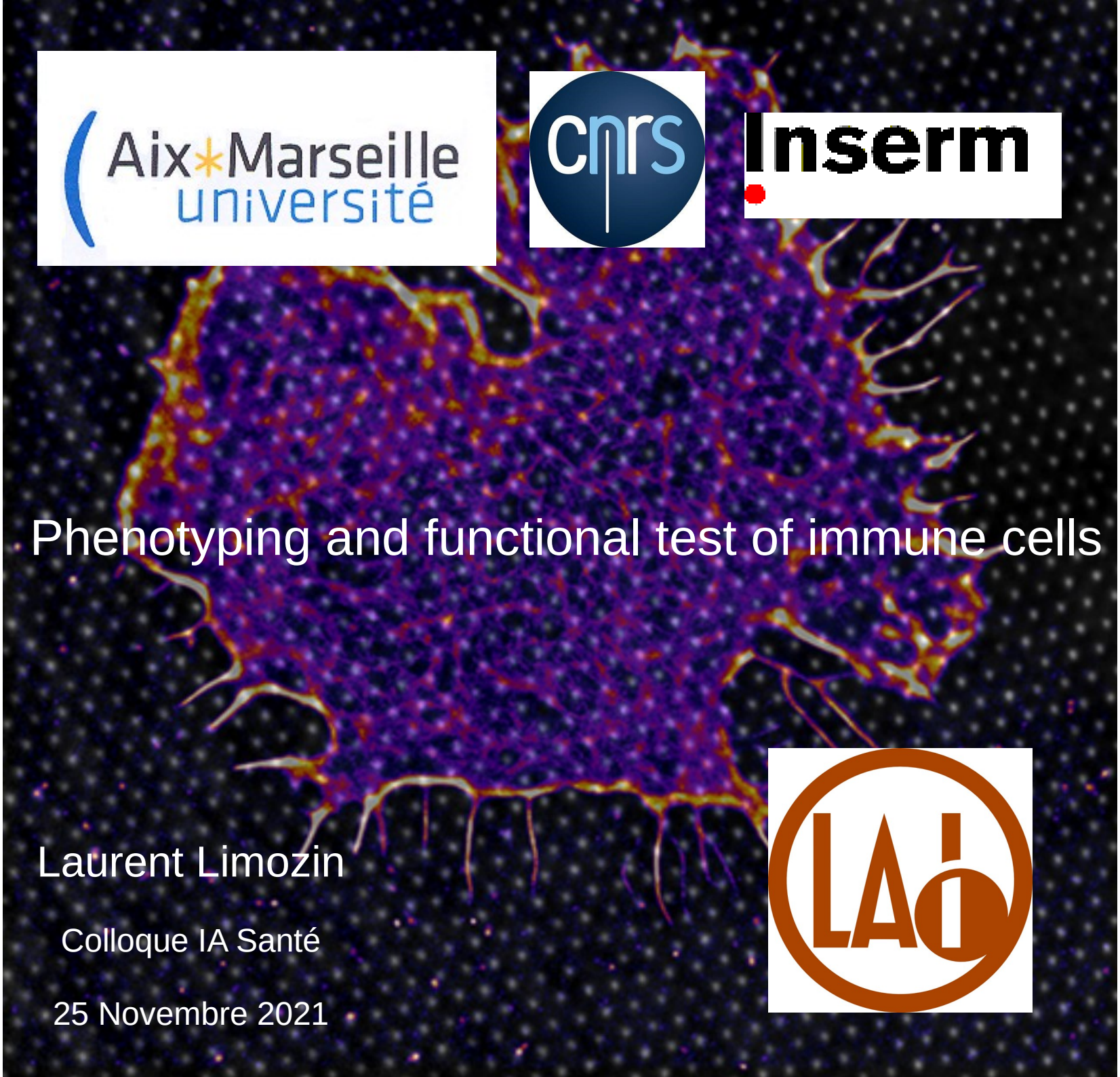


Phenotyping and functional test of immune cells

Laurent Limozin

Colloque IA Santé

25 Novembre 2021

Outline

Biophysics of immune cells function
- in vitro experiments
- cells from donors

Optical imaging:
- multimodal
- time-lapse sequences

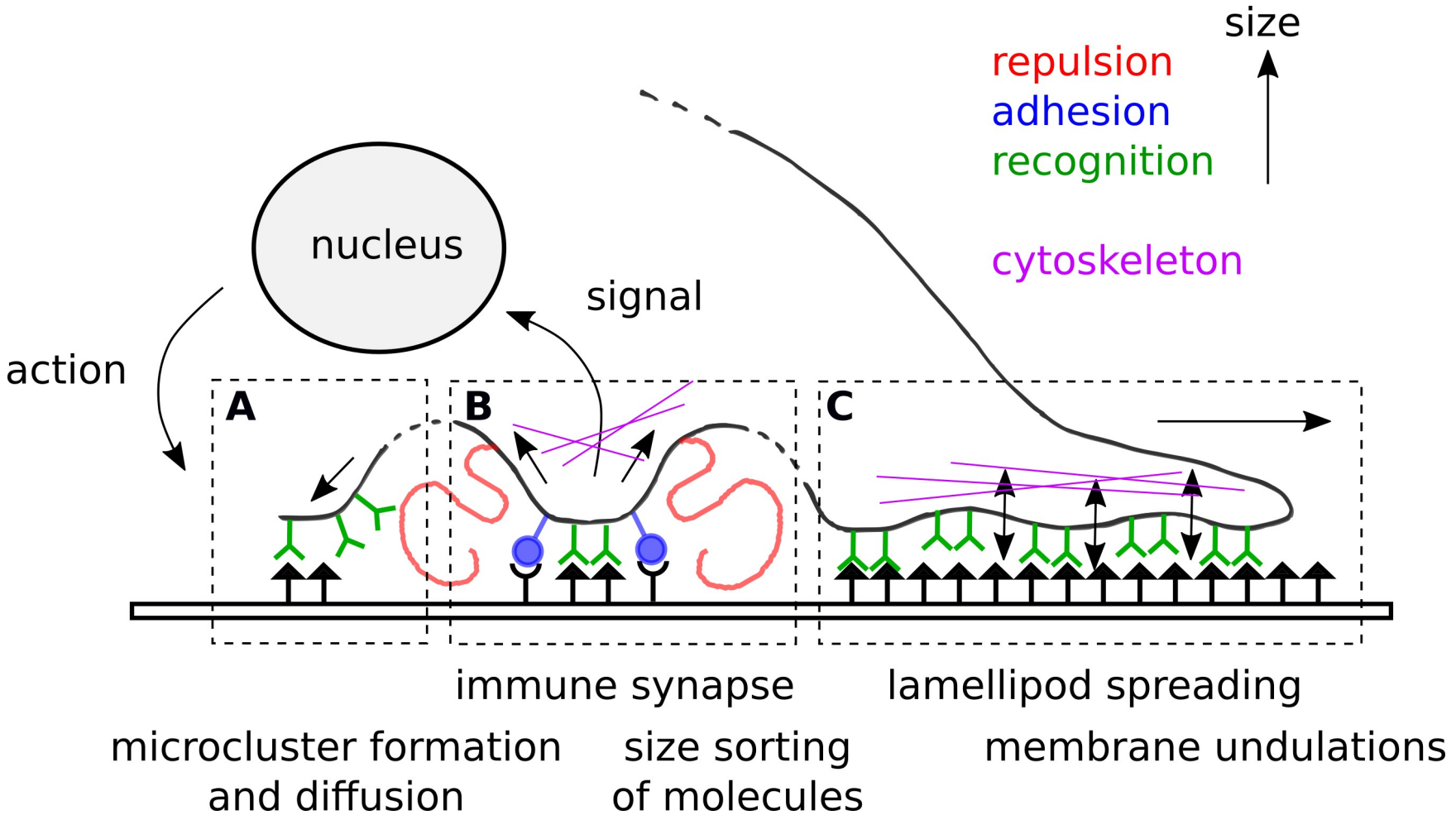
Health applications:
- test cells or active molecules (eg antibodies)

1. cell adhesion and identification: regression, generation

2. subcellular structure: classification, generation

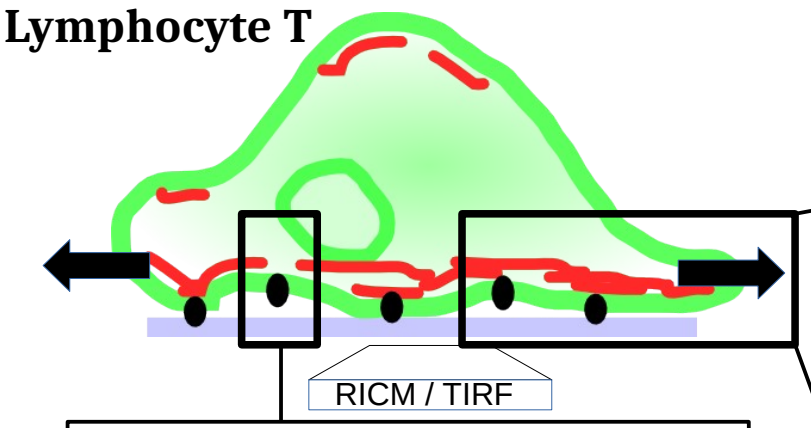
3. killing function: segmentation, classification

