WE ARE ON

OSA Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group

Optical Trapping of Deformable Material

Phil Jones, University College London

The OSA Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group Welcomes You!

OSA

Optical Trapping and Manipulation
in Molecular and Cellular Biology
Technical Group

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OSA Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group

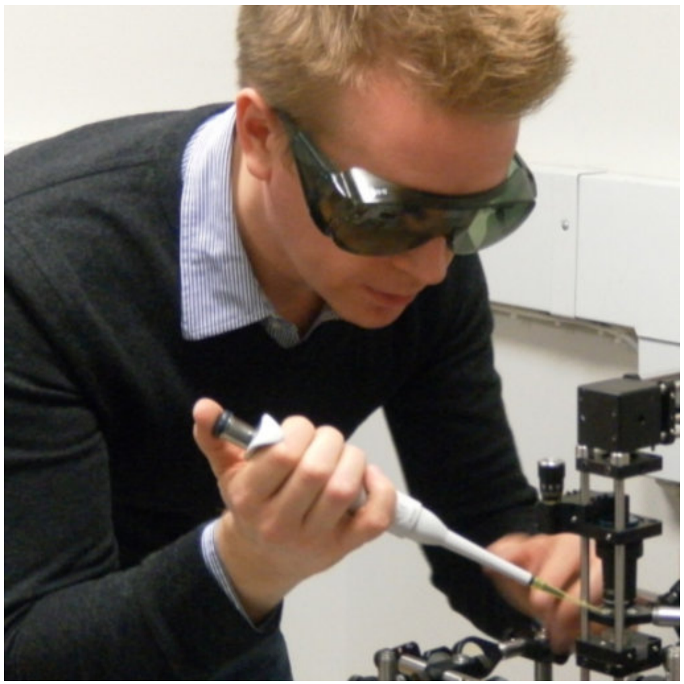
Technical Group at a Glance

- Focus
  - Development and application of novel optical trapping and manipulation techniques to biological problems
  - More than **600** members
- Mission
  - To benefit ***YOU***
  - Webinars, technical events, network events
  - Interested in presenting your research? Have ideas for technical group events? Contact us!
- Find us here
  - Website: www.osa.org/BT
  - Twitter: hashtag #BTTechGroupOSA

OSA Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group

Today’s Webinar:

Optical Trapping of Deformable Material



Prof. Phil Jones

University College London, UK

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Speaker’s Short Bio:

Natural Science at Cambridge University, UK
M.S. at Imperial College, UK
Ph.D. at Oxford University, UK
Postdoc at University College London, UK

Co-authored the book
“Optical Tweezers: Principles & Applications”

OSA Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group

Optical trapping of deformable material

P H Jones

Optical Tweezers Group

Department of Physics & Astronomy

UCL

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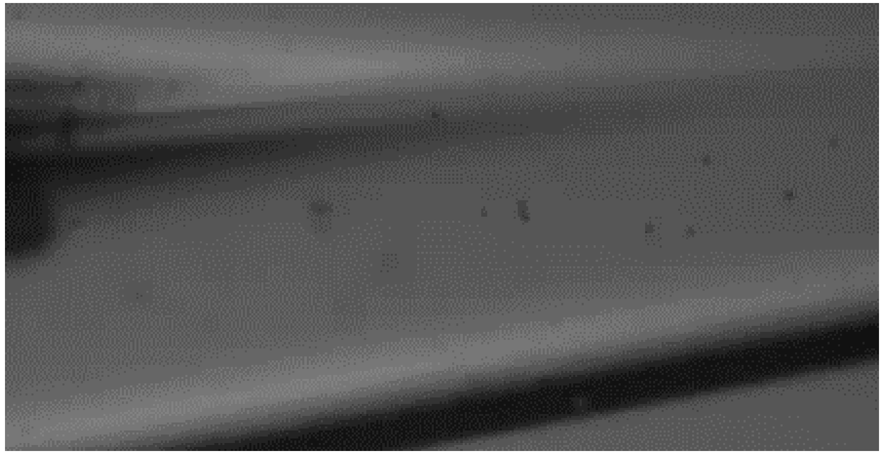
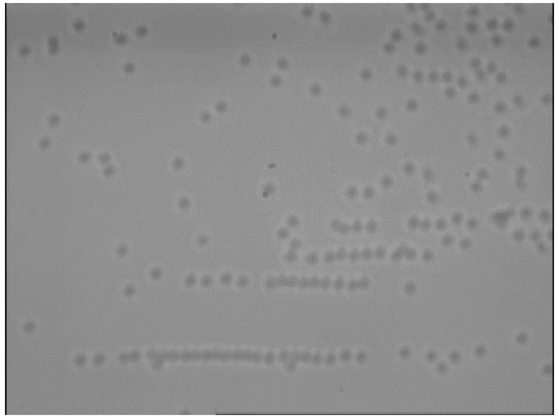
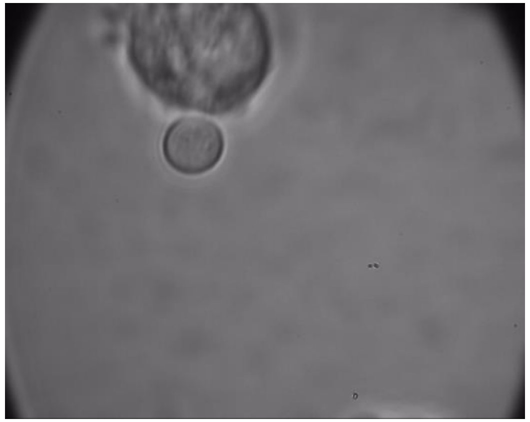
OSA Technical Group Webinar

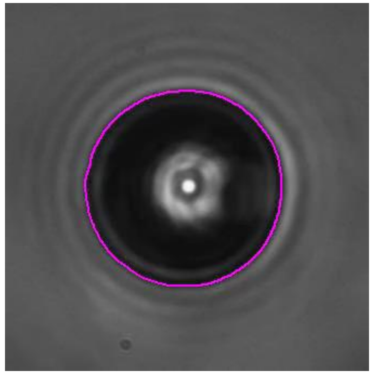
Tue 06 May 20

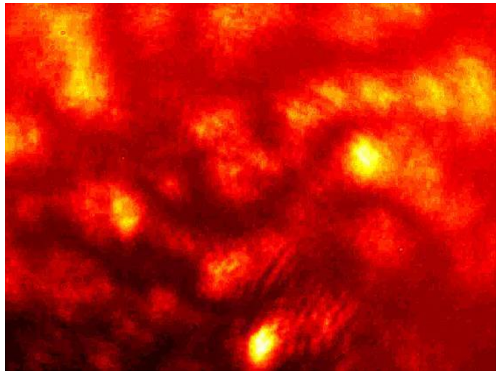
This talk is not about....

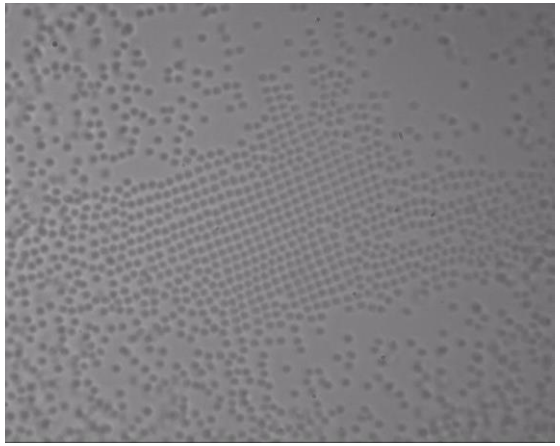


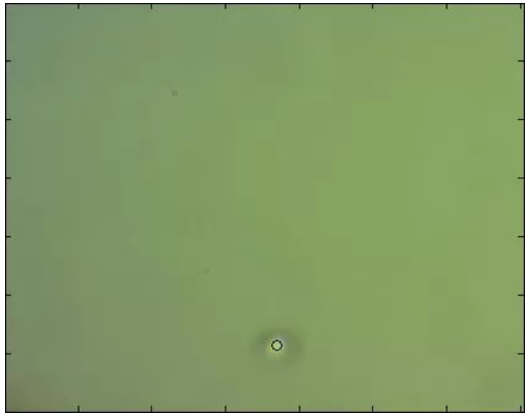




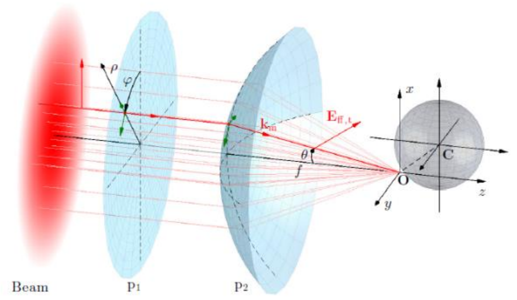




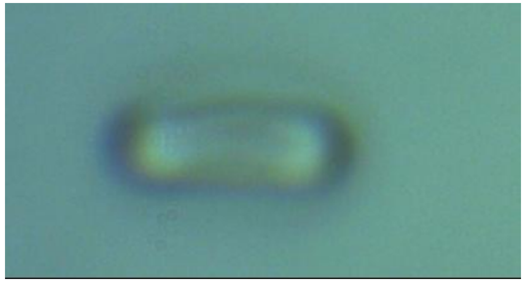




Optical trapping of deformable material



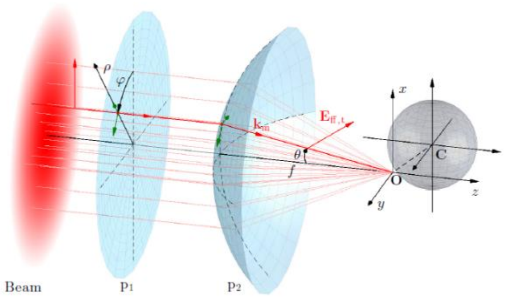
I. Optical tweezers



II. Red blood cells

III. Microemulsions

Optical trapping of deformable material



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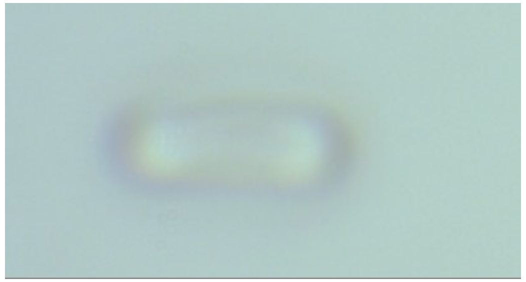
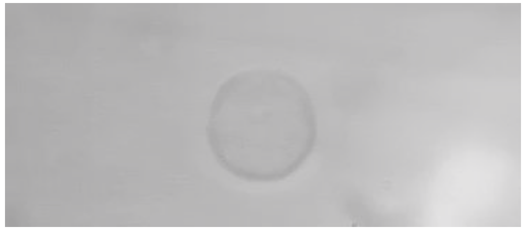
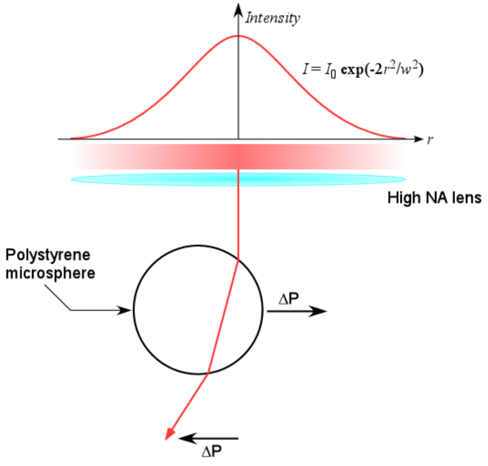


