

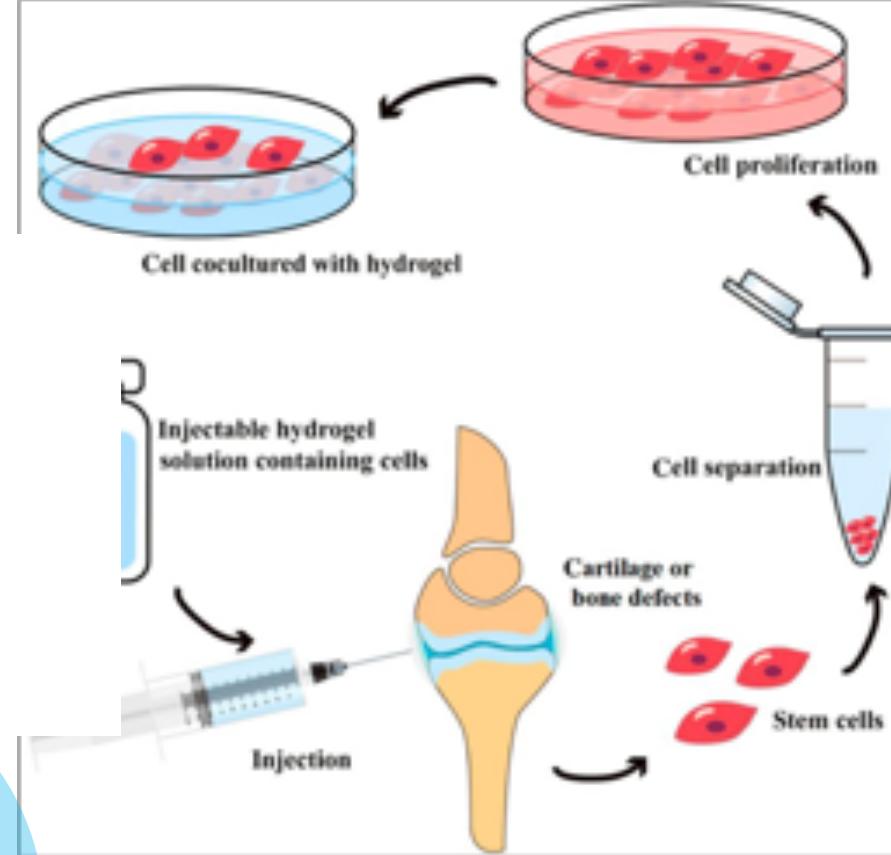
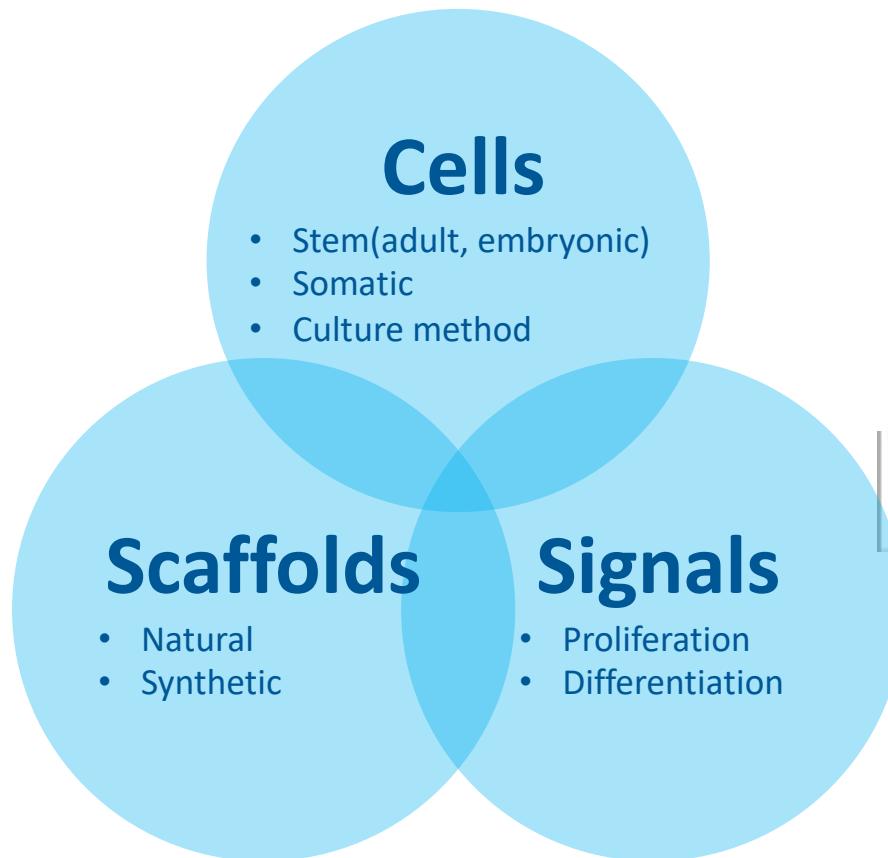


Microscopy toolbox for structural and mechanical characterisation of new biomimetic materials

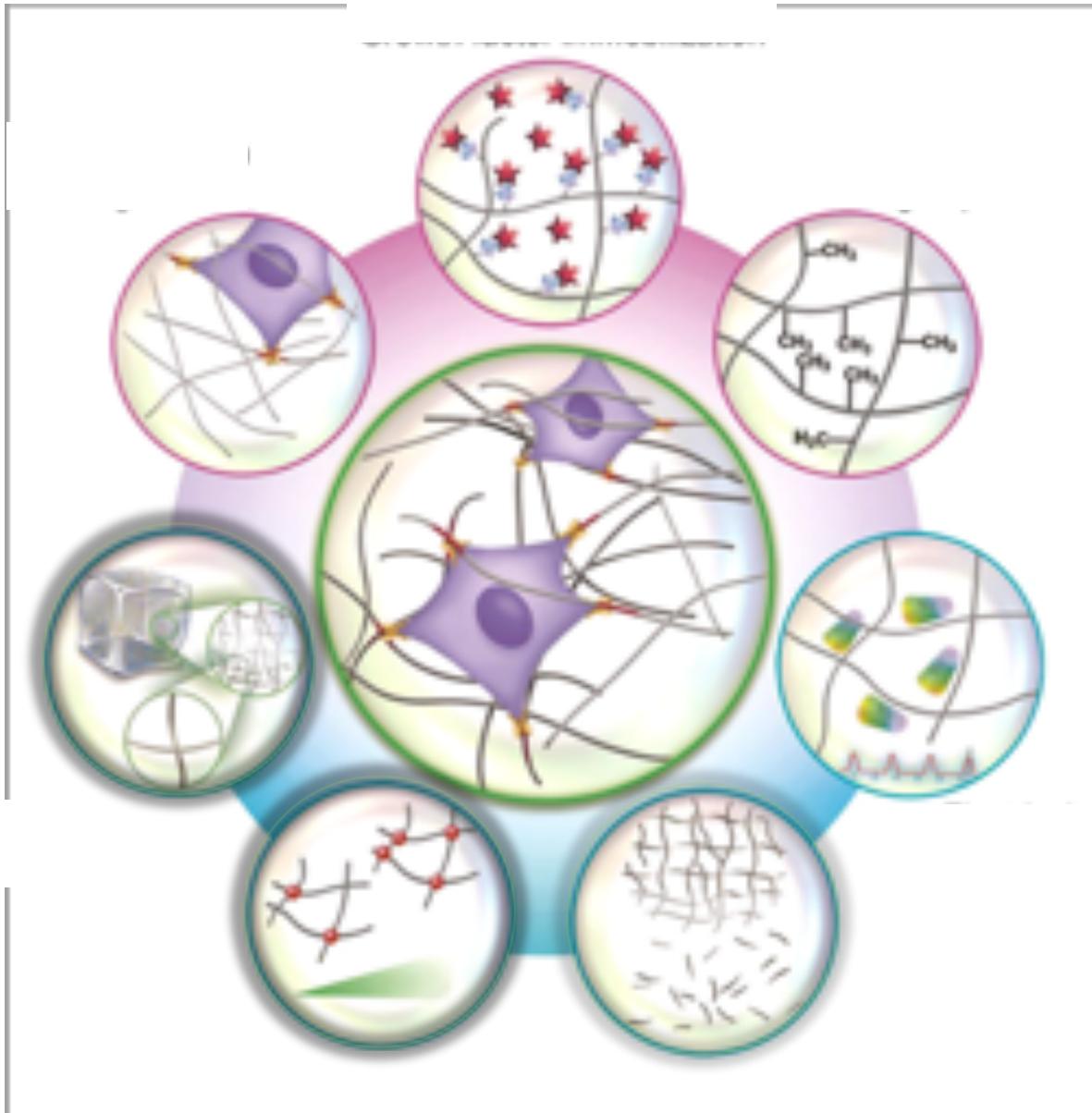
Susana Rocha
post-doctoral researcher
@ KU Leuven (BE)

Molecular biophysics goes chemistry, KULeuven
5th July 2019

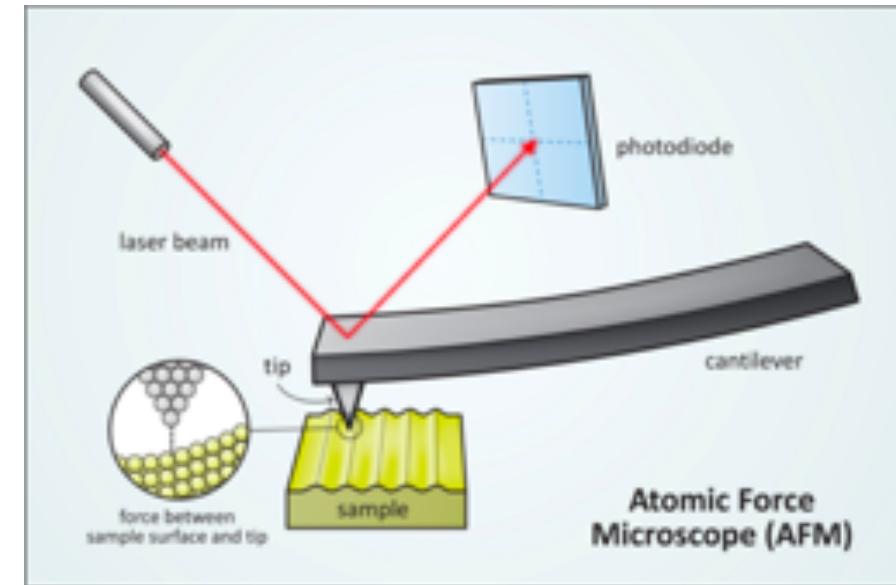
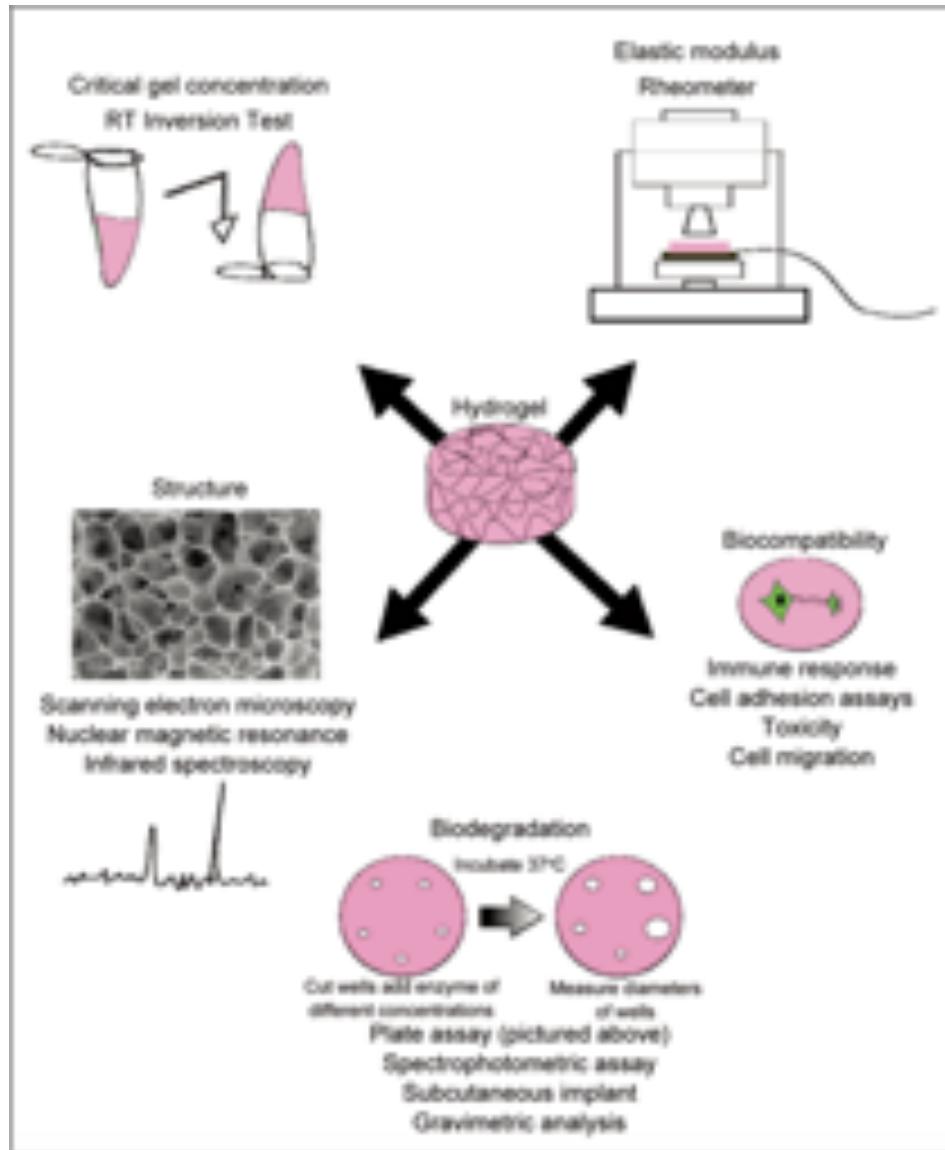
Tissue engineering and 3D cell culture



Design considerations for Biomimetic materials



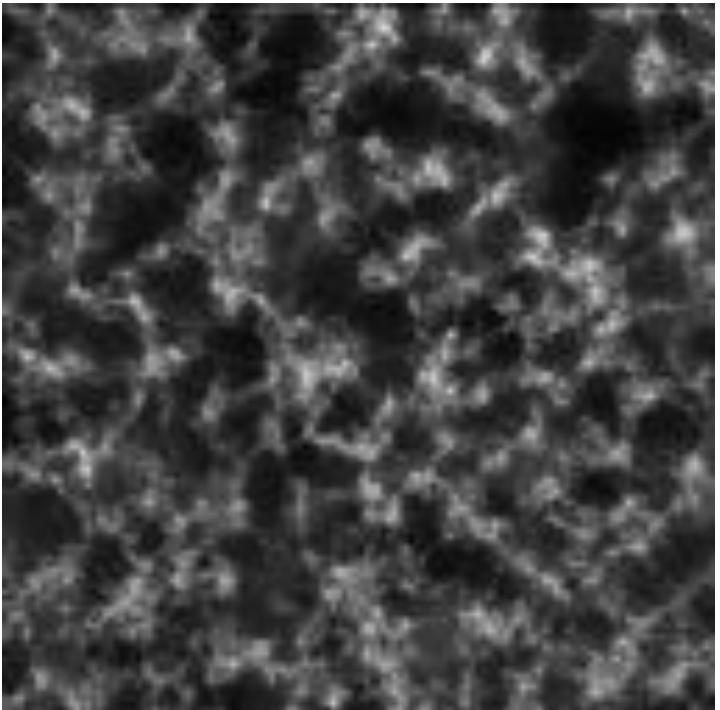
Techniques for characterization of hydrogels



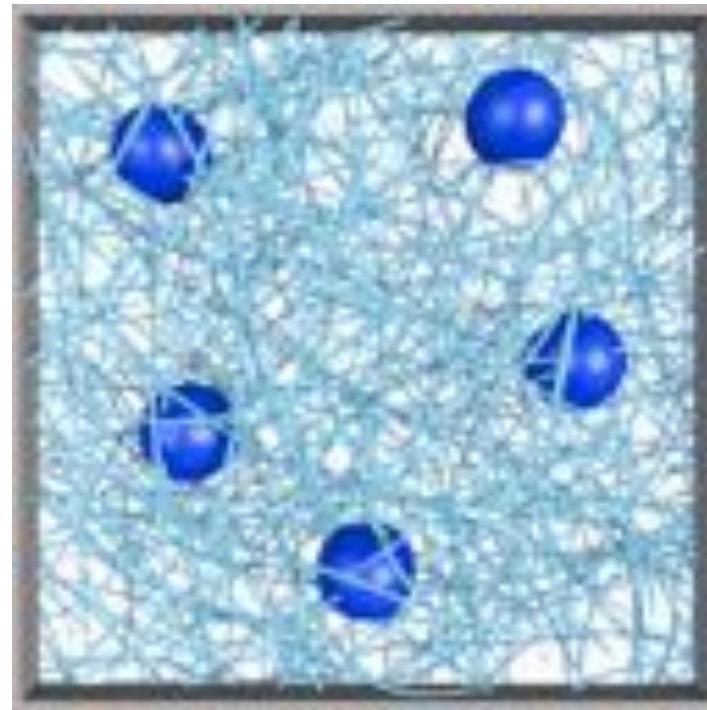
Lack of technologies that can investigate the dynamics of the mechanical and structural properties of biomaterials *in situ*, at the subcellular and molecular level

Material Characterization

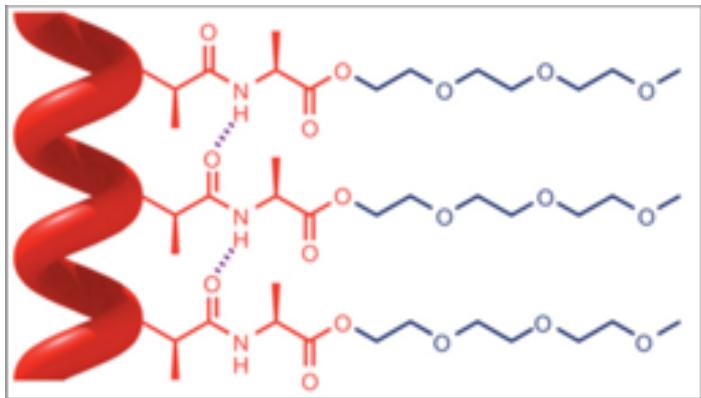
Structure using advanced fluorescence microscopy



