Optical Tweezers towards Single-Cell Analysis

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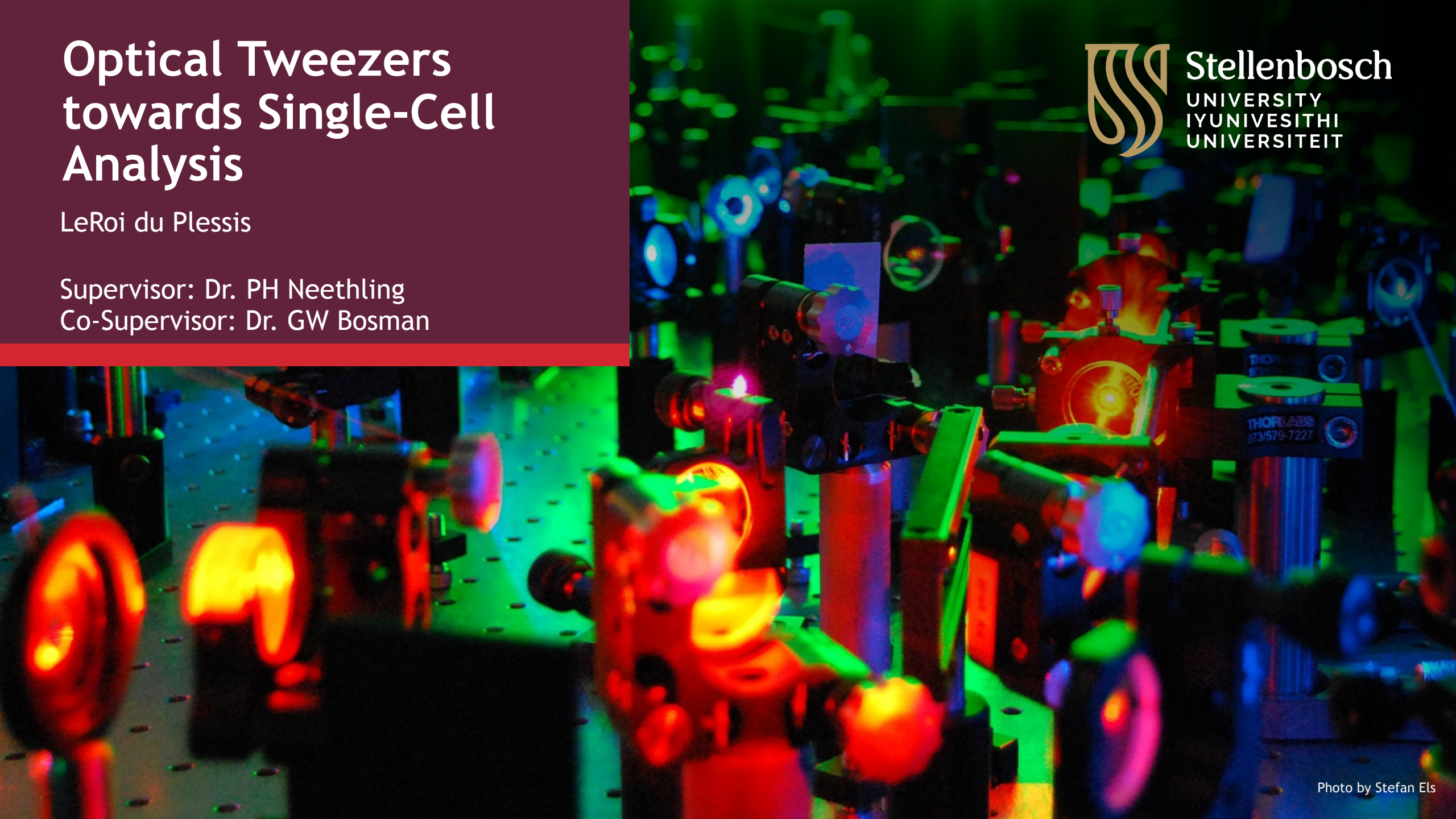


Photo by Stefan Els

Overview

- Optical tweezers Tutorial
- Experimental setups and observations
- Microfluidics
- Review of existing integrated system

Optical Tweezers

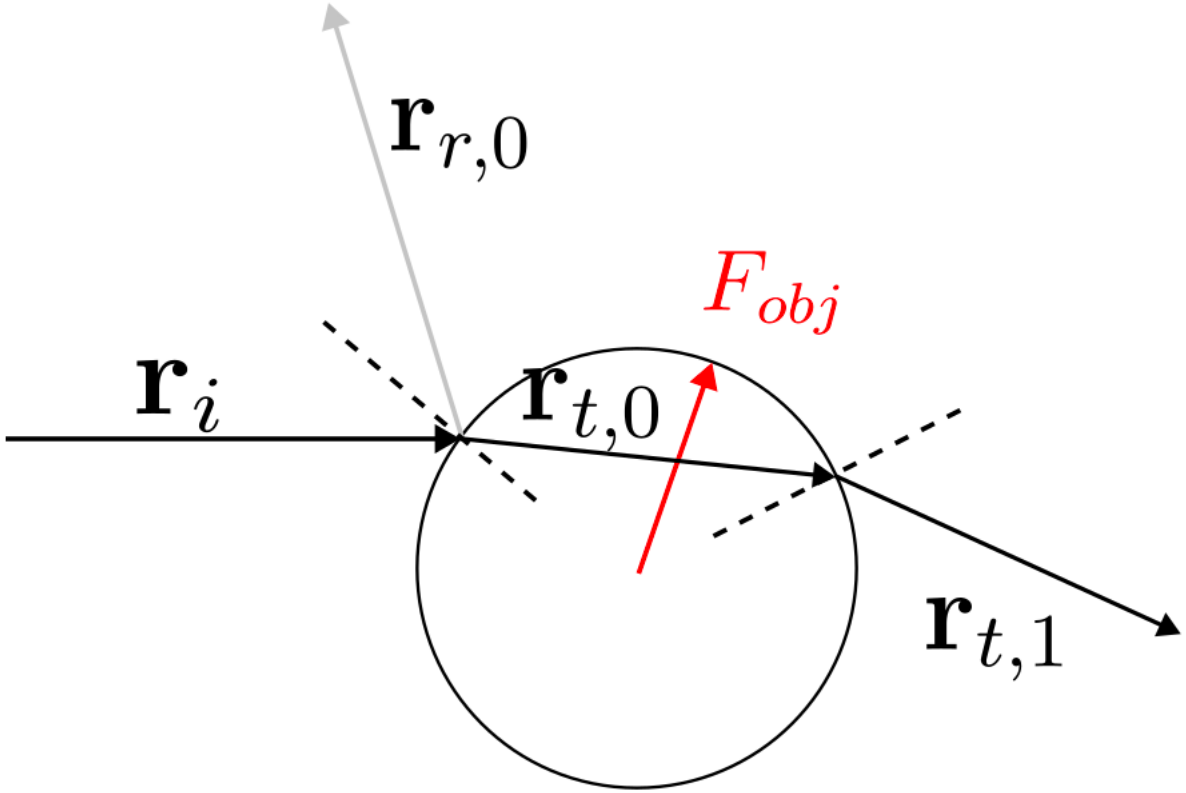
- Ray optics perspective with momentum consideration:

$$\boldsymbol{p_i} = \boldsymbol{p}_r + \boldsymbol{p}_t + \boldsymbol{p}_{obj}$$

Where $|\boldsymbol{p}_i| = \frac{h}{\lambda}$

- Beam with initial power P,photon energy $u = \frac{hc}{\lambda}$ and photon flux $N = \frac{P}{u}$ gives a resulting force to micro-object:

$$\boldsymbol{F}_{obj} = \frac{n_i P_i}{c}\hat{\boldsymbol{r}}_i - \sum_{\boldsymbol{m=0}}^{\boldsymbol{1}} \frac{n_t P_{t,m}}{c}\hat{\boldsymbol{r}}_{t,m} - \frac{n_r P_{r,\boldsymbol{0}}}{c}\hat{\boldsymbol{r}}_{r,0}$$



Jones, P. H., Maragò, O. M. and Volpe, G. (2015) *Optical Tweezers: Principles and Applications*. Cambridge University Press. doi: 10.1017/CBO9781107279711.

