

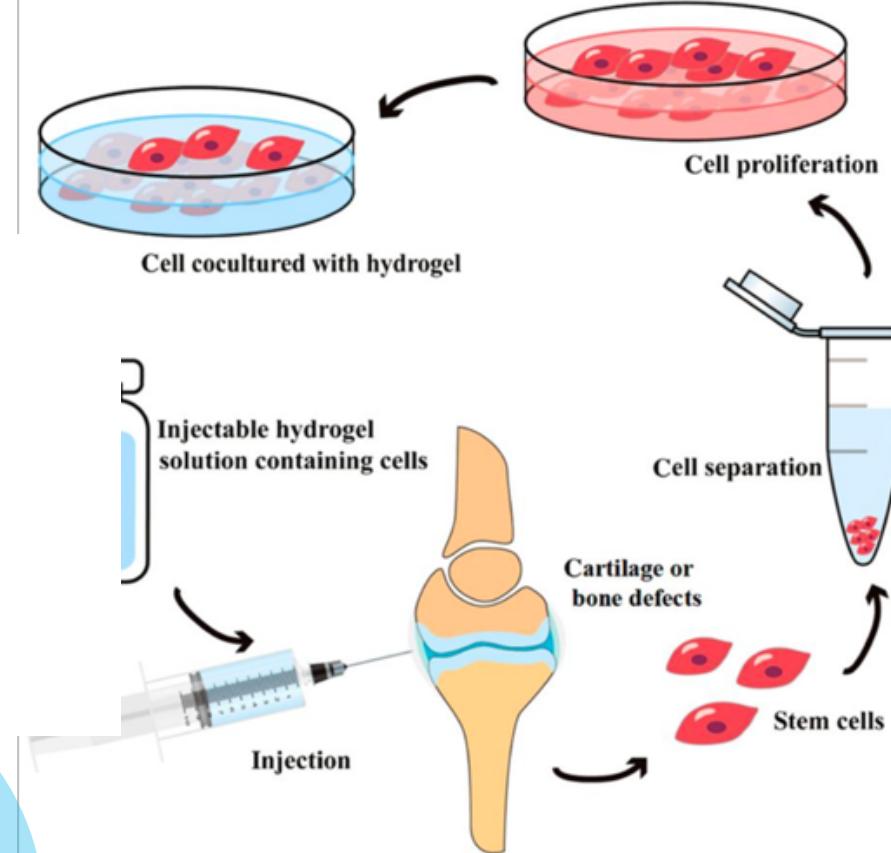
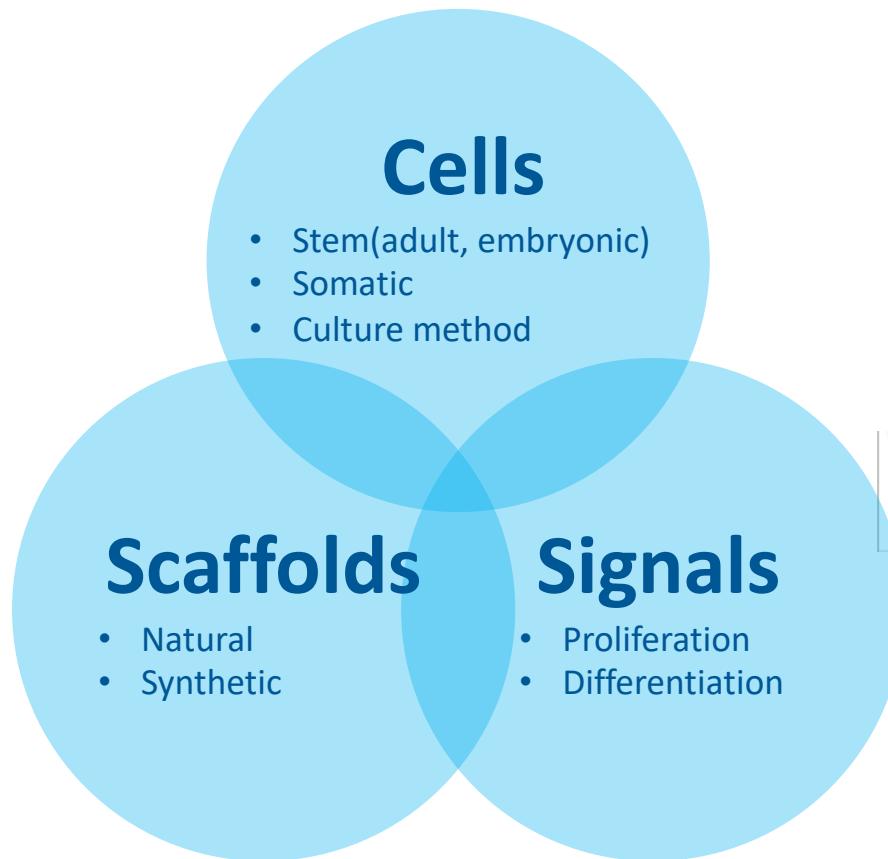


# Microscopy toolbox for structural and mechanical characterisation of new biomimetic materials

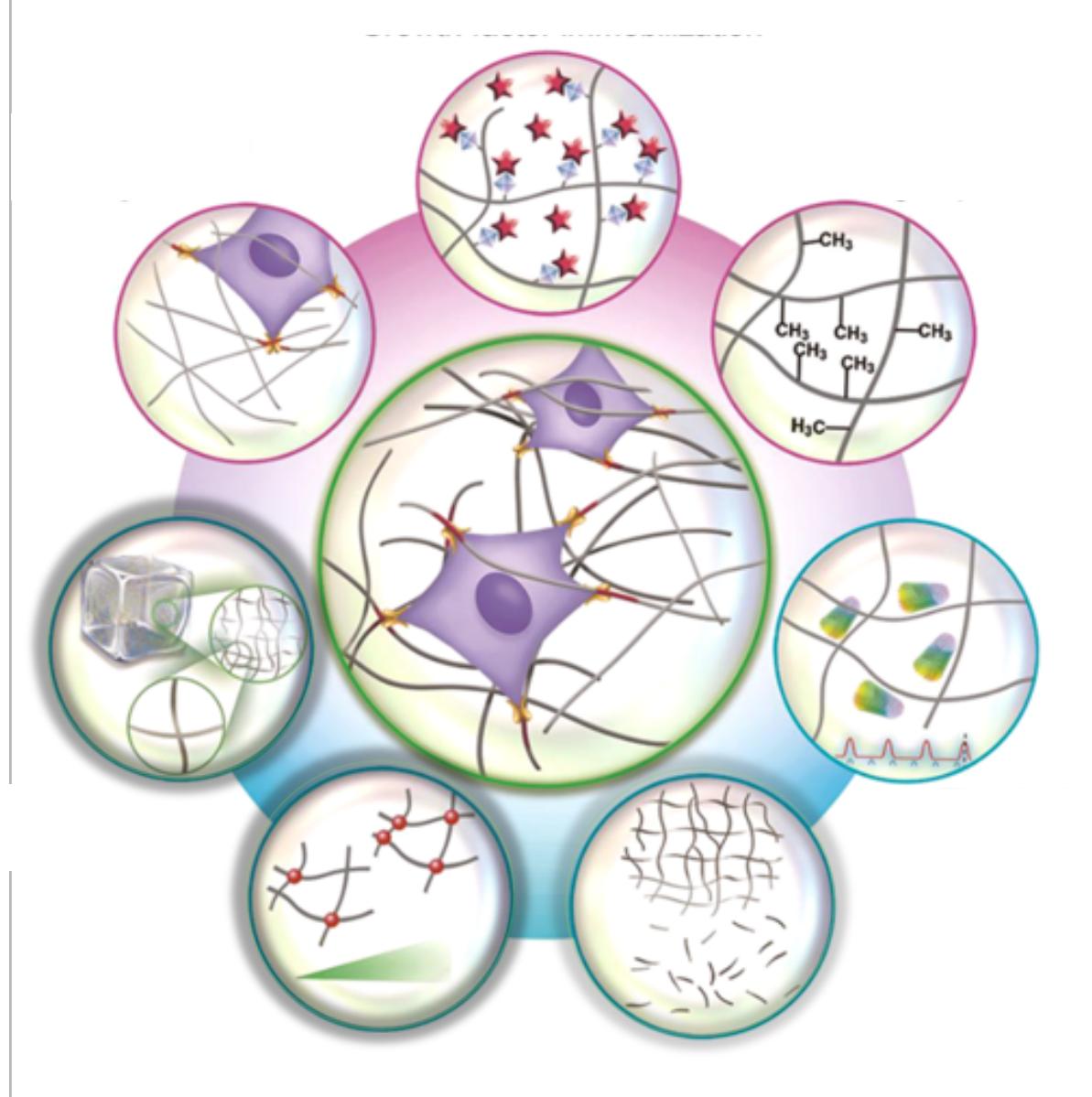
Susana Rocha  
post-doctoral researcher  
@ KU Leuven (BE)

Molecular biophysics goes chemistry, KULeuven  
5<sup>th</sup> July 2019

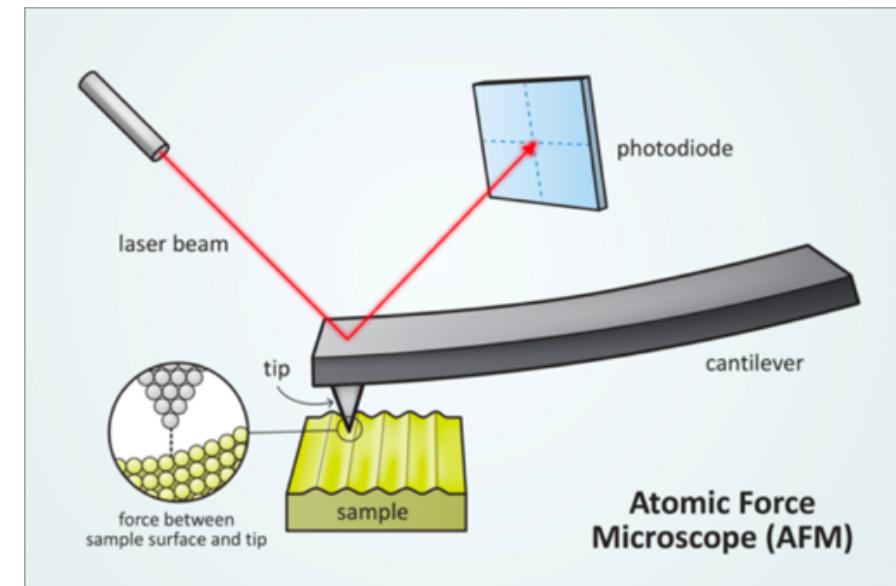
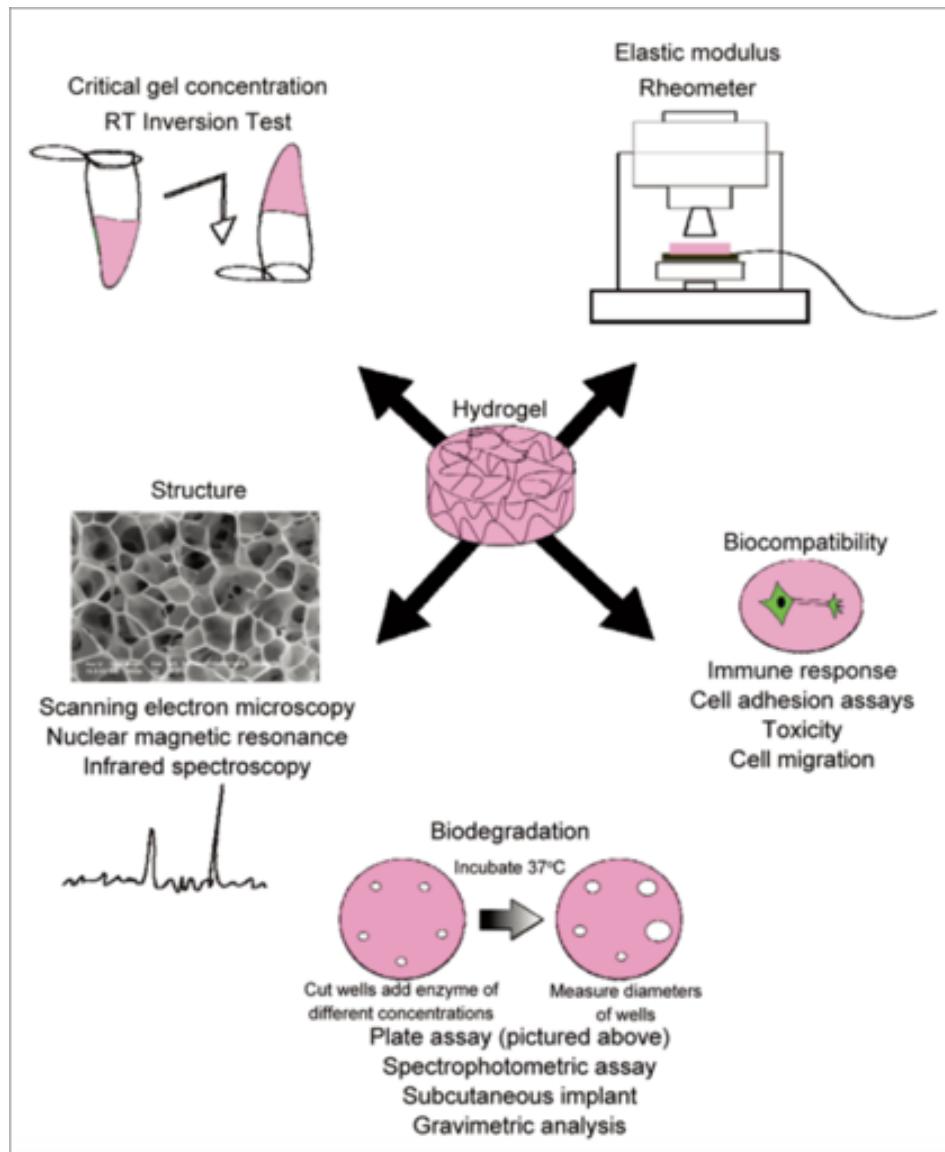
# Tissue engineering and 3D cell culture



# Design considerations for Biomimetic materials



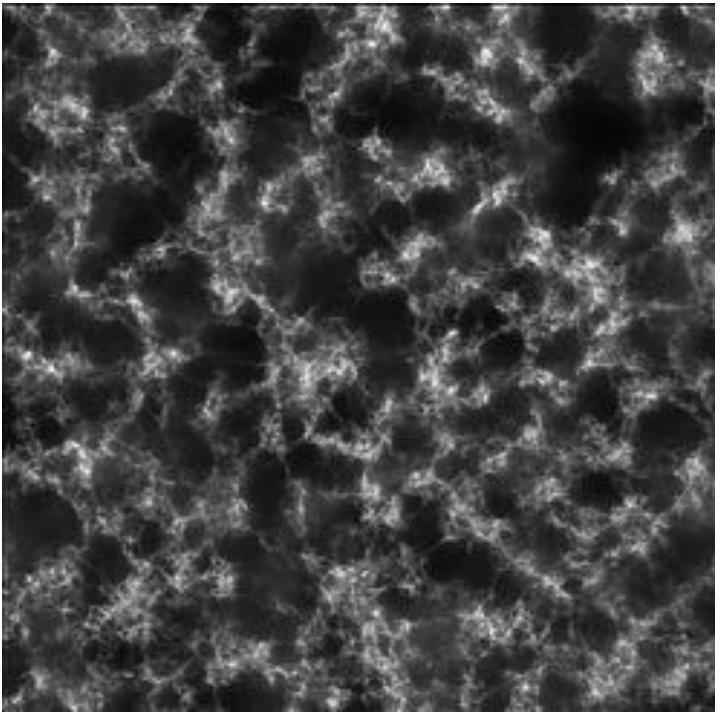
# Techniques for characterization of hydrogels



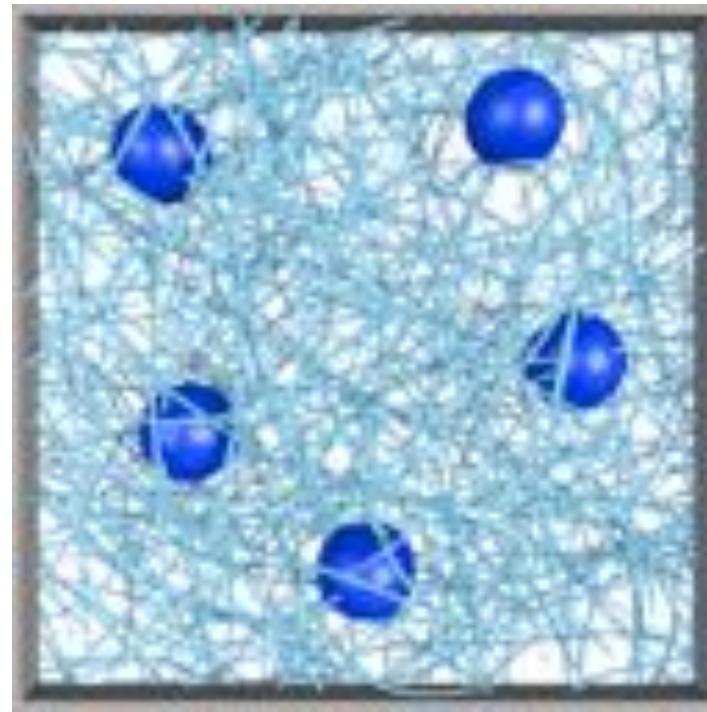
Lack of technologies that can investigate the dynamics of the mechanical and structural properties of biomaterials *in situ*, at the subcellular and molecular level

