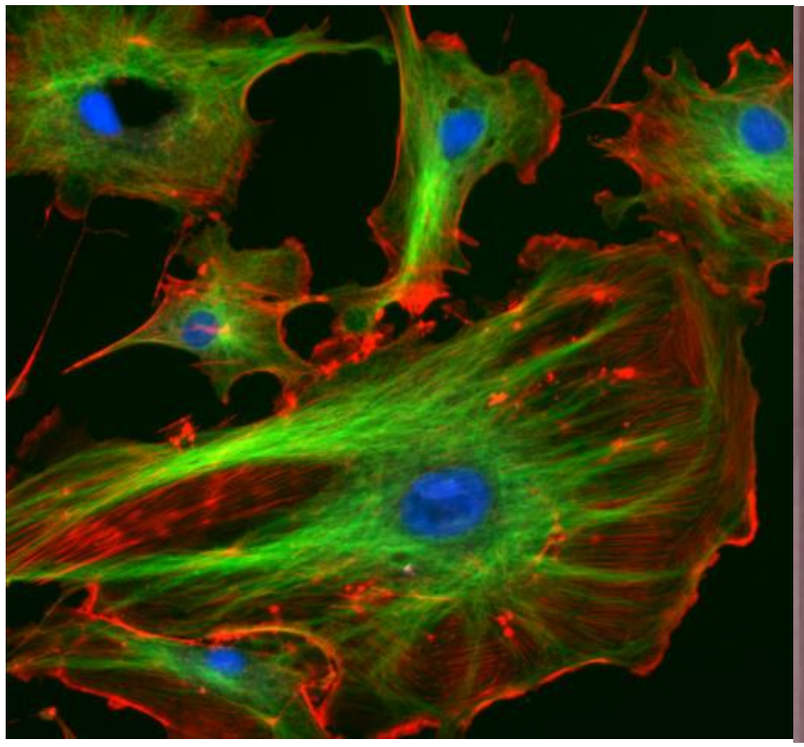
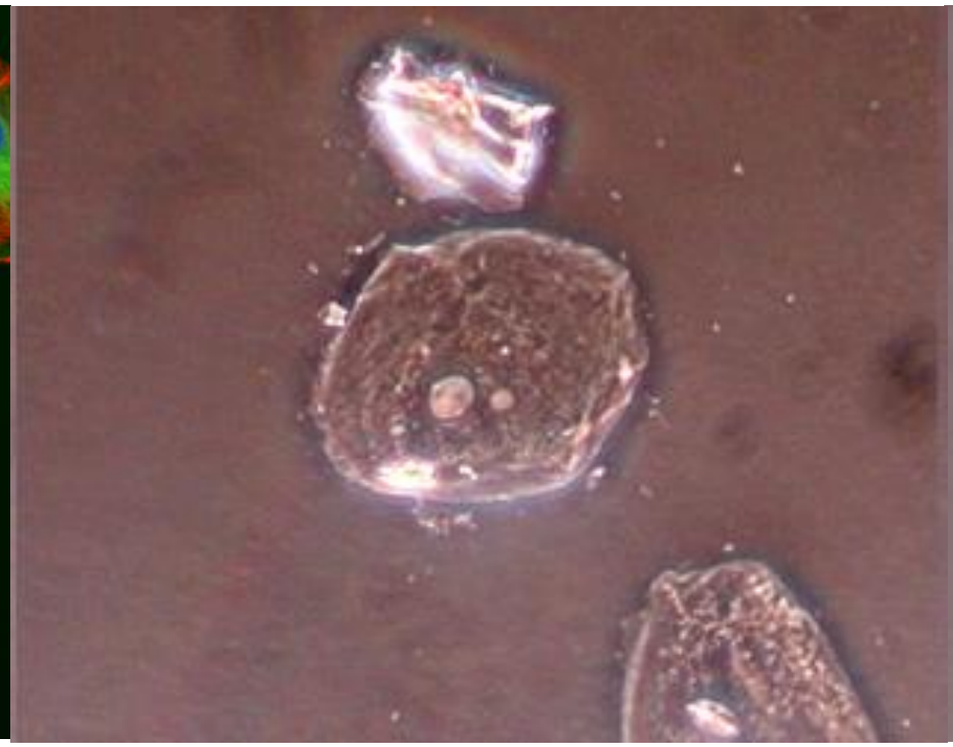


Fluorescence

+:
SR methods
Interpretation
Object localization

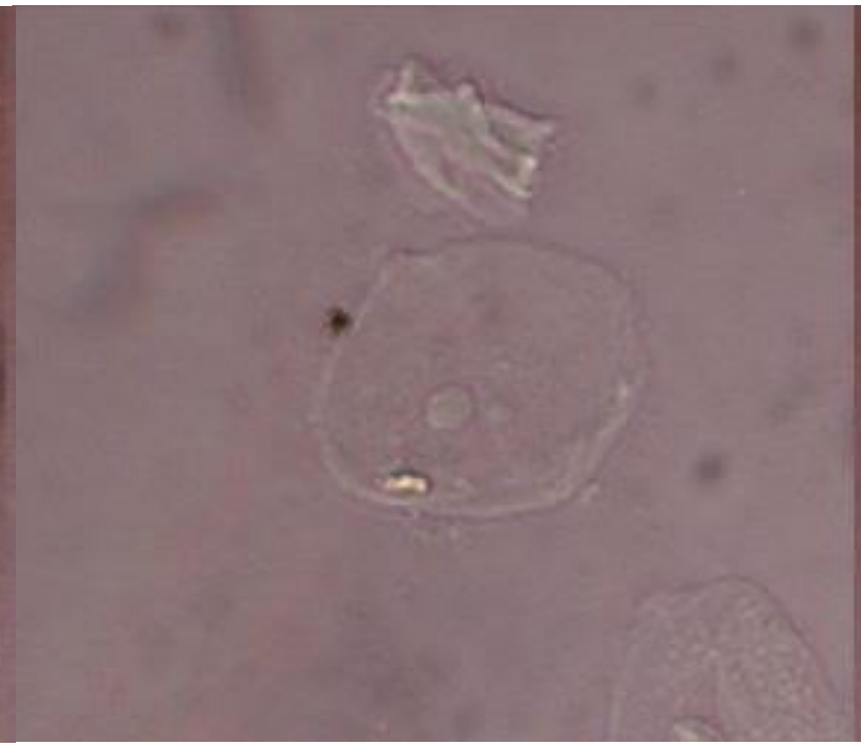
-:
staining



Phase contrast

+:
Cell in physiological state
visibility

-:
Artificial internsity interferences



Bright field

+:
Cell in physiological state
Natural Intensity interferences

-:
Visibility

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1. Theoretical assumptions

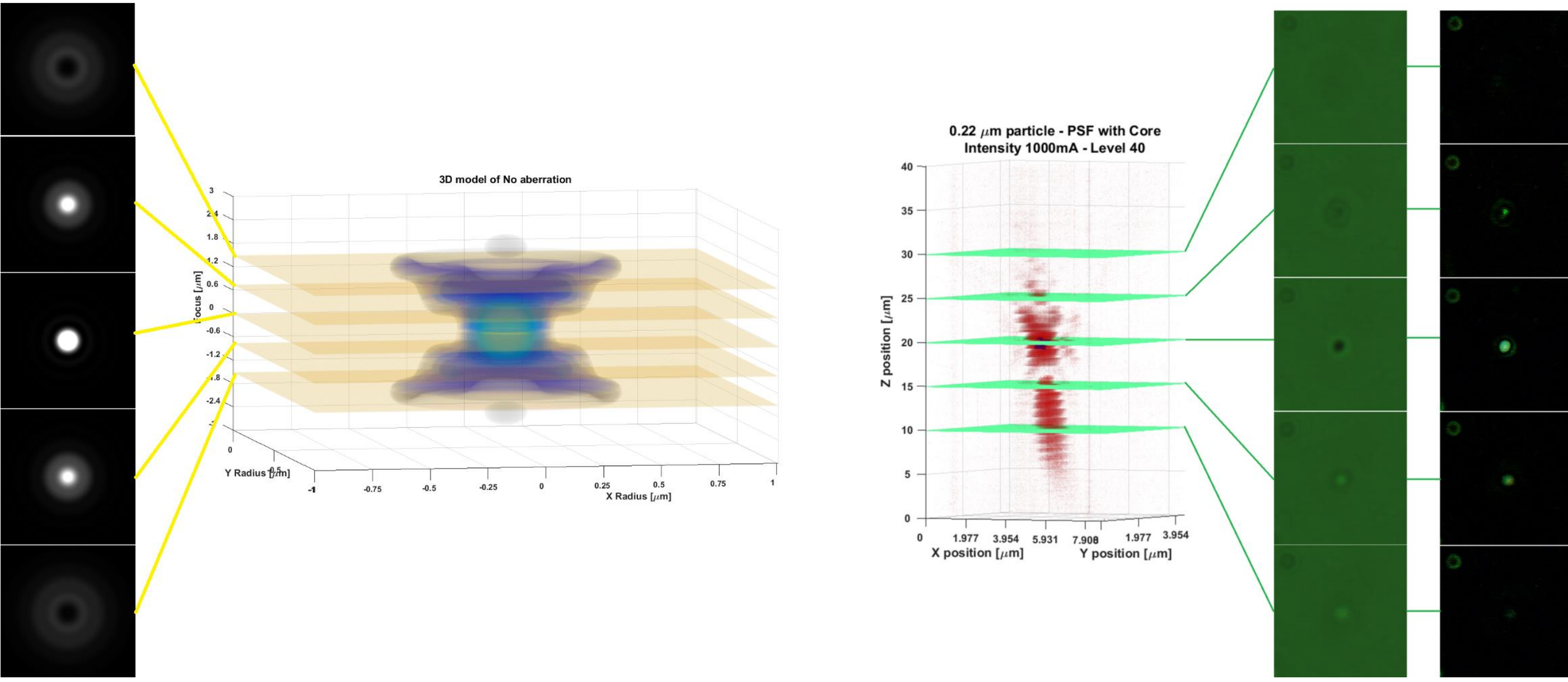
- Extended Nijboer-Zernike Theory
- Videoenhanced Microscopy