Optical Tweezers

- Most of the light exits the system after the first two scattering events, however some of the light remains in the particle.

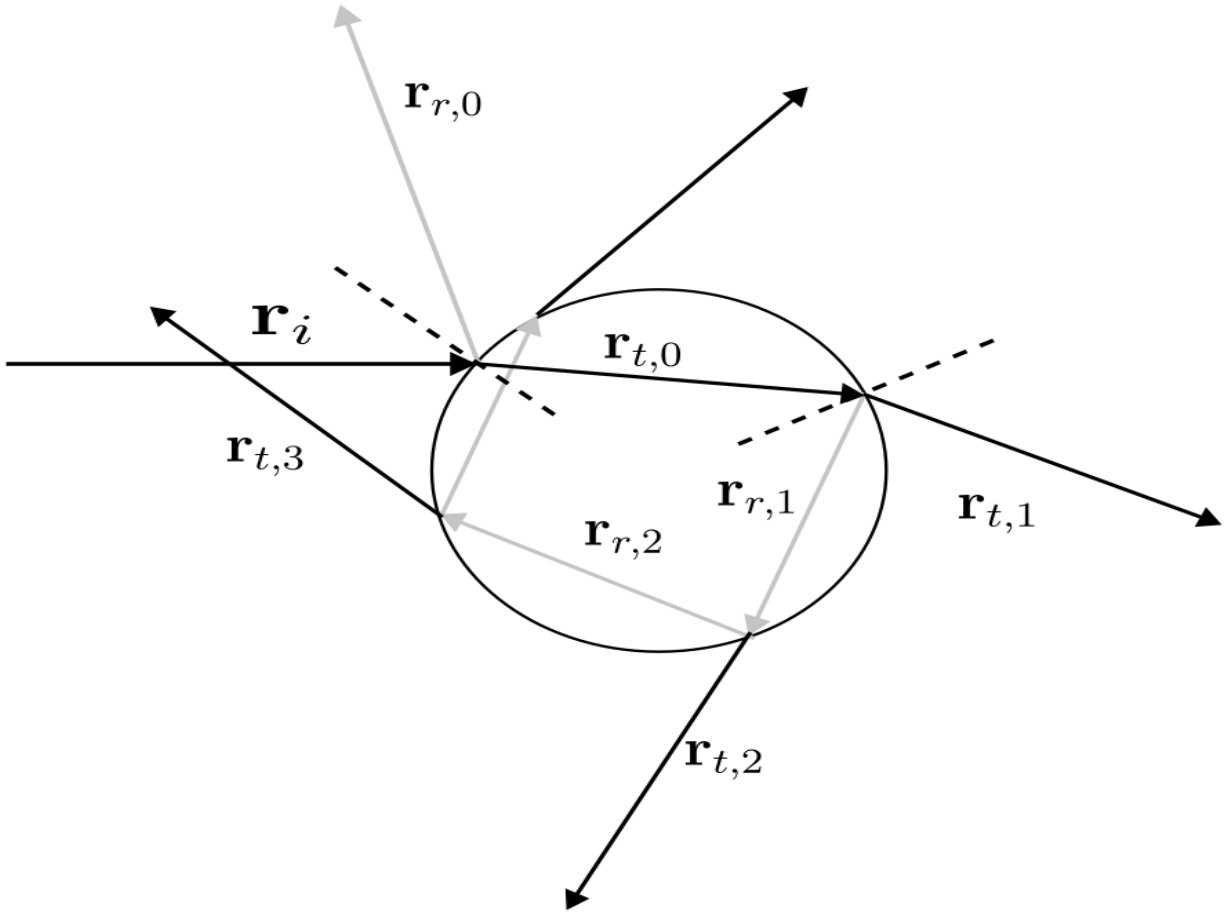
$$\boldsymbol{F}_{obj} = \frac{n_i P_i}{c}\hat{\boldsymbol{r}}_i - \sum_{m=0}^{\infty}\frac{n_t P_{t,m}}{c}\hat{\boldsymbol{r}}_{t,m} - \frac{n_r P_{r,0}}{c}\hat{\boldsymbol{r}}_{r,0}$$

$$\boldsymbol{F}_{obj} = \sum_{\boldsymbol{n}}\left[\frac{n_i P_i^{(n)}}{c}\hat{\boldsymbol{r}}_i{}^{(n)} - \sum_{m=0}^{\infty}\frac{n_t P_{t,m}^{(n)}}{c}\hat{\boldsymbol{r}}_{t,m}{}^{(n)} - \frac{n_r P_{r,0}^{(n)}}{c}\hat{\boldsymbol{r}}_{r,0}{}^{(n)}\right]$$

- The total force on the particle is broken up into the gradient- and scattering forces:

$$\boldsymbol{F}_{obj} = F_g\hat{\boldsymbol{r}}_\perp + F_s\hat{\boldsymbol{r}}_i$$

$$\boldsymbol{F}_{obj} = \frac{n_i P_i}{c}Q_g\hat{\boldsymbol{r}}_\perp + \frac{n_i P_i}{c}Q_s\hat{\boldsymbol{r}}_i$$



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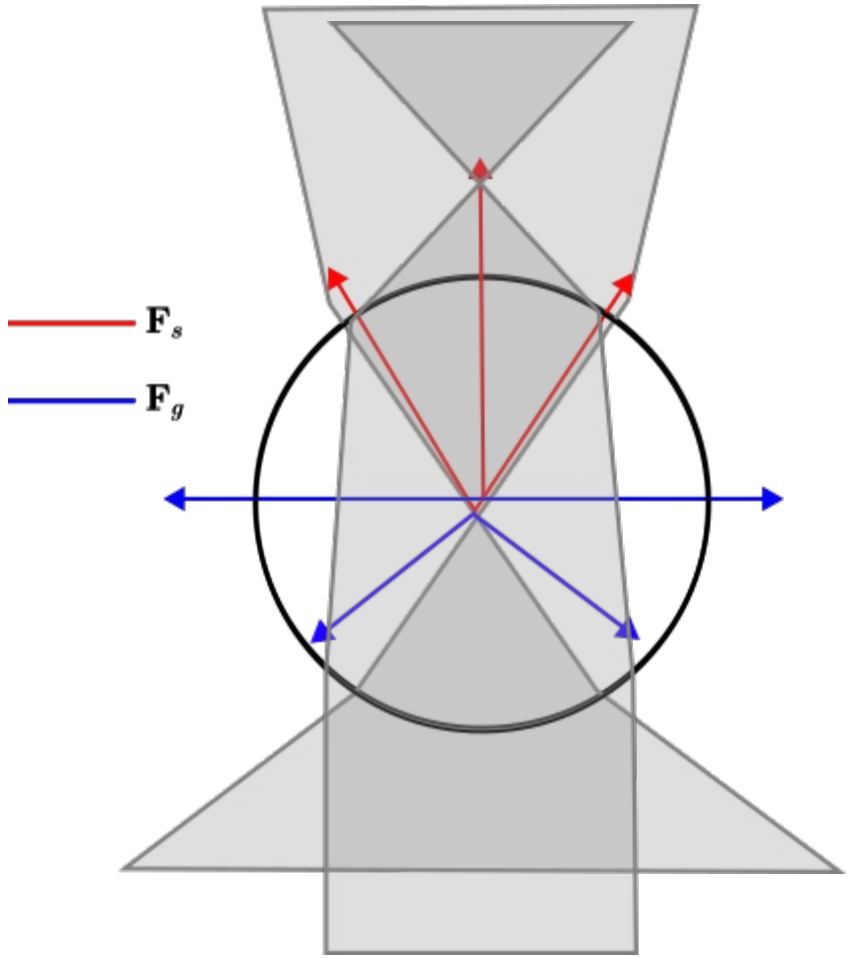
Creating a stable trap