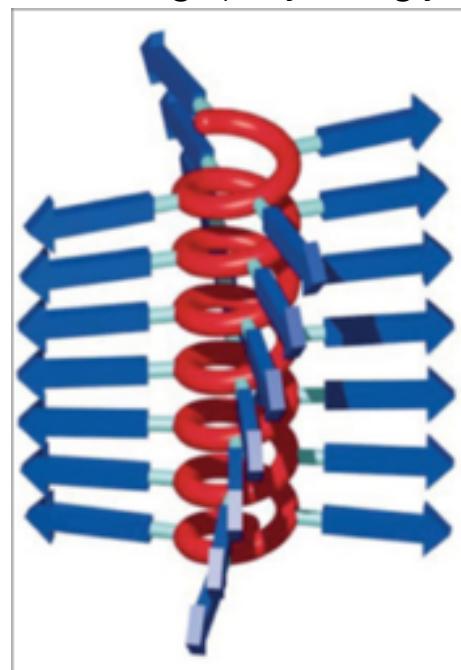
Local mechanical properties using 3D micro-rheology



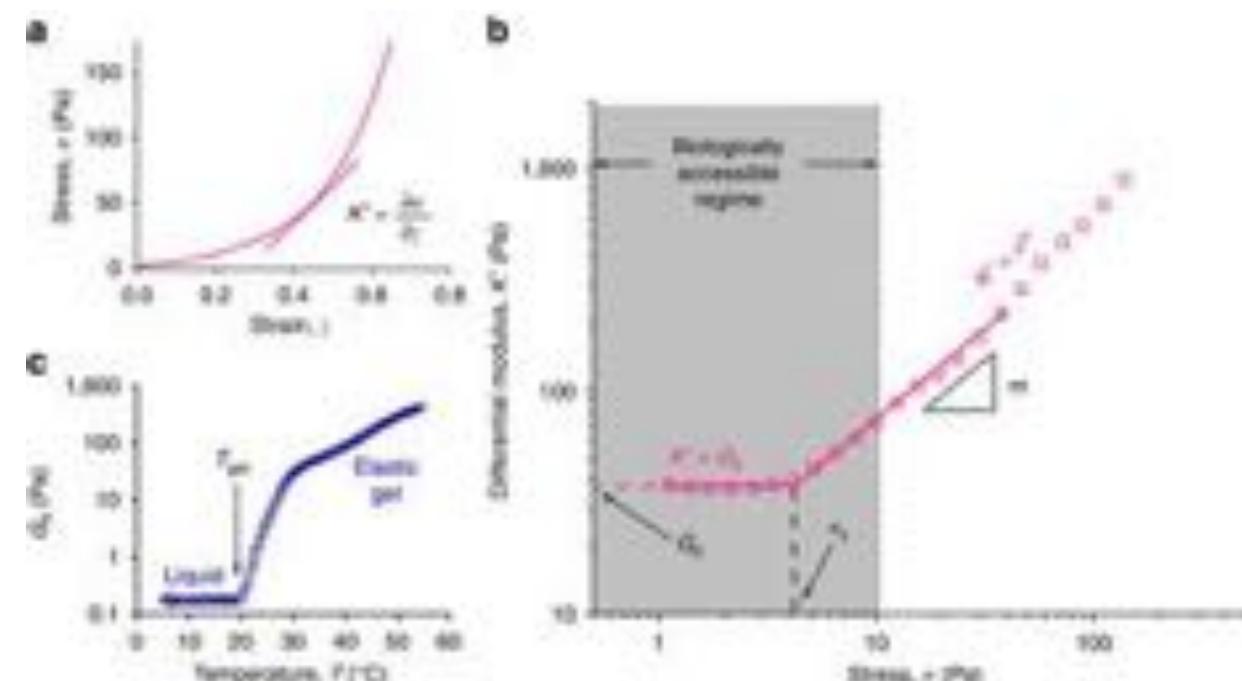
New polymer class for biomimetic 3D matrices



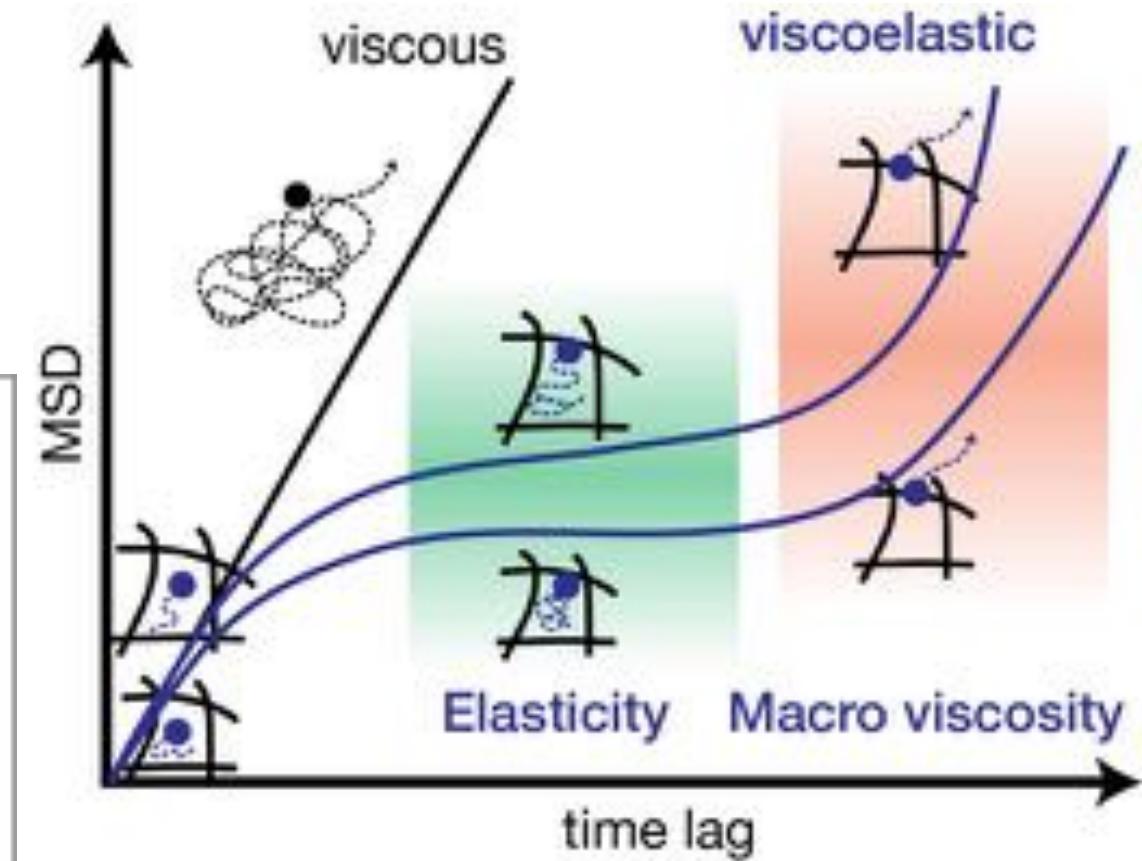
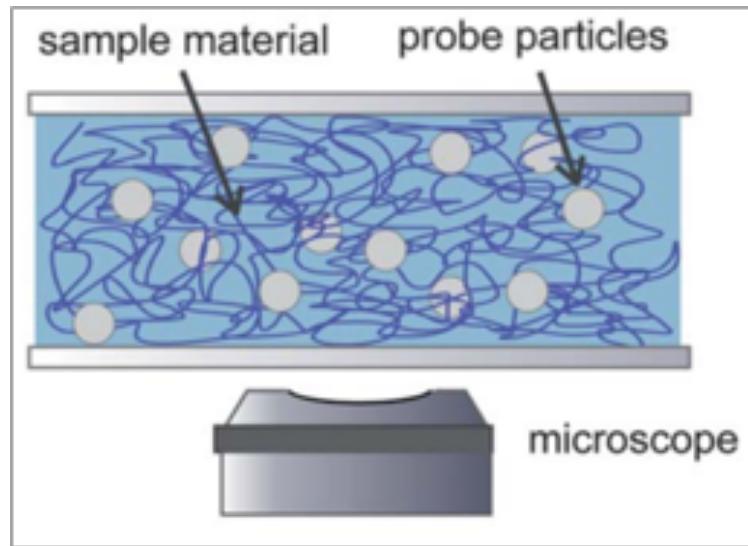
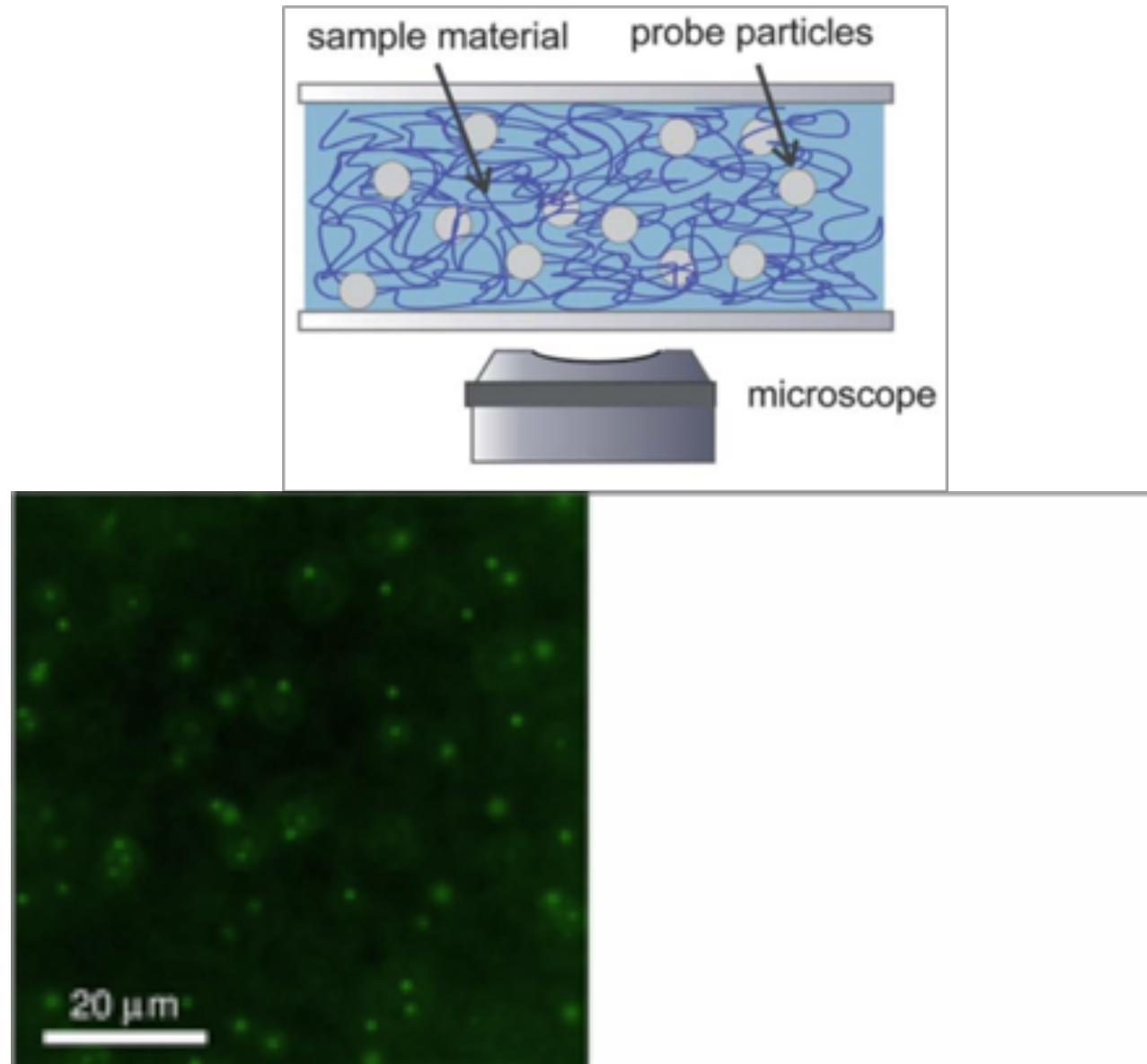
Polyisocyanopeptides (PICs)
grafted with oligo(ethylene glycol)



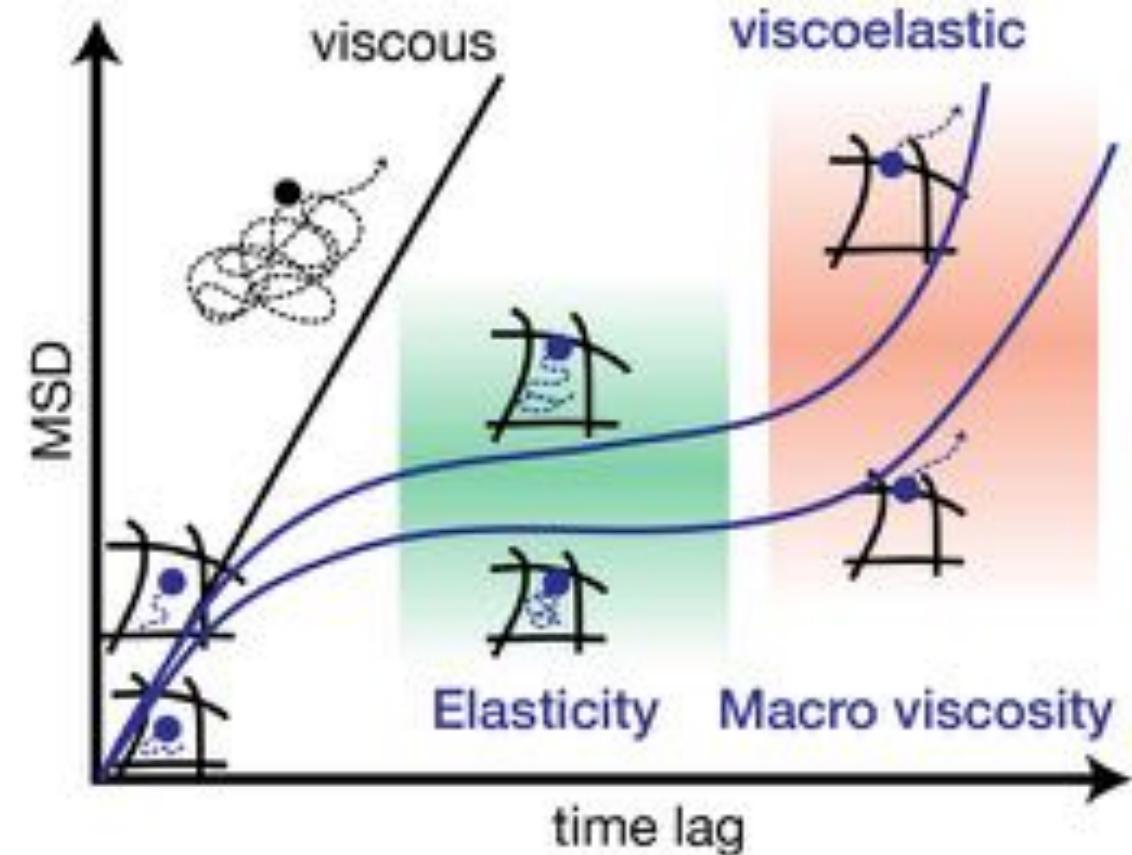
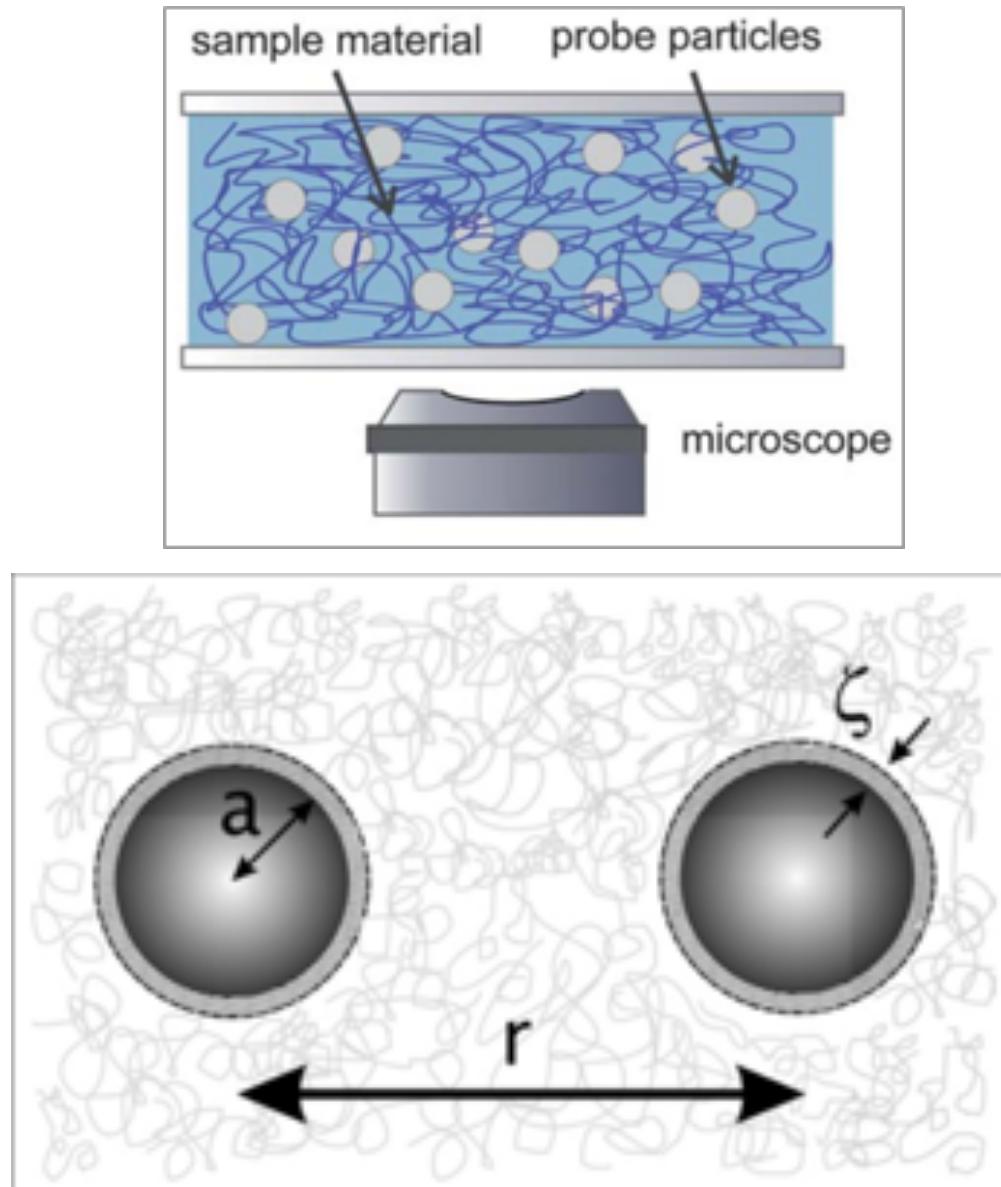
Collaboration with prof. Paul Kouwer,
Radboud University (Nijmegen, The Netherlands)