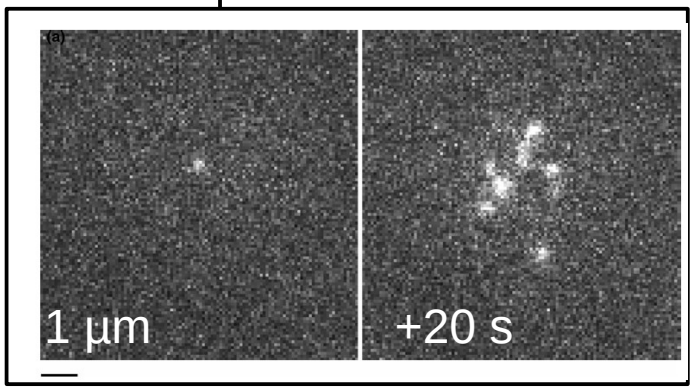
Biophysics of immune recognition



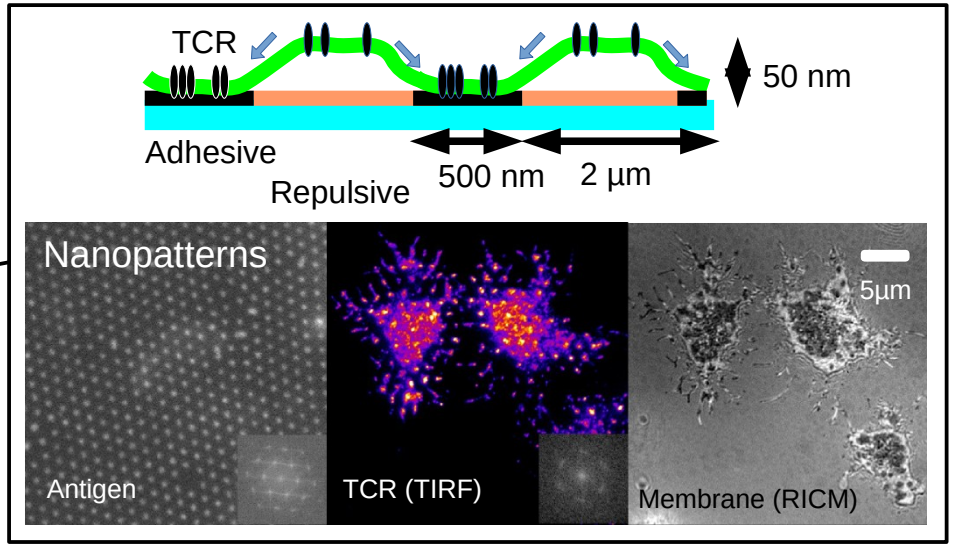
Limozin and Puech 2019

Surface stimulation and lymphocyte spreading

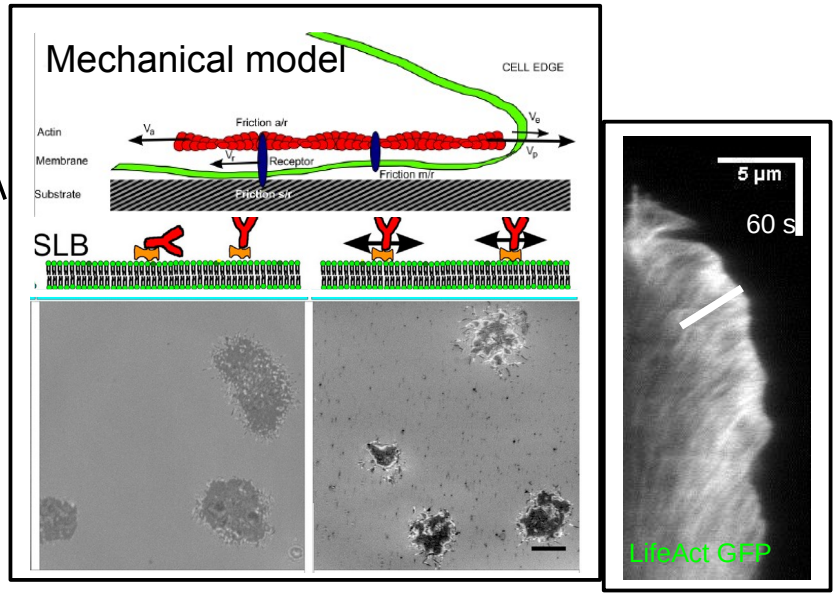




Homogeneous substrates

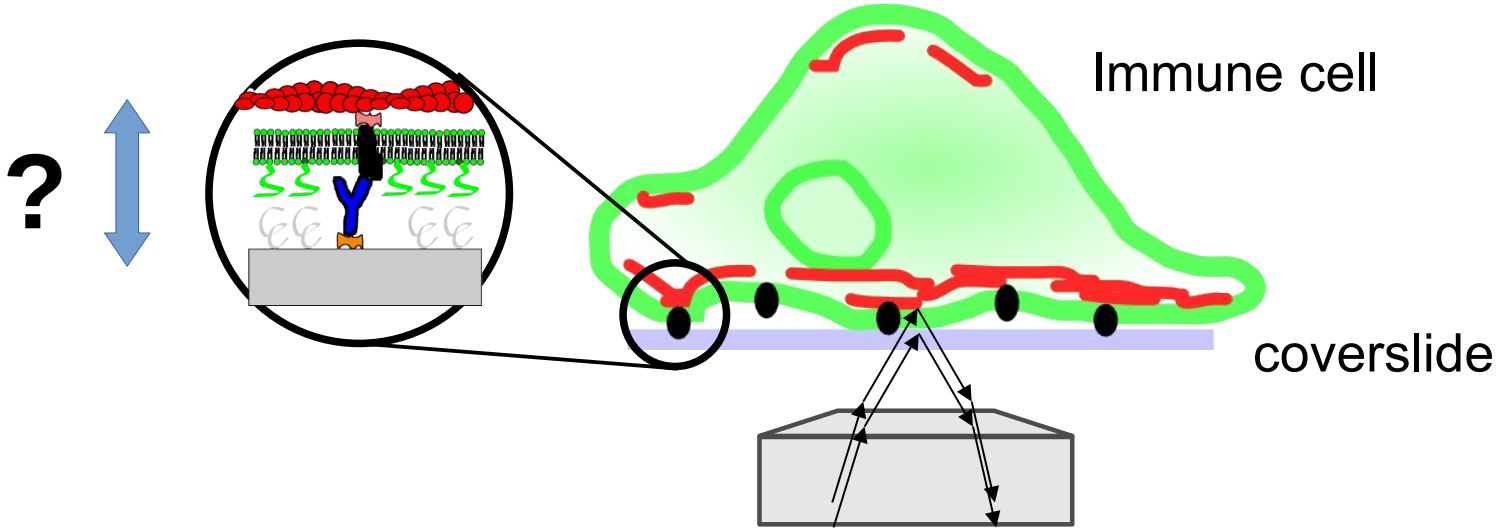


Textured substrates

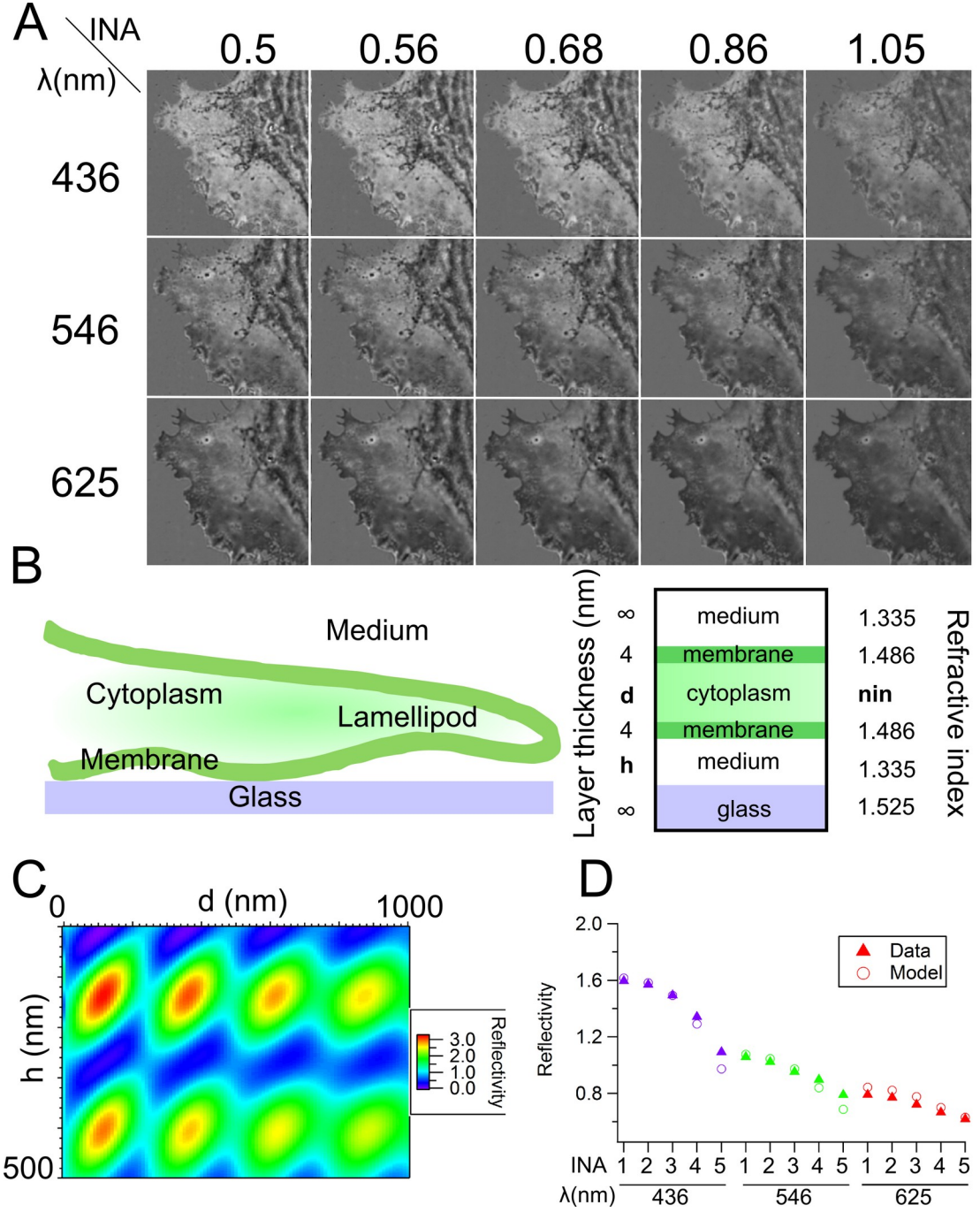


Fluid vs solid substrate

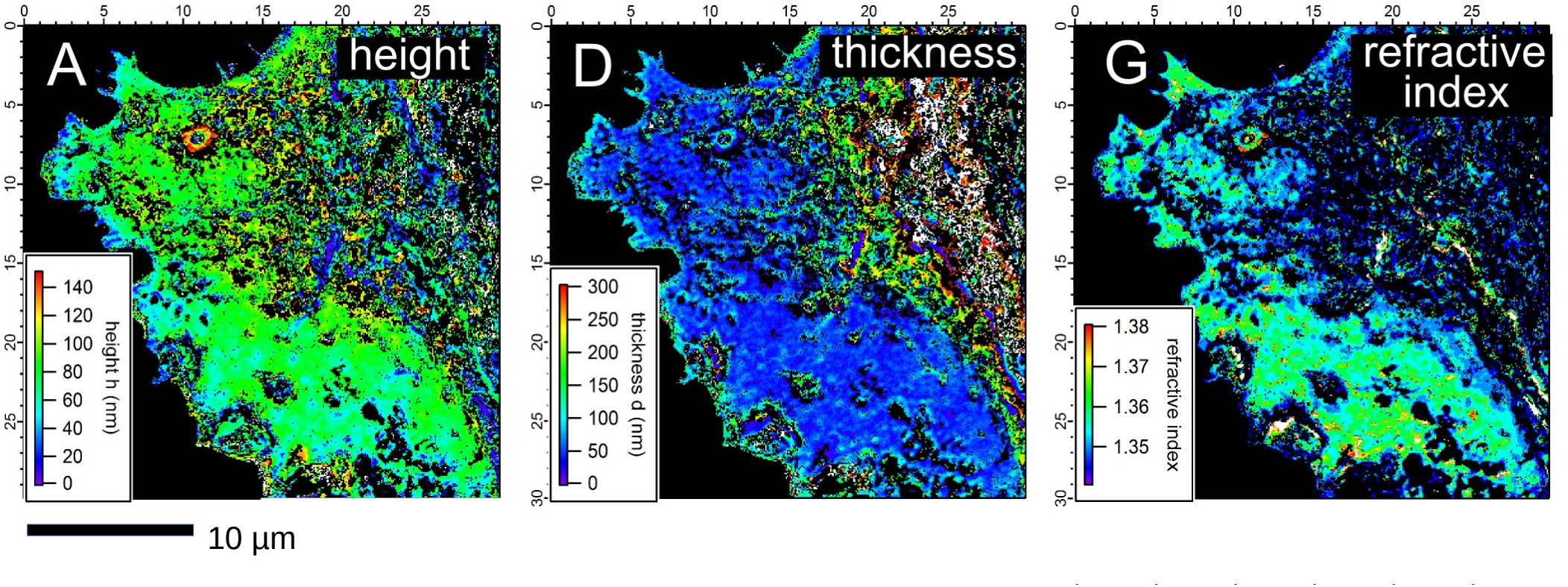
Quantitative surface optical microscopy

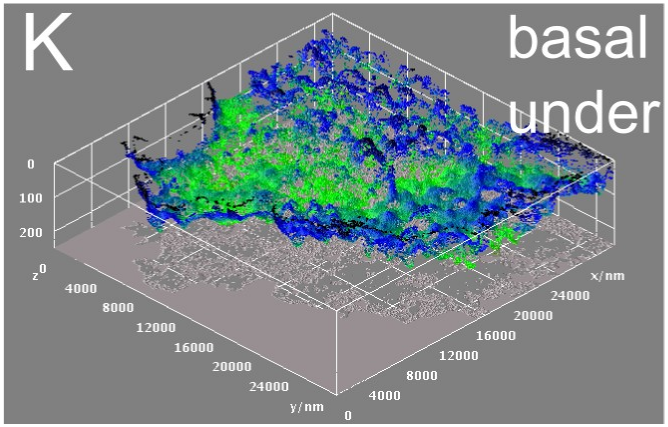


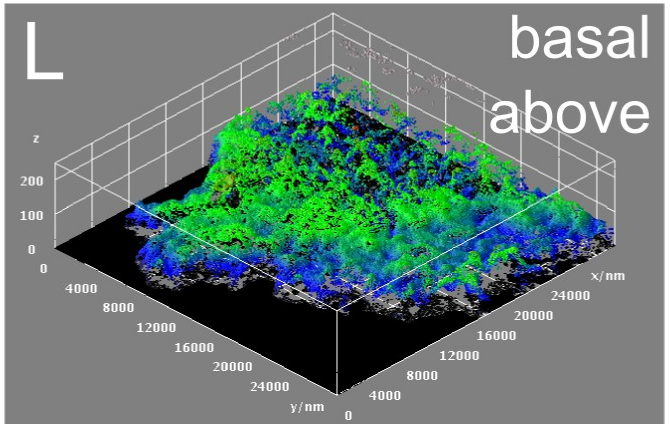
3D-Reflection Interference Contrast Nanoscopy (3D-RICN): an inverse problem

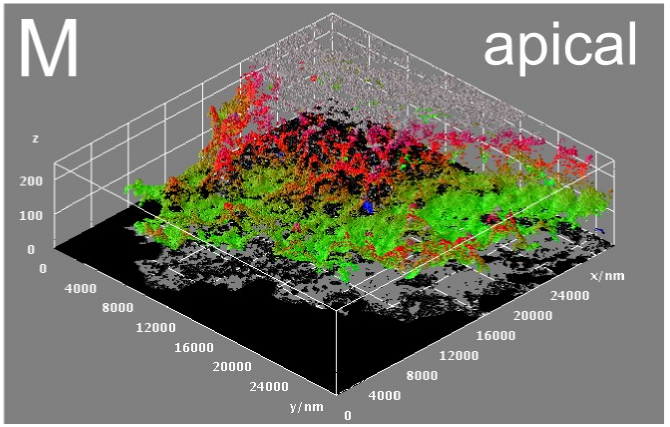


3D-RICN: reconstruction



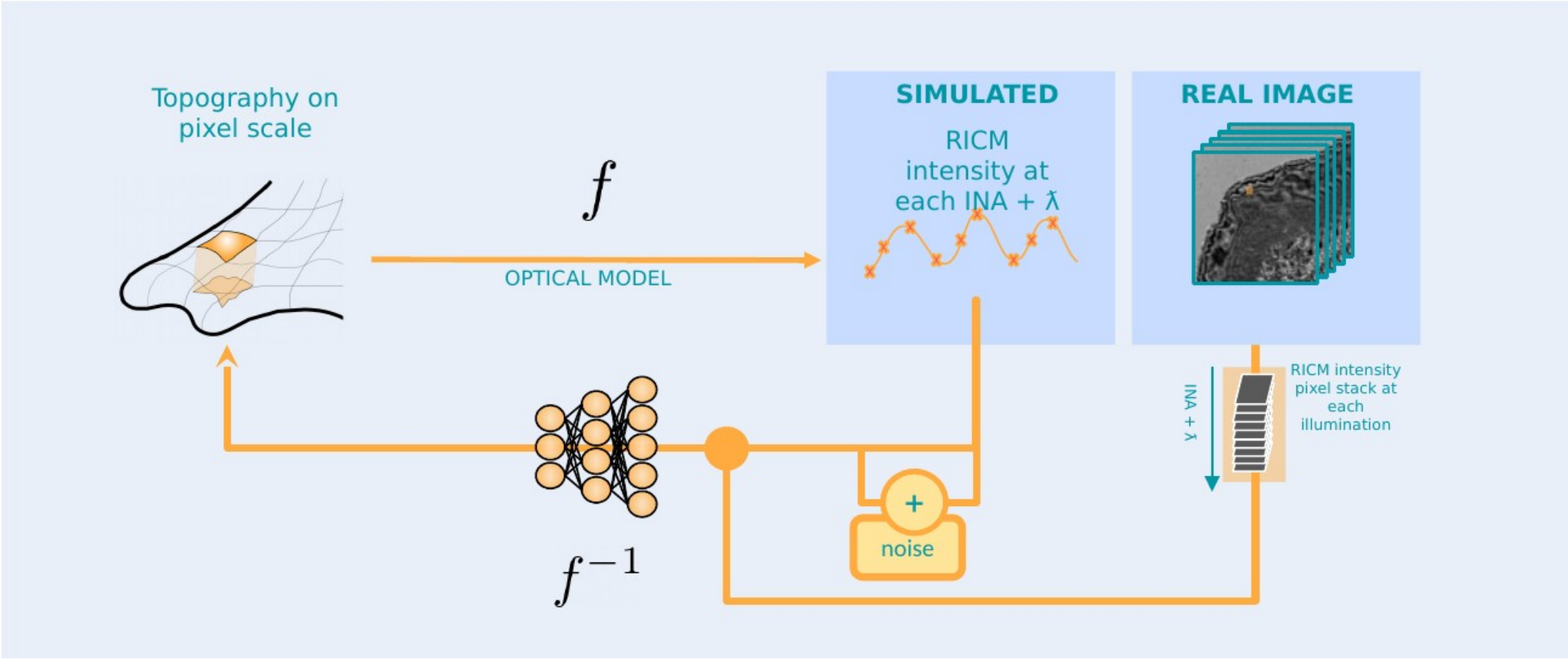






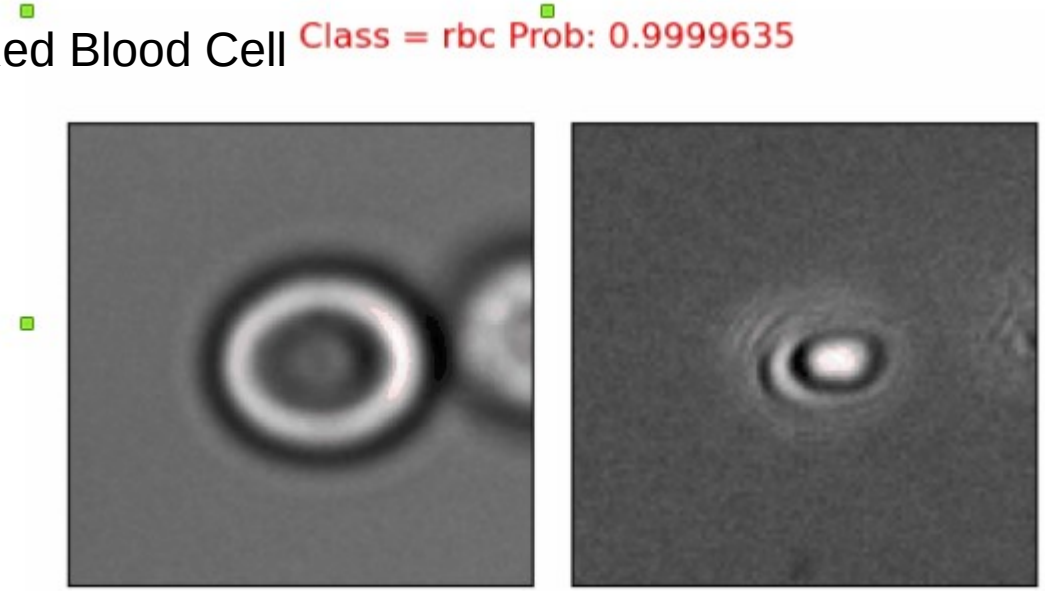
Dejardin, Hemmerlé … Limozin, Nanoletters (2019)

