Optimizing photoswitching performance of organic dyes for SMLM through a single MEMS mirror

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Introduction

Whilst SMLM is able to localize molecules with nanometre precision it is only able to achieve this if the imaging parameters have been properly optimised [1]. Key parameters we have investigated for optimisation are homogeneous excitation illumination and the optimal pH and thiol concentrations for photoswitching buffers.

Typical SMLM experiments make use of conventional Gaussian illumination modes meaning either a compromise in the excitation intensity is made due to overfilling of the objective lens, or an uneven illumination field of view (FOV) is observed which can cause intensity driven photoswitching differences in dye molecules located at different points in the FOV.

We demonstrate the use of a single microelectromechanical system (MEMS) mirror as a cost-effective method to generate a flat-field of illumination across the FOV resulting in consistent SMLM metrics [2]. We also show a workflow employing an intensity gradient through the MEMS in which we screen for optimal pH and thiol concentrations to obtain the best results for brightness and photoswitching performances of the carbocyanine dye Alexa Fluor 647. Finally, we have monitored the performance of the oxygen scavenger system based on glucose and glucose oxidase in open or closed environments, determining the amount of acidification present in prolonged imaging experiments [3].

Scanning MEMS mirror for tuneable wide-field illumination

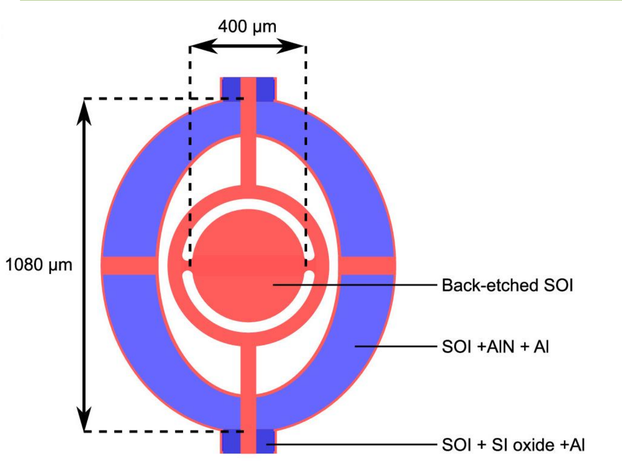

Fig. 1 2D MEMS micromirror is suspended by 4 thin film piezoelectric actuators allowing the mirror to rapidly tip-tilt. MEMS mirror actuators have found extensive use in optical microscopy such as allowing independent angular and phase control of two SIM excitation beams [4], and low-cost scanning optics in light sheet systems [5].

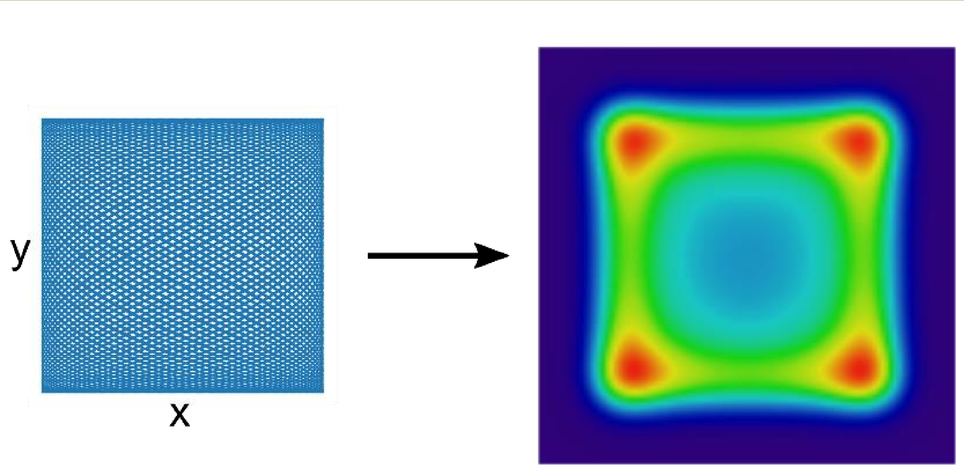

Fig. 2 Left: MEMS mirror scans illumination in a Lissajous pattern due to MEMS sinusoidal oscillation at a resonance frequency of 45.5 kHz vertically and 85.5 kHz horizontally. Right: The illumination pattern detected by the camera is a convolution of the 2D Gaussian model of the laser beam and the 2D histogram. The detected illumination can be changed by driving the MEMS at different voltages and hence altering the amplitude of the Lissajous pattern.