+

2. Technical solutions

- Calibration of optical path and camera chip
- Small camera pixel
- Primary vice-bit camera signal
- Short z-step
- Strong illumination
- Reading z-position

Extended Nijboer-Zernike Theory



+ Theory of Electromagnetic Centroid

Electromagnetic centroid:

- intensity extreme
- the same intensity in two consecutive images

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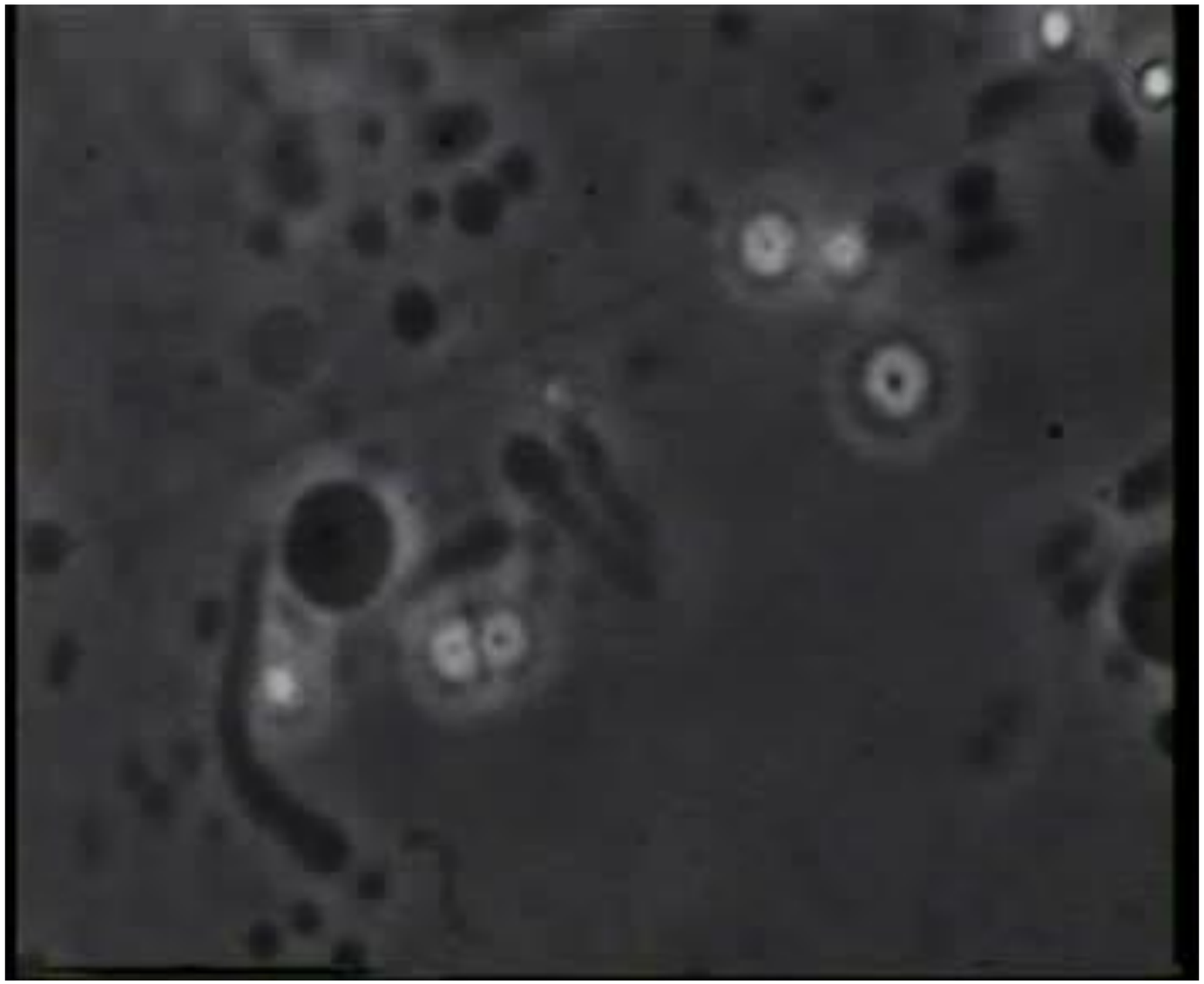
VIDEO-ENHANCED MICROSCOPY



SOURCE:
Irene Lichtscheidel
Uni. Vienna

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Calibration of Optical Path and Camera Chip

Why?

To remove image inhomogenities (spots on microscope optics and camera, optics vignetting, …)

Requirements:

1. Primary camera signal
2. Calibration of the whole optical path

Result:

image pixel intensity → total number of photons

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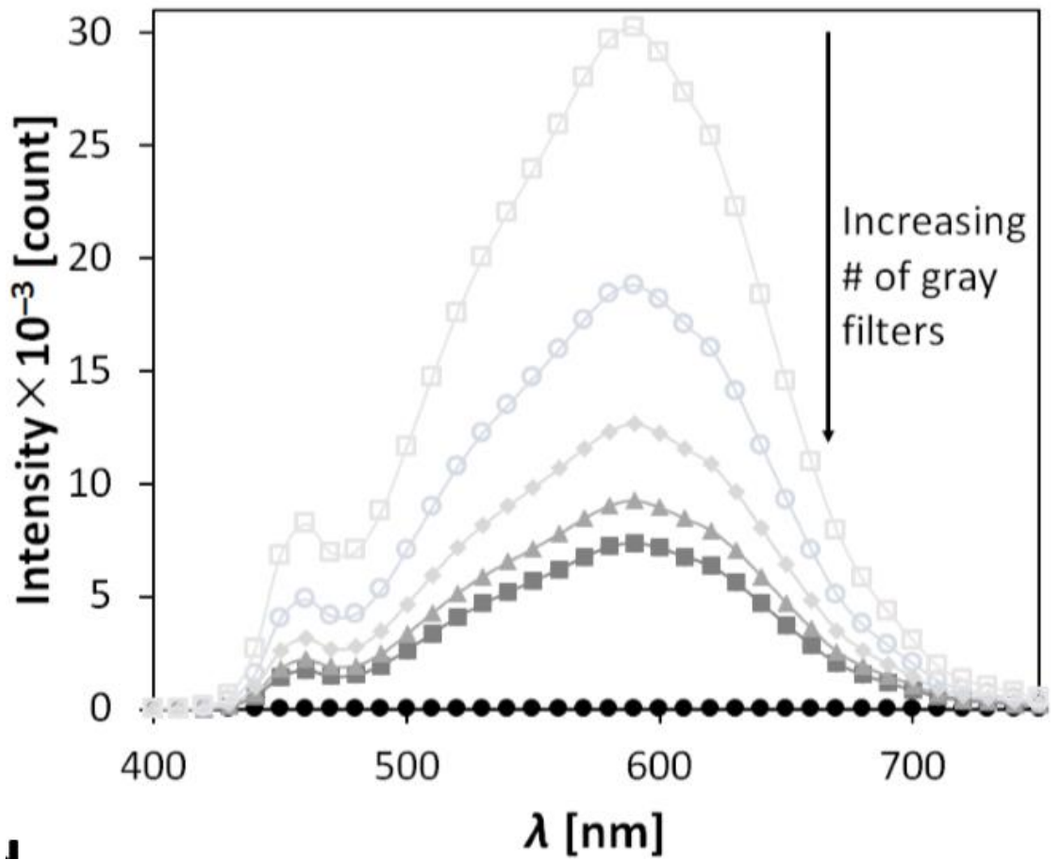
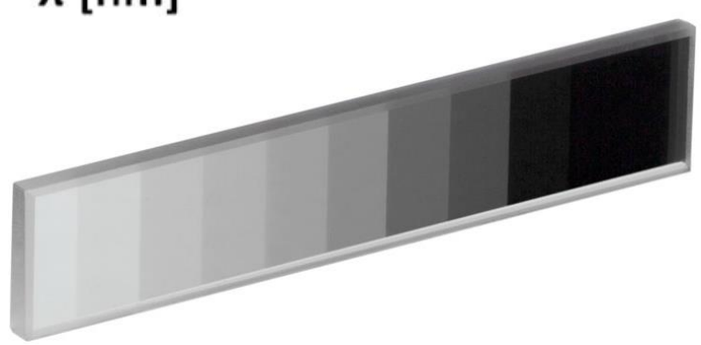
Calibration of Optical Path and Camera Chip

1. Experimental Part

- **Scan gray filters** in their focus.
- Measure **transparency spectra** of the filters in their focus.





