SAND2011-9142P

BioAnalytical Spectroscopy and Imaging of Cellular Response

Jerilyn A. Timlin
Principle Member of Technical Staff
Bioenergy and Defense Technologies
Sandia National Laboratories

Presented to CDC Visitors
December 6, 2011

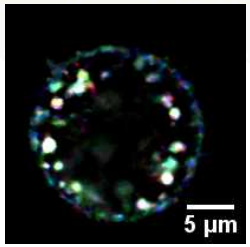
Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000.

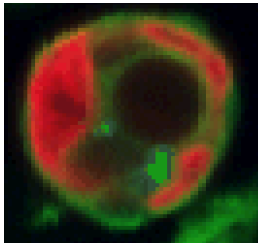
NNSA National Nuclear Security Administration

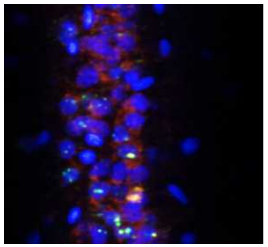
Sandia National Laboratories

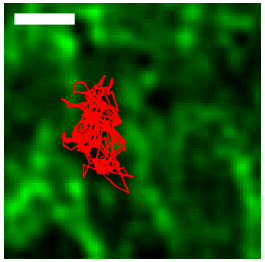
Research Focus

http://bio.sandia.gov/people/timlin.html







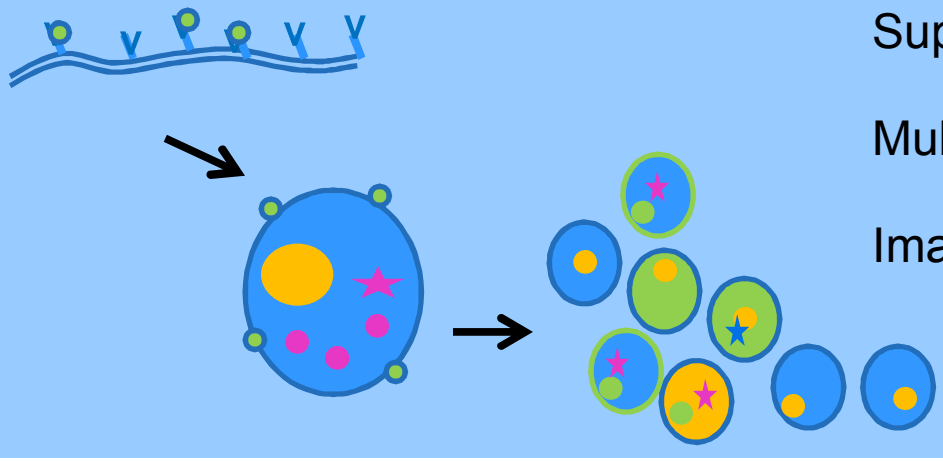


- Conduct multidisciplinary fundamental & applied research in cell biology, immunology, and microbiology
- Support biodefense, emerging infectious disease, and biofuels mission areas
  - Advanced spectroscopy
  - Innovative imaging technologies
  - Chemometric data analysis tools
  - Multicomponent biological systems
  - Multiscale
- Current projects include understanding receptor activation and cell signaling processes, viral and bacterial pathogenesis, plant physiology, cellulase enzymes, as well as algal biochemistry and cultivation characterization for biofuels applications.

