Simulation and experimental evidence for the decrease of platelet margination with an increase in volume fraction of stiffened red blood cells in flow

Benjamin Czaja[1], M. Gutierrez[3], G. Závodszky[1,2], D. de Kanter[1], A.G. Hoekstra[1,4], O. Eniola–Adefeso[3]

1: Computational Science Lab, Institute for Informatics, University of Amsterdam, Amsterdam, Netherlands

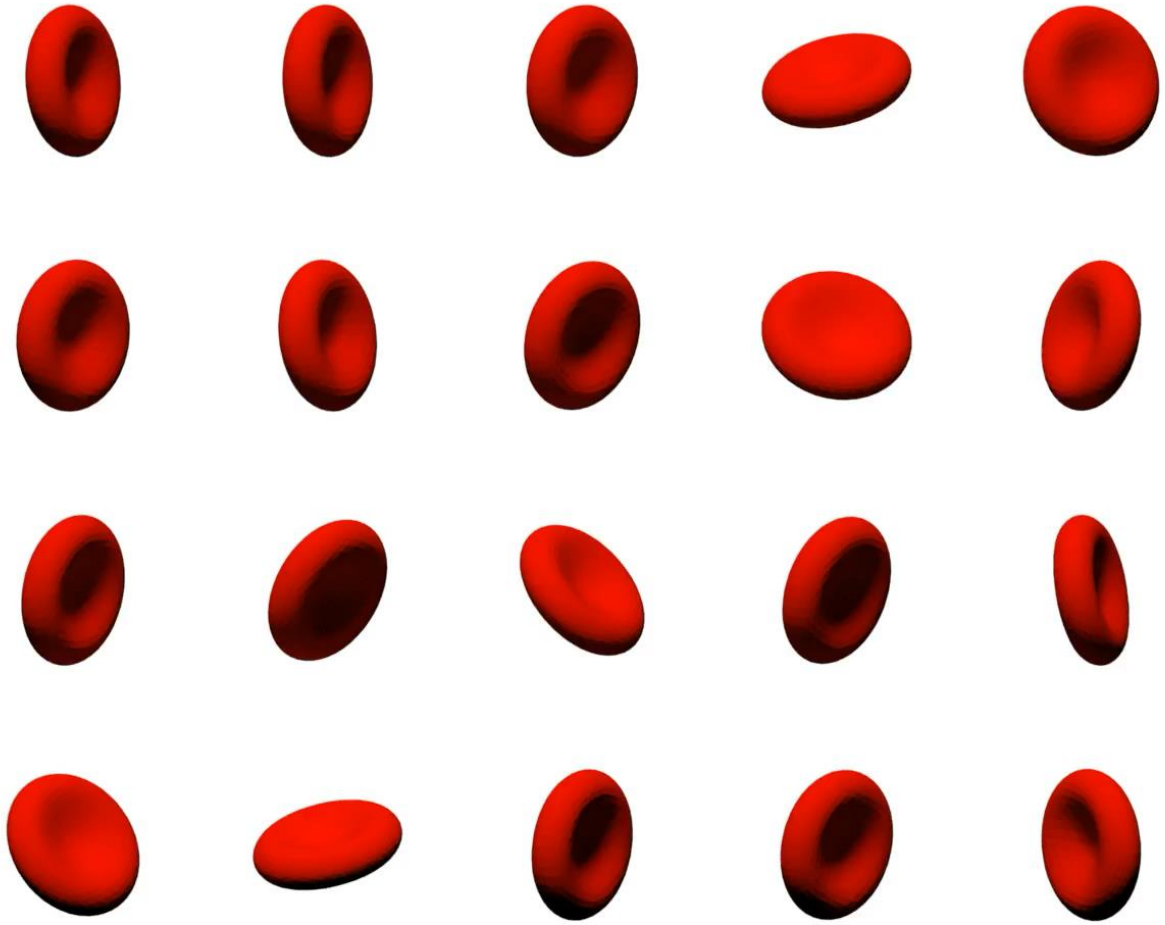
2: Department of Hydrodynamic Systems, Budapest University of Technology and Economics, Budapest, Hungary

3: Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan, United States

4: ITMO University, Saint Petersburg, Russia

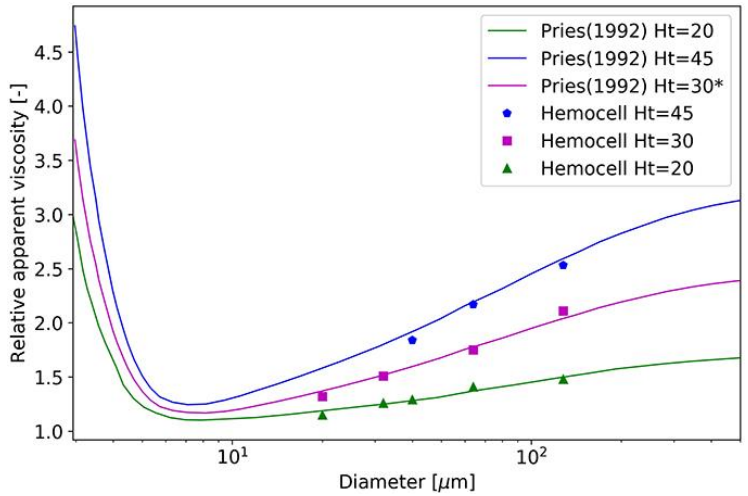
Motivation & background

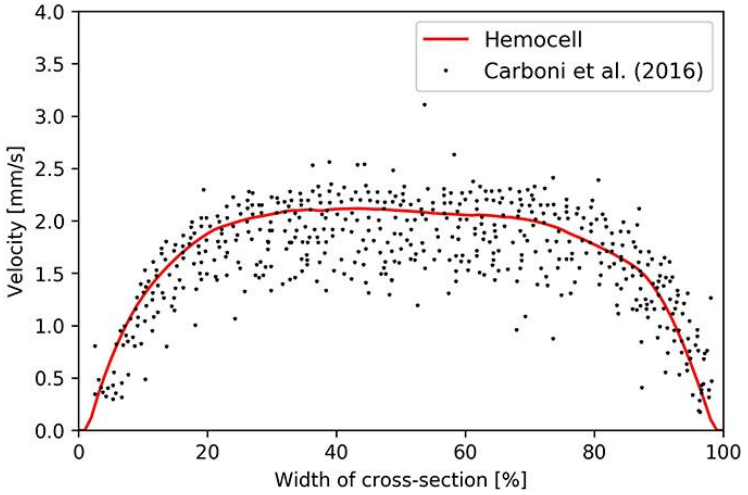
Red Blood Cell: deformability

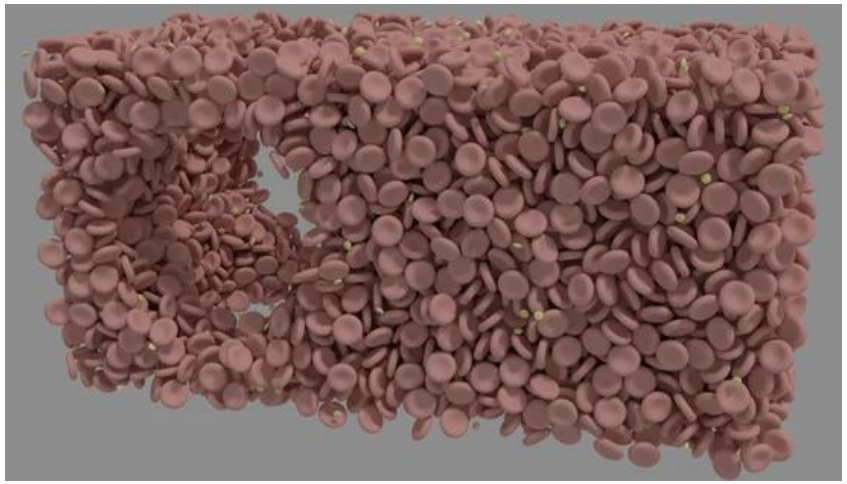


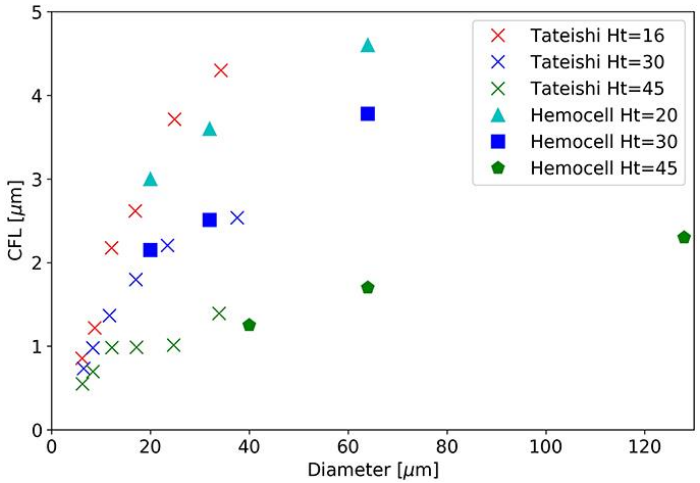
- The primary function of RBCs is to enable respiration in tissues by providing oxygen and removing carbon dioxide via gas exchange in the lungs
- During a typical 120 days lifespan of a RBC, it circulates through arteries, veins and small capillaries traveling –in total– a distance of 500 km (Lasch,2000)

HemoCell: bulk rheology validation





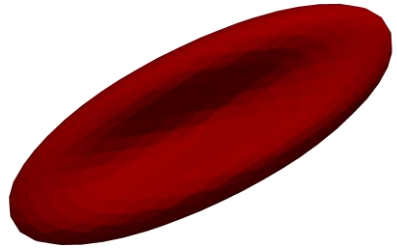


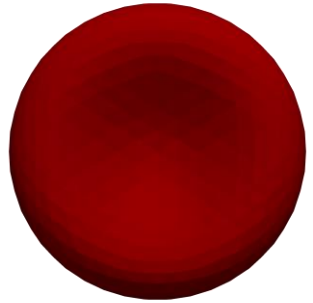


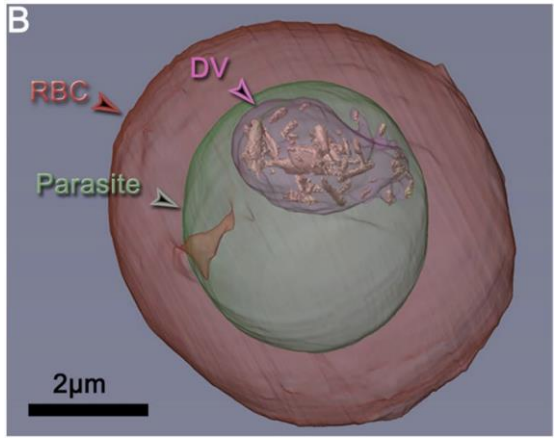
- Fåhræus–Lindqvist effect (reduction of relative apparent viscosity with reduction of vessel diameter)
- Plug-flow profile
- Red blood cell free layer

Changes in deformability

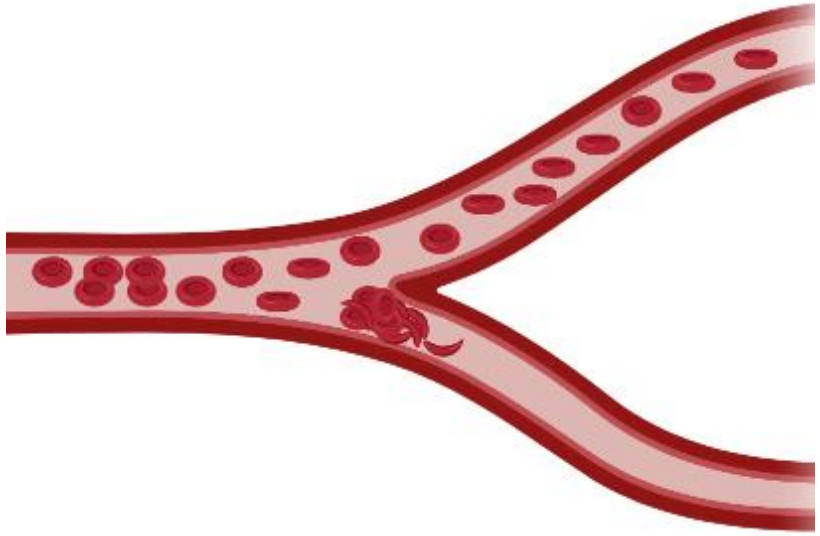
- Rigid Red Blood Cells
  - occluding microvasculature
  - depriving tissues of nutrients
  - damaging walls of the spleen/liver/lungs
- Diabetes
  - Oxidative stress due to high concentrations of glucose in the blood
- Sickle cell anemia
  - Dehydration of RBCs allowing polymerization of HbS molecules
- Malaria
- Hereditary spherocytosis
- HIV





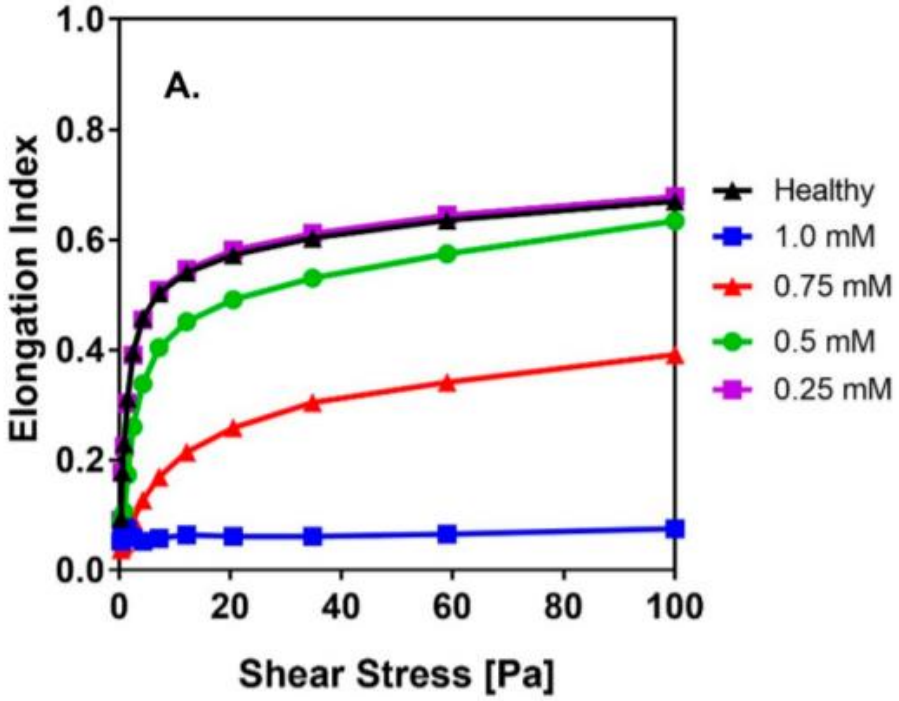


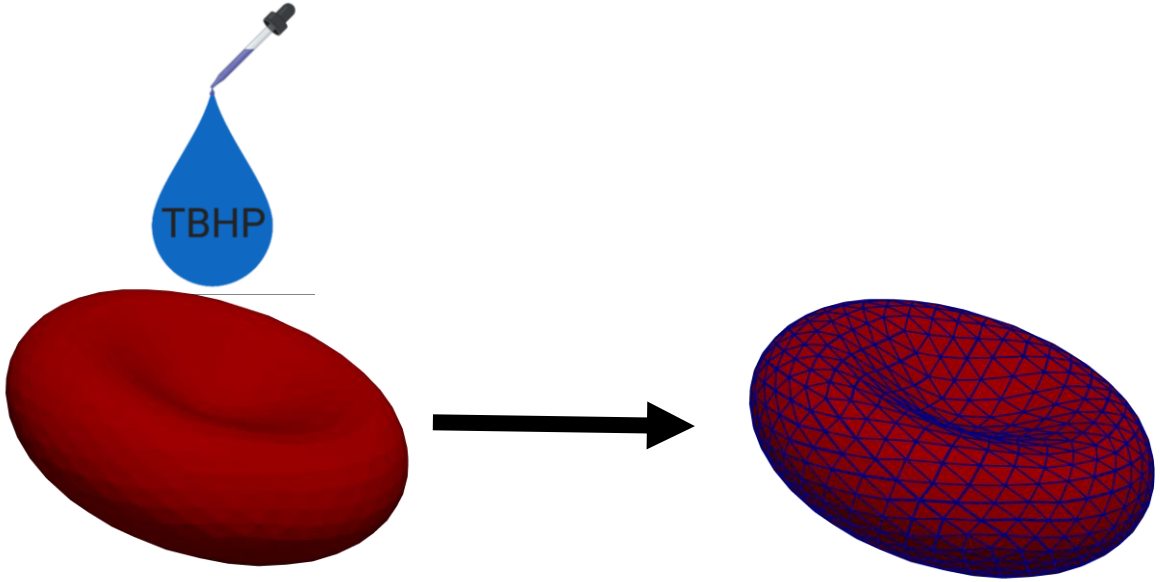
(Kapishnikov, 2017)