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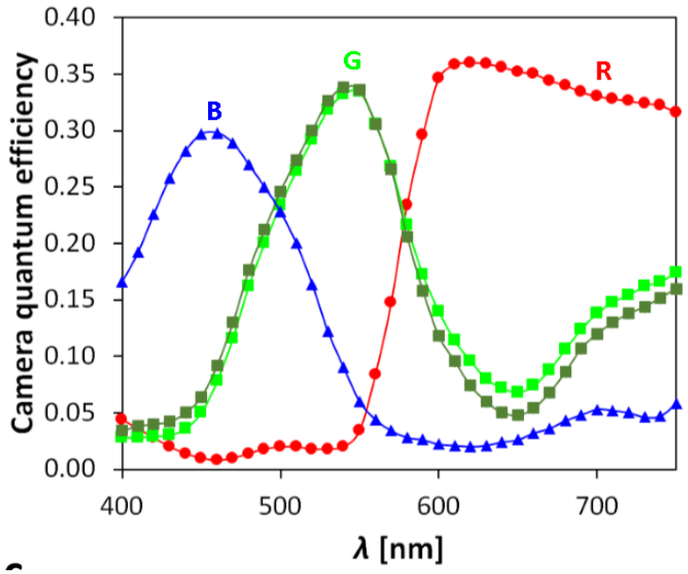
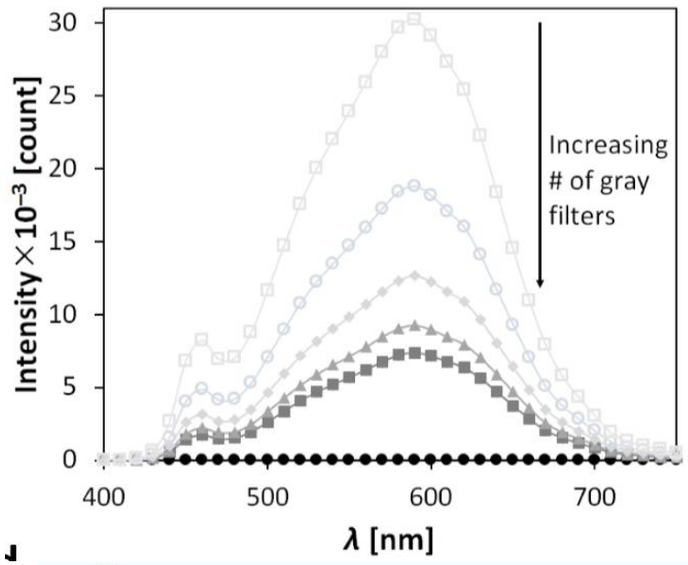
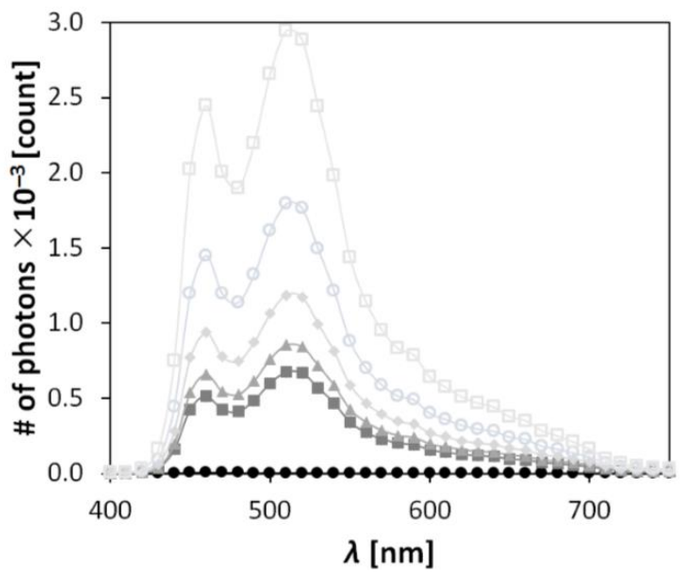
Calibration of Optical Path and Camera Chip

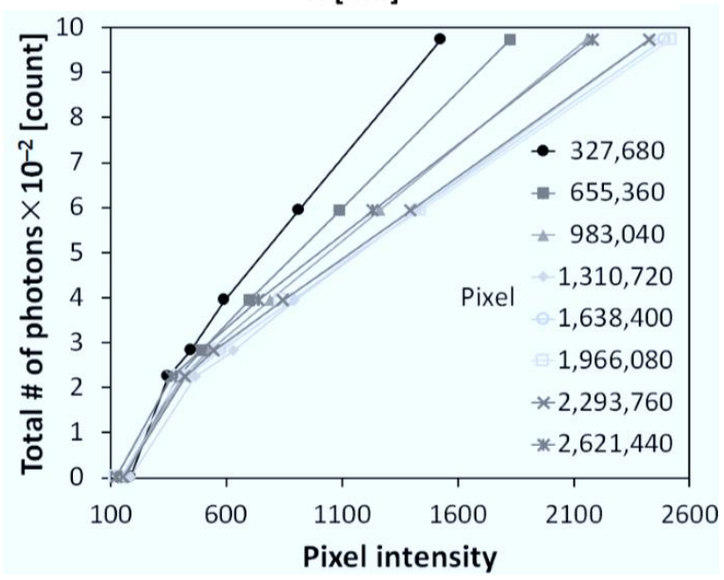
2. Computing Part

- For each pixel of image of filter and each relevant filter, **multiply the camera filter** transparency s**pectrum by the gray filter** transparency **spectrum**
  - ➢ Integral under the curve is a Total Number of Photons reaching the camera pixel
- Plot a calibration curve (image pixel intensity vs. TNP)
- For each pixel of a biological image, recalculate pixel intensity to TNP