Sandia National Laboratories

Advanced Analytical Imaging & Analysis Tools

Unraveling Spatial-Temporal Relationships in Complex Biological Systems

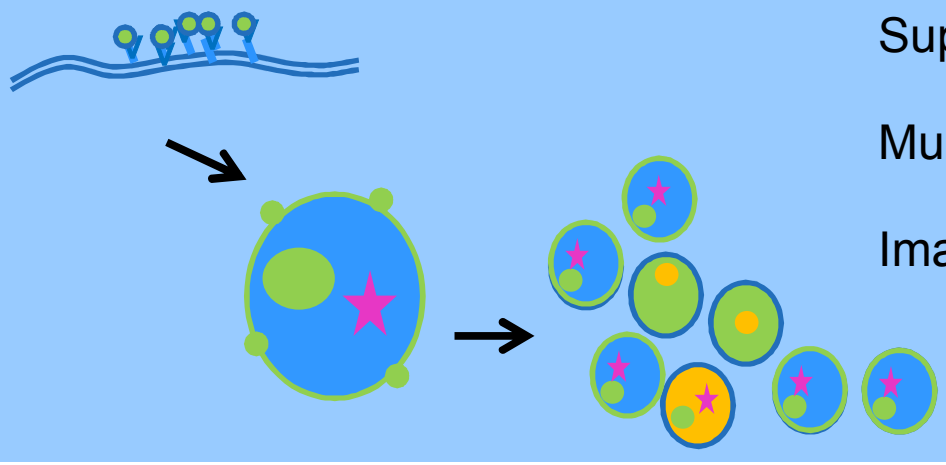


- Hyperspectral Fluorescence Imaging
- Vibrational Spectroscopic Imaging
- TIRF Microscopy
- Single-molecule Imaging
- Super Resolution Microscopy
- Multivariate Image Analysis
- Image Correlation, Particle Tracking

Sandia National Laboratories

Advanced Analytical Imaging & Analysis Tools

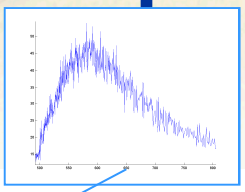
Unraveling Spatial-Temporal Relationships in Complex Biological Systems

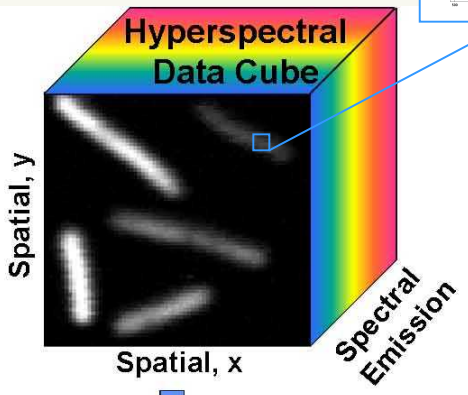


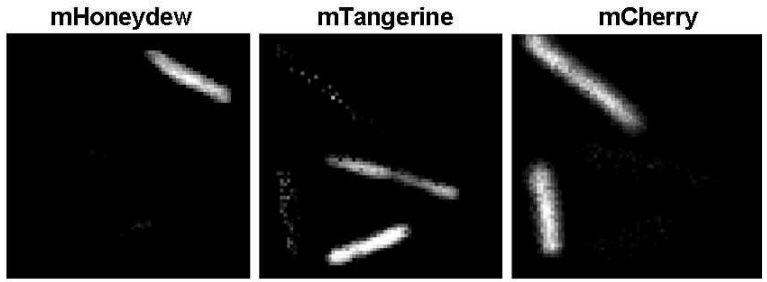
- Hyperspectral Fluorescence Imaging
- Vibrational Spectroscopic Imaging
- TIRF Microscopy
- Single-molecule Imaging
- Super Resolution Microscopy
- Multivariate Image Analysis
- Image Correlation, Particle Tracking