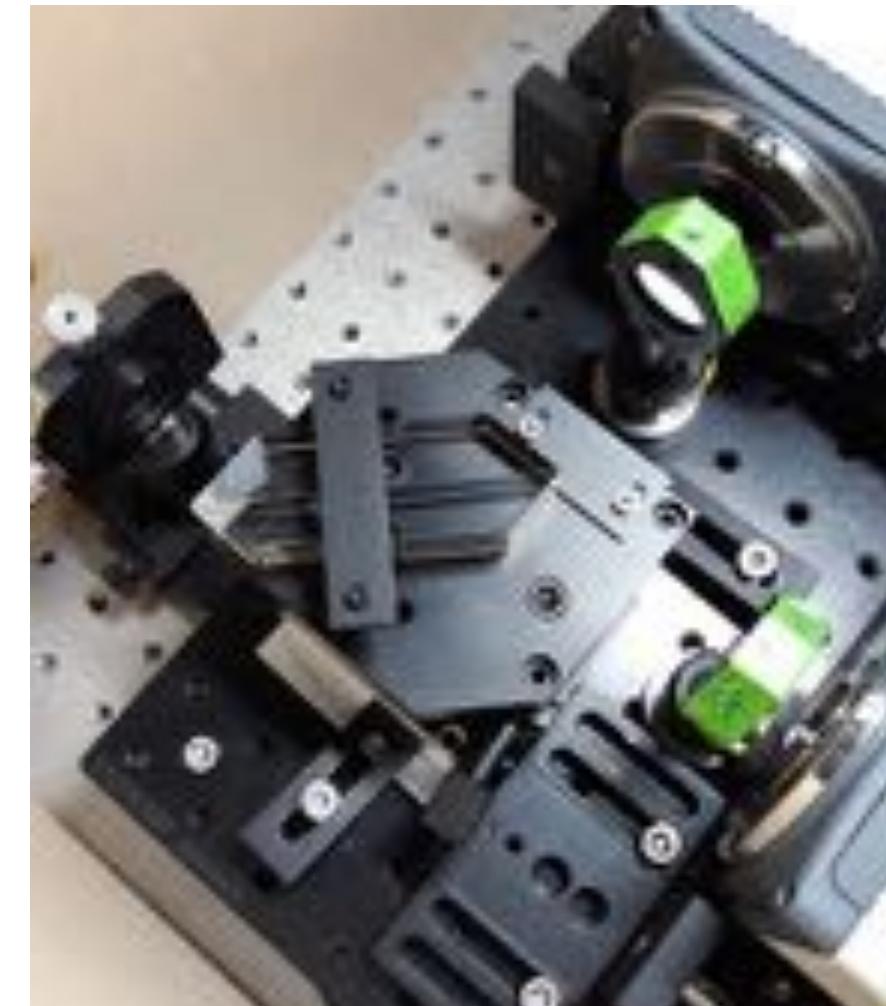
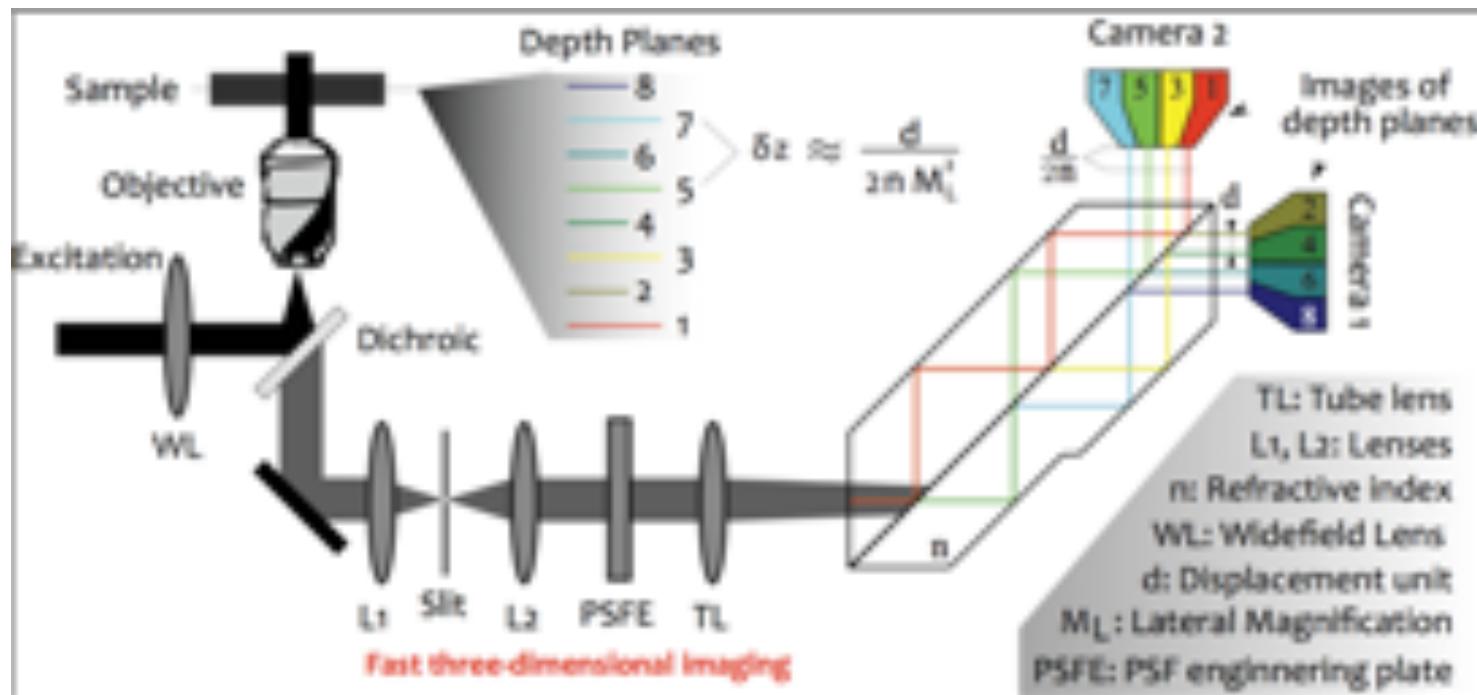
Mechanical Characterization



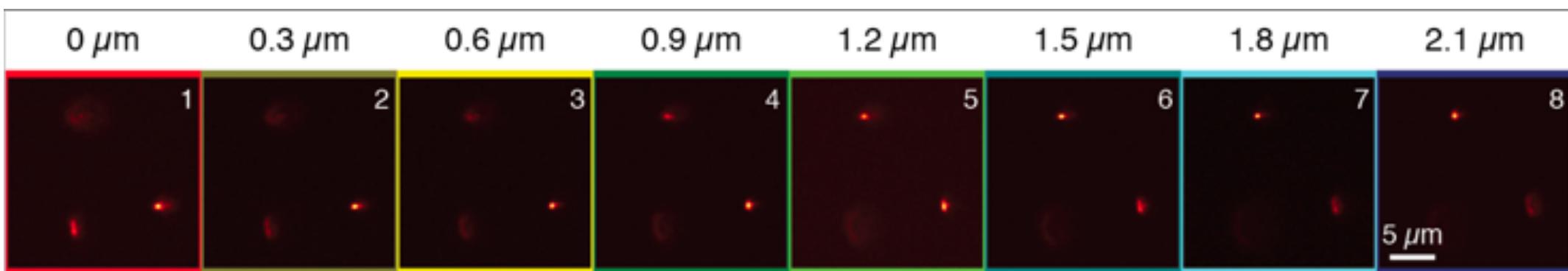
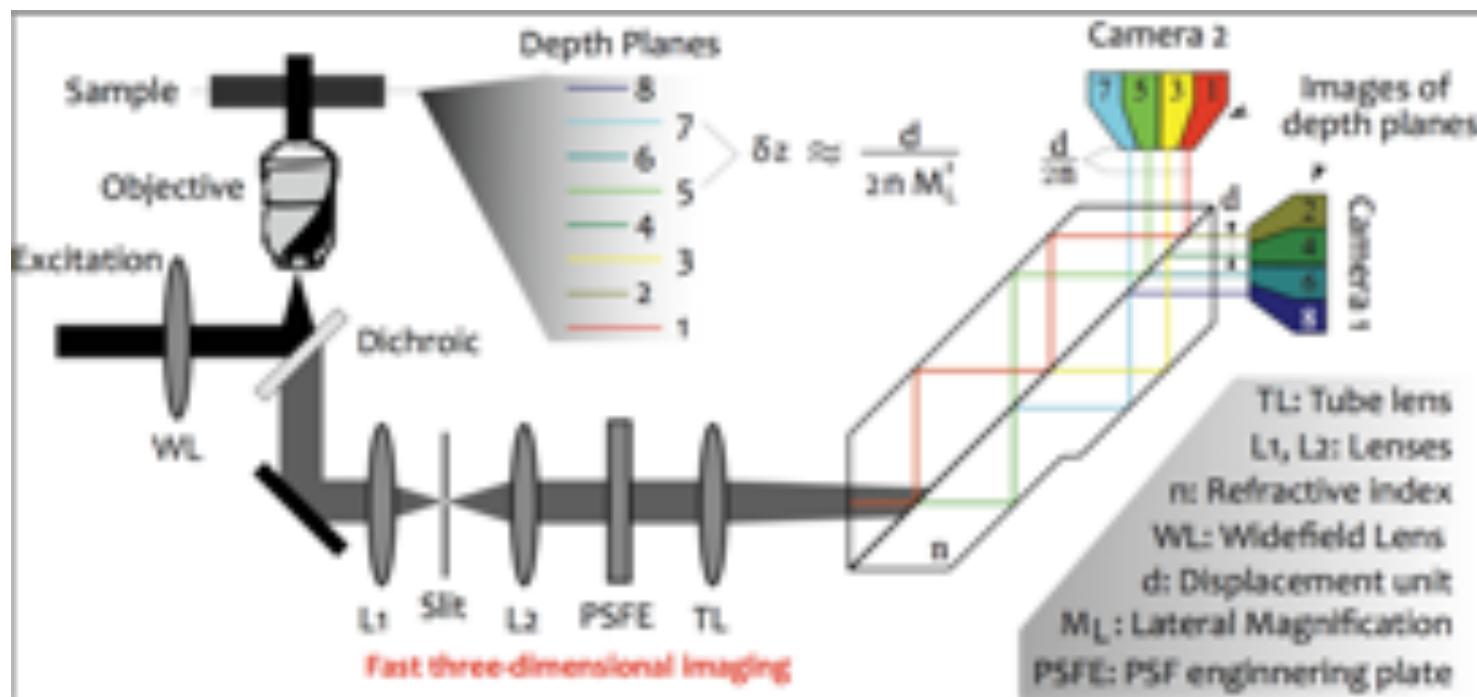
Mechanical Characterization @ the microscale



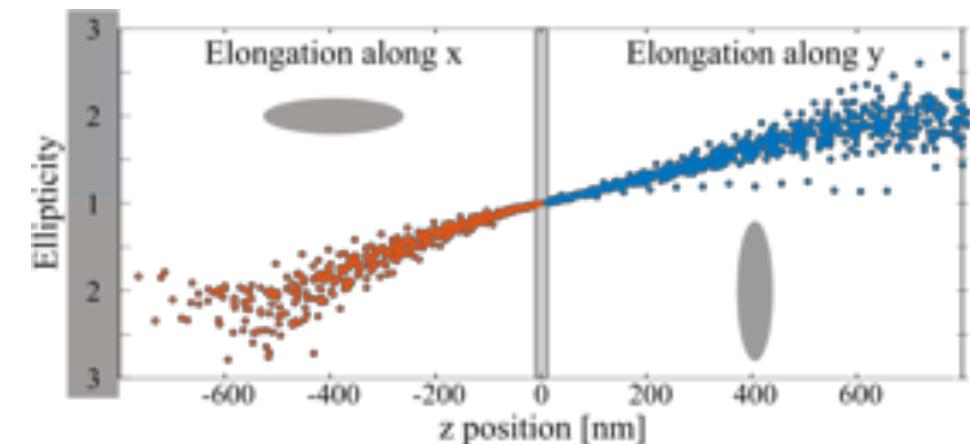
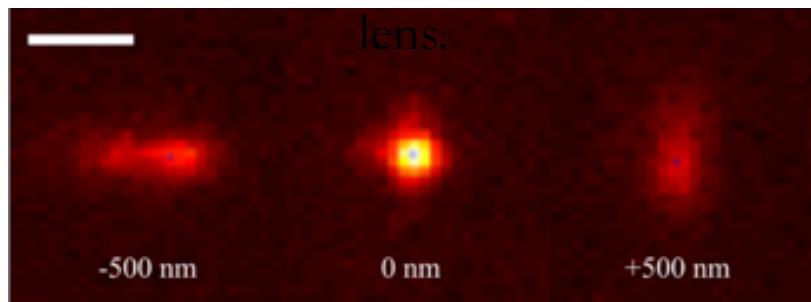
Multi-plane wide field microscope for 2-point micro-rheology



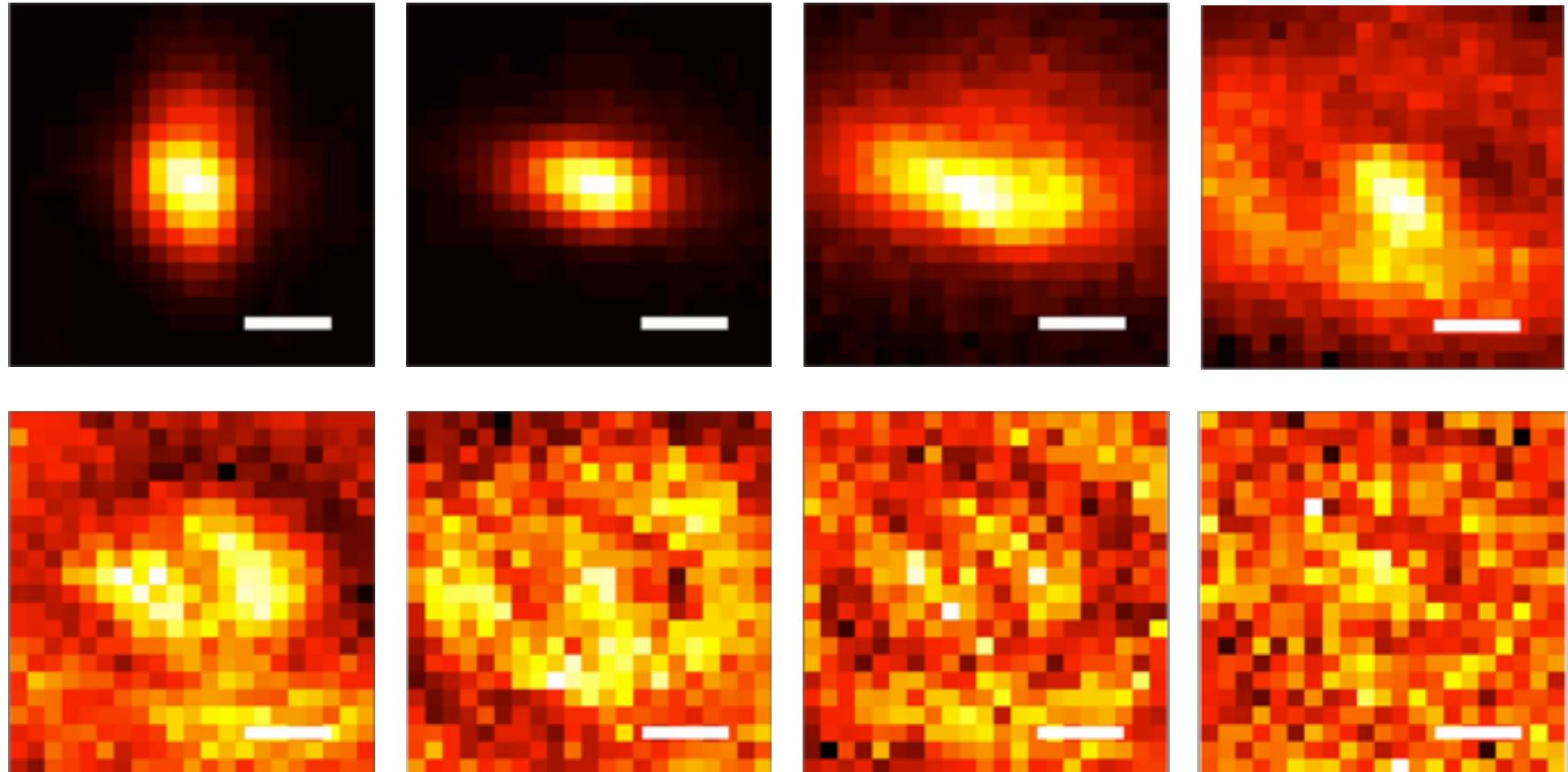
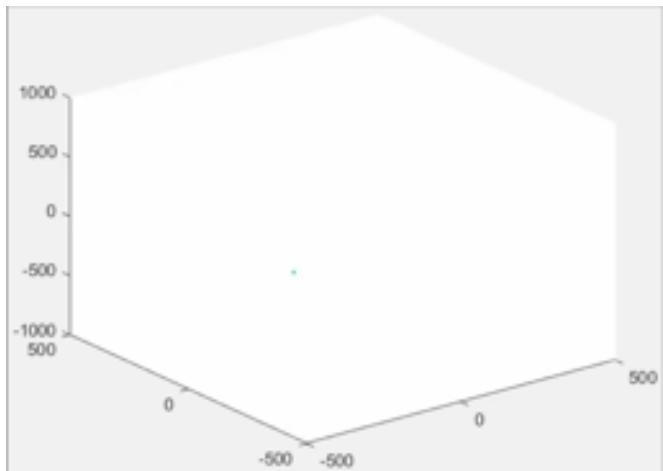
Multi-plane wide field microscope for 2-point micro-rheology