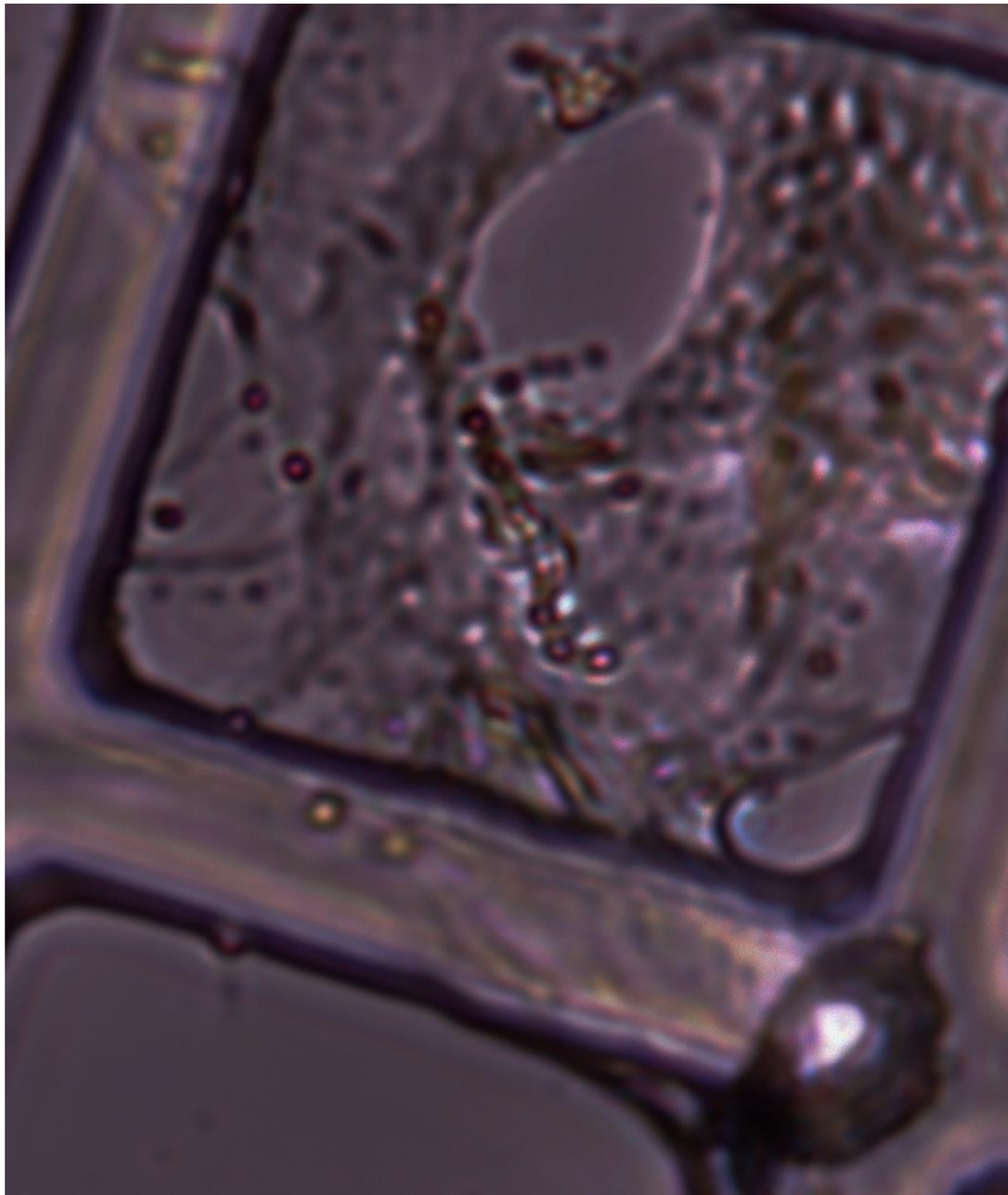
Resulted image correction

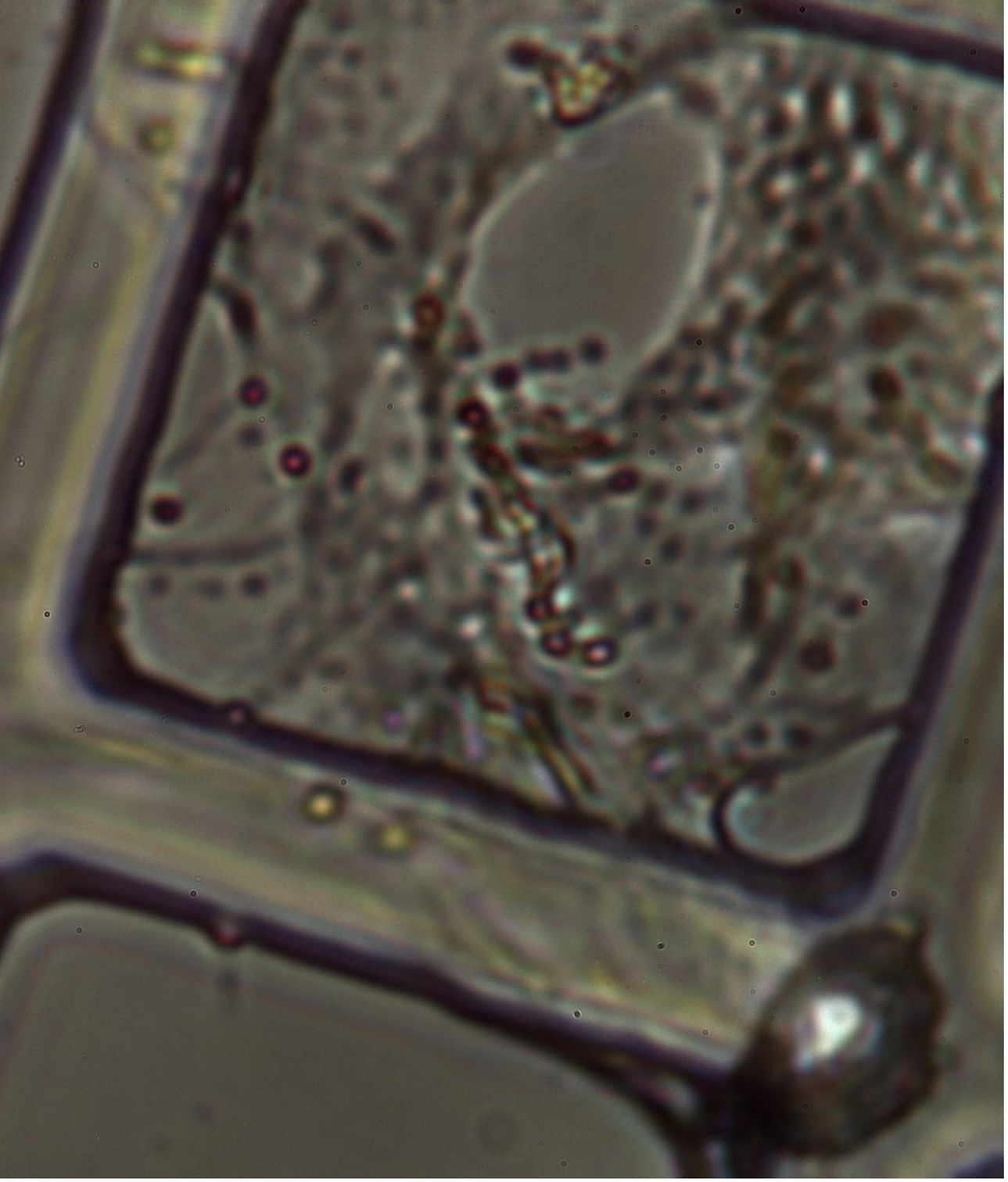
Uncalibrated

Calibrated

Resulted image correction

Calibrated





Uncalibrated

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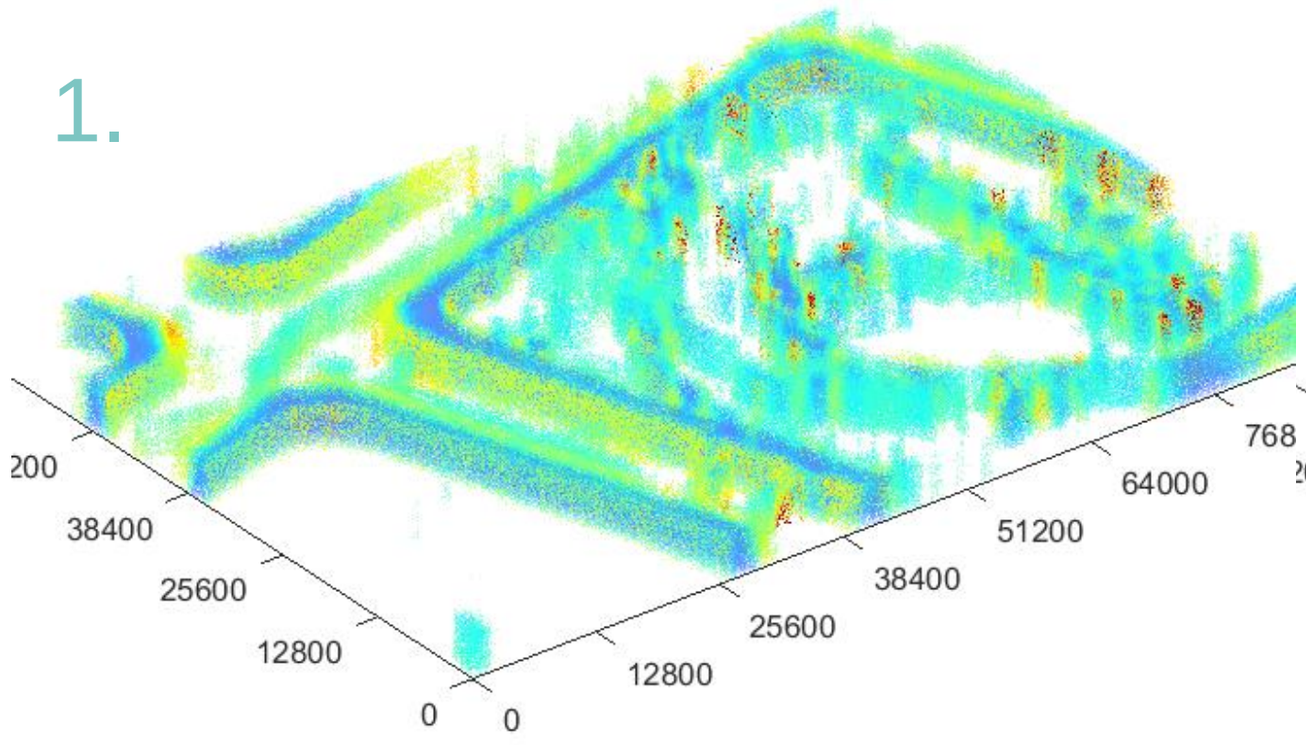
Final 3D image of Electromagnetic Centroids

B-channel with increasing intensity threshold

1.



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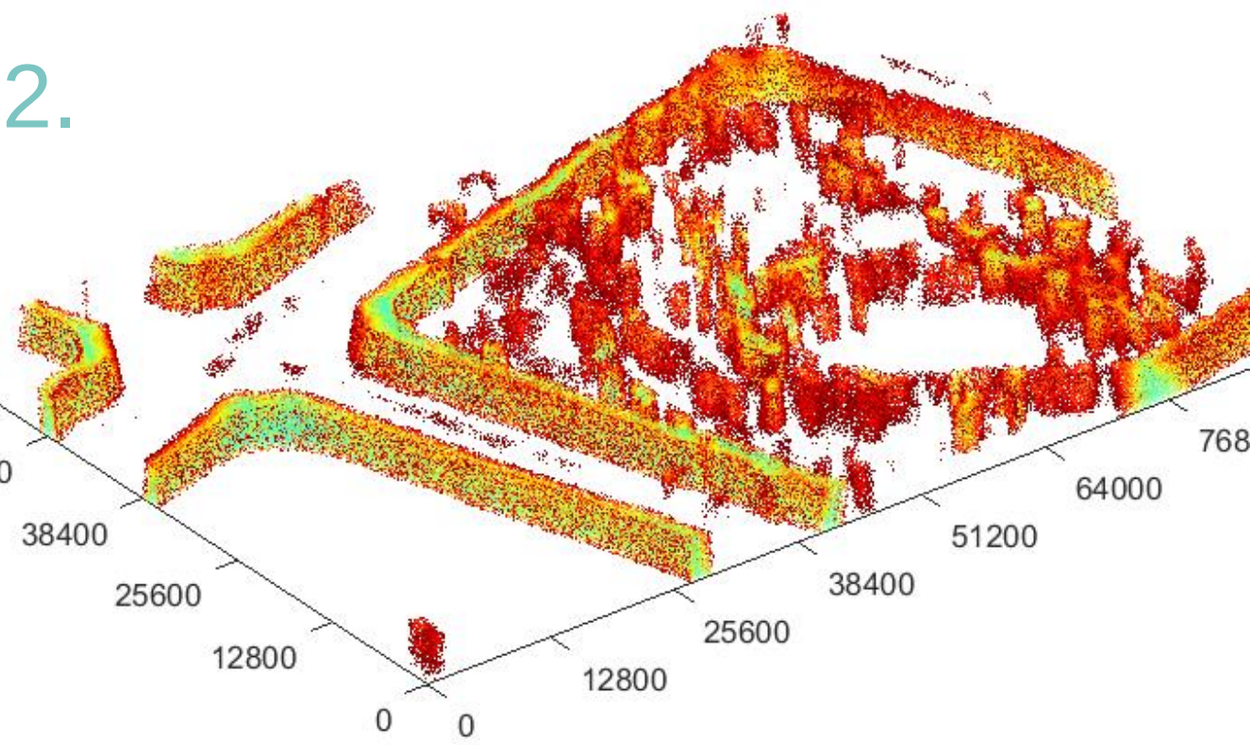
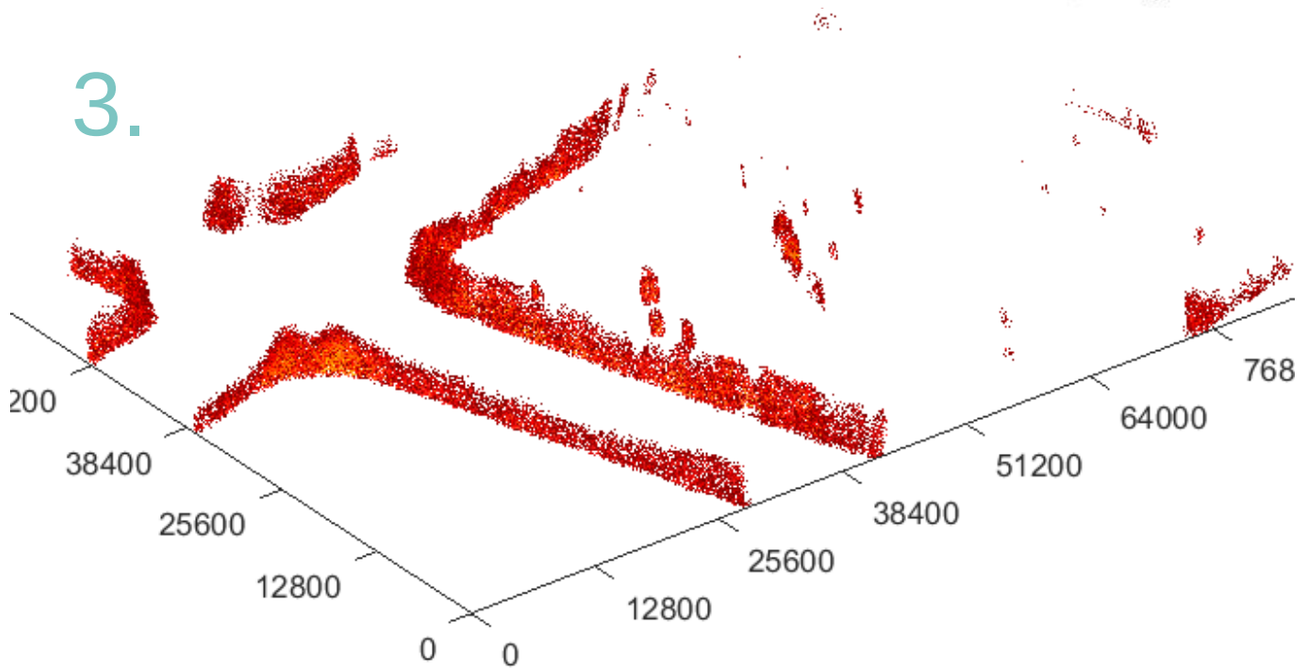
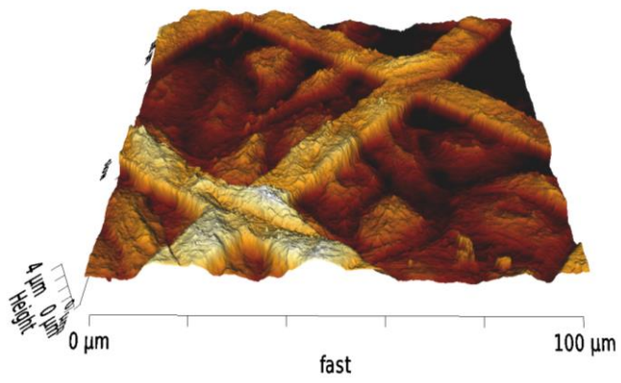