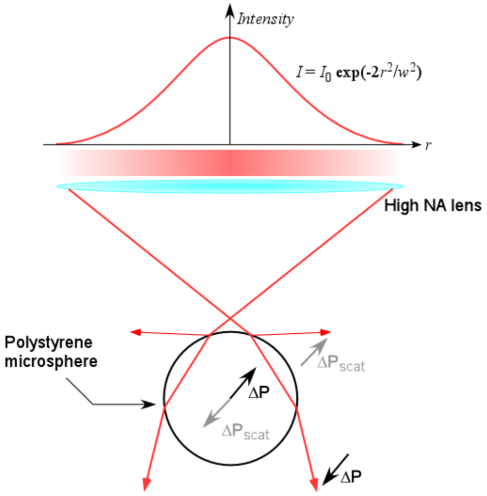
II. Red blood cells

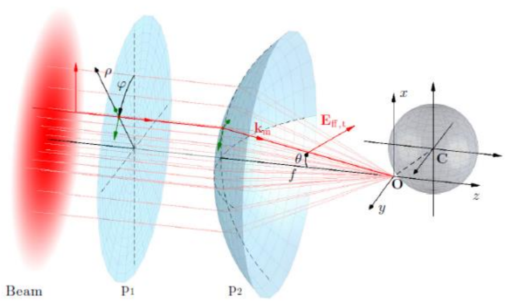


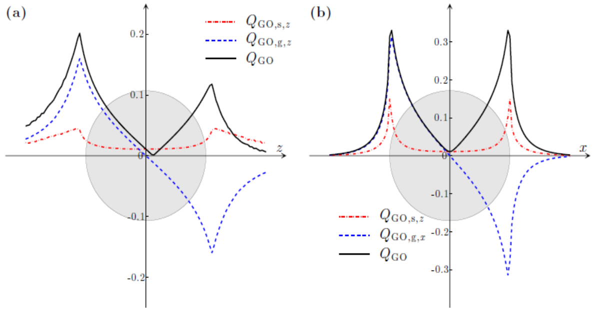
III. Microemulsions

Optical tweezers: trapping mechanism







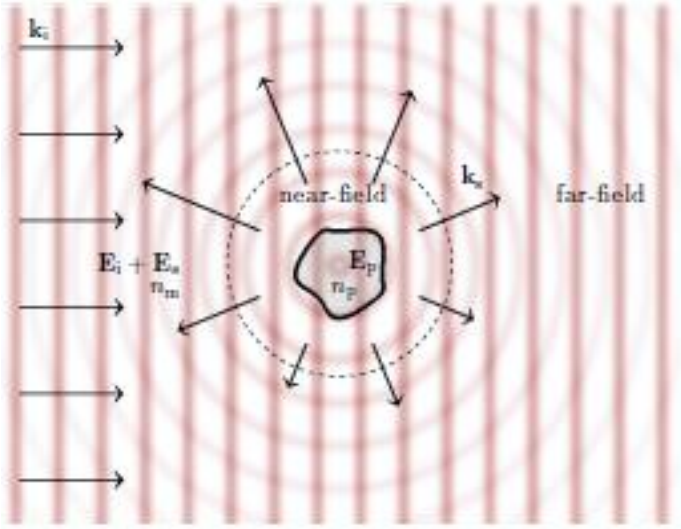


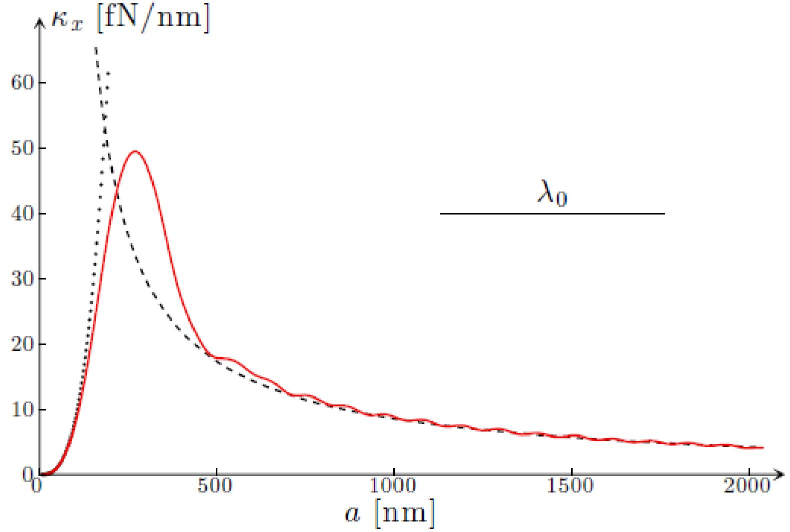
$$\mathbf{F}_{\mathrm{GO}} = \sum_m \mathbf{F}_{\mathrm{ray}}^{(m)} = \sum_m \left[\frac{n_{\mathrm{i}} P_{\mathrm{i}}^{(m)}}{c} \hat{\mathbf{r}}_{\mathrm{i}}^{(m)} - \frac{n_{\mathrm{i}} P_{\mathrm{r}}^{(m)}}{c} \hat{\mathbf{r}}_{\mathrm{r},0}^{(m)} - \sum_{n=1}^{+\infty} \frac{n_{\mathrm{i}} P_{\mathrm{t},n}^{(m)}}{c} \hat{\mathbf{r}}_{\mathrm{t},n}^{(m)} \right]$$

$$\mathbf{F}_{\mathrm{DA}} = \frac{1}{4} \alpha'_{\mathrm{d}} \nabla |E_{\mathrm{i}}|^2 + \frac{\sigma_{\mathrm{ext,d}}}{c} \mathbf{S}_{\mathrm{i}} - \frac{1}{2} \sigma_{\mathrm{ext,d}} c \nabla \times \mathbf{s}_{\mathrm{d}}$$

$$\boldsymbol{\mathcal{S}}(\mathbf{r}, t) = \frac{1}{\mu} \boldsymbol{\mathcal{E}}(\mathbf{r}, t) \times \boldsymbol{\mathcal{B}}(\mathbf{r}, t) = \boldsymbol{\mathcal{E}}(\mathbf{r}, t) \times \boldsymbol{\mathcal{H}}(\mathbf{r}, t)$$

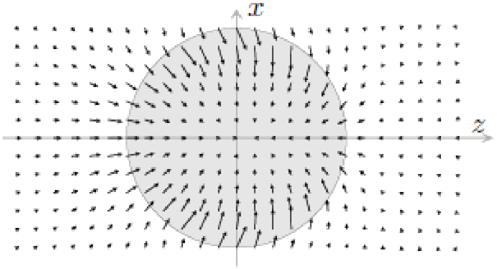
Optical tweezers: electromagnetic scattering theory

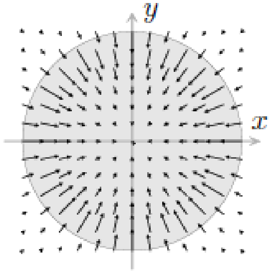




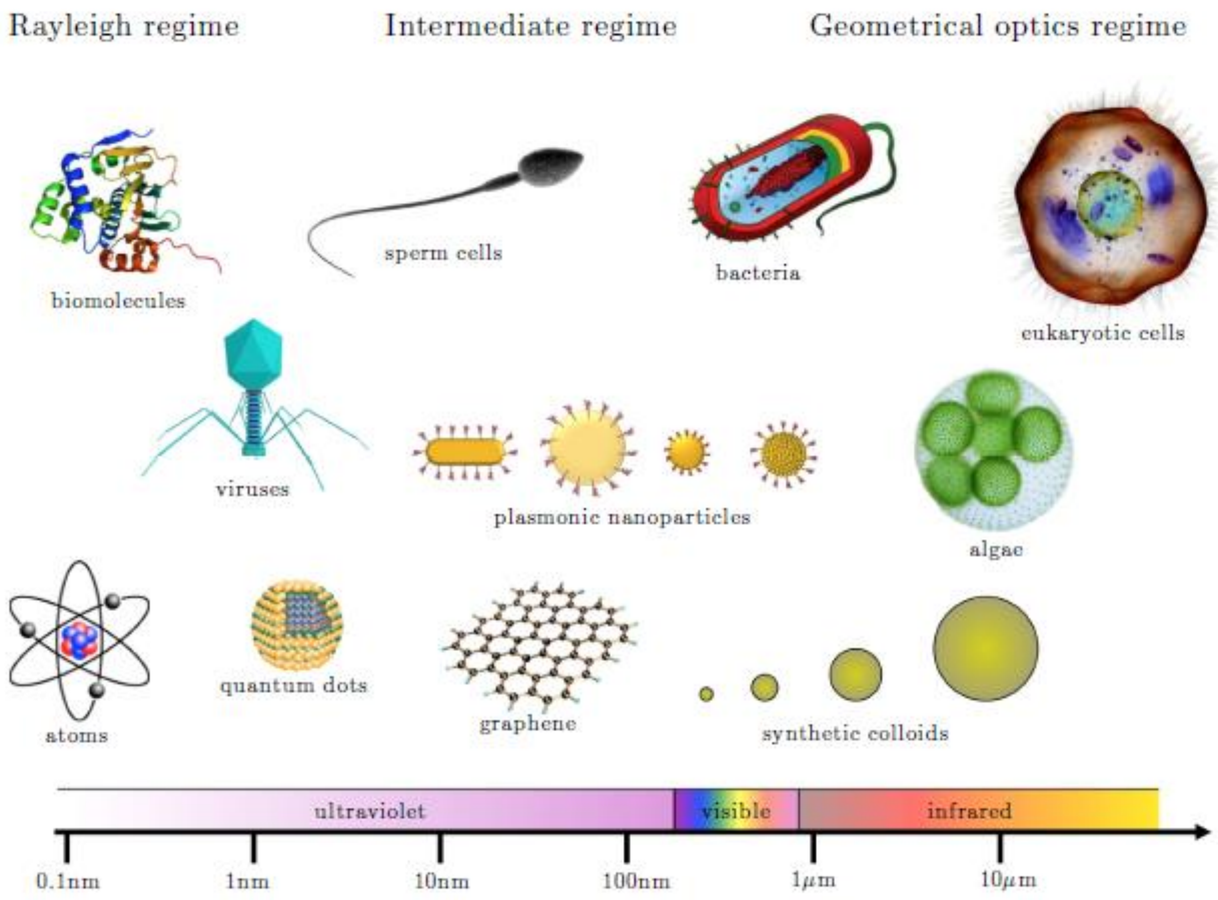
$$\mathsf{T}_{\mathrm{M}} = \varepsilon_0 \left[\boldsymbol{\mathcal{E}} \otimes \boldsymbol{\mathcal{E}} + c^2 \boldsymbol{\mathcal{B}} \otimes \boldsymbol{\mathcal{B}} - \frac{1}{2} \left(\boldsymbol{\mathcal{E}} \cdot \boldsymbol{\mathcal{E}} + c^2 \boldsymbol{\mathcal{B}} \cdot \boldsymbol{\mathcal{B}} \right) \mathsf{I} \right]$$

$$\mathbf{F}_{\mathrm{rad}} = \oint_S \overline{\mathsf{T}}_{\mathrm{M}} \cdot \hat{\mathbf{n}} \, dS \, .$$