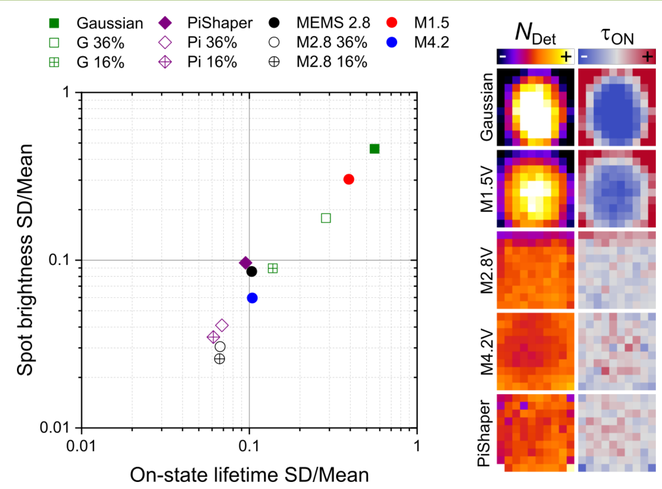

Fig. 3 MEMS modes for tuneable wide-field illumination. Left: Coefficient of variance plot of spot brightness and the on-state lifetime demonstrating that MEMS driven at 2.8/4.2 V results in far lower variation compared to Gaussian illumination. Right: Spot brightness and on-state lifetime over the entire FOV for different illumination modalities including three different MEMS modes including an induced Gaussian mode.

Photoswitching metrics for buffer pH and thiol concentration

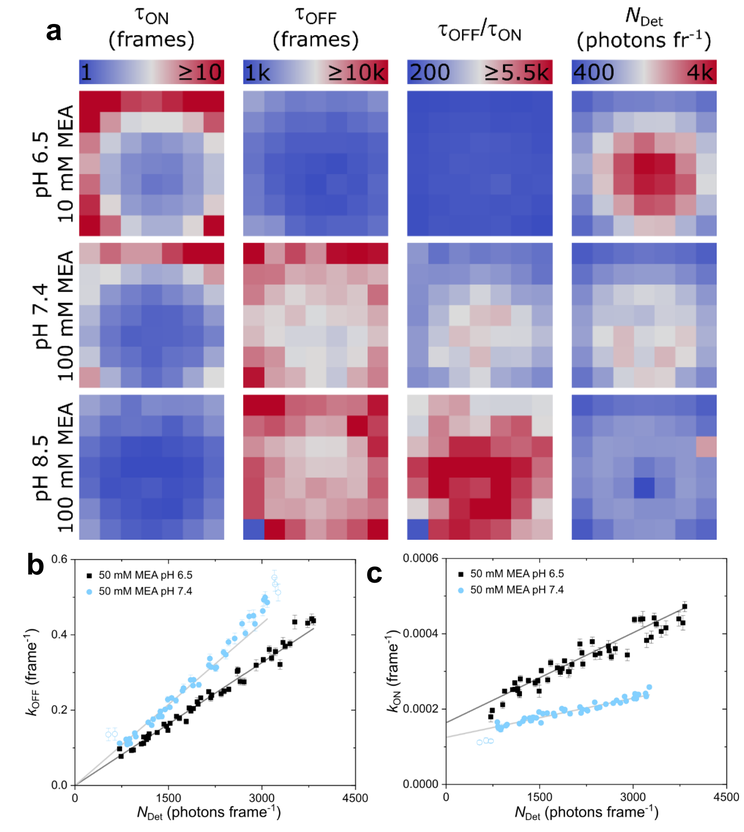

Fig. 4a Photoswitching metrics with different buffer conditions showing increasing pH and mM of MEA resulted in increased photoswitching ratios and decrease in spot brightness. **b** Plot demonstrating linear relationship between the inverse of the on-state and spot brightness. **c** Photoswitching rate constants for different buffer conditions.