3.





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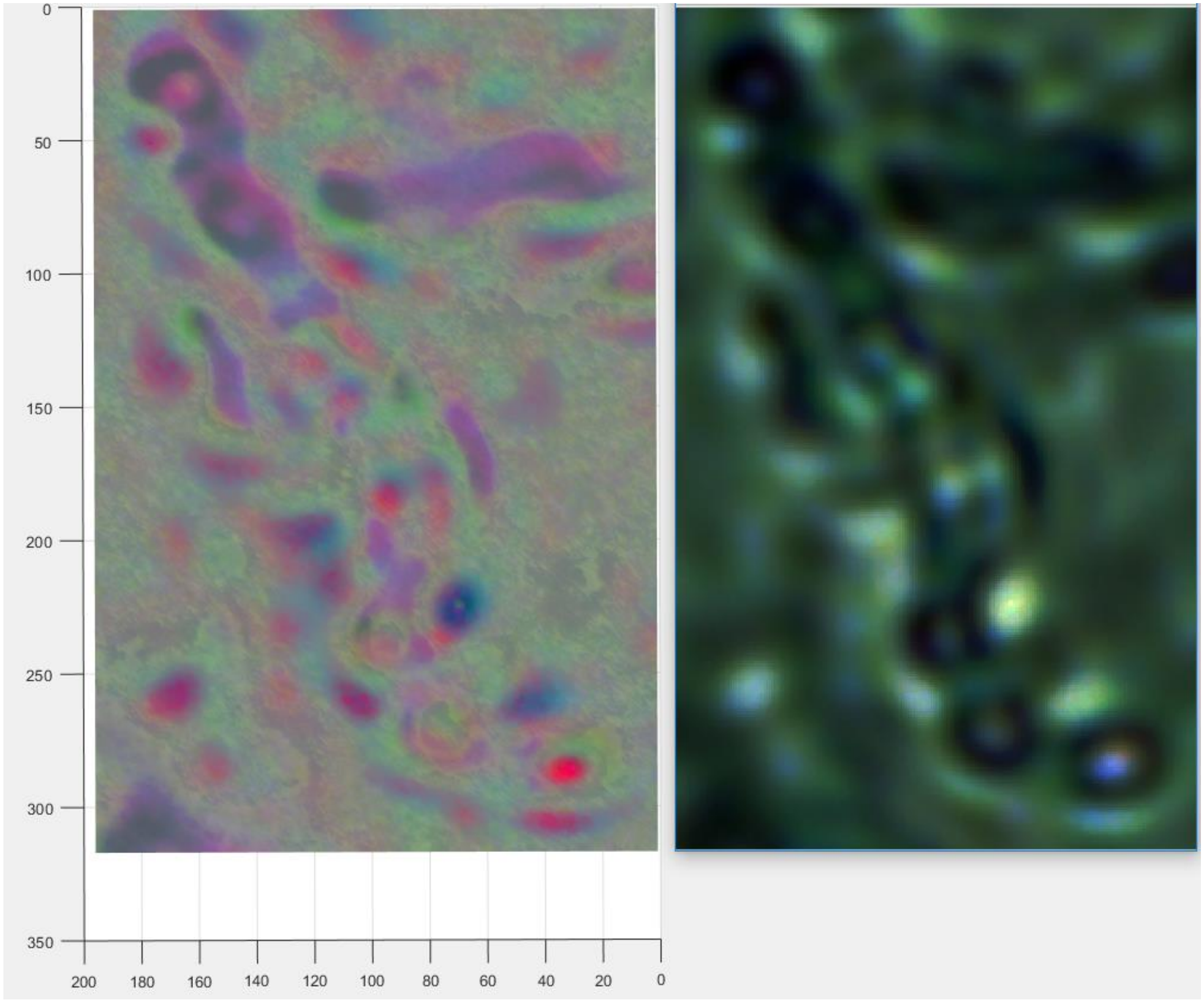
Diffraction Extraction



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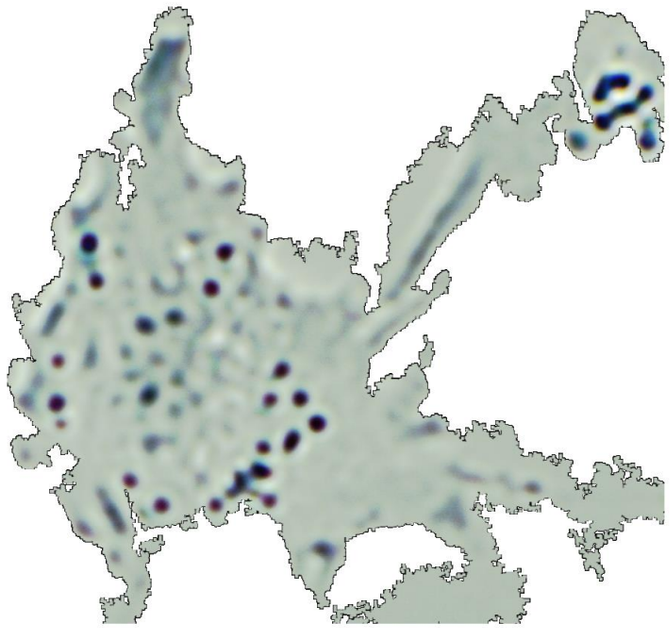
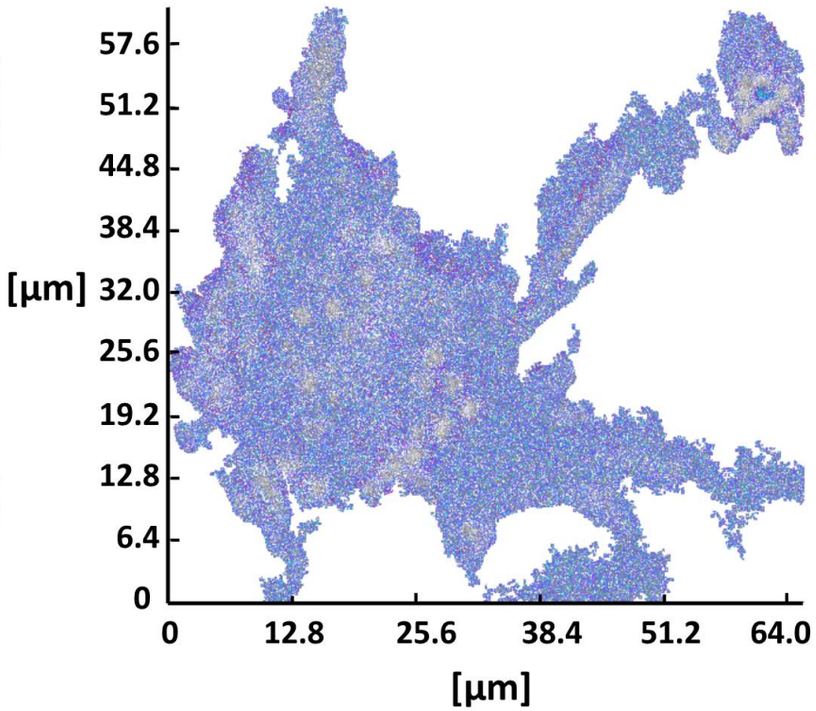
Final 3D image of Electromagnetic Centroids