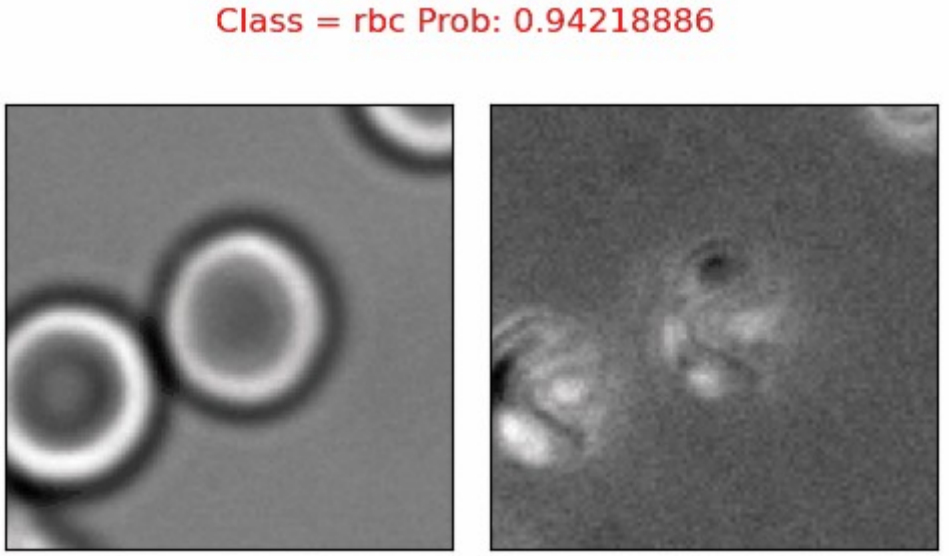
Inverting the topography to RICM problem