# Material Characterization

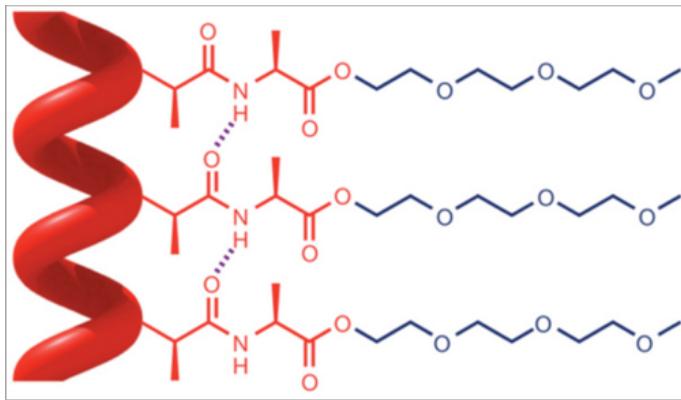
Structure using advanced fluorescence microscopy



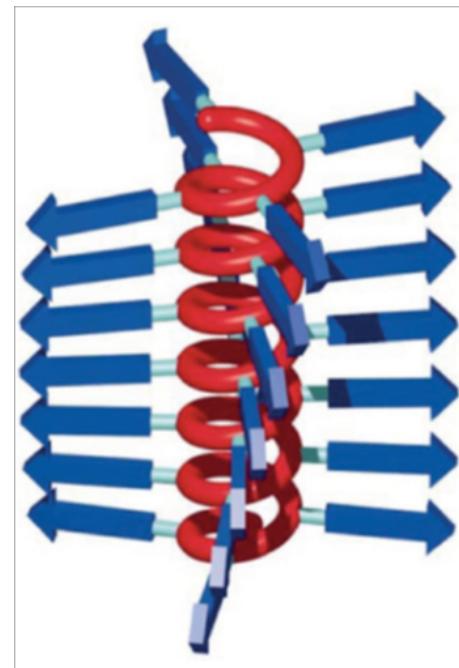
Local mechanical properties using 3D micro-rheology



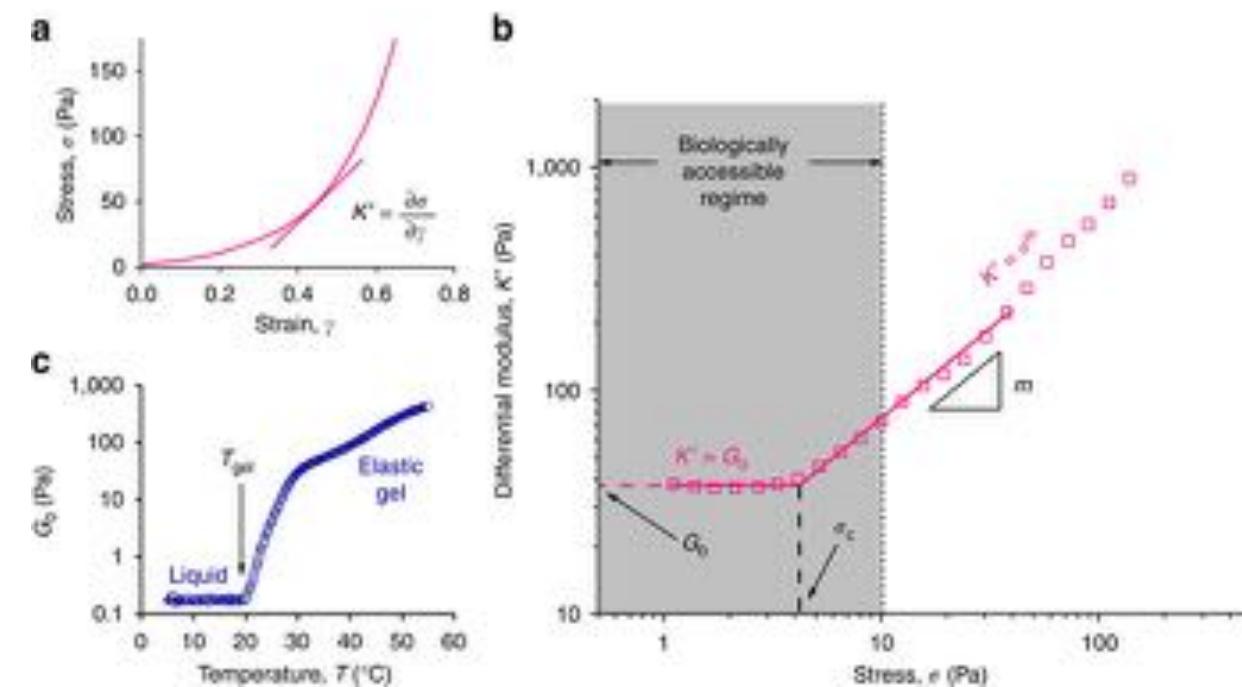
# New polymer class for biomimetic 3D matrices



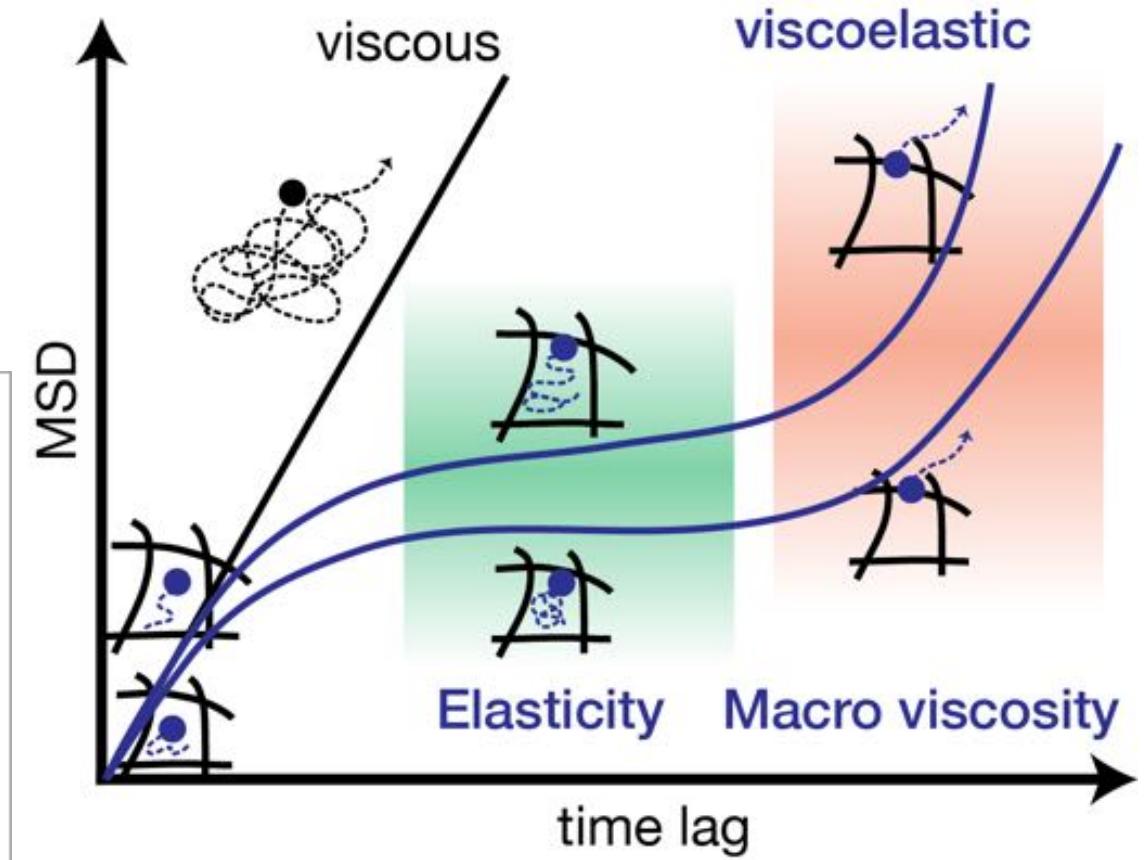
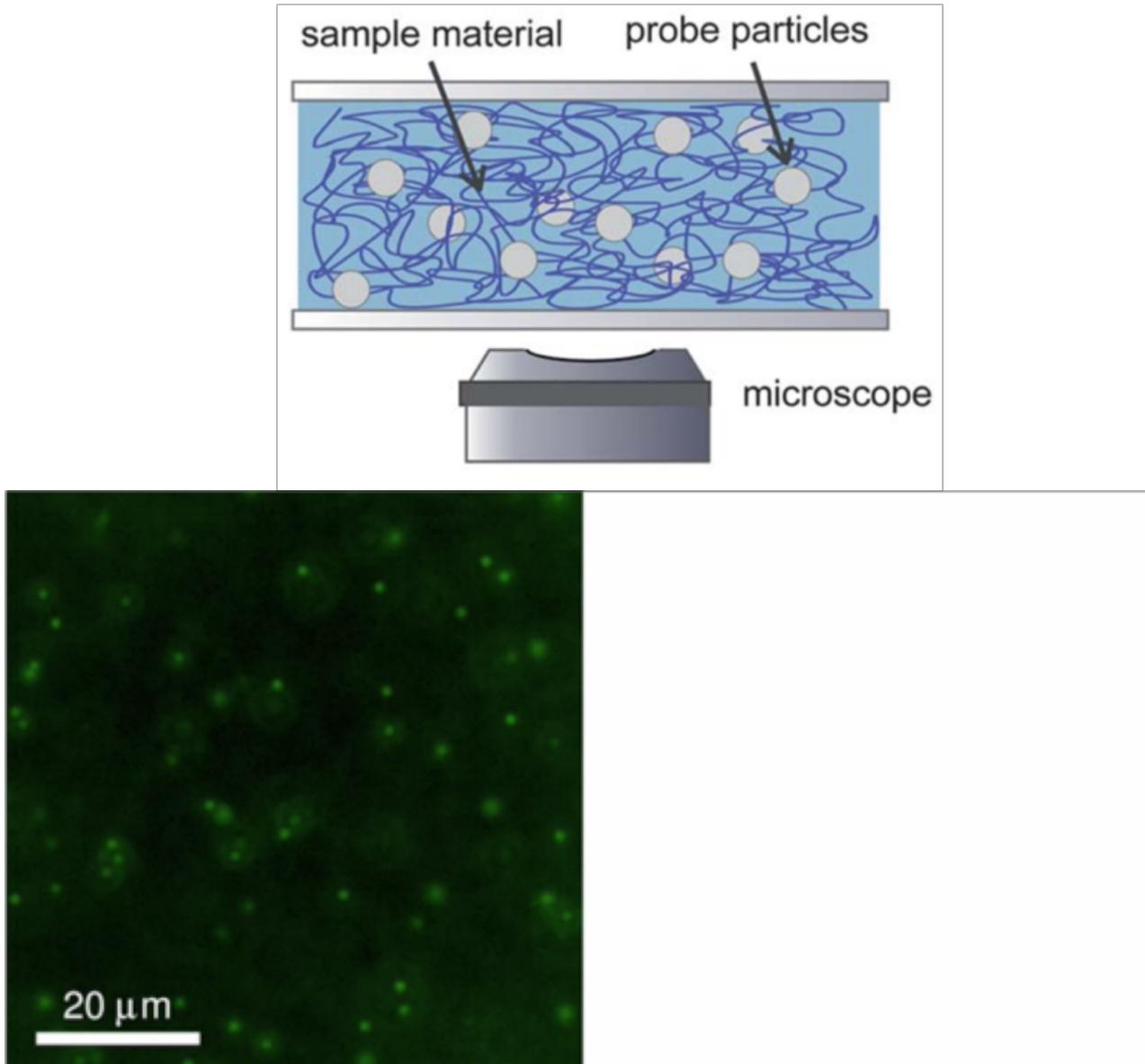
Polyisocyanopeptides (PICs)  
grafted with oligo(ethylene glycol)



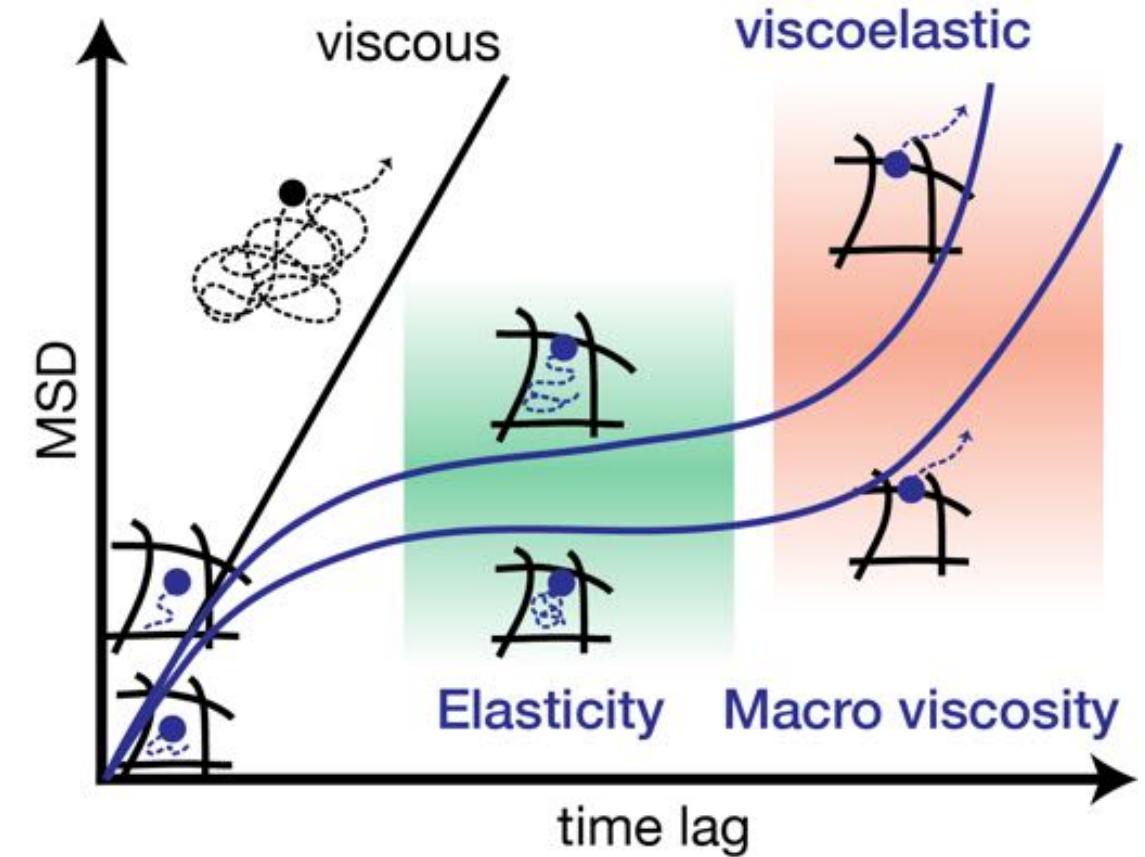
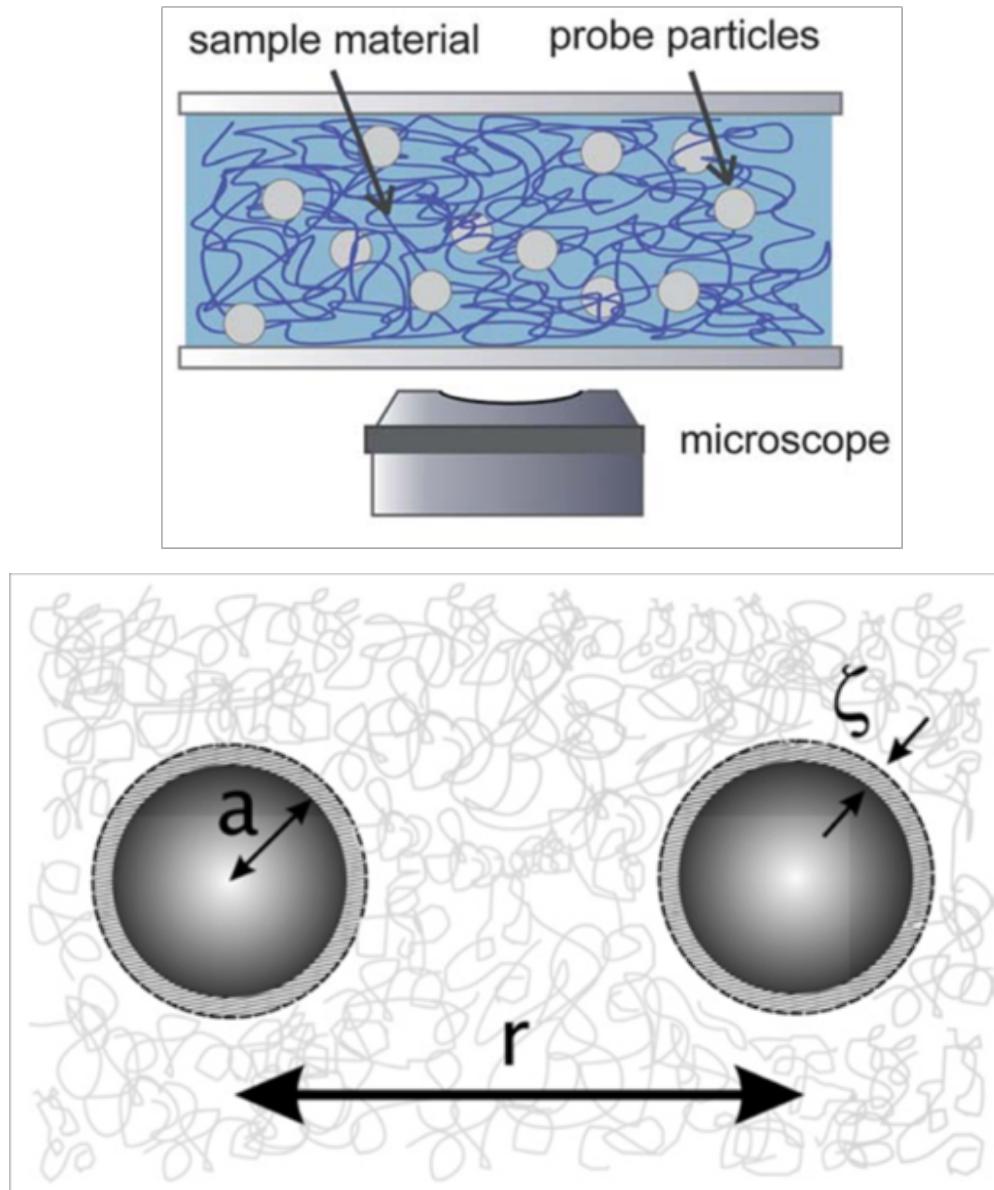
Collaboration with prof. Paul Kouwer,  
Radboud University (Nijmegen, The Netherlands)