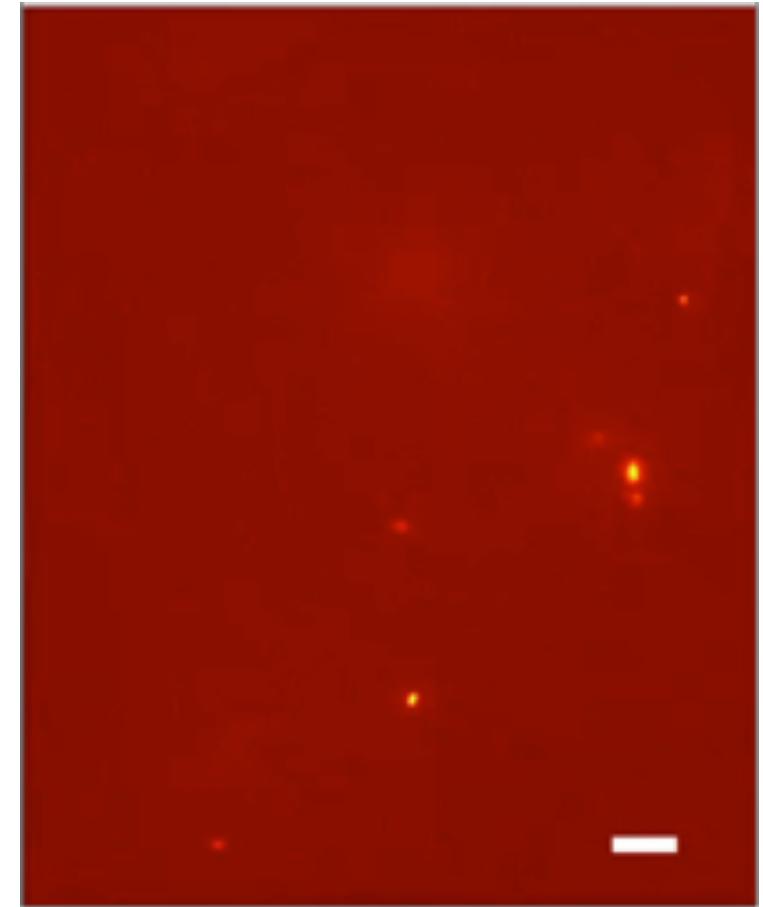
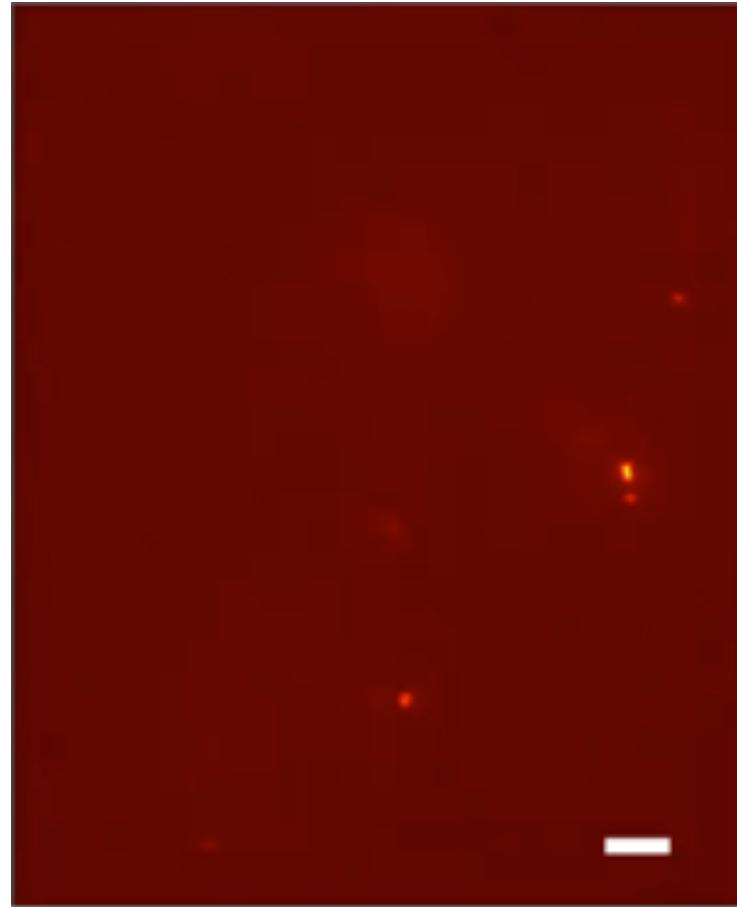
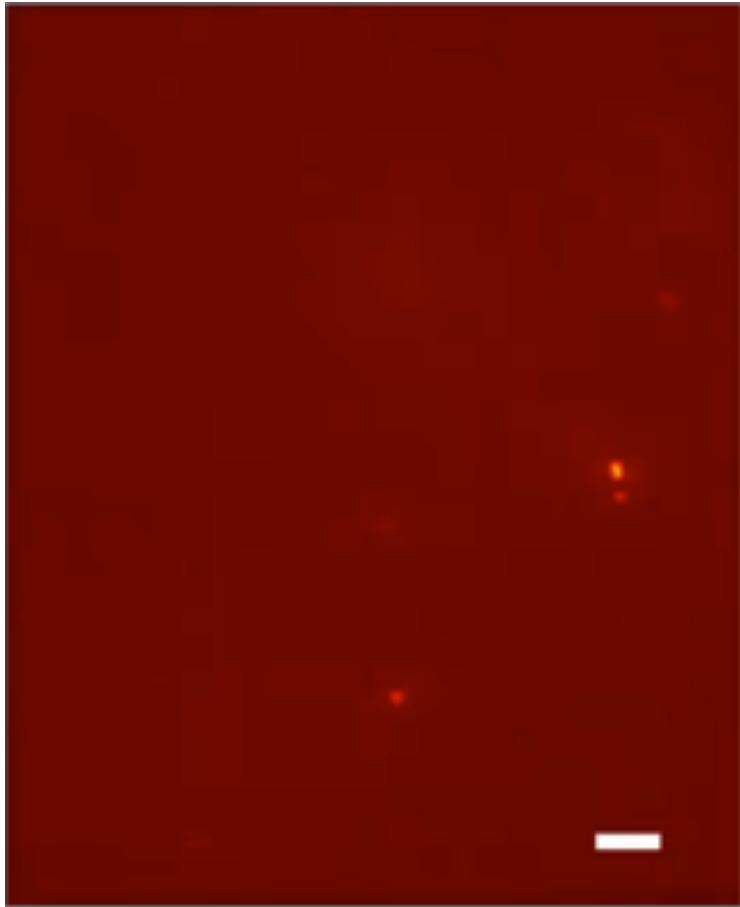
PSF engineering via cylindrical
lens.



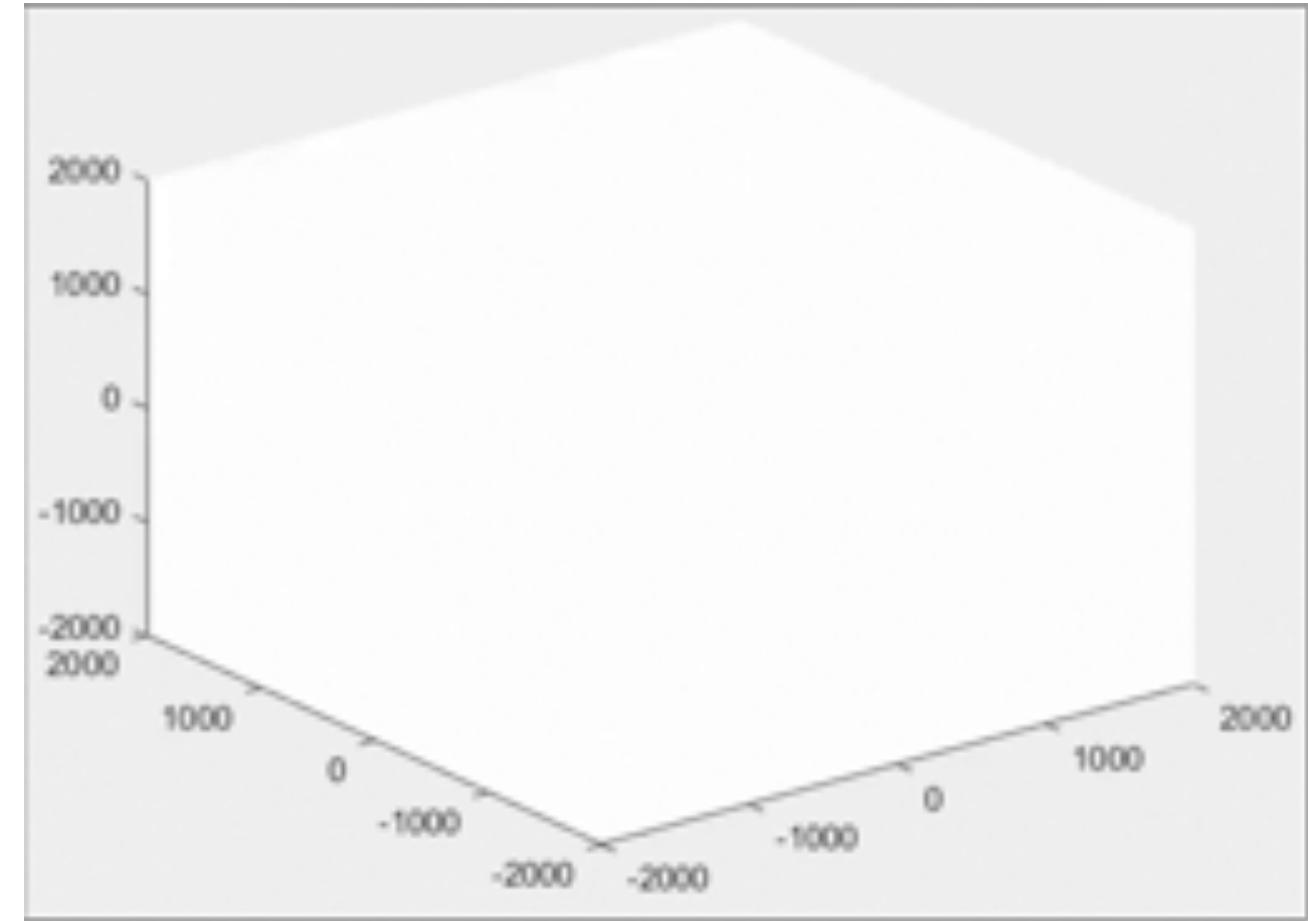
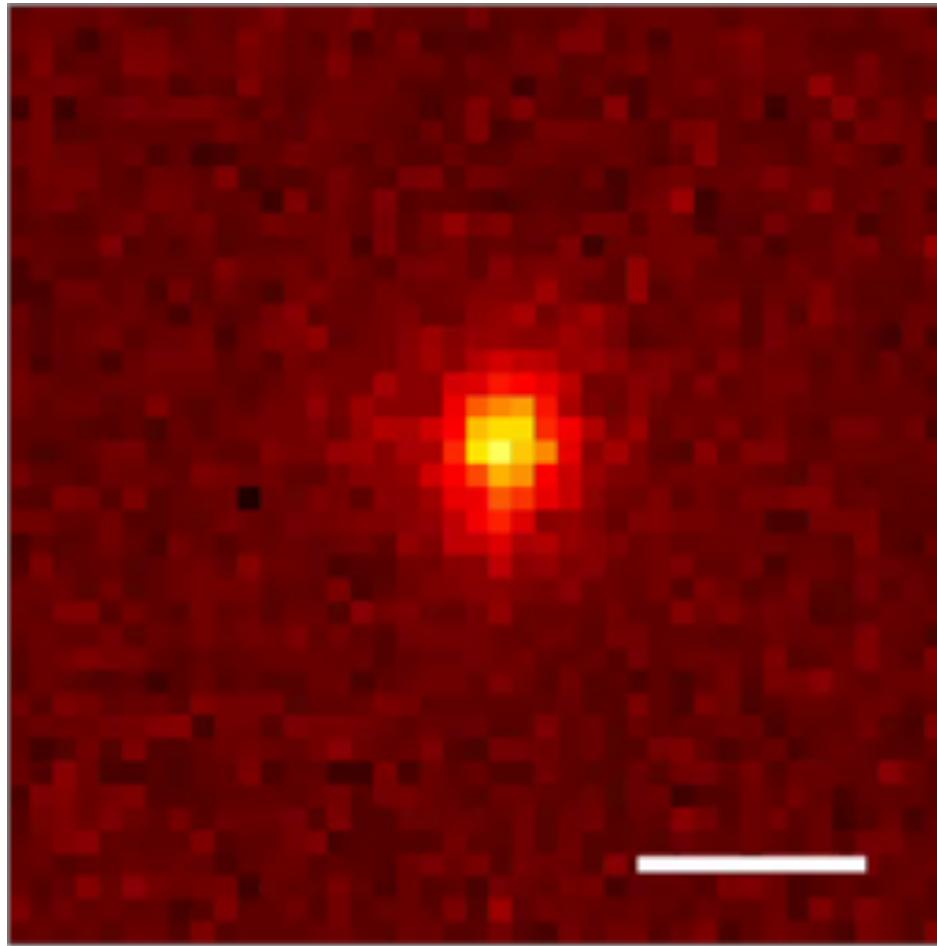
Multi-plane wide field microscope for 2-point micro-rheology