Confocal microscopy measurements of platelet distributions

Oxidative stress on cell membrane





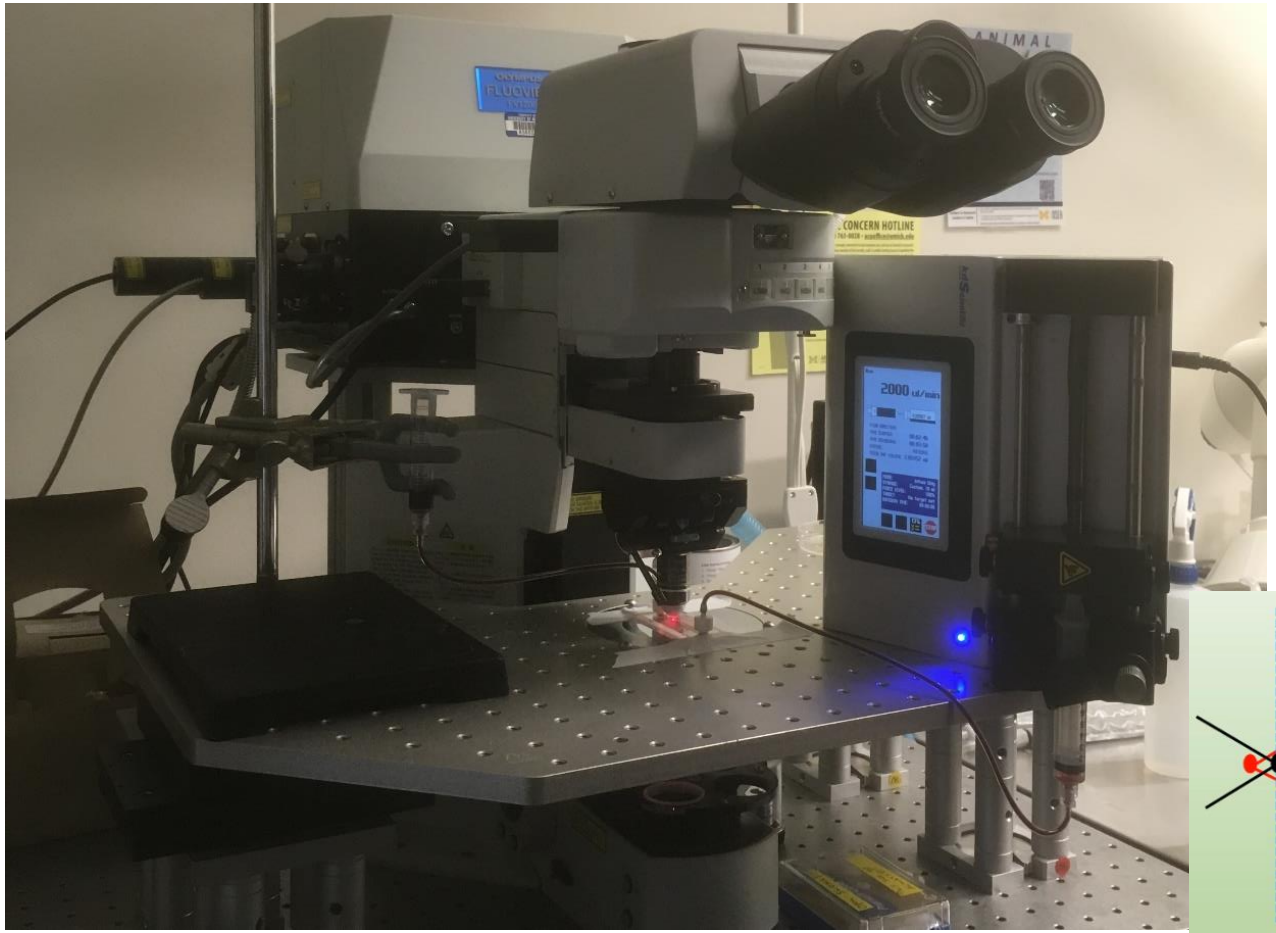
- tert-Butyl hydroperoxide (TBHP) to induce oxidative stress and rigidify red blood cell membranes (Gutierrez,2018).

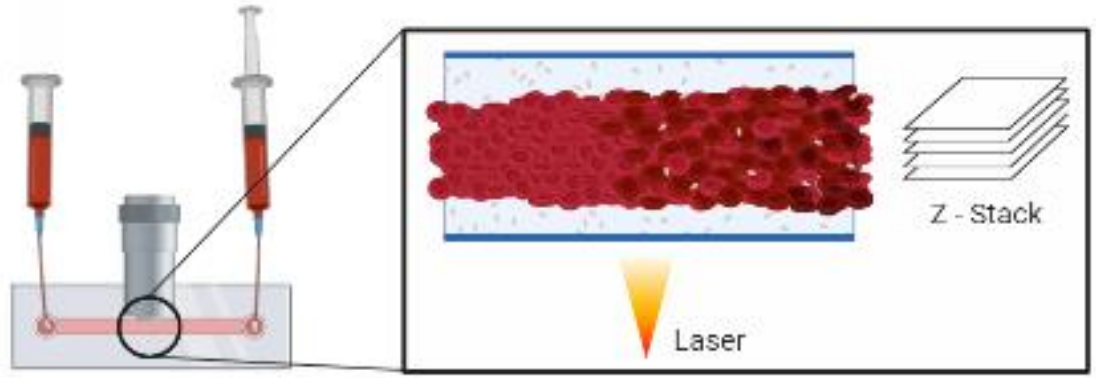
UNIVERSITY OF MICHIGAN

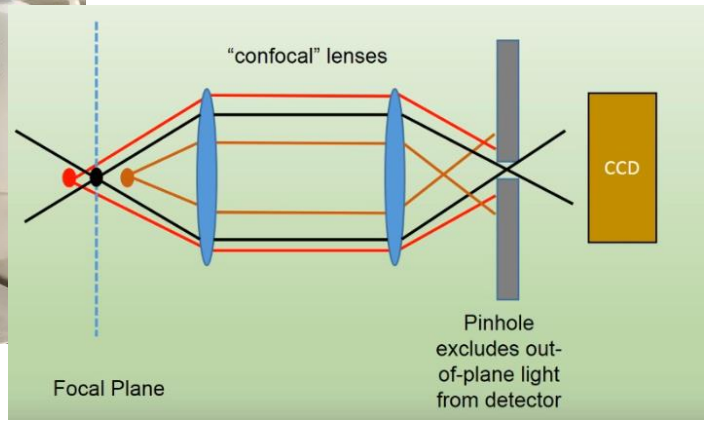
Eniola Research Group in the Department of Chemical Engineering

cell adhesion and drug delivery

Experimental setup





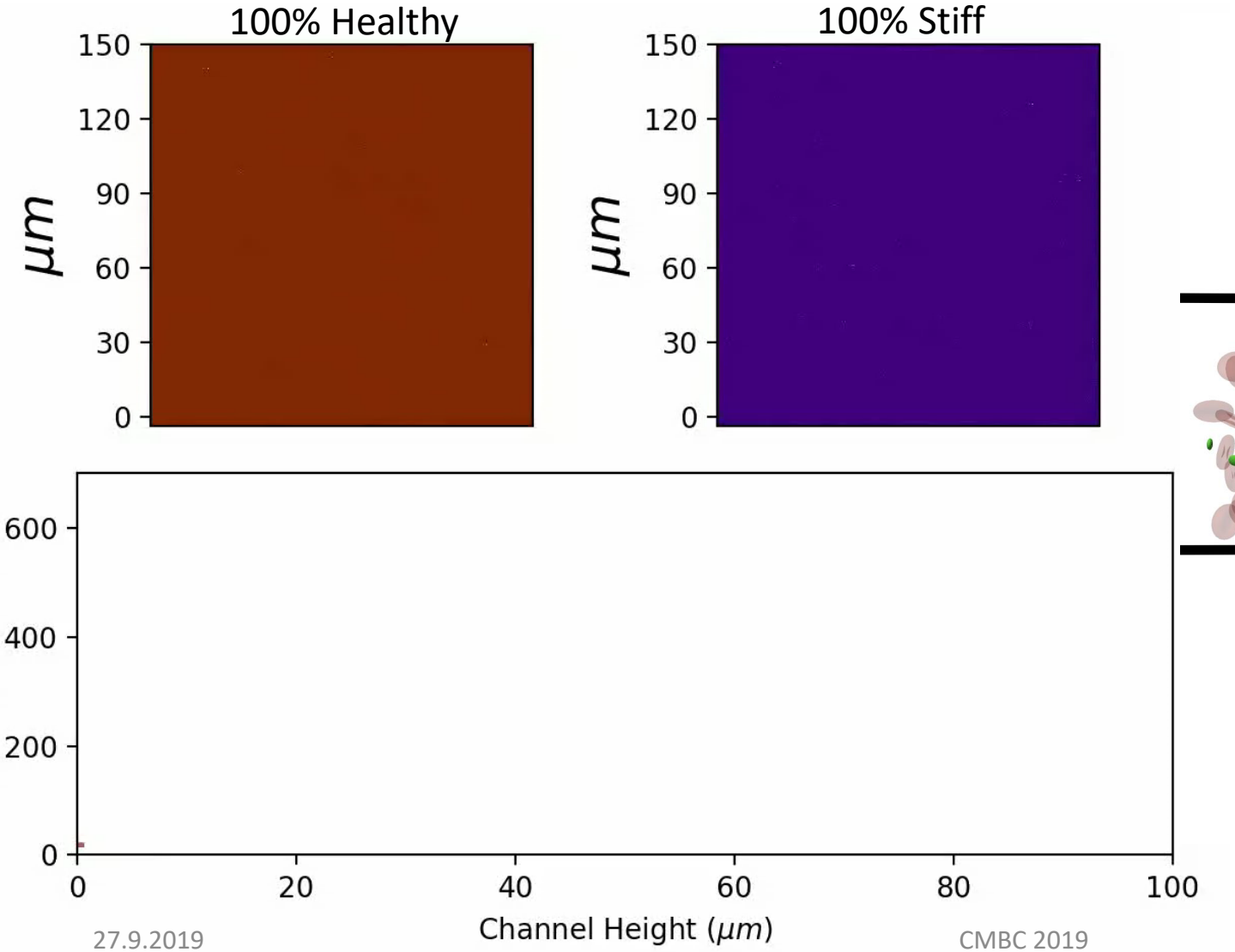


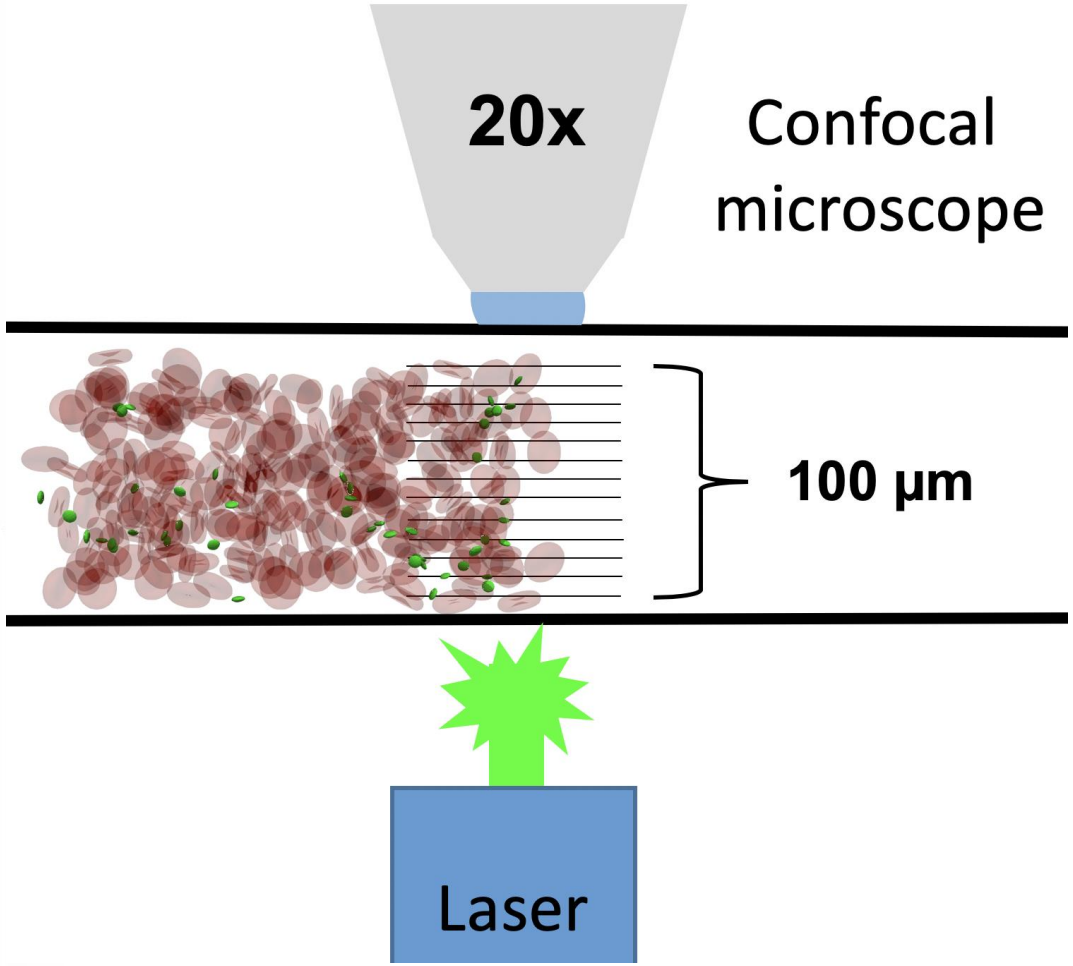
Platelet

anti-human CD41/CD61 Allophycocyanin

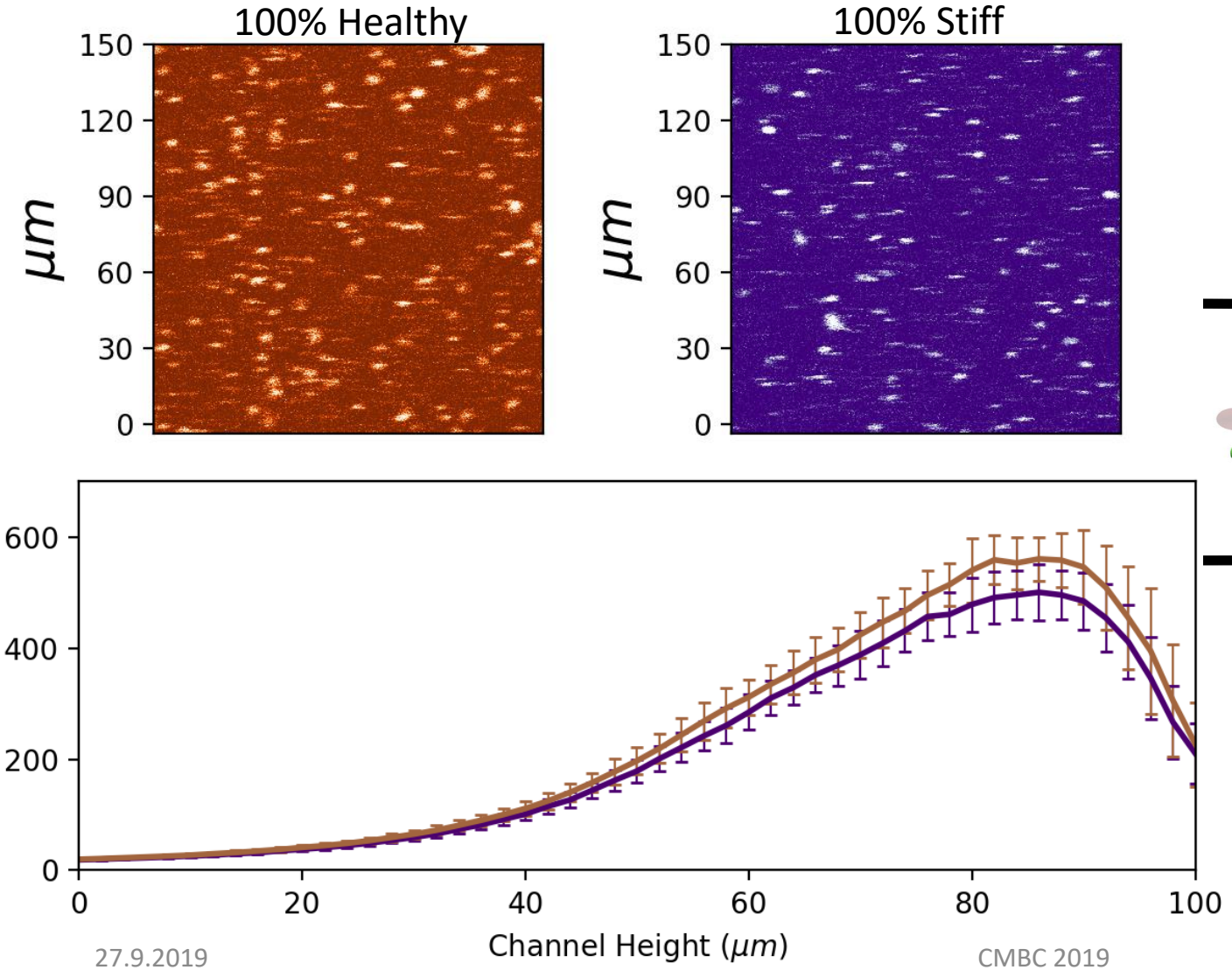
633nm laser
peak emission wavelength
660nm

