Towards routine fluorescence-free imaging of biomimetic cell membranes

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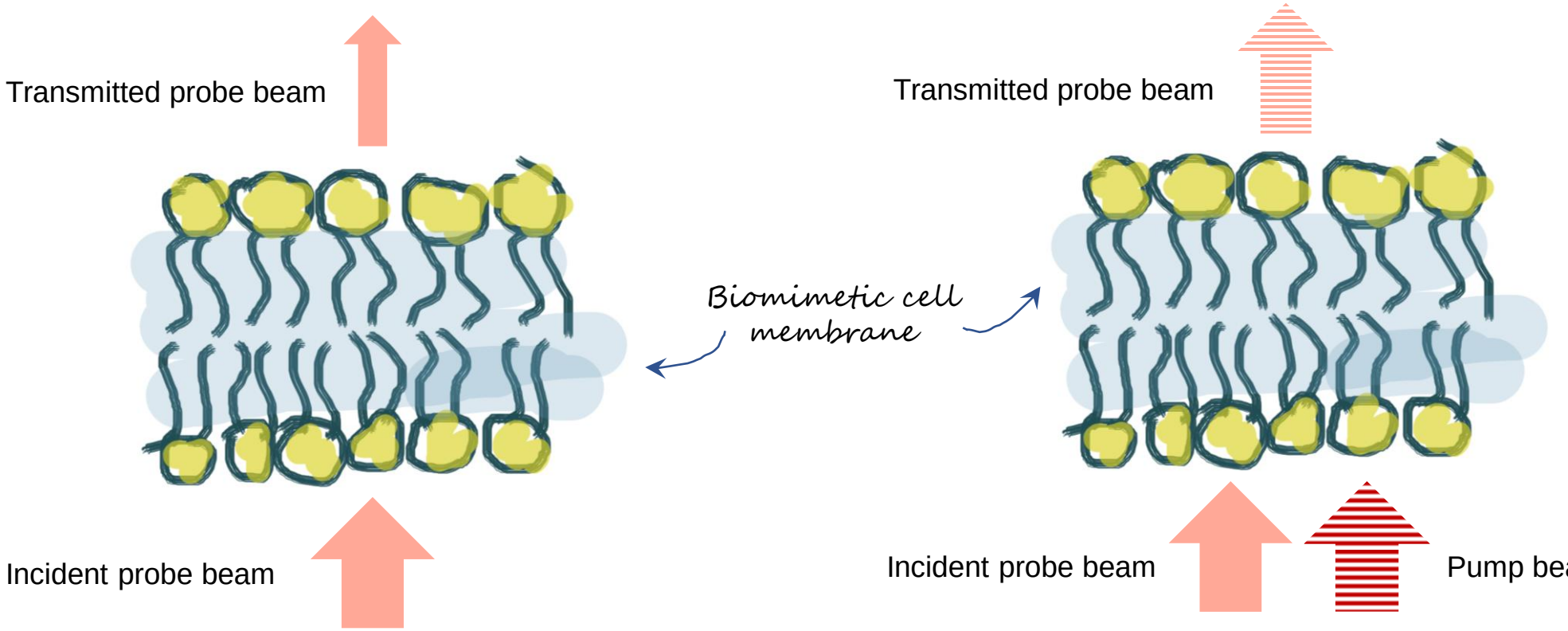


1. Background

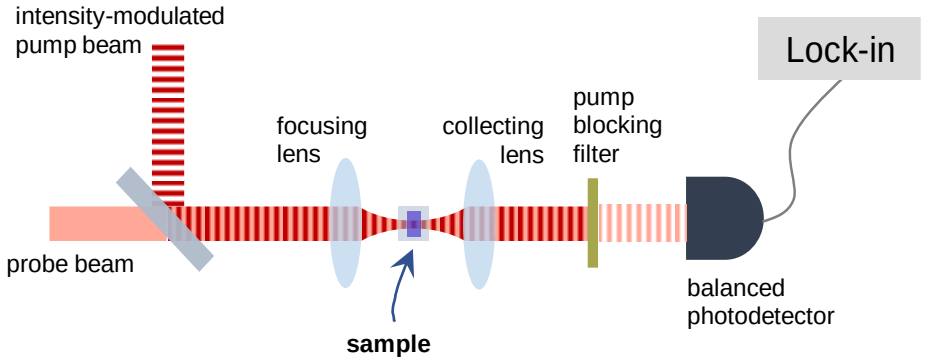
Although fluorescence spectroscopy and microscopy remain the most widely used tools in biological research, **they have significant limitations.** They require fluorescent samples and are prone to photobleaching. Hence, a great effort was made to develop approaches bringing all the benefits of fluorescence techniques while not relying on fluorescent signal.