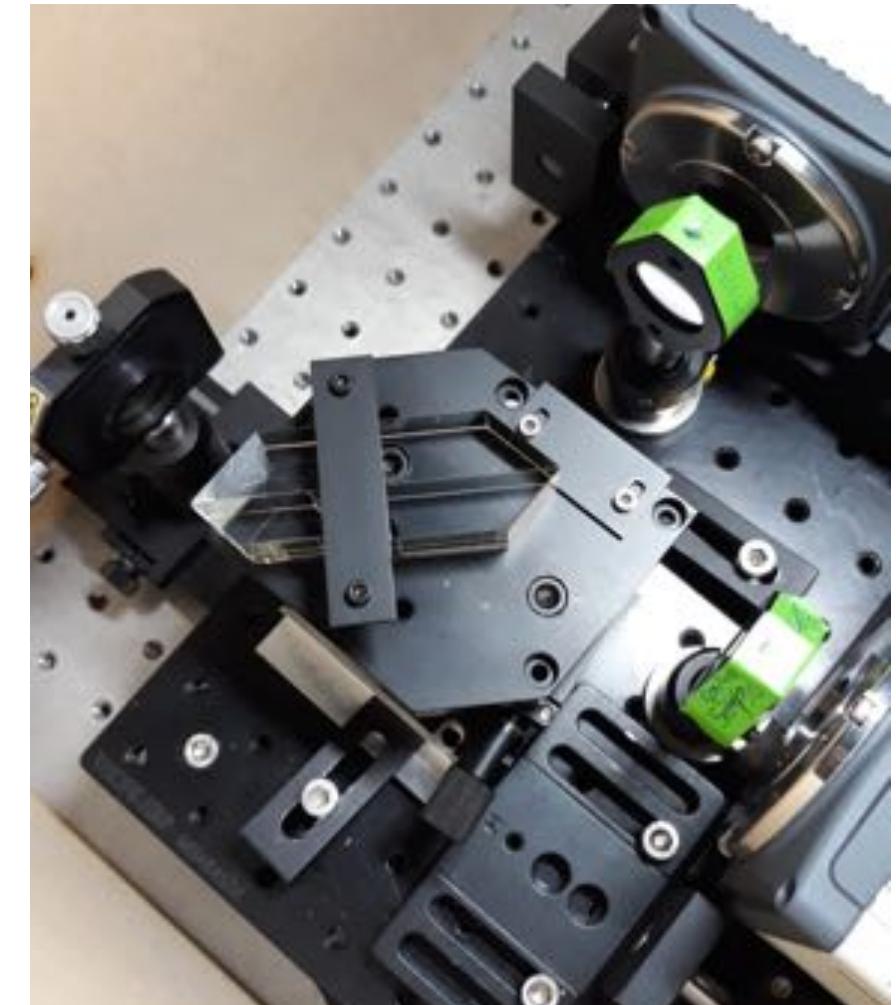
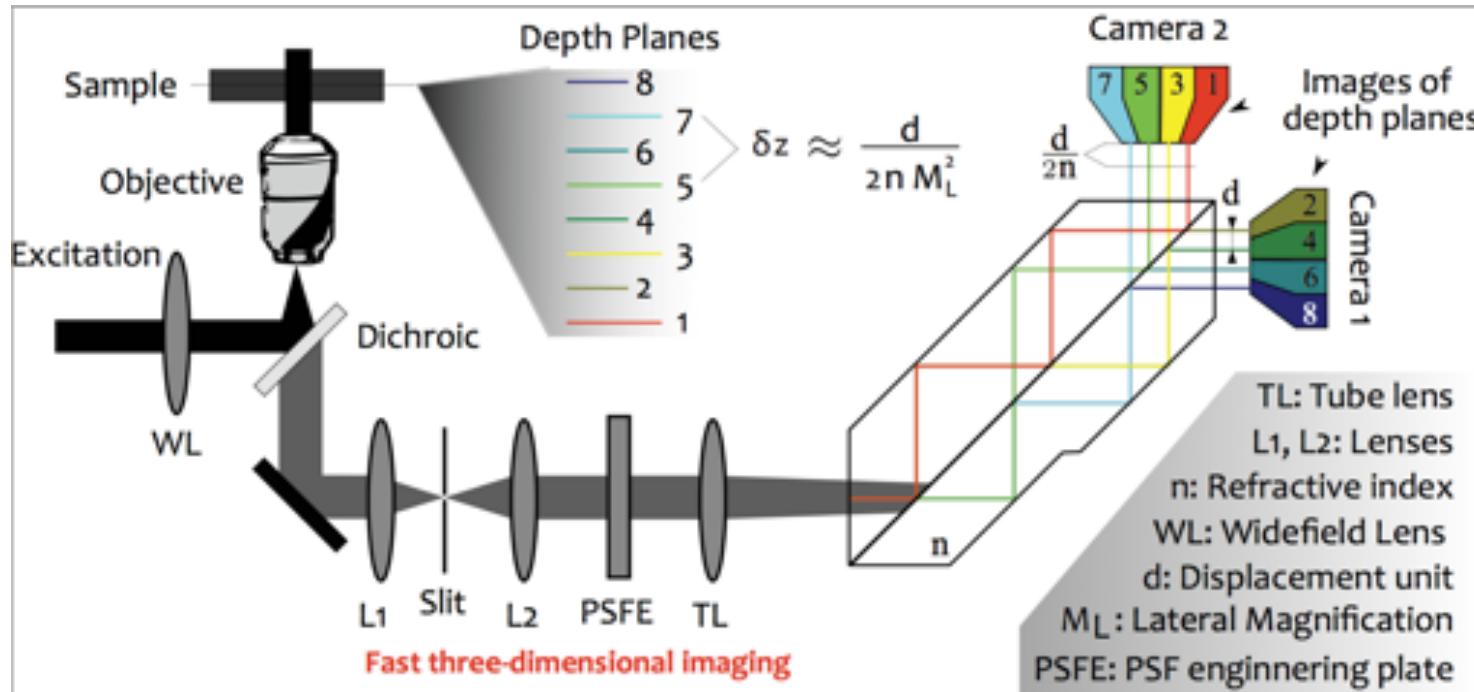
# Mechanical Characterization



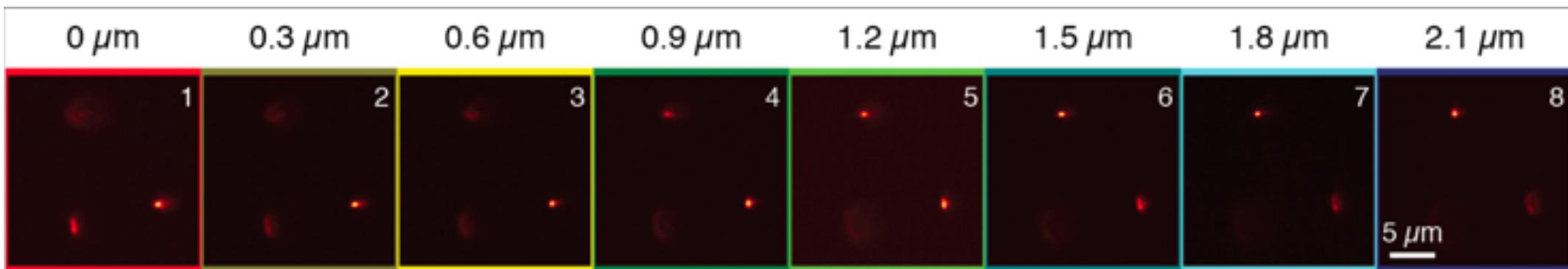
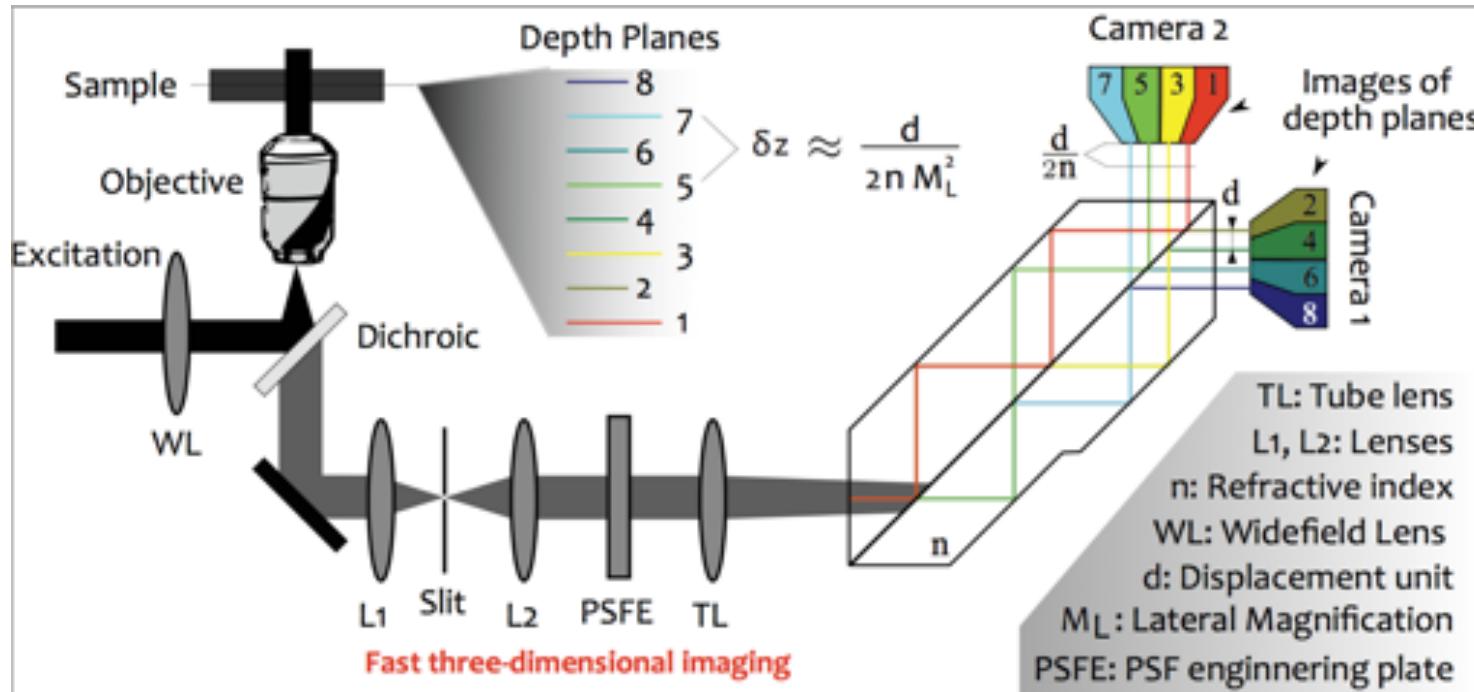
# Mechanical Characterization @ the microscale



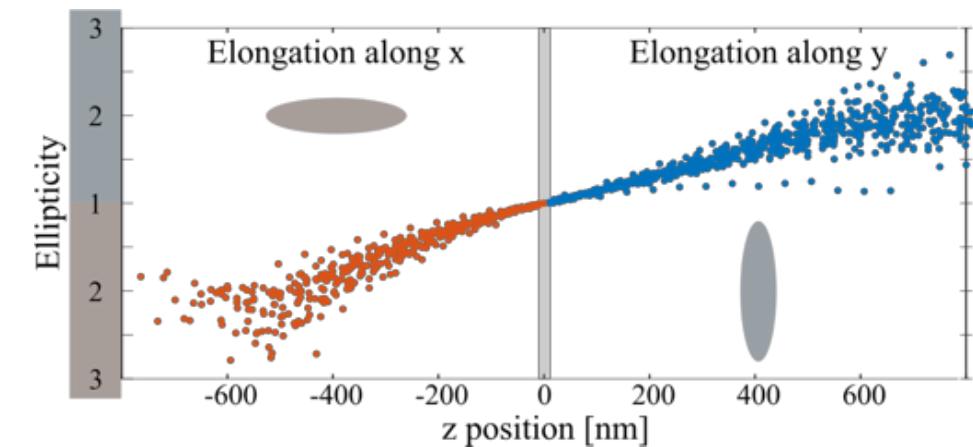
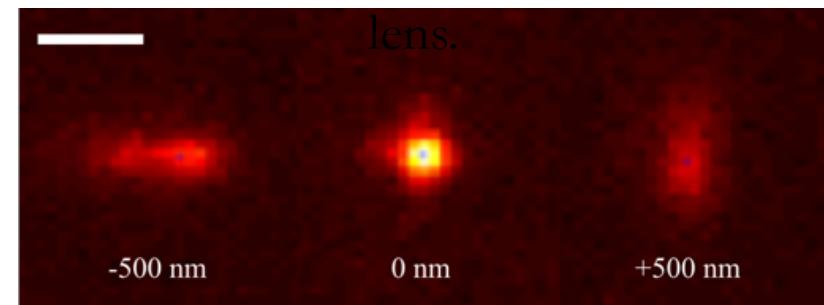
# Multi-plane wide field microscope for 2-point micro-rheology



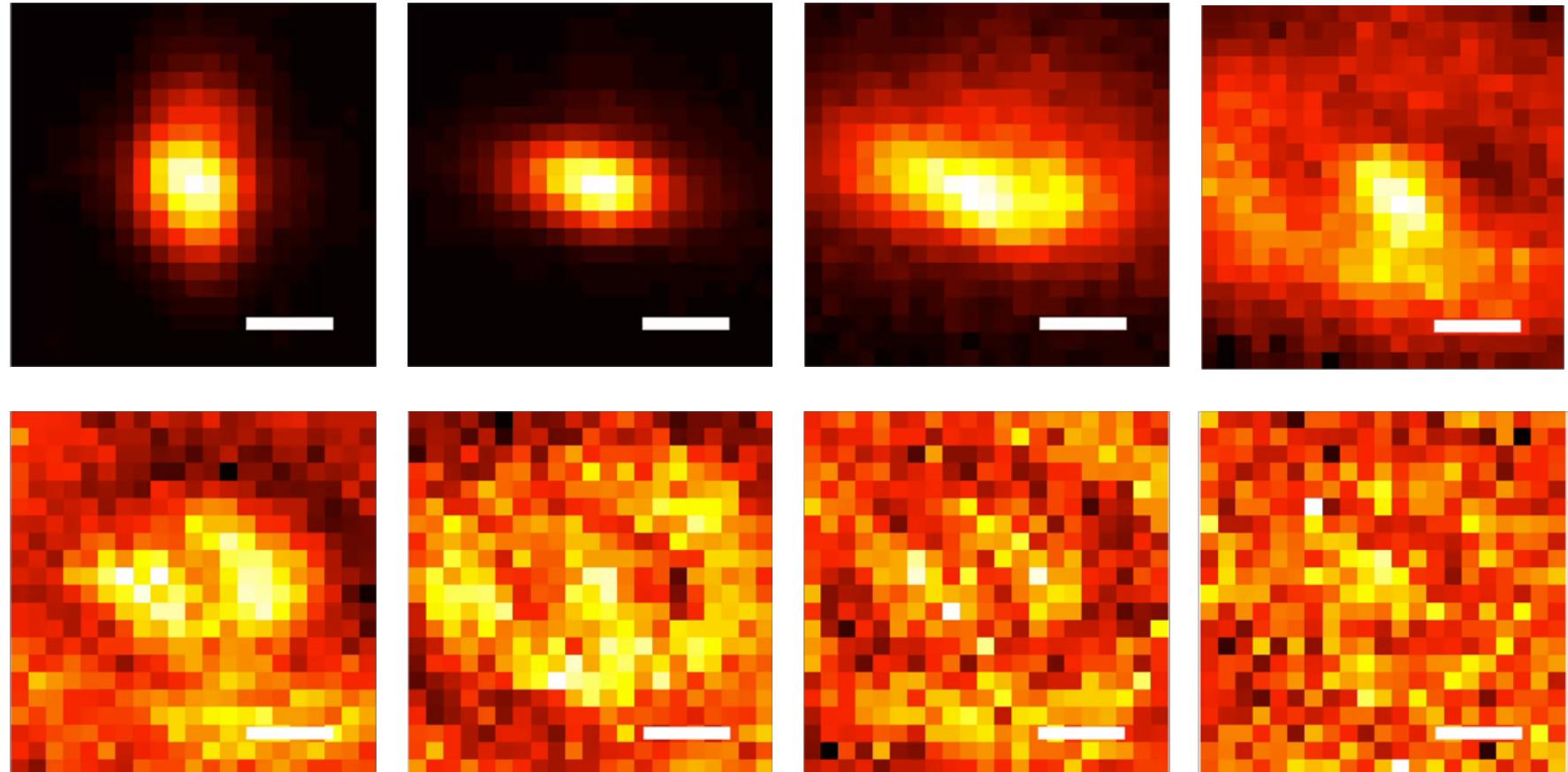
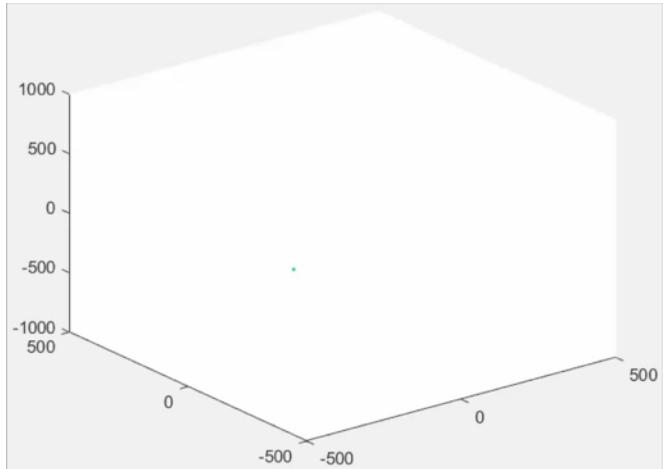
# Multi-plane wide field microscope for 2-point micro-rheology