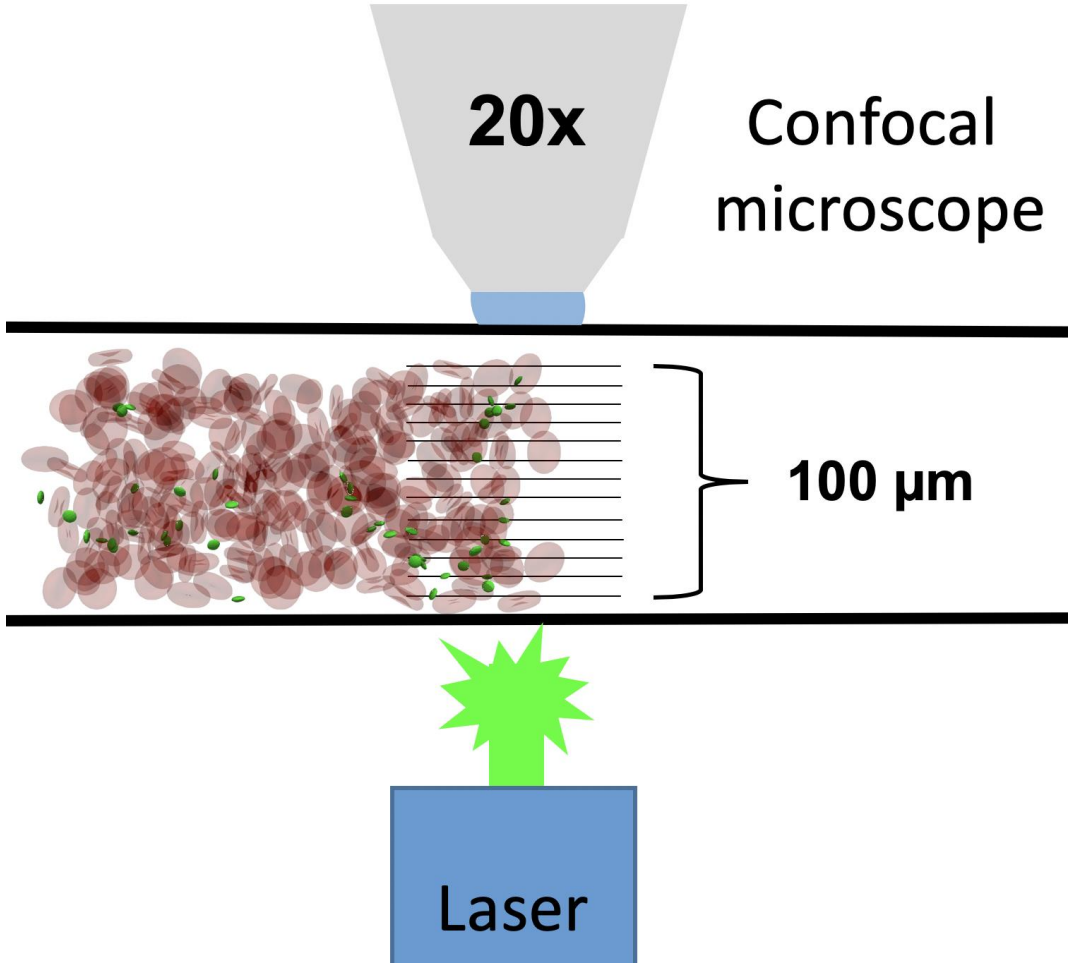
Platelet Zscans



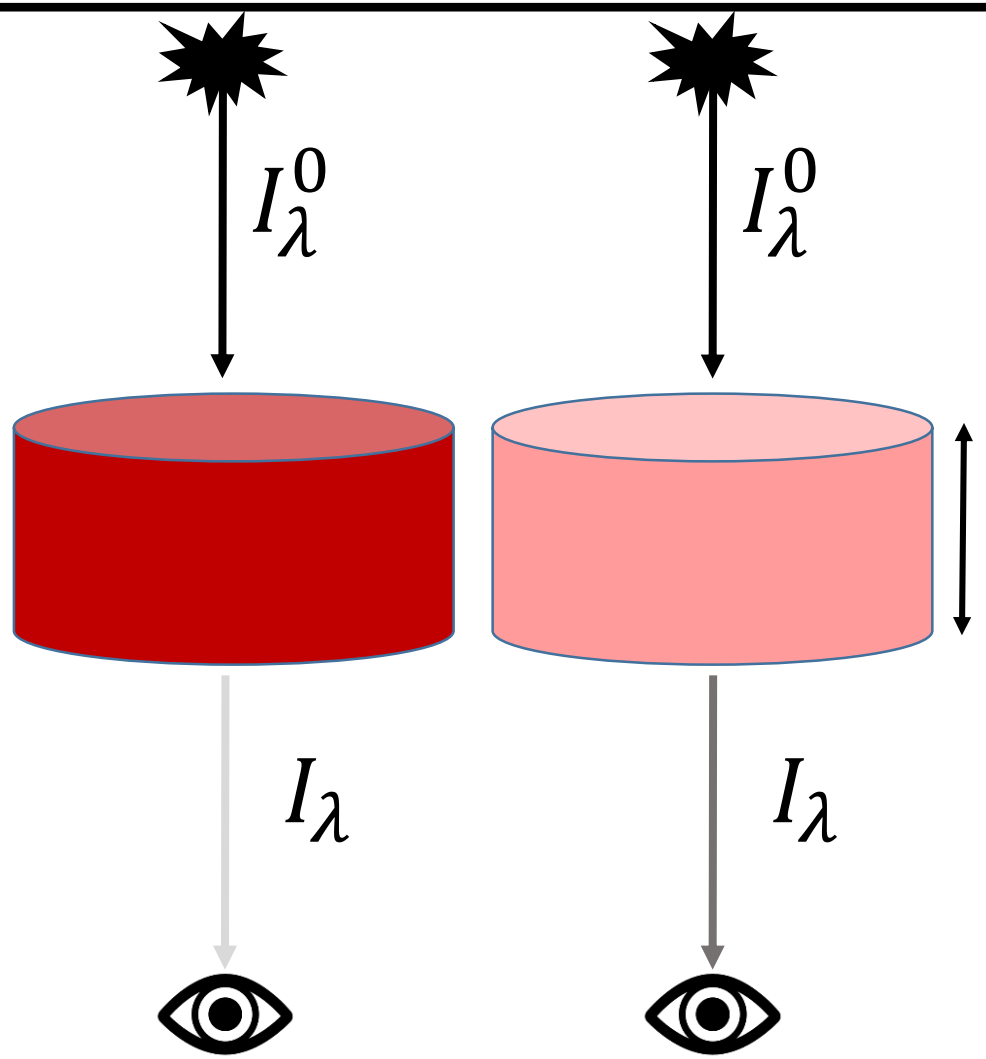


Platelet Zscans

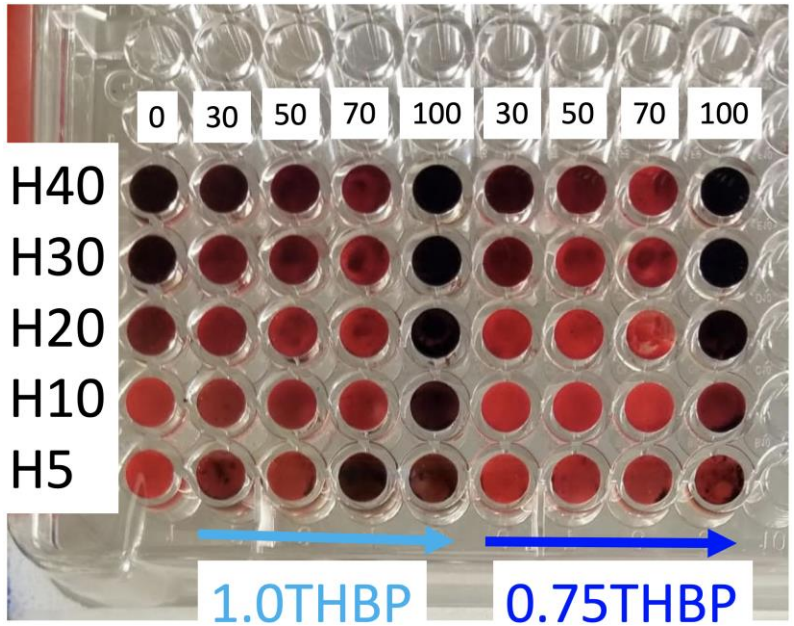




Absorbance



Height
$3100\mu m$



$$A(\lambda) = \log \frac{I_\lambda^0}{I_\lambda} = \varepsilon(\lambda) l c$$

$$a(\lambda) = \frac{A(\lambda)}{l}$$

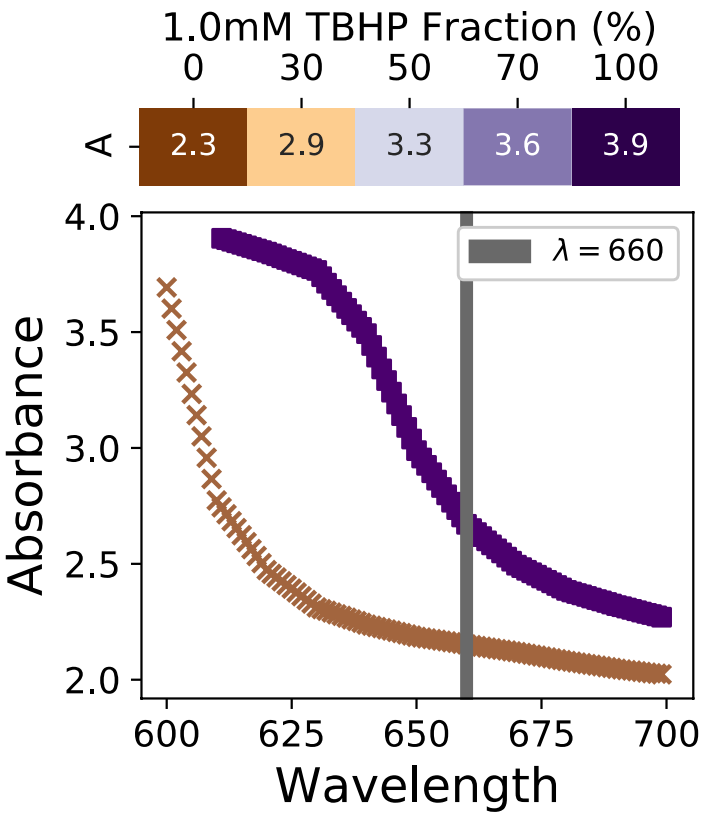
$$I_\lambda = I_\lambda^0 10^{-a(\lambda) l}$$

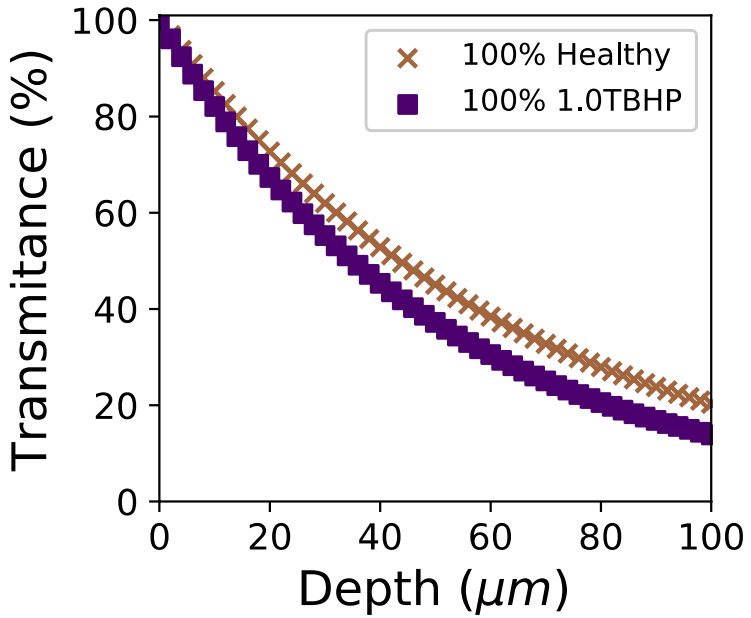
Absorbance & Normalization

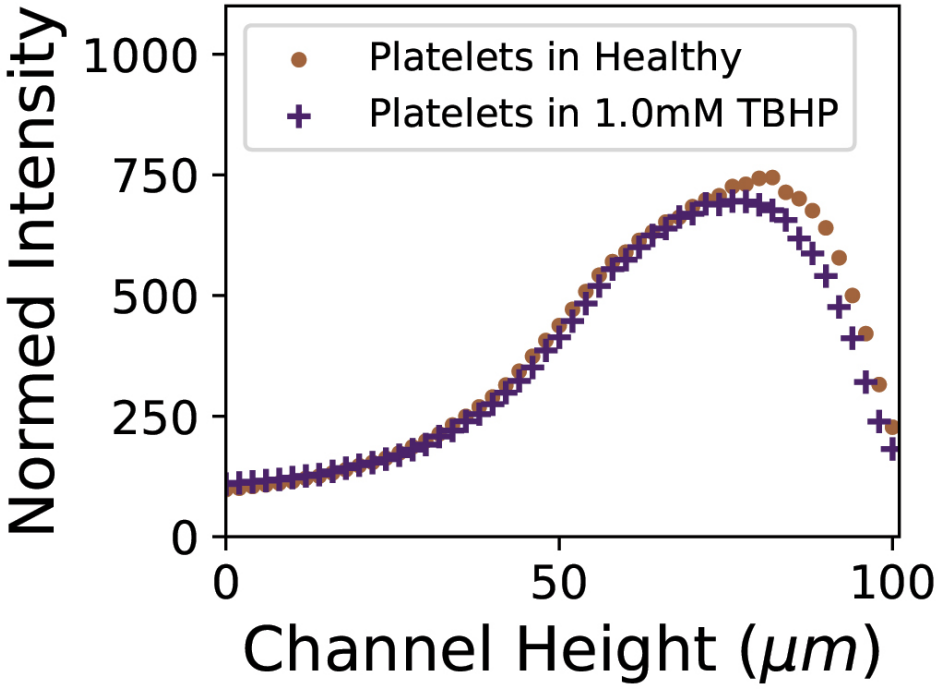
$$A(\lambda) = \log \frac{I_\lambda^0}{I_\lambda} = \varepsilon(\lambda) l c$$

