Load Response of the Flagellar Beat

investigated by means of micropipettes and microfluidics

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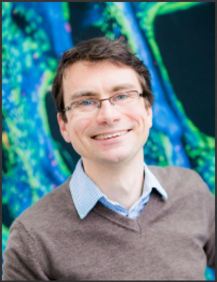
Dynamics of Fluids

UNIVERSITÄT DES SAARLANDES

FR PHYSIK

Our collaborative research team



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Load Response of the Flagellar Beat

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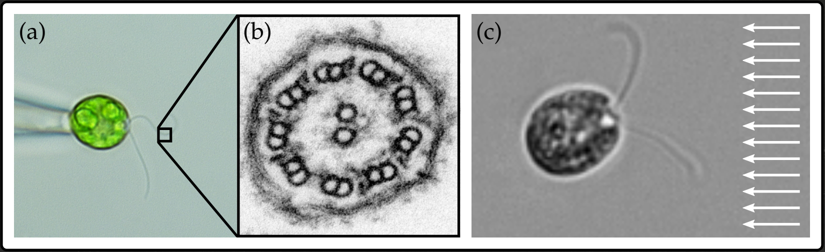
($\star$ equal contribution)

The eukaryotic flagellum:

a very complex machine on a very small scale

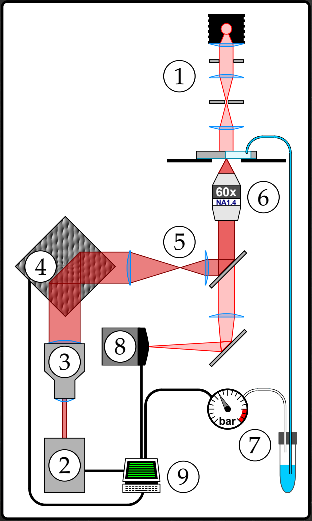


How does it perform under load?

electron micrograph taken from Pazour et al., *Proteomic analysis of a eukaryotic cilium*, J Cell Biol. **170**(1):103-13 (2005)

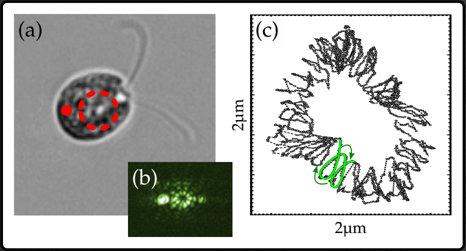
Holographic optical tweezers:

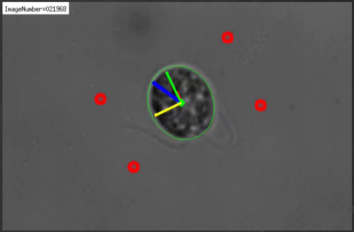
contactless fixation and force sensing capabilities



1. custom-made Köhler illumination
2. Nd:YAG laser, 1064 nm 1.5 W
3. 8× beam expander
4. phase modulator (PAL-SLM)
5. 2× telescope
6. 60× oil-immersion objective, NA 1.4
7. pressure controller
8. high-speed camera
9. PC

Reliable orientation of optically trapped *Chlamydomonas reinhardtii* is impossible

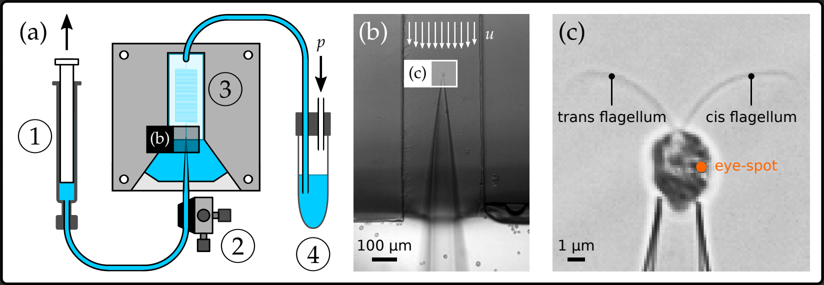




- cell rotates out of the focal plane
- optical forces just strong enough for trapping under no flow conditions