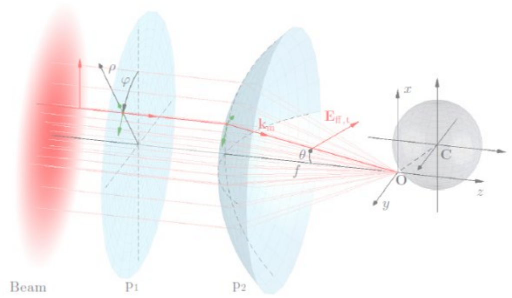




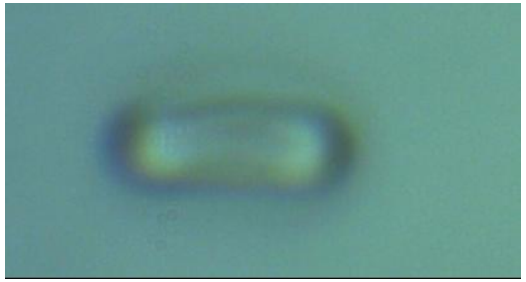
Optical tweezers



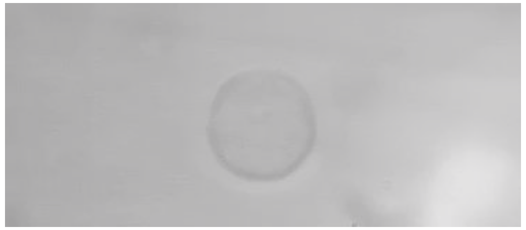
Optical trapping of deformable material



I. Optical tweezers



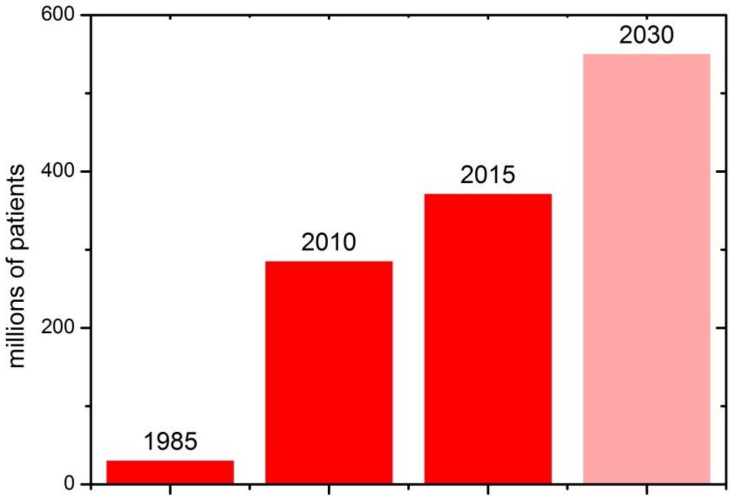
II. Red blood cells

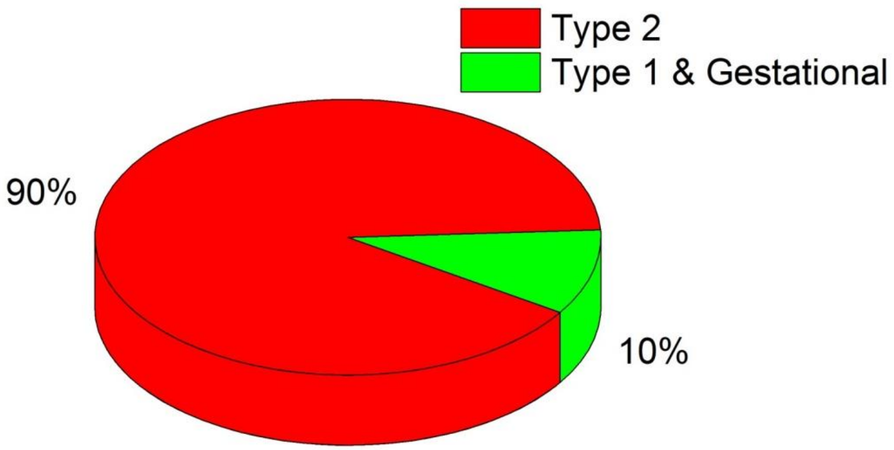


III. Microemulsions

Motivation: Diabetes Mellitus & Diabetic Retinopathy

- Diabetic retinopathy (DR): deterioration of eyesight caused by hyperglycaemic damage to the retinal microvasculature
- DR affects ~80% of all patients who have had diabetes for 10 years or more
- First stage *non-proliferative DR* causes narrowing or blocked retinal arteries. Microaneurysms form which leak fluid into the retina
- In later stage *proliferative DR*, vascular occlusion causes retinal ischaemia and abnormal new blood vessels (neovascularisation) grow out of the retina. These can burst and bleed into the vitreous humour (vitreous haemorrhage) obscuring vision



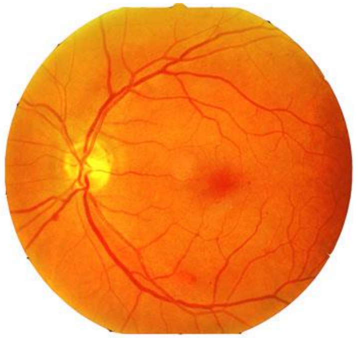


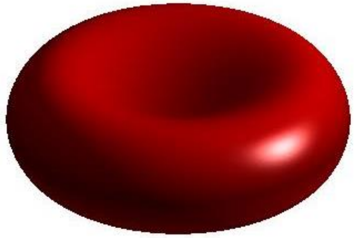
Hypothesis

- Hypothesis:

reduced RBC deformability leads to retinal ischaemia and subsequent diabetic retinopathy

- Red blood cell diameter ~8 μm
- Microvasculature in the retina ~5 μm
- Adult human has 20-30 trillion red blood cells
- Blood takes ~1 minute to circulate around the body
- Lifetime of red blood cells is ~120 days





Protocol

- Blood was taken from three groups of patients presenting at Moorfields Eye Hospital:
  - Patients with T2DM but no DR
  - Patients with T2DM and associated DR
  - Healthy patients with no T2DR
- Groups were age- and gender-matched
- Routine blood and liver function tests, cholesterol profile, renal function test and coagulation profile were performed and compared between the control groups and test group
- There was *no statistical significant difference* in the study groups for any of the biochemical or haematological parameters in the blood.

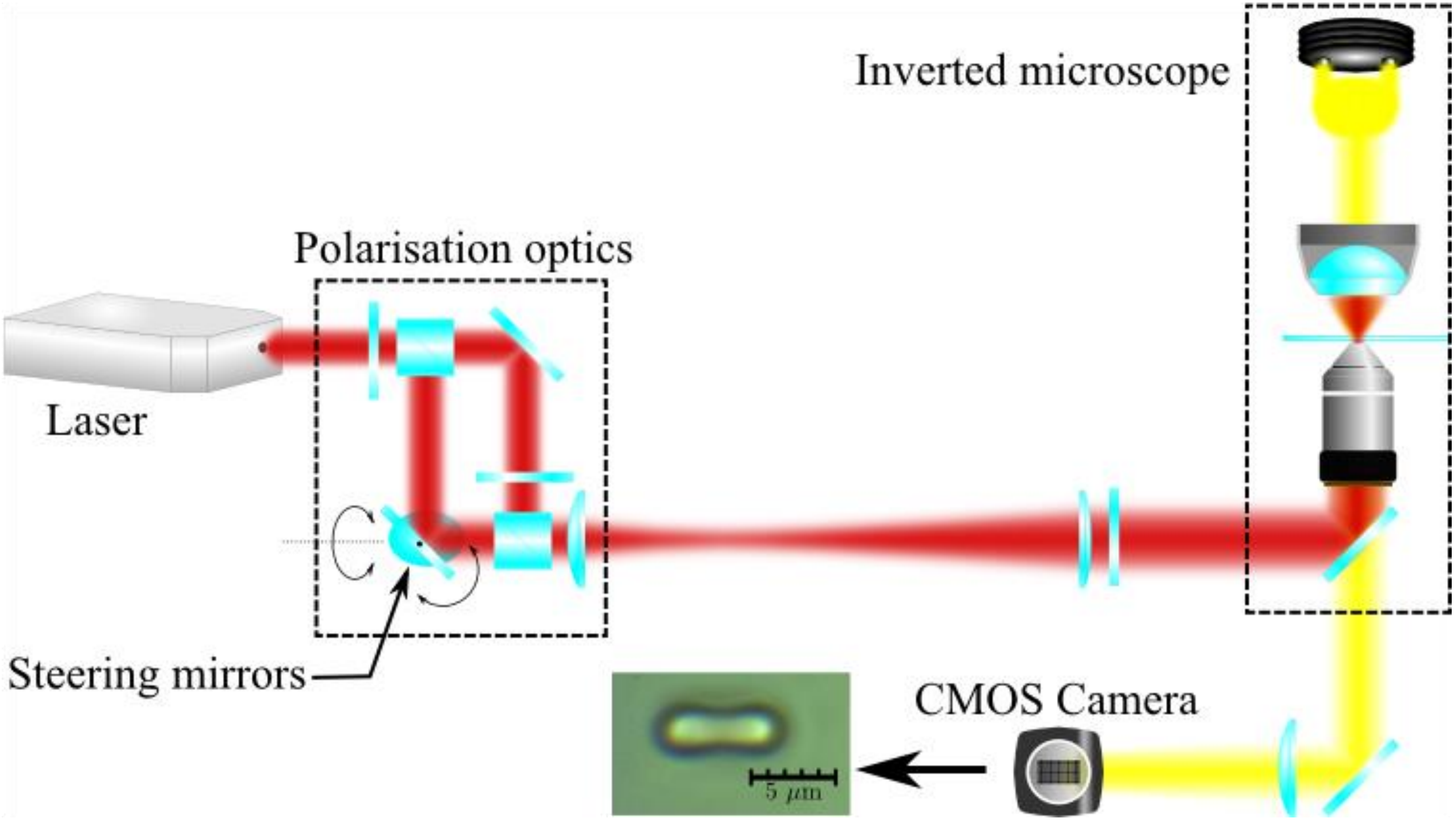
Biochemical & haematological tests

Hematological/Biochemical variables	Normal range (units)	Controls (SD, 95% CI)	Patients (SD, 95%CI)	pvalue (t test)
Hemoglobin (Hb)	13.0–17.0(g/dL)	14.27(1.64,12.90–15.64)	13.34(1.80, 11.67–15.01)	0.31
Hematocrit (Hct)	37–50%	42.12(0.04, 0.38–0.45)	39.47(0.04, 0.35–0.43)	0.27
Red blood cell count (RBC)	4.40–5.80(x10^12/L)	4.8(0.51, 4.37–5.22)	4.71(0.45, 4.51–5.01)	0.74
Mean corpuscular volume (MCV)	80–99	**87.81(2.81, 85.45–90.16)**	**83.48(3.22, 80.50–86.47)**	**0.01**
Mean corpuscular hemoglobin (MCH)	26.0–33.5(pg)	**29.68(1.04, 28.81–30.56)**	**27.84(1.82, 26.15–29.52)**	**0.02**
Mean corpuscular hemoglobin concentration (MCHC)	30.0–35.0(g/dL)	29.90(10,72, 20.94–38.87)	36.63(11.84, 32.53–34.72)	0.38
RBC distribution width (RDW)	11.5–15.0	13.58(1.48, 12.34–14.82)	13.65(1.37, 12.41–14.90)	0.92
Platelet count (PC)	150–400(x10^9/L)	270.5(76.63, 206.42–334.57)	317.57(69.54, 253.25–381.8)	0.23
Mean platelet volume (MPV)	7–13(fL)	10.73(1.41, 9.55–11.91)	10.88(0.77, 10.17–11.60)	0.81
White blood cell count (WBC)	3.0–10.0(x10^9/L)	7.07(1.63, 5.71–8.44)	7.79(1.25, 6.64–8.95)	0.36
Neutrophils	2.0–7.5(x10^9/L)	3.67(1.00, 2.83–4.51)	4.40(0.68, 3.76–5.03)	0.12
Lymphocytes	1.2–3.65(x10^9/L)	2.45(0.86, 1.73–3.18)	2.55(0.71, 1.89–3.21)	0.82
Monocytes	0.2–1.0(x10^9/L)	4.32(1.4, 3.11–5.53)	4.71(1.95, 2.90–6.52)	0.66
Eosinophils	0.0–0.4(x10^9/L)	3.5(0.9, 2.7–4.2)	3.14(0.2, 0.12–0.50)	0.66
Basophils	0.0–0.1(x10^9/L)	3.28(1.81, 1.77–4.80)	3.4(3.6, 0.67–6.73)	0.94
Erythrocyte sedimentation rate (ESR)	1–20(mm/hr)	7.87(6.26, 2.63–13.11)	21.14(28.81, –5.50–47.78)	0.22
Sodium (Na)	135–145(mmol/L)	142.62(2.26,140.73–144.51)	140(3.60, 136.66–143.33)	0.11
Potassium (K)	3.5–5.1(mmol/L)	4.46(0.36, 4.15–4.76)	4.77(0.18, 4.32–5.22)	0.18
Chloride (Cl)	98–107(mmol/L)	102.37(1.76, 100.89–103.85)	99.85(2.47, 97.56–102.14)	0.04