- Collimated beam with Gaussian distribution.
- Focusing the beam causes longitudinal component of gradient force to cancel the scattering force.
- Stable trapping near the focus where $F_{g,z} = F_{s,z}$

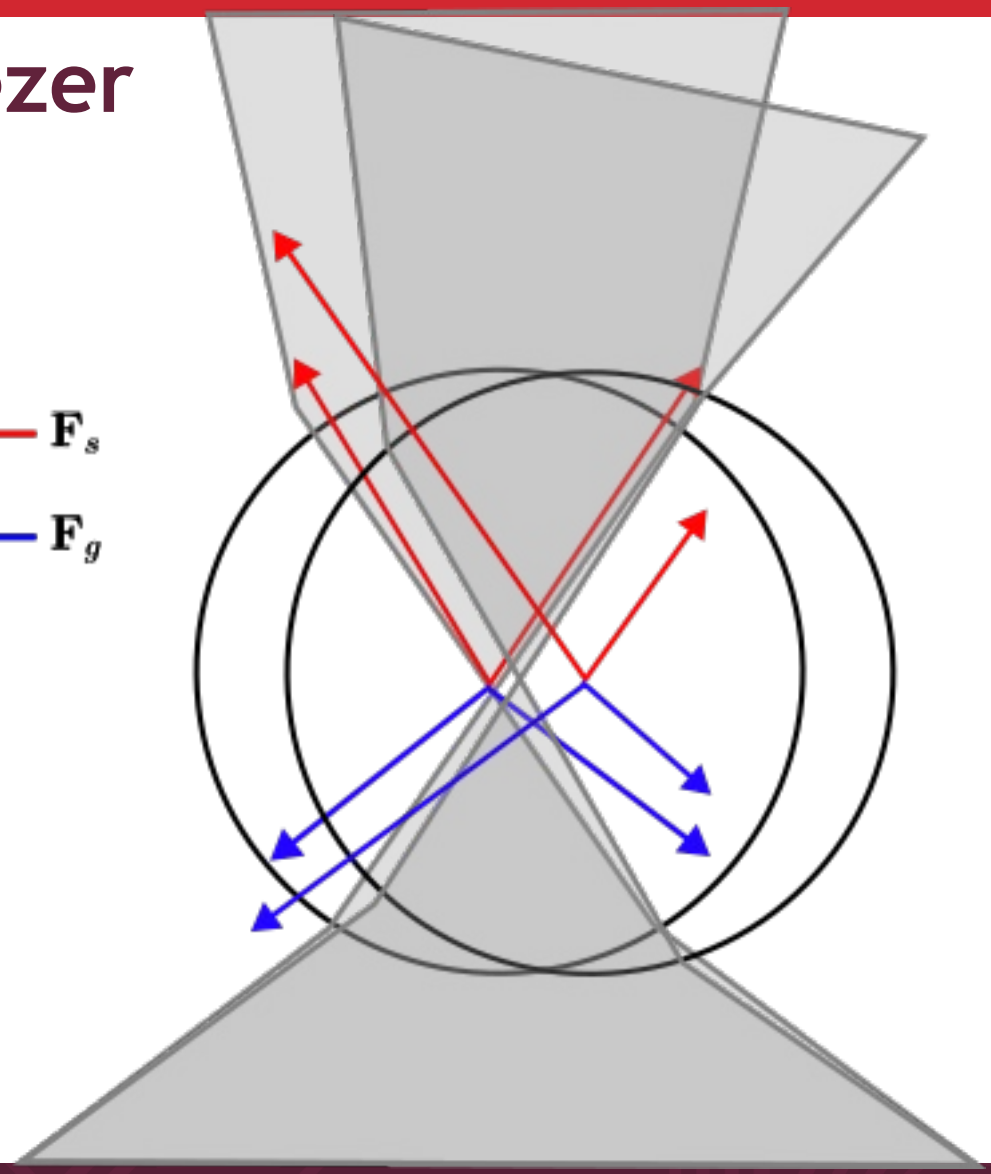


Characterization of an Optical Tweezer

- A trapped particle which is displaced from the center of the trap experiences and restoring force.