Manifold optical imaging methods has been proposed, including ground state depletion,[1] stimulated emission[2,3] and photothermal microscopy.[4] All these approaches, based on modulation transfer scheme, has shown the potential of detecting individual quantum emitters, yet so far **they remain in the state-of-art state rather than routine experiments.**

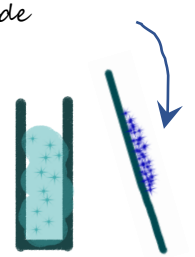
2. Methodology

Experimental setup (simplified)

CW lasers employed

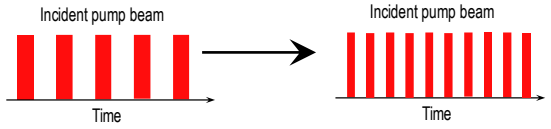


Solid film of analyte on the cover slide

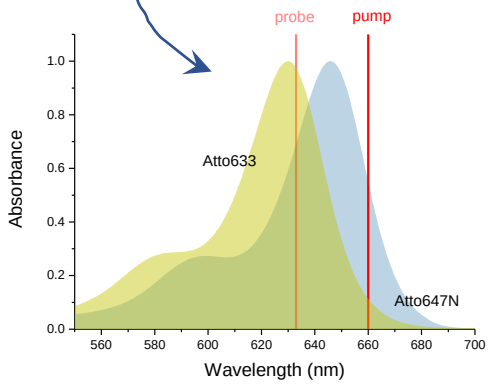


A **Effect of molecular environment**

> Modulation frequency dependence

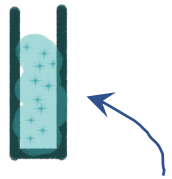


Both pump and probe in resonance with chromophores' absorption spectra – ground state depletion (GSD) scheme



B **Sensitivity and detection limit of the experimental setup**

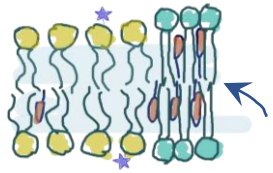
> Analyte concentration dependence



Spectroscopy cuvette with an analyte solution

C **Liquid-disordered phase in biomimetic membrane detection**

> Pump and probe intensity dependence



Solid supported lipid bilayer as a biomimetic membrane

3. Results

A Modulation transfer signal vs. modulation frequency

Contribution of ground state depletion and photothermal effect depends on the nature of the sample

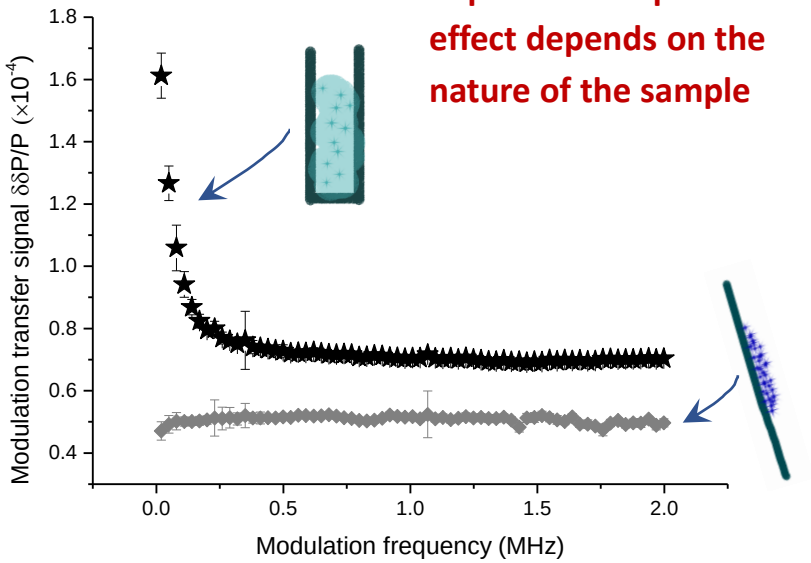


Figure 1. Modulation transfer signal from 3.3 mM aqueous Atto647N solution and from solid Atto647N film as a function of the modulation frequency. The power level of the pump and probe beams is 2.0 mW and 2.7 mW, respectively. The beam waist area is ca. 1.3×10^{-5} cm^2.