Biochemical & haematological tests

Hematological/Biochemical variables	Normal range (units)	Controls (SD, 95% CI)	Patients (SD, 95%CI)	pvalue (t test)
Bicarbonate	22–29(mmol/L)	23.62(2.50, 21.53–25.71)	24.14(2.11, 22.18–26.09)	0.67
Urea	1.7–8.3(mmol/L)	4.61(1.59, 3.28–5.94)	6.31(2.90, 3.62–8.99)	0.17
Creatinine	66–112(mmol/L)	83(24.85, 62.22–103.77)	92.71(22.42, 71.97–113.45)	0.44
Bilirubin	0–20(umol/L)	5.375(2.77, 3.05–7.69)	6(3.10, 3.12–8.87)	0.69
Alkaline phosphatase (ALP)	40–129(IU/L)	64(12.4,53.51–74.48)	89.71(18.10, 72.96–106.46)	<0.001
Aspartate aminotransferase (AST)	0–37(IU/L)	22.62(5.65, 17.89–27.35)	23.71(15.25, 9.61–37.81)	0.85
Alanine transaminase (ALT)	10–50(IU/L)	23.12(10.80, 14.09–32.15)	37.71(23.68, 15.81–59.61)	0.14
Protein	63–83(g/L)	**67.37(3.50, 64.44, 70.30)**	**73.28(3.77, 69.79–76.77)**	**0.007**
Albumin	34–50(g/L)	43.62(2.77, 41.30–45.94)	43.57(3.35, 40.46–46.67)	0.97
Globulin	19–35(g/L)	**23.75(2.54, 21.61–25.88)**	**29.71(1.88, 27.96–31.46)**	**<0.001**
C-reactive protein (CRP)	0–5.0(mg/L)	2.18(2.33, 0.23–4.14)	2.12(2.34, 0.04–4.29)	0.962
Random blood glucose (RBG)	3.9–6.9(mmol/L)	**4.91(0.40, 4.58–5.24)**	**10.04(3.67, 6.64–13.43)**	**0.001**
Glycosylated hemoglobin (HbA1C)	4.5–6.0%	**5.82(0.34, 5.54–6.11)**	**9.77(2.64, 6.17–9.15)**	**0.001**
Triglycerides (TG)	0.0–2.2(mmol/L)	1.62(0.98, 0.80–2.44)	2.31(2.10, 0.36–4.26)	0.42
Cholesterol	2.3–4.9(mmol/L)	4.93(1.18, 3.94–5.93)	4.47(1.18, 3.94–5.93)	0.38
High density lipoprotein (HDL)	0.9–1.5(mmol/L)	1.34(0.31, 1.05–1.63)	1.17(0.29, 1.08–1.42)	0.29
Low density lipoprotein (LDL)	0–3.0(mmol/L)	2.82(0.84, 2.12–3.52)	2.76(0.72, 2.00–3.53)	0.89
Angiotensin converting enzyme (ACE)	8–52 Units/liter	35.87(11.63, 26.15–45.60)	31(26.41, 6.56–55.43)	0.64
Fibrinogen	2–4 (g/L)	3(0.59, 2.50–3.5)	3.12(0.89, 2.29–3.95)	0.74

Methodology: Optical Tweezers



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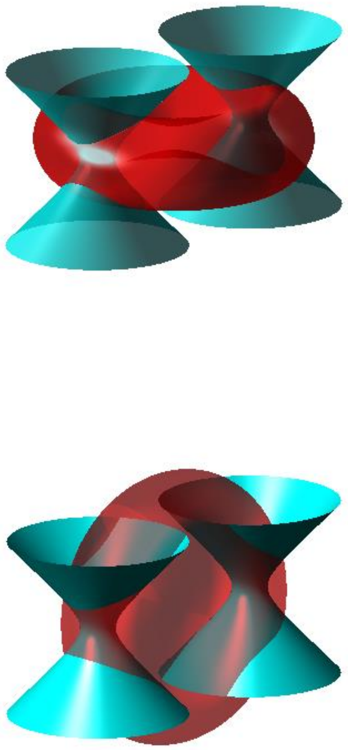
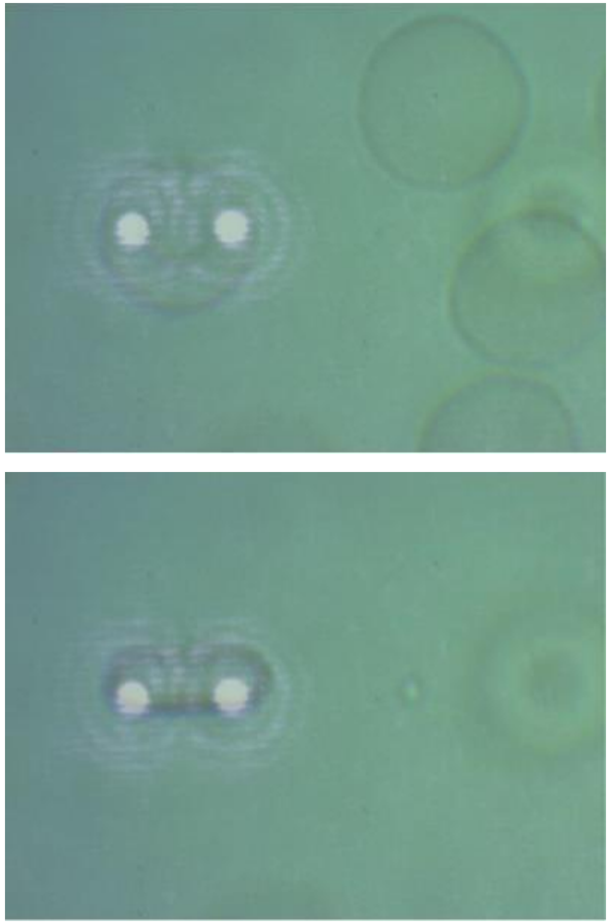
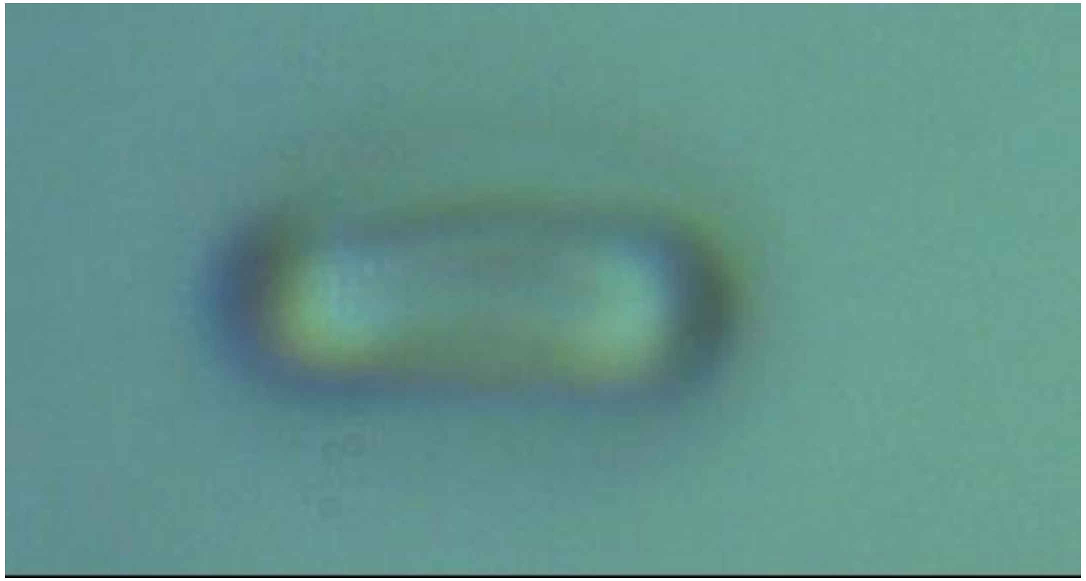




Analysis: video microscopy



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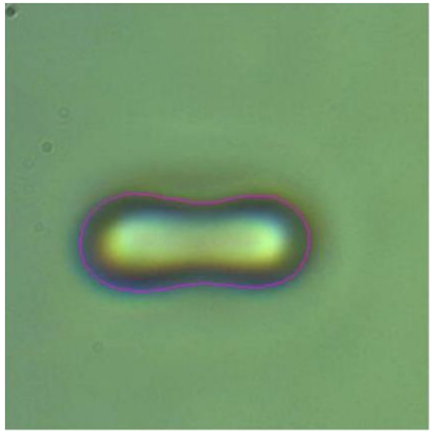
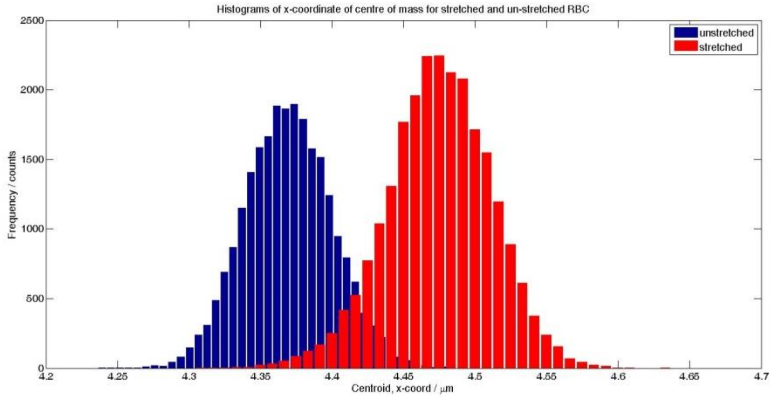
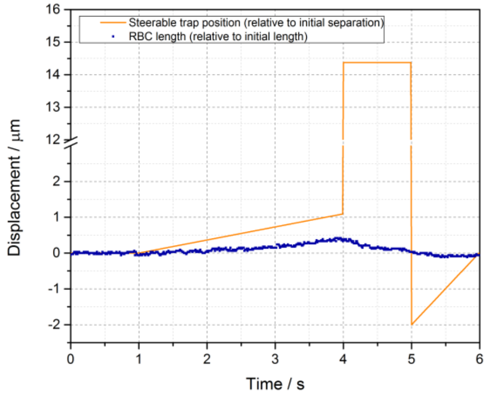
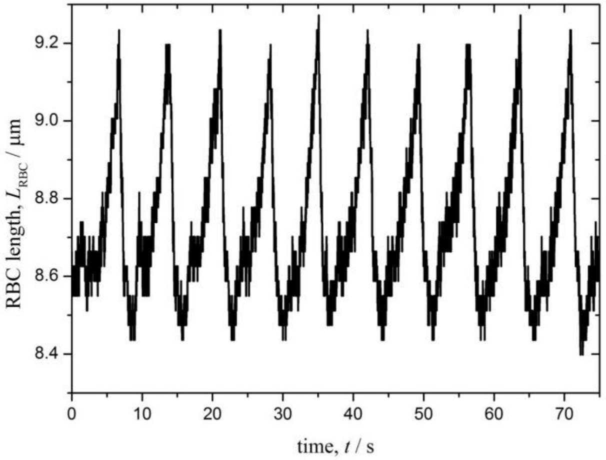
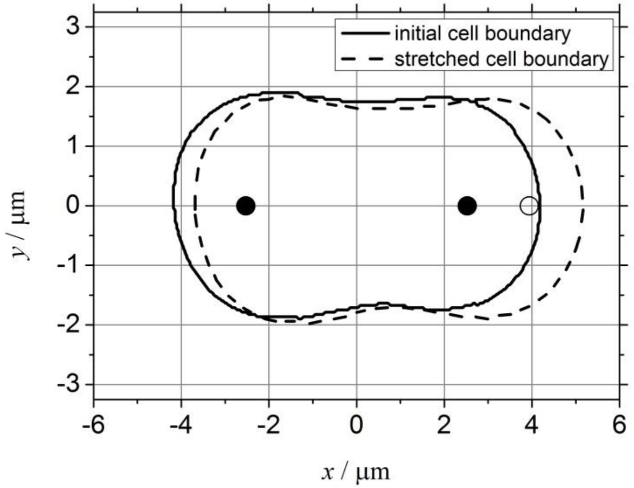