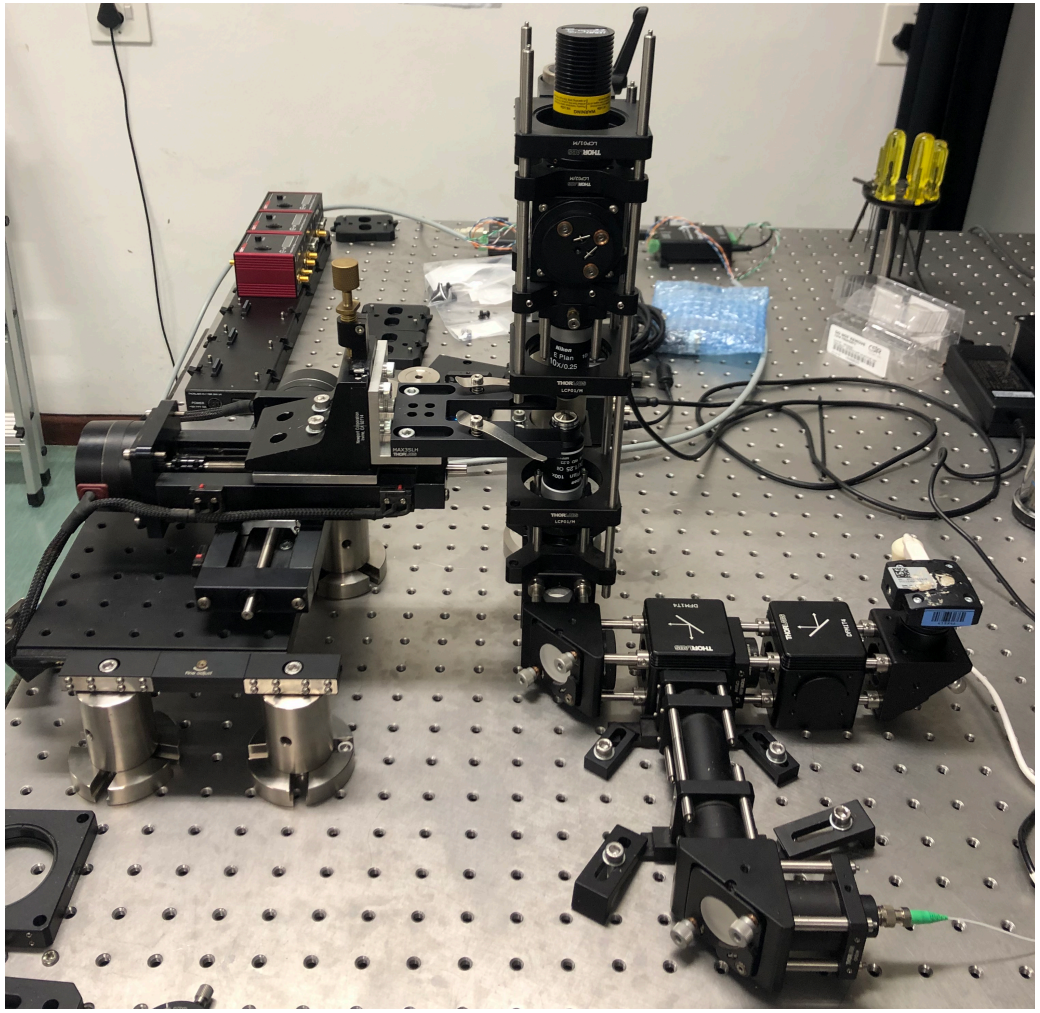
$$F = -kx$$

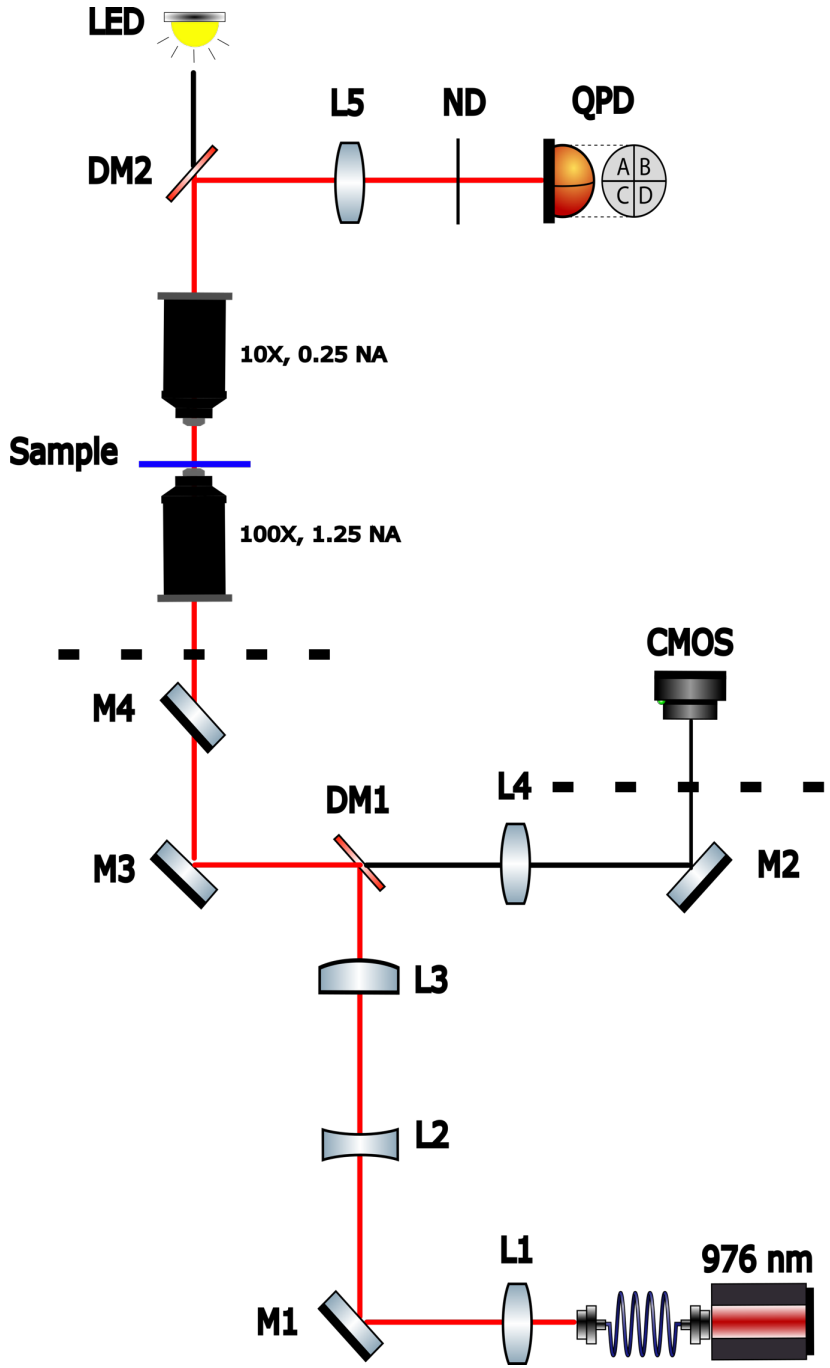
- A force calibration is achieved through comparison to thermodynamic or hydrodynamic forces.



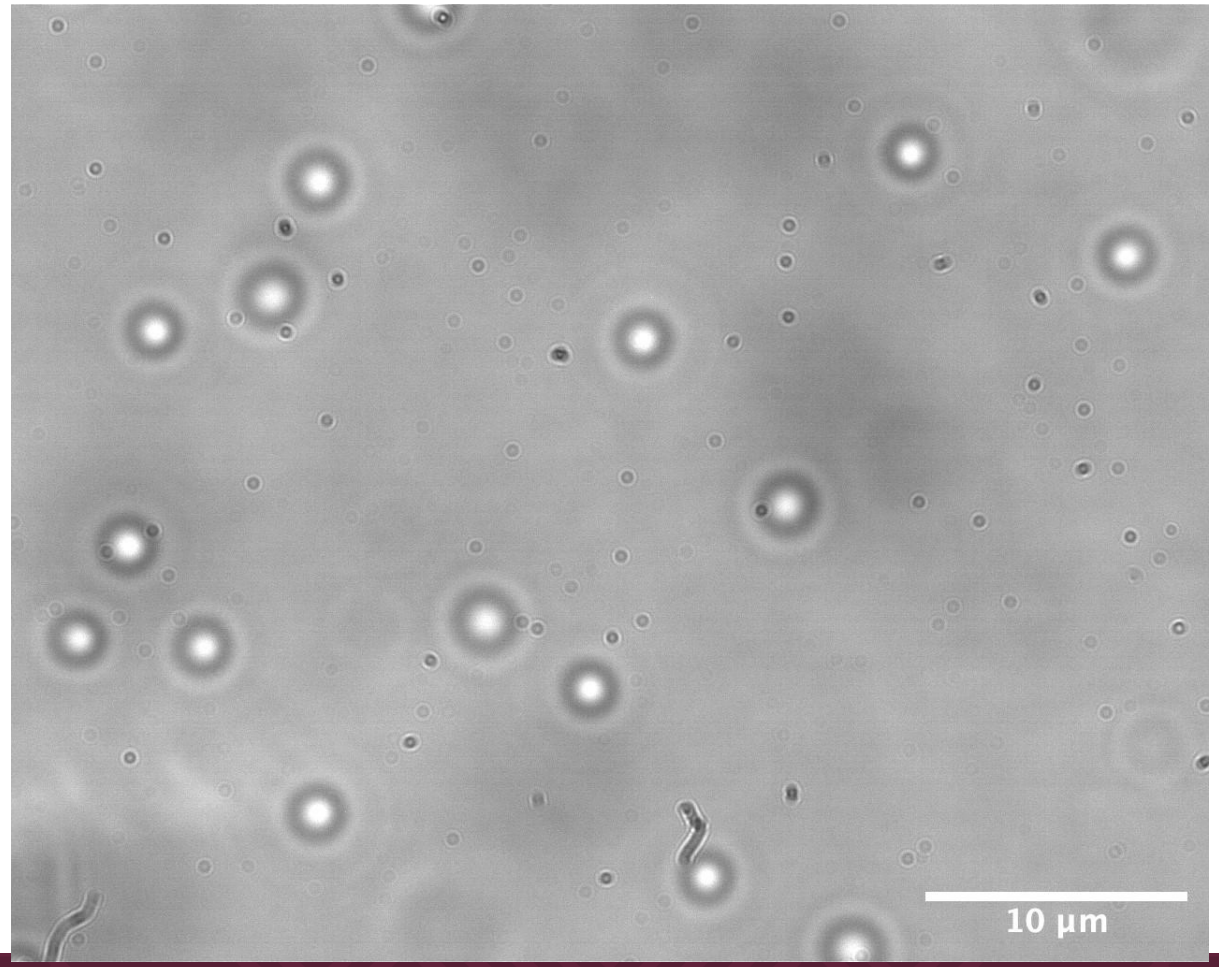
Sarshar, M., Wong, W. T. and Anvari, B. (11 2014) 'Comparative study of methods to calibrate the stiffness of a single-beam gradient-force optical tweezers over various laser trapping powers', *Journal of Biomedical Optics*, 19, p. 115001. doi: 10.1117/1.JBO.19.11.115001.