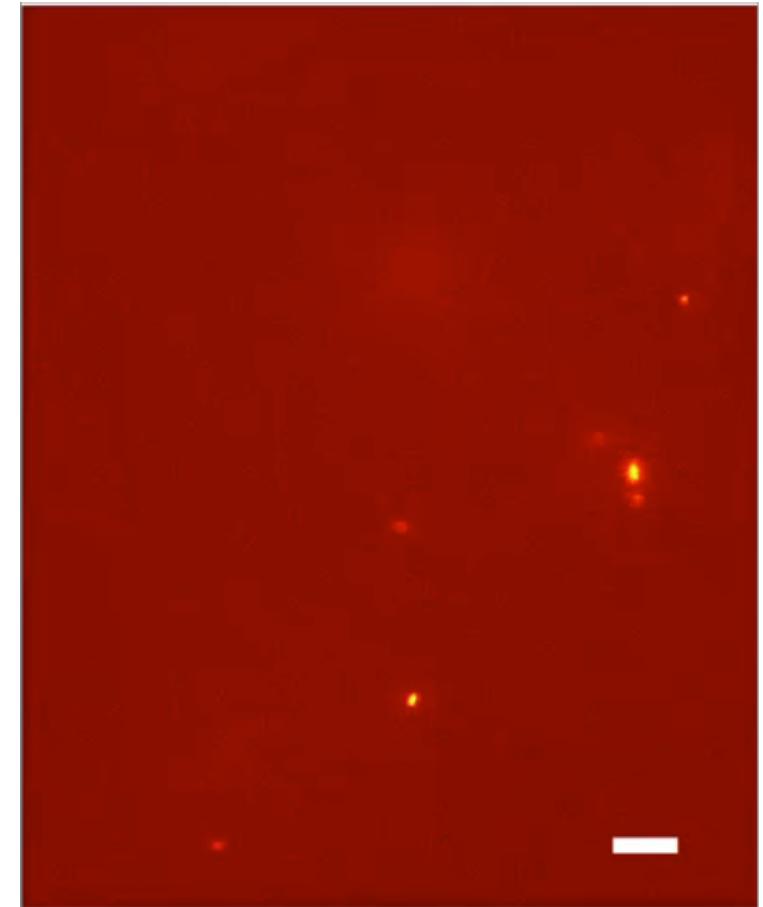
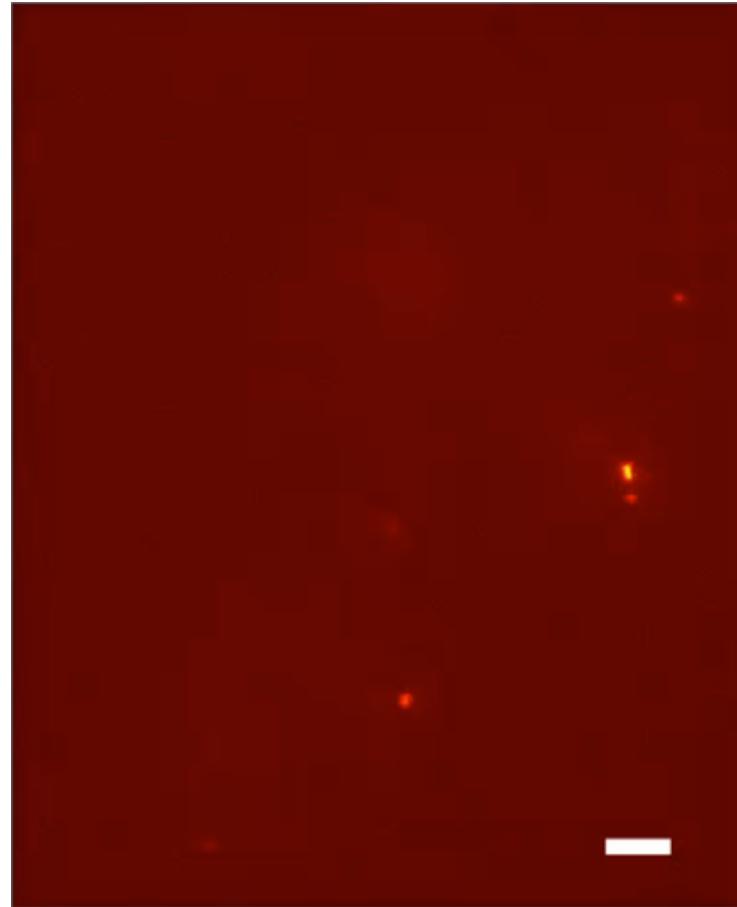
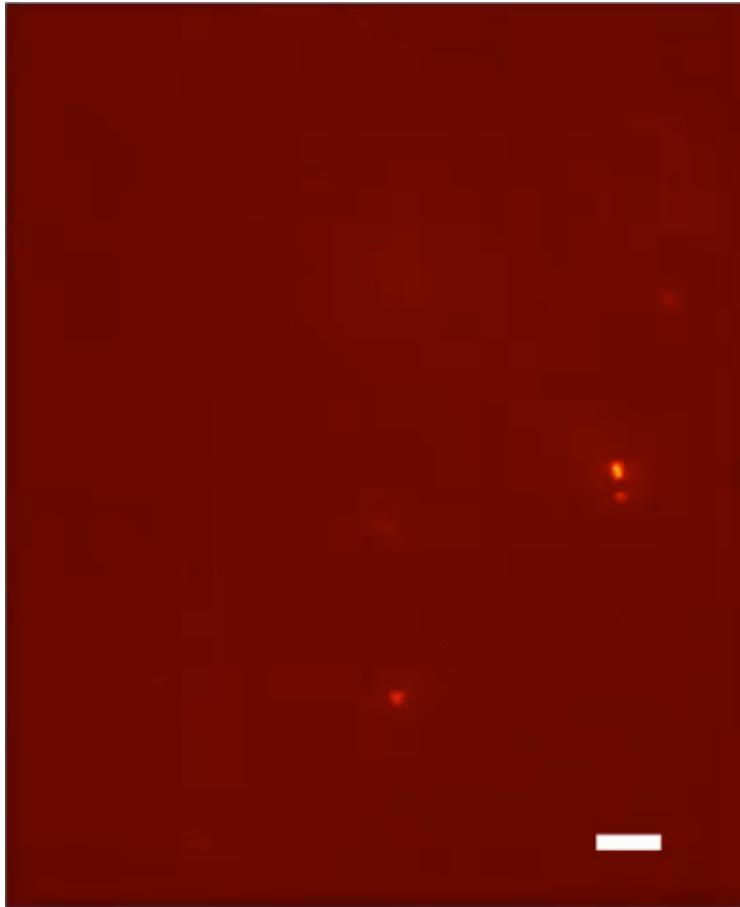
PSF engineering via cylindrical  
lens.



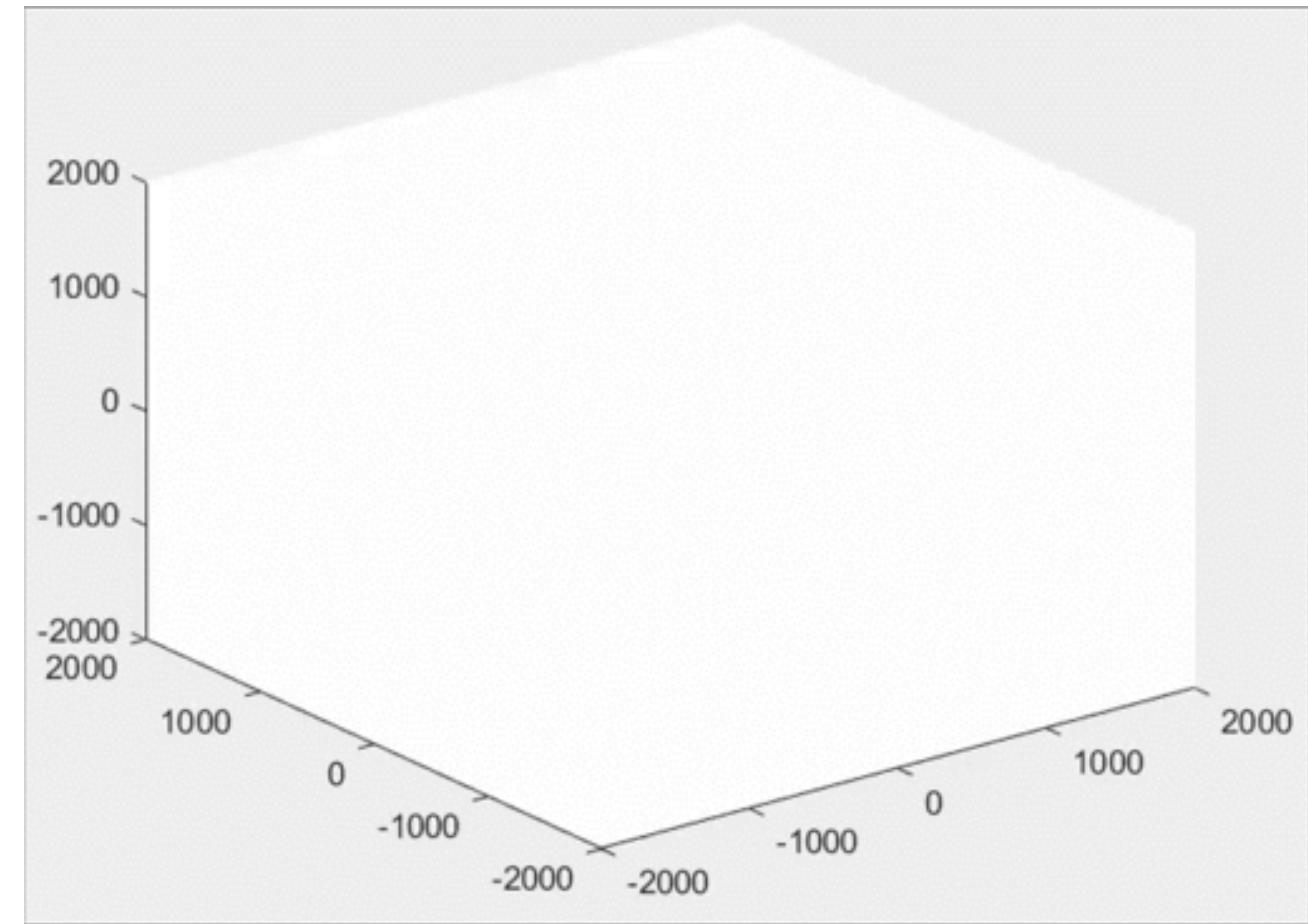
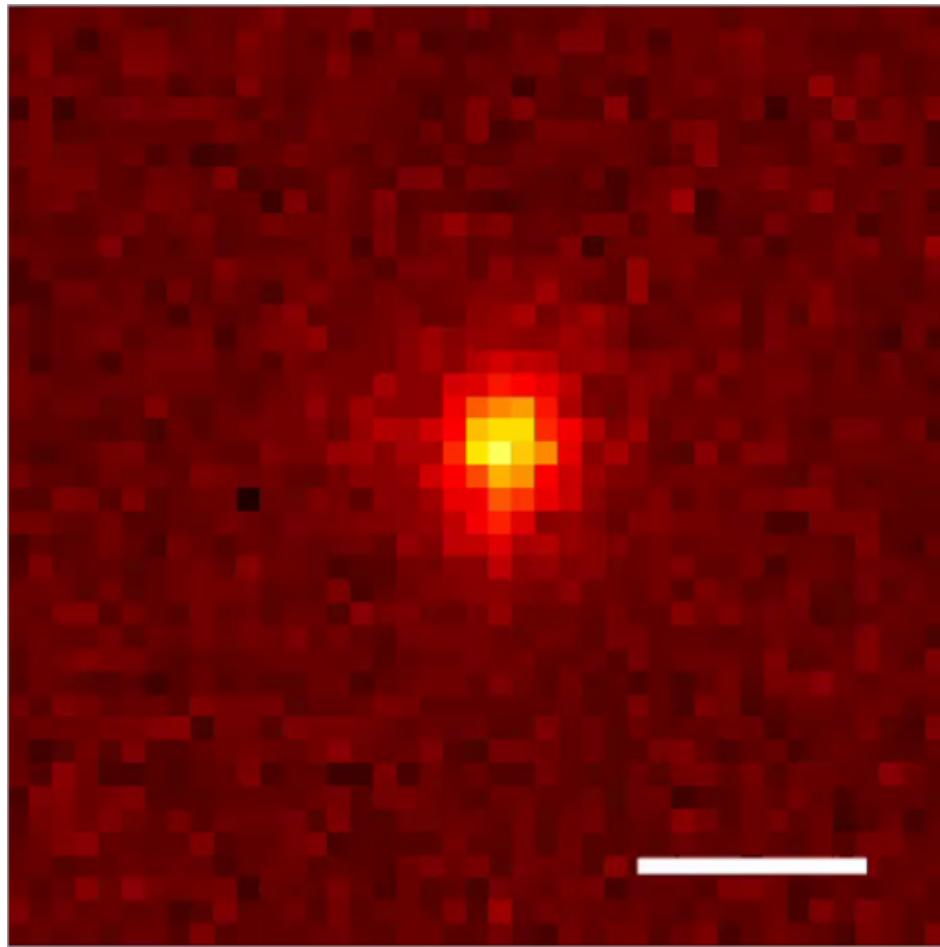
# Multi-plane wide field microscope for 2-point micro-rheology