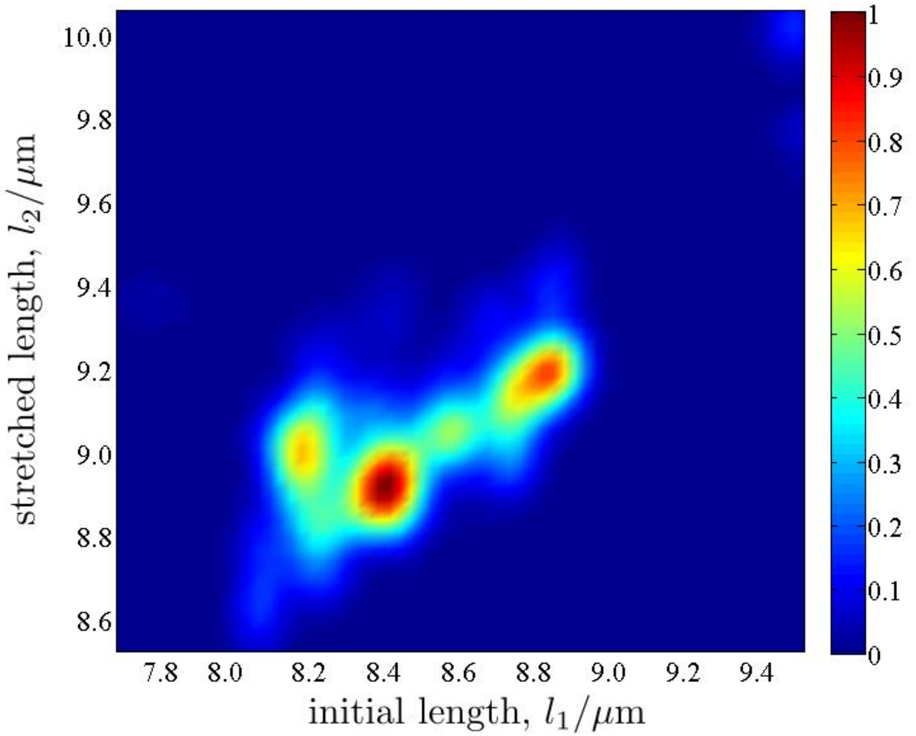


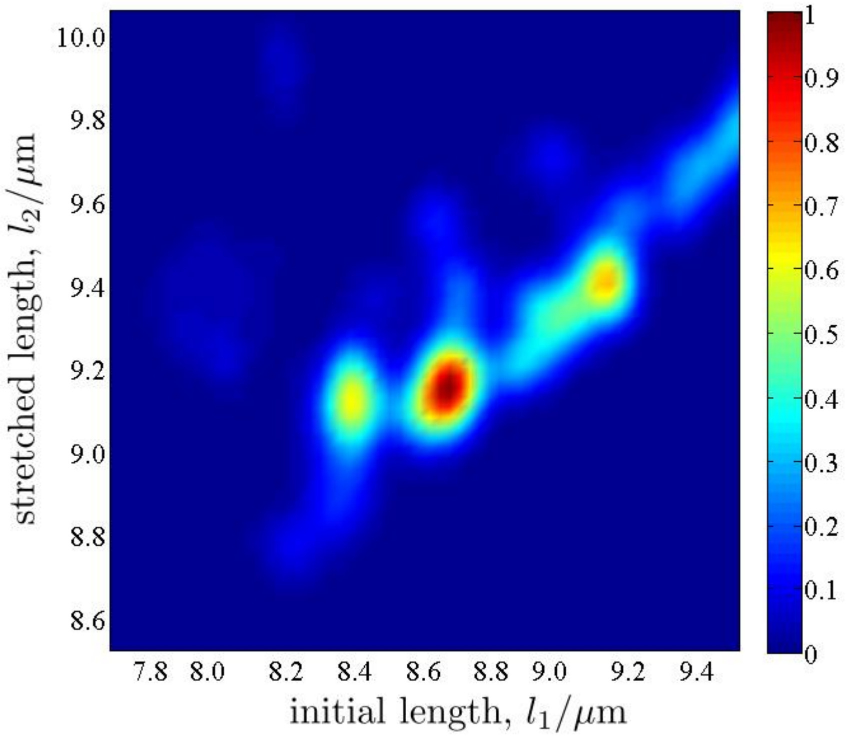






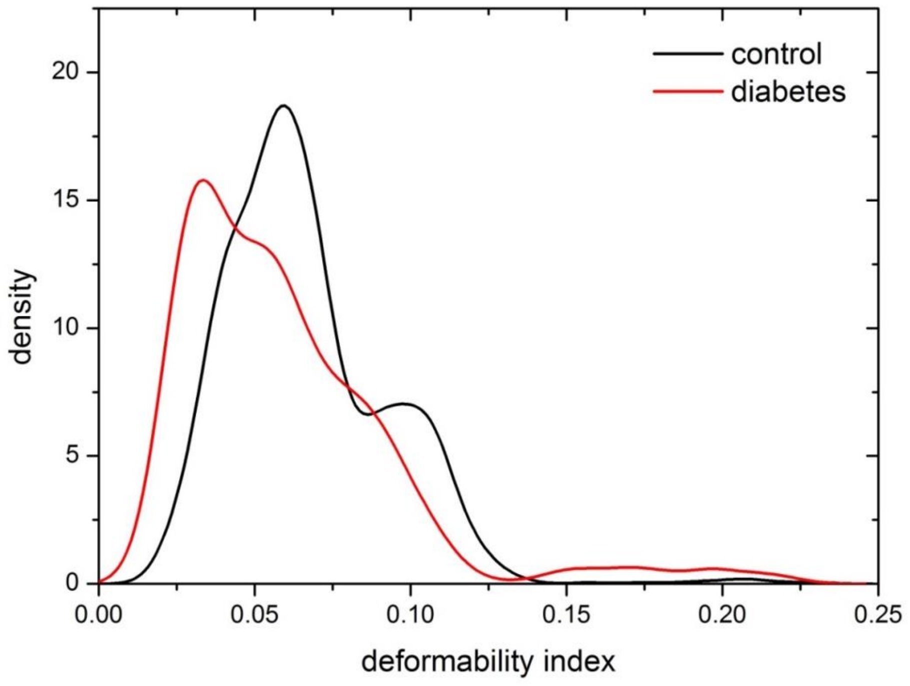
Results: RBC deformability





Results: RBC deformability

- Define a deformability index, which is the fractional change in cell length when subject to this stretching protocol: $(l_2 - l_1)/l_1$
- DR patients were found to have a **statistically significant decrease in deformability** of red blood cells compared to patients from the control groups



References

- R. Agrawal, et al. '*Assessment of red blood cell deformability in type 2 diabetes mellitus and diabetic retinopathy by dual optical tweezers stretching technique*', Scientific Reports **6** 15873, doi:10.1038/srep15873 (2016)
- T. J. Smart, et al. '*A study of red blood cell deformability in diabetic retinopathy using optical tweezers*', Proc SPIE **9548**,, 945820, doi 10.1117/12.2191281 (2015)

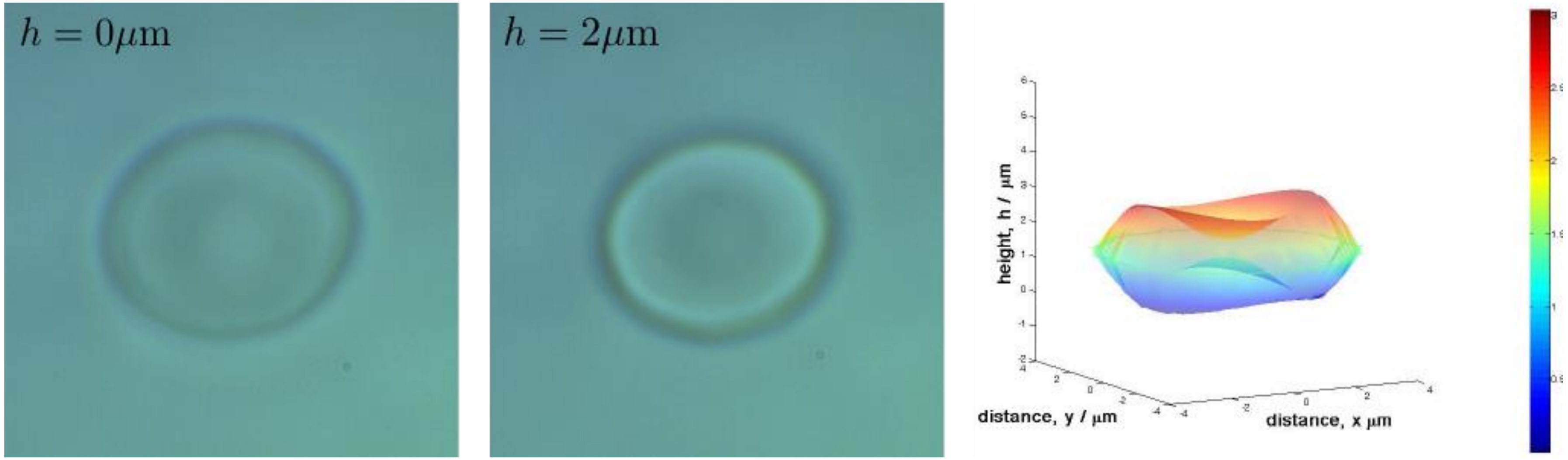
Other eye conditions

- Birdshot chorioretinopathy (BCR) is an uncommon, bilateral inflammatory disease affecting the choroid layer of the eye, thought to be caused by an inherited immune dysfunction
- Unlike diabetic retinopathy, BCR is not reportedly associated with thrombosis and vascular occlusion
- For cells from these patients we found no significant change in cell deformability
- We have also tested cells from patients with Behçet's disease (but patient numbers are very small)

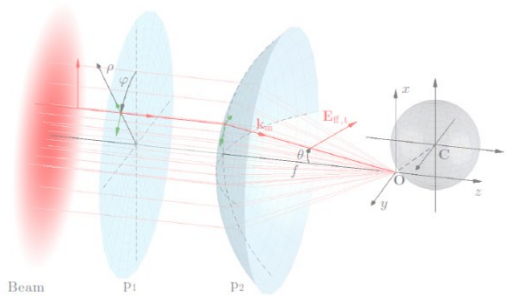
References:

- R. Agrawal, et al. '*Non-occlusive retinal vascular inflammation and role of red blood cell deformability in birdshot chorioretinopathy*', Ocular Immunology and Inflammation **6** 978-986 (2019)
- R. Agrawal, et al. '*Red blood cells in retinal vascular disorders*', Blood Cells, Molecules and Diseases **56** 53-61 (2016)