Experimental Setup

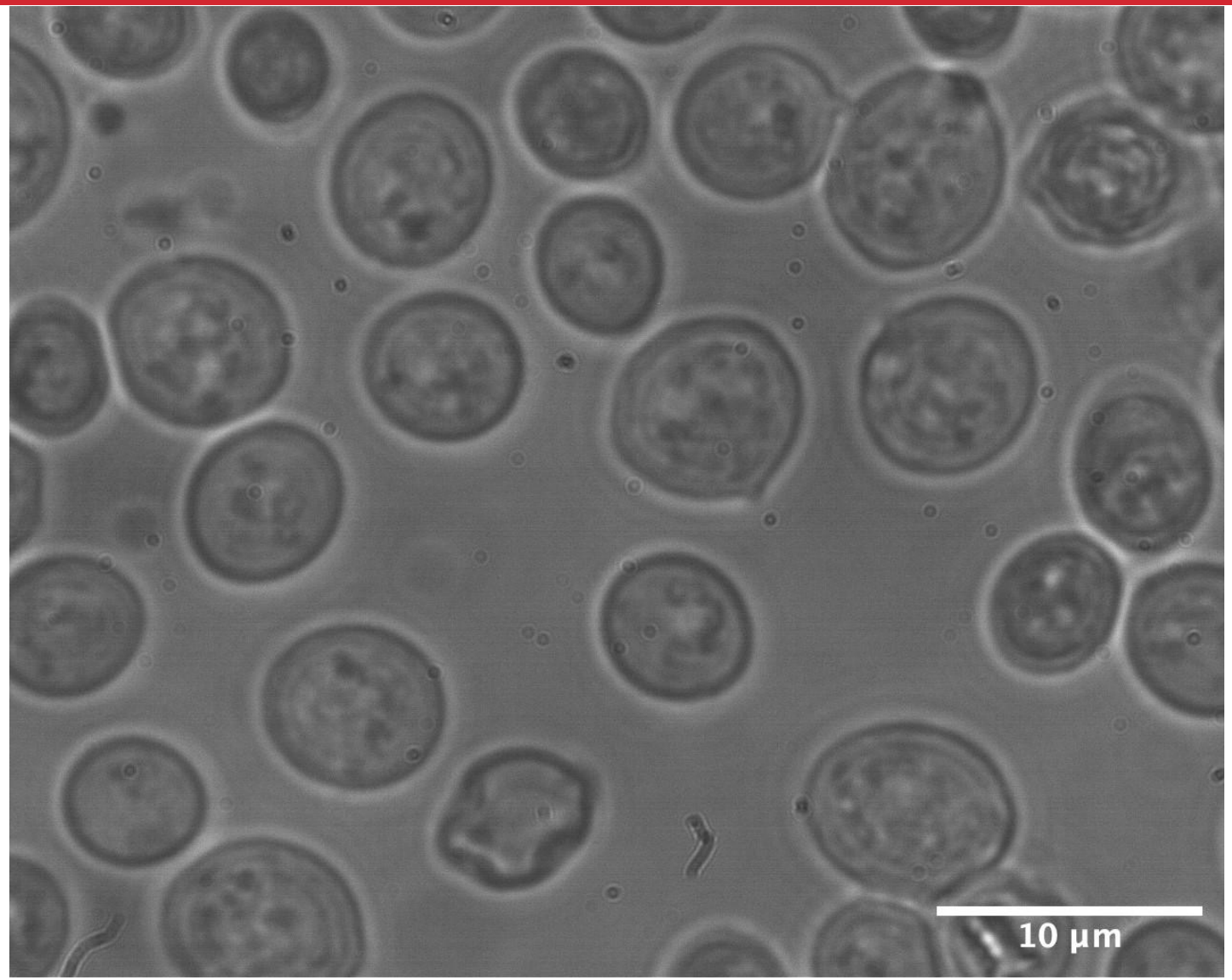




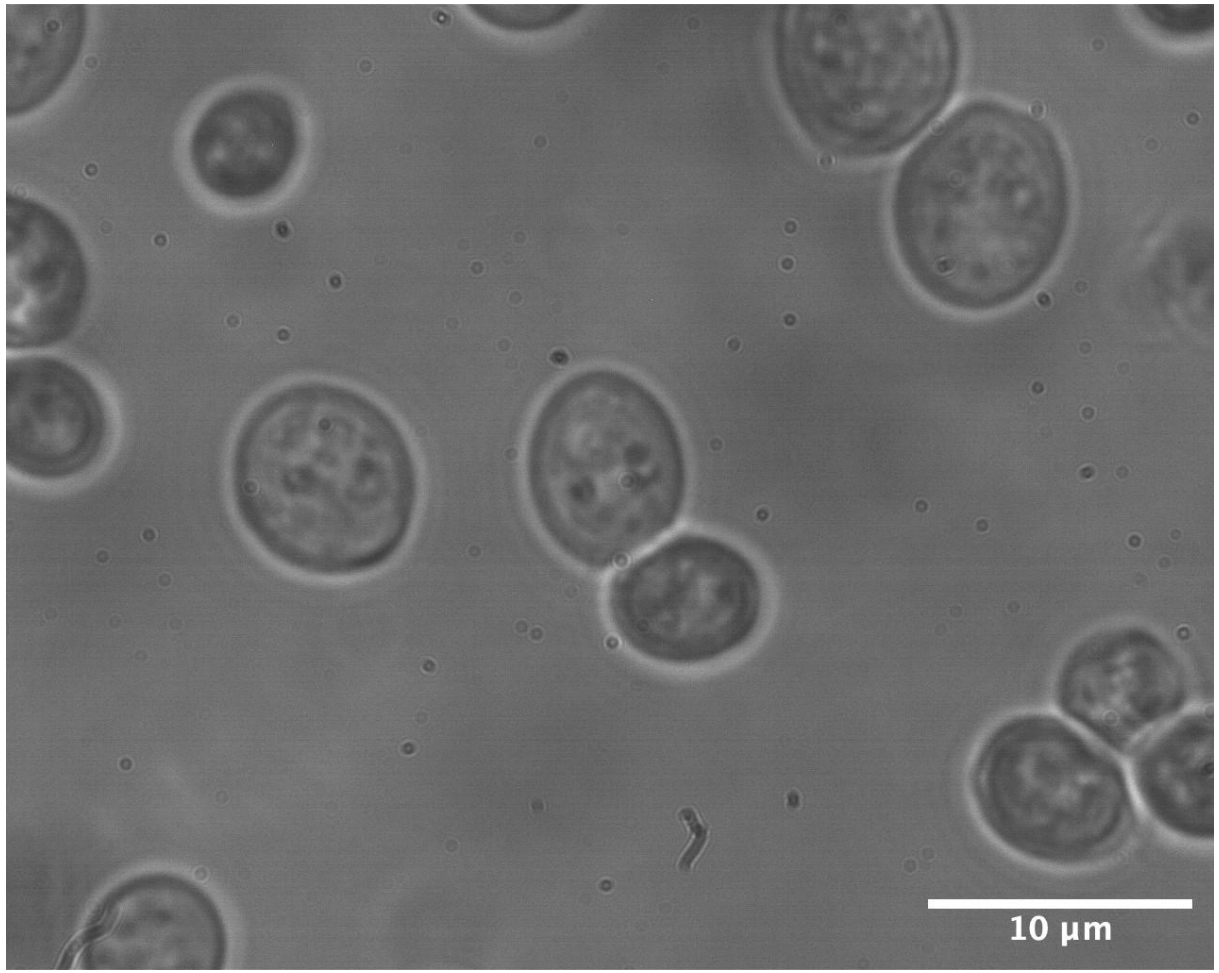
Trapping of 1 µm silicon beads

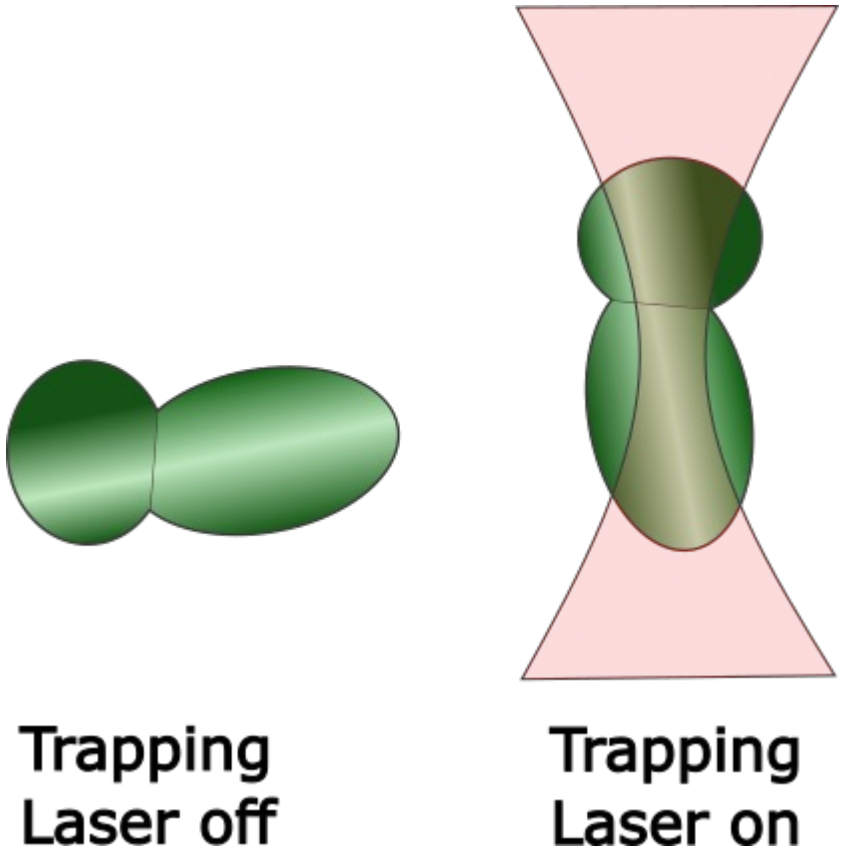