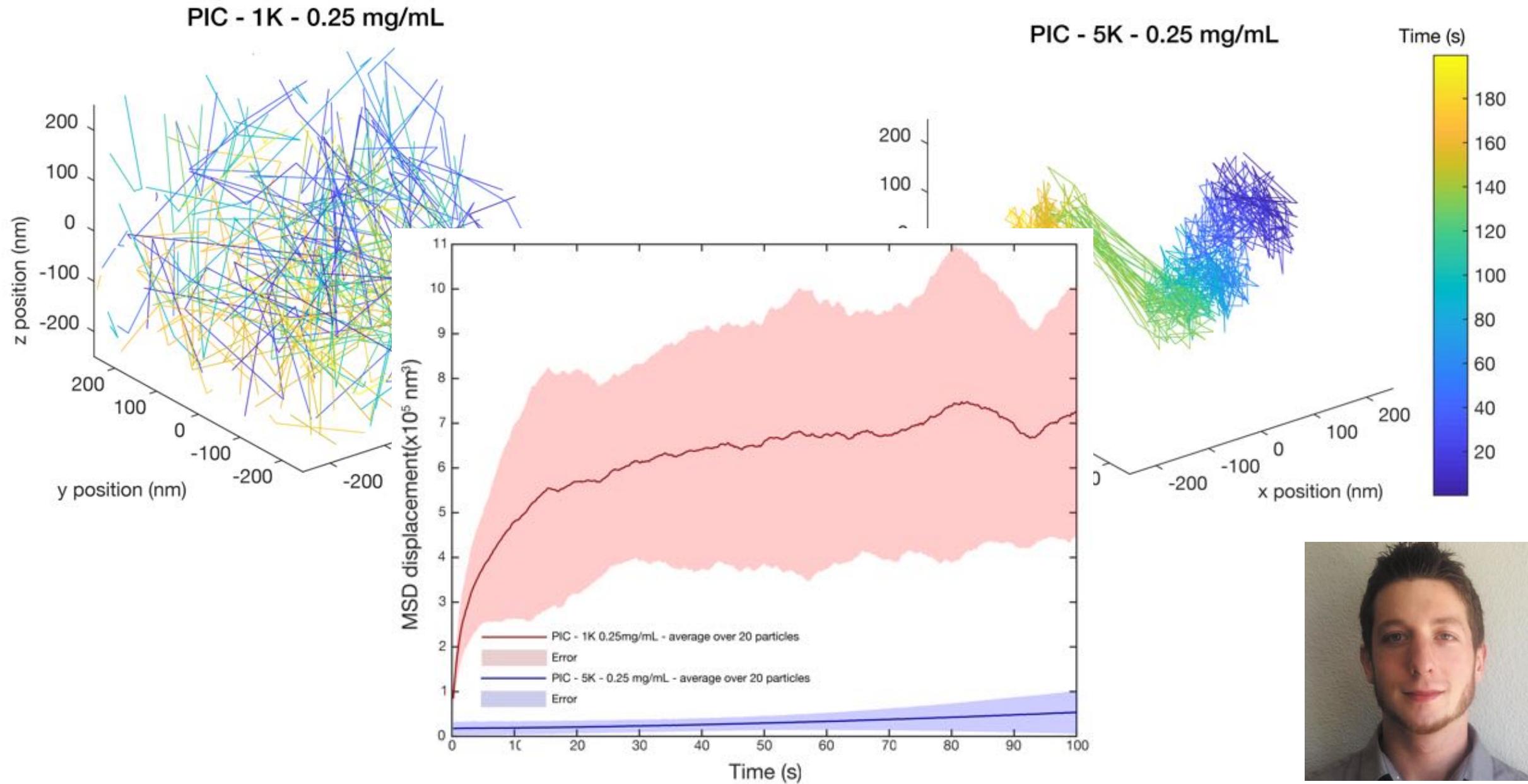
# 3D SPT in PIC hydrogel



# 3D SPT in PIC hydrogel

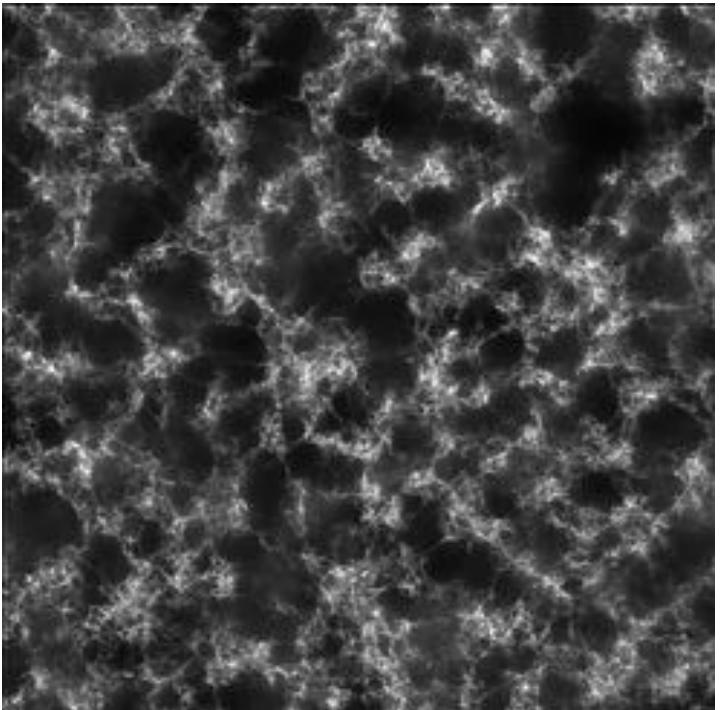


# 3D SPT in PIC hydrogel

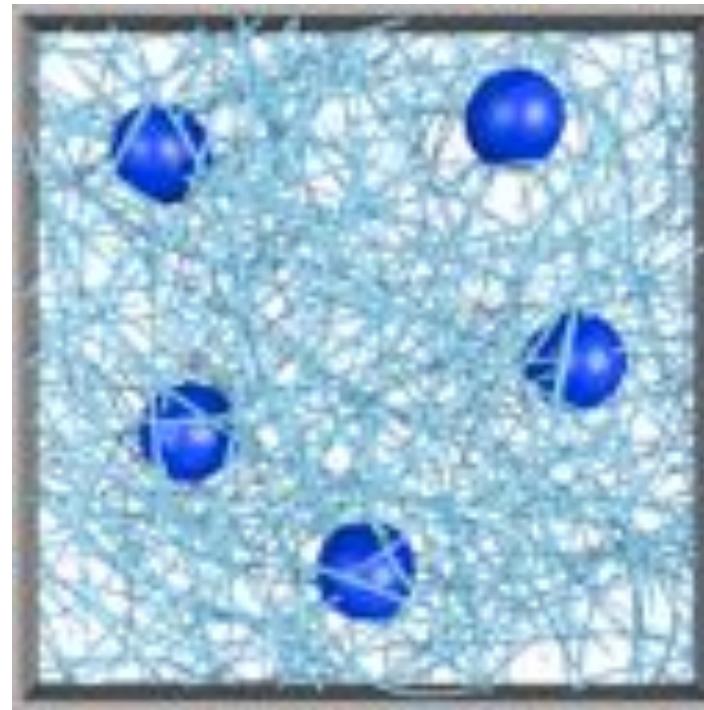


# Material Characterization

Structure using advanced fluorescence microscopy

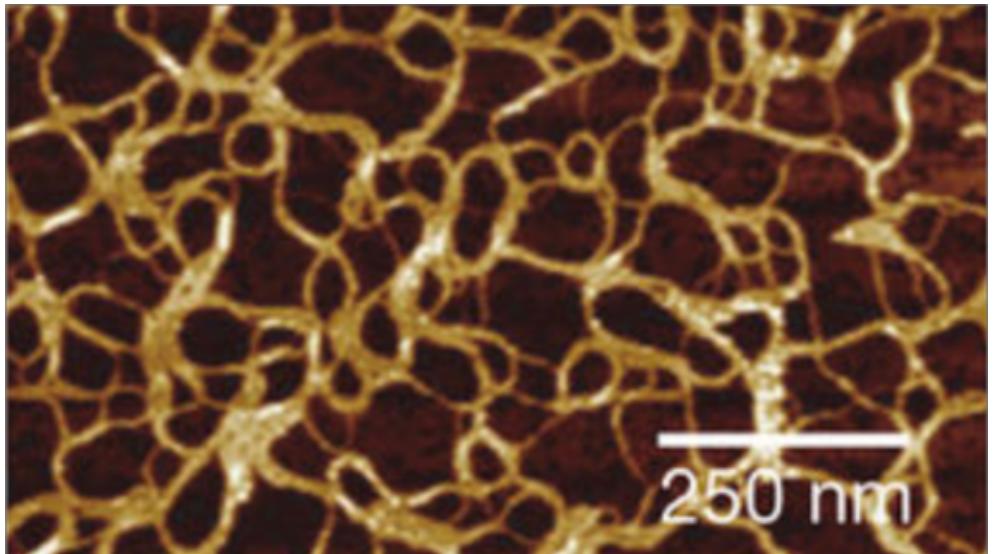


Local mechanical properties using 3D micro-rheology

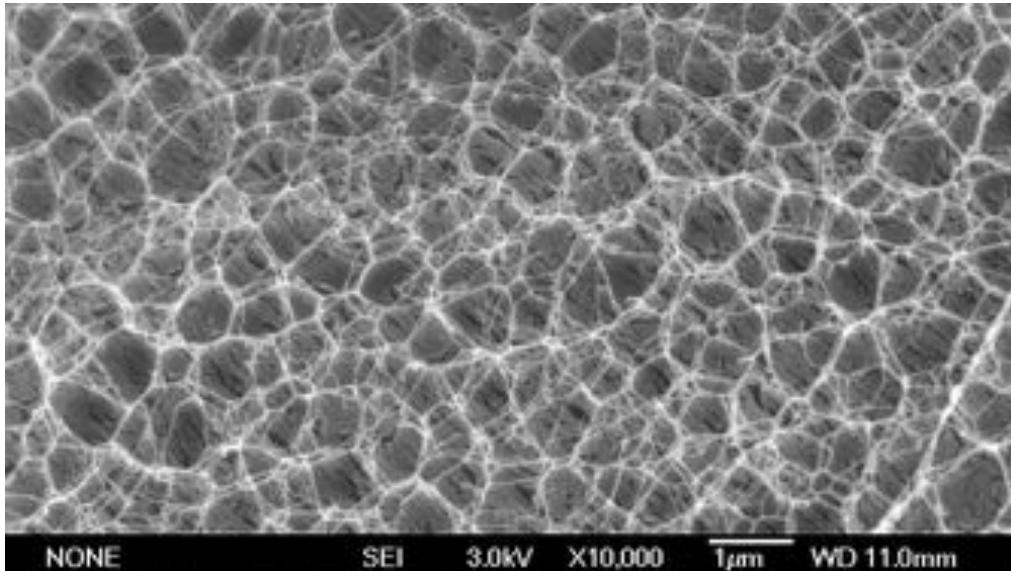


# Structural Characterization of PIC-based hydrogels

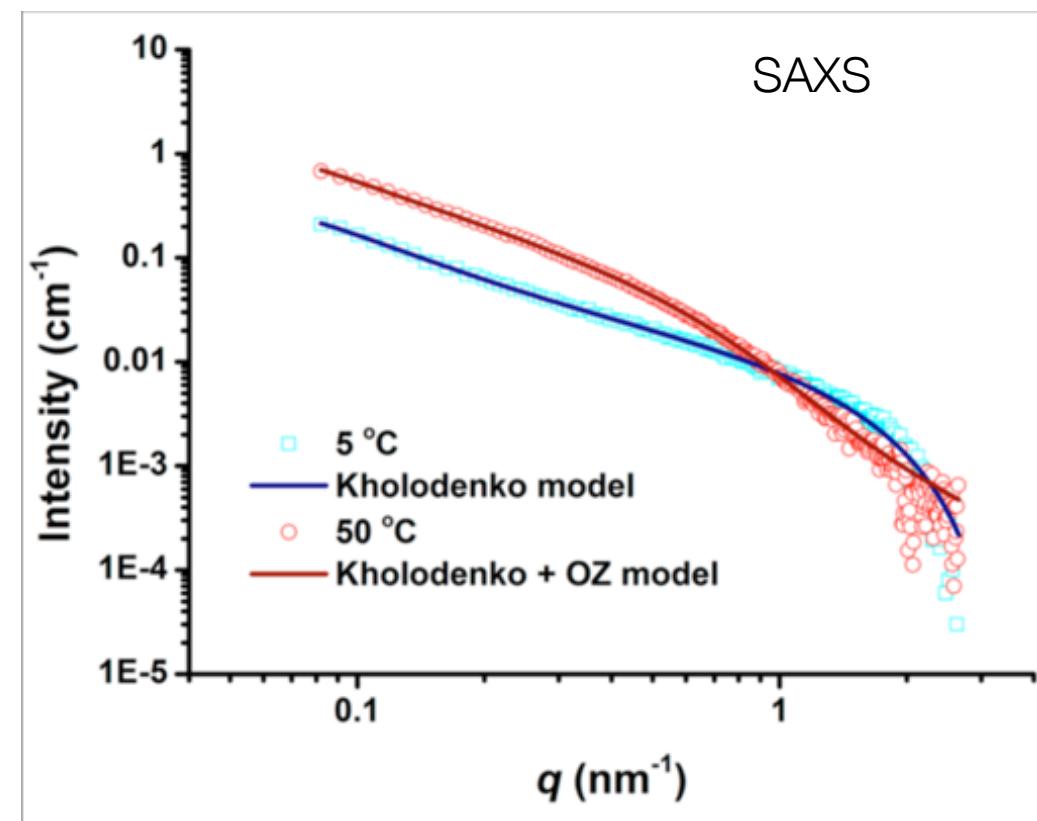
AFM



CryoEM



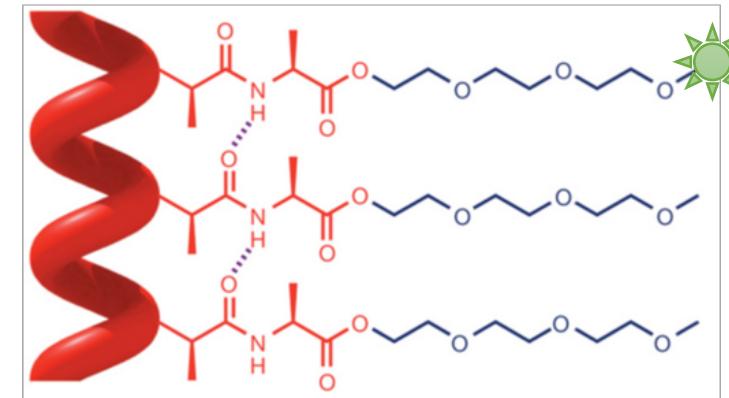
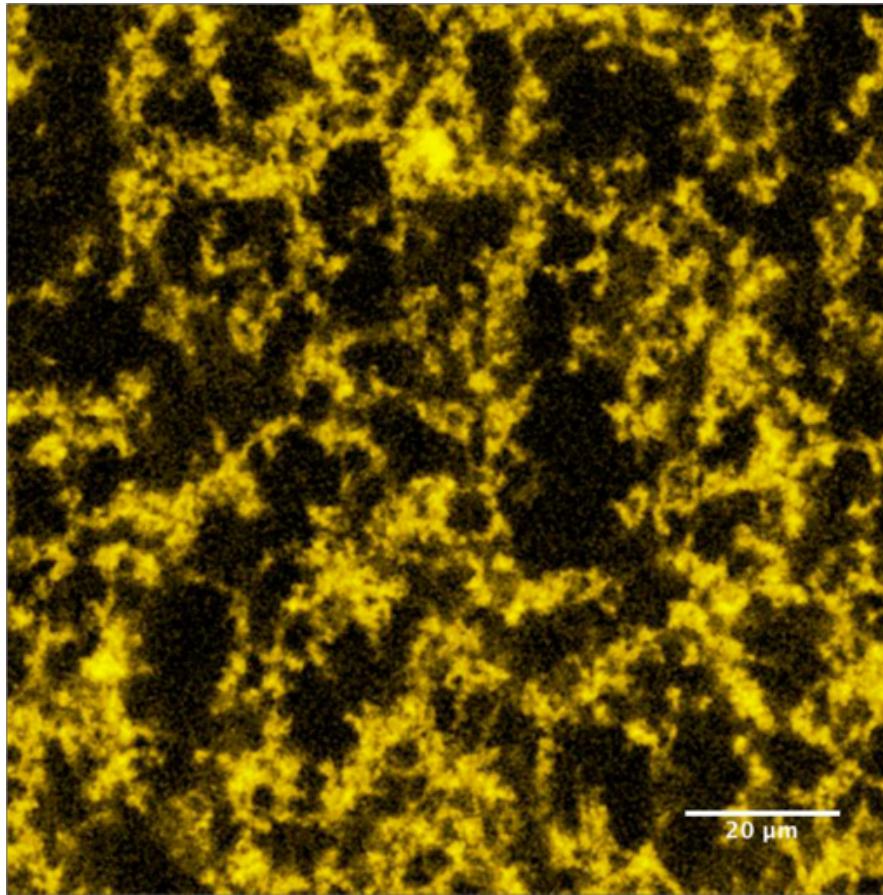
SAXS



PIC	$L$ (nm)	$l_p$ (nm)	$R$ (nm)	$\xi_{\text{OZ}}$ (nm) <sup>b</sup>	$R_B$ (nm)
P1a	55	$13 \pm 7$	$1.1 \pm 0.1$	$>80^{\text{c}}$	$5.0 \pm 0.2$
P1b	77	$10 \pm 1$	$1.1 \pm 0.1$	$>80^{\text{c}}$	$4.7 \pm 0.2$
P1c	110	$9 \pm 2$	$1.2 \pm 0.1$	$>80^{\text{c}}$	$3.3 \pm 0.1$
P1b	134	$9 \pm 1$	$1.1 \pm 0.1$	68	$3.0 \pm 0.1$
P1e	160	$12 \pm 2$	$1.1 \pm 0.1$	30	$3.0 \pm 0.2$

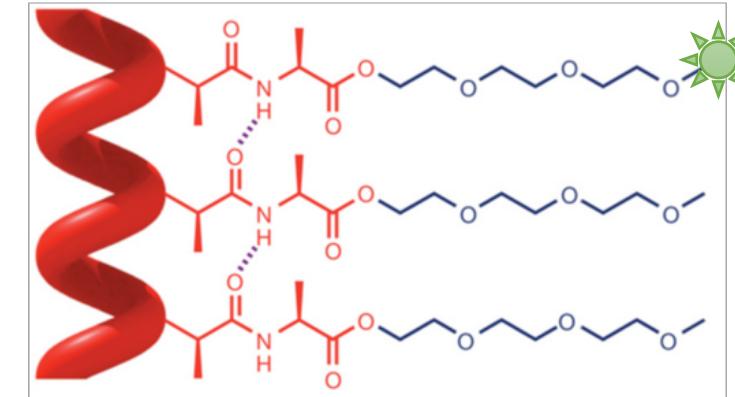
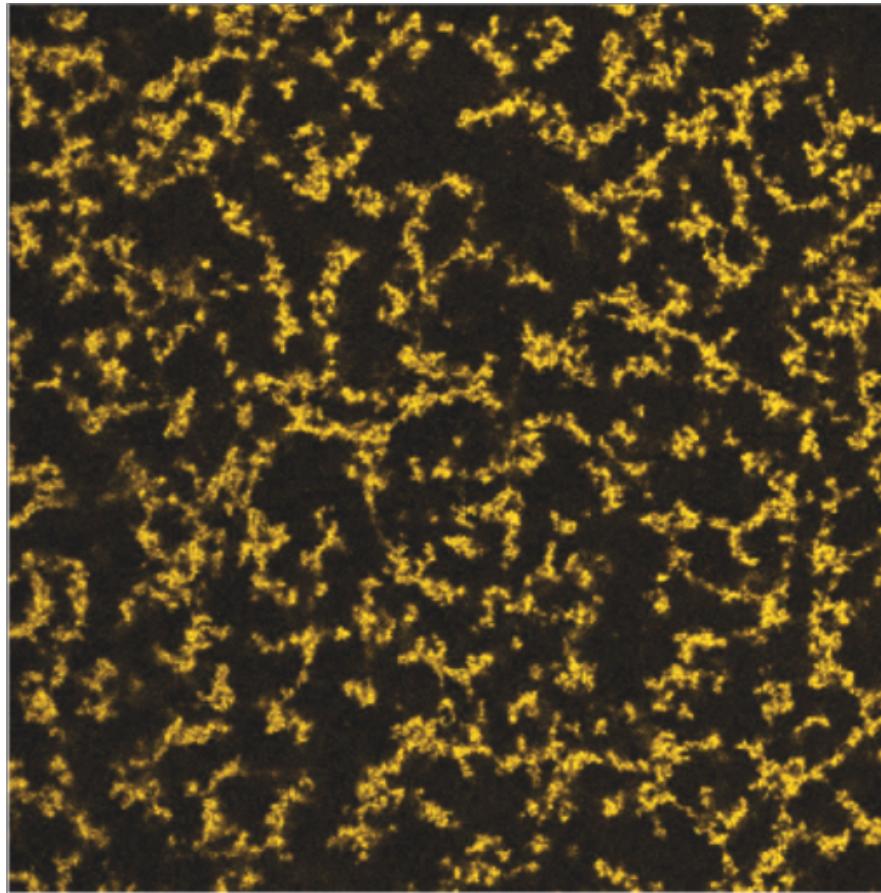
# Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy



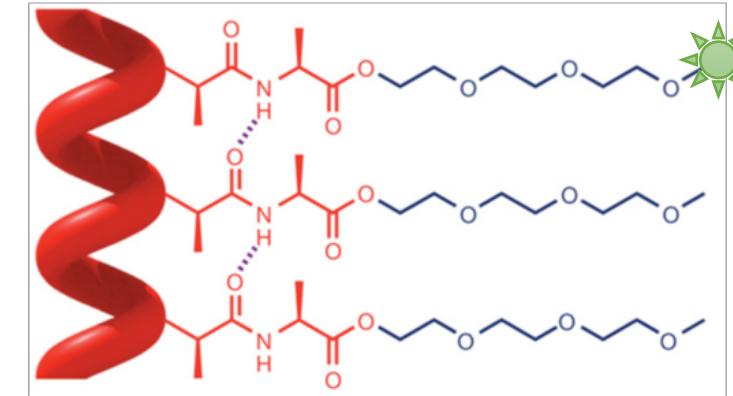
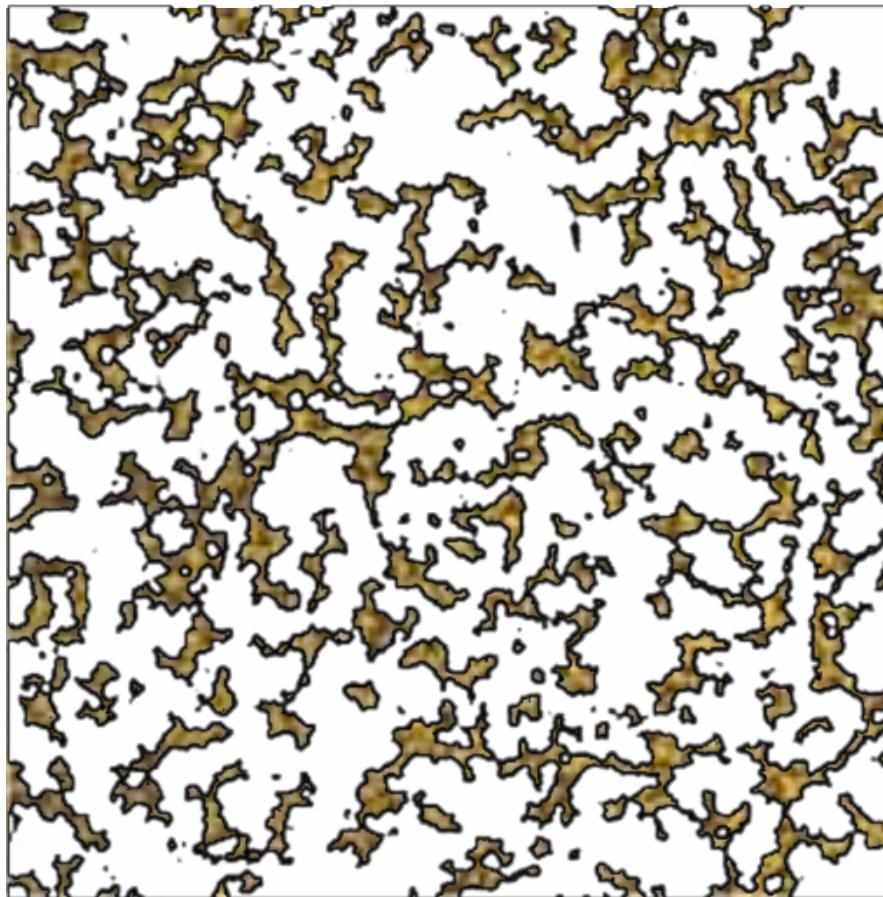
# Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy

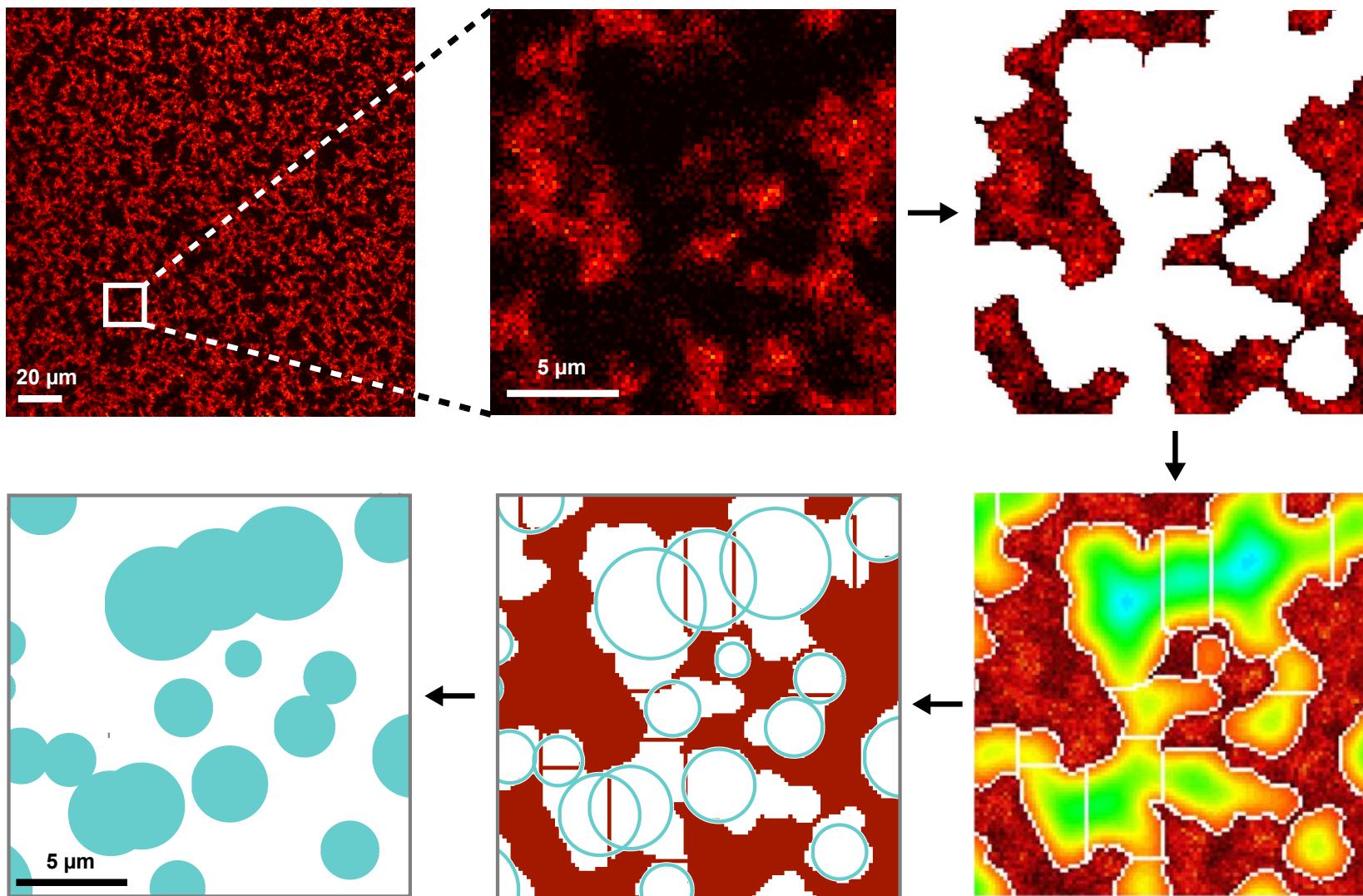


# Structural Characterization of PIC-based hydrogels

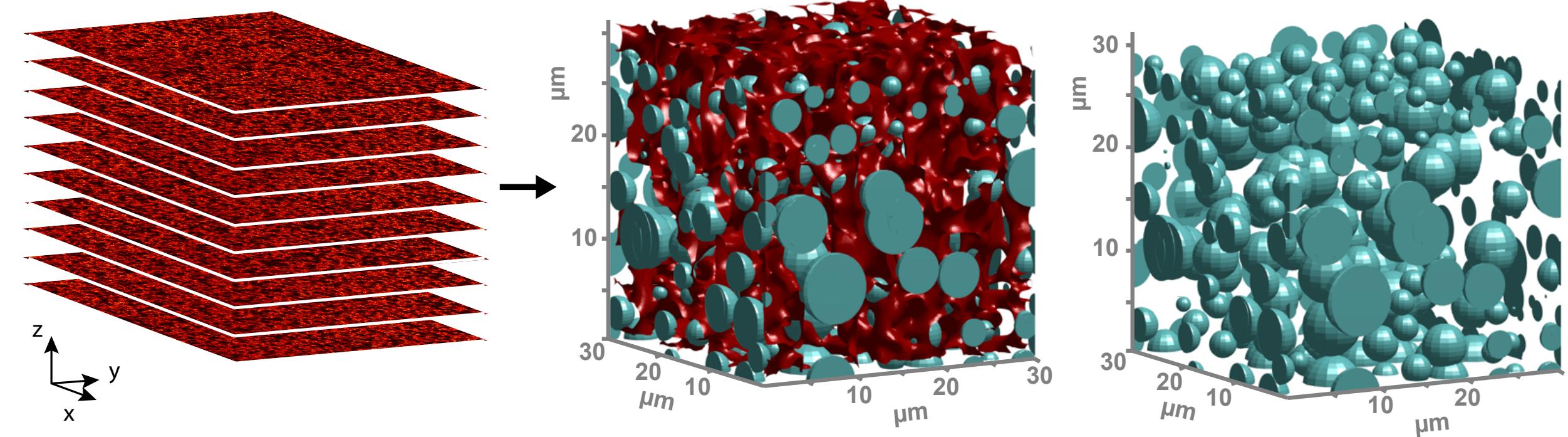
Fluorescence Microscopy



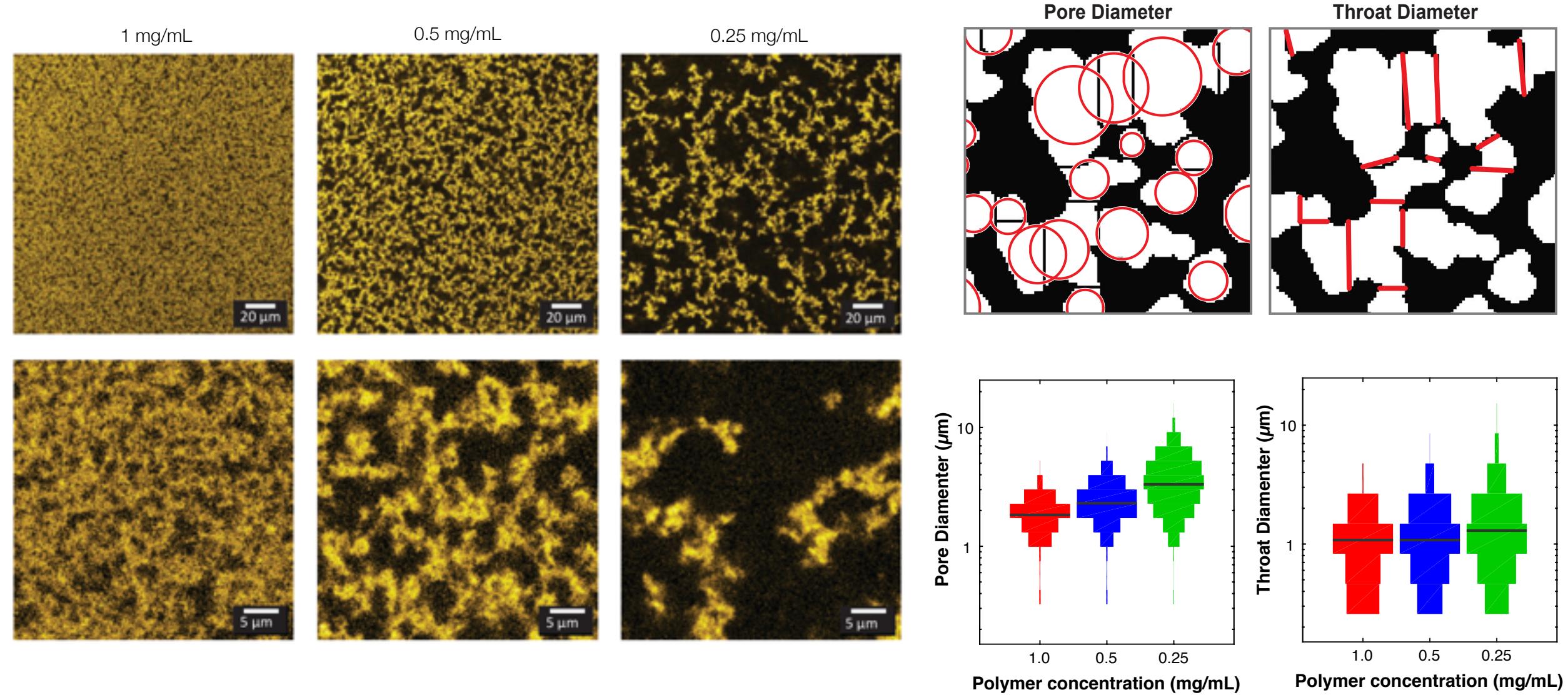
# Structural Characterization of PIC-based hydrogels



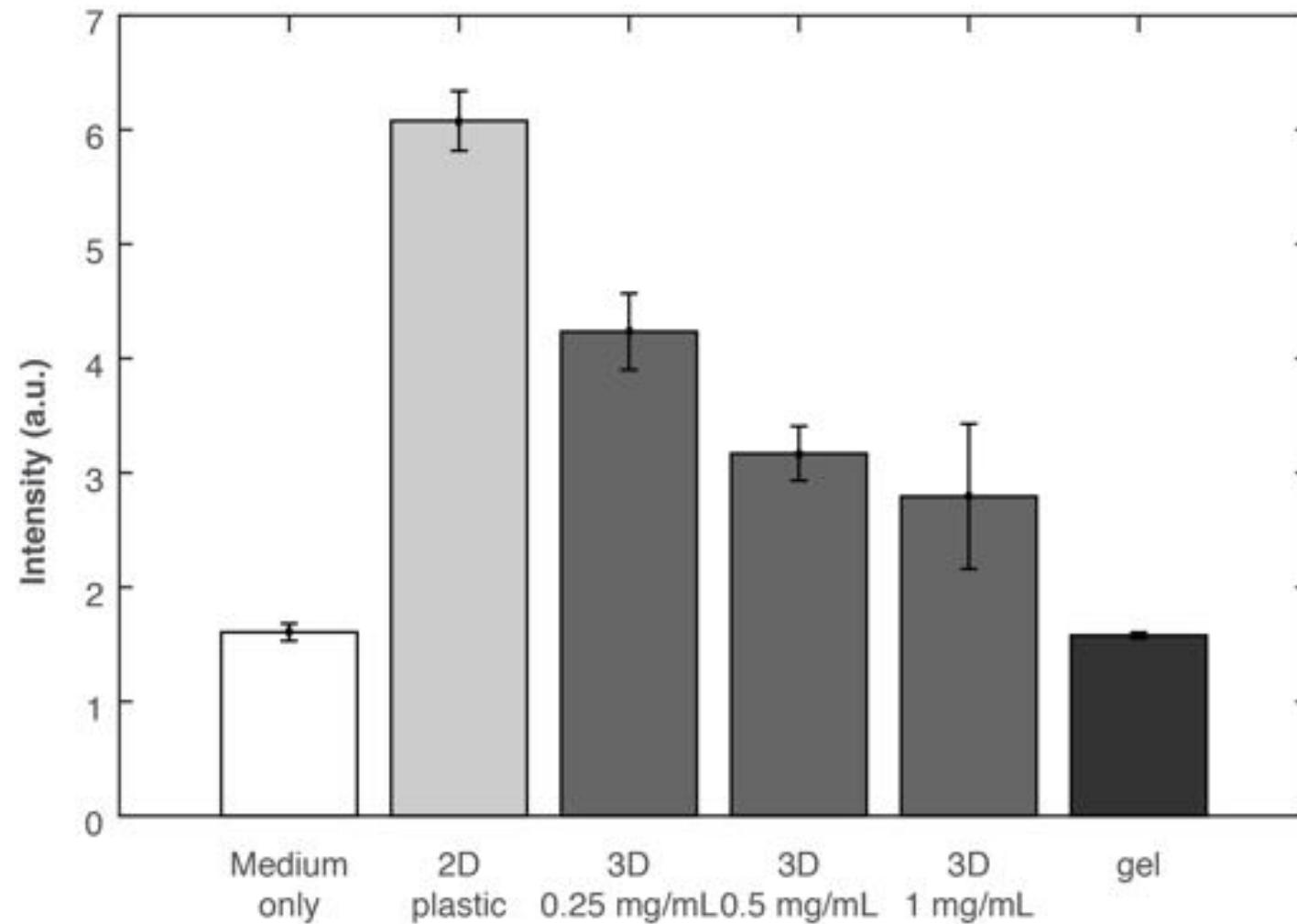
# Structural Characterization of PIC-based hydrogels in 3D