L929 mouse fibroblast

In-focus image

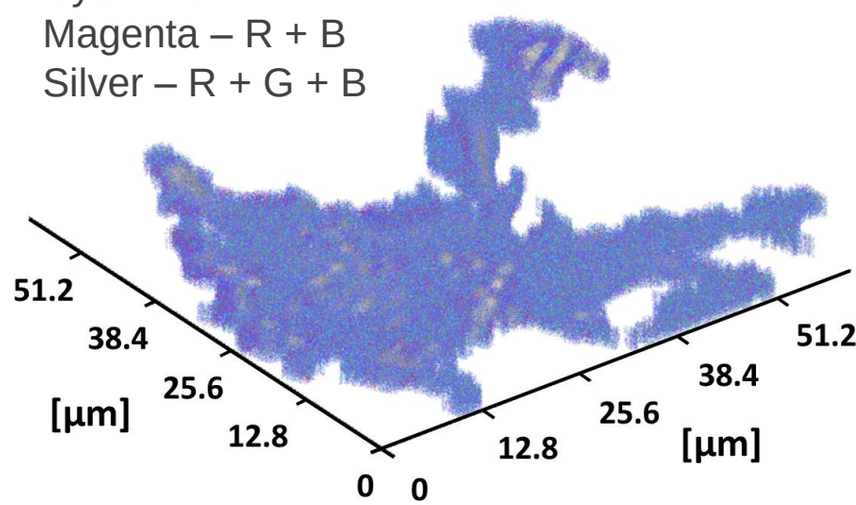


ECs – RGB 2D projection

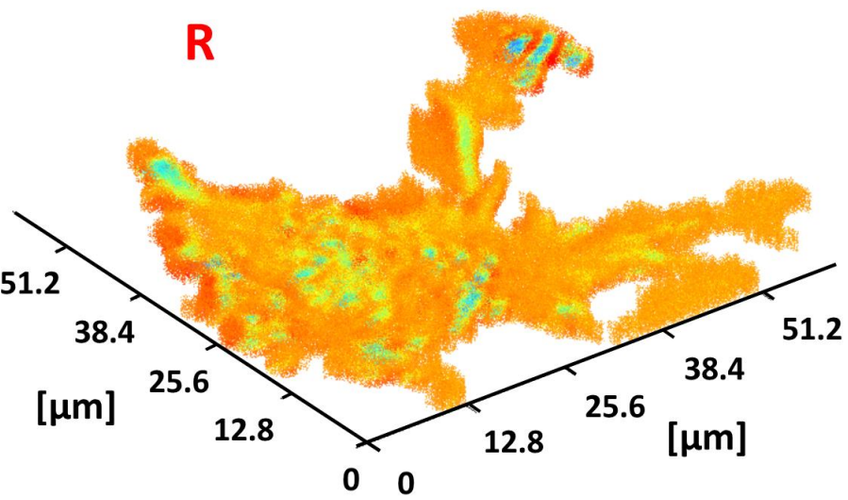


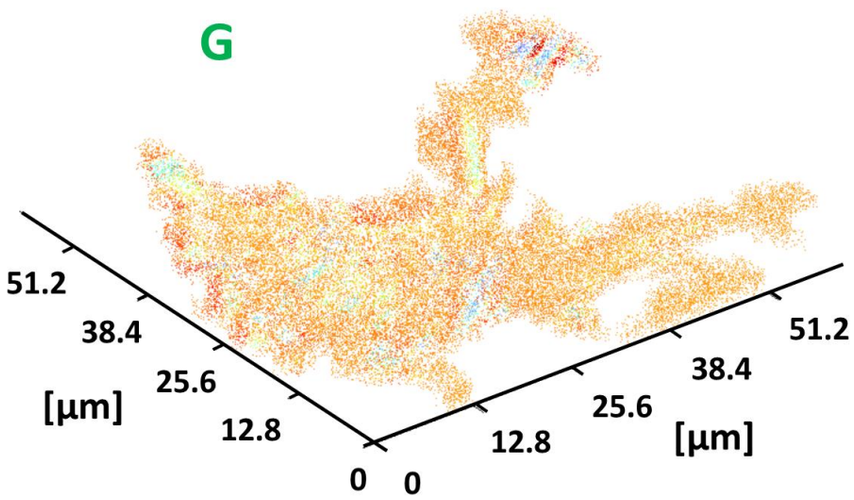
ECs – RGB

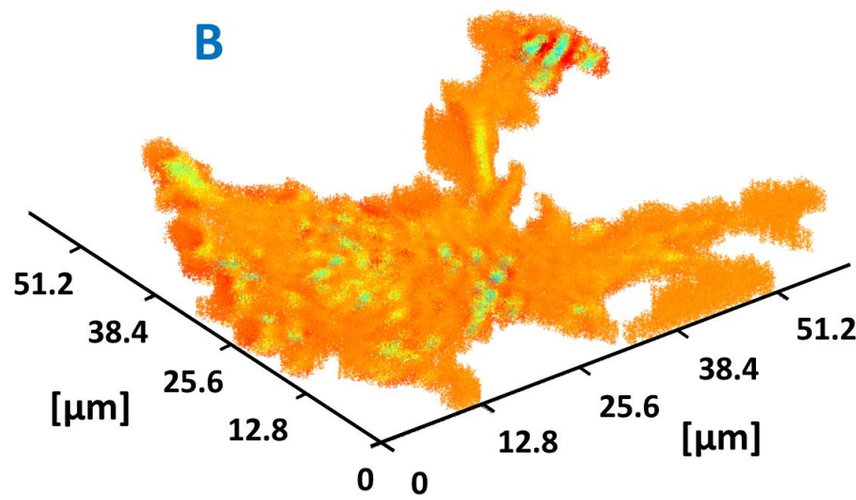
Yellow – R+G,
Cyan – G+B
Magenta – R + B
Silver – R + G + B



ECs for each colour channel







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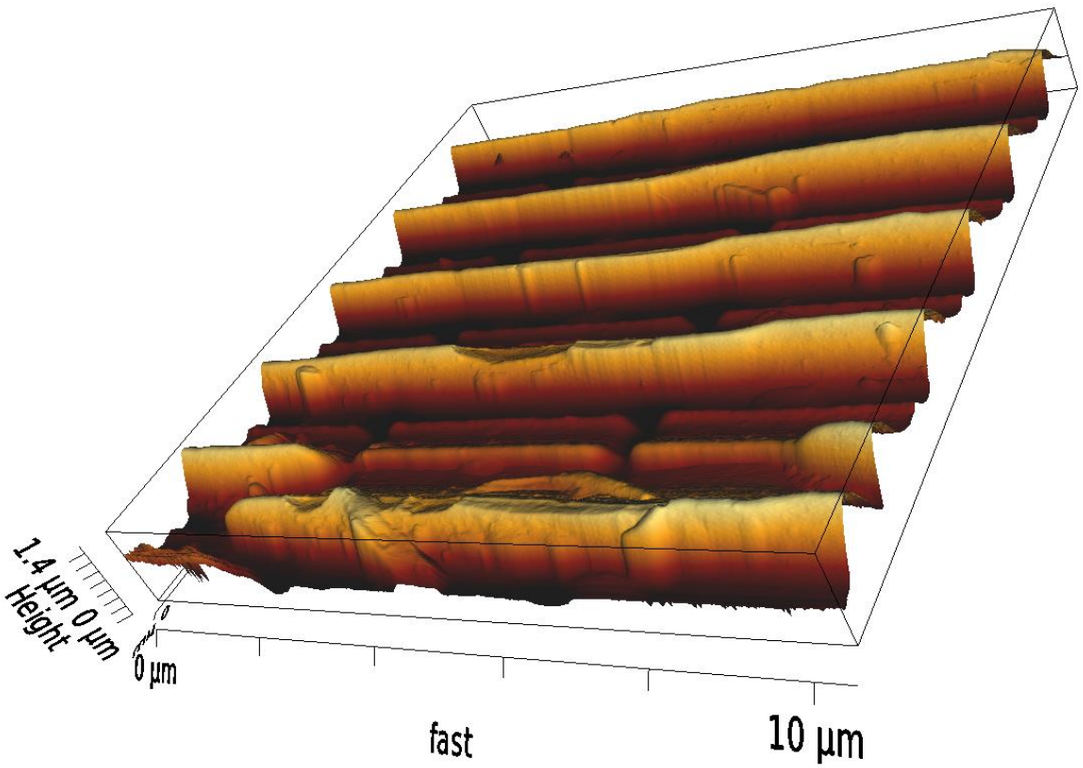
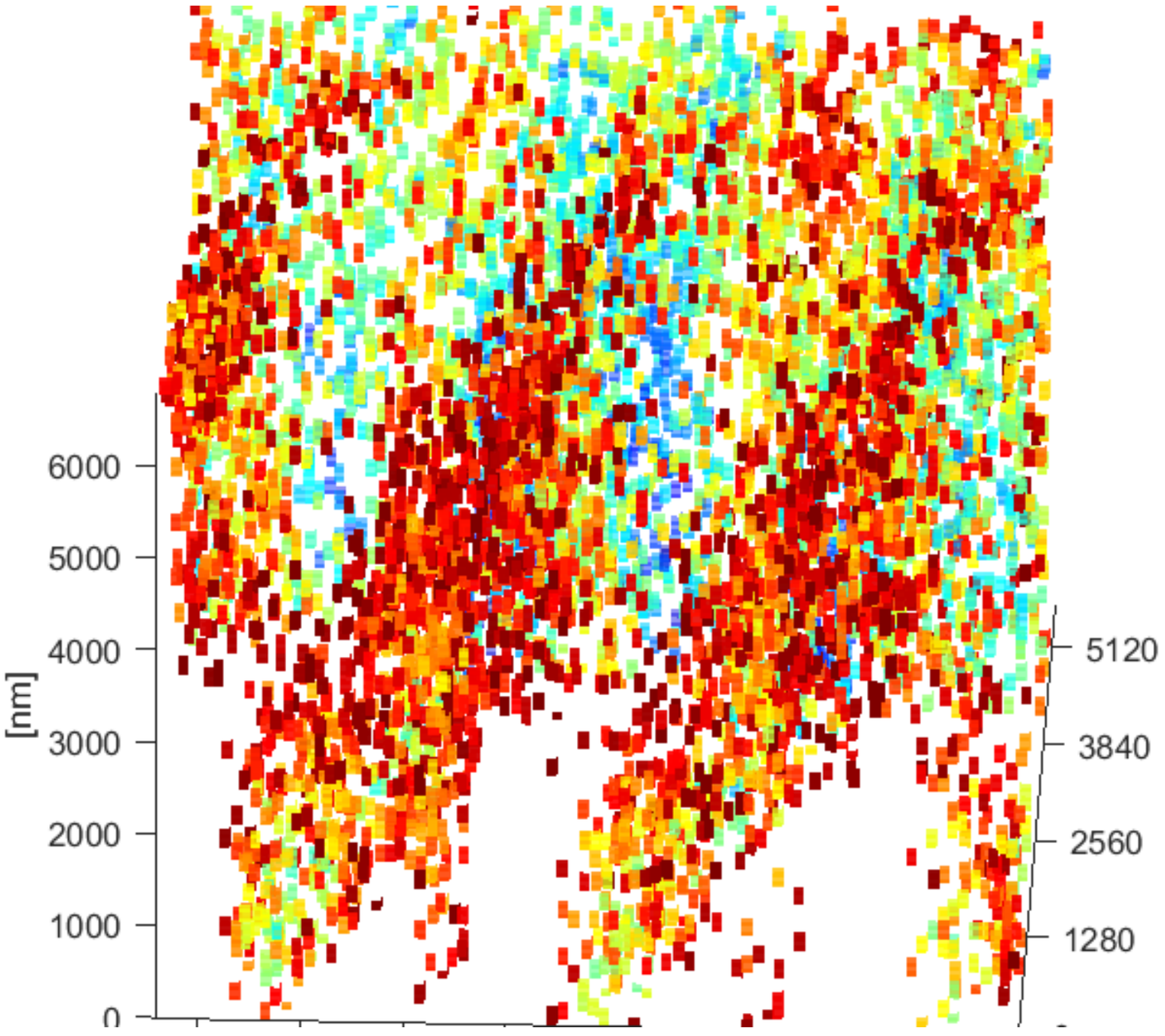
Nanoroofs