B Ground state depletion signal vs. analyte concentration

The detection limit is ~**9 nM** corresponding to ~**200,000** Atto647N molecules in the probe volume (~8.2×10^{-11} liter)

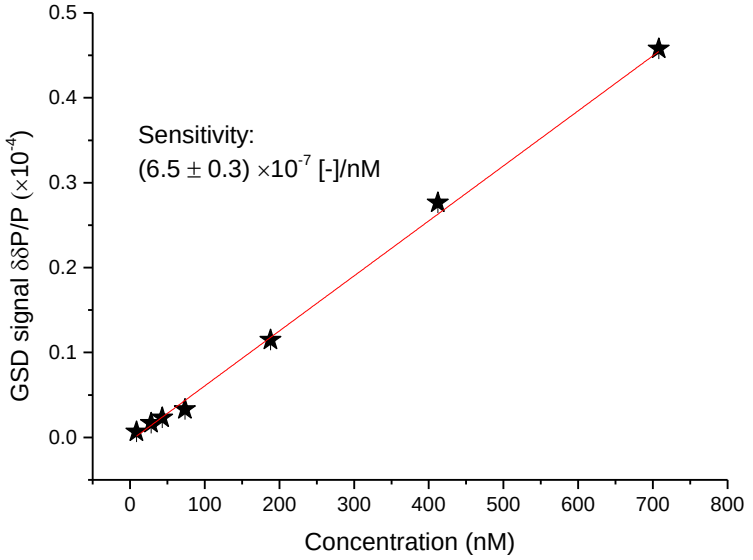
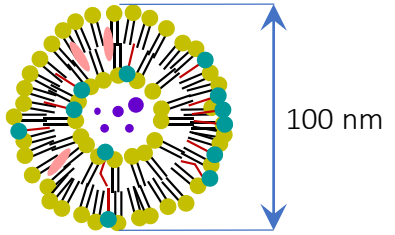


Figure 2. Ground state depletion signal as a function of concentration of aqueous Atto647N solution. The modulation frequency is 1 MHz. The power level of the pump and probe beams is 4.2 mW and 3.4 mW, respectively. The beam waist area is ca. 3.1×10^{-6} cm^2.

For the reference: the number of lipid molecules in a **100 nm** size vesicle is about **80,000**



3. Results

C Liquid-disordered phase in biomimetic membrane detection

Dividing the acquired GSD signal by the theoretical **contrast for 1 Atto633 molecule** gives ~**400,000 molecules** in the probe volume

IT IS CONSISTENT

Taking into account the area per lipid parameter and the concentration of labeled lipid: **430,000 DOPE-Atto633 molecules** within the probe volume