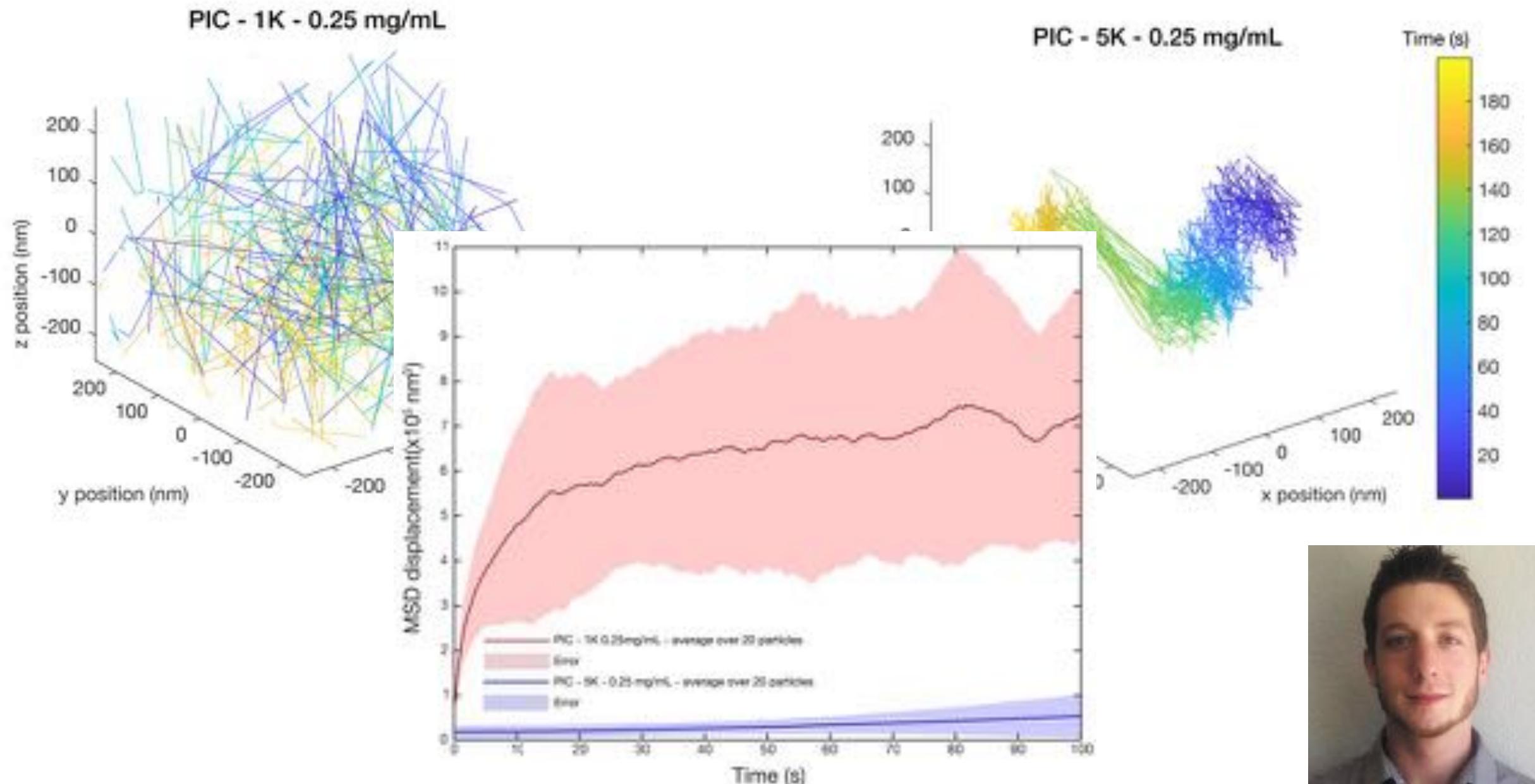
3D SPT in PIC hydrogel



3D SPT in PIC hydrogel

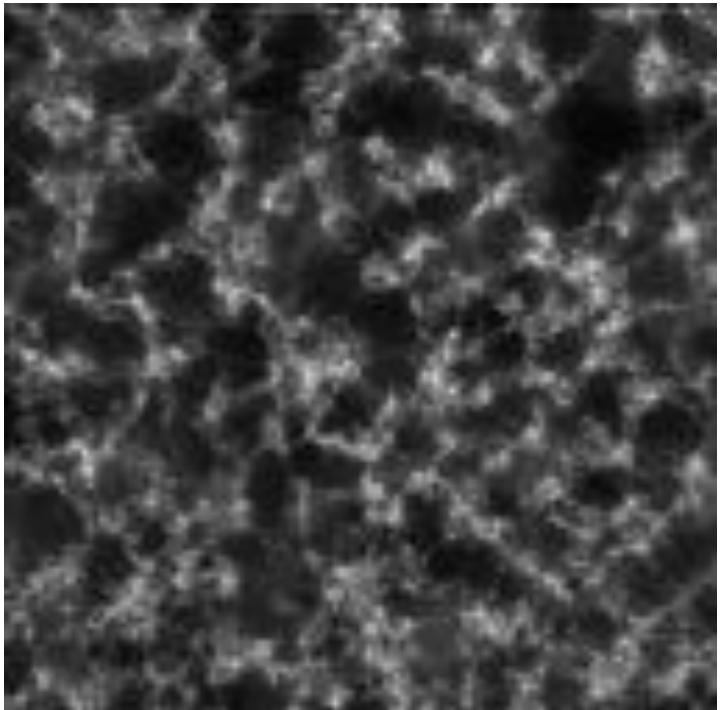


3D SPT in PIC hydrogel

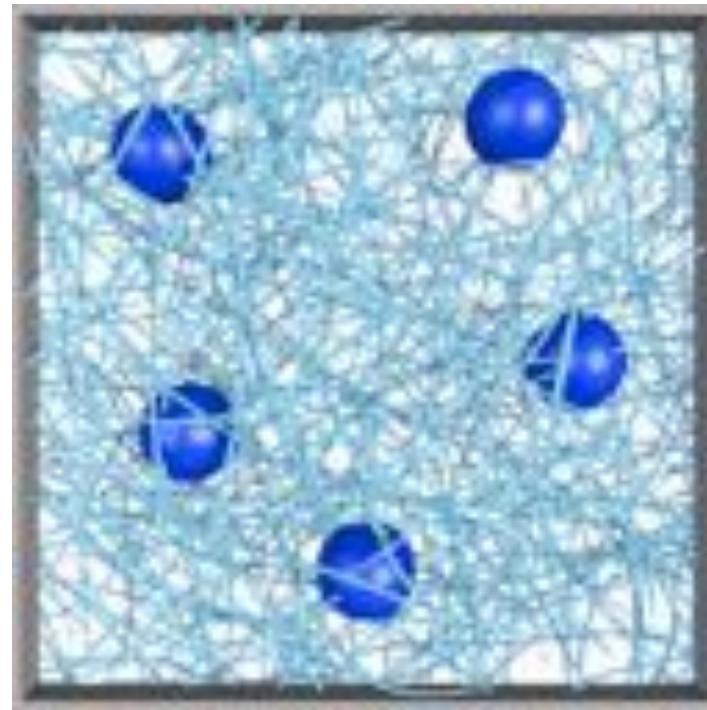


Material Characterization

Structure using advanced fluorescence microscopy

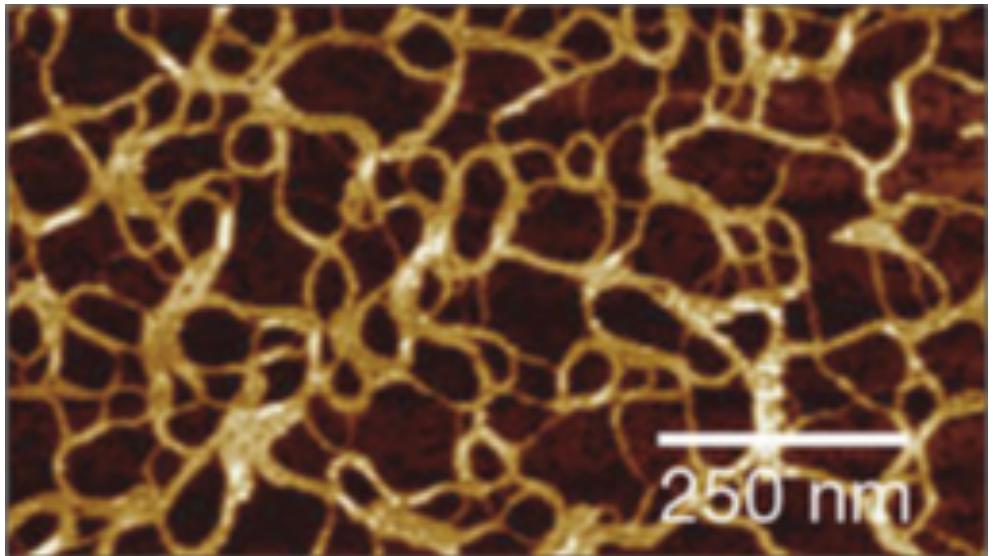


Local mechanical properties using 3D micro-rheology

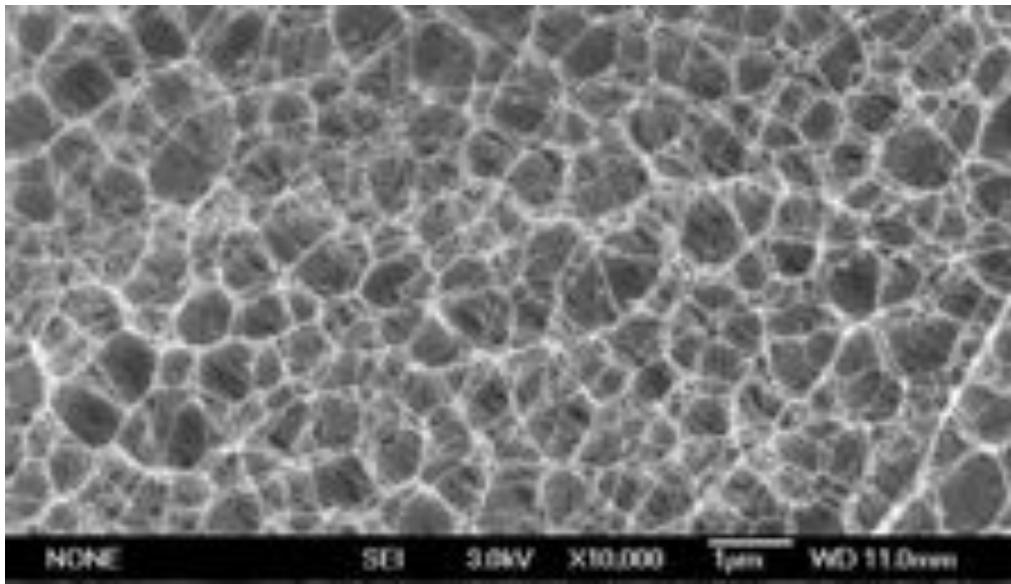


Structural Characterization of PIC-based hydrogels

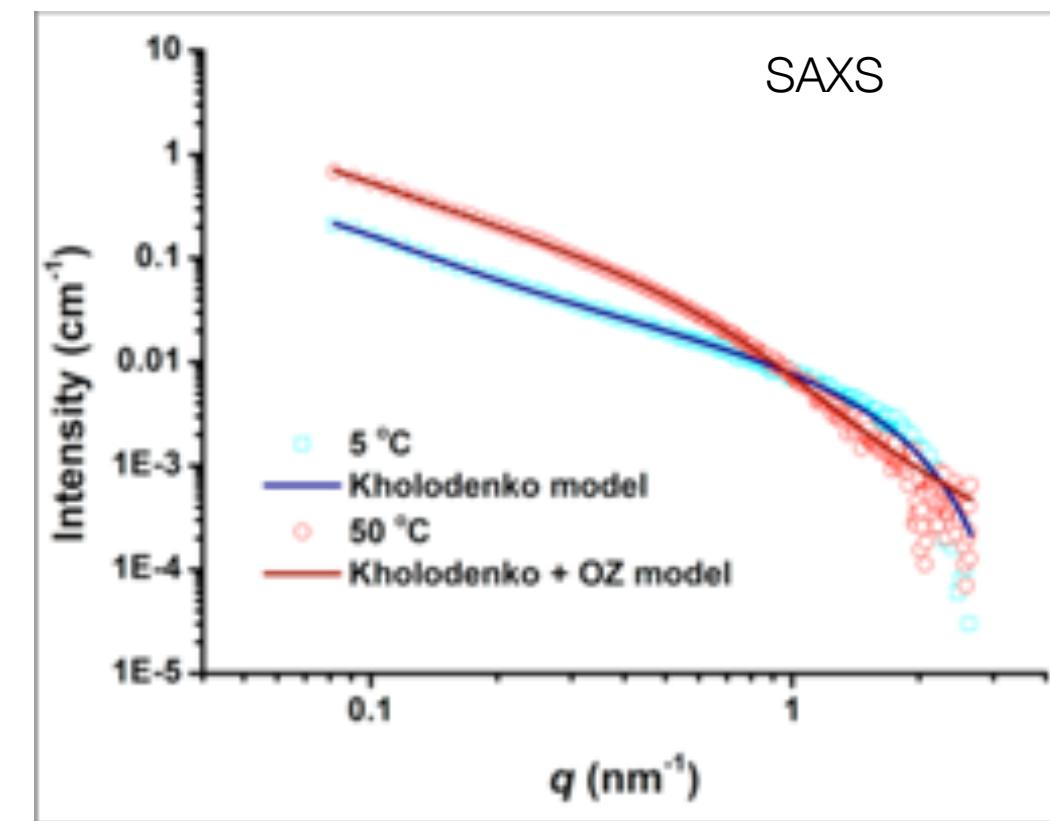
AFM



CryoEM



SAXS



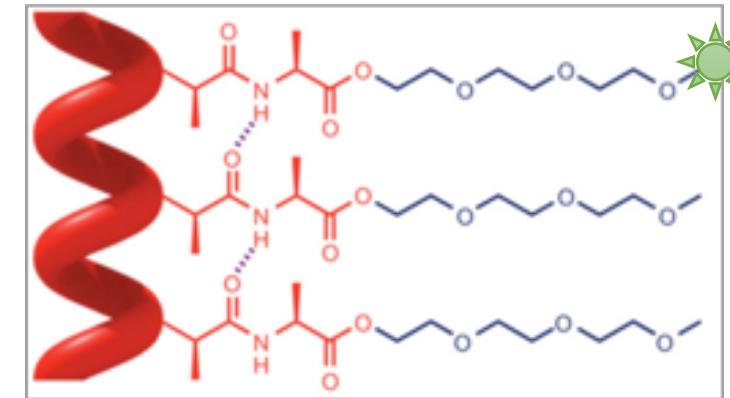
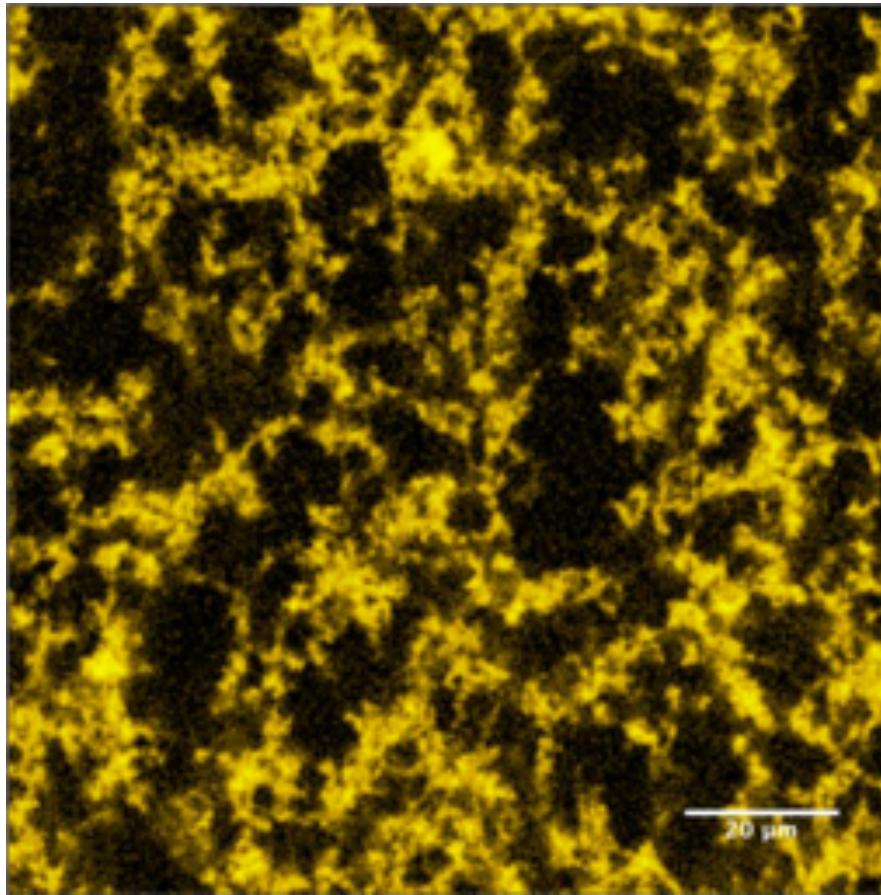
PIC	L (nm)	l_p (nm)	R (nm)	ξ_{OZ} (nm) ^b	R_B (nm)
P1a	55	13 ± 7	1.1 ± 0.1	$>80^{\text{c}}$	5.0 ± 0.2
P1b	77	10 ± 1	1.1 ± 0.1	$>80^{\text{c}}$	4.7 ± 0.2
P1c	110	9 ± 2	1.2 ± 0.1	$>80^{\text{c}}$	3.3 ± 0.1
P1b	134	9 ± 1	1.1 ± 0.1	68	3.0 ± 0.1
P1e	160	12 ± 2	1.1 ± 0.1	30	3.0 ± 0.2

Nature, 493, 651-655 (2014), doi: 10.1038/nature11839

Nature Communications, 5, 5808 (2014), doi: 10.1038/ncomms6808

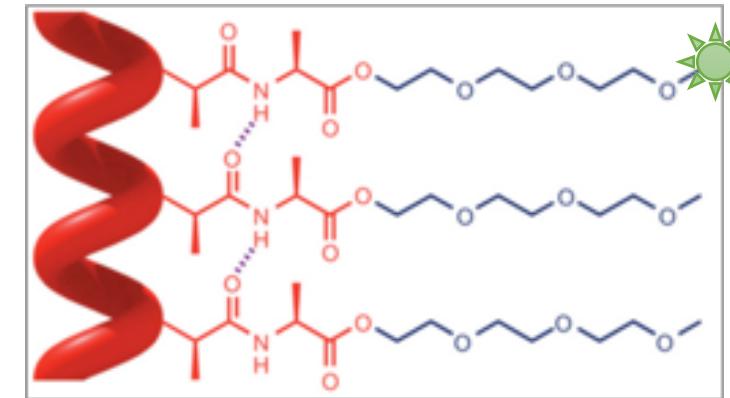
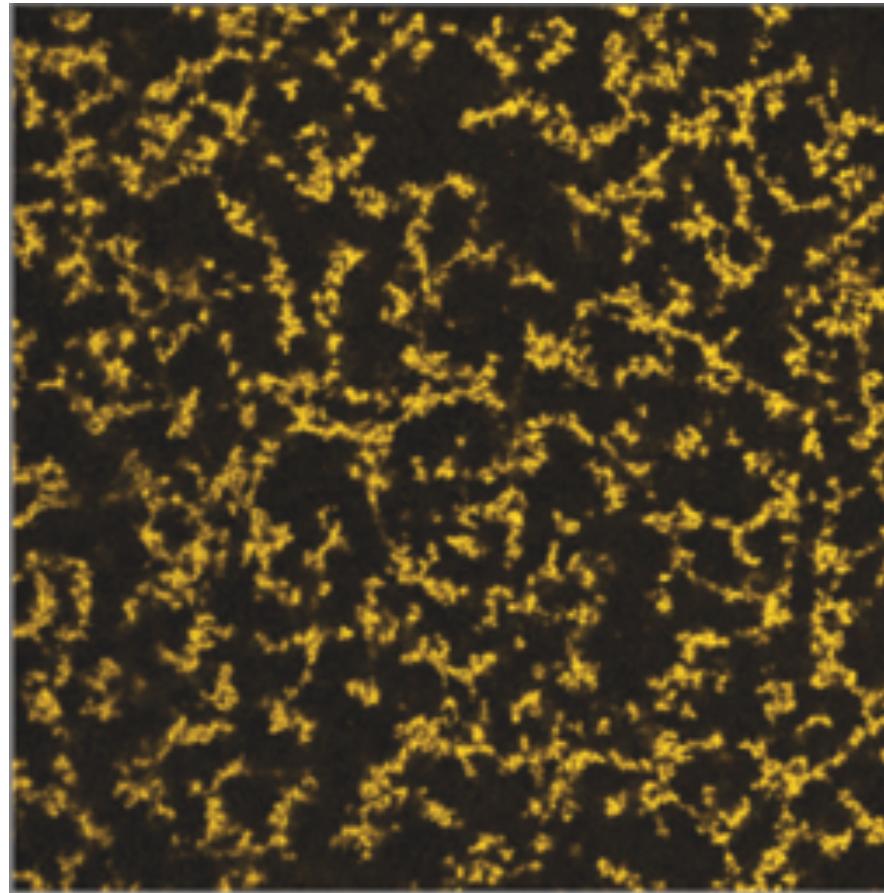
Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy



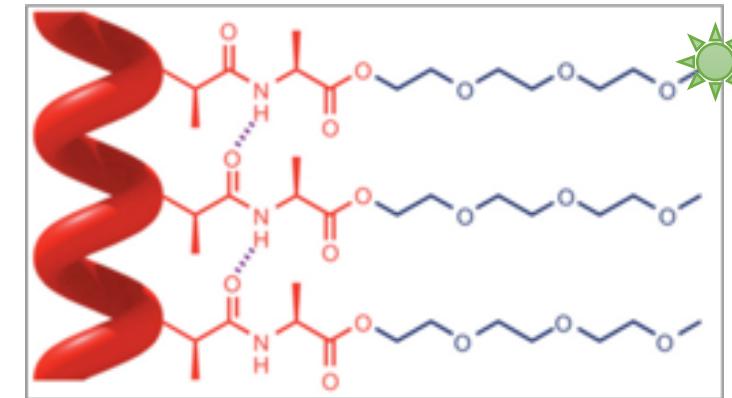
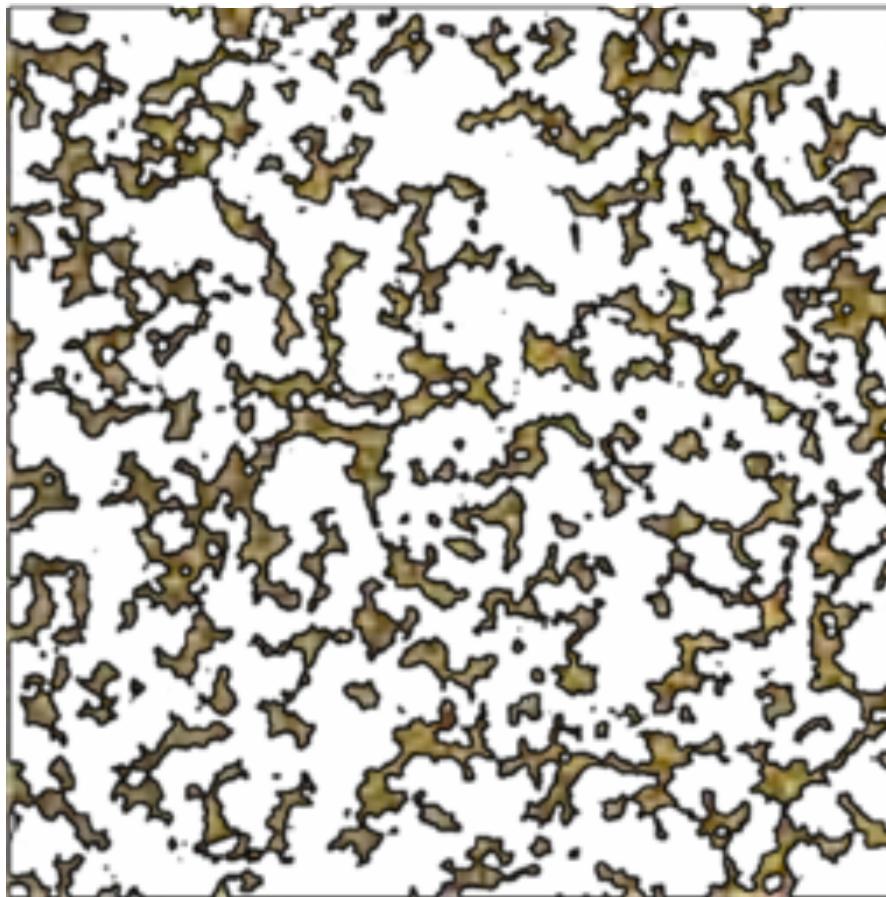
Structural Characterization of PIC-based hydrogels

Fluorescence Microscopy

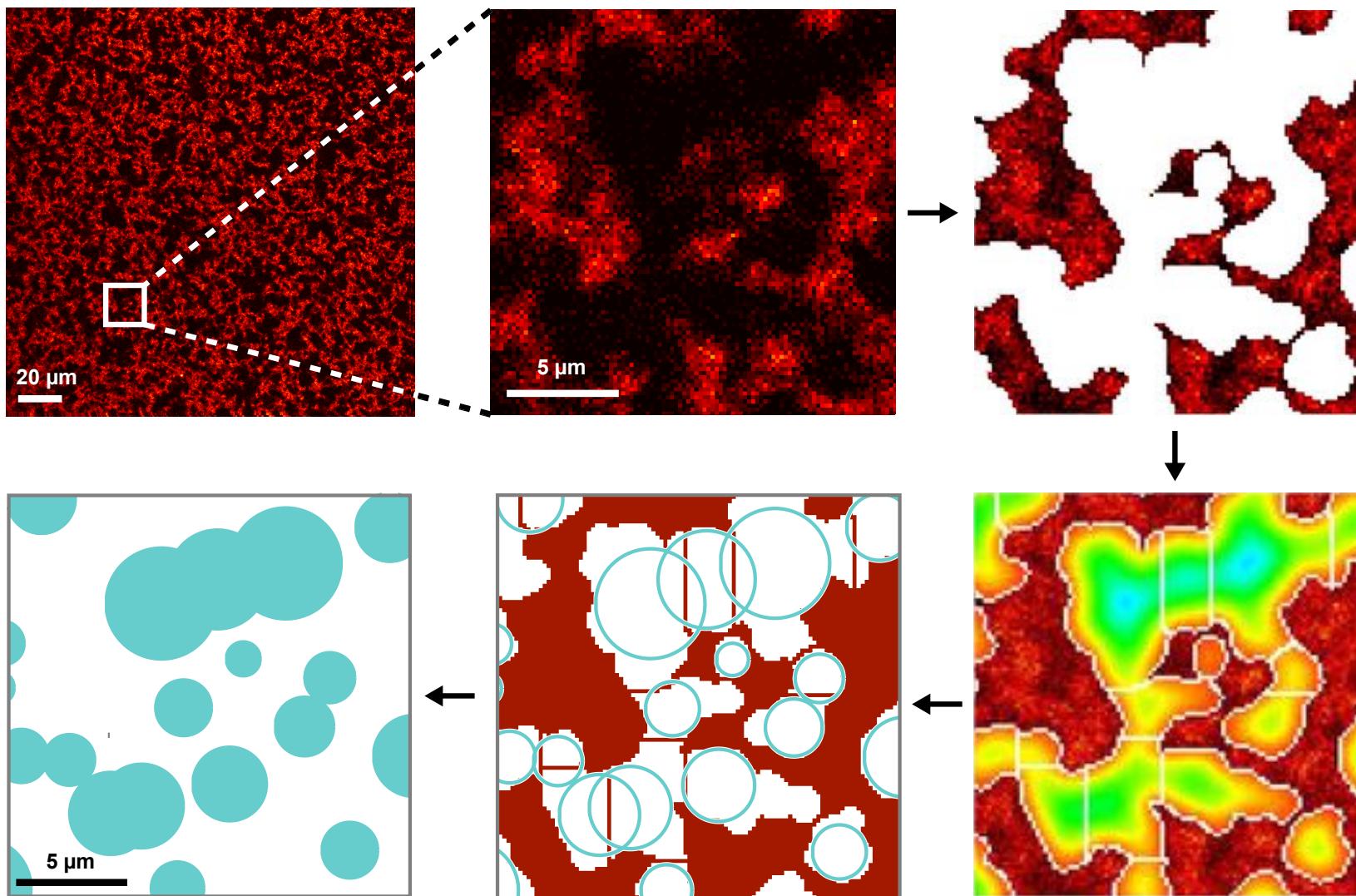


Structural Characterization of PIC-based hydrogels

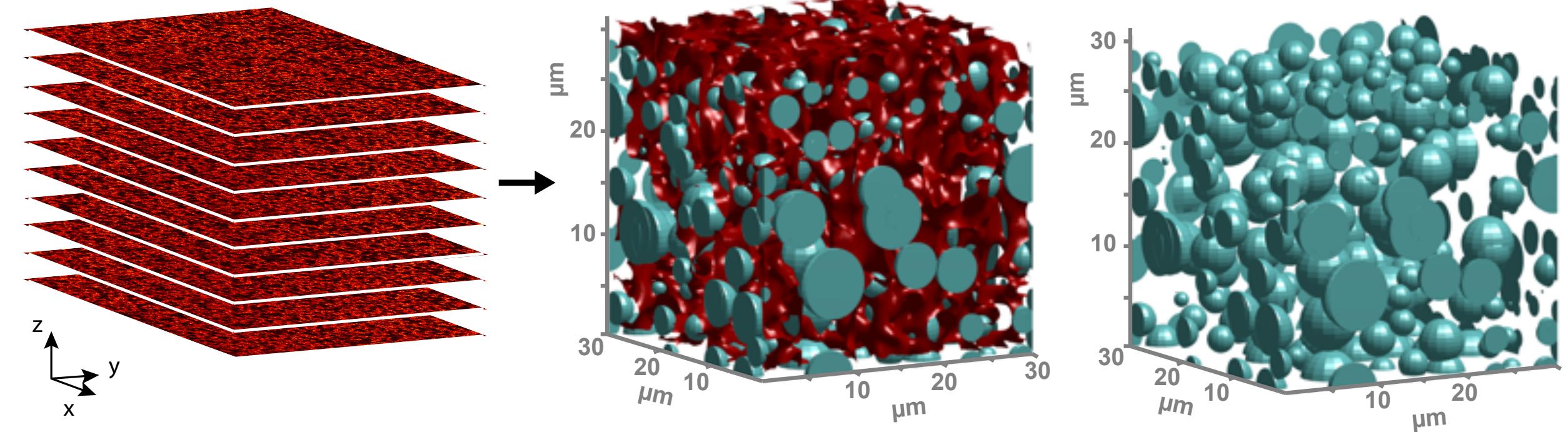
Fluorescence Microscopy



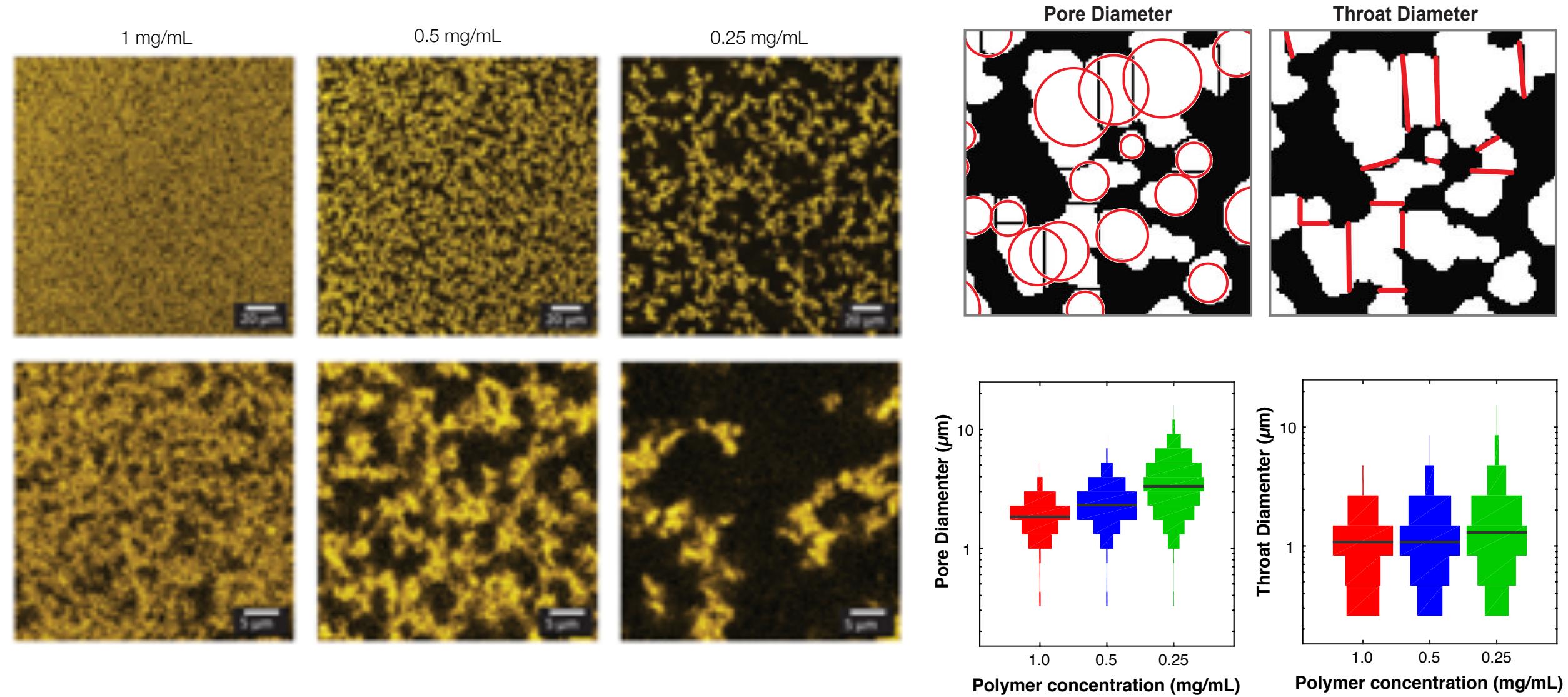
Structural Characterization of PIC-based hydrogels



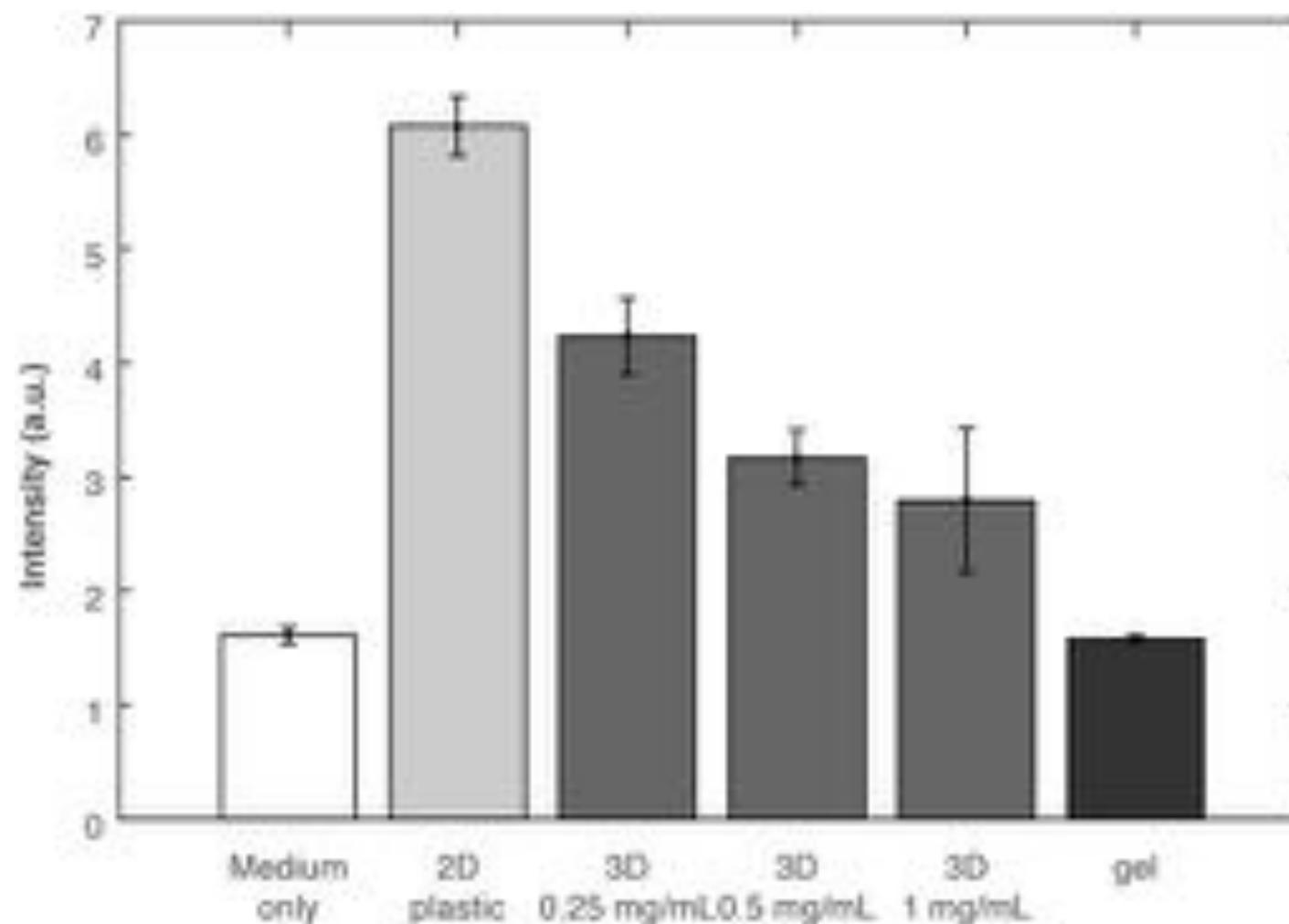
Structural Characterization of PIC-based hydrogels in 3D



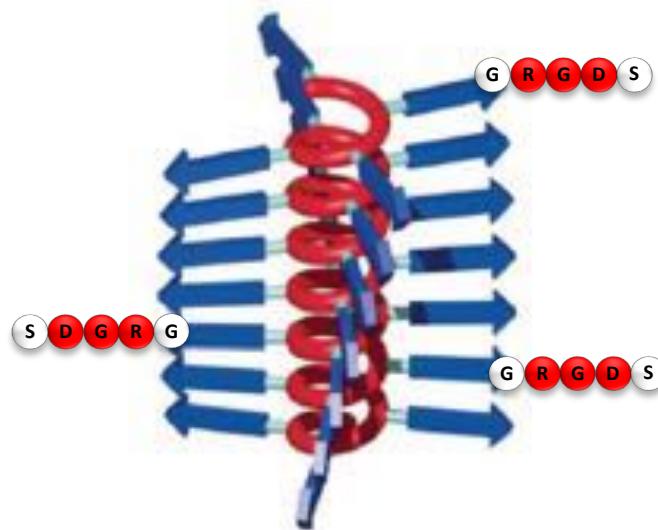
Structural Characterization of PIC-based hydrogels