Trapping of single yeast cell:

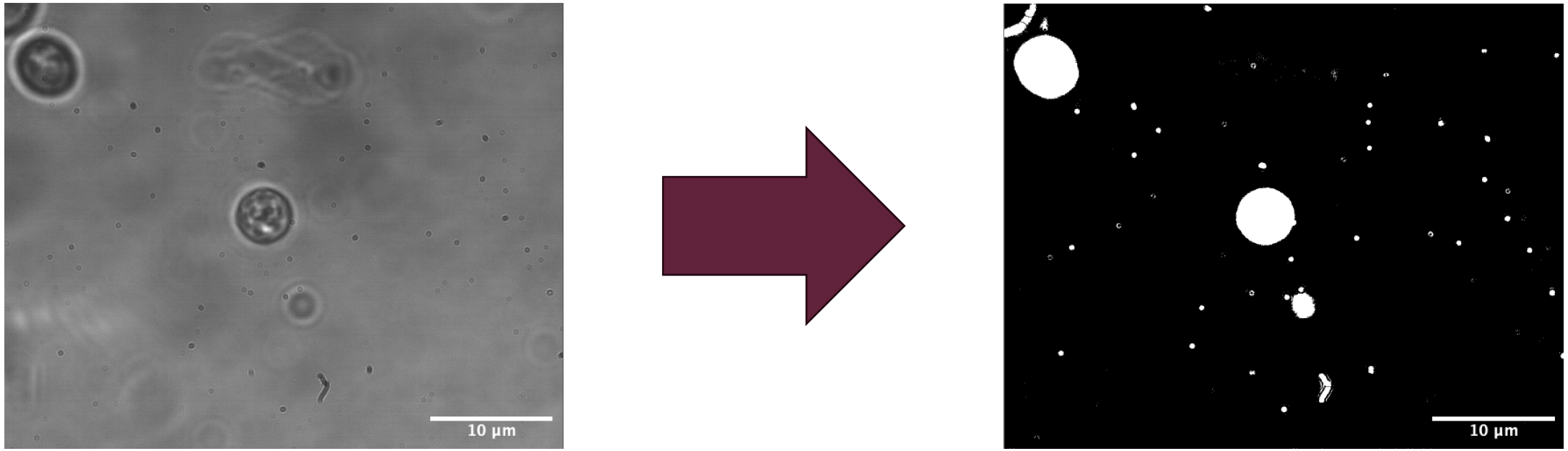


Trapping of single yeast cell





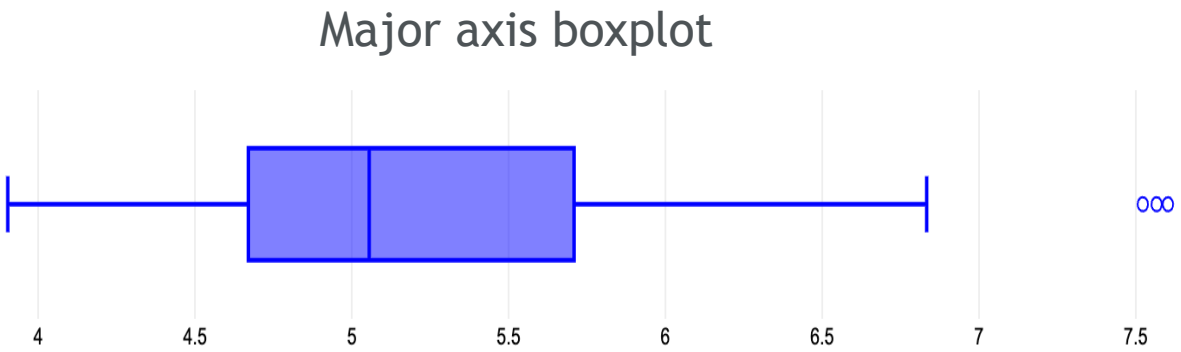
Size measurements of trapped yeast cells