$$a(\lambda) = \frac{A(\lambda)}{l}$$

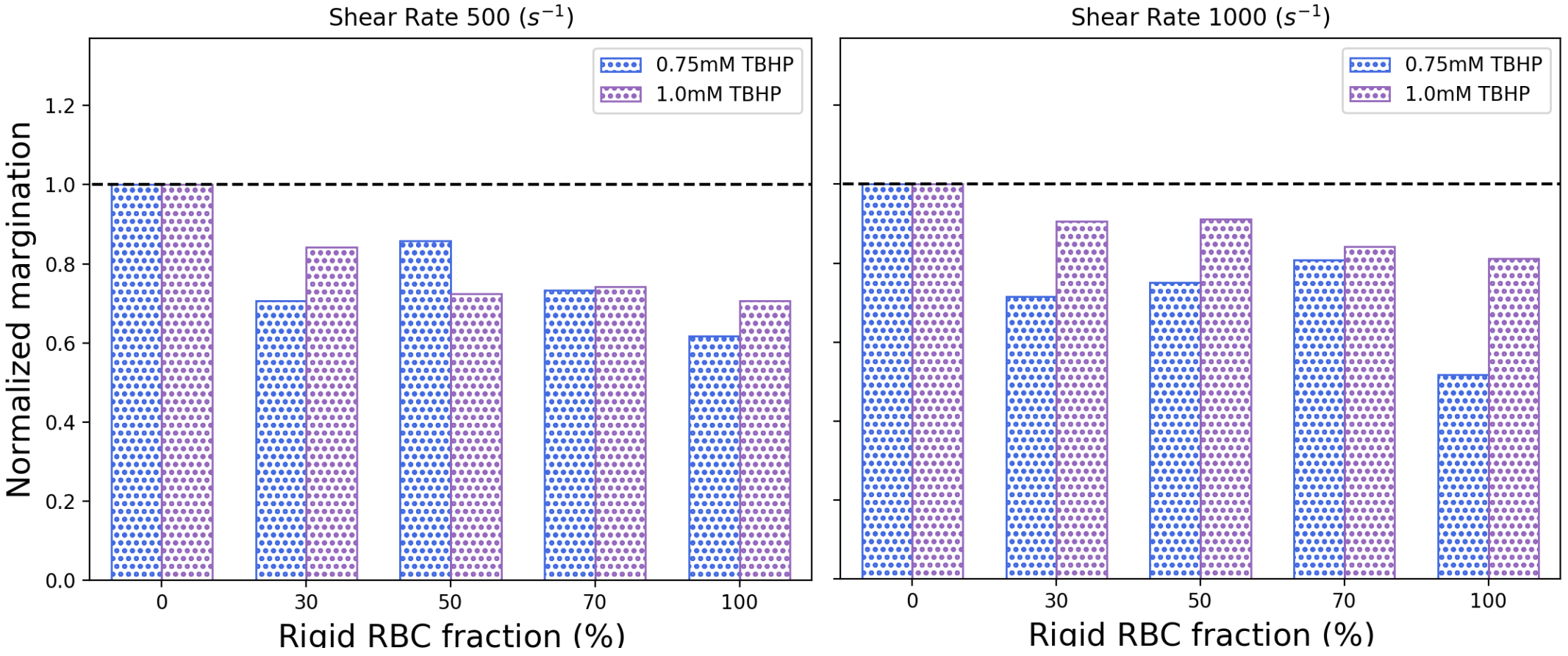
$$I_\lambda = I_\lambda^0 10^{-a(\lambda) l}$$





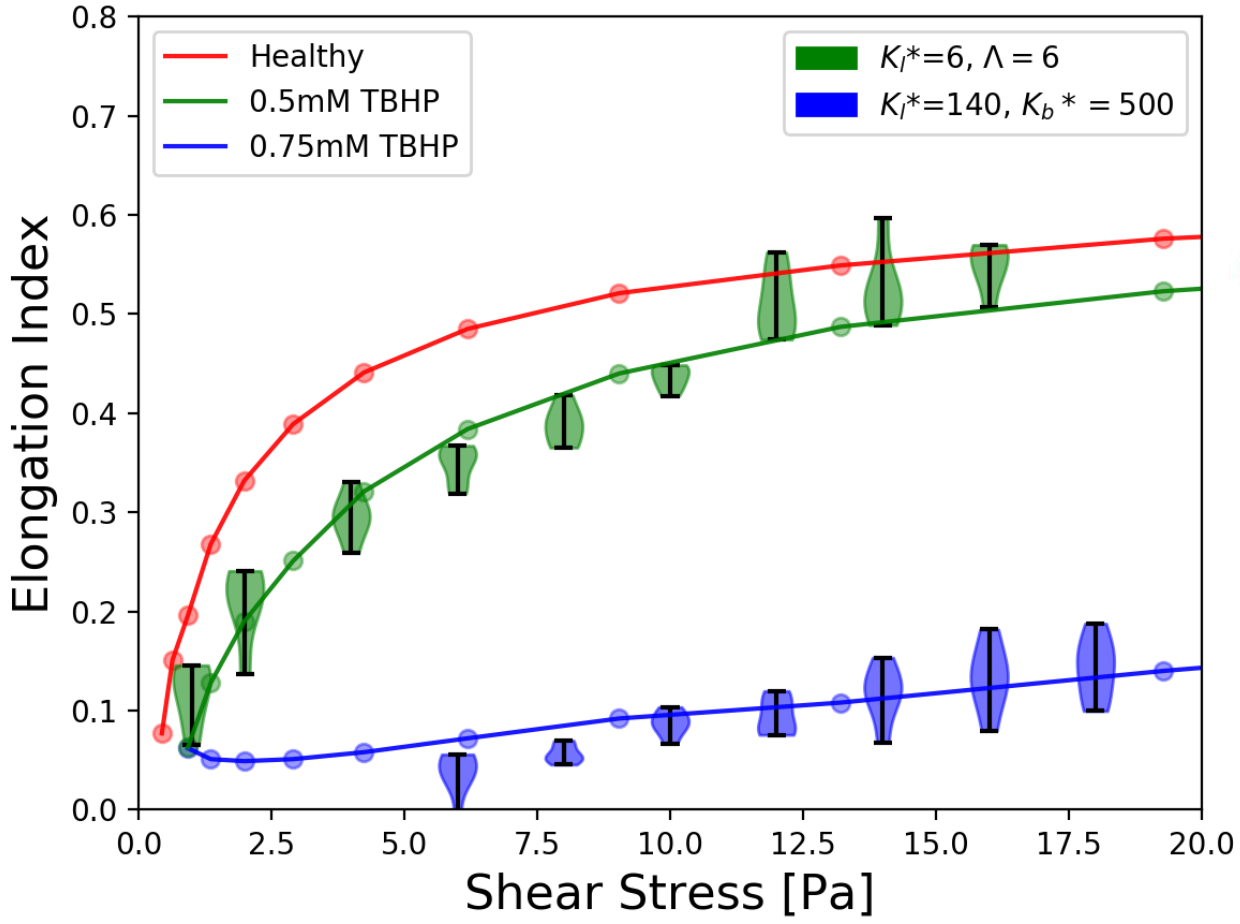


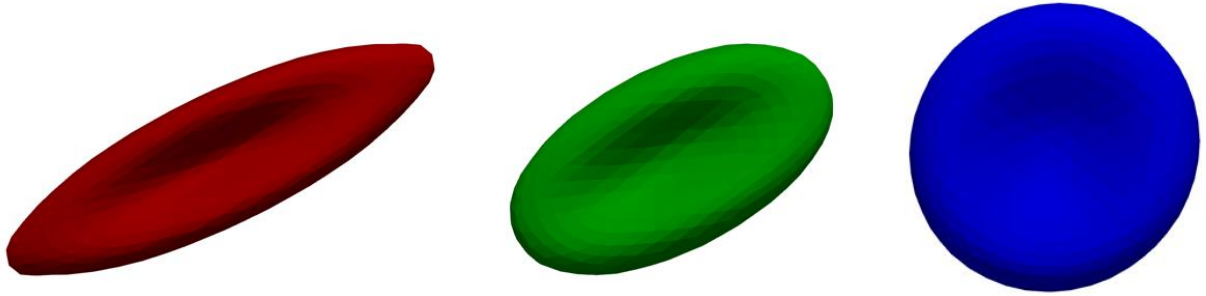
Platelet concentration at the wall



Single cell simulations

Stiffened HemoCell RBC Model

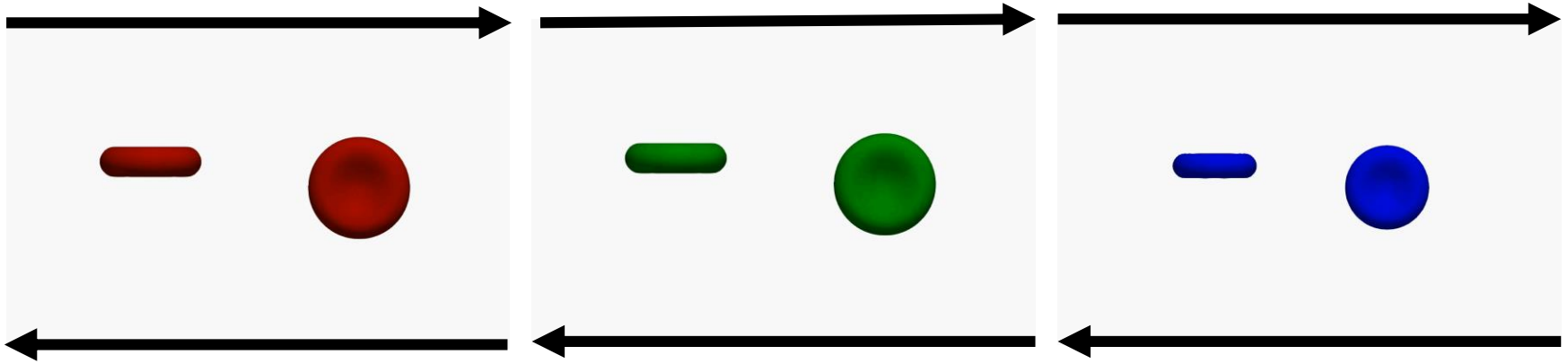


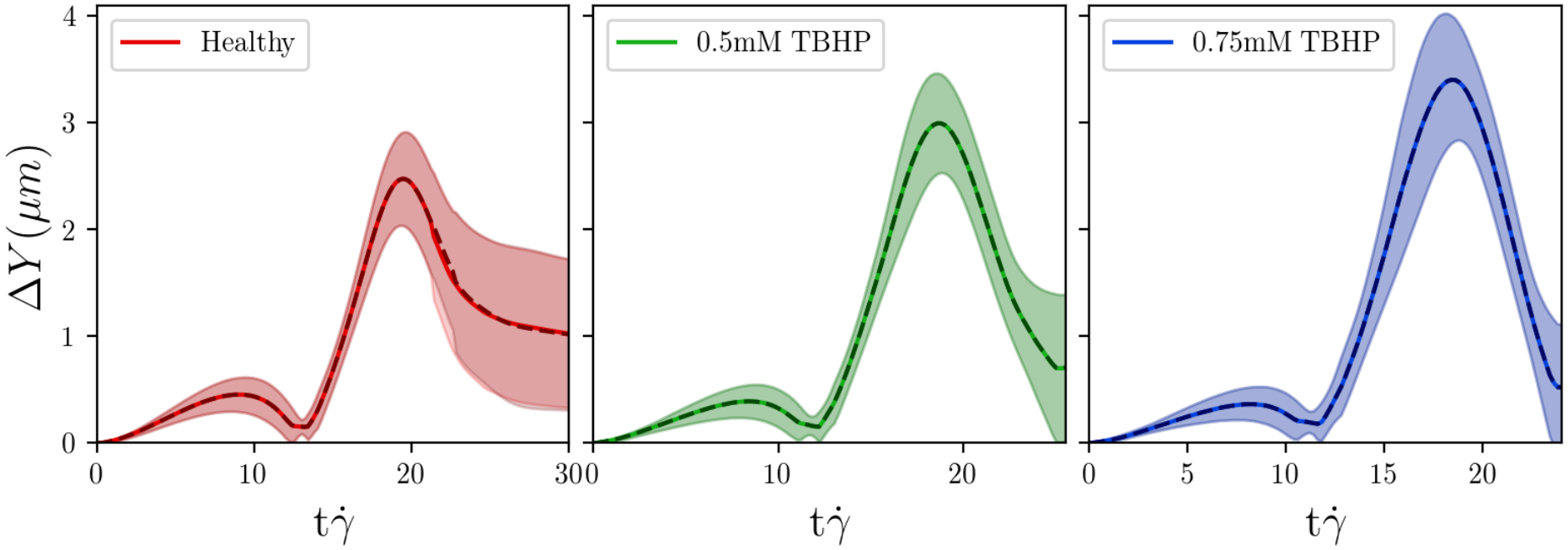


$$F_{link} = -\frac{\kappa_l dL}{p}\left[1 + \frac{1}{\tau_l^2 - dL^2}\right], \quad F_{bend} = -\frac{\kappa_b d\theta}{L_0}\left[1 + \frac{1}{\tau_b{}^2 - d\theta^2}\right],$$

- Stiffness is achieved by increasing the link force coefficient, bending force coefficient, and the interior viscosity ratio.

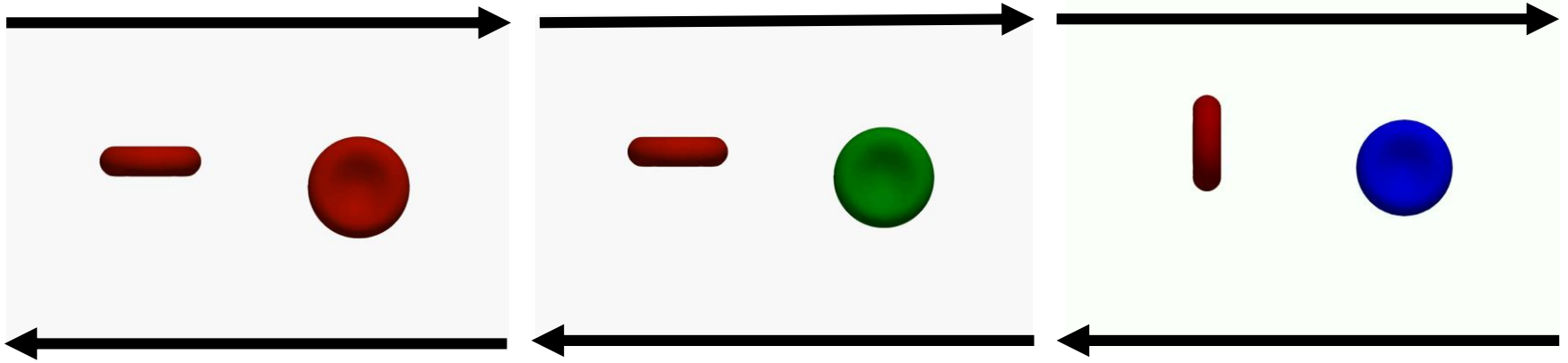
Homogenous RBC Collisions

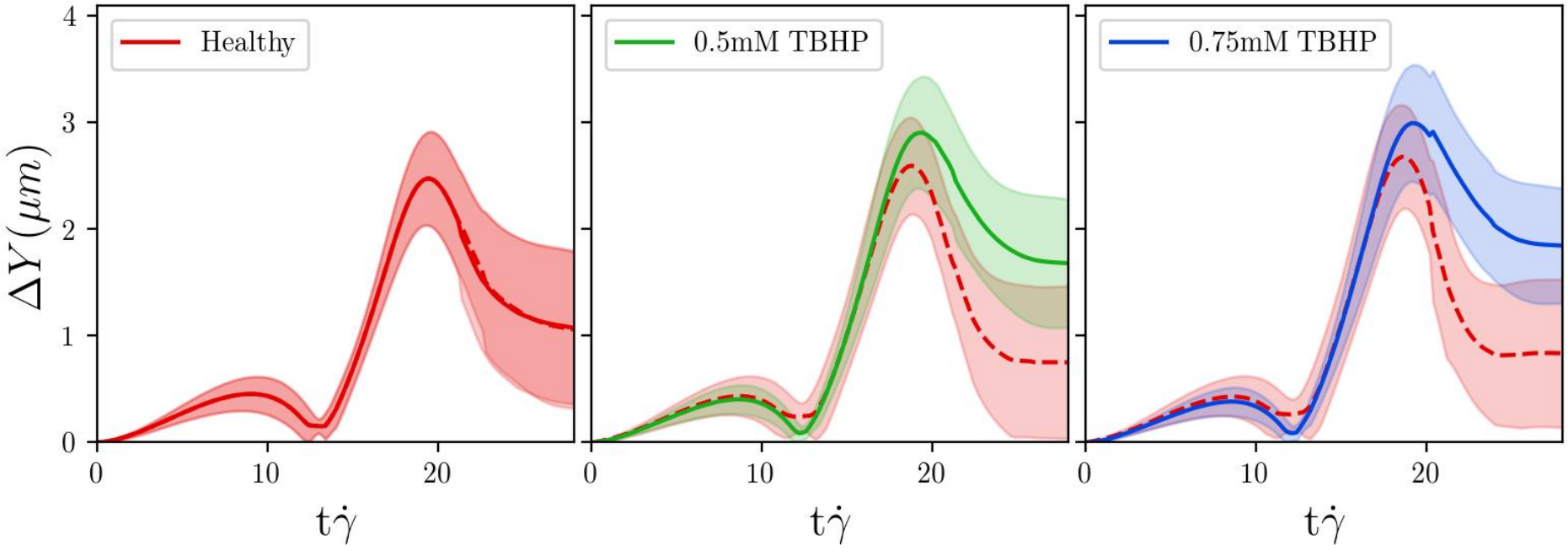




- Collisions approach stokes regime for two colliding rigid spheres as RBC stiffness increases