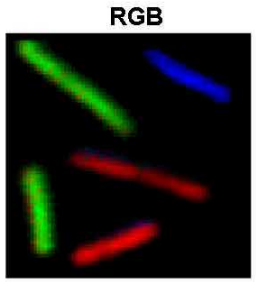
Sandia National Laboratories

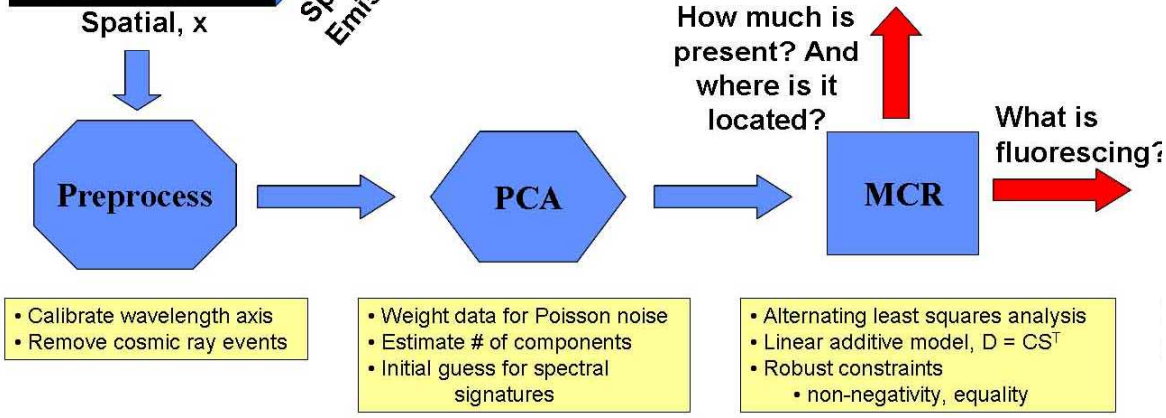
Hyperspectral Confocal Fluorescence Microscopy & Analysis