AFM

R-channel



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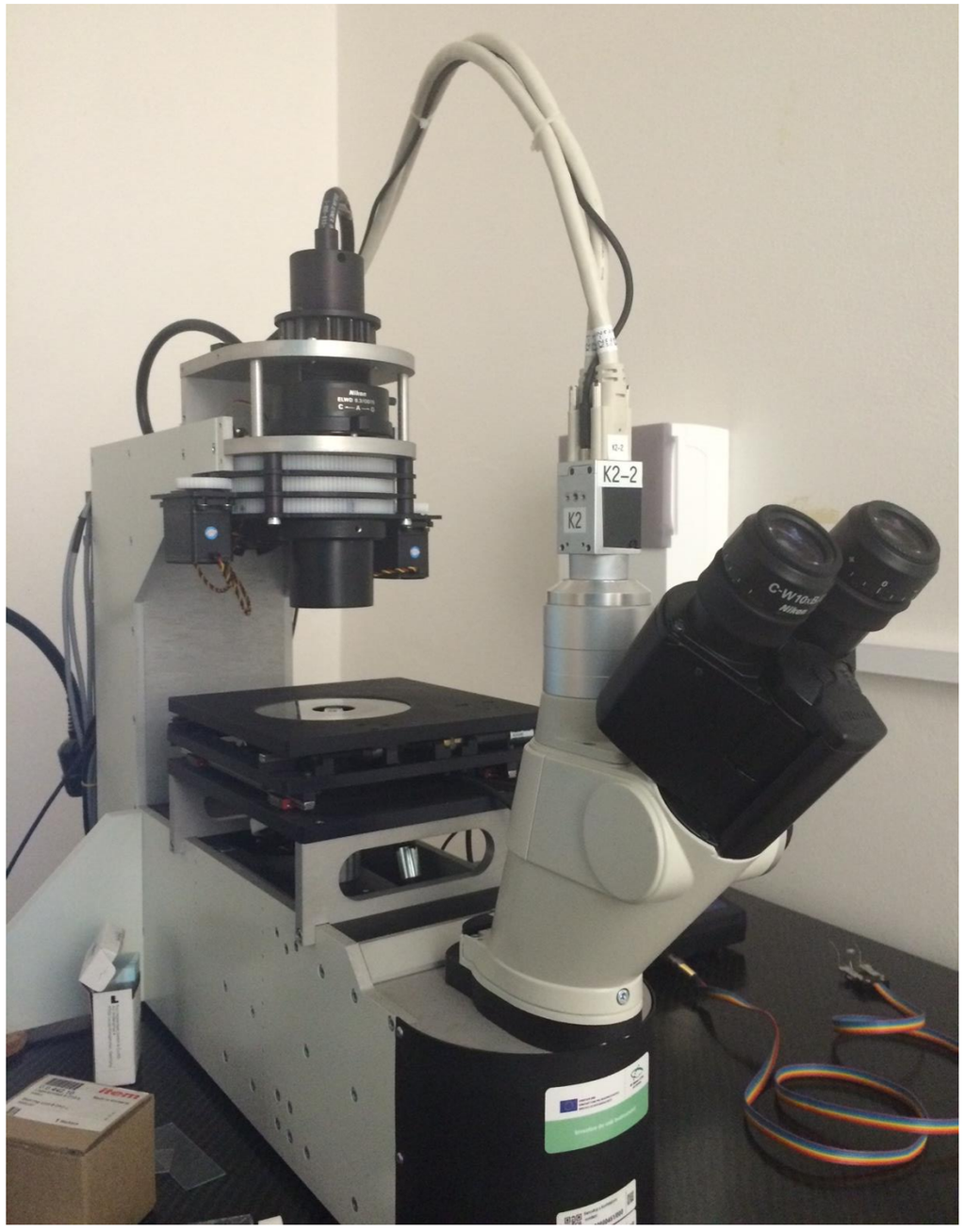
Thanks to:

- prof. Dalibor Štys
- Ing. Pavel Souček
- Vladimír Kotal
- Ing. Petr Macháček
- Ing. Petr Tax
- Anna Platonova
- and others



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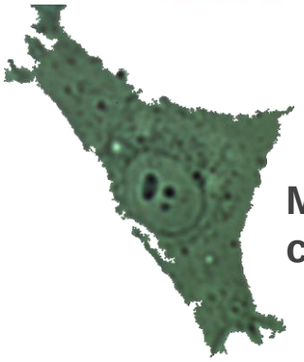
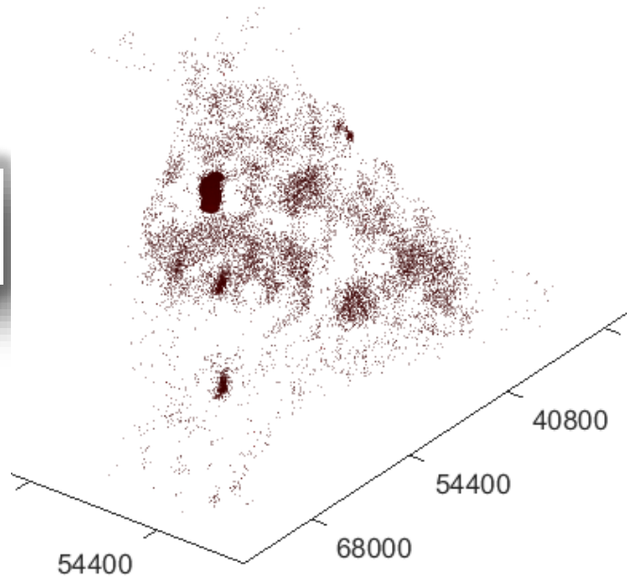
3D reconstruction