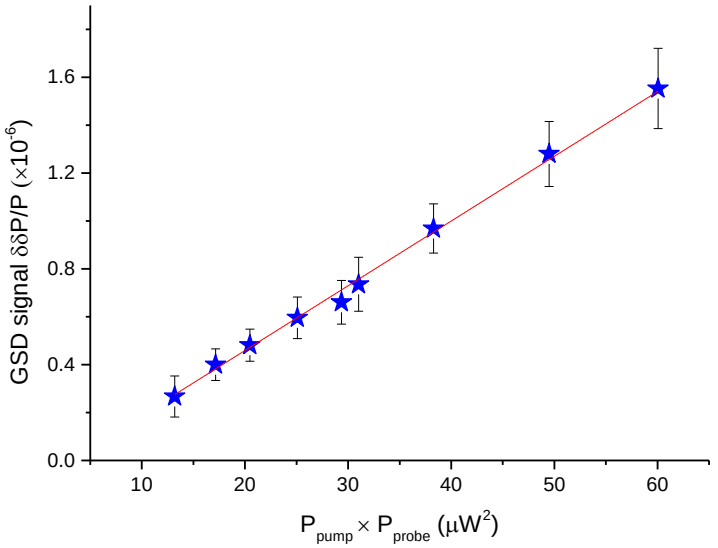
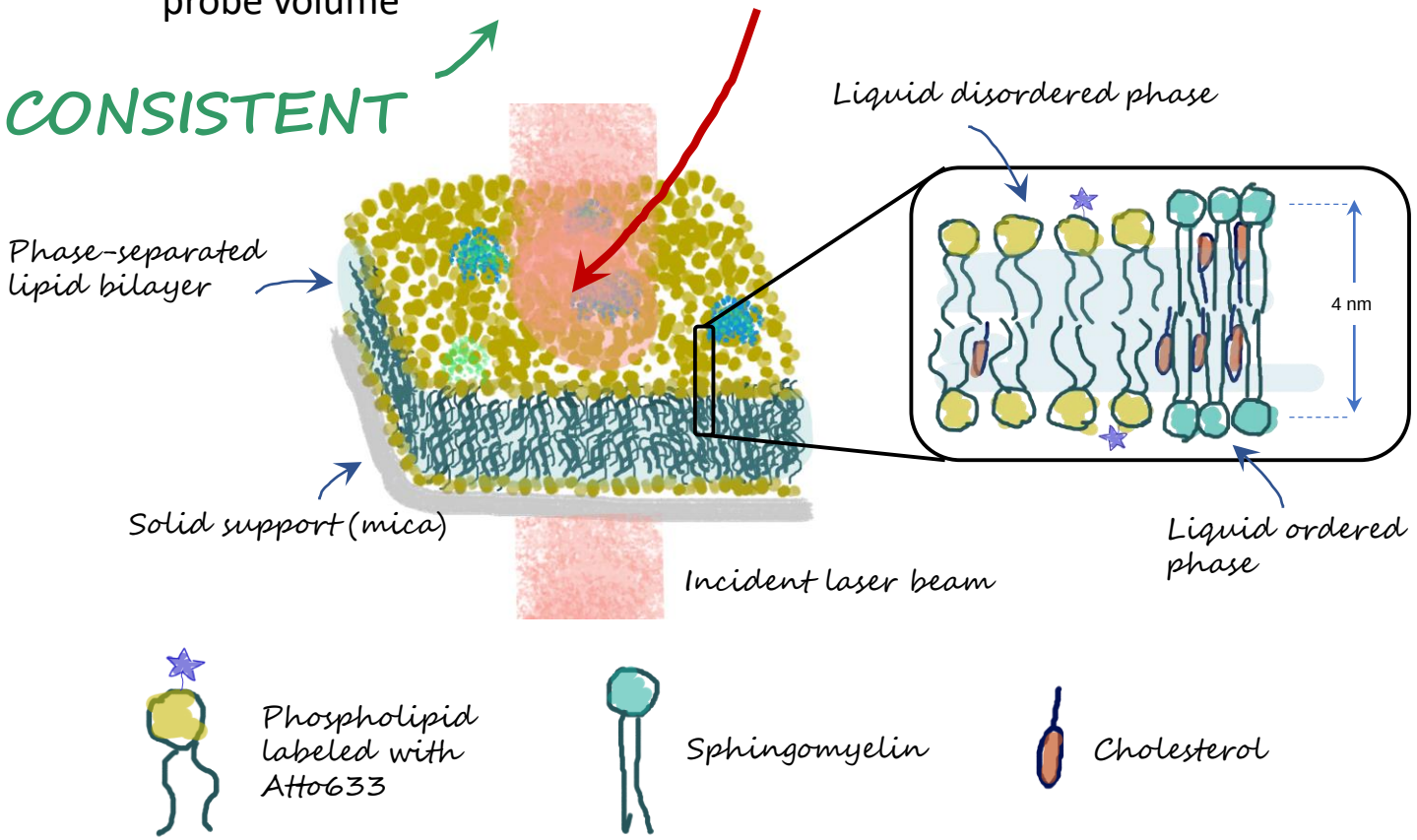


Figure 3. Ground state depletion signal from biomimetic membrane containing Atto633 labeled phospholipids as a function of the product of the pump beam power level and the probe beam power level. The beam waist area is ca. 3.1×10^{-6} cm^2.