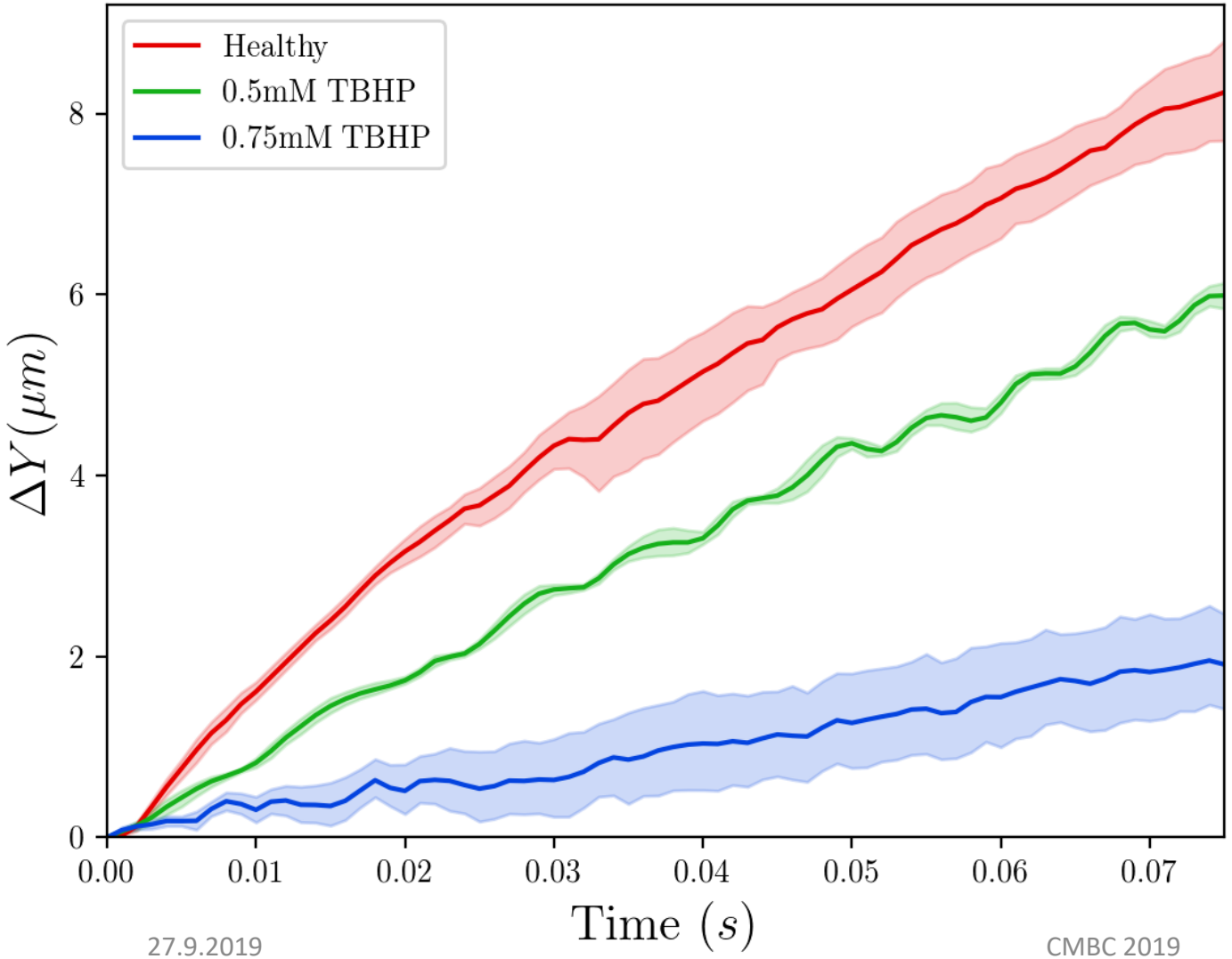
Heterogeneous RBC Collisions



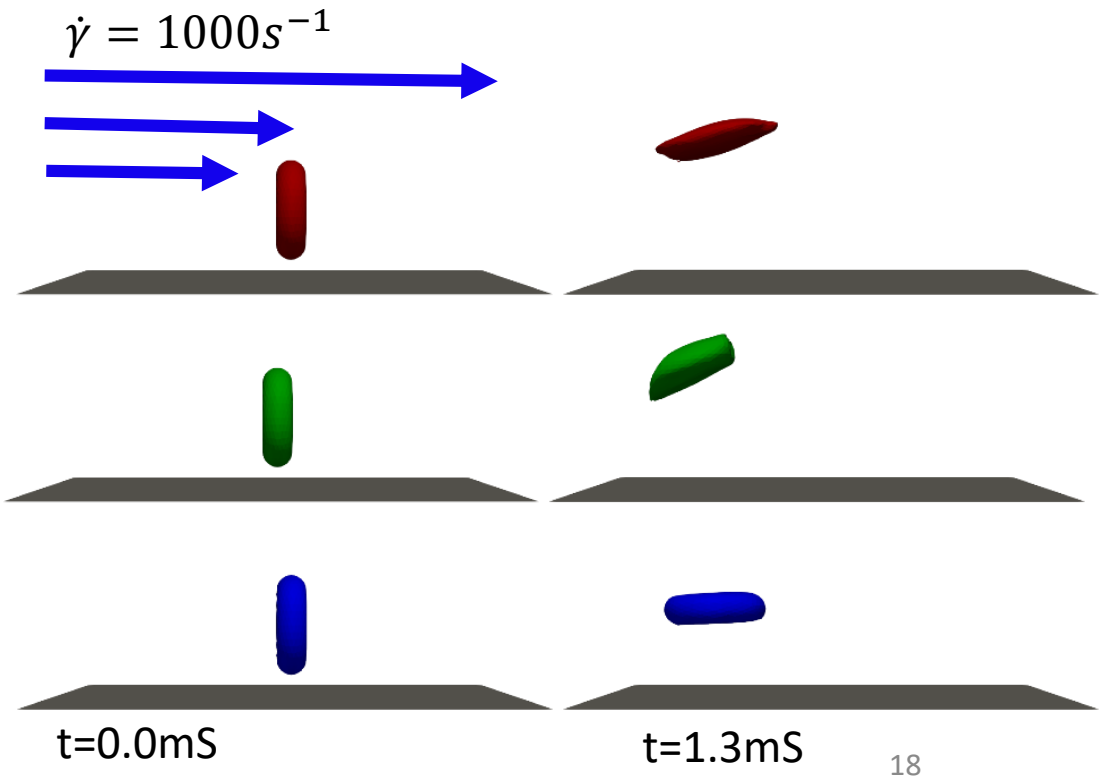


- Collisions approach stokes regime for two colliding spheres as RBC stiffness increases

Lift Force

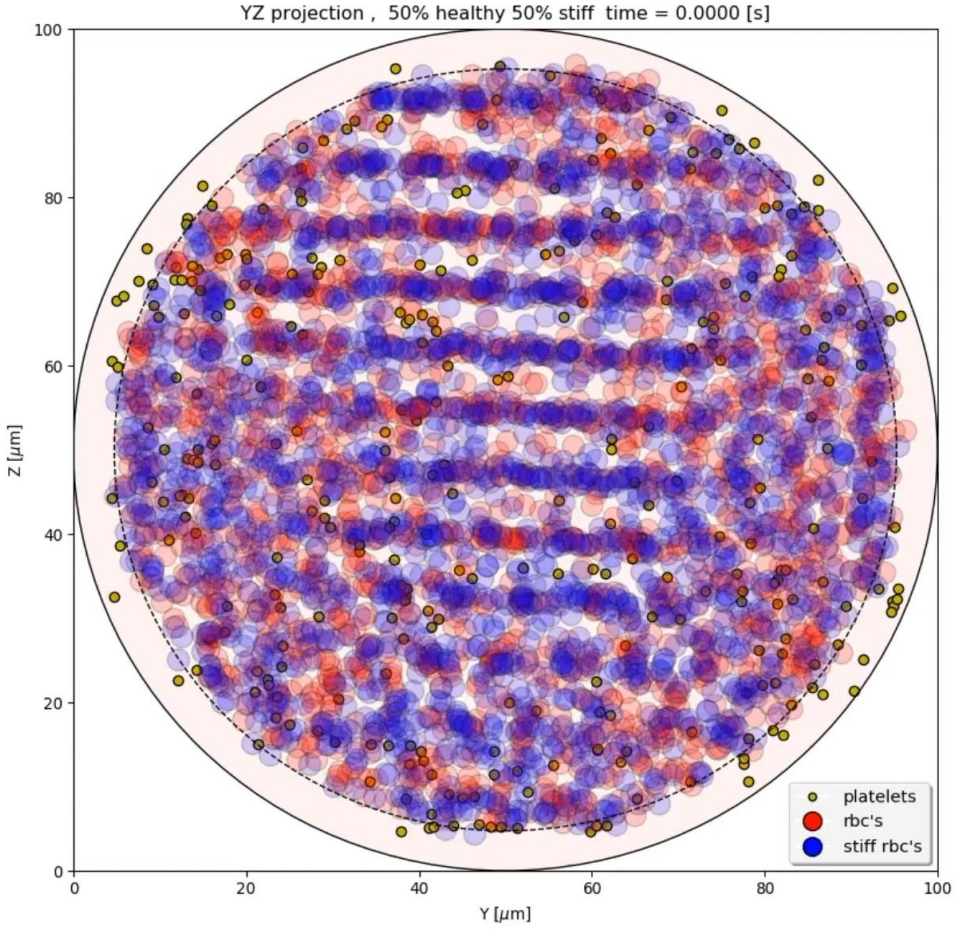


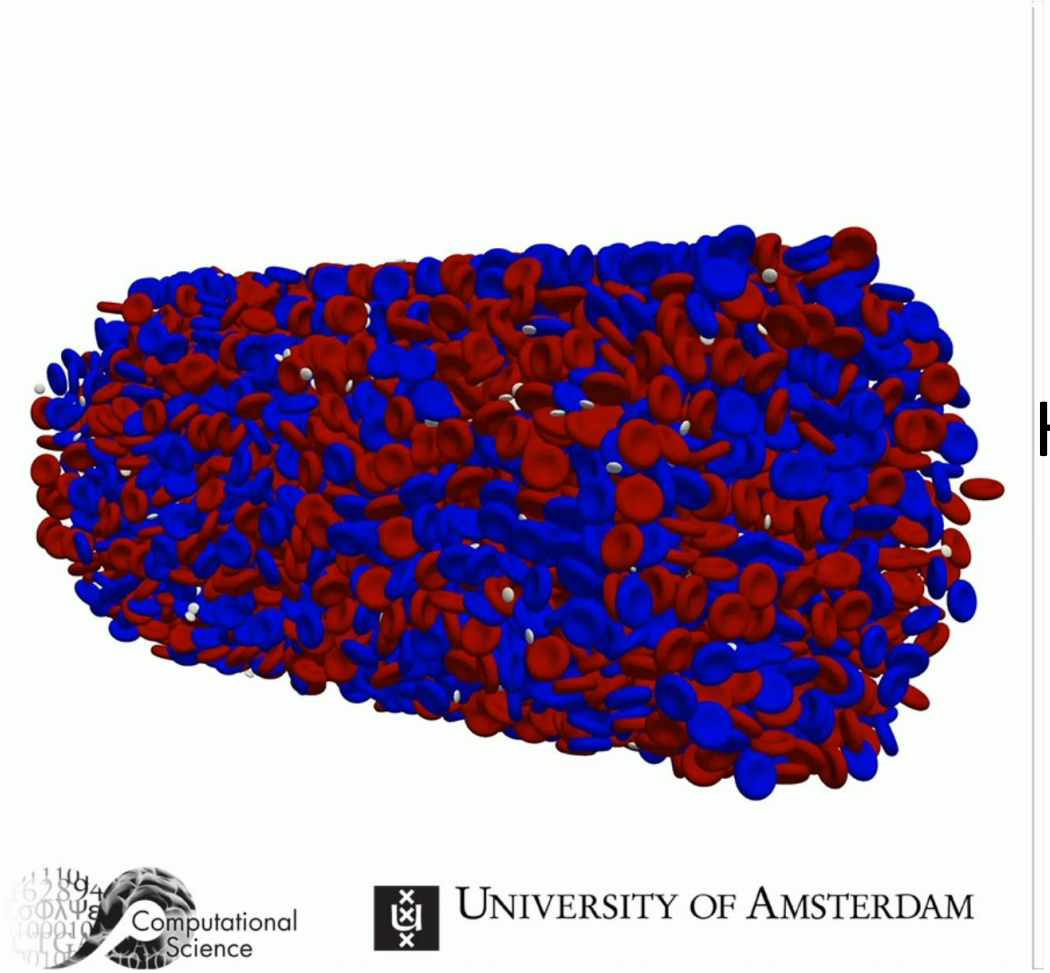
- Lift force effects deformable RBCs greater