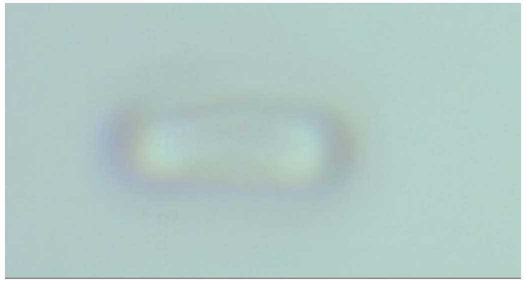
3D defocussing microscopy



Optical trapping of deformable material



I. Optical tweezers

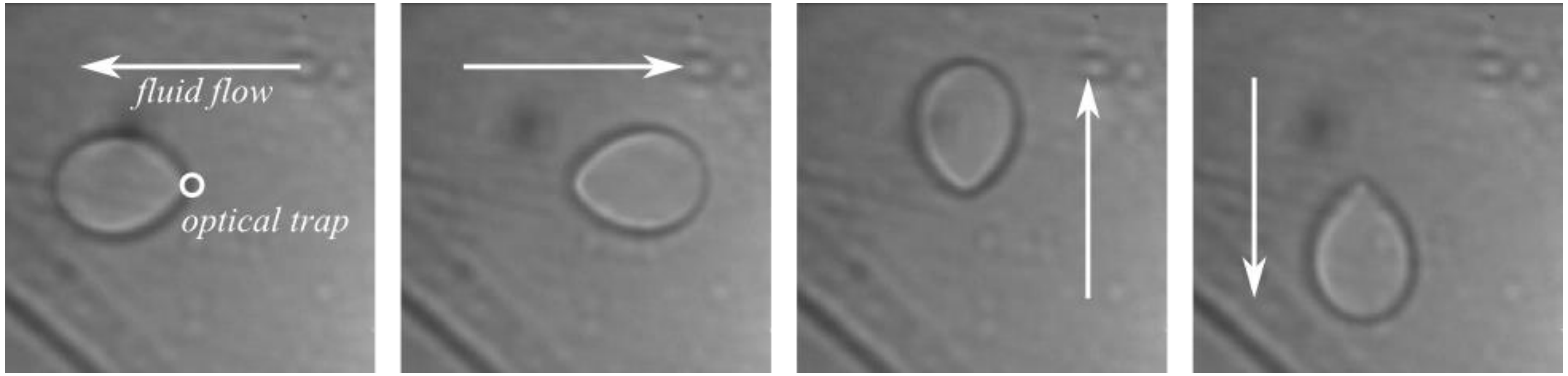


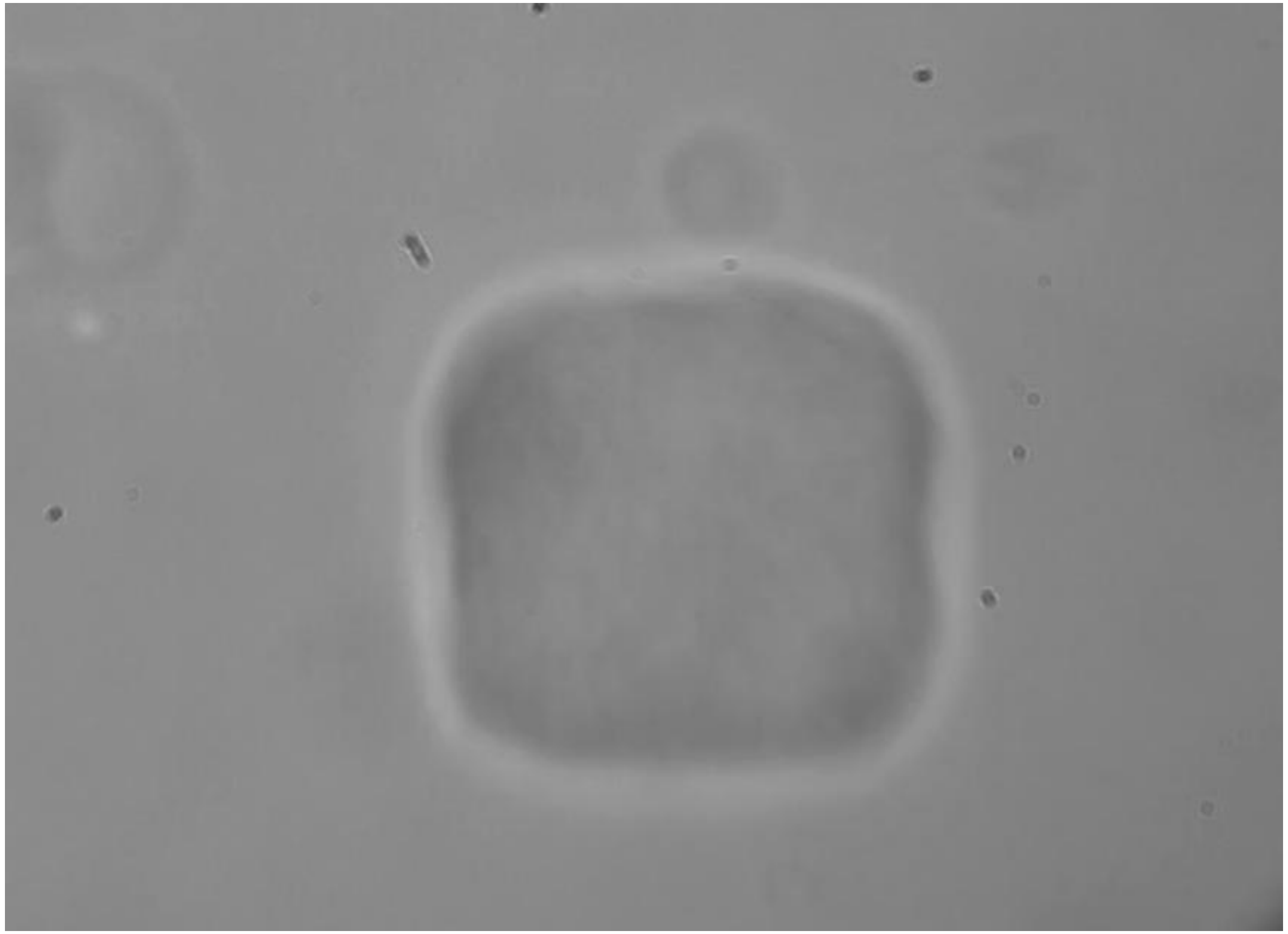
II. Red blood cells

III. Microemulsions

Microemulsions

- Dispersed phase = n-Heptane (1 part in 400)
- Continuous phase = Water
- Surfactant = 2mMol Docusate Sodium (AOT)
- Interfacial tension, $\gamma \sim 70\mathrm{mN} \cdot \mathrm{m}^{-1} \equiv 70 \times 10^{3} \mathrm{pN} \cdot \mathrm{\mu m}^{-1}$
- Addition of 40-60mMol NaCl can reduce interfacial tension by several orders of magnitude
- Strong temperature dependence: minimum γ achieved at phase inversion temperature
- Interfacial tension can be low enough that drop is deformed by optical stress





Deformation under optical stress

Laplace-Young equation

$$-\gamma \nabla \cdot \hat{\boldsymbol{n}} = \Delta\sigma = \frac{2\gamma}{a}$$

Define functional $f = r - R(\theta)$

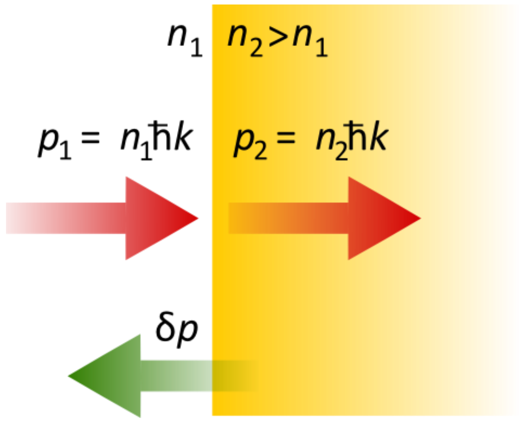
$$\hat{\boldsymbol{n}} = \frac{\nabla f}{|\nabla f|}$$

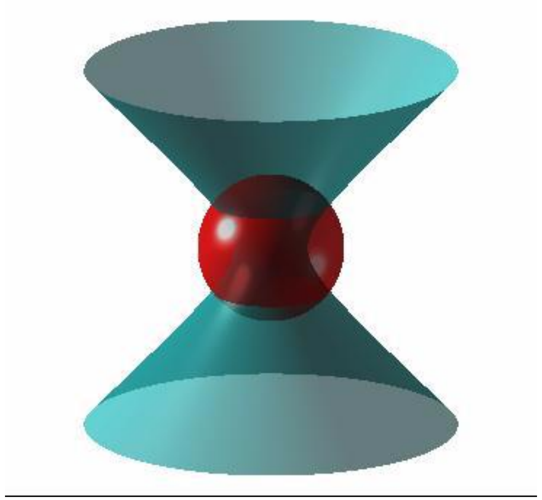
$$\nabla \cdot \hat{\boldsymbol{n}} = \frac{1}{R^2} \sum_{n=0} a_n (n^2 + n + 2) P_n(\cos\theta)$$

$$R(\theta) = \sum_{n=0} a_n P_n(\cos\theta)$$

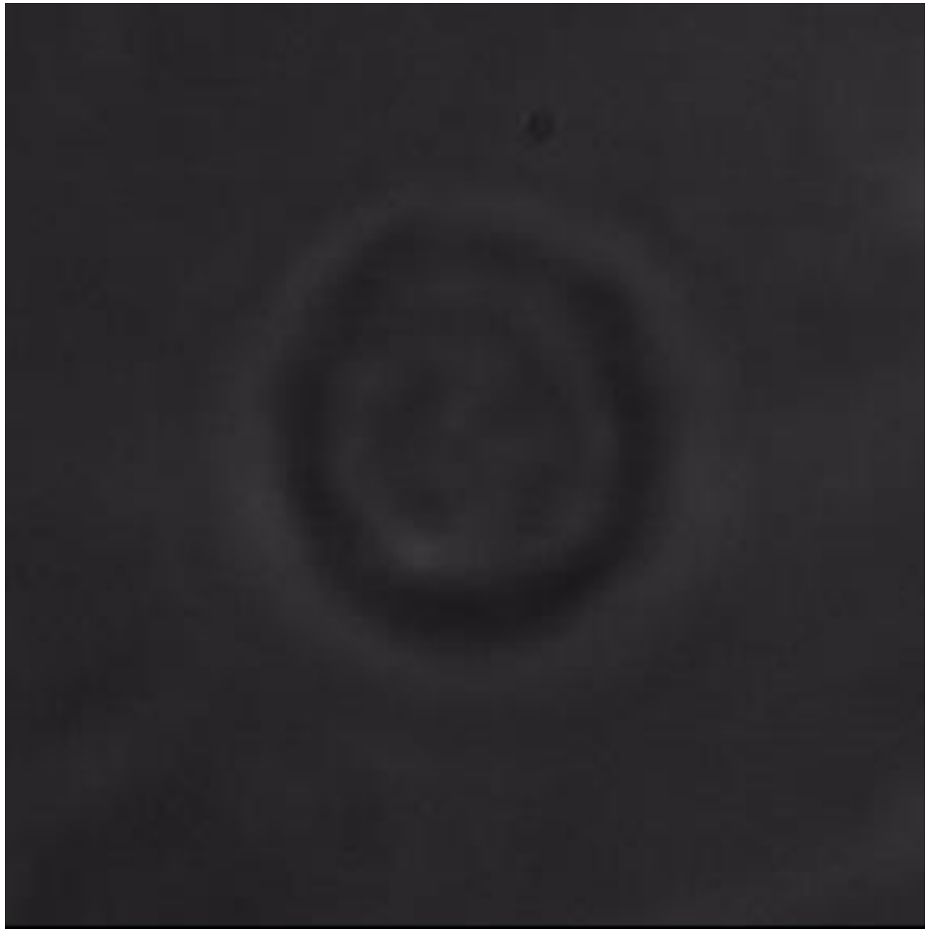
Assume deformations small and volume conserved

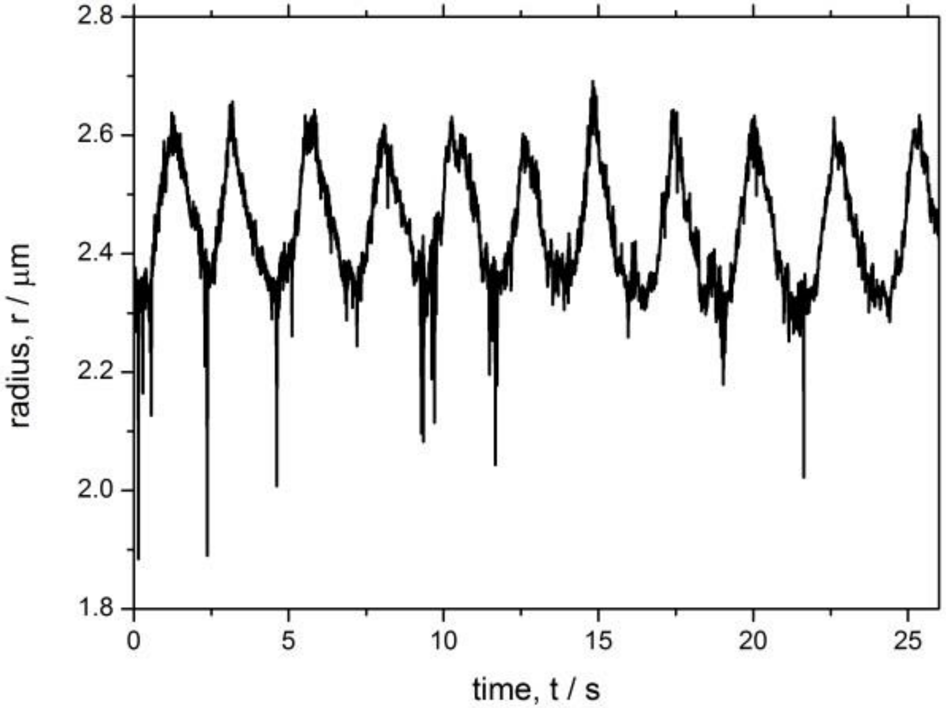
$$-\gamma \nabla \cdot \hat{\boldsymbol{n}} = \frac{2\gamma}{a_0}\left\{1 + \frac{1}{2}\sum_{n>0} \frac{a_n}{a_0}(n+2)(n-1)P_n(\cos\theta)\right\} = \frac{2\gamma}{a} + \sigma_{opt}$$