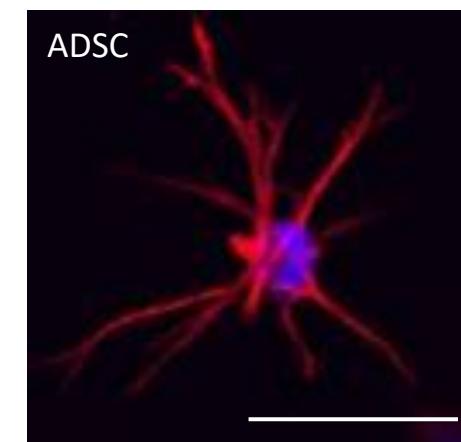
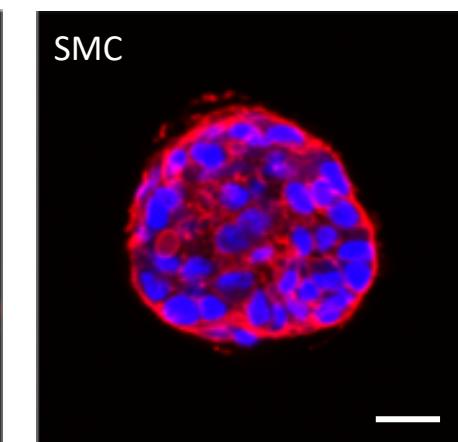
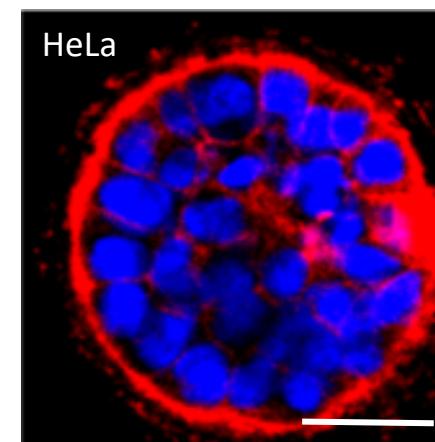
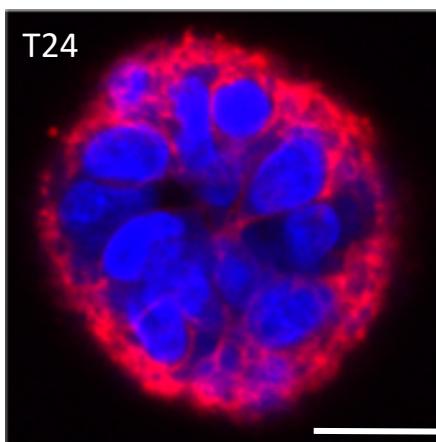
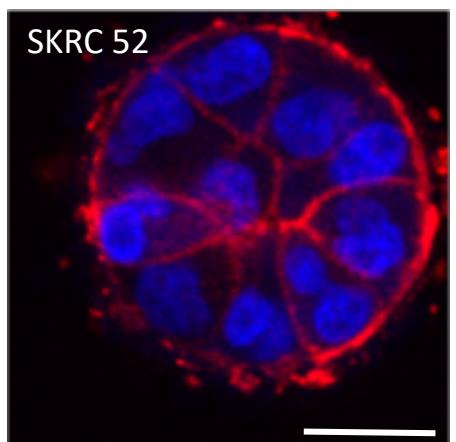
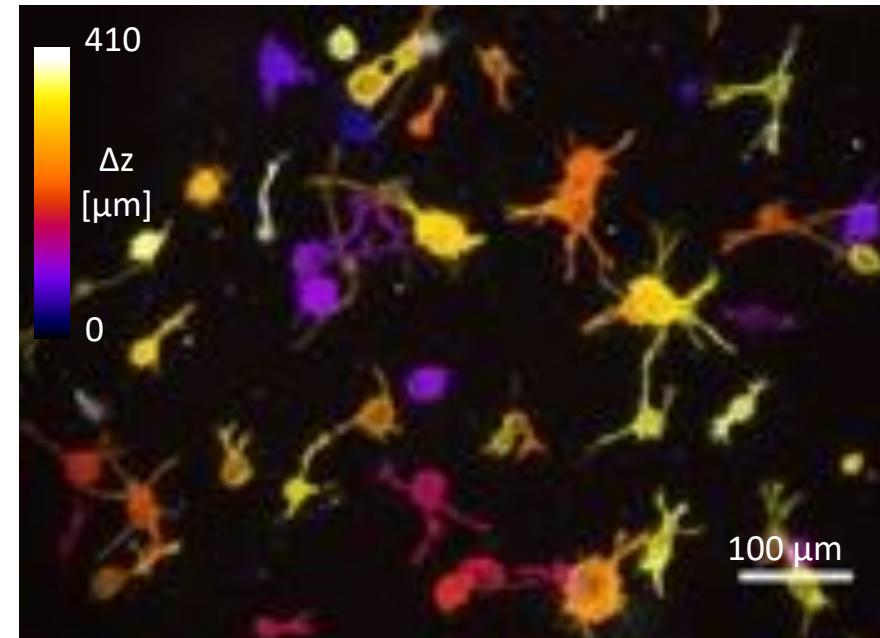
Hydrogel structure and cell proliferation



Minimalist matrix: Polyisocyanopeptides (PIC)



Different cell types → Different behavior

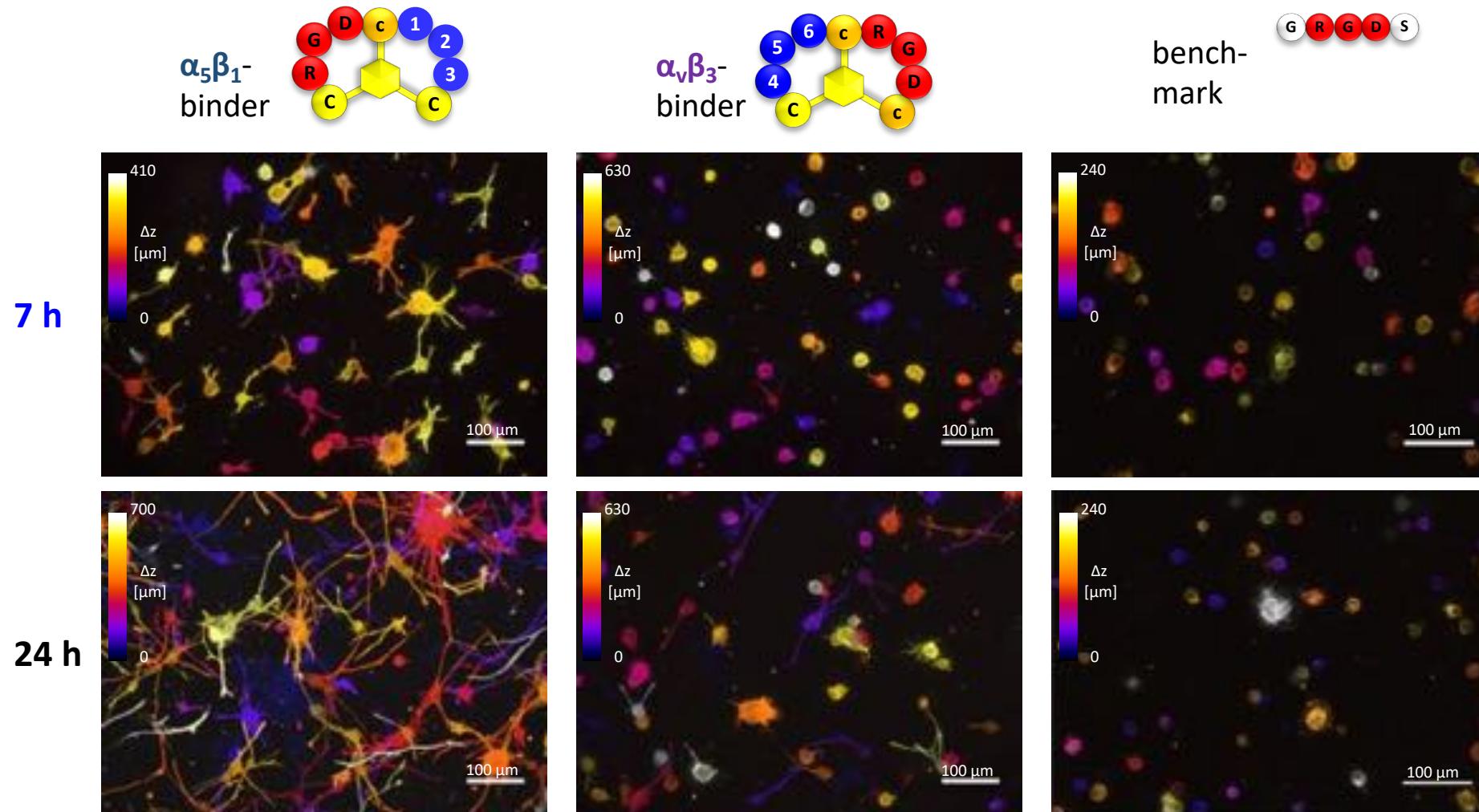


Phalloidin

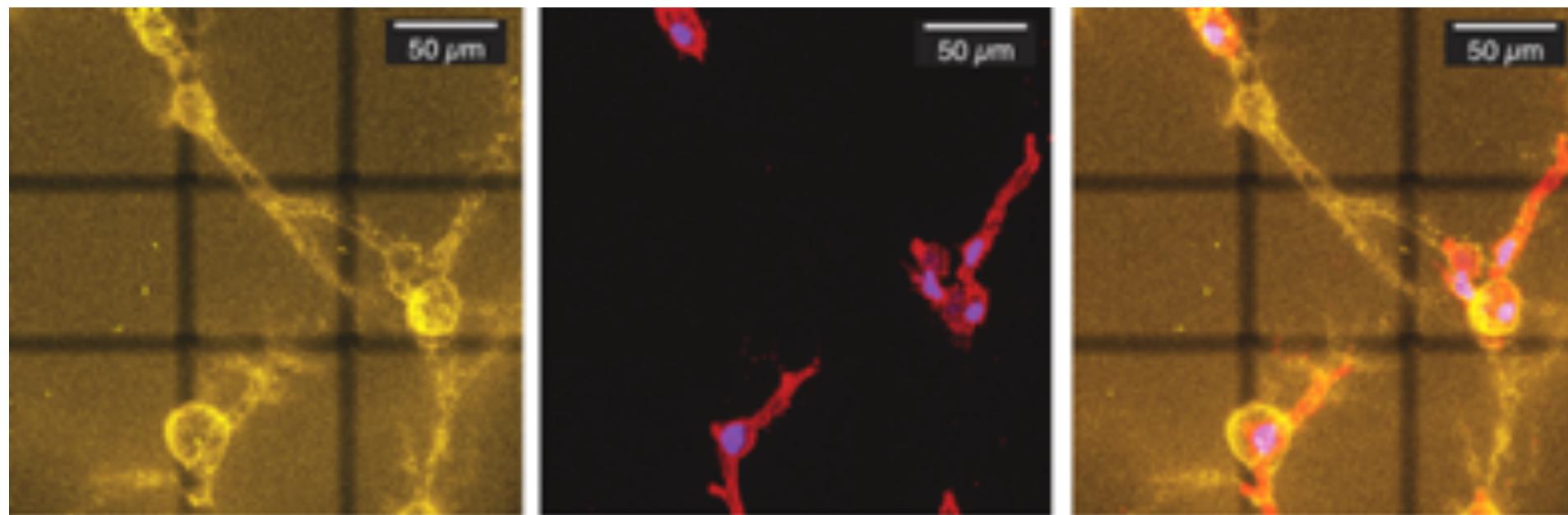
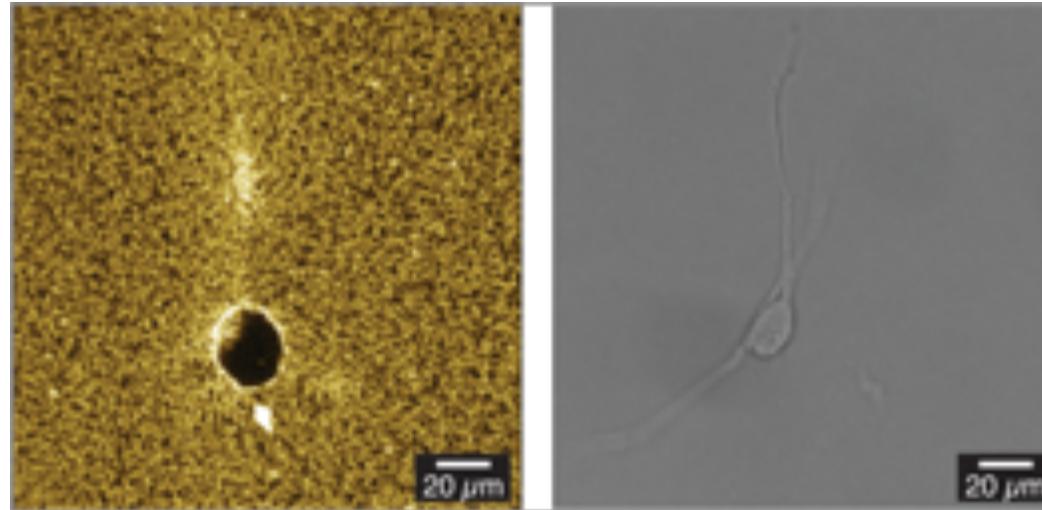
DAPI

Spreading of human Adipose Stem Cells

Peptides with different integrin affinities influence stem cell spreading

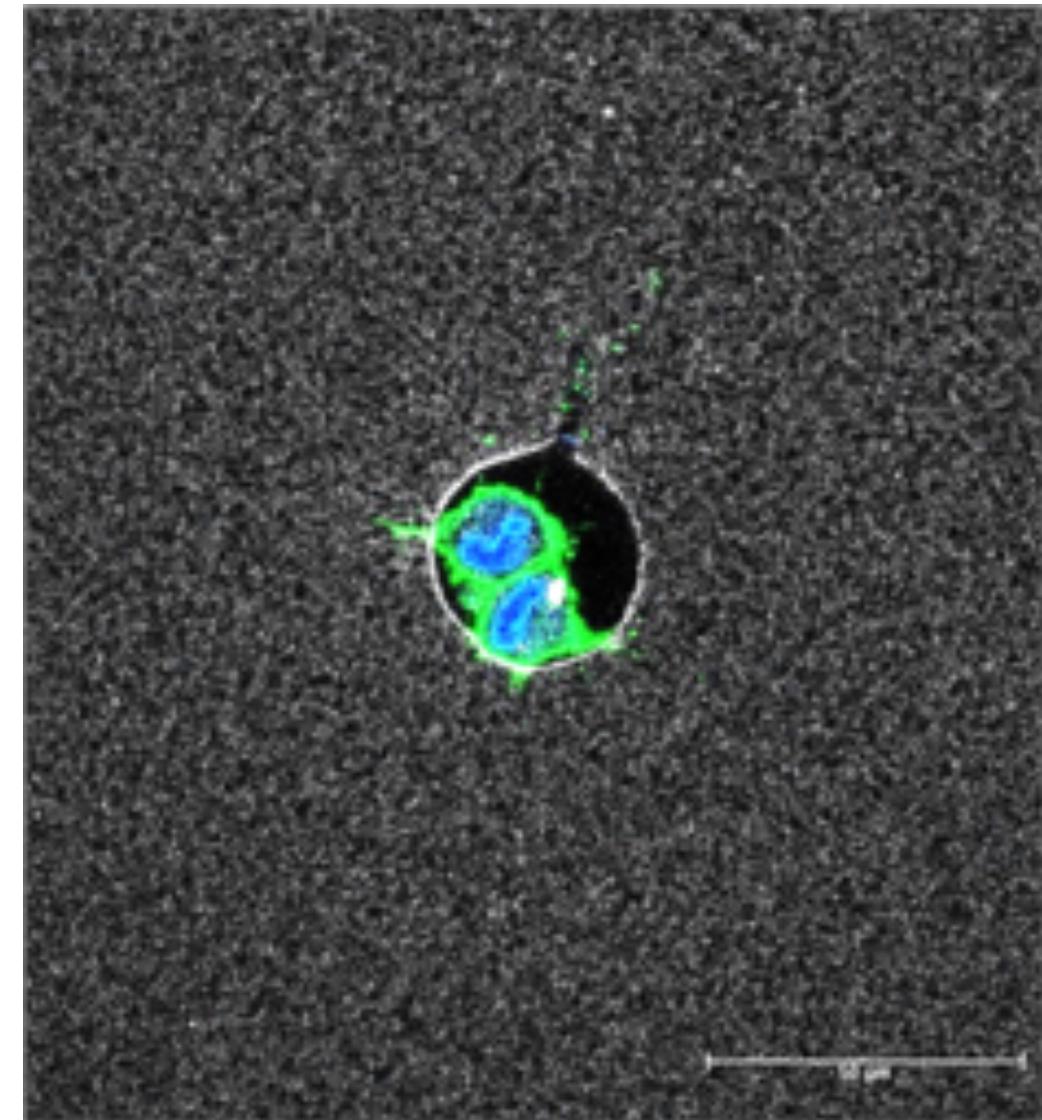
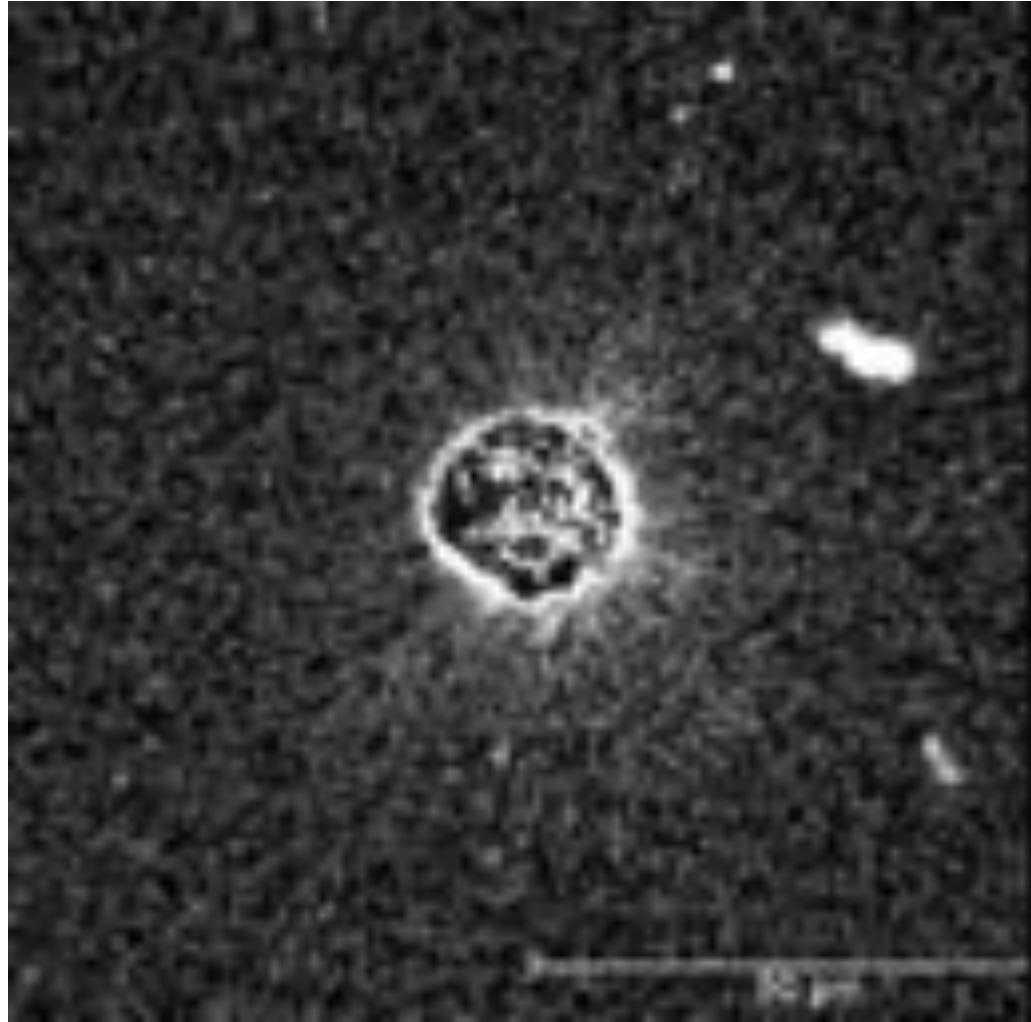