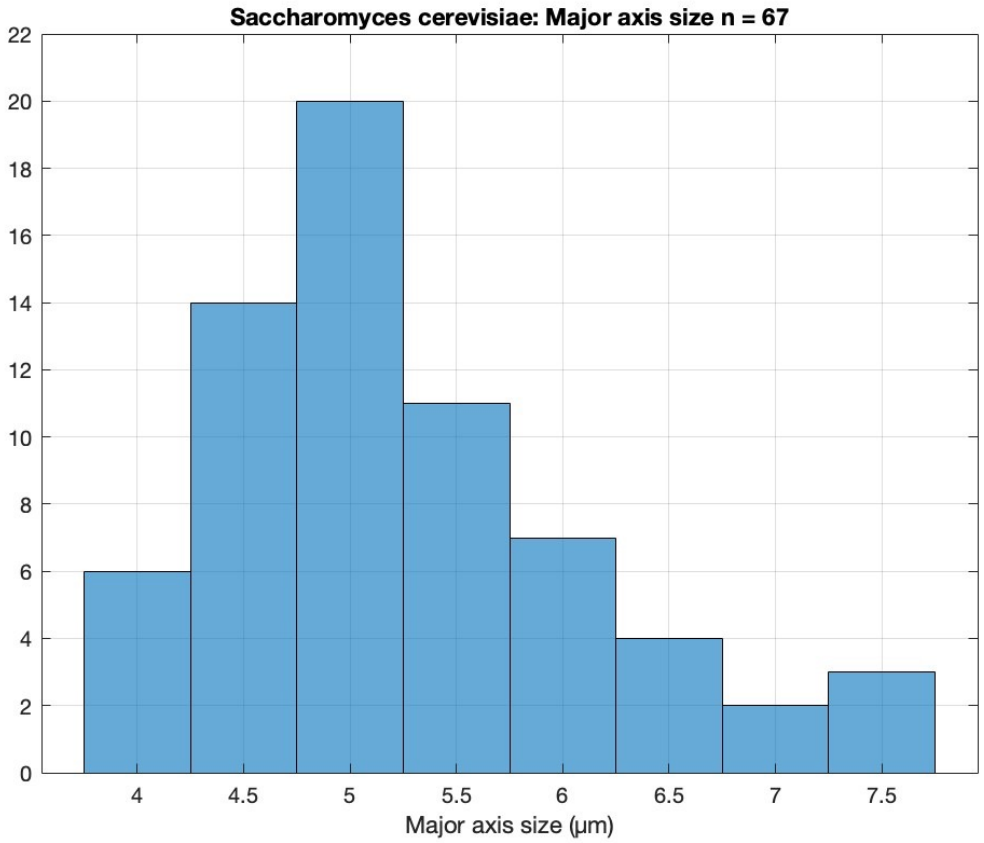


- Change in cell-volume can be induced by changing environmental conditions.
- Using image processing tools such as ImageJ, cell dynamics can be analyzed.
- Average Individual cell error: 0.03 µm

Trapped cell size measurements

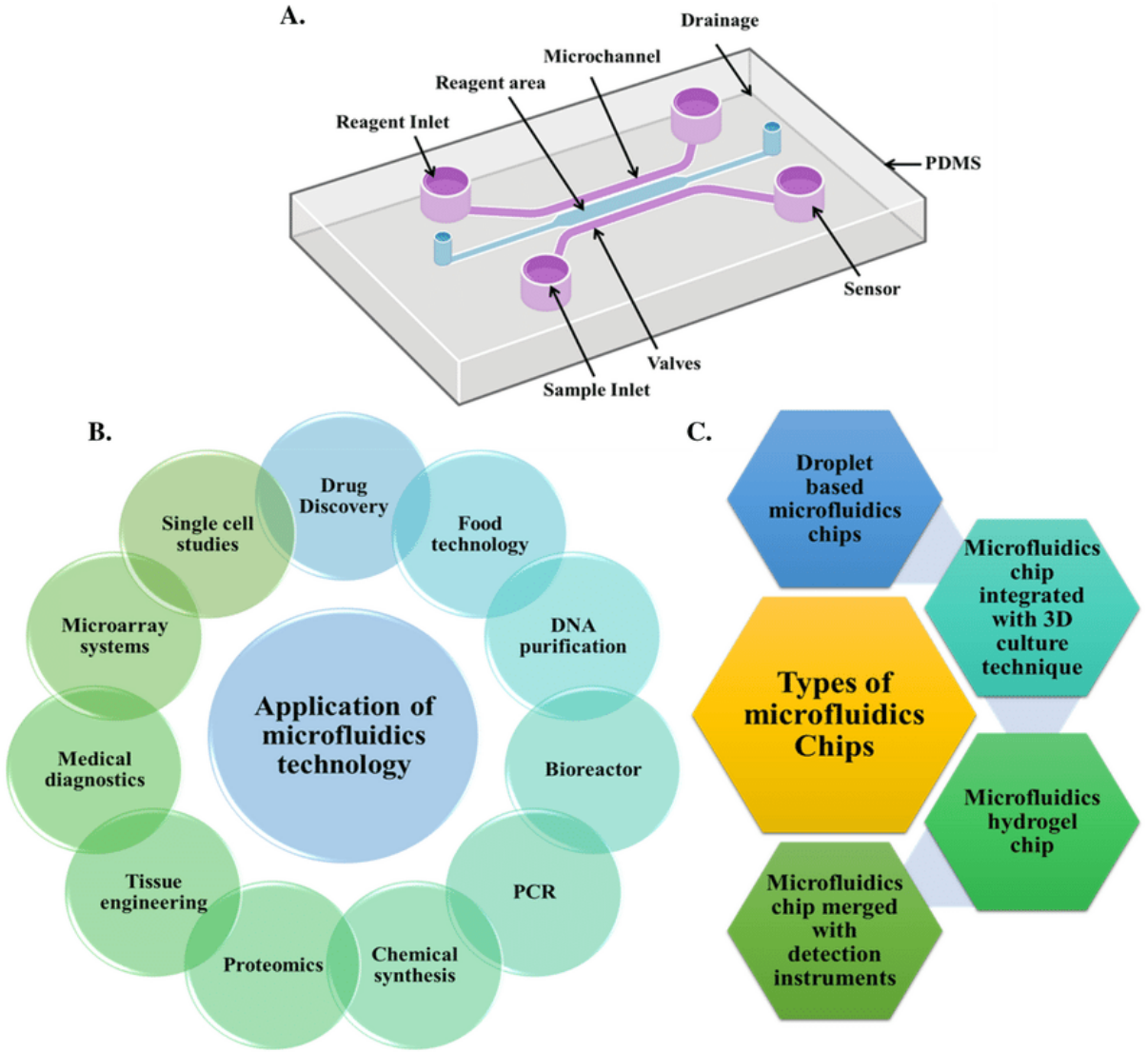
- Average major axis length: 5.27 ± 0.86 µm
- Average minor axis length: 5.07 ± 0.81 µm





Microfluidics

- Chips typically made of polymers such as PDMS and PMMA with groove and channels used to accurately control environment in chemical reactions.
- Compact size and high throughput screening.
- Bioinert and hydrophobic



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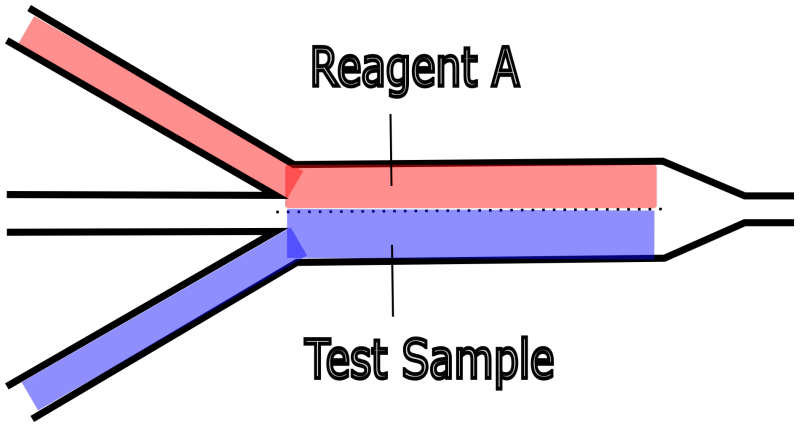
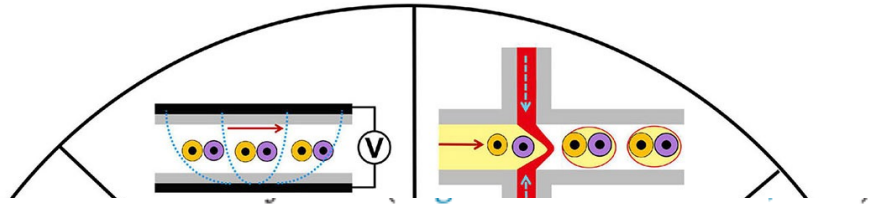
Cell-manipulation in Microfluidics