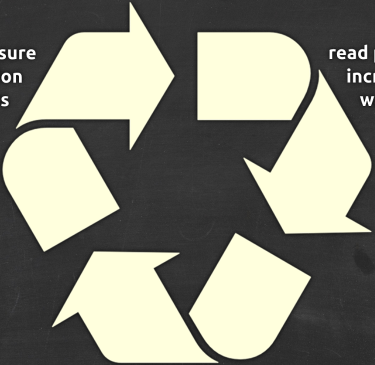
Micropipettes:

strong fixation and reliable orientation



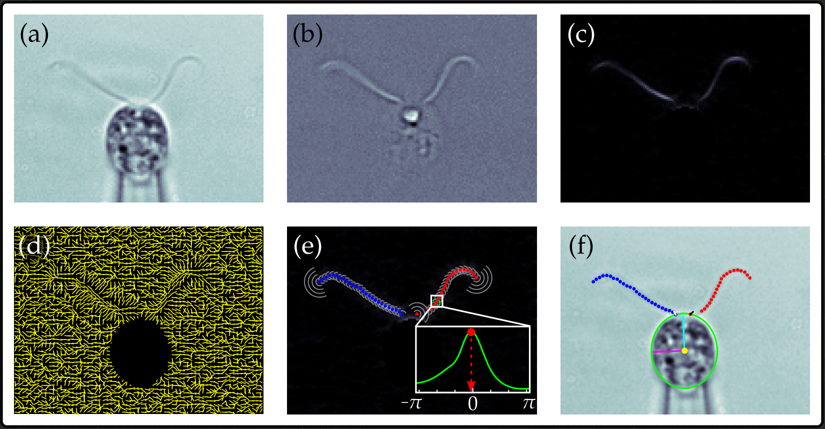
1. manually operated syringe
2. custom-made translational and rotational holder for micropipettes
3. microfluidics chip (pre-resistor channel and sample channel, sequential)
4. fluid reservoir to control the flow speed by means of a pressure device

Fully automated measurements are possible due to
device control toolboxes

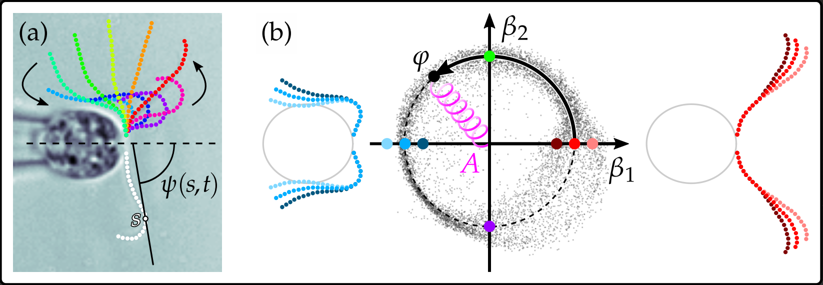


High precision flagellar tracking

reveals full dynamics of flagellar beating

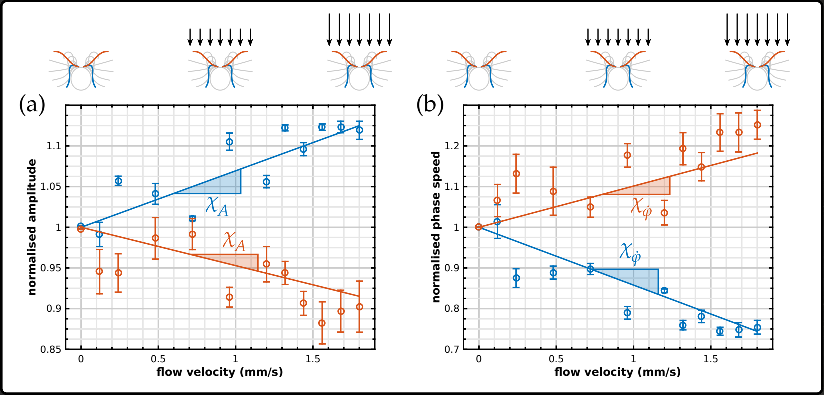


High-dimensional measurement data is reduced to a two-dimensional
limit cycle representation



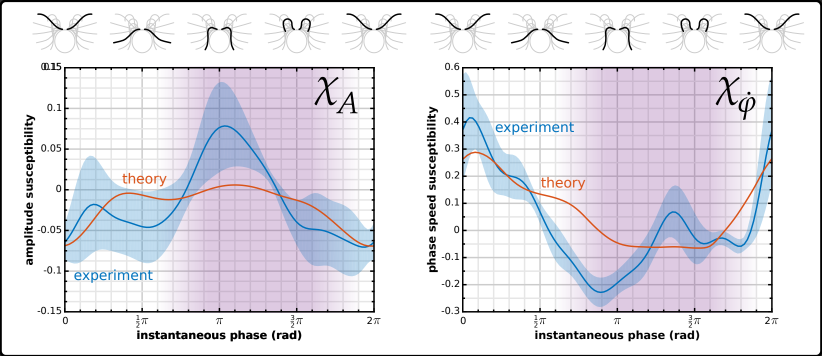
- tangent angle representation $\psi(s,t)$ for flagellar point $s = 1 \ldots S$ at times $t = 1 \ldots T$
- principle component analysis: $\psi(s,t) = \sum_{n=1}^{S} \beta_n(t)\, \tilde{\psi}_n(s)$
- $Z(t) = \beta_1(t) + \mathrm{i}\beta_2(t) = A(t) \exp(\mathrm{i}\varphi(t))$