Matrix remodeling by human Adipose Stem Cells

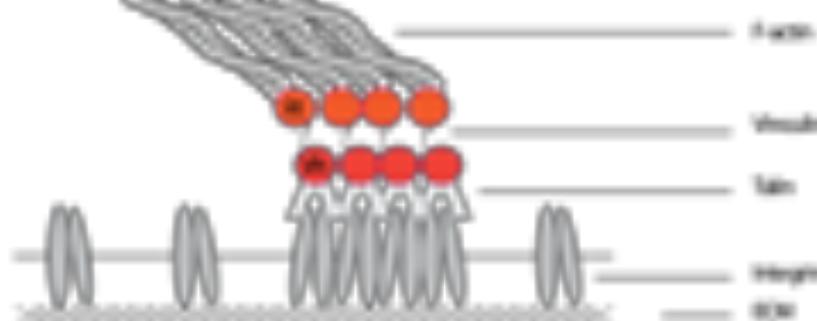
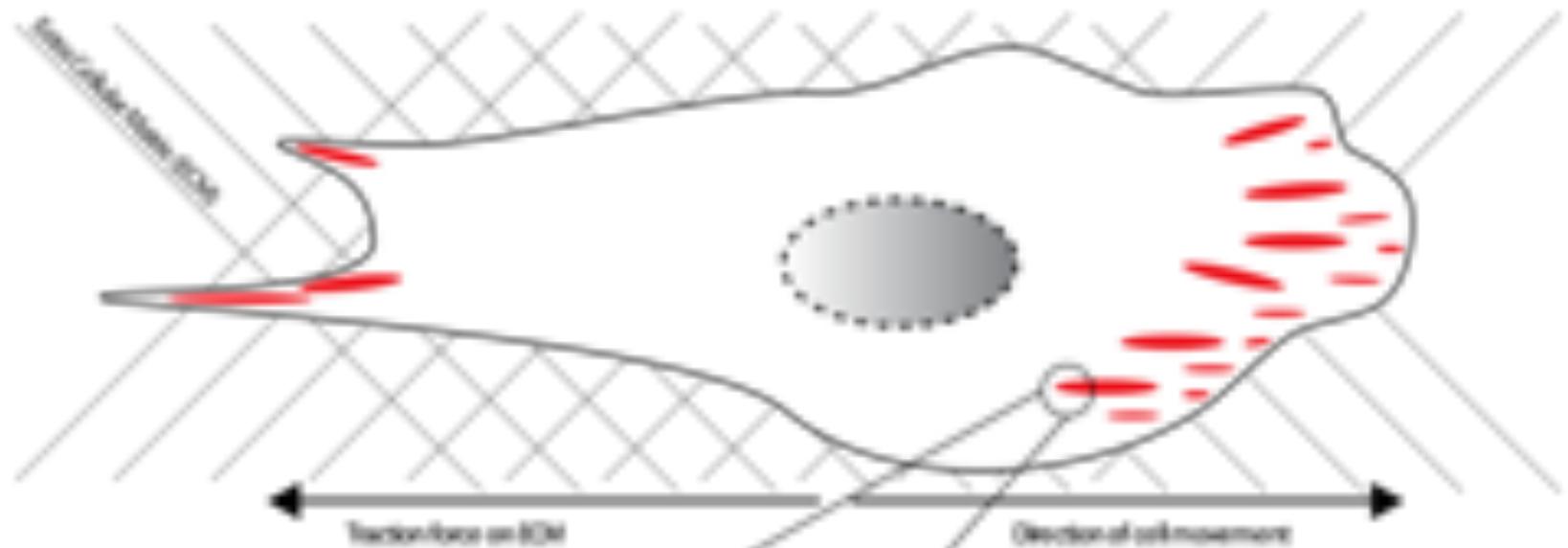
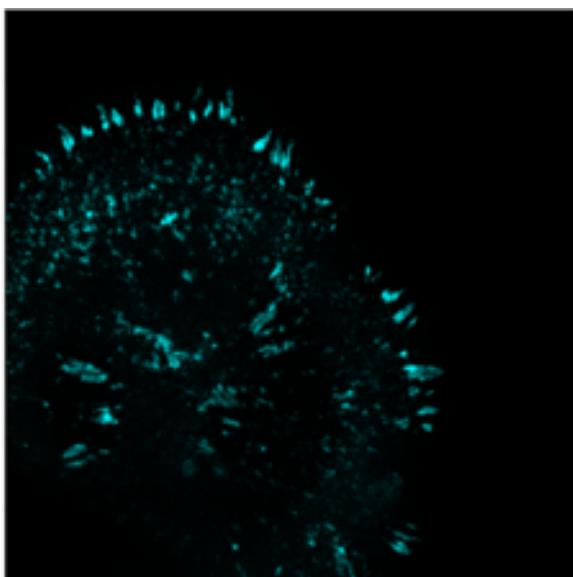
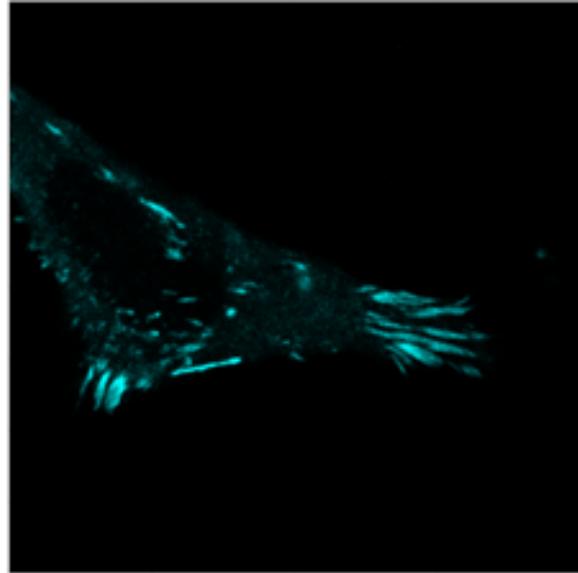


PIC F-actin Nucleus

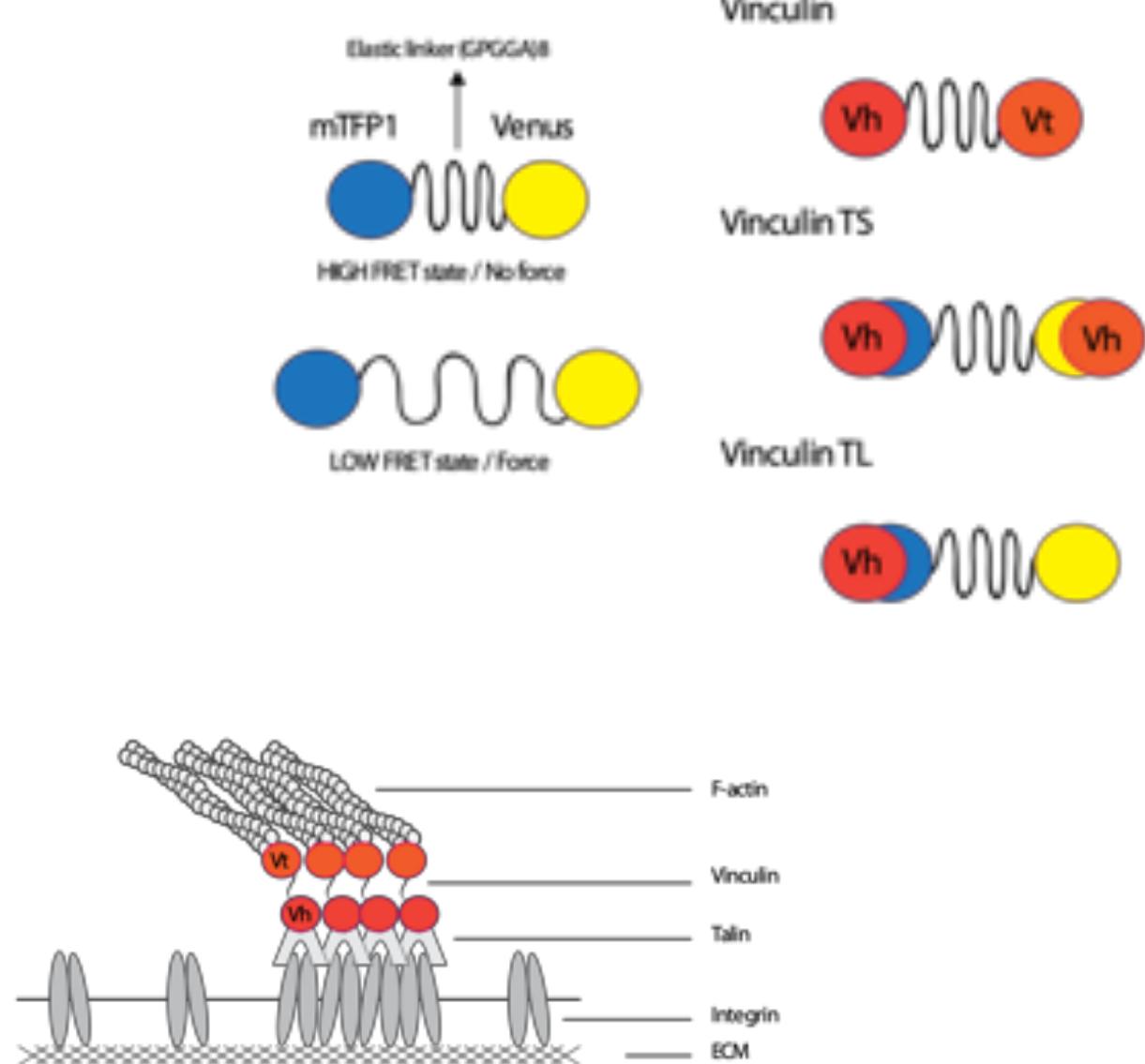
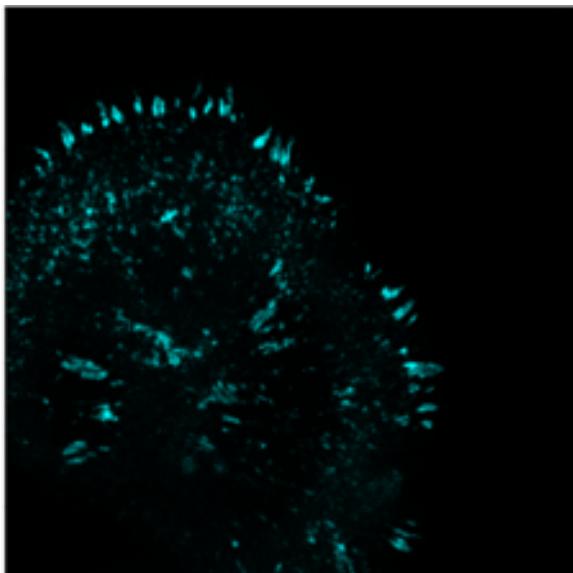
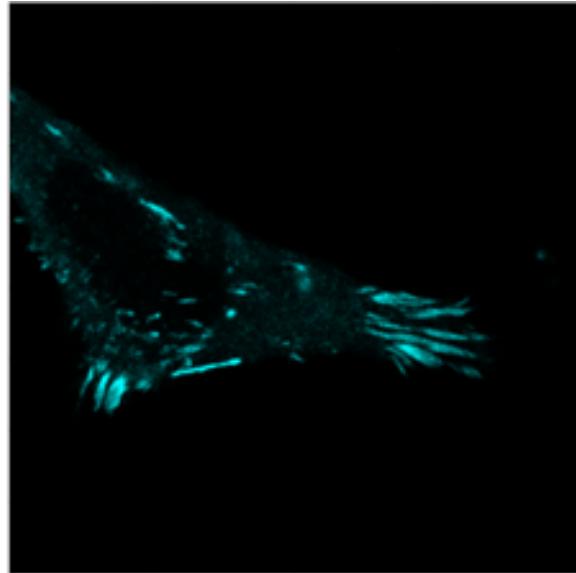
Matrix remodeling by human Adipose Stem Cells



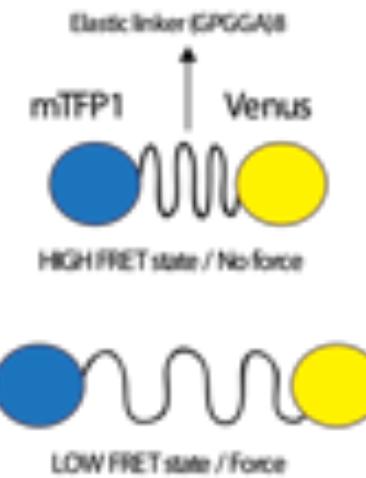
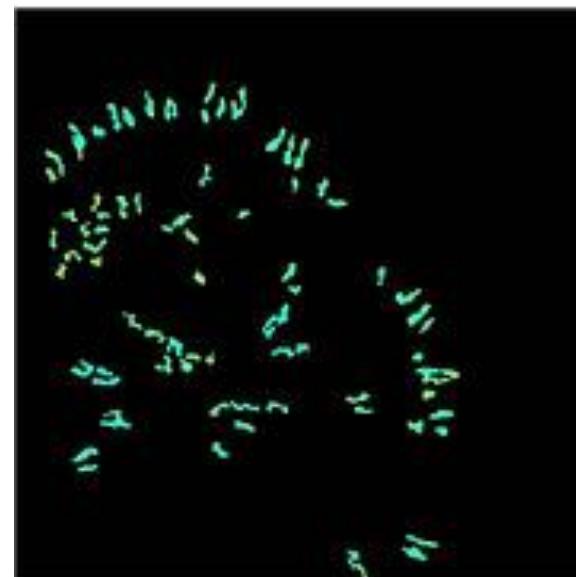
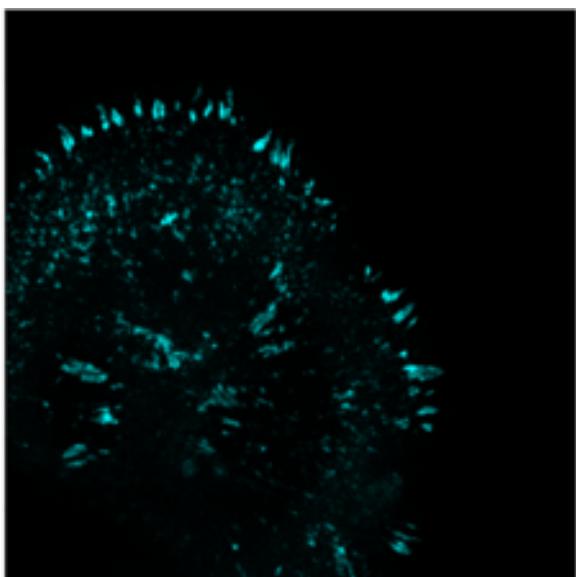
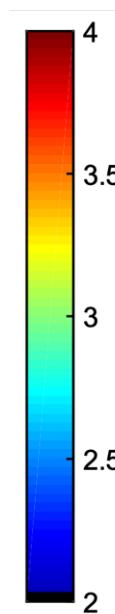
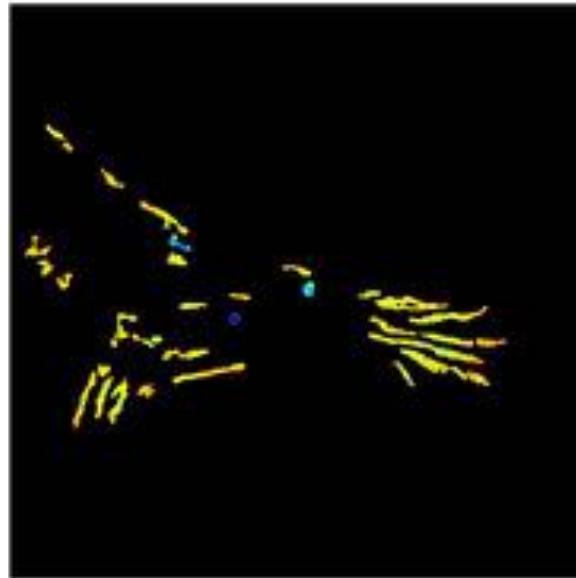
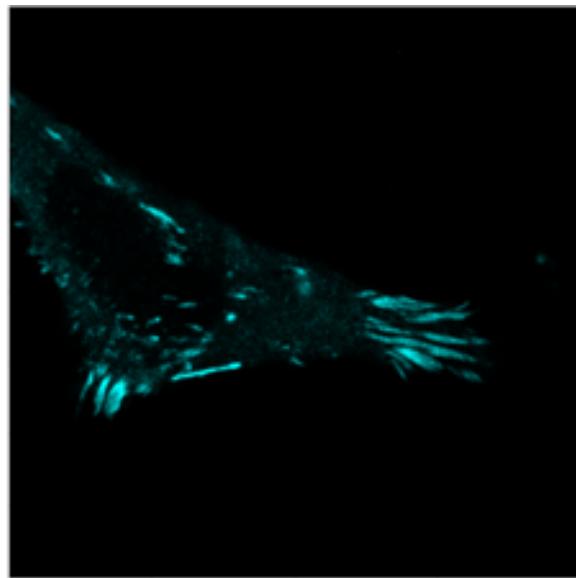
Cellular Focal Adhesions



Cellular Focal Adhesions



FRFT-based force sensor



Vinculin



Vinculin TS



Vinculin TL



Acknowledgments

Molecular Imaging and Photonics

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Dr. Beatrice Fortuni

Drs. Monica Ricci

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Prof. Dr. Hiroshi Uji-i

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Hasselt University

Prof. Dr. Jelle Hendrix

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Dr. Rodrigo Barderas

Drs. Guillermo Solis



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