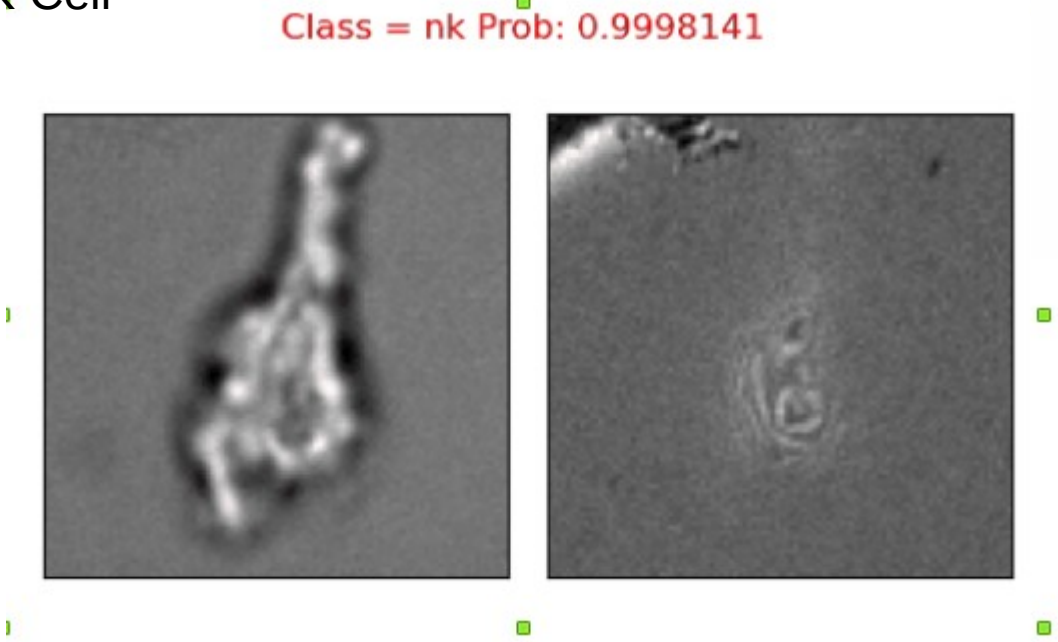
Blood cell classification

Red Blood Cell

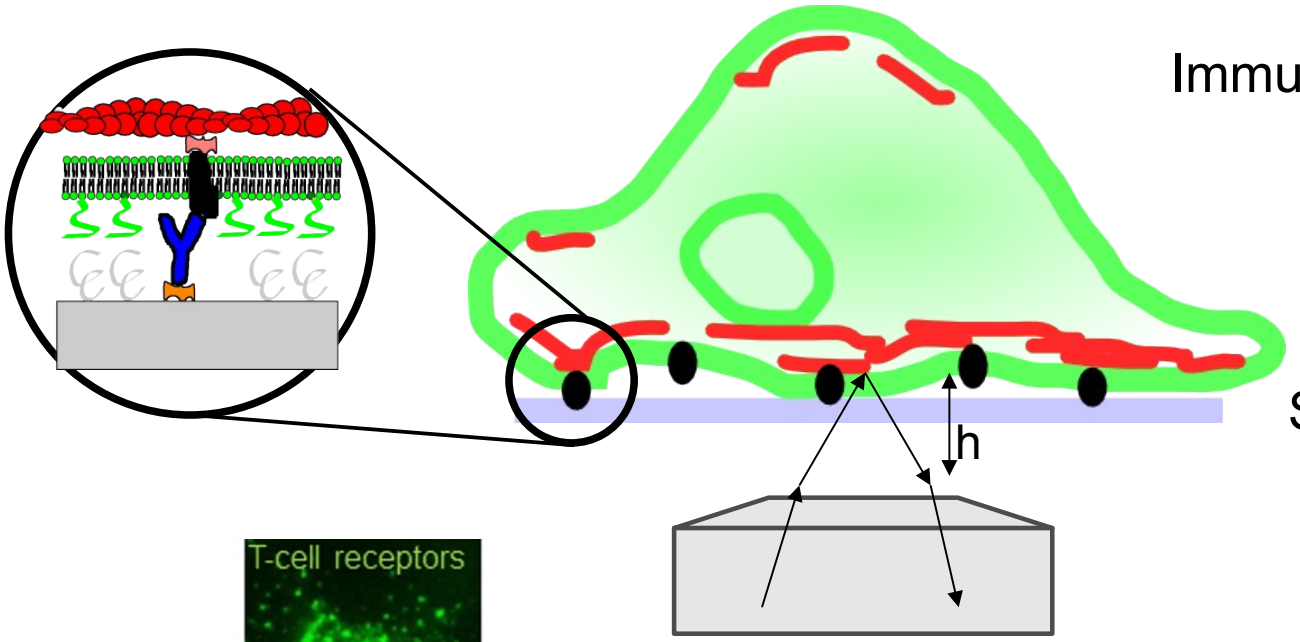


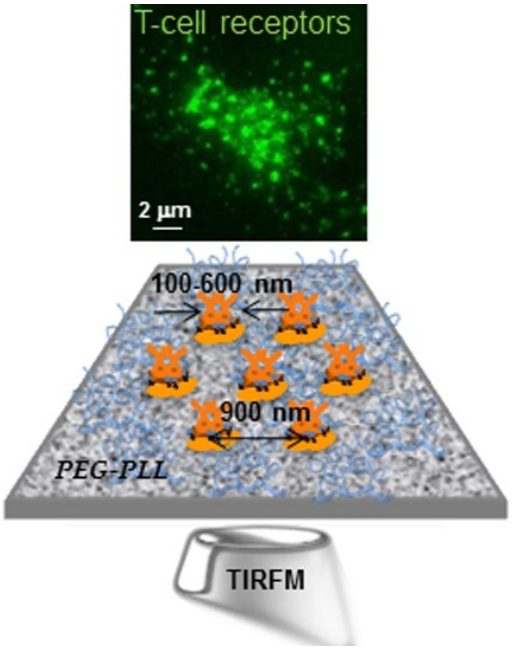


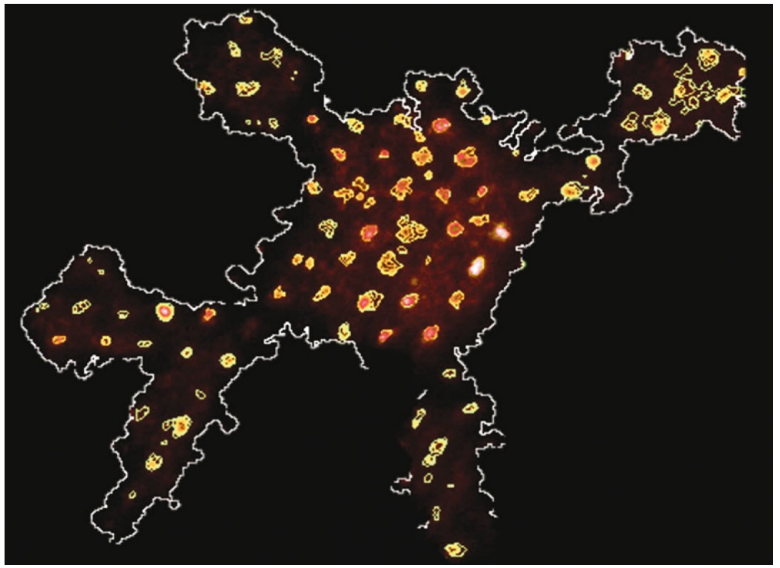
NK Cell



Surrogate cell and nanopatterning

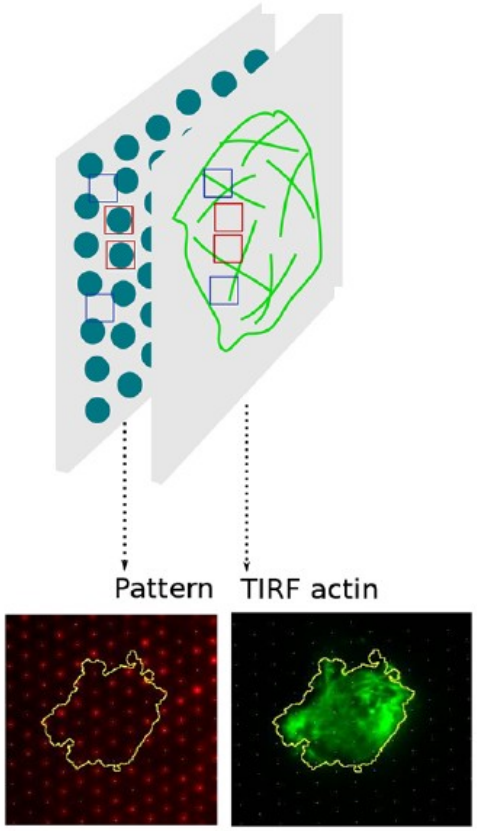




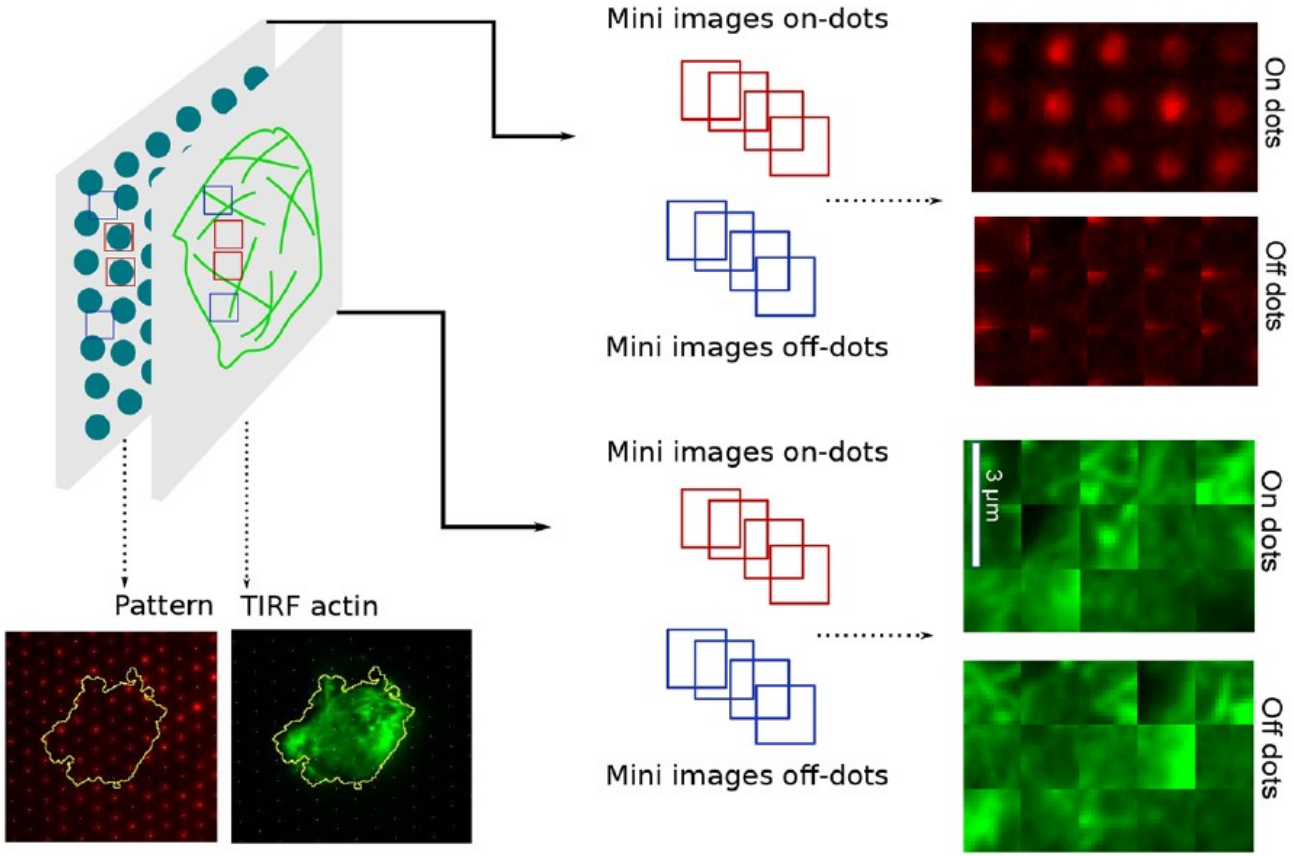


Pi et al. 2013, 2015
Dillard et al. 2016

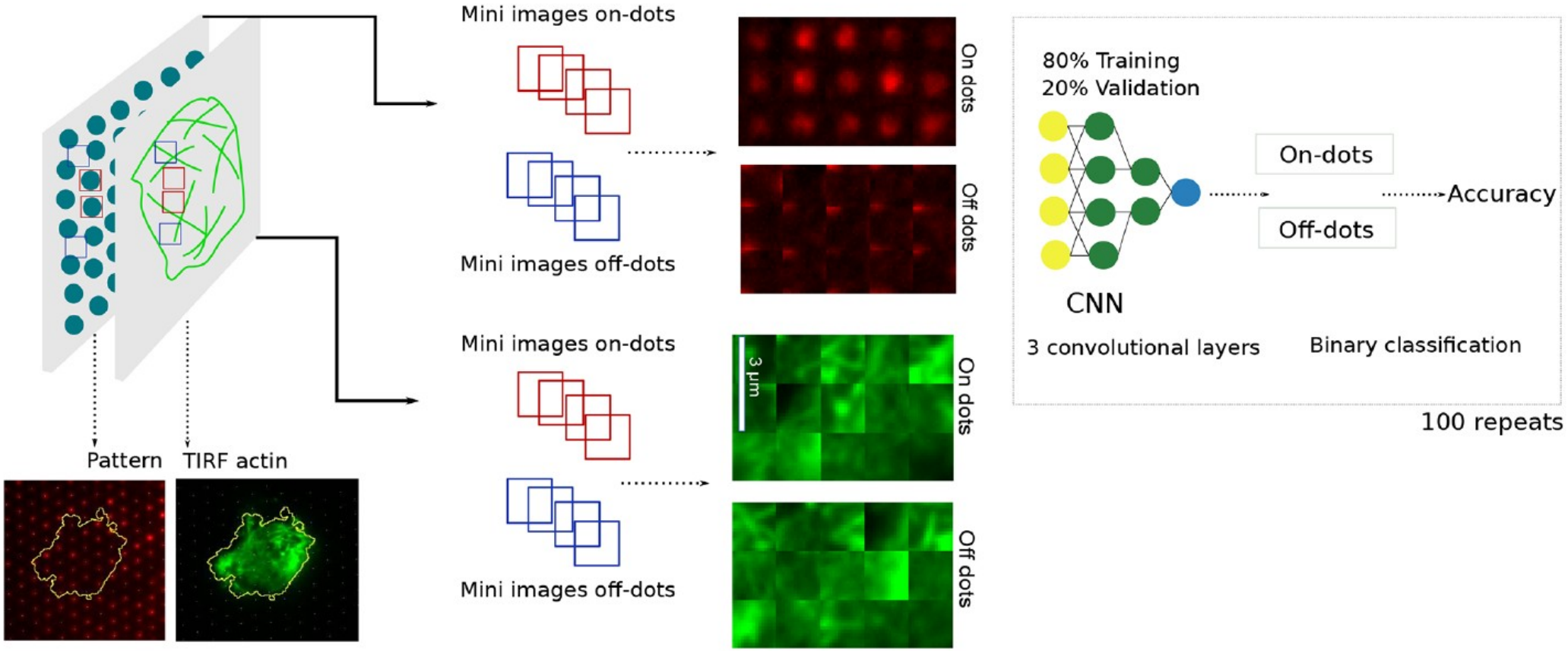
Decipher subcellular actin organisation

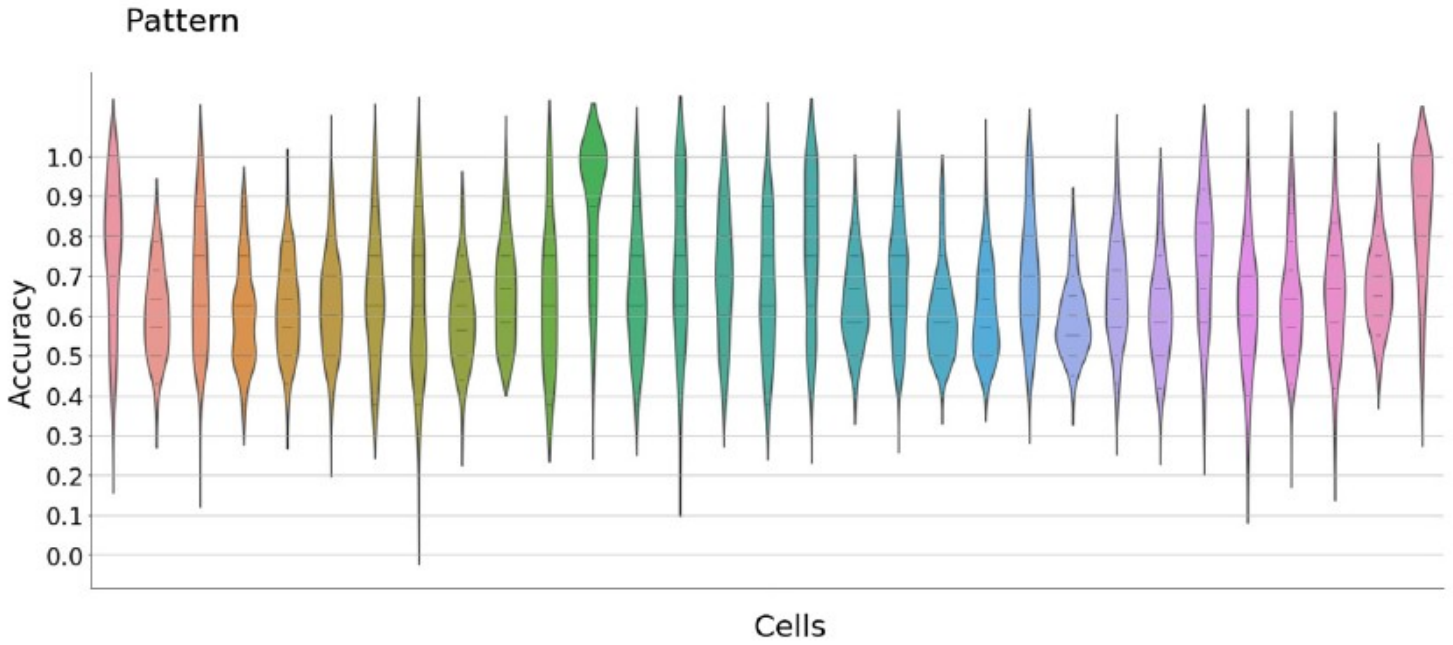


Decipher subcellular actin organisation



Decipher subcellular actin organisation

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0.3
0.2
0.1
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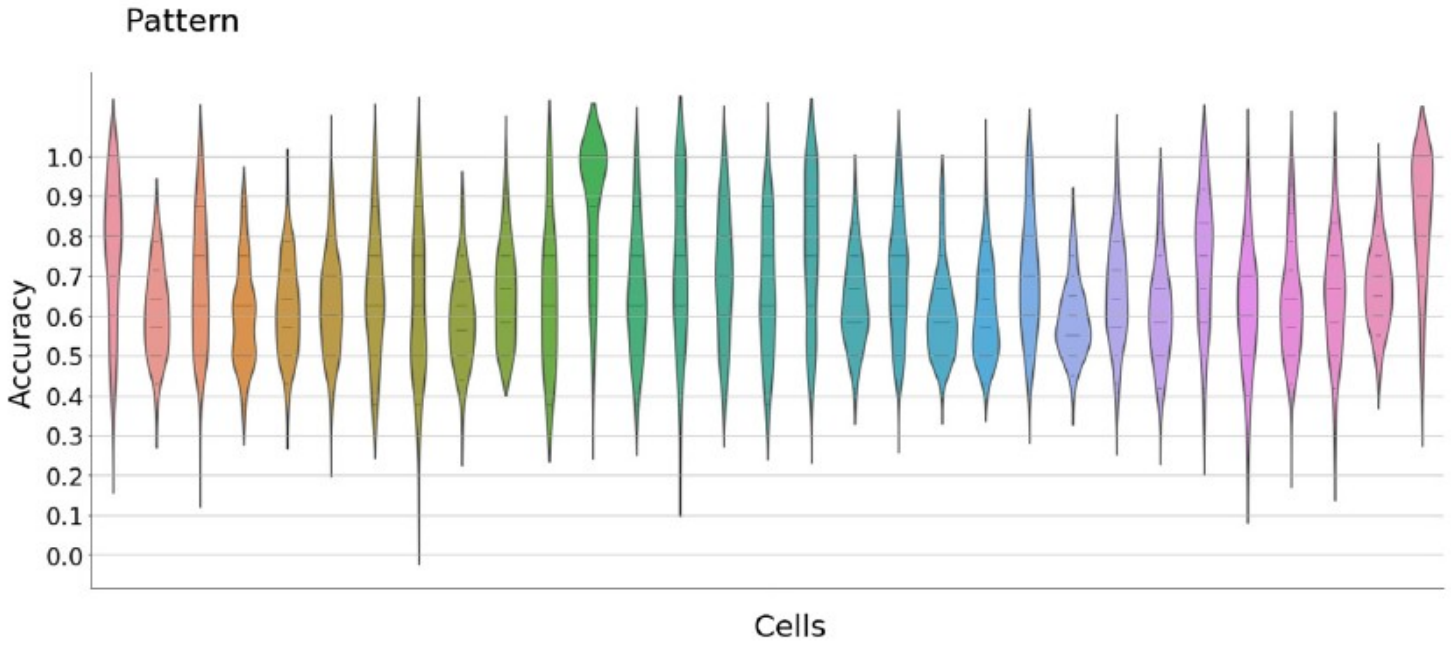
Cells


Pattern
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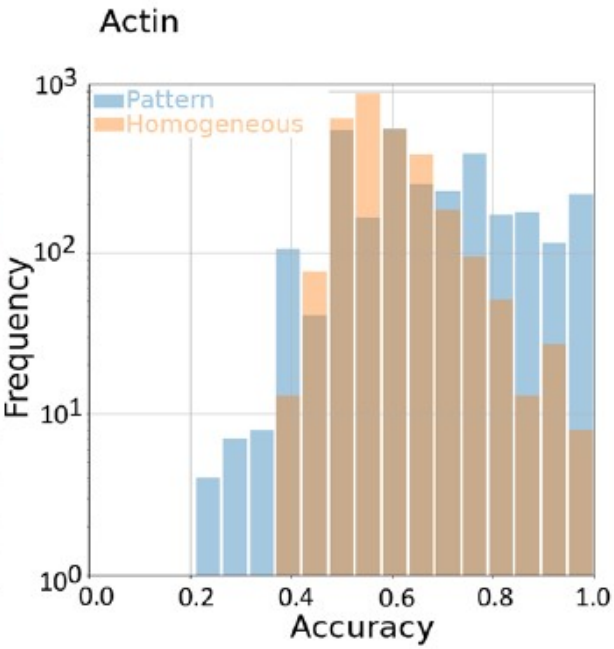
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Pattern
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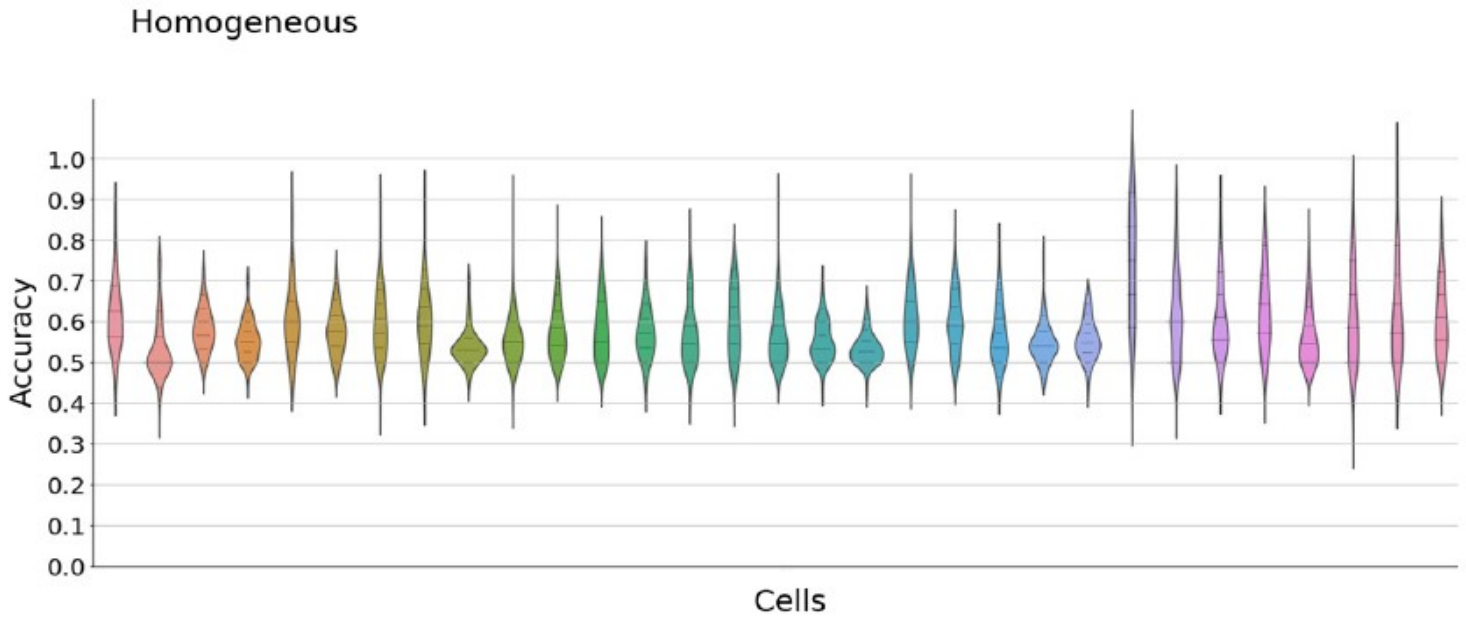
Accuracy


