# Stochastic Optical Reconstruction Optimization for Investigating Innate Immune Response

Quinton Smith, Jesse S. Aaron, Jerilyn A. Timlin[1]

[1] Bioenergy and Defense Technologies Dept., Sandia National Laboratory, Albuquerque, NM 87185

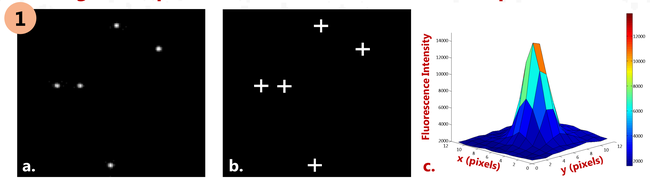
SAND2012-6278P

## Introduction

**Although optical microscopy drastically improved our understanding of biological processes, the diffraction barrier limits optical resolution of cellular features to ~400nm. To combat this limitation, imaging techniques such as stochastic optical reconstruction microscopy (STORM) have evolved, achieving resolutions to the nanometer scale[1].**

**Blinking Fluorophore** **Localization** **Point Spread Function** **2D Gaussian Approximation**

1




$$PSF(x,y) = Ae^{-\left(\frac{(x-x_o)^2}{2\sigma_x^2}+\frac{(y-y_o)^2}{2\sigma_y^2}\right)}$$

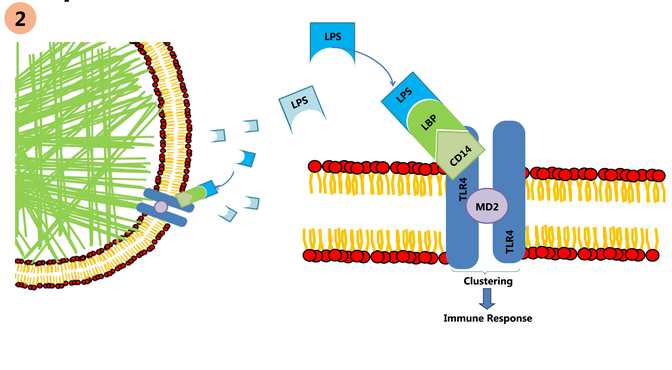
$$A = intensity \quad x_o, y_o = center$$

$$\sigma_X, \sigma_Y = spread$$

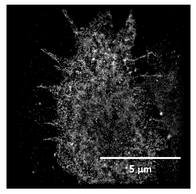
**Figure 1. The basis of single molecule localization methods relies on the detection of a small, random isolated subset of single fluorophores at any given time (termed photo-switching), permitting their localization to within 10-50 nm (a,b). The process of imaging and localization is then repeated many thousands of times, where each localized point is approximated to a Gaussian function and used to reconstruct a super-resolution image (c,d). This work addresses a particularly important challenge in multi-color super-resolution imaging: the need to image samples that contain both highly dense and relatively sparse fluorophores.**

## Experiment

2




**Dense Actin**




**Sparse TLR4**

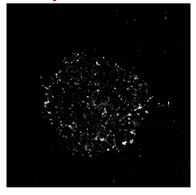
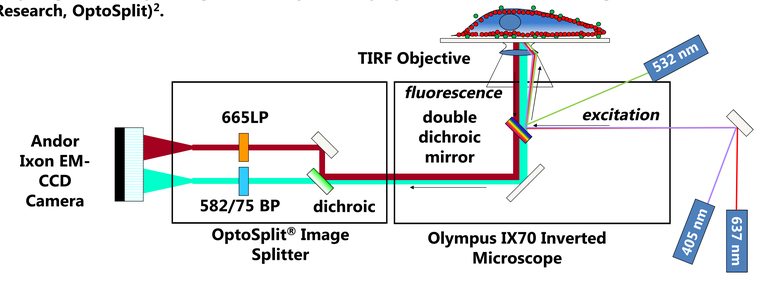


**Figure 2. We imaged the high-density actin cytoskeletal morphology in combination with relatively low density toll-like receptor 4 (TLR4) expression in P388D1 macrophage cells**

**Figure 3. 20,000 to 80,000 images were captured and projected for each cell at approximately 0.05 – 0.1 seconds per frame using an objective based TIRF-microscope (Olympus, IX -71) with an electron multiplied charge coupled device (Andor Technologies, iXon). Illumination was achieved by solid-state 405 nm, 532 nm and 637 nm emitting lasers in which lasers emitting at 637 nm and 405 nm were consolidated into one fiber. The laser emitting at 405 nm was used for photon reactivation, while the 532 nm and 637 nm lasers were used to excite Atto532 and Alexa647 dyes, respectively. Signal corresponding to each dye were projected and isolated through a dual-channel image-splitter (Cairns Research, OptoSplit)[2].**

3




## Data

4

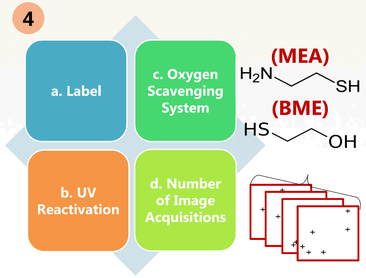
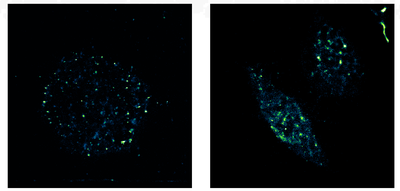



**Figure 4. We investigated the influence of four important STORM imaging parameters on overall image quality. Fluorescent localization and Gaussian approximations were performed in the Matlab 2011b environment, utilizing a 256 CUDA Core NVIDIA Quadro 4000 Graphic Processing Unit[3].**