The flagellar load response is characterised by the amplitude susceptibility χ_A and the phase speed susceptibility $\chi_{\dot{\varphi}}$



$$\frac{A}{A_0} \approx 1 + \chi_A \cdot u \quad \text{and} \quad \frac{\dot{\varphi}}{\dot{\varphi}_0} \approx 1 + \chi_{\dot{\varphi}} \cdot u$$

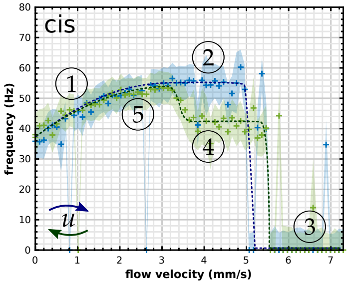
The flagellar load response is highly phase-dependent



- during the recovery stroke, the flagella are closer to the cell body
- the recovery stroke slows down
- the power stroke speeds up

The frequency response of the flagellar beat

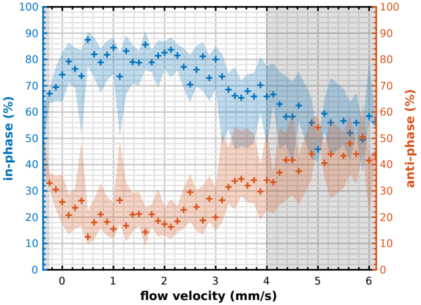
reveals dynamic beating modes and shows hysteresis



- low load: almost linear frequency response
- intermediate load: chiral beating and tremor-like beating
- high load: stalling

Flagellar synchronisation changes with external load

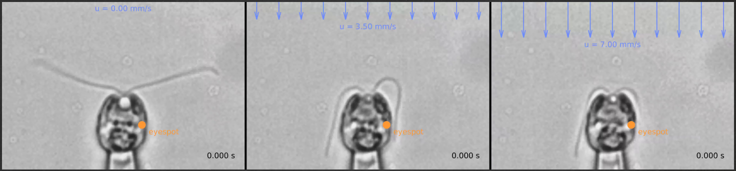
but does not switch from in-phase to anti-phase



- ▶ low positive load: almost no change in flagellar synchrony
- ▶ intermediate positive load: reduced flagellar synchrony
- ▶ negative load: loss of flagellar synchrony

Two dynamic modes of beating for positive load...

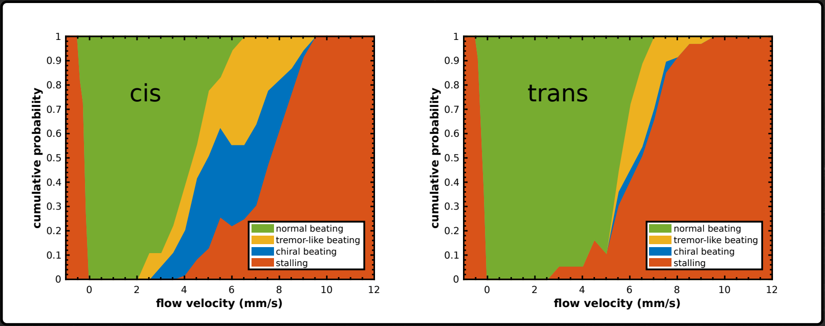
(chiral and tremor-like beating)



...which do not exist for negative load

Different response of cis and trans flagellum

to external load



- chiral beating is almost exclusive to the cis flagellum
- tremor-like beating is less pronounced for the trans flagellum
- trans flagellum stalls at lower flow speeds than cis flagellum

Technical summary

- optical tweezers are versatile but lack trapping power and orientational control
- micropipettes are not contactless but guarantee strong and stable orientational fixation
- device control toolboxes enable fast prototyping & reliable, fully automated measurements
- high precision flagellar tracking permits new insights into the dynamics of flagellar beating

Scientific summary

- flagellar load response is phase-dependent
- load response is different for cis and trans
- different dynamic modes of beating
- pronounced stalling hysteresis under positive load
- no stalling hysteresis under negative load
- flagella can sustain very high positive load but only small negative load

Thank you for your attention!