Homogeneous
Accuracy
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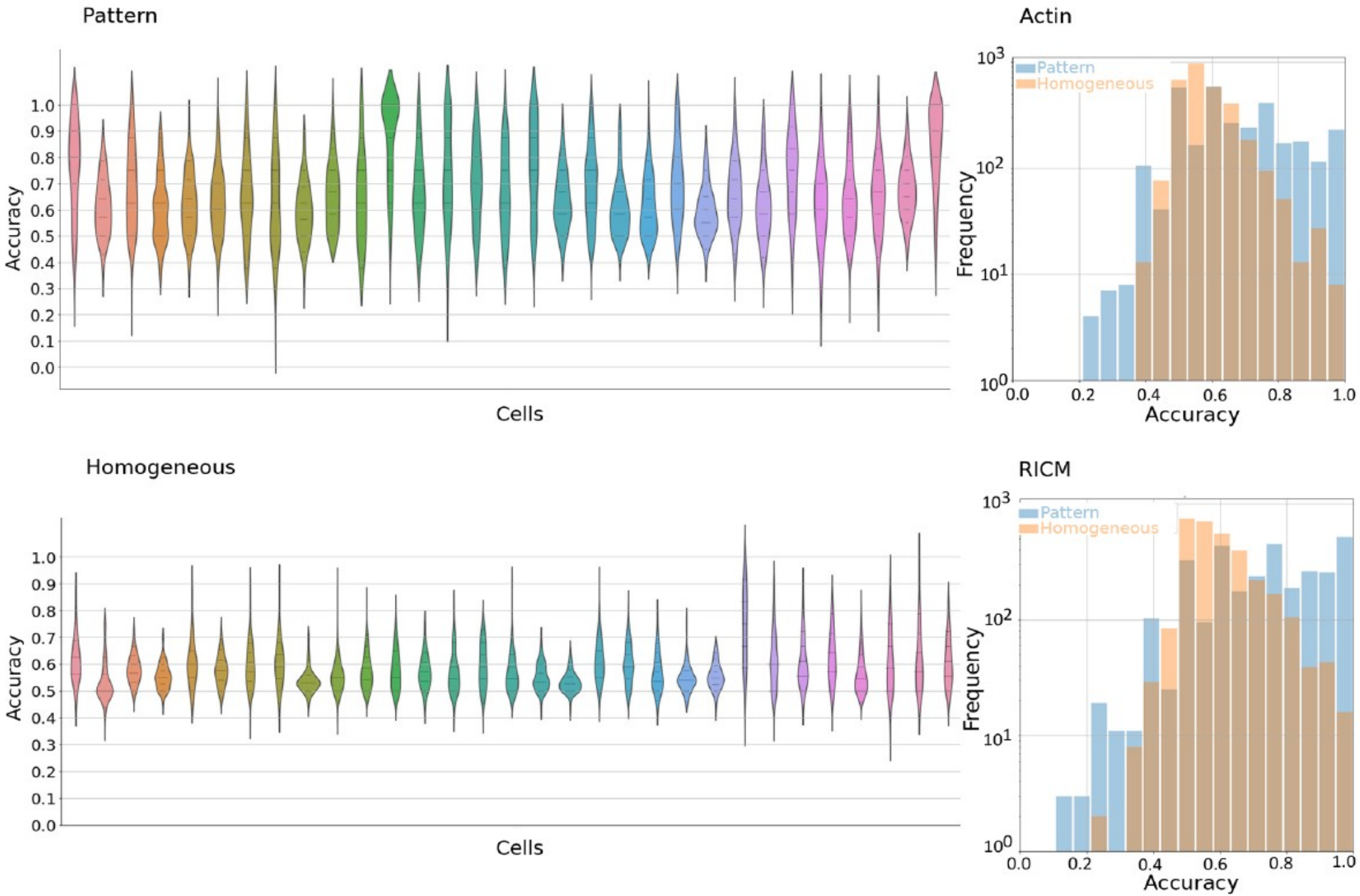
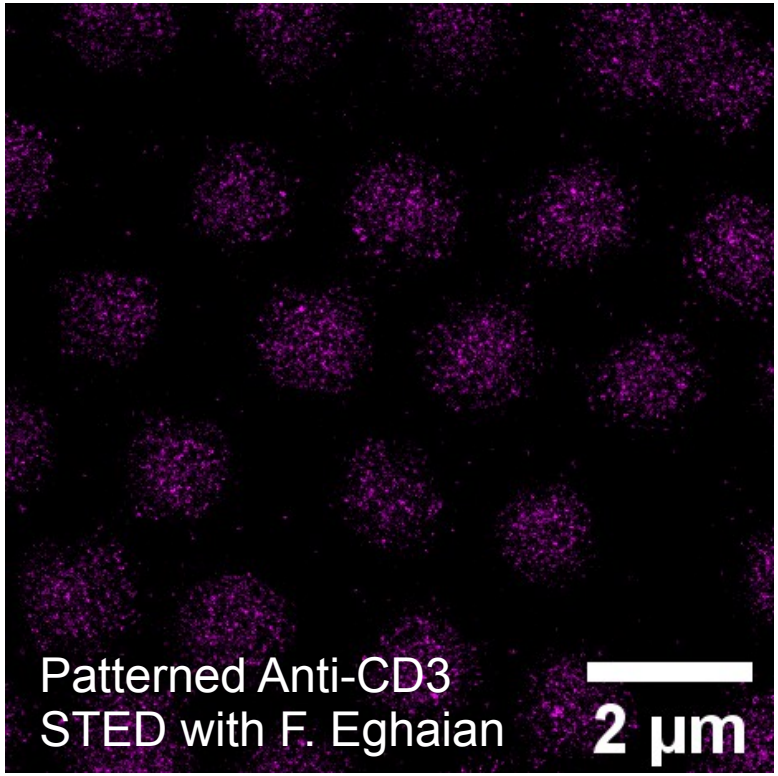
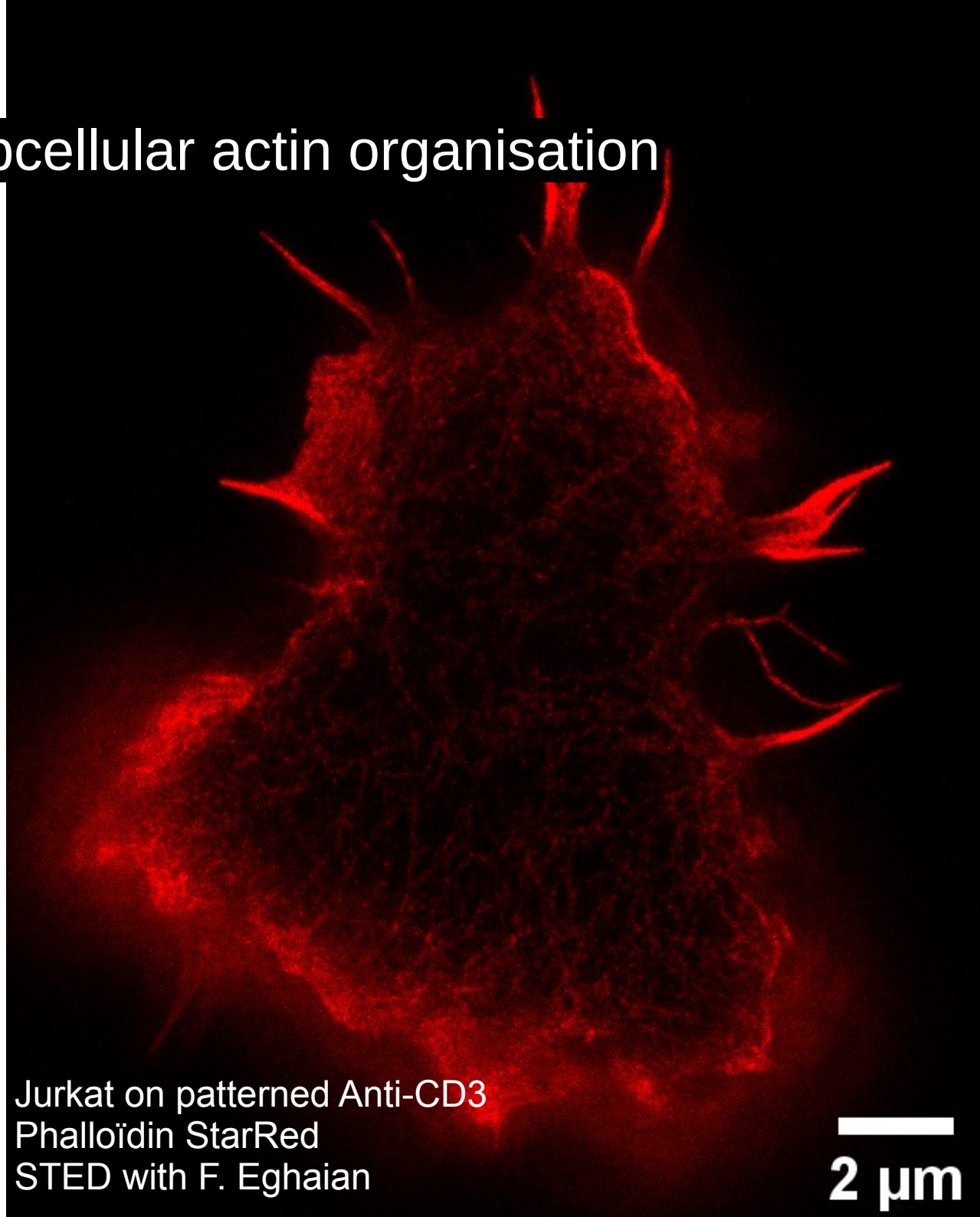


Nasseredine, … Limozin, Sengupta, Nanoletters (2021)