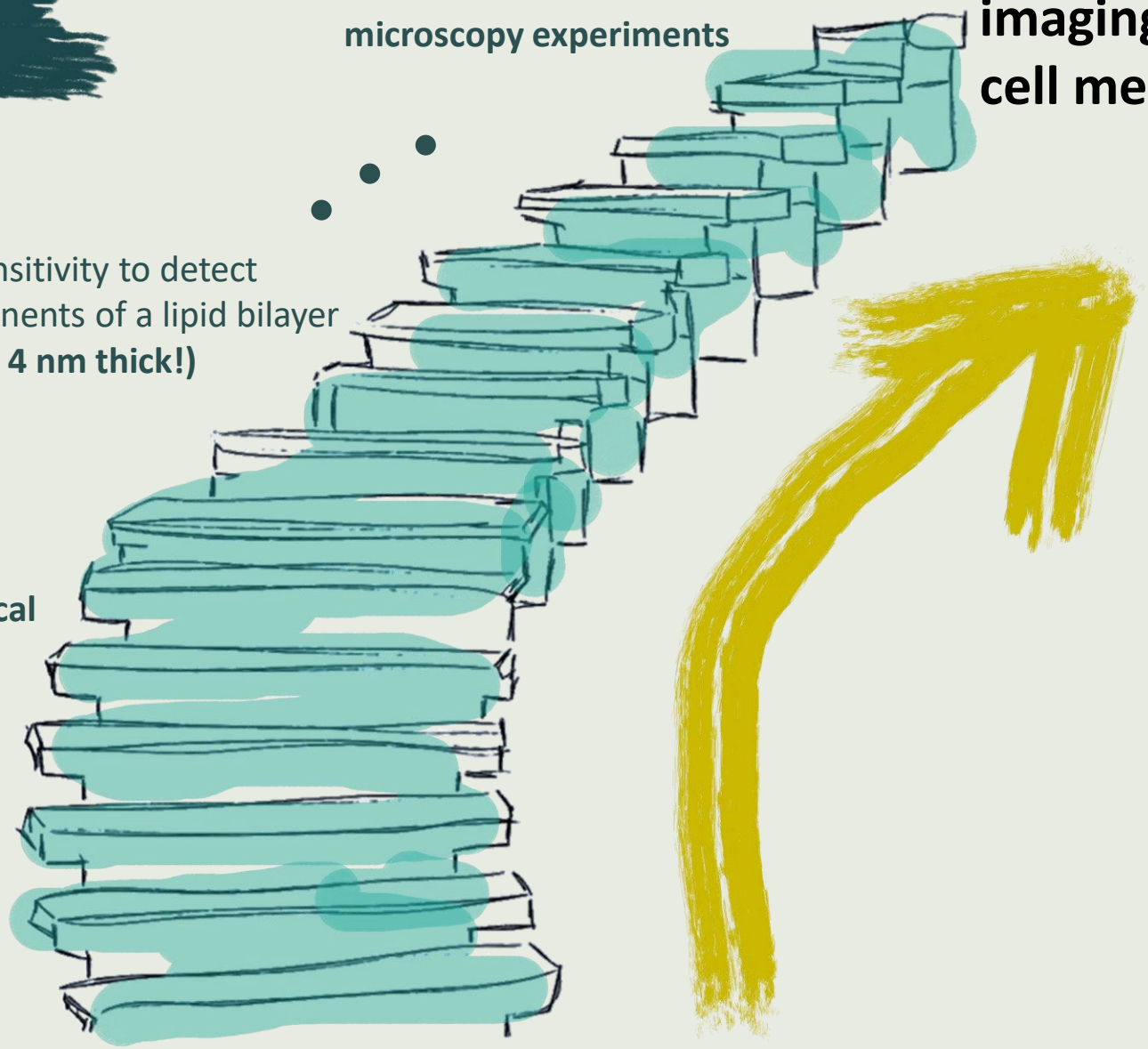


4. Conclusions

Towards routine fluorescence-free imaging of biomimetic cell membranes

microscopy experiments

- has sensitivity to detect components of a lipid bilayer (**about 4 nm thick!**)
- it enables the fluorescence-free investigation the **model biological system**
- Employing the modulation transfer detection scheme, we constructed the **relatively low-cost and simple experimental setup with CW lasers**



References

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2. W. Min et al., *Nature*, **461**, 1105–1109 (2009).
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4. A. Gaiduk, M. Yorulmaz, P. V. Ruijgrok, and M. Orrit, *Science*, **330**, 353–356 (2010).
5. A. Bonvicini et al., *Phys. Chem. Chem. Phys.*, **21**, 15487–15503 (2019).

Acknowledgments

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