Deformation under optical stress

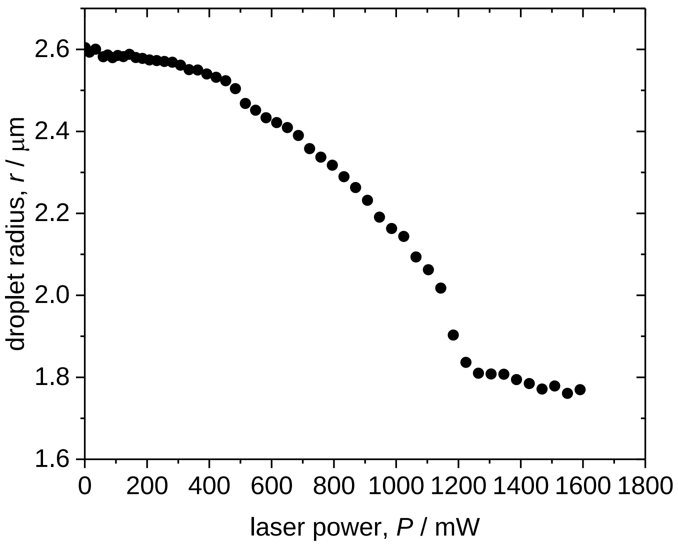


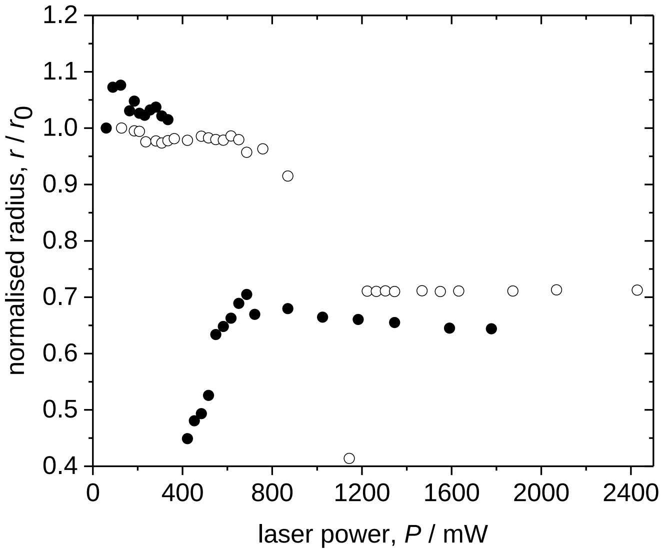


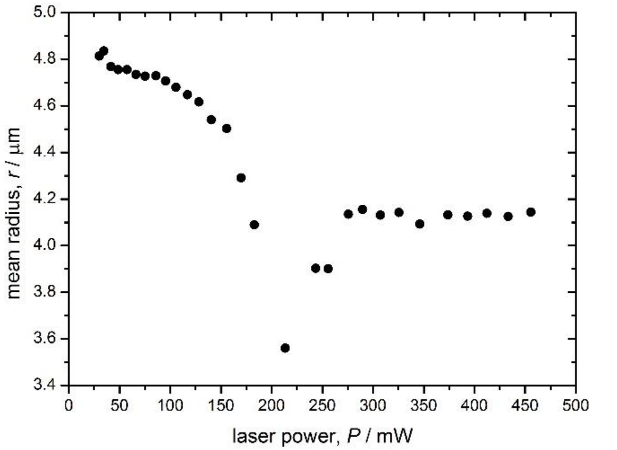
Deformation under optical stress

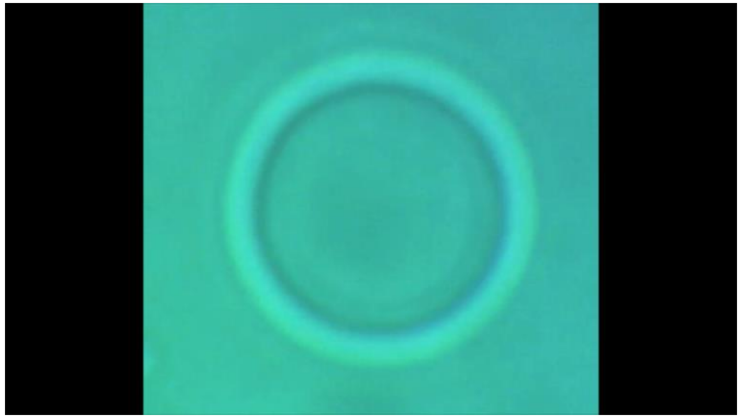
- We measure the droplet radius as a function of trapping laser power and observe decrease in radius to a ‘plateau’, consistent with earlier theoretical work
- We only observe this behaviour if the temperature of the sample is stablilized
- Without temperature stabilization the droplet passes through phase inversion and we see a sudden decrease in radius followed by a quick recovery
- We attribute this to laser heating of the droplet and use it to measure a heating rate:

$$C = (0.17 \pm 0.05)°C \text{ per } 100 \text{ mW}$$

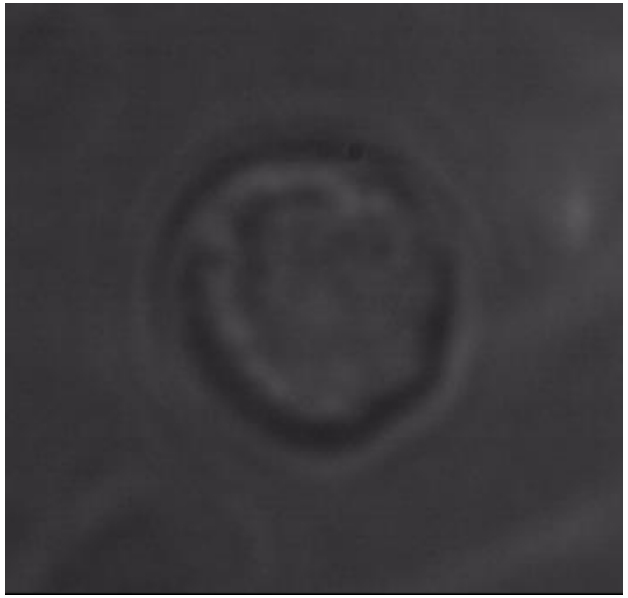


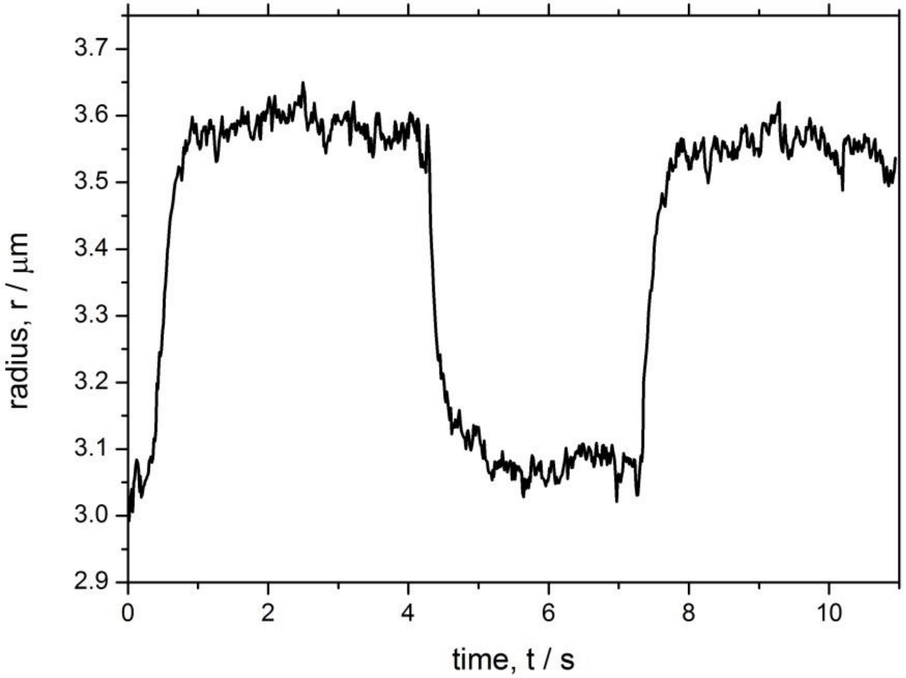






Measure interfacial tension





$$\gamma = \frac{2R_0}{\tau}\eta_c \frac{(19\mu + 16)(2\mu + 3)}{80\mu + 80}$$

$$\tau = (180 \pm 30)\ \text{ms}$$
$$\gamma = (58 \pm 9) \times 10^{-6}\ \text{mN} \cdot \text{m}^{-1}$$

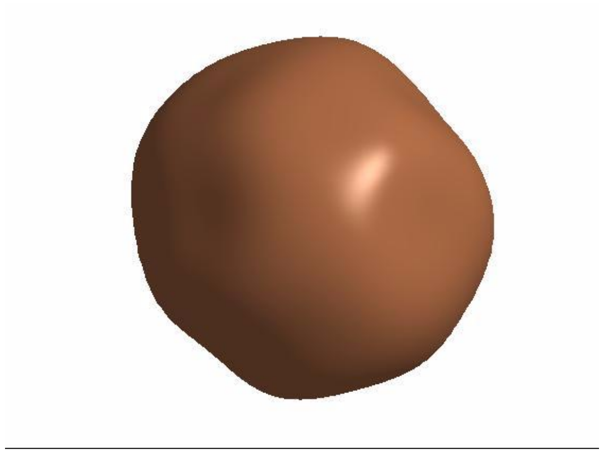
Surface fluctuations

Helfrich Hamiltonian:

$$F = \int_S [2\kappa(\Delta H)^2 + \gamma] dS - p \int_V dV;$$

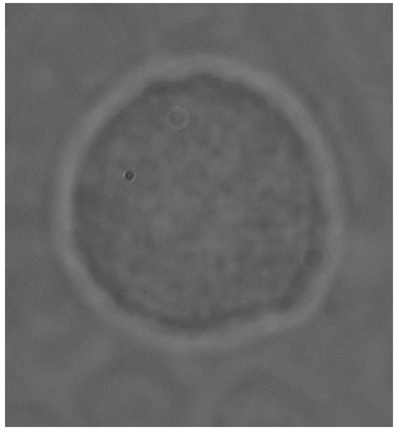
$\Delta H = H - C_0$

H = mean curvature; C_0 = spontaneous curvature



To second order:

$$\Delta F = \sum_{lm} \frac{{U_{lm}}^2}{2a^2} (l-1)(l+2) [a^2\gamma + l(l+1)\kappa]$$

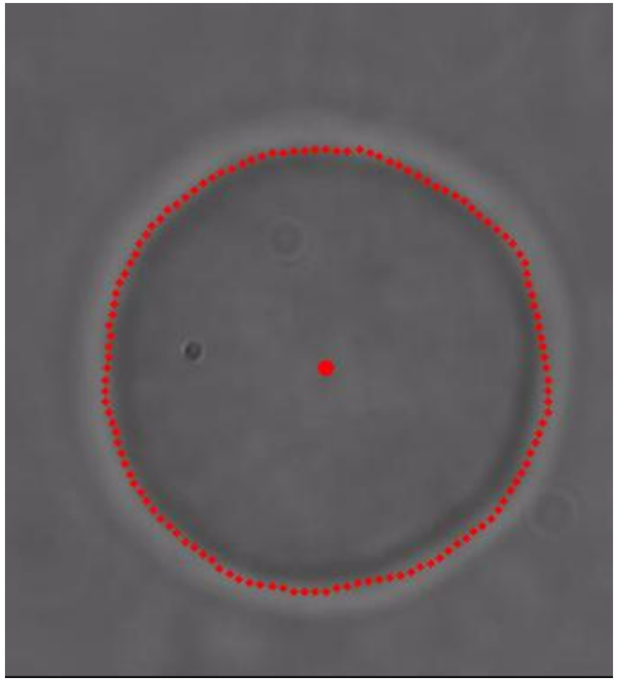


Surface fluctuations

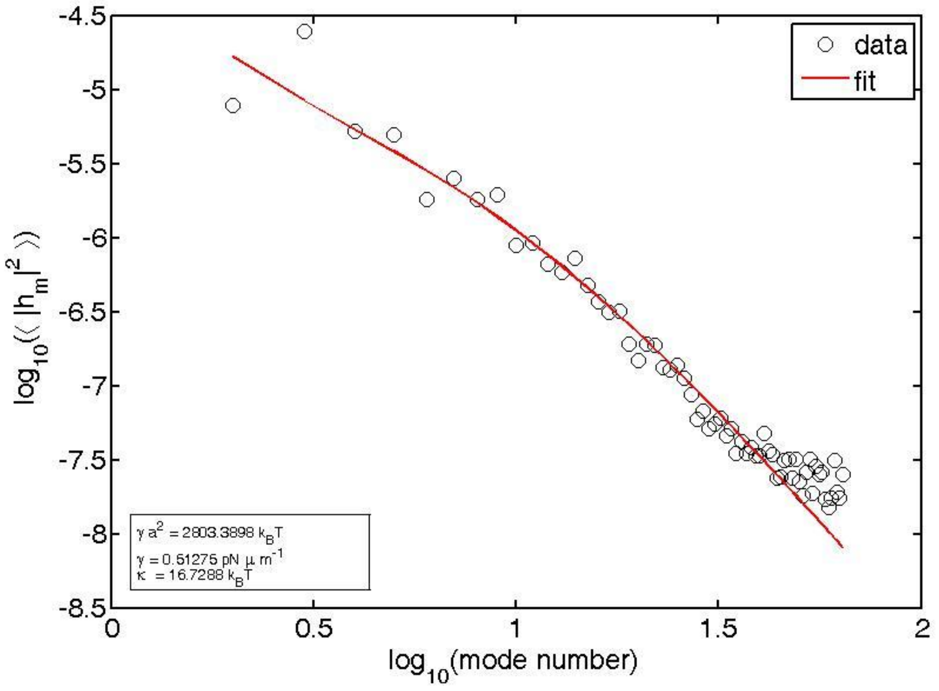
$$\frac{\langle |h_m|^2 \rangle}{a^2} = \sum_l \frac{b_{lm}^2 k_B T}{(l-1)(l+2)[a^2\gamma + l(l+1)\kappa]}$$