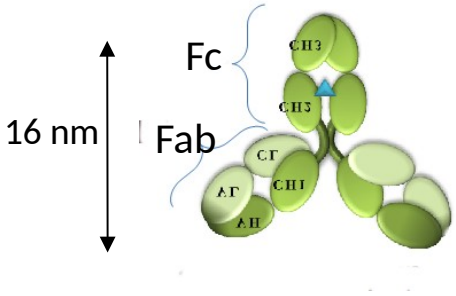
Decipher subcellular actin organisation

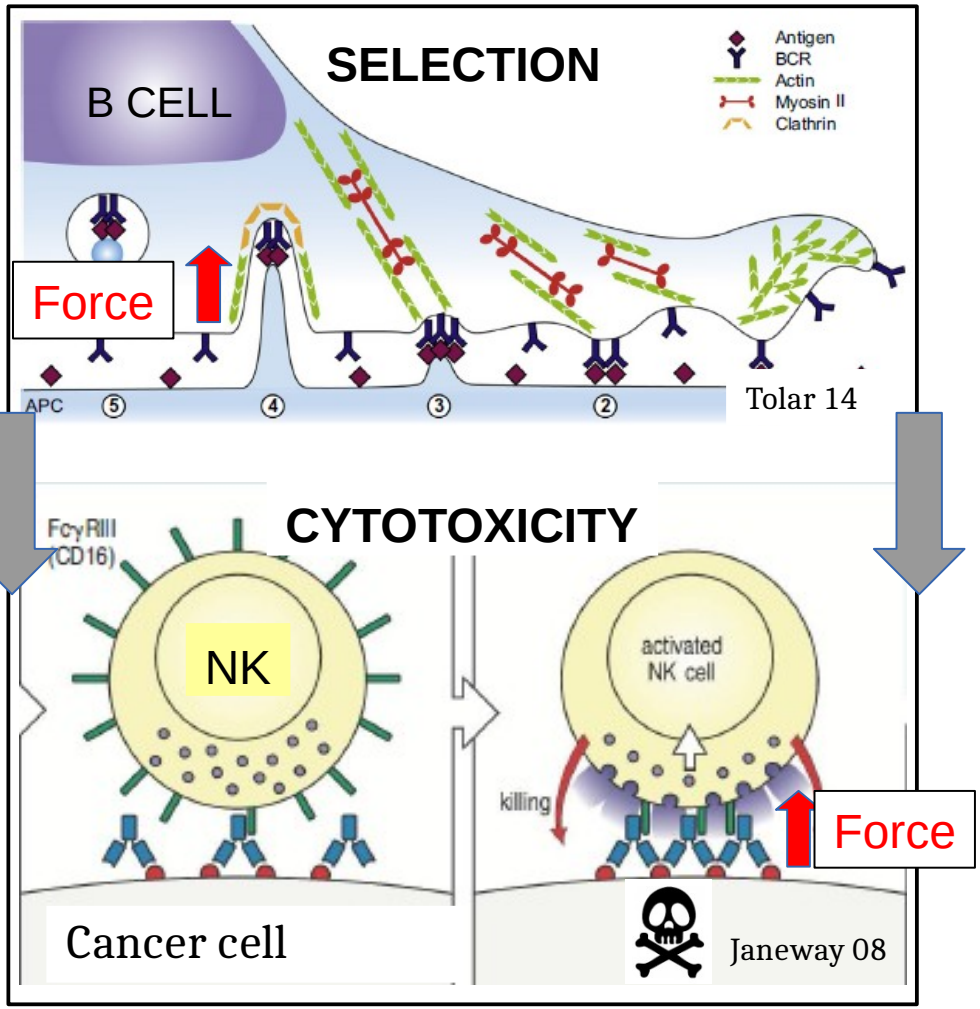




Antibodies and immunotherapy

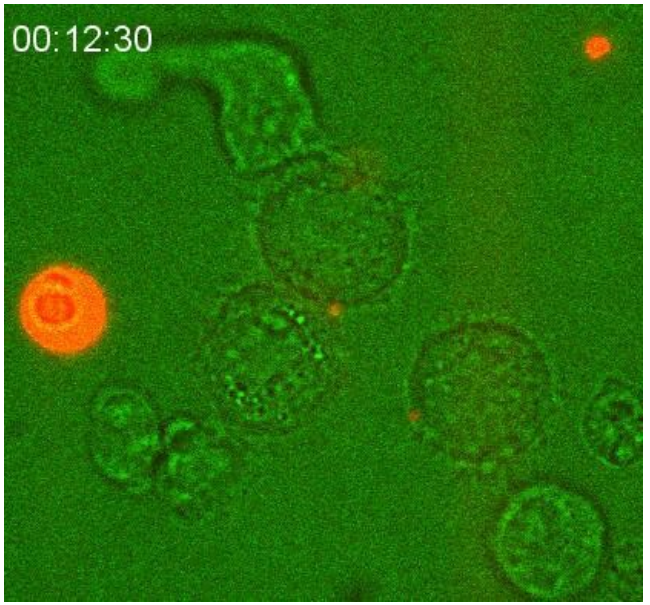
Conventionnal Antibodies

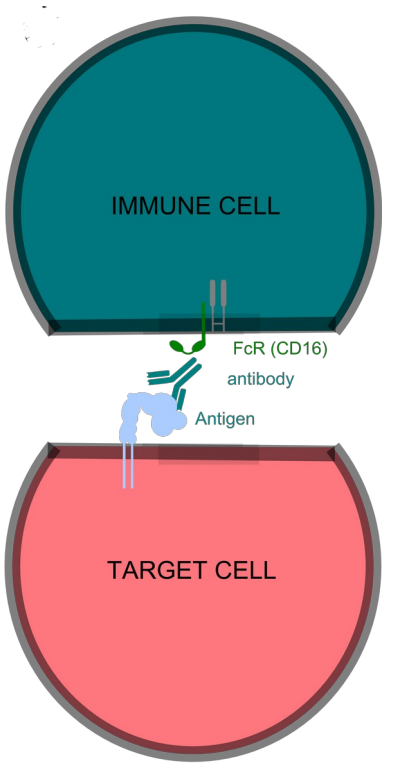


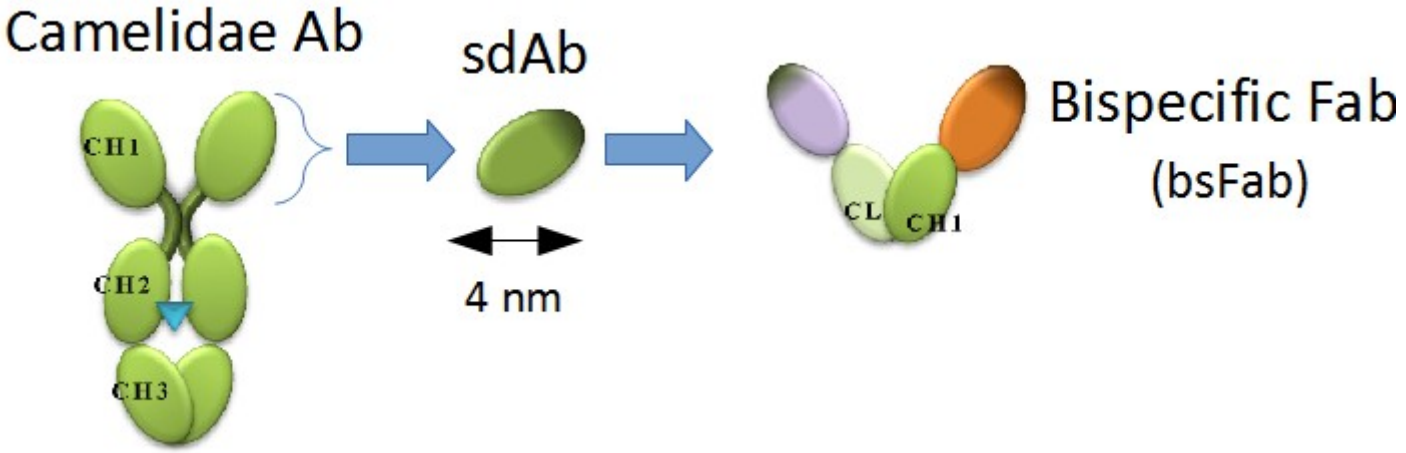


Bispecific Abs

Antibody dependent cell cytotoxicity







Synthesis of 3x2 new bispecific antibodies

Collab. P. Chames (CRCM)