Bulk suspension rheology

3D pipe flow simulations



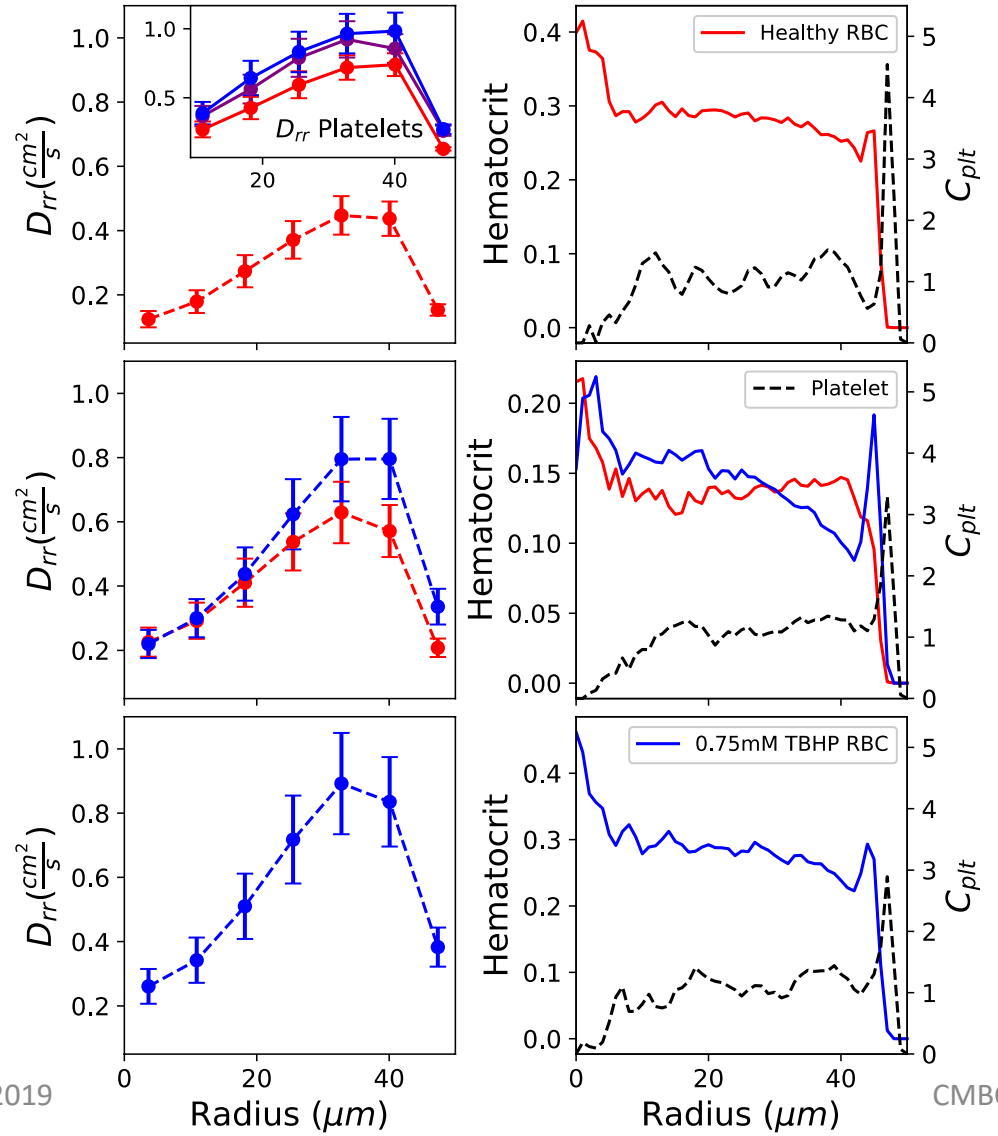


WSR:
1000 s^{-1}

Hematocrit:
30%

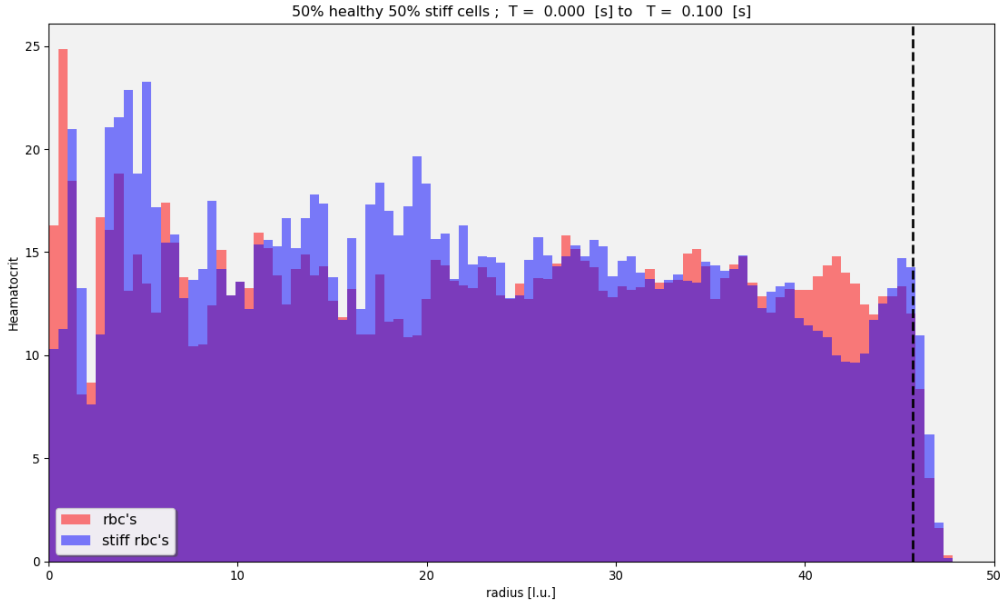
Diameter:
100 µm

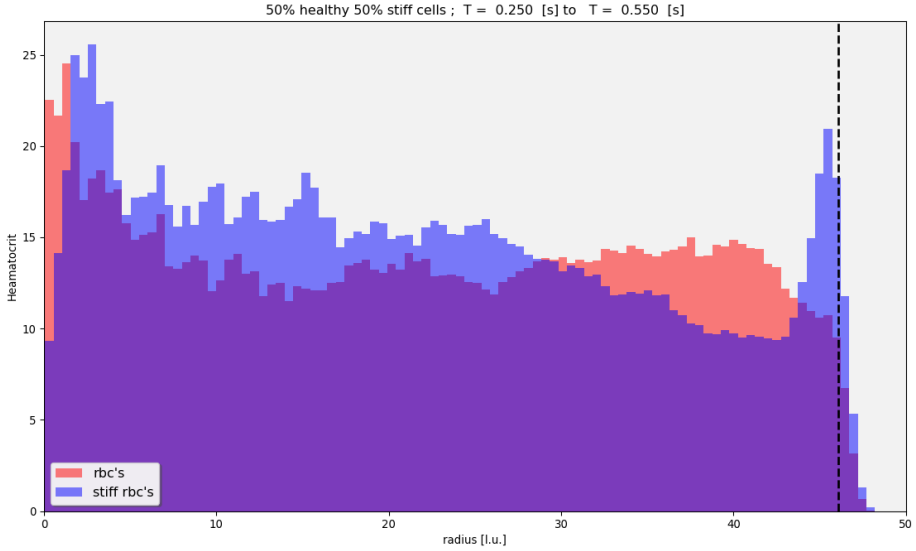
Diffusivities and cell distributions



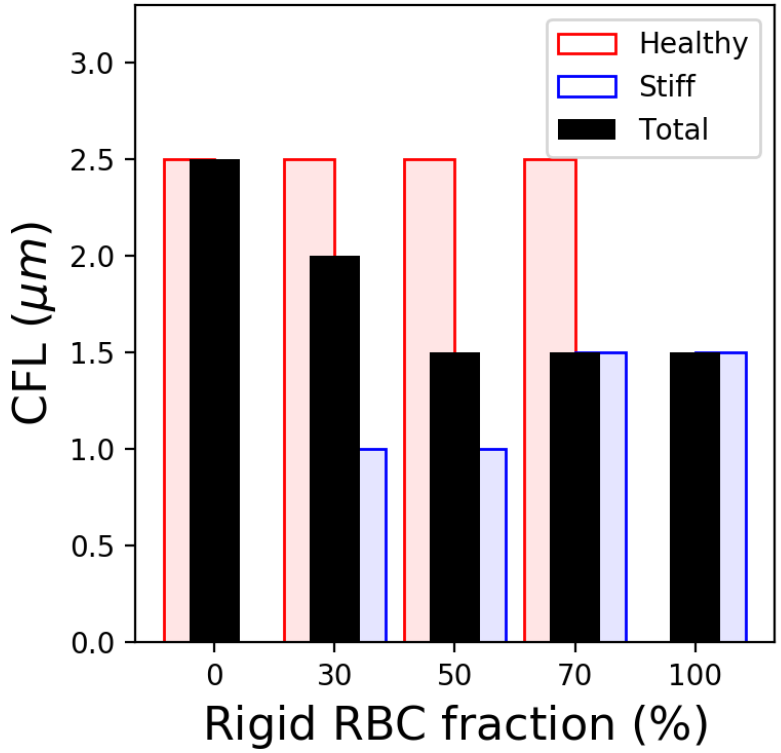
- Platelet margination decreases with increase of stiff cell fraction.
- Stiffened RBCs dominate close to vessel wall (R > 45).
- Healthy RBCs dominate in higher shear rate region (28 < R <45).

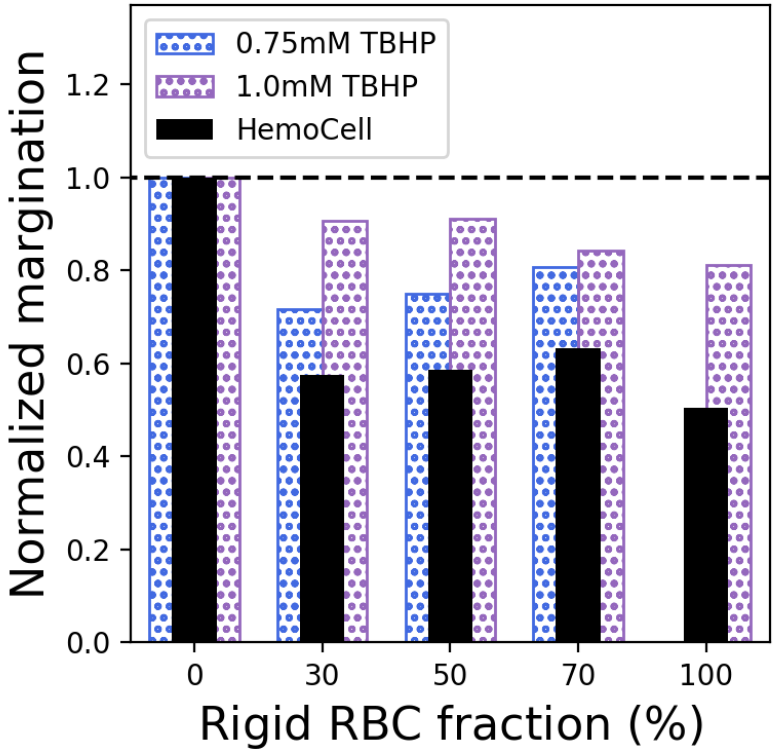
50/50 Mixture





Cell free layer and platelet margination





- Red blood cell free layer decreases as stiff RBC fraction increases.
- Margination of platelets decreases as stiff RBC fraction increases.

Conclusions

Single Cell Phenomena	Bulk Rheology Phenomena
A. Stiffened RBCs have lessened lift force and stay closer to vessel wall.	1. Cell free layer decreases with increasing stiff RBC fraction. 2. Margination decreases with increasing stiff RBC fraction.
B. Healthy Vs Stiff RBC collisions result in larger displacement of stiffened RBC as cell stiffness increases.	3. Healthy RBCs dominate higher shear region in pipe (28 < R <45).

Thank you!

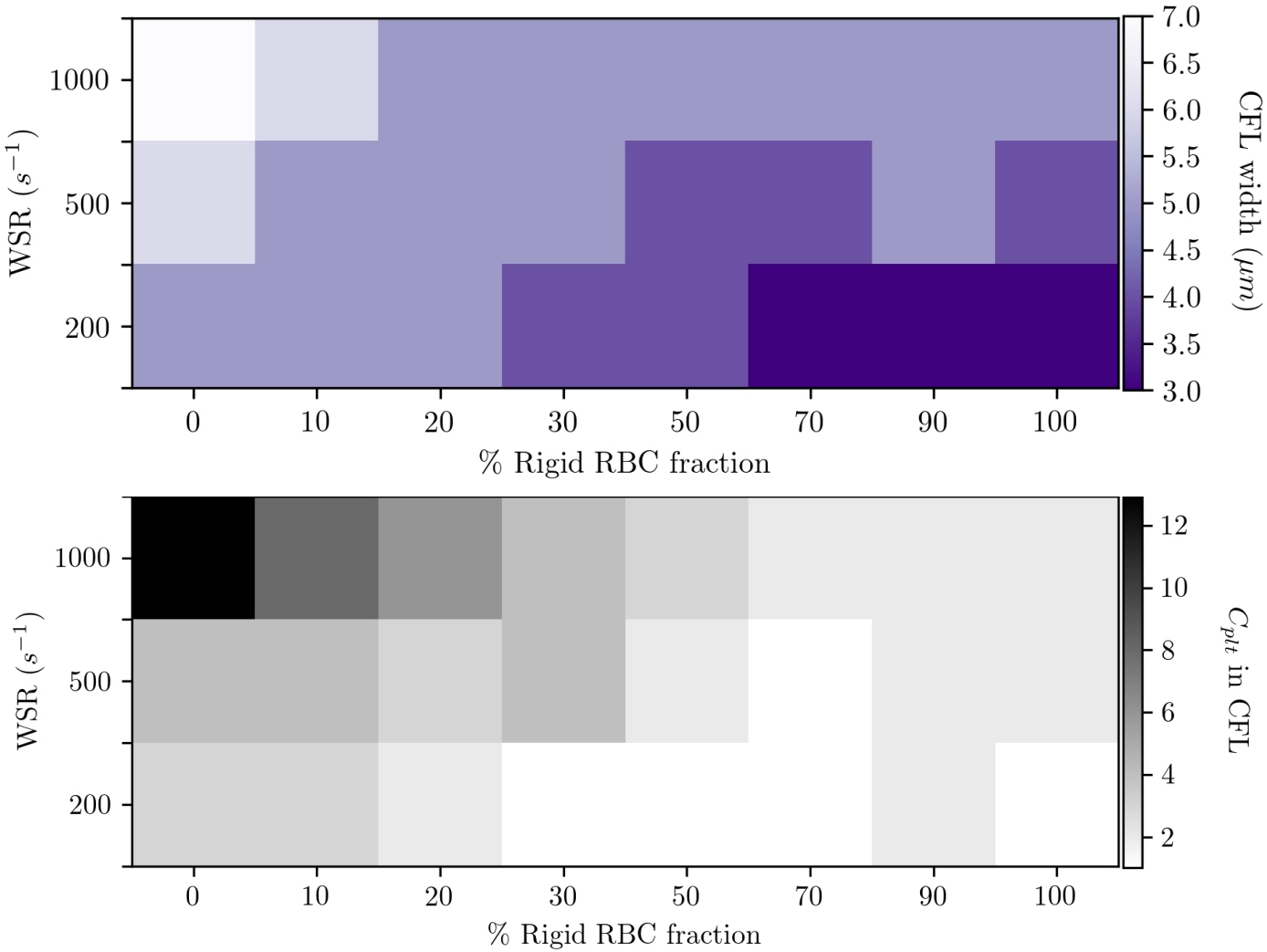
- Computational Science Lab at the University of Amsterdam
- Eniola-Adefeso Lab in the Chemical Engineering Dept. at the University of Michigan
- www.hemocell.eu

UNIVERSITY OF AMSTERDAM

References

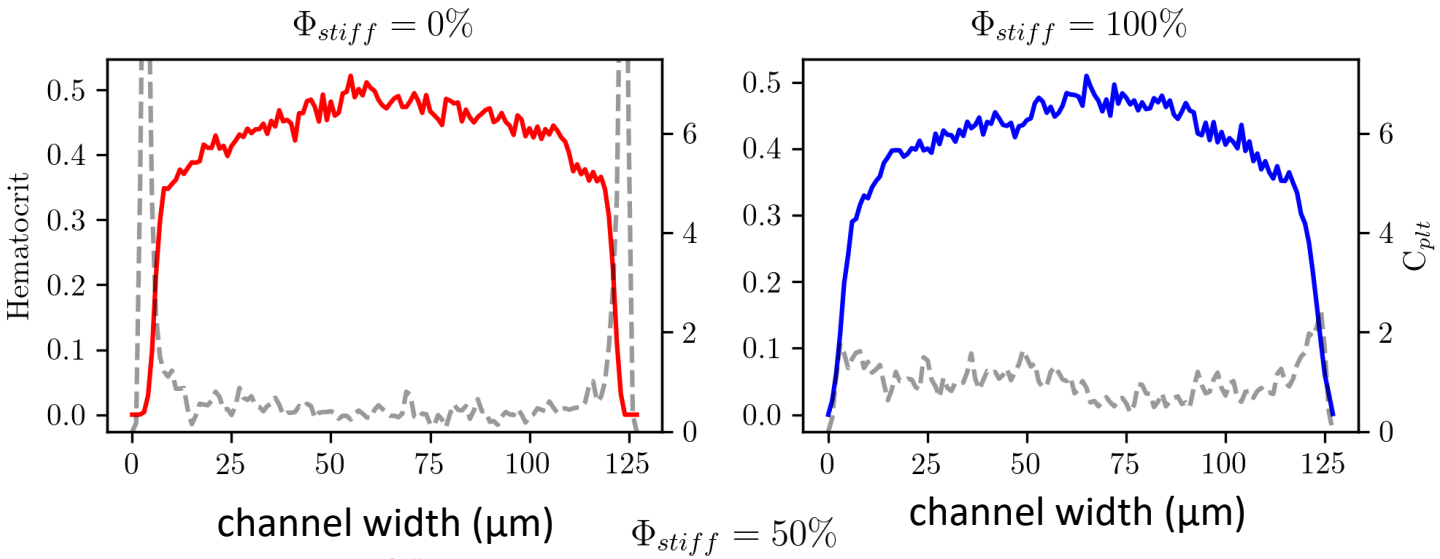
- Lasch, Jürgen, Gerhard Küllertz, and Jens Rüdiger Opalka. "Separation of erythrocytes into age-related fractions by density or size? Counterflow centrifugation." *Clinical chemistry and laboratory medicine* 38.7 (2000): 629-632.
- Gutierrez, M., Fish, M. B., Golinski, A. W., & Eniola-Adefeso, O. (2018). *Langmuir*, *34*(6), 2363-2372.
- Kapishnikov, Sergei, et al. "Biochemistry of malaria parasite infected red blood cells by X-ray microscopy." *Scientific reports* 7.1 (2017): 802.