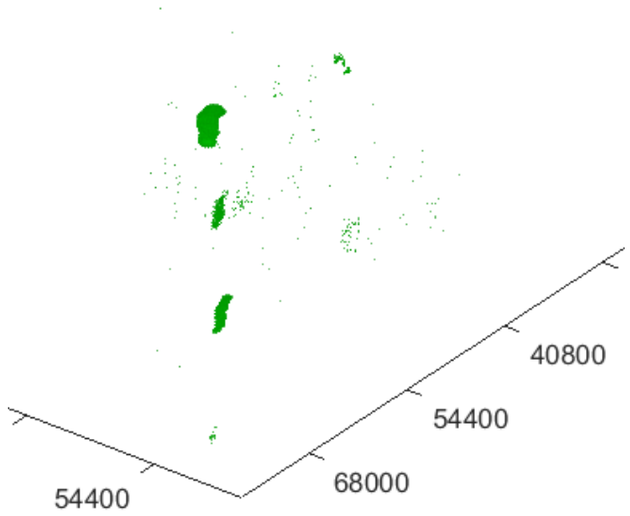
MG-63
cell line

Autofluorescence
Positive interference

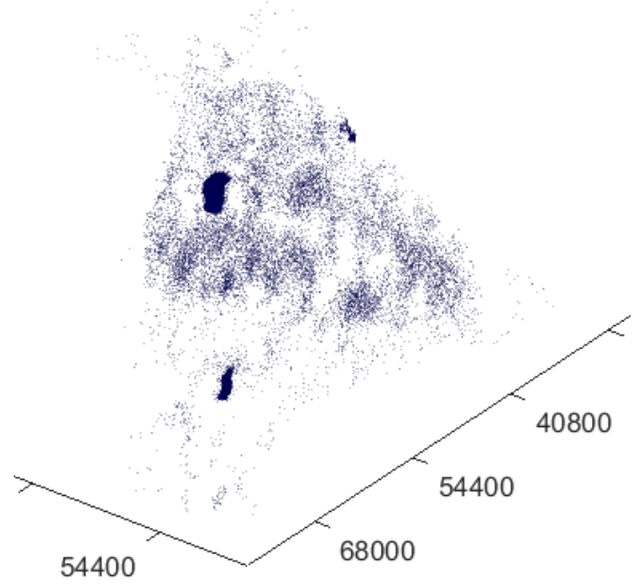
R bright



G bright

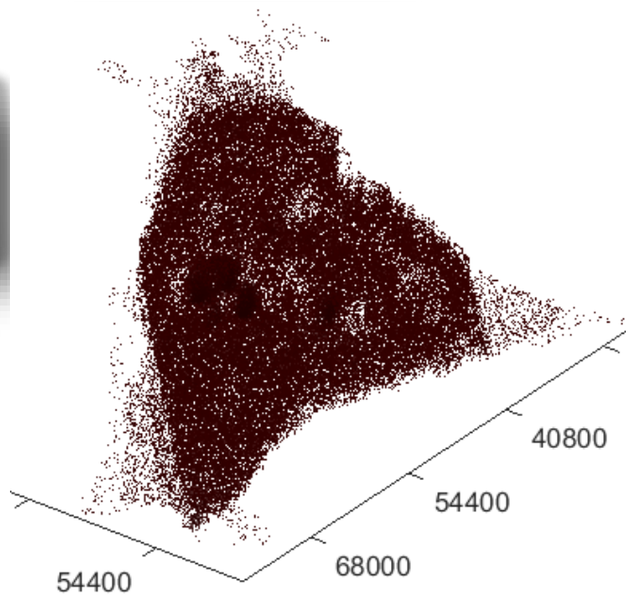


B bright

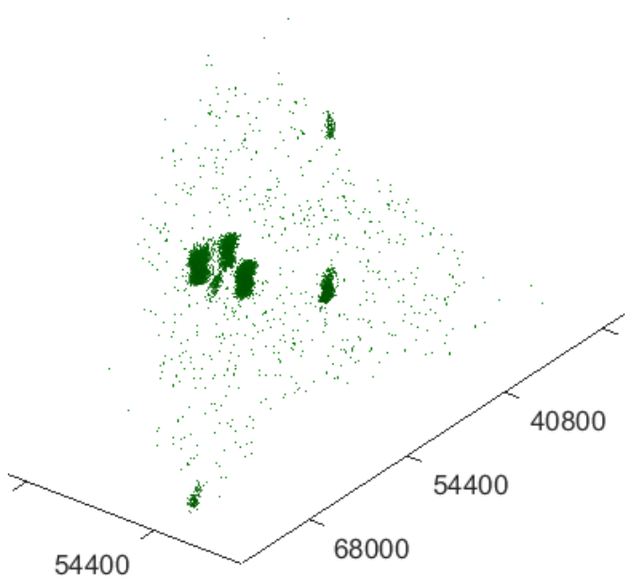


Diffraction
Absorption
Negative interference

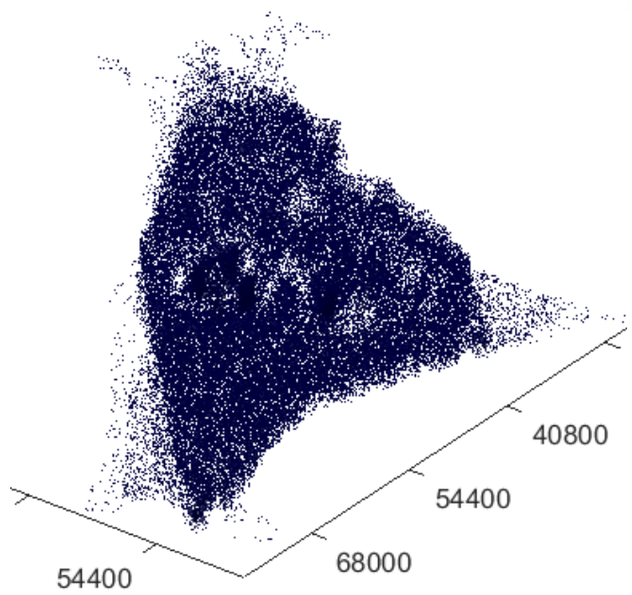
R dark



G dark



B dark



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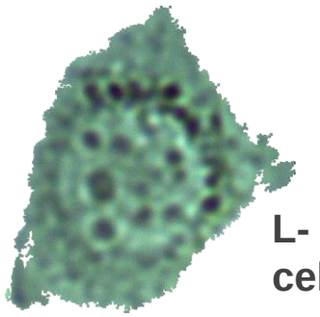
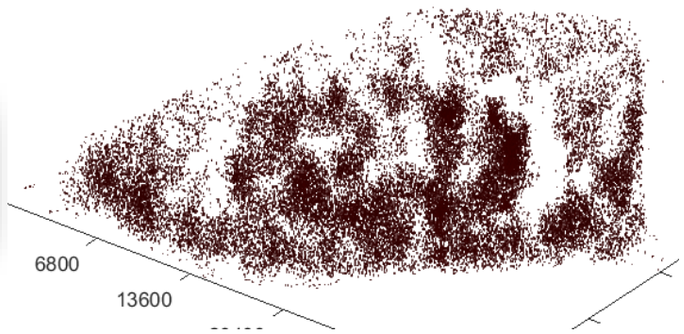
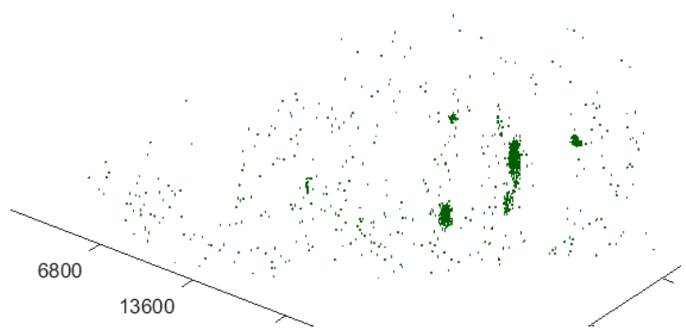


L- 929
cell line

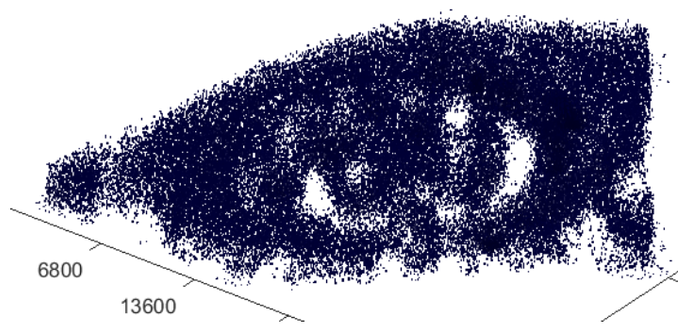
R bright



G bright

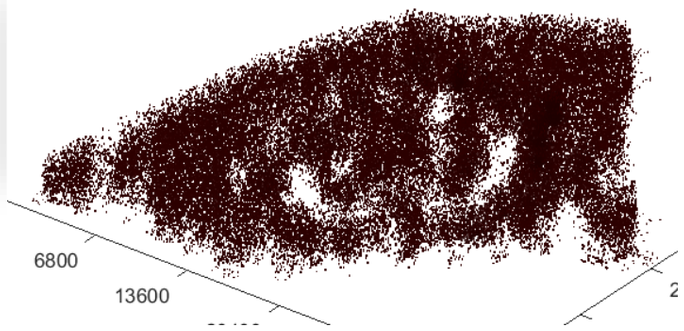


B bright

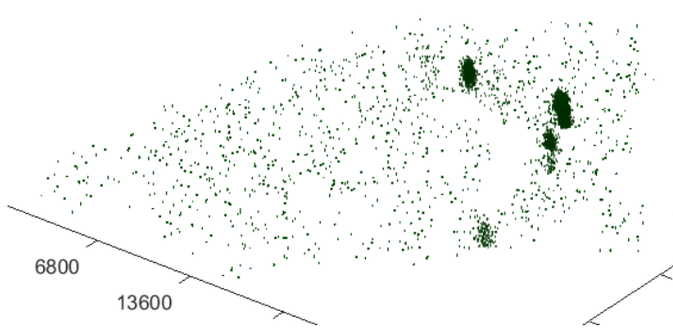


Autofluorescence
Positive interference

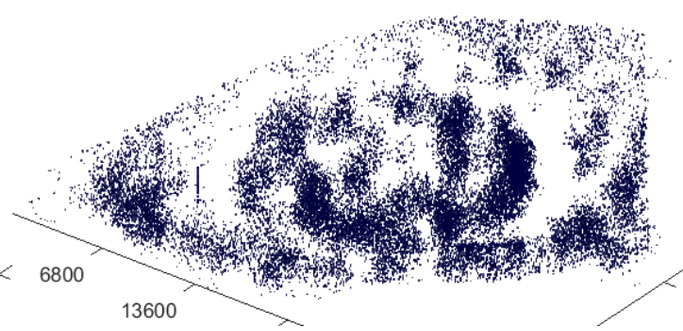
R dark



G dark



B dark



Diffraction
Absorption
Negative interference

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