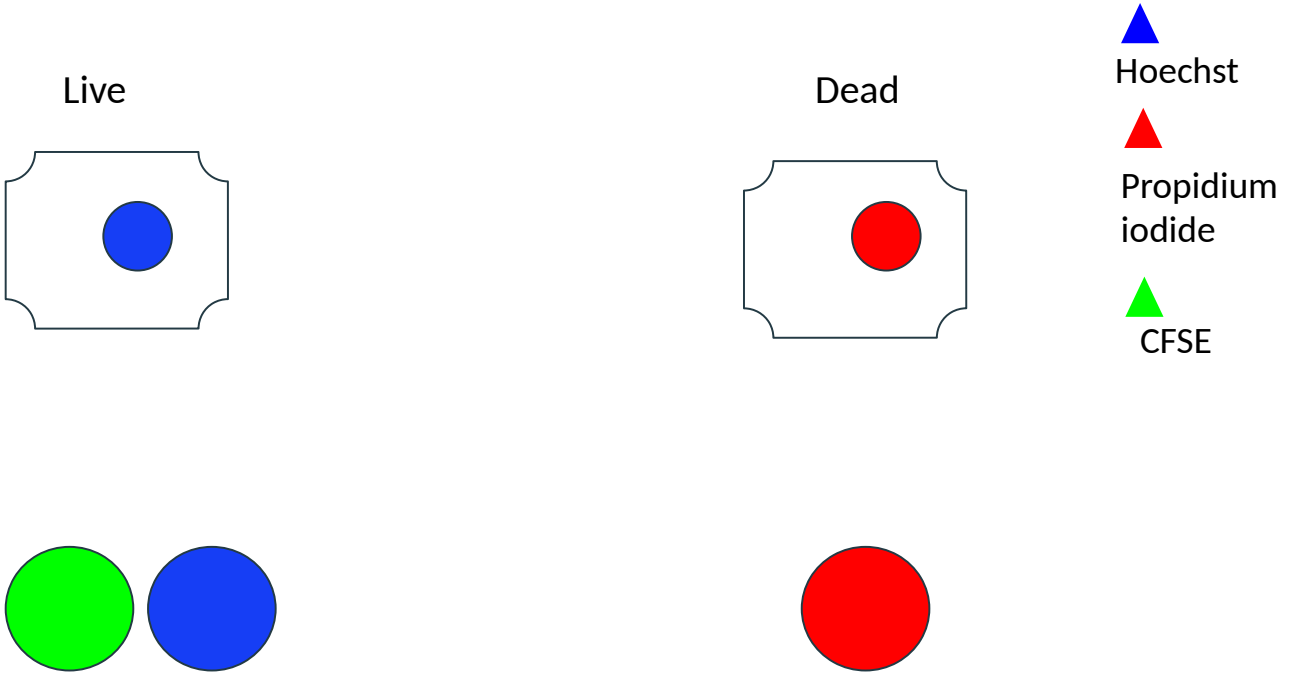
Antibody dependent cell cytotoxicity

ADCC

bsAb CE4-28

- **MCF7 cells**

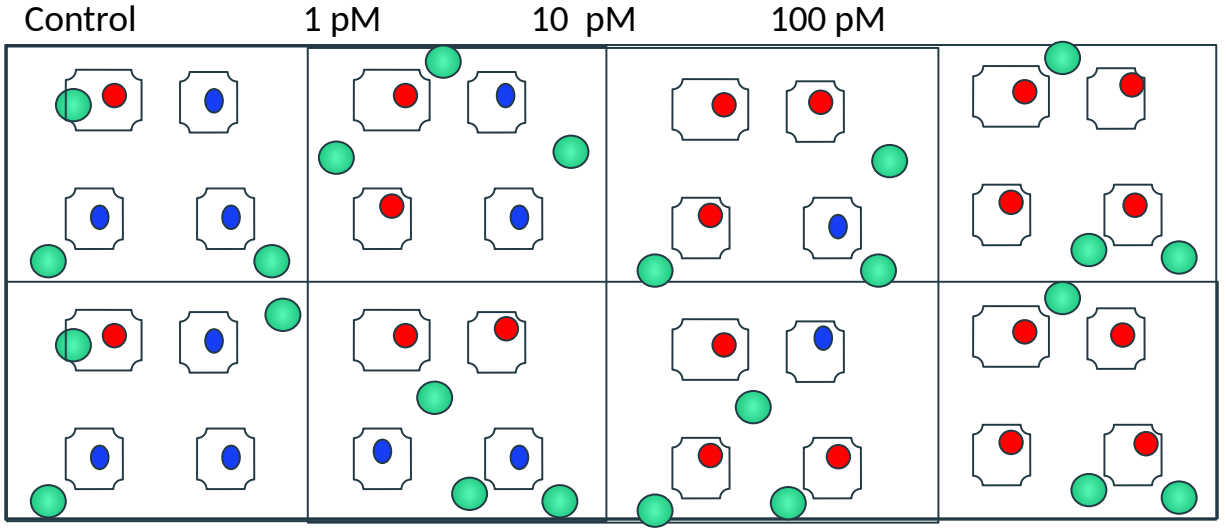
- **NK cells**



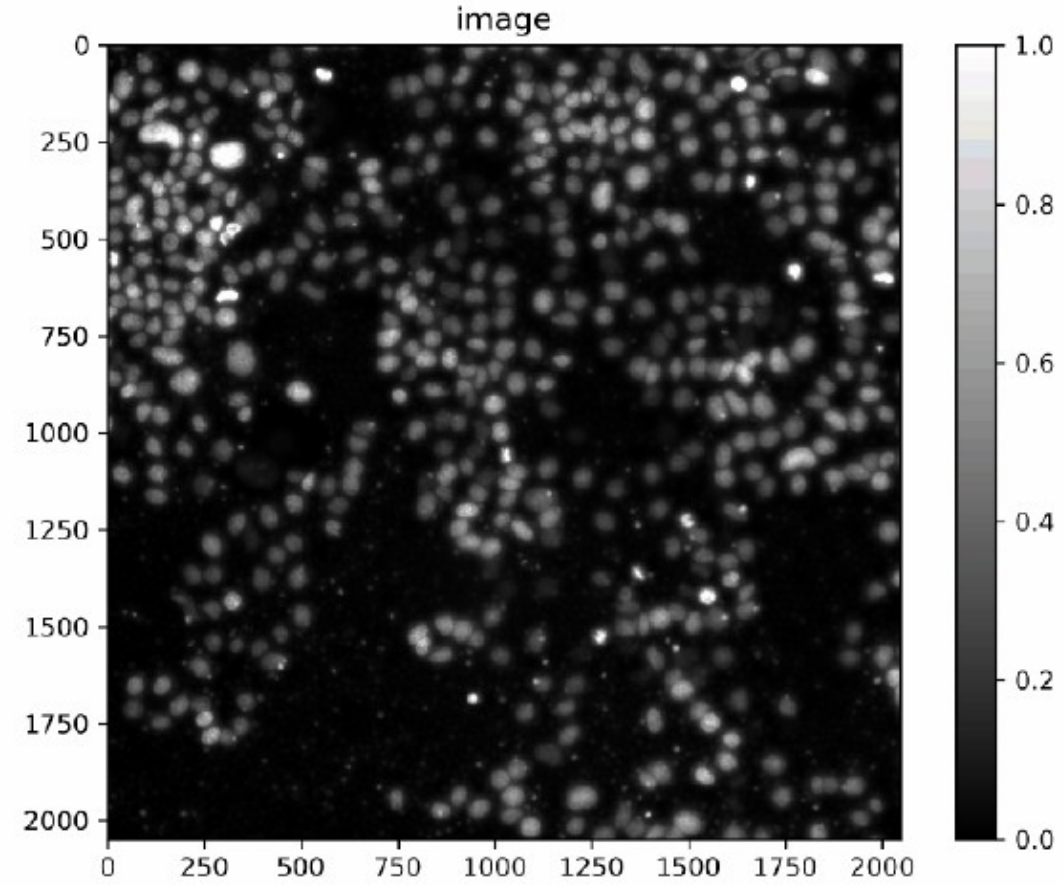
Antibody dependent cell cytotoxicity

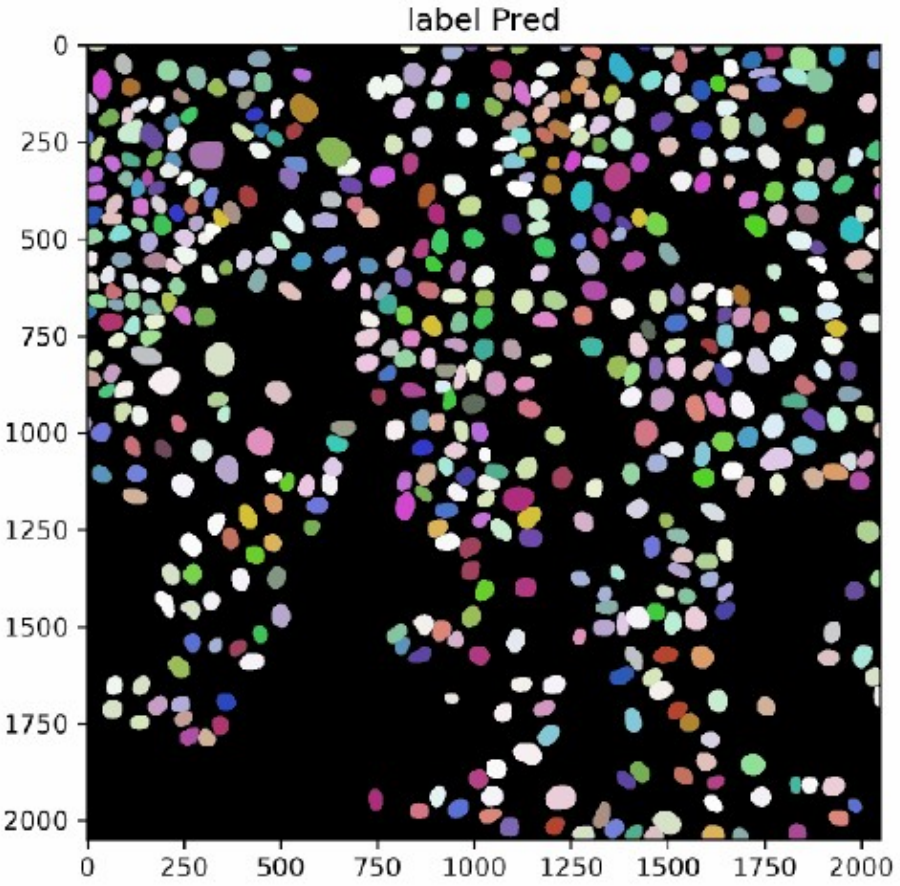
MCF7-WT
+
Primary NK
(NK92-CD16+)
1:5

MCF7-Her2+
Primary NK/
(NK92-CD16+)
1:5



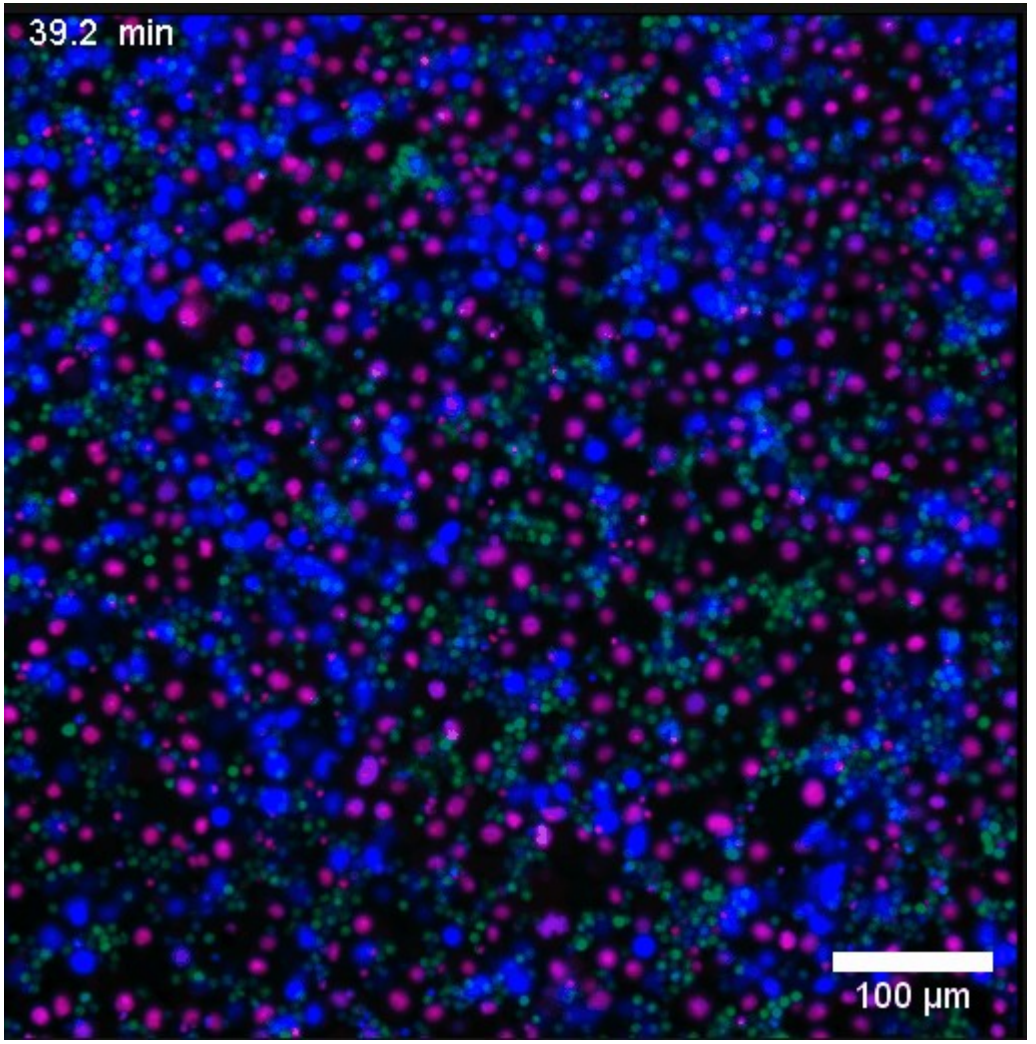
Segmentation and tracking

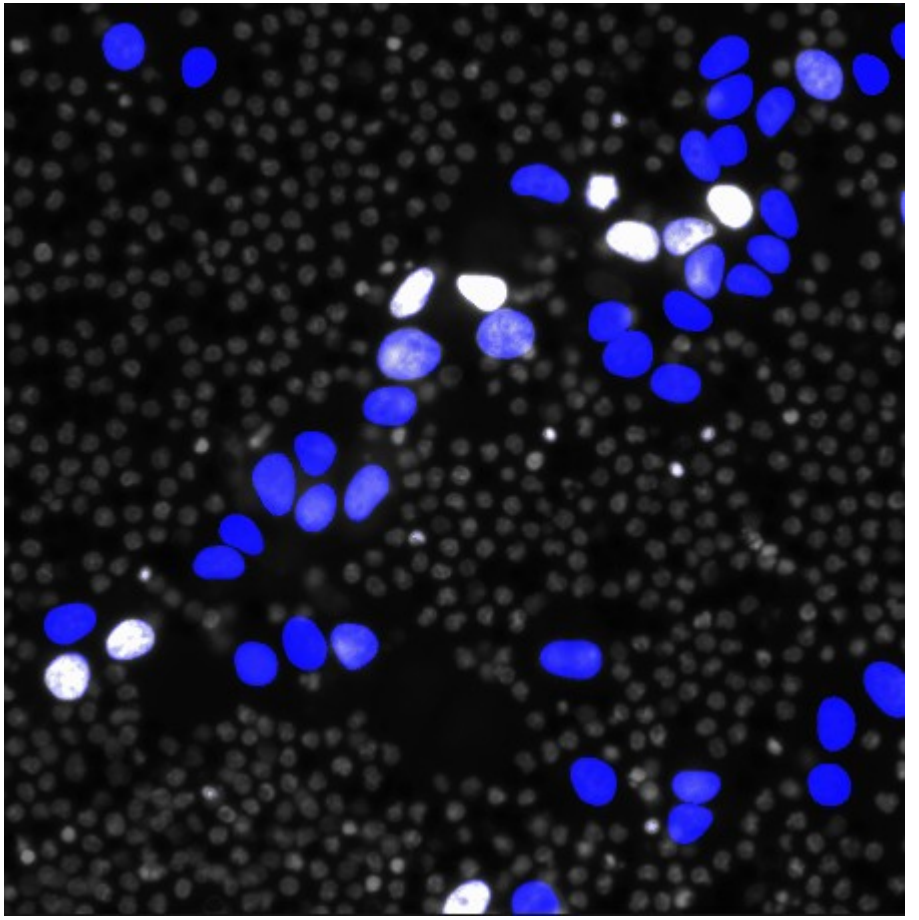




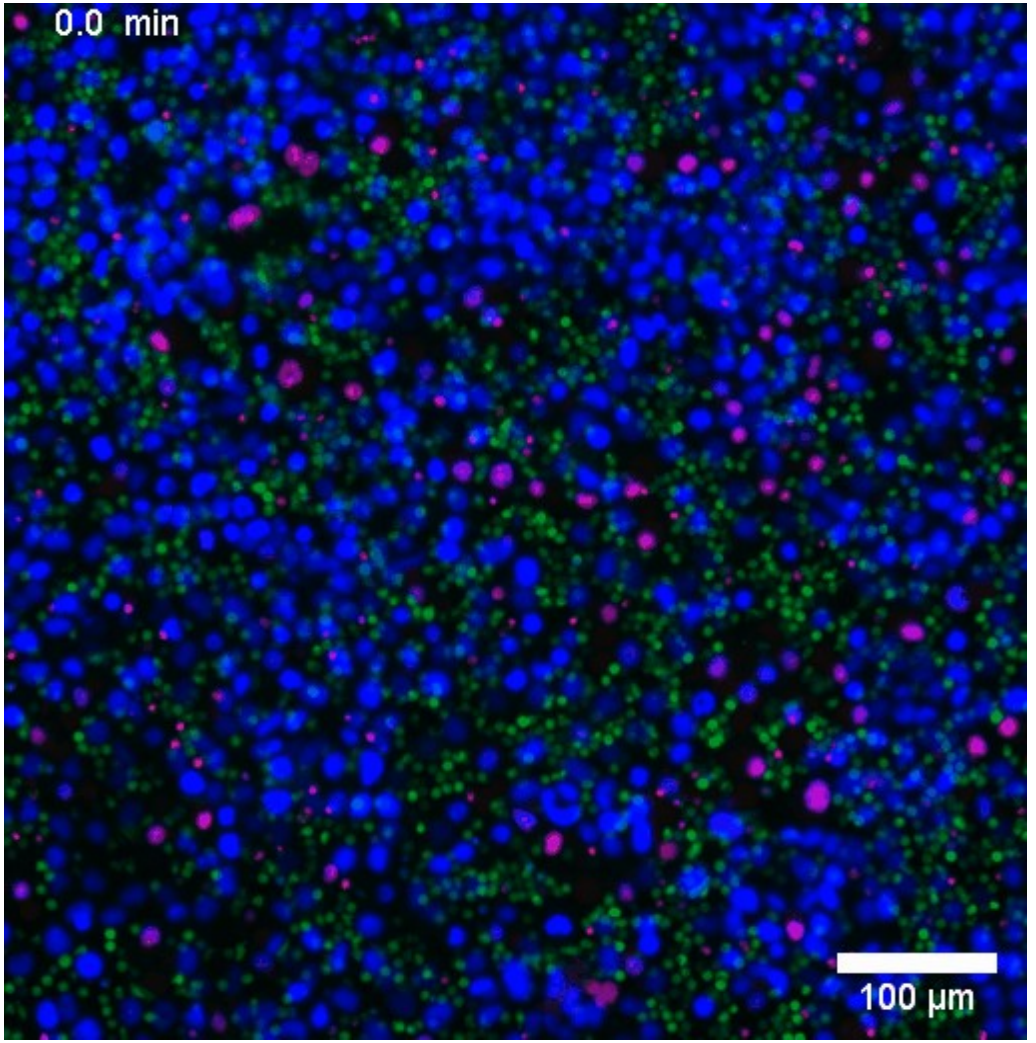
Customized Stardist

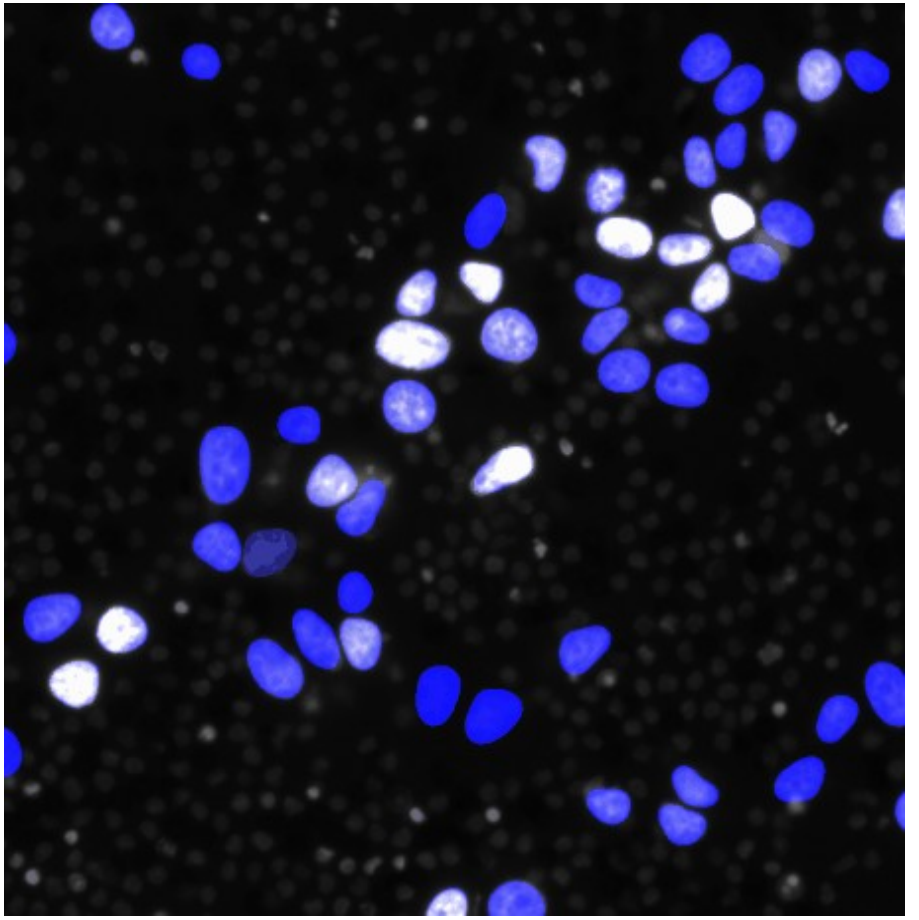
Segmentation and tracking



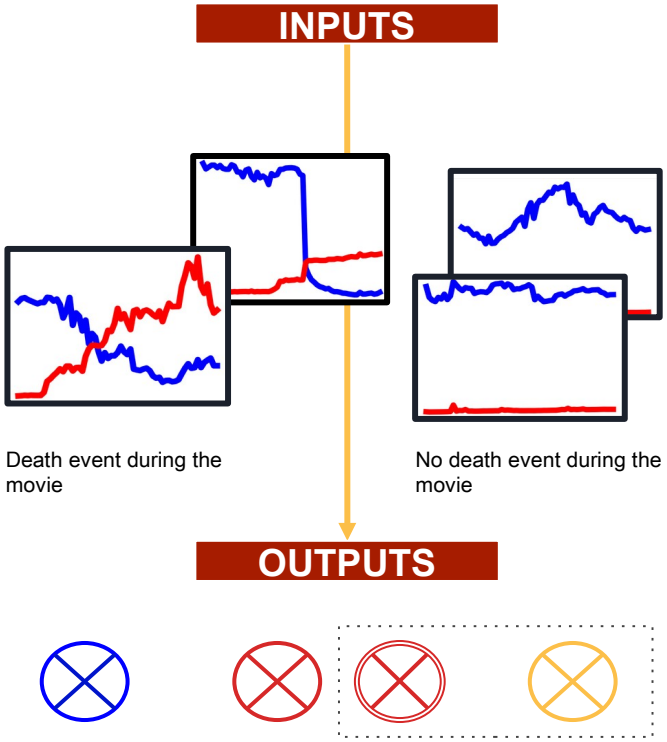


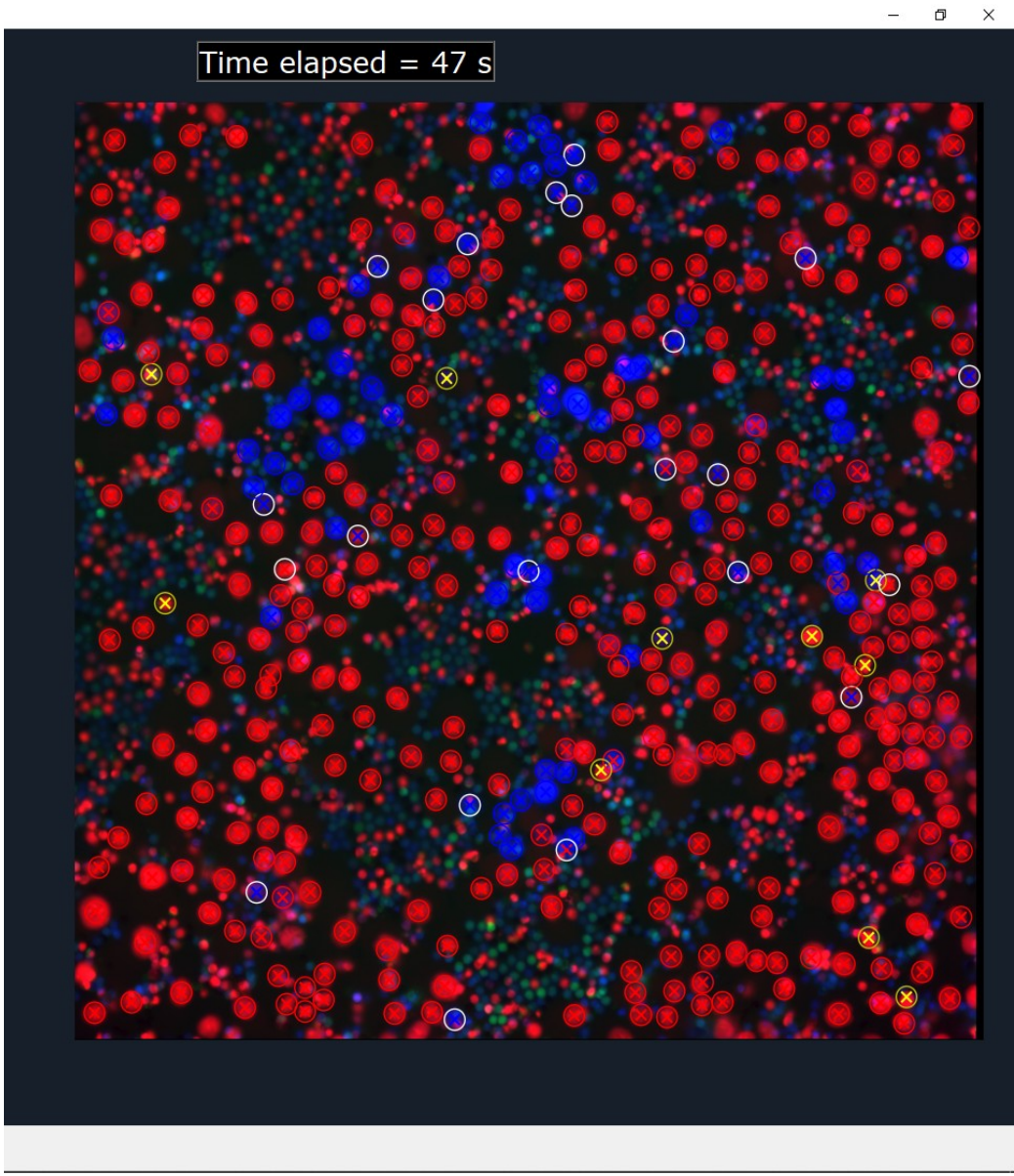
Segmentation and tracking





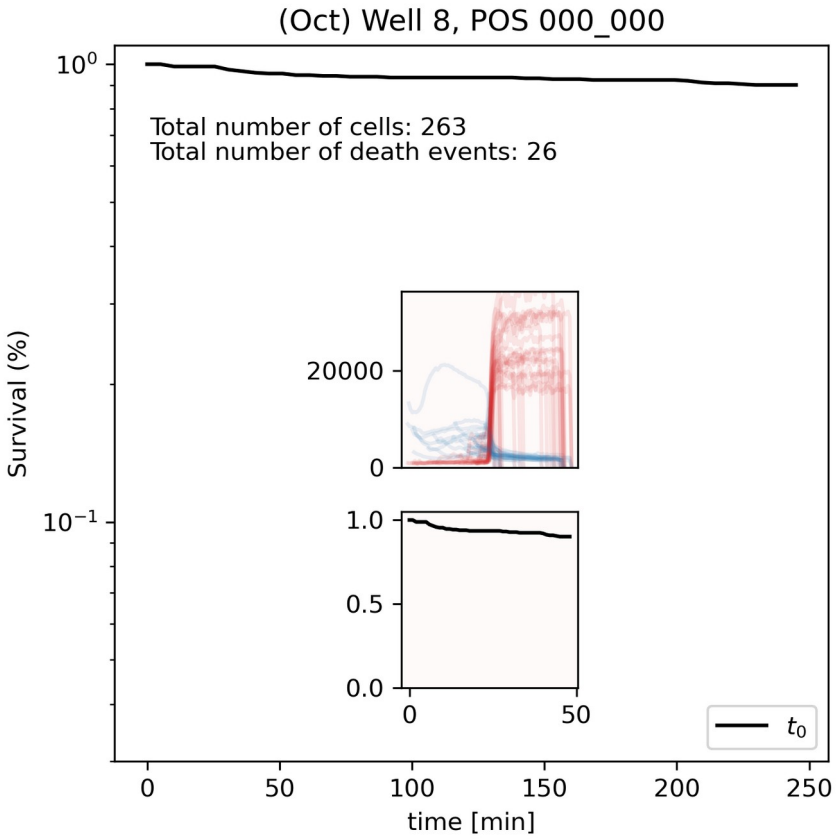
Retrieve killing time



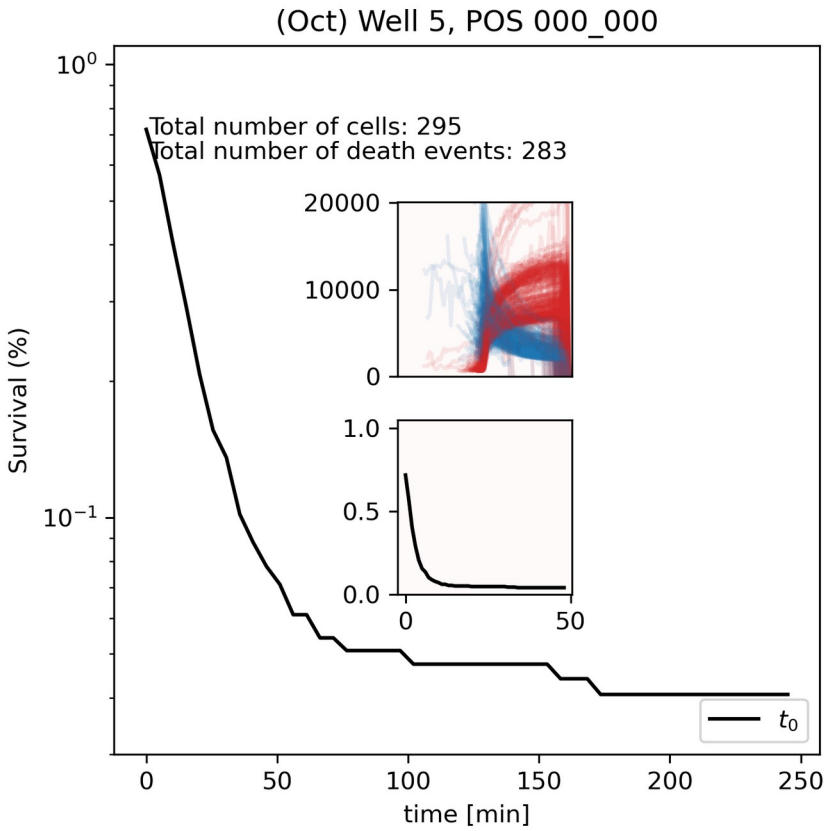


Survival curve to measure antibody efficiency

MCF7-HER2+