- Flow type is typically characterized by the Reynolds number:

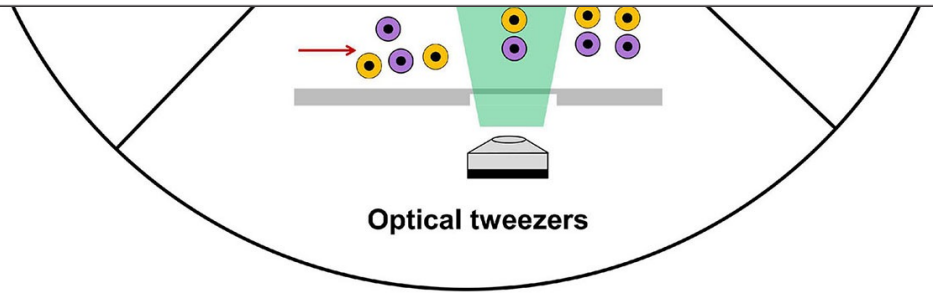
$$Re = \frac{f_i}{f_v} = \frac{\rho U_0 L_0}{\eta}$$

- Microfluidic channels have very low Reynolds numbers ($Re \sim 10^{-6}$)





Re range	Flow regime
$Re < 1$	Creeping flow
$3–5 < Re < 30–40$	Steady separation (Foppl vortices)
$30–40 < Re < 150–300$	Laminar periodic shedding
$150–200 < Re < 1.4 \times 10^5$	Subcritical
$1.4 \times 10^5 < Re < 1 \times 10^6$	Critical
$1 \times 10^6 < Re < 5 \times 10^6$	Supercritical
$5 \times 10^6 < Re < 8 \times 10^6$	Transcritical
$8 \times 10^6 < Re$	Postcritical



Pang, L., Ding, J., Liu, X.-X., Kou, Z., Guo, L., Xu, X., & Fan, S.-K. (2021). Microfluidics-Based Single-Cell Research for Intercellular Interaction. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.680307

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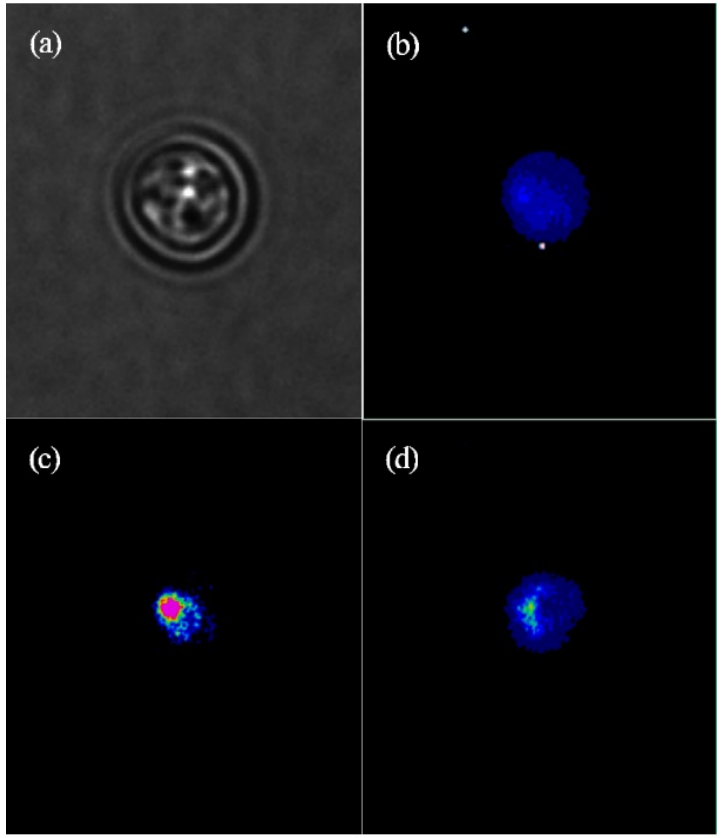
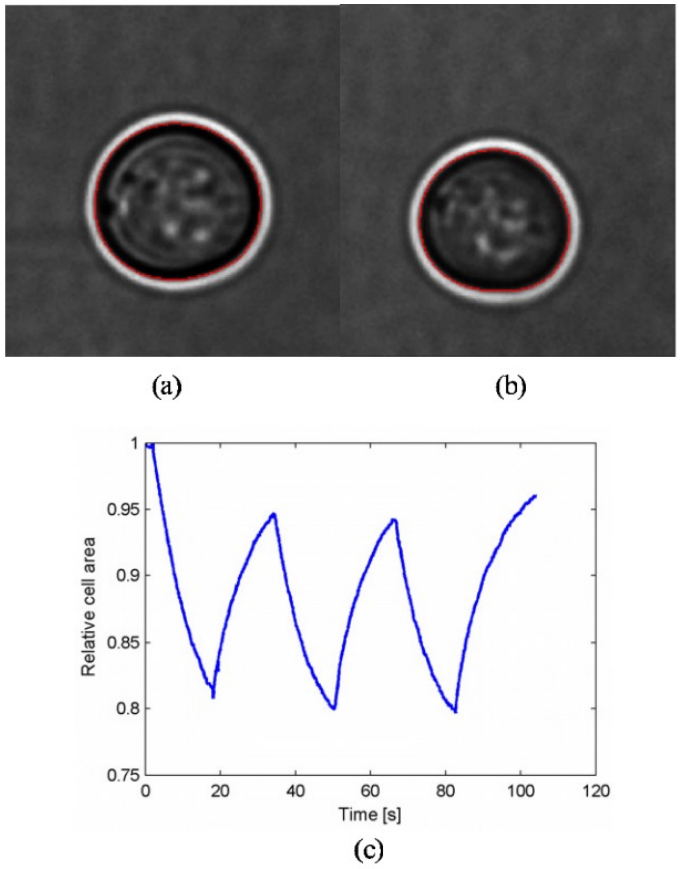
Review of existing integrated setups: Single-Cell analysis of *Saccharomyces cerevisiae* under different stress environments

(a) Transmission image of an optically trapped yeast cell before being exposed to oxidative stress. (b) Spatial distribution of Yap1p-GFP proteins in the cytosol as the cell is held in a neutral environment. (c) Accumulation of Yap1p-GFP proteins in the nucleus after approximately 30 min in a peroxide-rich environment. (d) Spatial distribution of Yap1p-GFP in the cytosol as the stress response is down-regulated 20 min after returning the cell back to the flow with a pure growth medium.



(a) Transmission image of an optically trapped yeast cell in neutral buffer. (b) The same cell after exposure to an environment of higher osmotic pressure. (c) Graphical presentation of the changes in cross section as the cell is repeatedly moved between the two different media.



Eriksson, E., Scrimgeour, J., Granéli, A., Ramser, K., Wellander, R., Enger, J., Hanstorp, D., & Goksör, M. (2007). Optical manipulation and microfluidics for studies of single cell dynamics. *Journal of Optics A: Pure and Applied Optics*, *9*(8), S113–S121. https://doi.org/10.1088/1464-4258/9/8/S02

What's next?

Characterization of optical tweezer by calibrating the stiffness with respect to input power.

Addition of microfluidic slide and for environmental control and characterization of concentration profile and shear forces.

Adding fluorescence detection. NADH works as a biomarker due to its autofluorescence at an excitation wavelength of 380 nm.

Possible addition of spatial light modulator for multi-trapping.

Stellenbosch
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Thank you
Enkosi
Dankie

Photo by Stefan Els