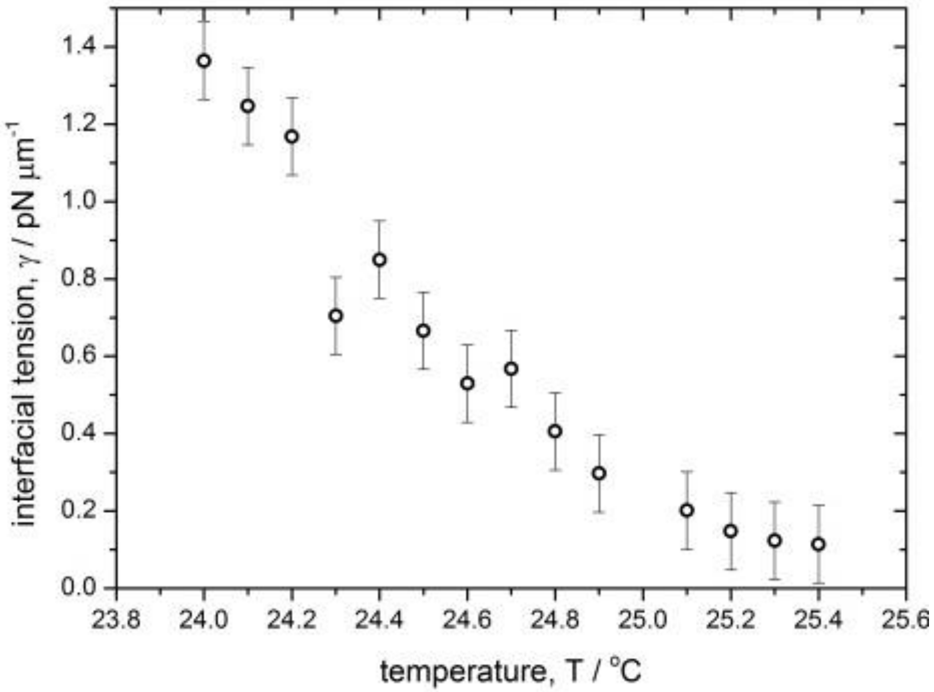
$$b_{lm} = Y_l^m\left(\frac{\pi}{2}, 0\right)$$

$$\frac{\langle |h_m|^2 \rangle}{a^2} = \frac{1}{4\pi}\sum_{k=0}^{k_{max}} \left\{\frac{1}{m+2k-1} + \frac{1}{m+2k+2}\right\}\left(\frac{1}{2^{2m}}\right)\binom{2k}{k}\left(\frac{1}{2^{2(m+k)}}\right)\binom{2(m+k)}{m+k} \times \left(\frac{1}{\frac{a^2\gamma}{k_B T} + (m+2k)(m+2k+1)\frac{\kappa}{k_B T}}\right)$$

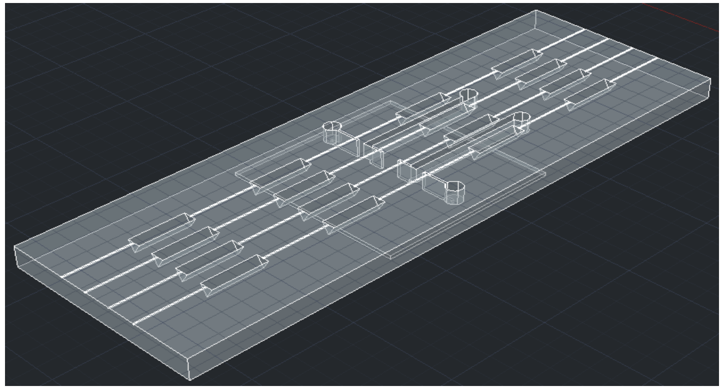


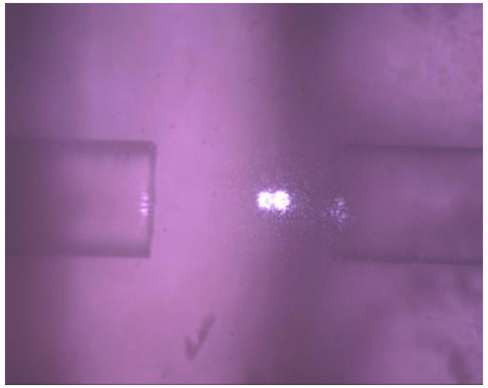
Measure interfacial tension

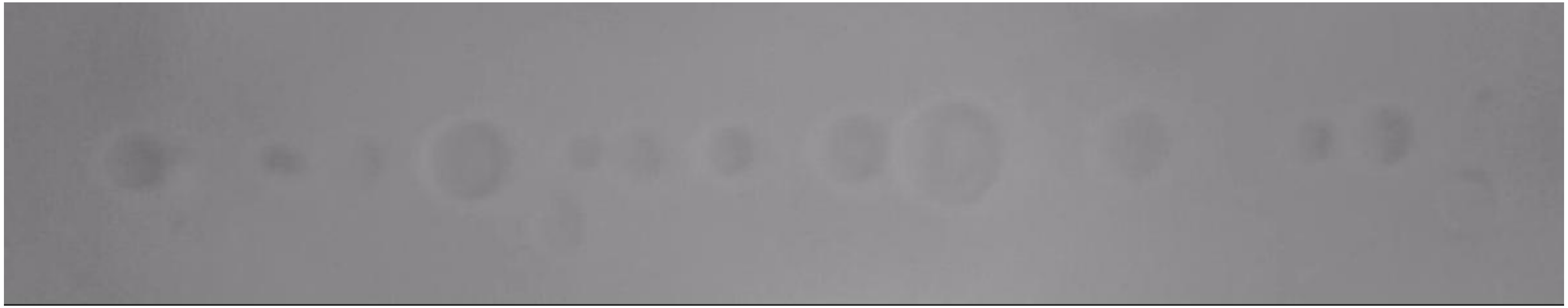




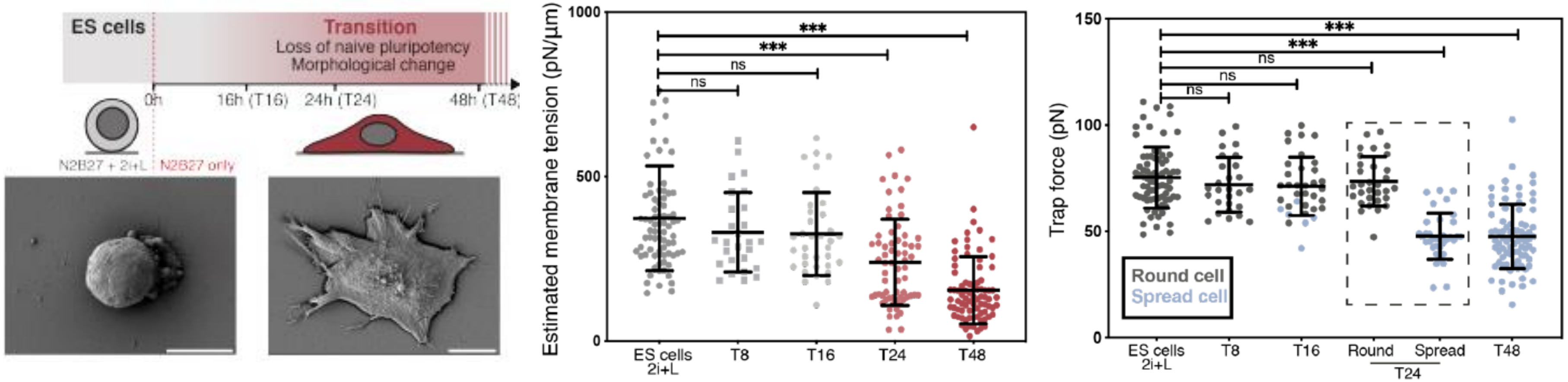
Future work: fibre trap / optical stretcher

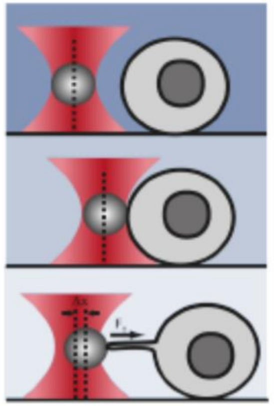


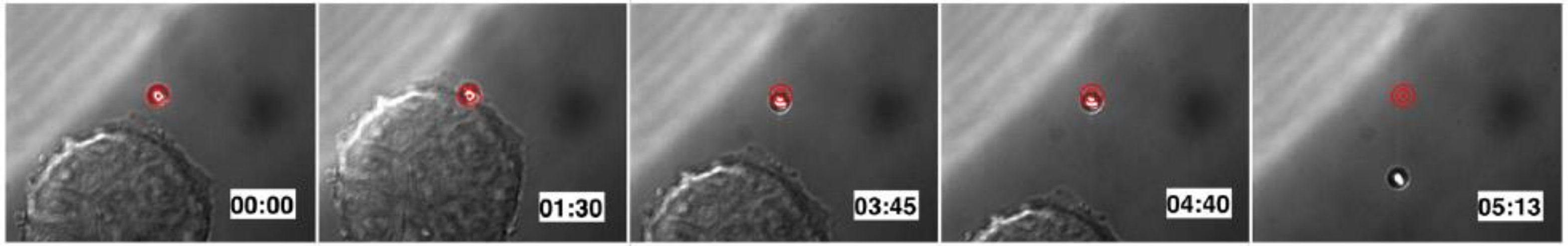




Pulling membrane tethers







H. De Belly, et al. ‘*Membrane tension regulates FGF-driven fate choice in embryonic stem cells*', bioRxiv 798959; doi: https://doi.org/10.1101/798959 (2019)

Thanks to…

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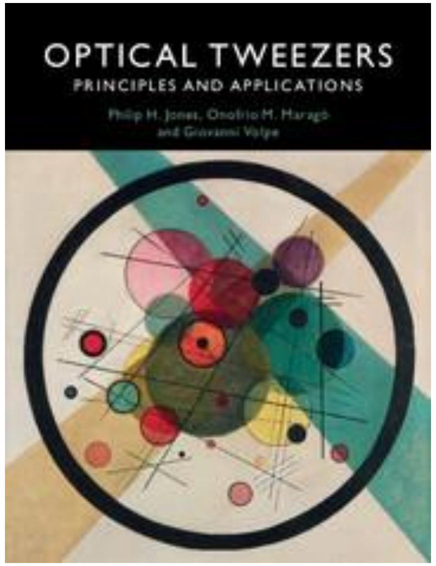

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P H Jones, O M Marago & G Volpe
Cambridge University Press (2015)
www.opticaltweezers.org