MCF7-HER2+ [100 pM CE4-28]



Perspectives / collaborations

- Standardize and optimize 3D-RICN (opticians)
- Apply RICM / 3D-RICN for non-labelled cell characterization (biologists/doctors)
- Train RICM and TIRF with deep learning (computer scientists: Stephane Ayache, LIS)

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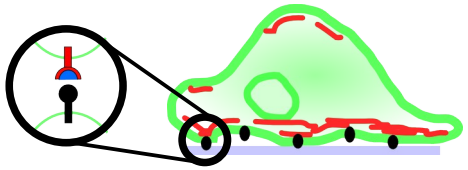
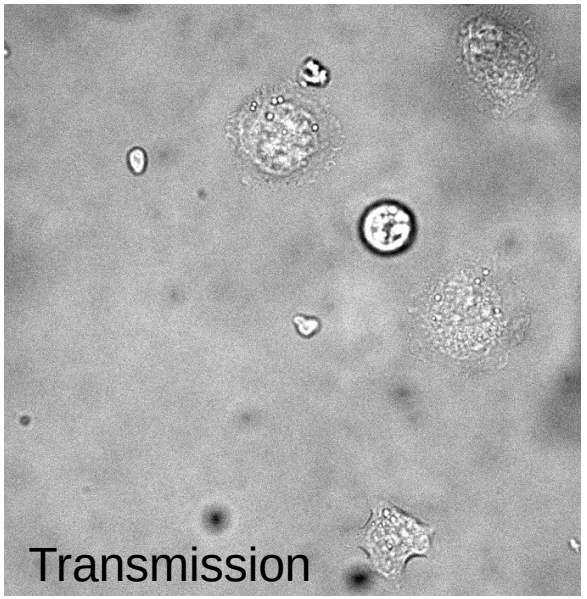
Acknowledgements

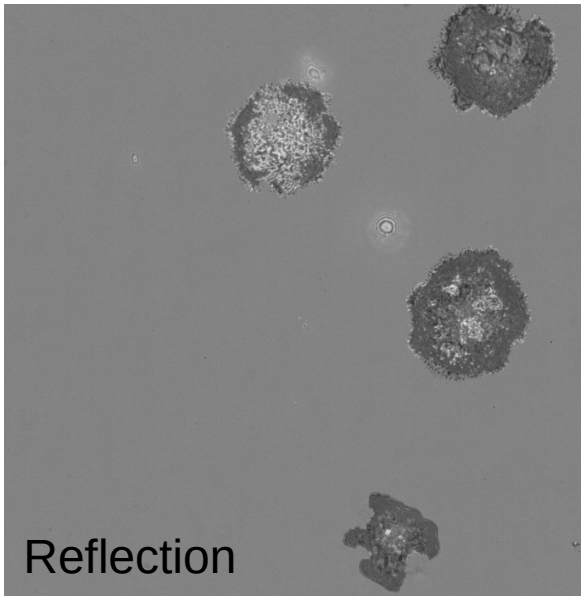
- **3D-RICN**: Marie-Julie Dejardin, Arnaud Hemmerlé, Rémy Torro
- **Nanopatterns & Fluorescence**: Aya Nassereddine, Ahmed Sharaf
- **ADCC:** Beatriz Diaz, Rémy Torro
- Kheya Sengupta
- Patrick Chames
- Ressources: ZeroCostDL4Mic, Google Colab, Keras

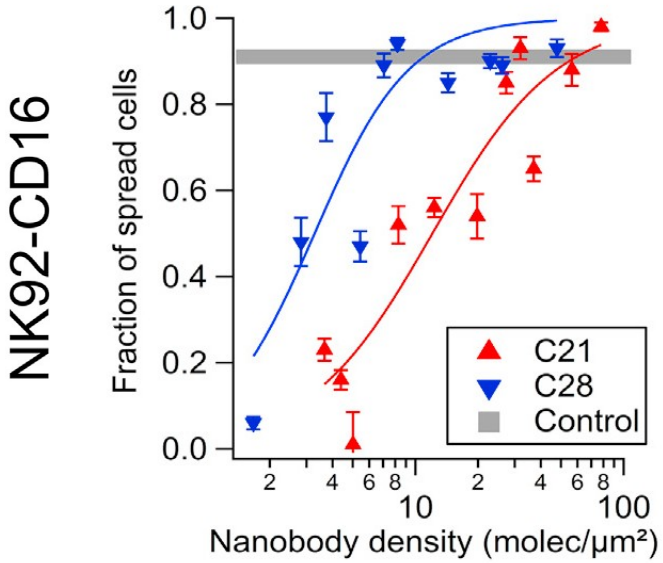
Nanobody mediated NK cell adhesion (2D bipartite)

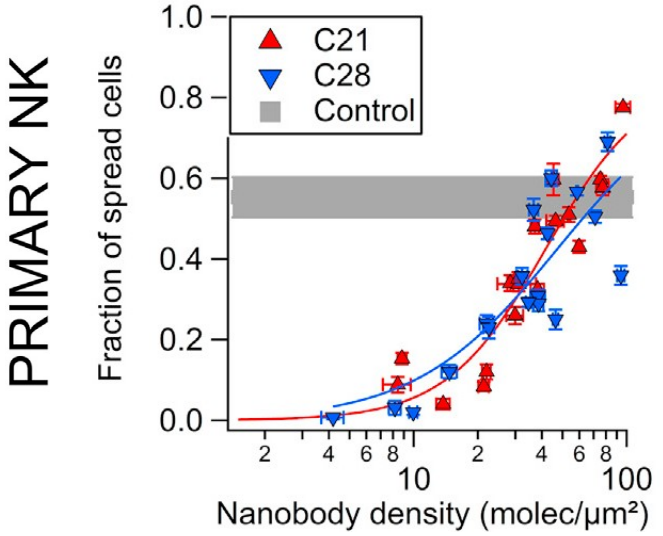


NK cells on Anti-CD16 coated surfaces









Gonzalez 2019 Biophys. J