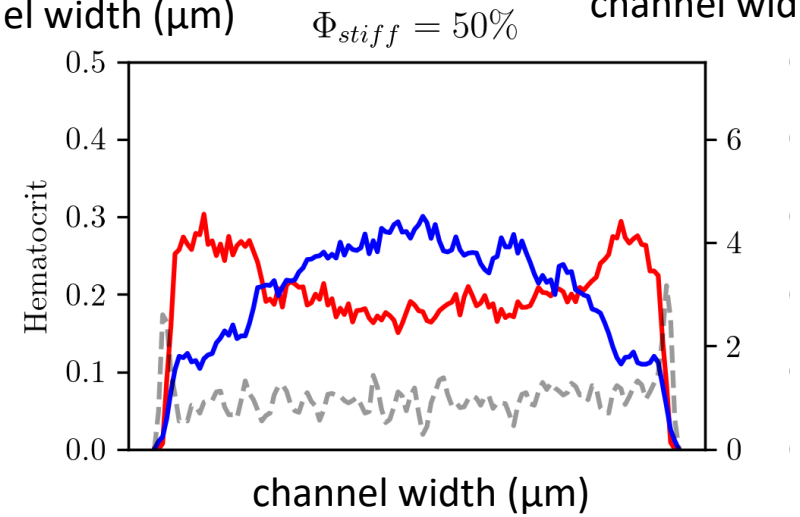
HemoCell2D suspension simulations

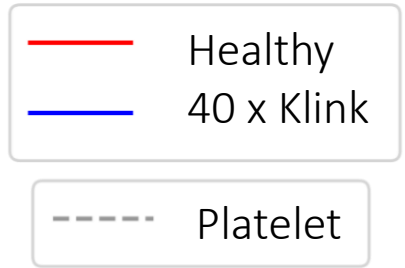


- Red blood cell free layer decreases as stiff RBC fraction increases.
- Margination of platelets decreases as stiff RBC fraction increases.

