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Super-Resolution Imaging of Murine Macrophage Cells

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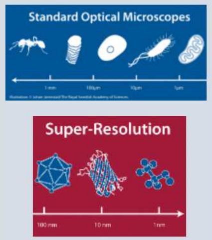
Microscopy is a critical technique in order to fully understand the unique pathways of many biological organisms and the functionality of cellular mechanisms. Optical microscopy techniques fail to show nanoscale resolution due to the fundamental limit of diffraction. By the use of TIRF and dSTORM imaging, images can be taken at a super-resolution level, thus beating the diffraction limit. The purpose of this study is to examine the immune response of RAW 264.7 macrophage cells using optical reconstruction microscopy to give precise detail of the organization of the molecules at a nanoscale level. Total internal reflection fluorescence microscopy (TIRF) uses the properties of a wave in a specimen that is adjacent to the interface between 2 different medias to look specifically at cell membranes.. TIRF-based excitation will be used to preform dual color super-resolution on the receptors to effectively use this new technique for future studies for the understanding of TLR receptor and its importance in the innate immune response. This poster shows preliminary work towards that aim.



Salacerria, Pablo. (2017, June 28). *Super-resolution Structed illumination microscopy (SR-SIM).* Retrieved from URL.

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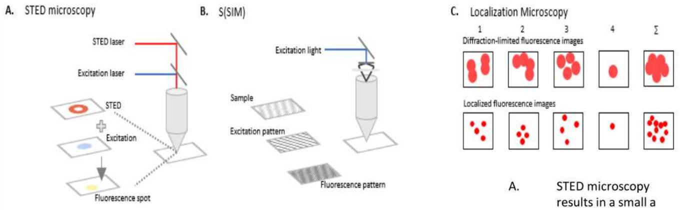
Exceptional service in the national interest

- TIRF uses the properties of a wave in a specimen that is adjacent to the interface between 2 different medias that allow for the refraction of light.
- A focused laser beam is aimed directly to the coverslip while the specimen is positioned opposite of the objective optics

Super-Resolution Microscopy

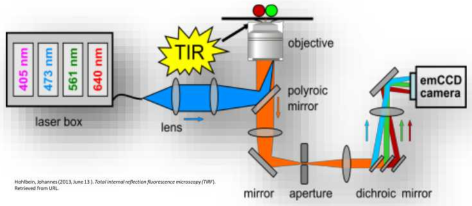


Habuchi S. Super-resolution molecular and functional imaging of nanoscale architectures in life and materials science. Frontiers in Bioengineering and Biotechnology. 2014;2

A. STED microscopy results in a small a small fluorescence spot due to over saturation.

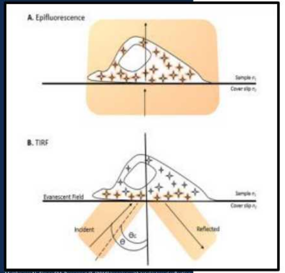
B. S(SIM) leads to better spatial resolution by which the fluorophores emit a color by excitation light.

C. Localization Microscopy leads to a finer and more defined image compared to the diffraction-limited fluorescence images.



Hohlbein, Johannes (2013, June 13). *Total internal reflection fluorescence microscopy (TIRF).* Retrieved from URL.

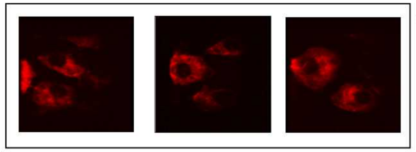
TIRF Excitation



Mattheyses AL, Simon SM, Rappoport JZ. (2010) Imaging with total internal reflection fluorescence microscopy for the cell biologist. J Cell Sci 123:3621–3628.

Functionality of EPI and TIRF illumination
- A. Epifluorescence; all fluorophores are emit light.
- B. TIRF; only fluorophores in the evanescent field emit light.

Murine Macrophage Cells in TIRF



- Cellmask™ Deep Red cell membrane stain in TIRF using 638nm on RAW 264.7 murine macrophage cells

Future Directions
- Use techniques shown to preform super-resolution microscopy
- Understand the interaction between TLR4/MHC complex and the LPS molecule on murine macrophage cells by super resolution microscopy.
- Analyze the difference between TLR2 and TLR4 to LPS by dual color super resolution microscopy.

Methods:

Cell culture: RAW 264.7 murine macrophage cells were cultured in DMEM, 10% FBS and 1% Pen/Strep at 37◦C in a 5% CO_2 environment.

Image Acquisition: Samples were imaged using a custom TIRF microscope (Olympus 1X-71), illumination provided by fiber-coupled solid-state lasers emitting at 638 nm. Images were projected simultaneously on an electron-multiplied charge coupled device.

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