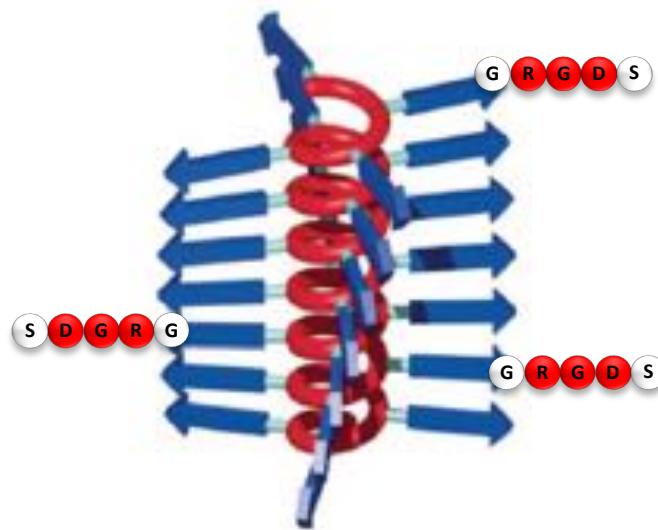
# Structural Characterization of PIC-based hydrogels



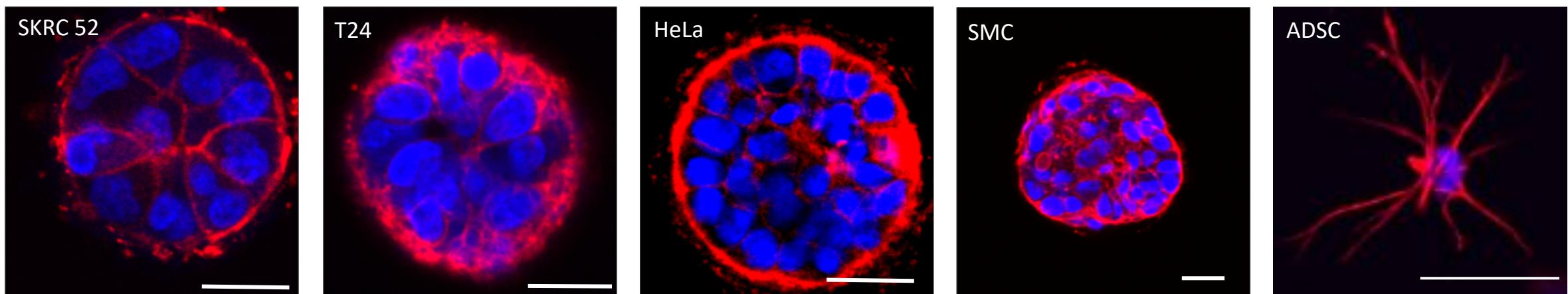
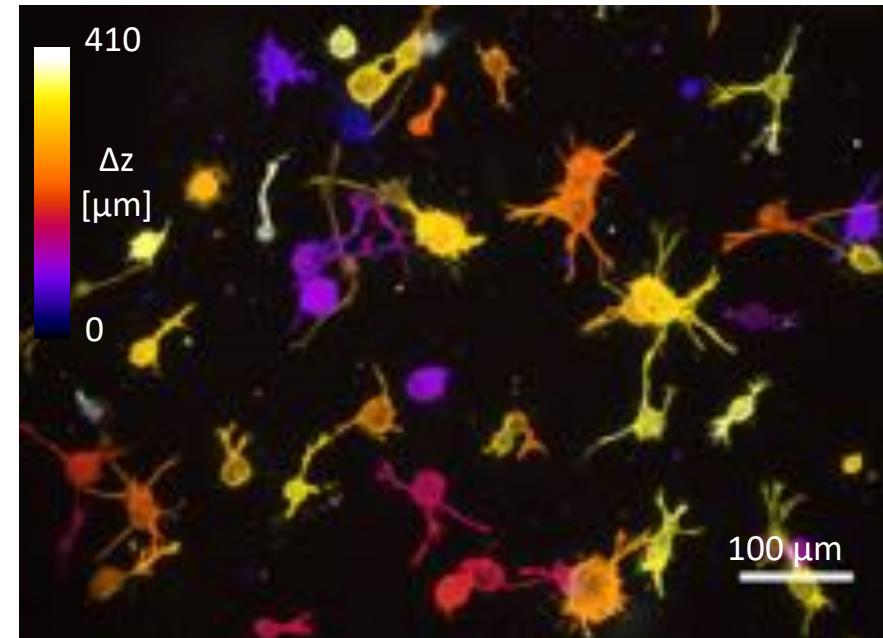
# Hydrogel structure and cell proliferation



# Minimalist matrix: Polyisocyanopeptides (PIC)



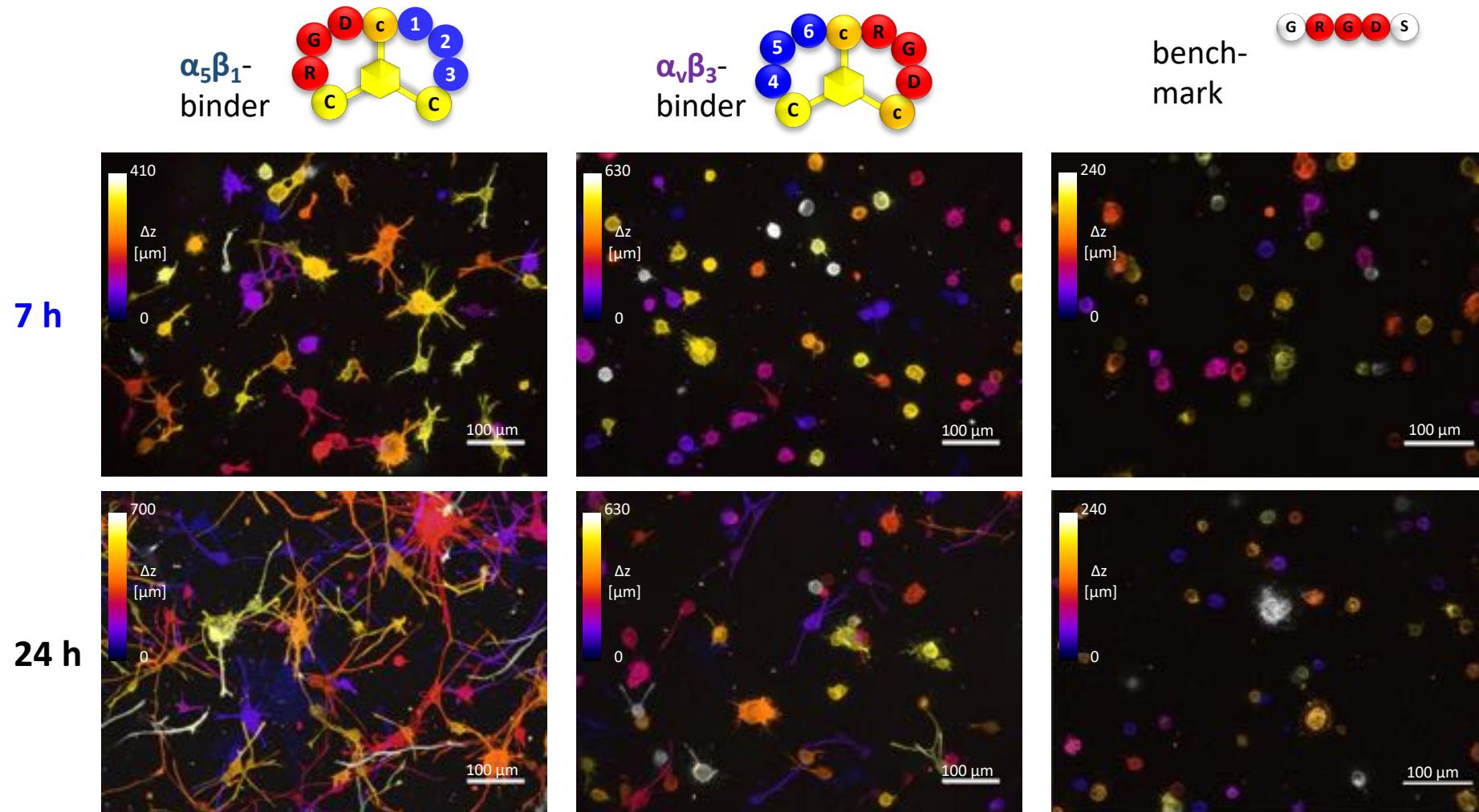
Different cell types → Different behavior



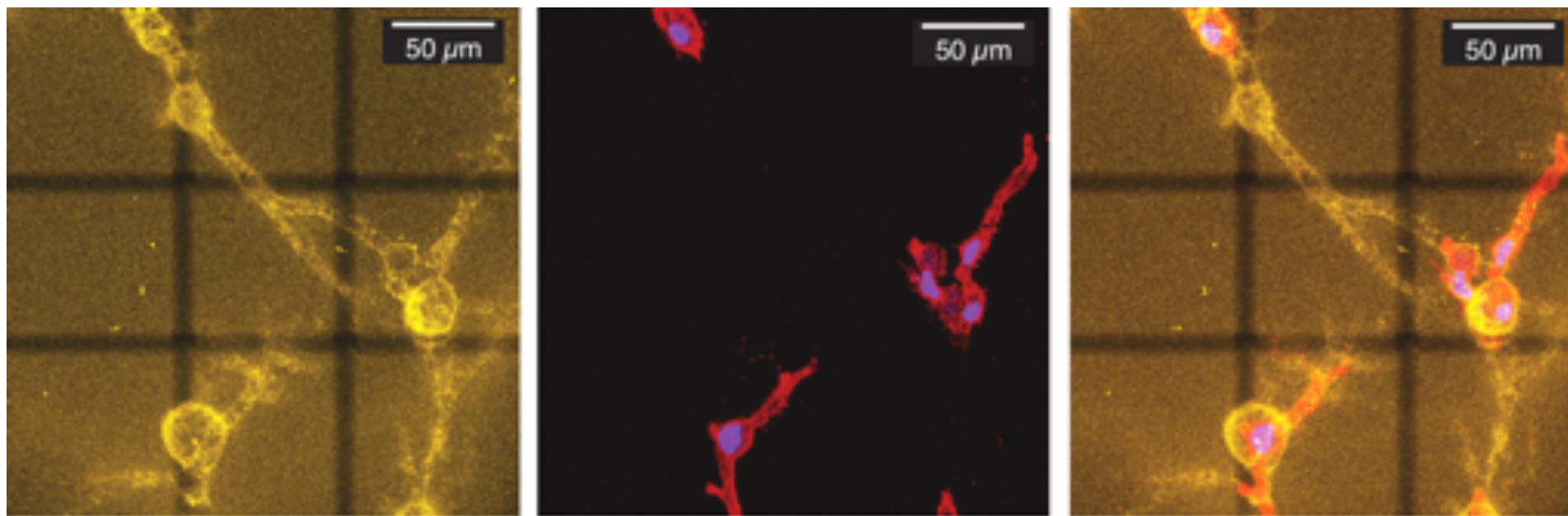
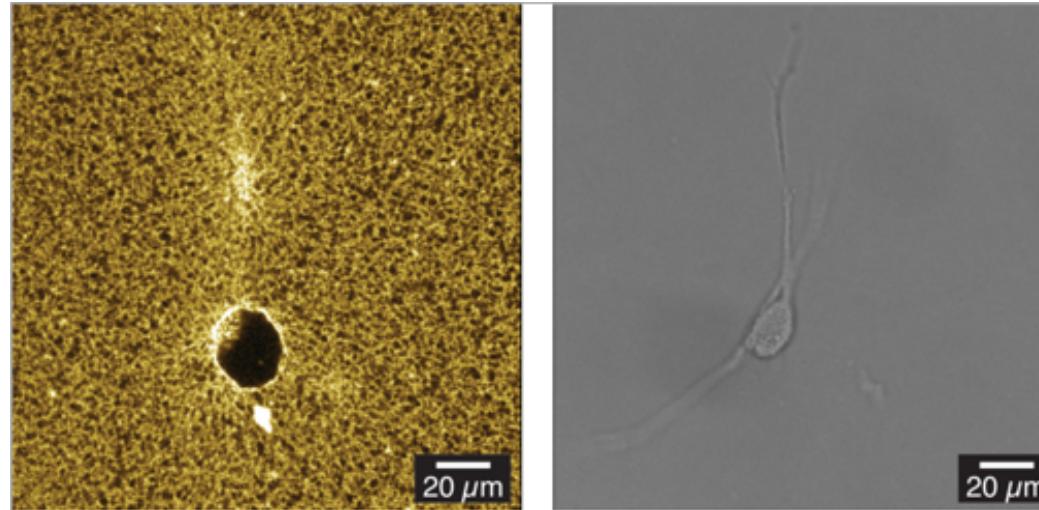
Phalloidin  
DAPI

# Spreading of human Adipose Stem Cells

Peptides with different integrin affinities influence stem cell spreading

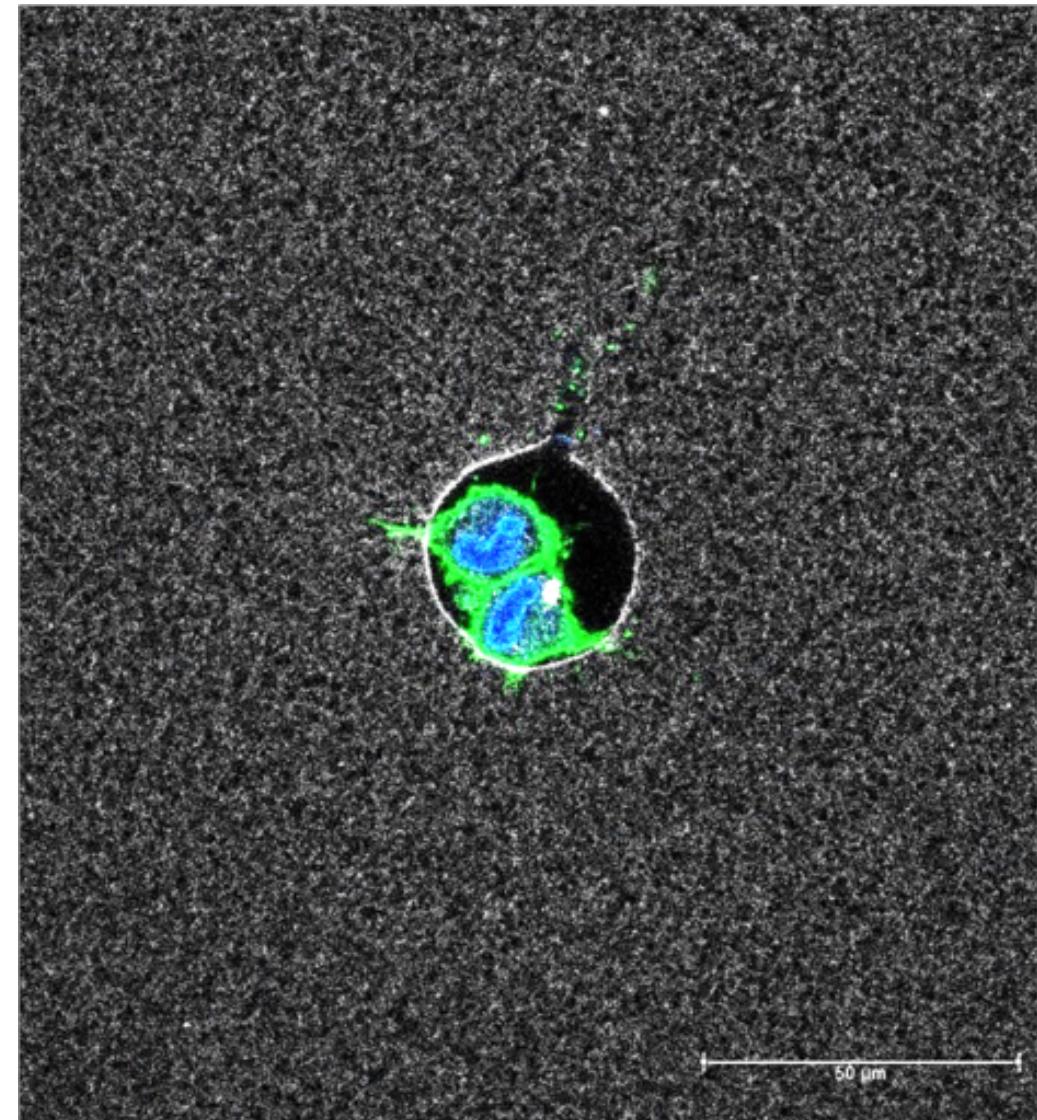
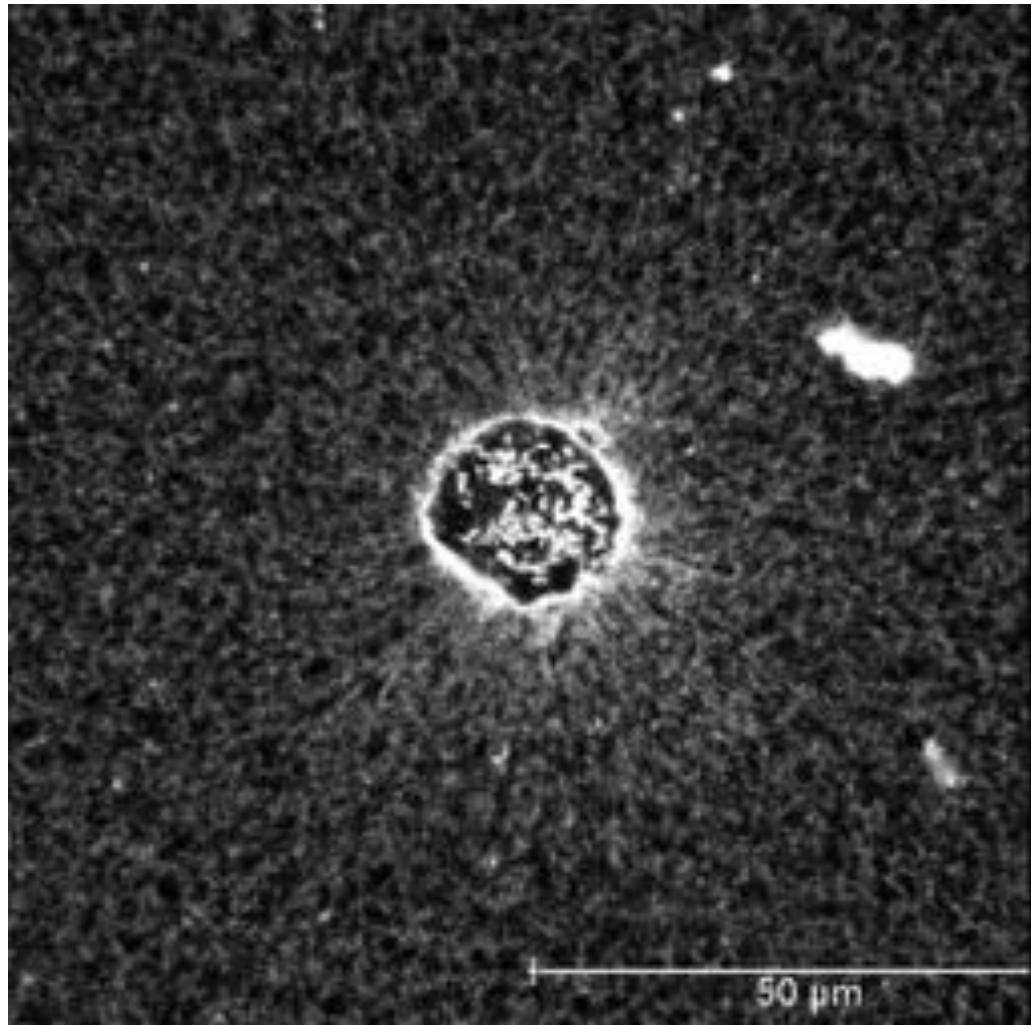