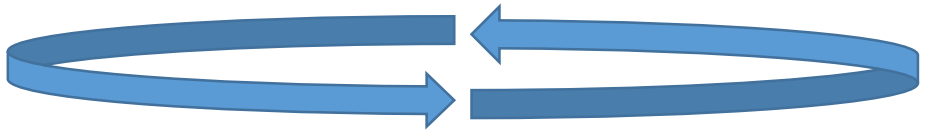
HemoCell2D suspension simulations

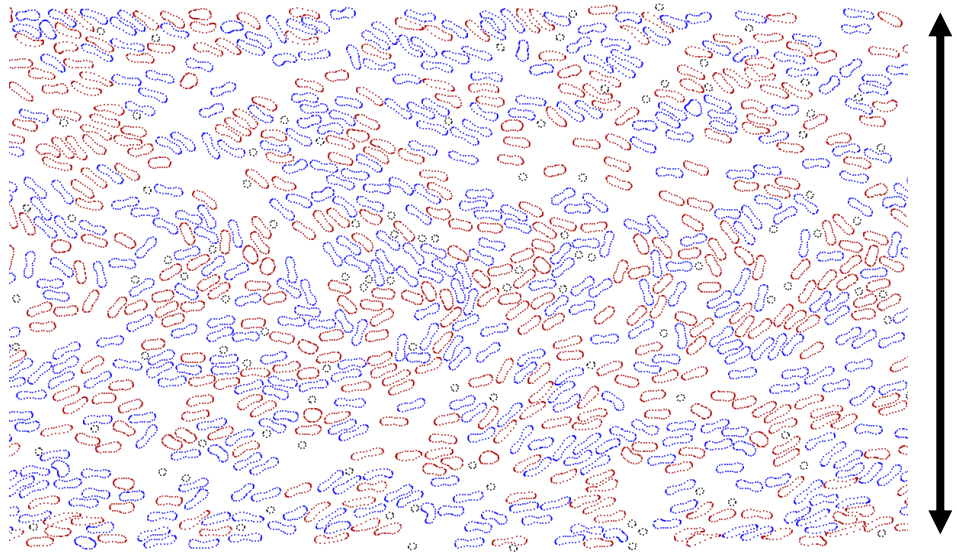






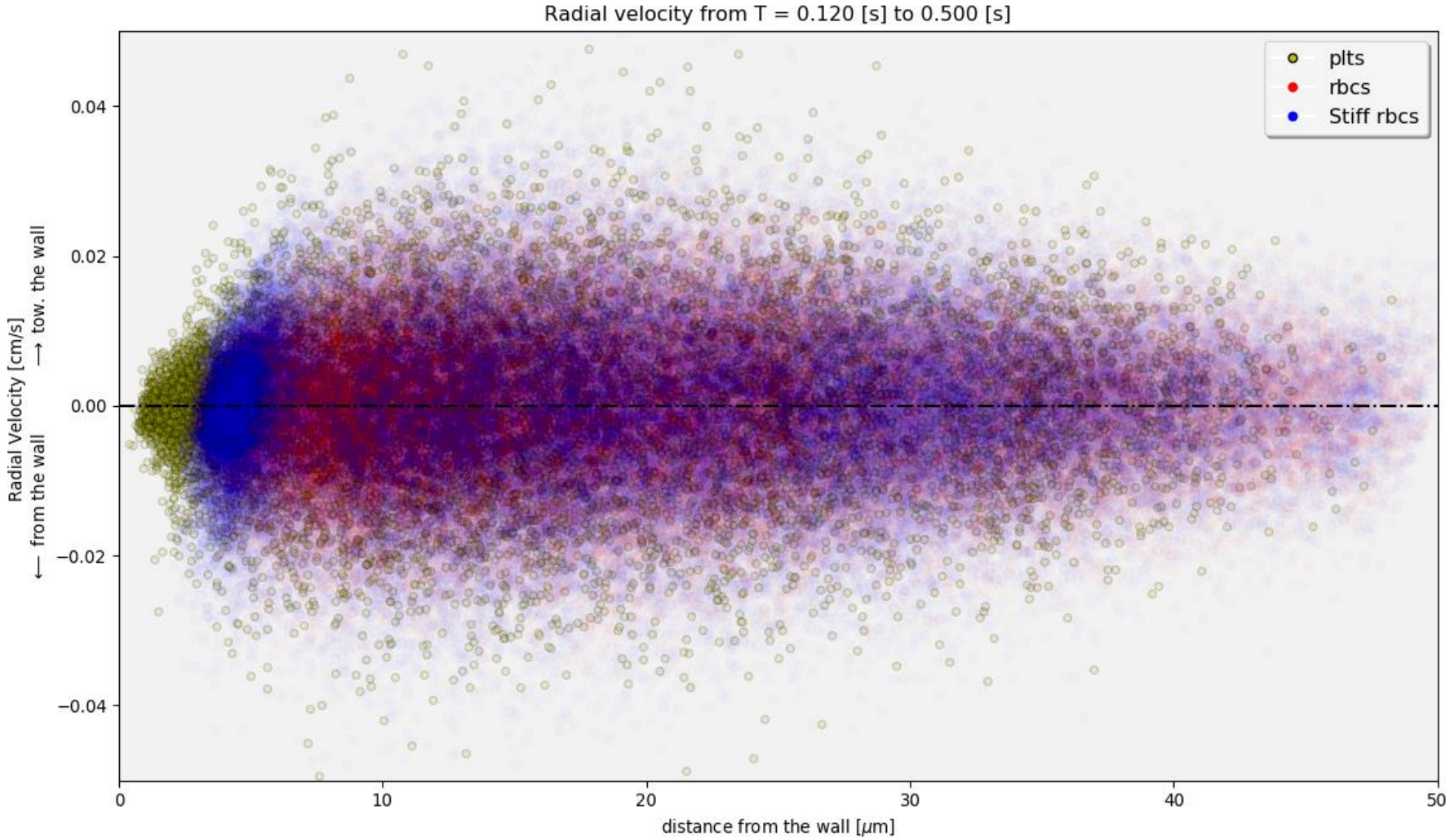
Periodic --- WSR 200, 500, 1000s^{-1}



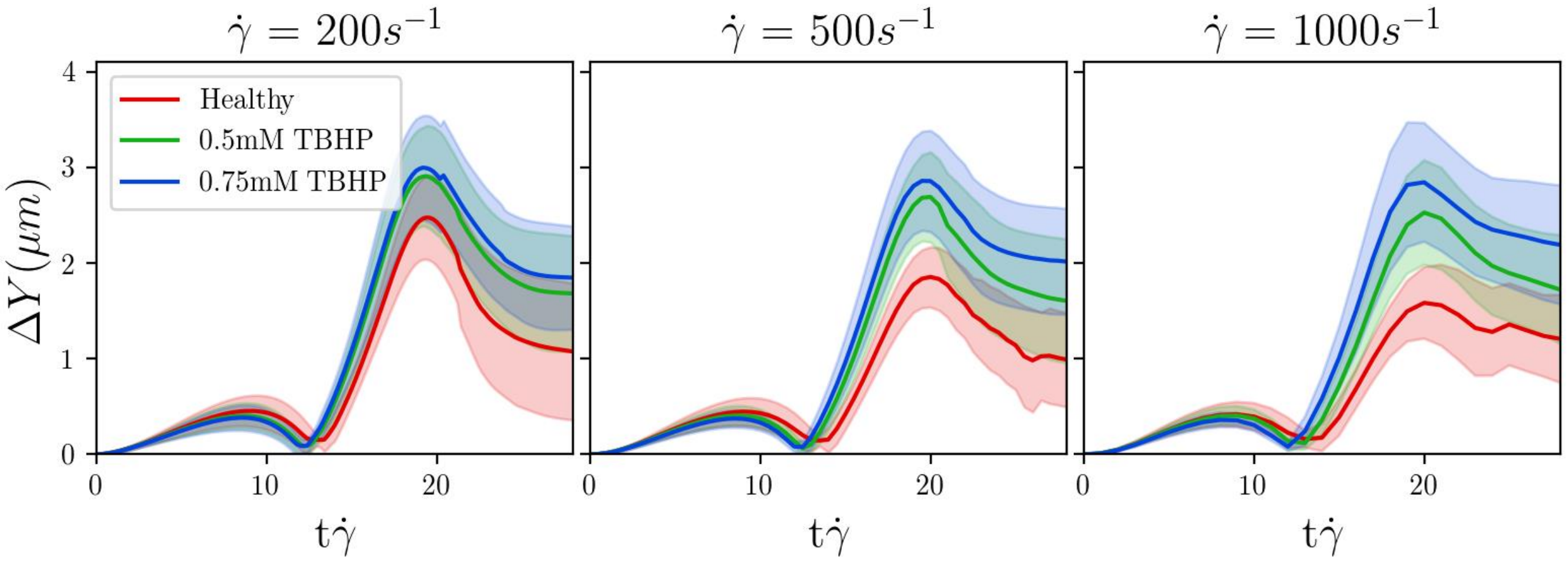


Width: 127 microns

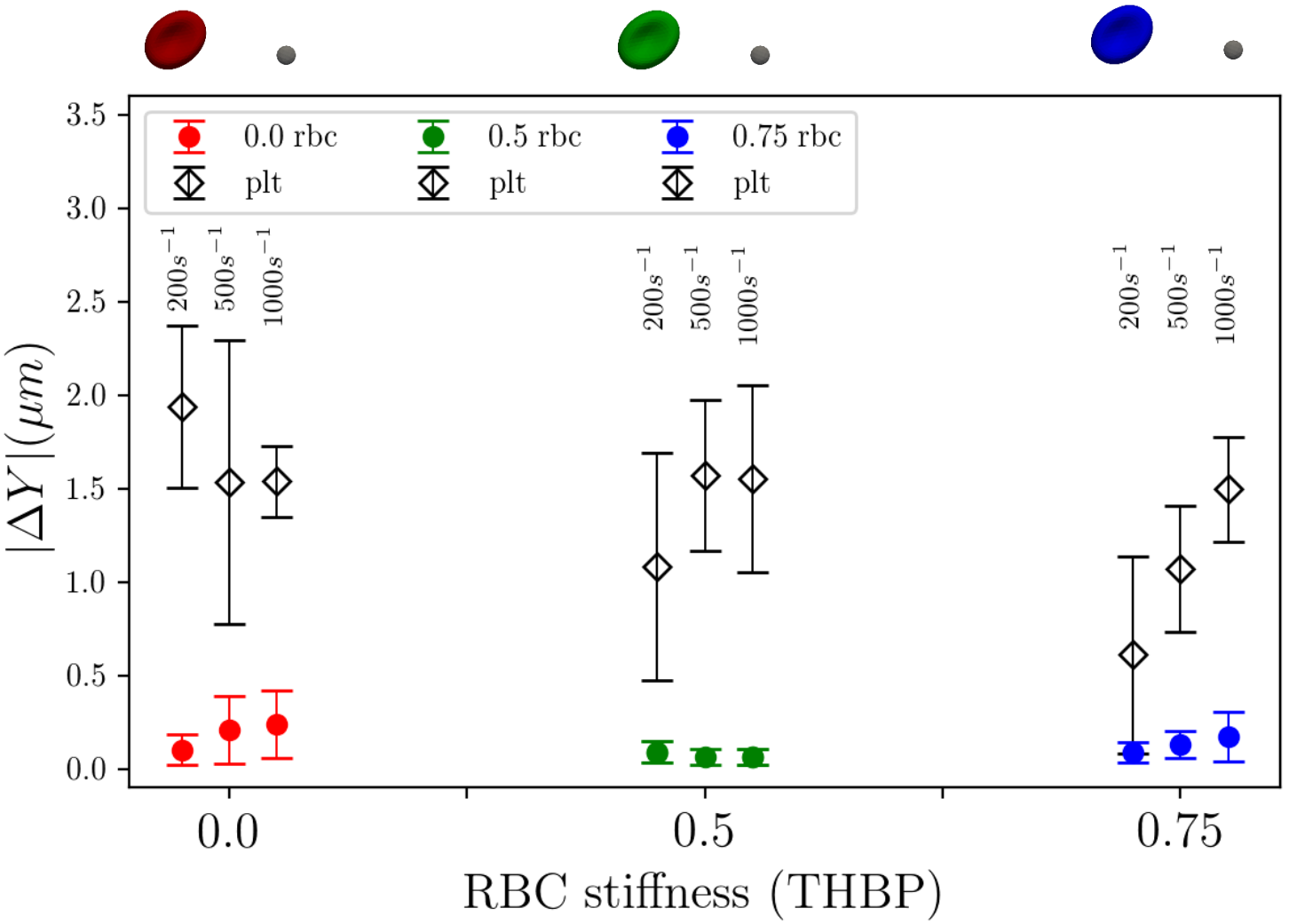
3D pipe flow simulations



Heterogeneous RBC Collisions

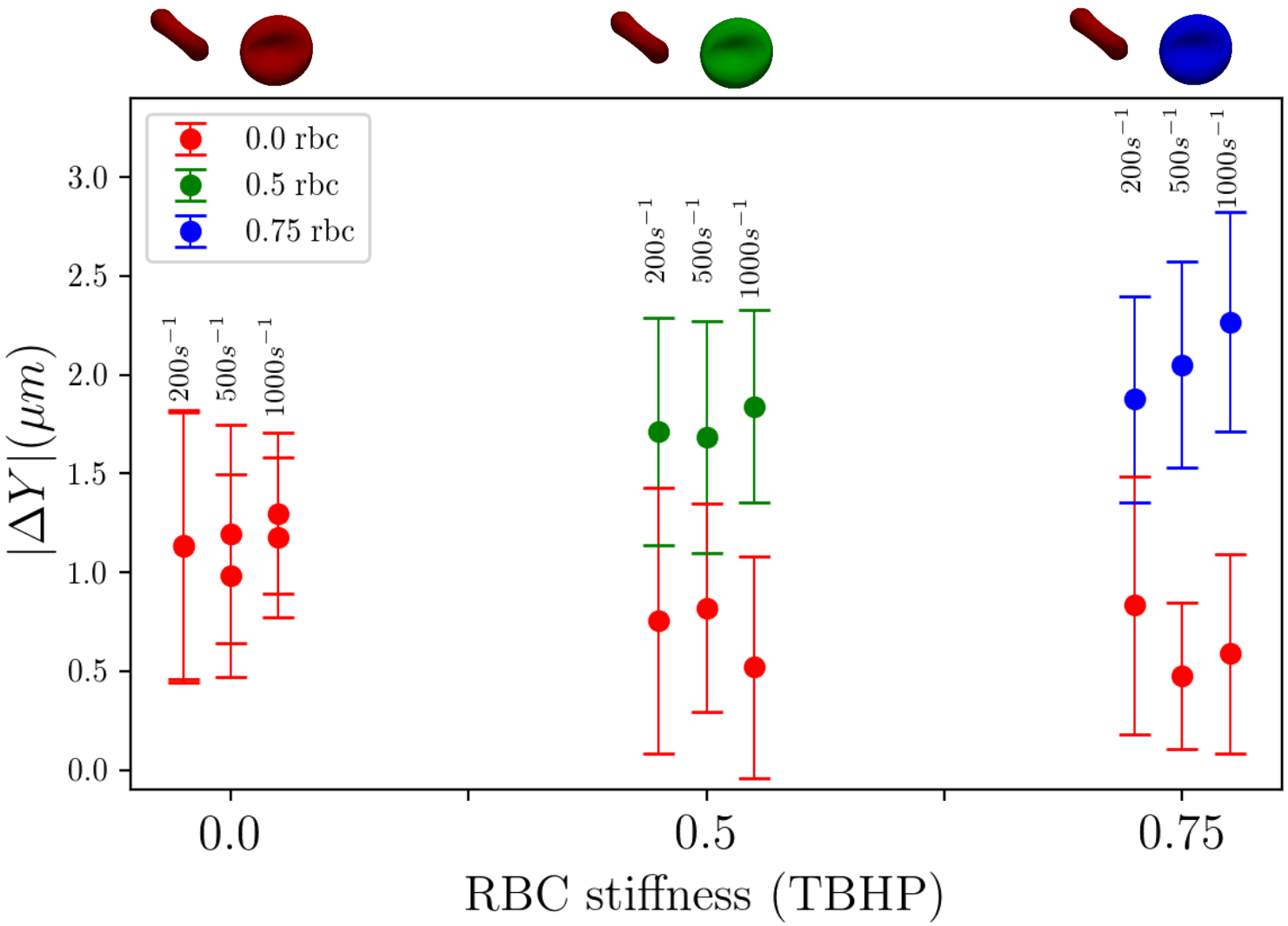


RBC-Platelet collisions



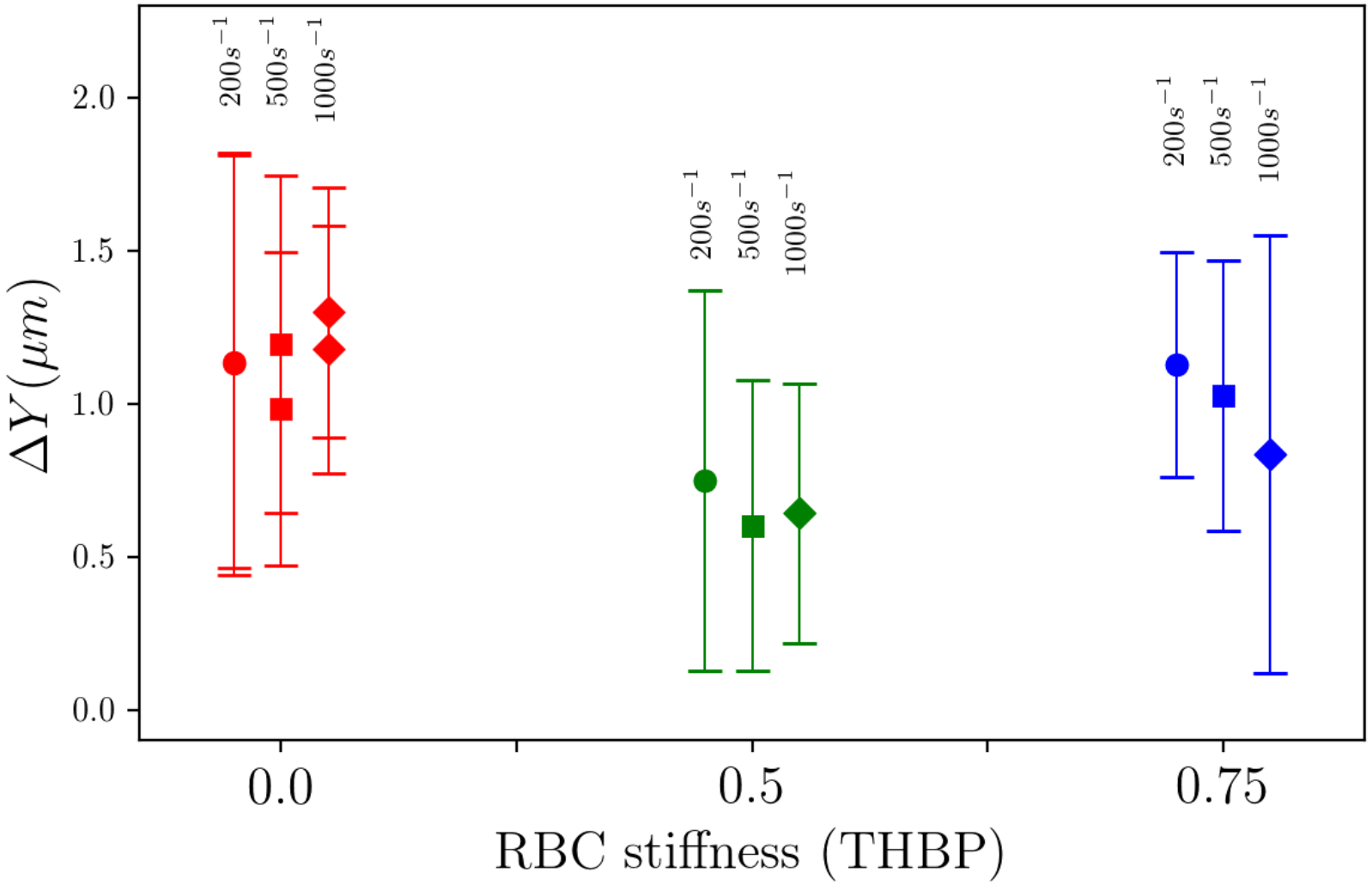
- Slight decrease in displacement of platelet with increasing red blood cell stiffness.

Heterogeneous RBC Collisions



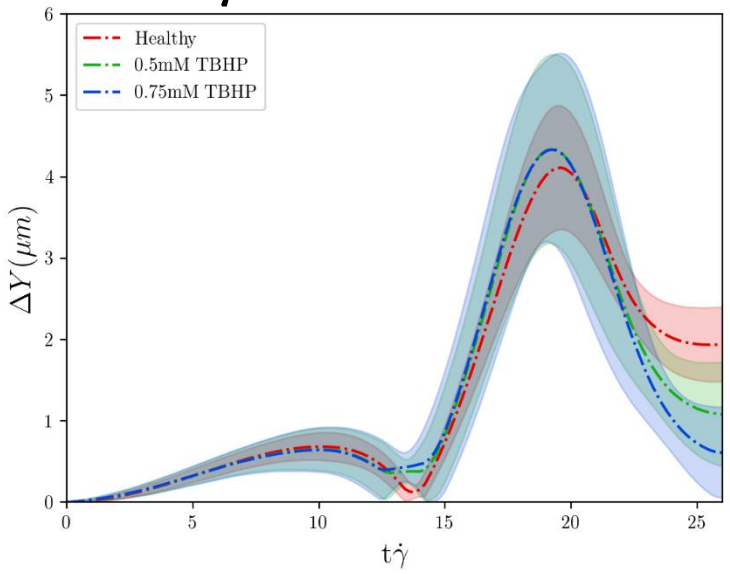
- Stiff RBC displacement increases as cell stiffness increases.
- This effect is increased with increasing shear rate.

Homogenous RBC Collisions

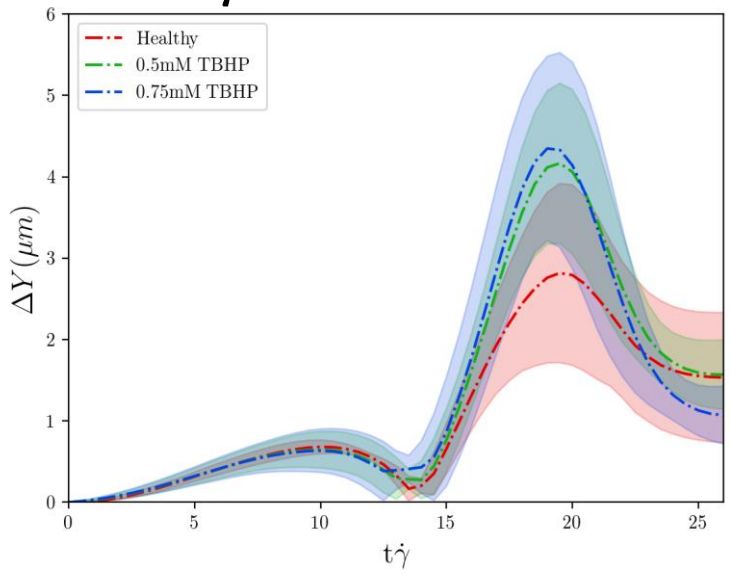


RBC vs. Platelet

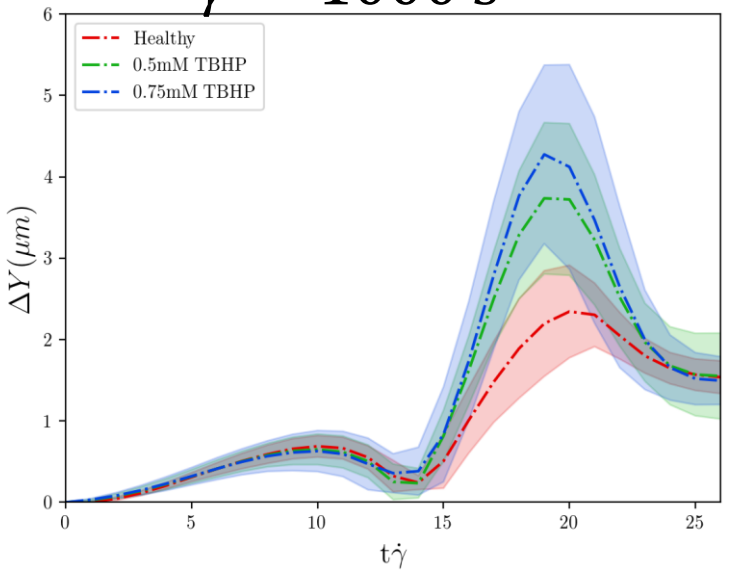
$\dot{\gamma} = 200\ s^{-1}$



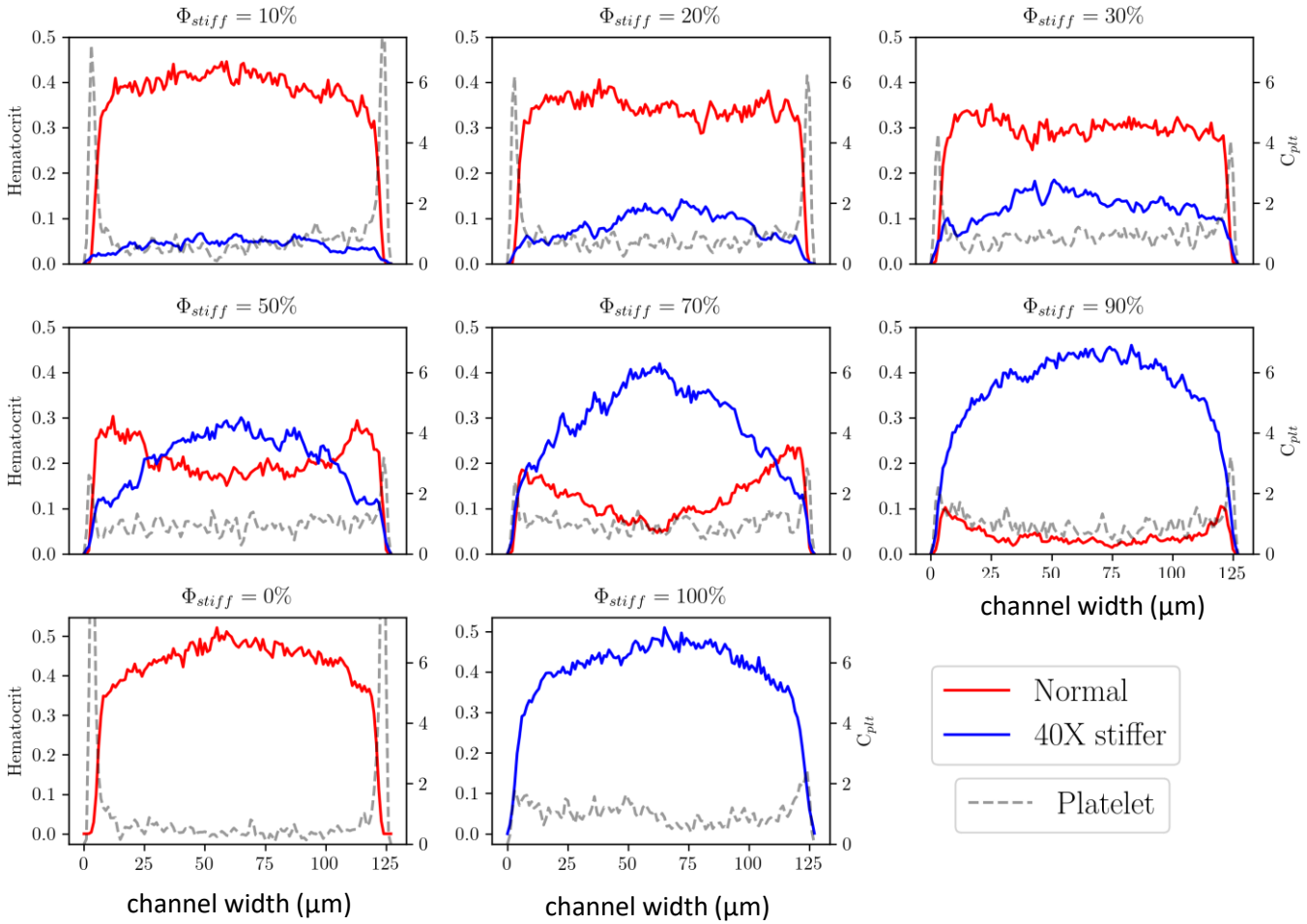
$\dot{\gamma} = 500\ s^{-1}$



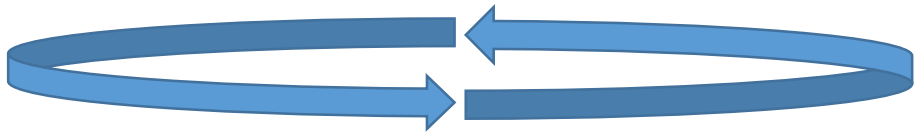
$\dot{\gamma} = 1000\ s^{-1}$

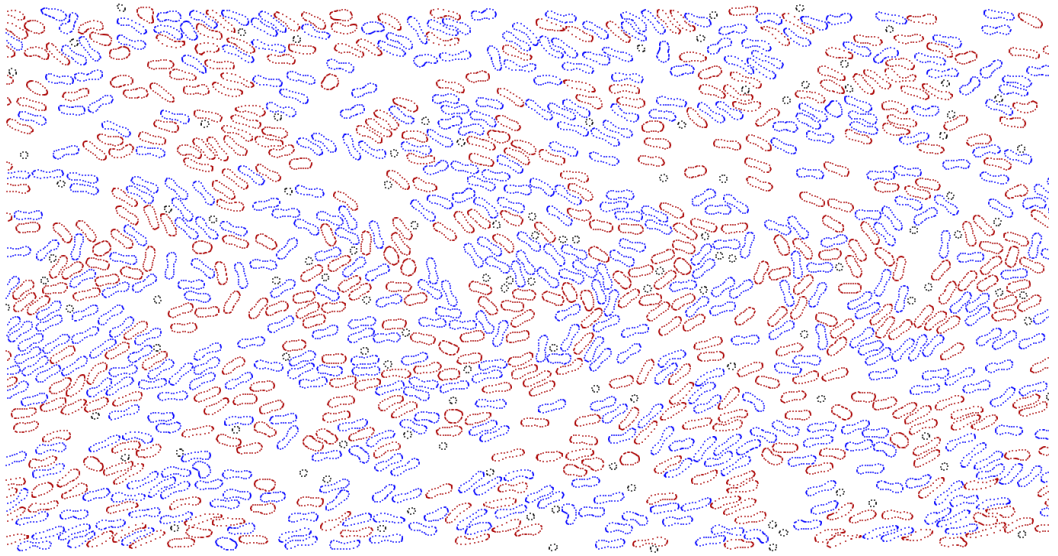


HemoCell2D suspension simulations



Periodic --- WSR 200, 500, 1000s^{-1}





Width: 127 microns