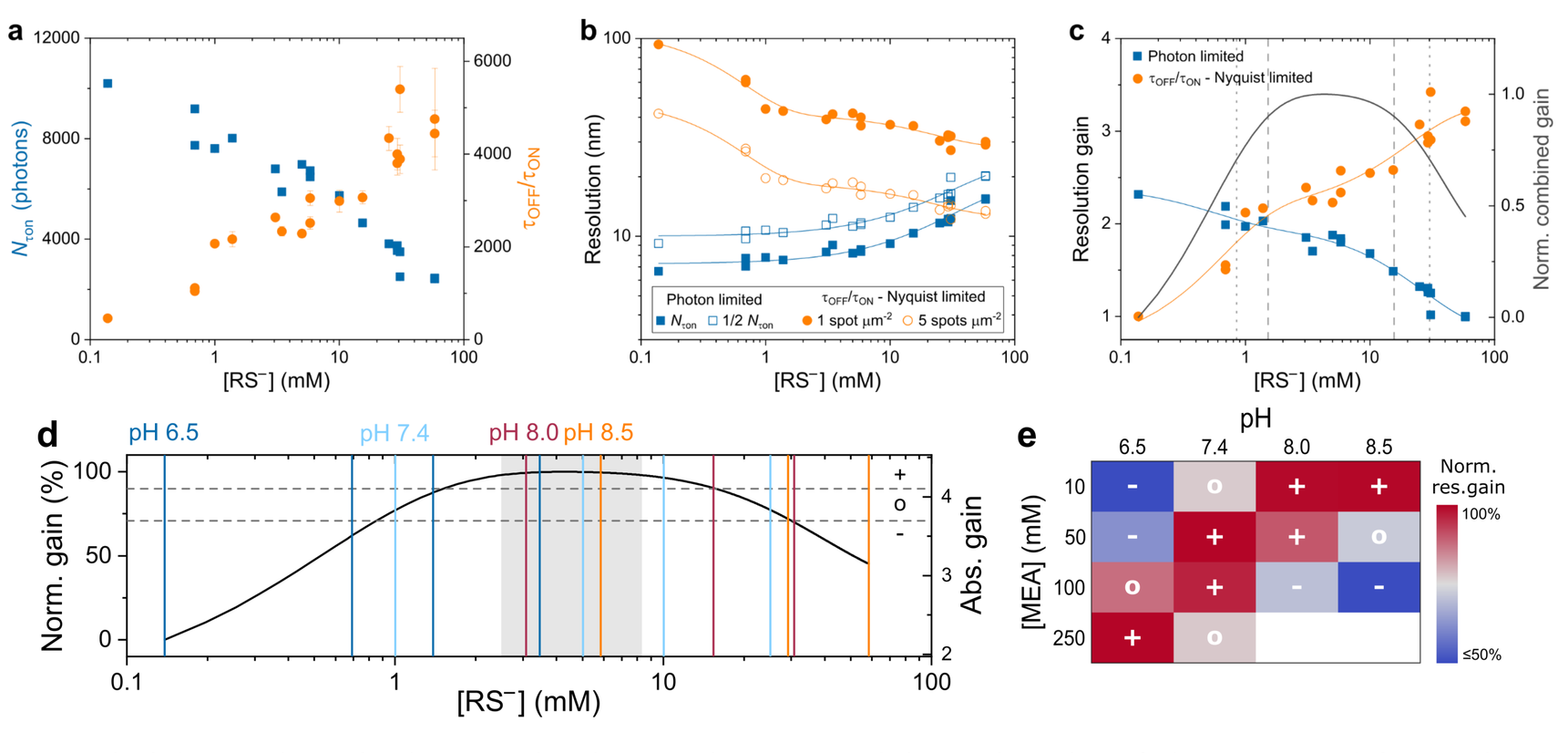

Fig. 5a Thiol driven relationship between the photoswitching ratio and the total number of photons emitted. **b** Optical and Nyquist resolutions as a function of the thiol concentration. **c** Resolution gain determined from **b** as a function of the thiol concentration. **d** Linear gain in resolution determined as a product of the Nyquist and photon based resolution. This demonstrates that the concentration of thiol can be used as a resolution bandpass filter. **e** A rating of the tested buffer conditions. A working thiolate concentration between 1 and 16 mM offers the best working resolution gain. The thiol concentration can be used to tailor the molecular brightness and the photoswitching ratio whilst maintaining high spatial resolution. The pH can be tailored in this range to accommodate and optimise for other pH dependent parameters.

Acidification of photoswitching buffers

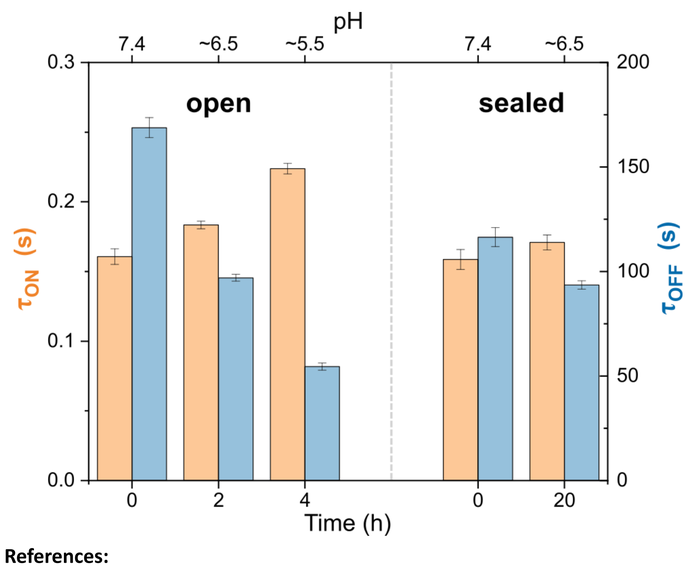

Fig. 6 Evaluating the stability of the photoswitching buffer (50 mM MEA, pH 7.4, GOC system) in sealed and unsealed sample chambers.
Simply removing oxygen head space in an imaging chamber with a coverslip significantly stabilises buffer switching parameters and acidification over long imaging durations.

Conclusions

- **MEMS mirror integration into a dSTORM microscope allows for tuneable illumination from flat-field to Gaussian modes to be generated.**
- **MEMS induced intensity gradients allows for investigation of photoswitching metrics within a single acquisition to be obtained.**
- **Systematic study of MEA concentrations and buffer pH underpins the versatile role of thiolate in single-molecule photoswitching in which it provides bright spots due to photo-stabilisation and the inefficient generation of long lasting off-states.**
- **Optimal thiolate concentration bandwidth of 1 – 16 mM allows for tailoring of photoswitching performance of cyanine dyes.**
- **Maximum combined gain in resolution is achieved at 4.3 mM thiolate, which can be achieved with a imaging buffer at pH of 7.4 and a MEA concentration of 43 mM.**
- **Acidification of the glucose oxidase catalase system can be stabilised for long imaging durations when sealing a imaging chamber is done.**

References:
[1] S. van de Linde et al. *Nat. Protoc.* **6**, 991 [2] L. Herdly et al. *ACS Photonics* **8**, 2728 [3] L. Herdly, P. W. Tinning et al. in prep. 2022 [4] P. W. Tinning, et al. *SPIE* 2022 [5] S. Bakas et al. *Sci. Rep.* 2021