# Matrix remodeling by human Adipose Stem Cells

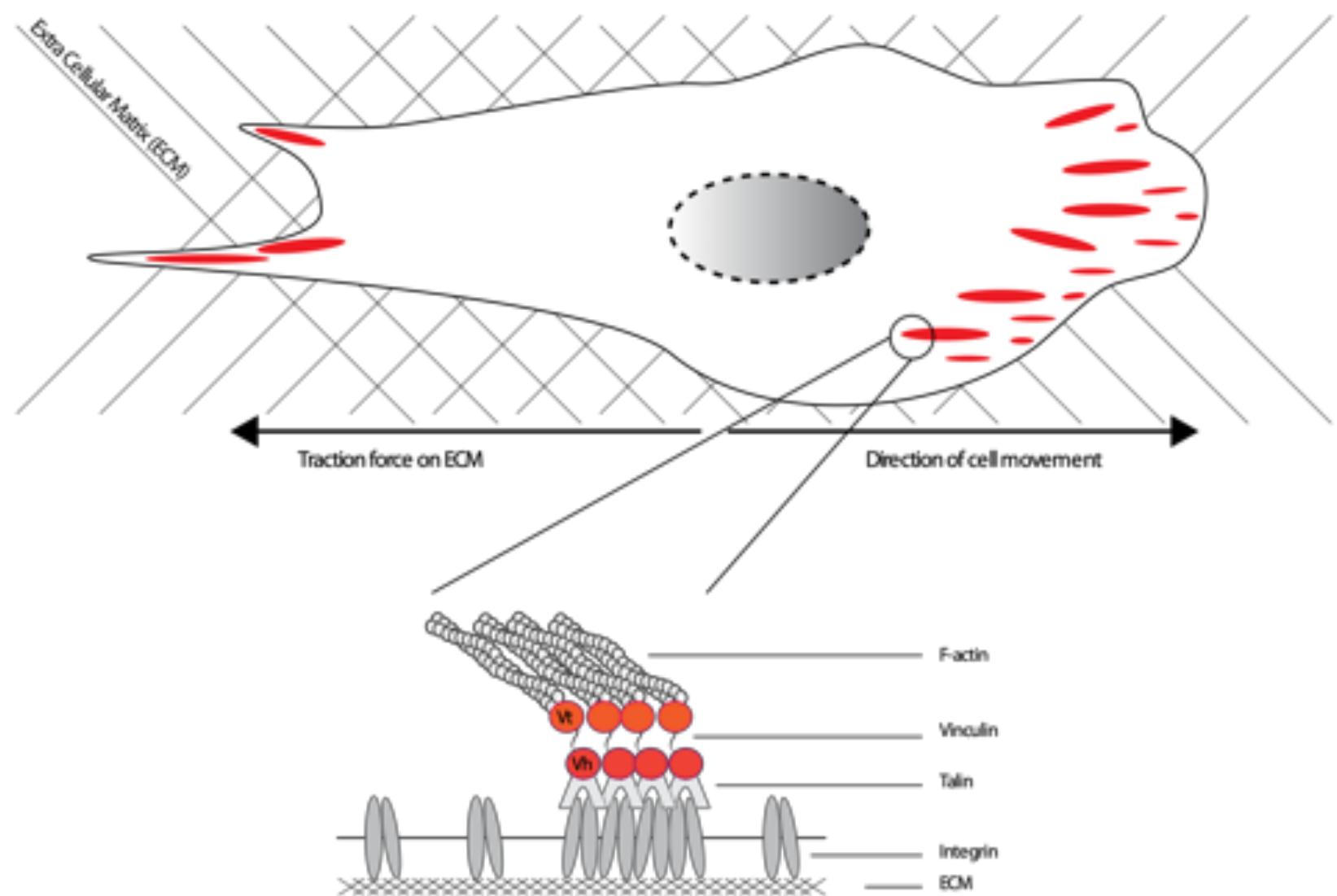
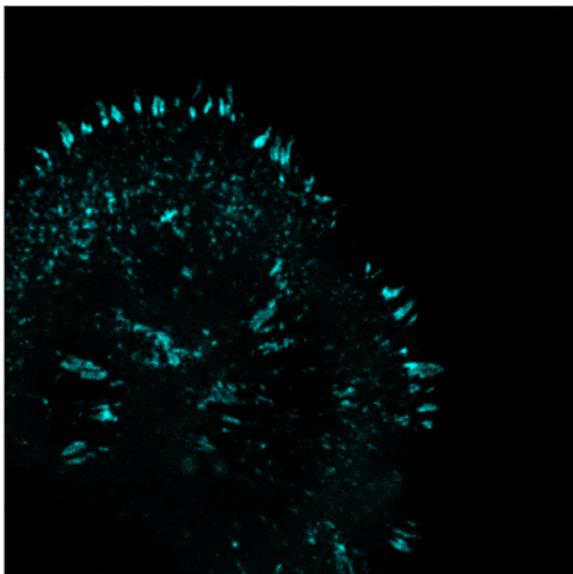
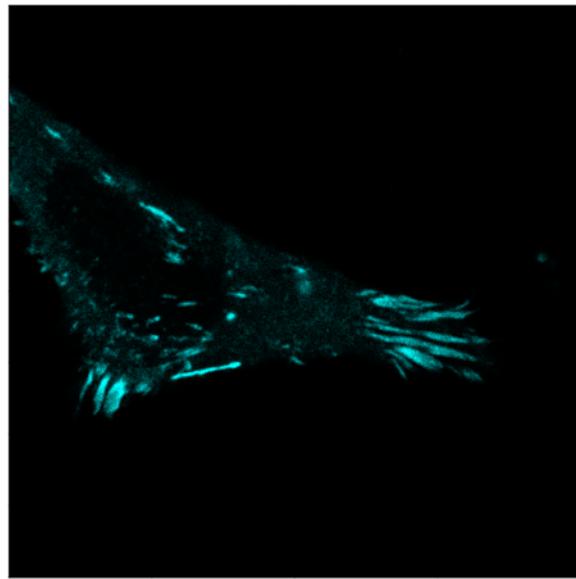


PIC F-actin Nucleus

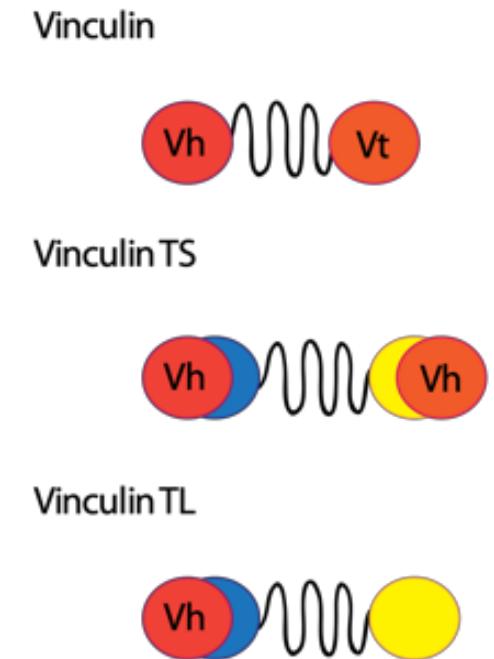
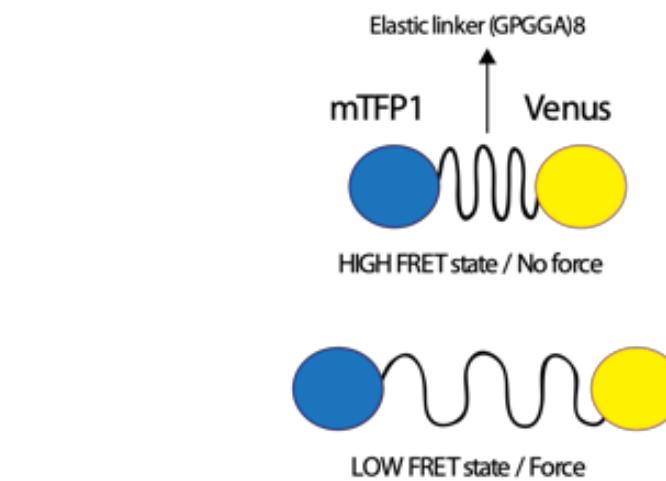
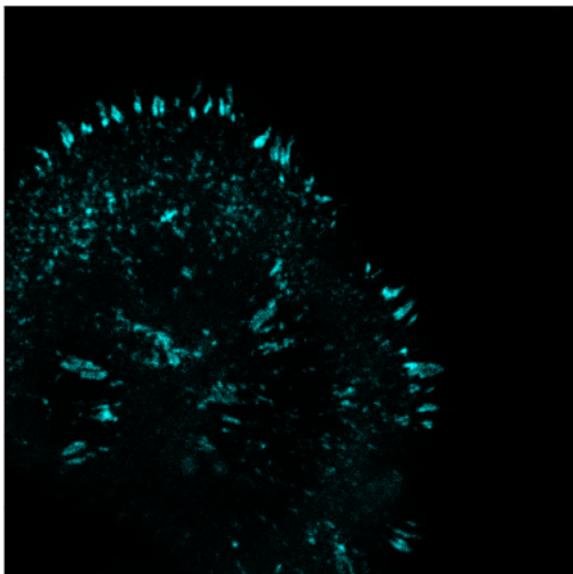
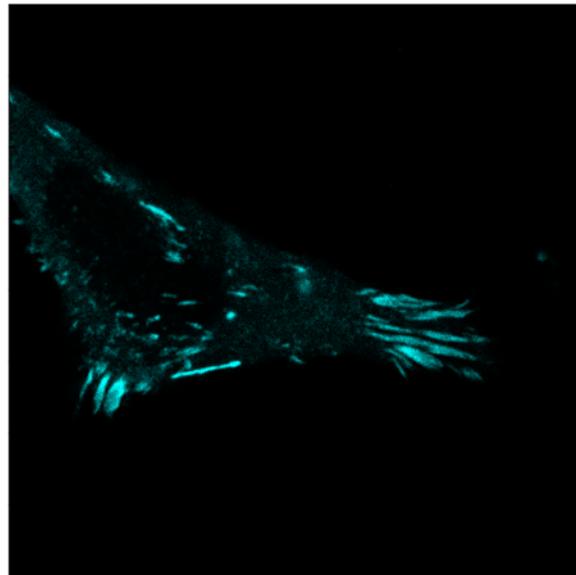
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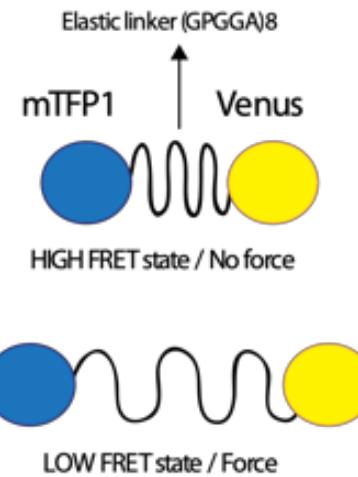
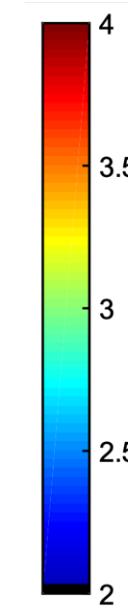
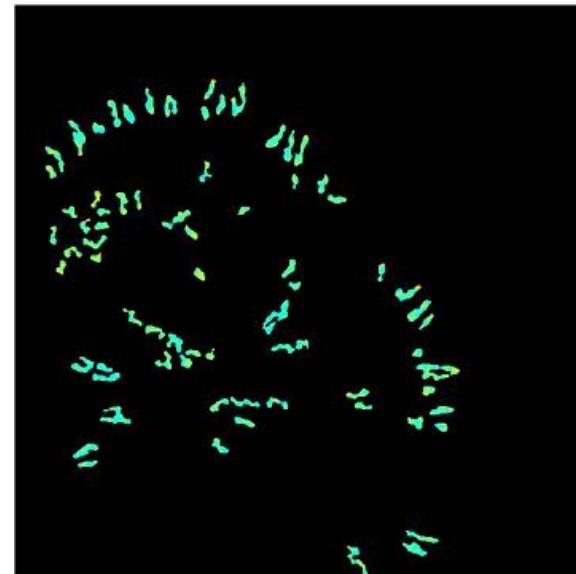
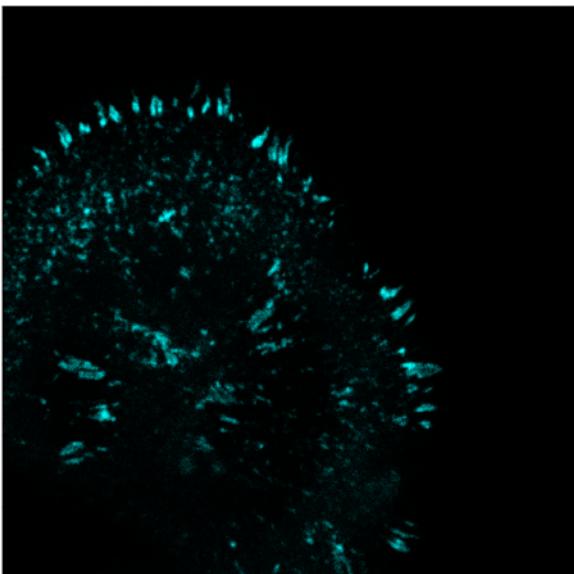
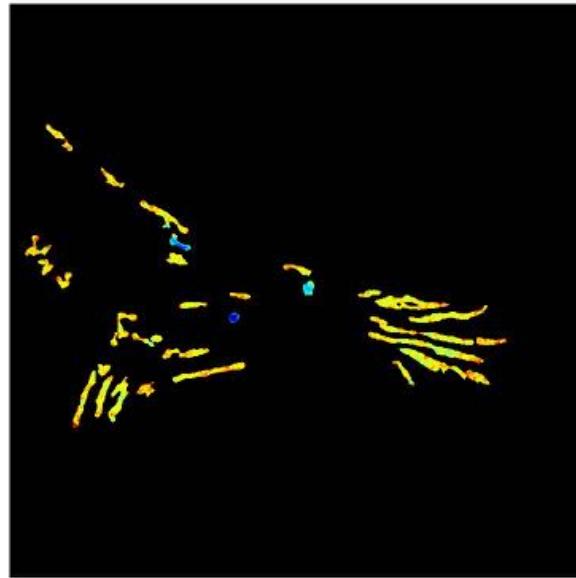
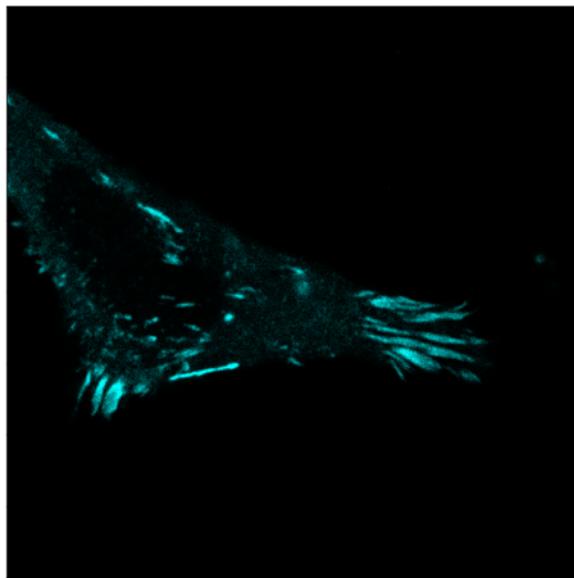
# Cellular Focal Adhesions



# Cellular Focal Adhesions



# FRFT-based force sensor



Vinculin



Vinculin TS



Vinculin TL



# Acknowledgments

## Molecular Imaging and Photonics

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Dr. Rafael Camacho

Drs. Quinten Coucke (FRET sensors)

Drs. Indra van Zundert

Dr. Beatrice Fortuni

Drs. Monica Ricci

Prof. Dr. Johan Hofkens

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