DPG 2022
SKM22 Regensburg
BP 12.19

Institute for Experimental Physics

universität ulm

Optical Stretcher for Adherent Cells

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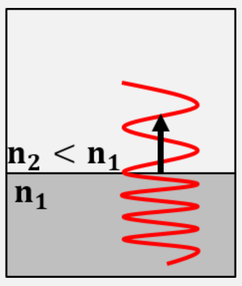
Introduction

We have demonstrated a method to stretch adherent cells with a parallel laser beam to characterize their viscoelastic properties [1].
In the recent work presented here, a new method for the detection of the membrane displacement was developed. It relies on off-axis interferometry, which allows for high precision as well as arbitrary positioning of the probed spot and makes the method completely contact-free.

Force generation



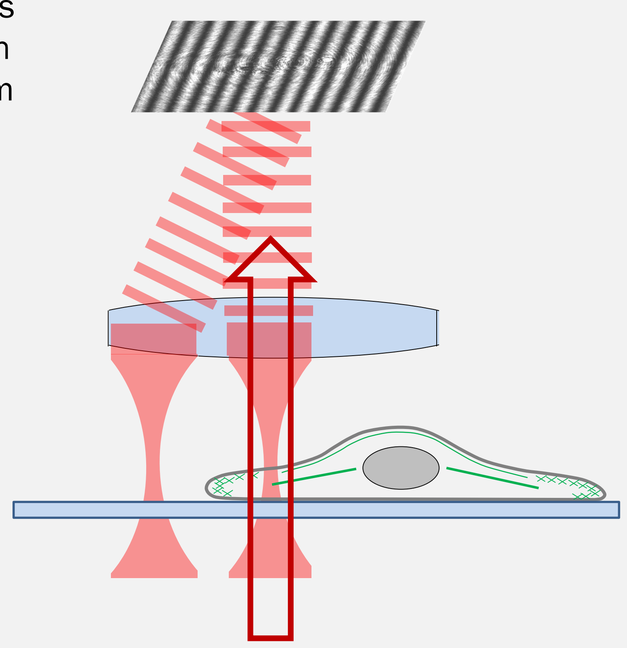
- Photons passing an interface to a lower refractive index medium transfer part of their momentum to the boundary:
  $\mathbf{F} \approx \text{photon rate} \cdot \Delta\mathbf{p}_{\text{ph}}$
- $n_{cytoplasm} \approx 1.365, n_{medium} \approx 1.335$, therefore the cell membrane gets pulled upwards

Interferometric measurement of the strain

- A membrane displacement leads to a relative phase shift between probe beam and reference beam
- Fitting the interference pattern yields $\Delta z = \frac{\Delta\phi}{2\pi} \cdot \lambda_0/\Delta n$

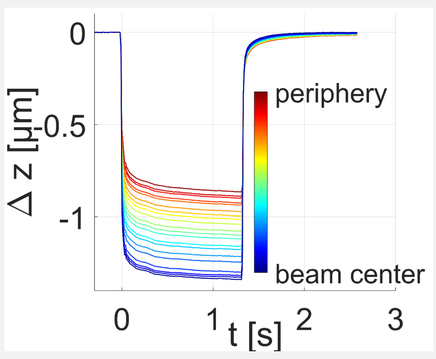
800 nm stretch beam
$P \approx 1.16\ \text{W}, \emptyset_{\text{beam}} \approx 2.43\ \mu\text{m}$

633 nm probe beam and reference beam

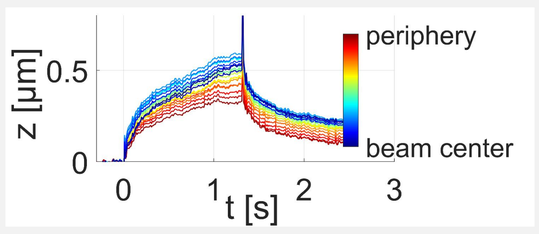


Signal and Resolution

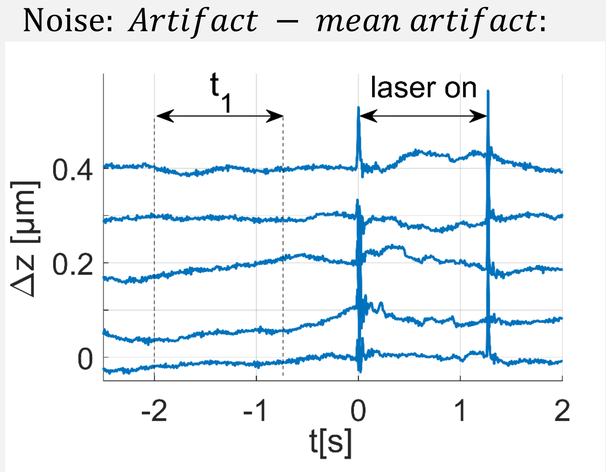
Artifact: Heating of a 0.5mm water column w/o a cell



Stretching of a 3T3 cell treated with Cytochalasin B, artifact subtracted



Noise: $Artifact - mean\ artifact$:

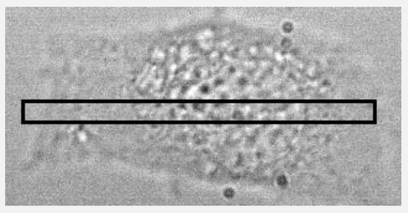


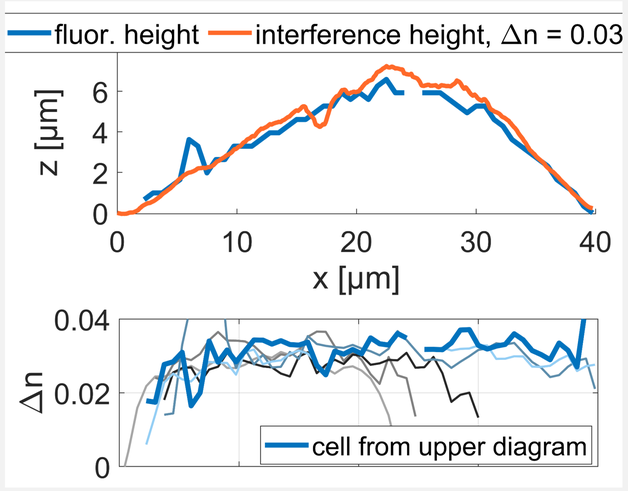
$\overline{\boldsymbol{STD}(\boldsymbol{\Delta z})}$ (n ≥ 50):

- $laser\ on$: (8.8 ± 5.5) nm
- t_1: (6.5 ± 4.5) nm
- $t = 1.3\ \text{s}, setup\ idle$: 2.6 nm

Does the phase shift agree with direct height measurements?

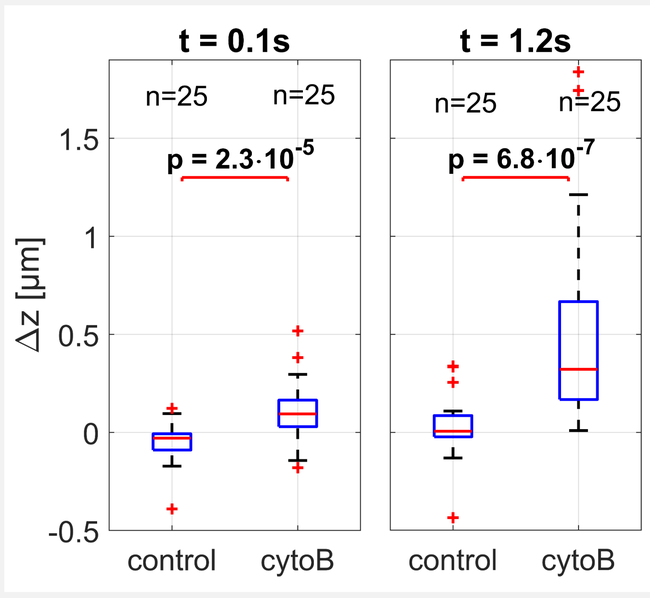
Confocal fluorescence imaging of the cell membrane while recording the phase shift:





- $\Delta n_{mean,3T3} = 0.0305 \pm 0.020$
- Comparison of phase shift and fluorescence cross section yields a consistent and plausible [2] n, confirming the validity of the height measurement

Stretching of 3T3 cells



- Cells treated with 10µM Cytochalasin B show significant softening
- Instantaneous deformation as well as creep behavior

- $F = \Delta n \cdot \frac{P}{c} = 118\ \text{pN};\ \ \sigma \approx 25.4\ \text{N/m}^2;$
- $|G*|(1.2s, \text{CytoB}) \approx {}^{\sigma}/_{\frac{\Delta z}{z}} \approx 370\ \text{Pa}$
- AFM-indentation, untreated 3T3 cells: $\emptyset$ 1.8µm, 0.2 nN: $E_{eff} = 468$ Pa [3]
  Guck stretcher: Signal from softer HeLa cells buried in noise at $\lambda = 780$ nm [4]

Summary

- Contact-free measurement, where the wavelength of 800 nm reduces photodamage and avoids excessive heating [4].
- High resolution phase measurement enables the clear distinction of untreated and Cytochalasin B treated cells, even on a short timescale
- Challenges: Quantitative measurement of the stiffness of stiff, untreated, adherent cells (e.g. 3T3); Uniform phase shift across the beam

Acknowledgement

The authors wish to thank Dorothee Erz and Carolin Grandy for the cell preparation, and all other colleagues at the Institute of Experimental Physics. Financial support by the DFG (GRK 2203) is gratefully acknowledged.

References

[1] Neckernuß, T. (2018), Phdthesis, Ulm University
[2] Steelman, Z.A. (2017), J. Biophotonics 10, 1714
[3] Chiou Y.W. et al (2013), PLoS ONE 8(10): e77384
[4] Huster, C. et al (2020), New J. Phys. 22, 085003

Poster link:

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