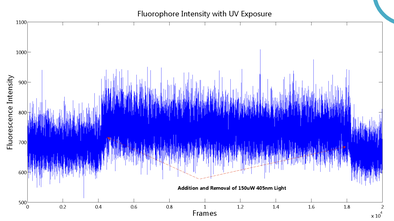
a

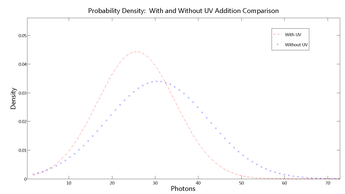
**TLR4 /Atto532** **TLR4 /Alexa647**



**The first parameter analyzed was the influence of Alexa647 and Atto532 conjugated dye on TLR4 fluorescence integrity in 140mM BME buffer.**

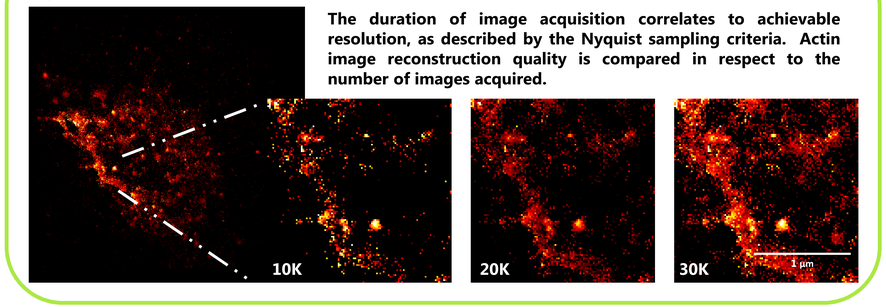
b




**UV light acts to re-activate fluorophores in the dark state, allowing the detection of more photons.**

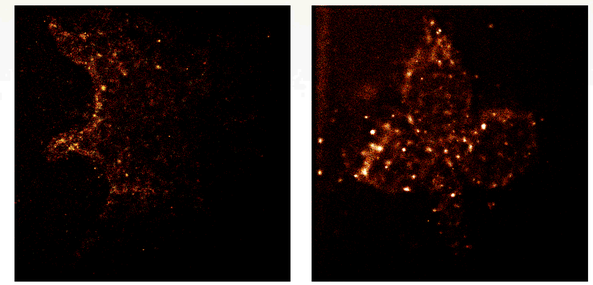
d

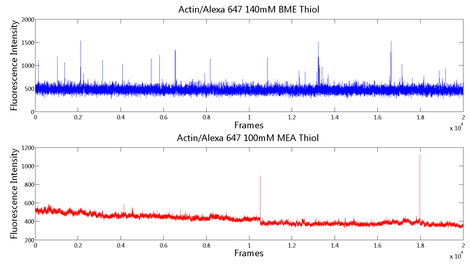


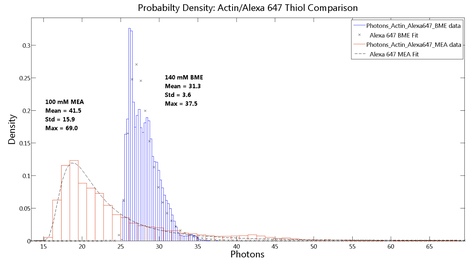

**The duration of image acquisition correlates to achievable resolution, as described by the Nyquist sampling criteria. Actin image reconstruction quality is compared in respect to the number of images acquired.**

c

**Actin/Alexa647 140mM BME** **Actin/Alexa647 100mM MEA**



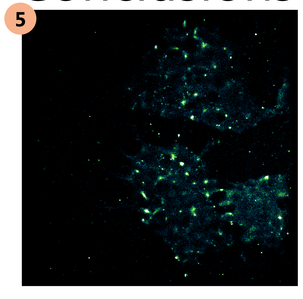



**Oxygen scavenging systems containing thiols act to sustain fluorophore photo-switching capabilities. Two common reagents, BME and MEA, were compared on the basis of both the number of photons excited and photo-switching events induced.**

## Conclusions

5



- **A compilation of imaging techniques were used to explore parameters influencing image quality in low and high density cellular features below the optical diffraction limit.**
- **Super-resolution image quality improved with TLR4 conjugated to Atto532 dye in comparison to Alexa647.**
- **Although 405 nm light reactivates fluorophores in the "off" state, dye performance was most sensitive to the removal of oxygen in the imaging media. Oxygen scavenging via 100mM MEA as opposed to 140mM BME, results in increased fluorophore blinking performance, evidenced by higher photon generation in the "on" states.**
- **Super-resolution imaging provides a platform to investigate, the interplay amidst cytoskeletal configuration and lipid raft organization/receptor clustering in immune signal transduction[4,2]. Figure 5: TLR4 configuration in macrophage cells treated with latrunculin A, a marine toxin which disrupts actin polymerization.**

## References

[1] Dempsey, Graham T, et al. "Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging." Nature Methods (2011): 1027-1036.
[2] Aaron, Jesse S., Bryan D. Carson and Jerilyn A. Timlin. "Characterization of Differential Toll-like Receptor Responses below the Optical Diffraction Limit." Small (2012): 1-9.
[3] Smith, Carlas S., et al. "Fast, single-molecule localization that achieves theoretically minimum uncertainty." Nature Methods (2010): 373-375.
[4] Kobayashi, Makiko and Kensuke Miyake. "Regulatory Roles for MD-2 and TLR4 in Ligand-Induced Receptor Clustering." The Journal of Immunology (2006): 6211-6218.
[5] This work was supported by the National Institutes of Health Director's New Innovator Award Program, 1-DP2-OD006673-01.
[6] Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

National Nuclear Security Administration · U.S. DEPARTMENT OF ENERGY · Sandia National Laboratories