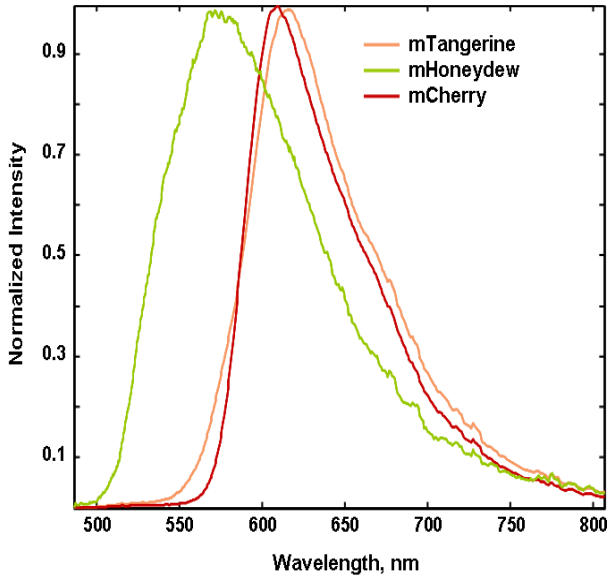












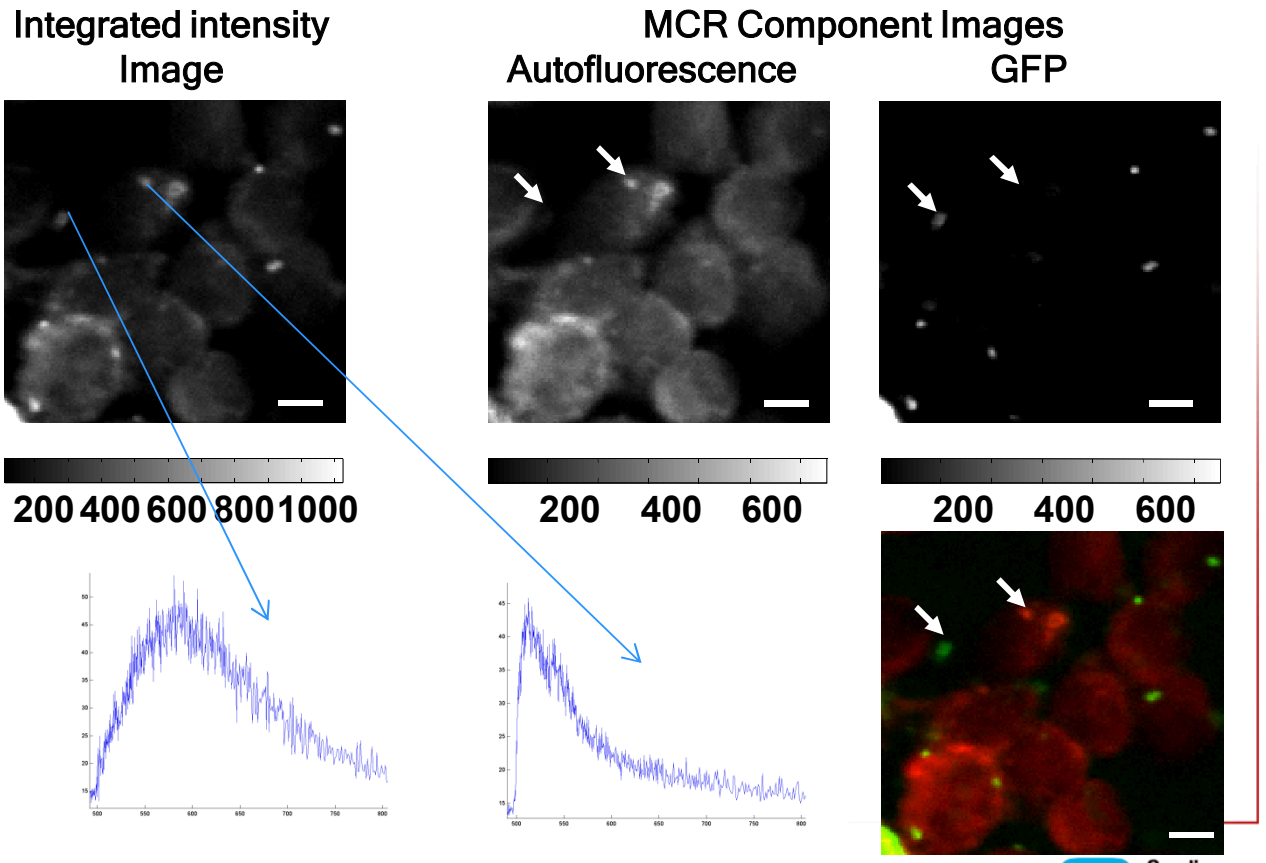
Example: E.coli DH5α expressing pRSETB-mCherry, -mHoneydew, and -mTangerine

Microscope details: *Sinclair, et al., Applied Optics, 45, 3283-3291, (2006).*
Algorithim details: *Haaland, et al., Proc. SPIE, Vol. 4959, 55 (2003)*

Sandia National Laboratories

Advantages of a Spectral Dimension

- Increase throughput – multiple overlapping dyes
- Improved background/interference correction
  - Accuracy
  - Reliability
  - Quantitation
  - Specificity



Macrophage cells infected w *F. novicida* expressing GroE:GFP higlight the criticality of spectral imaging for detection of low levels of protein expression in host cells.

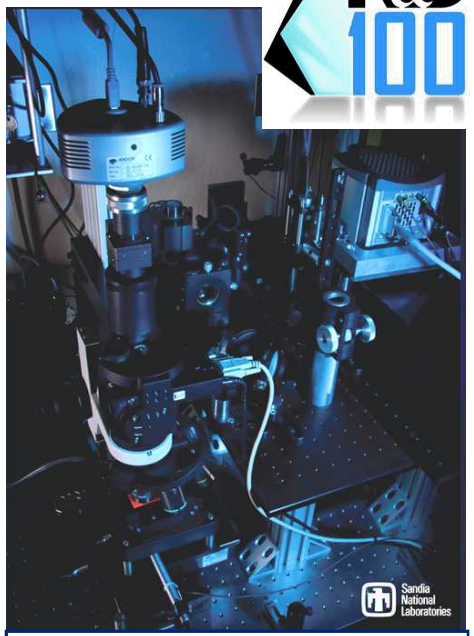
Davis RW, Timlin JA, Noek R, Kaiser JN, Jones HDT (2010) Accurate detection of low levels of fluorescence emission in autofluorescent background. Microsc. Microanal.16: 478-487.

Sandia National Laboratories

Applications

- Multiplexed, high information content imaging of single-cell response to
  - Therapuetics, vaccines
  - Infectious disease
  - Environment
- Population dynamics
- Label-free detection
- Not limited to fluorescence, also Raman, IR, etc.

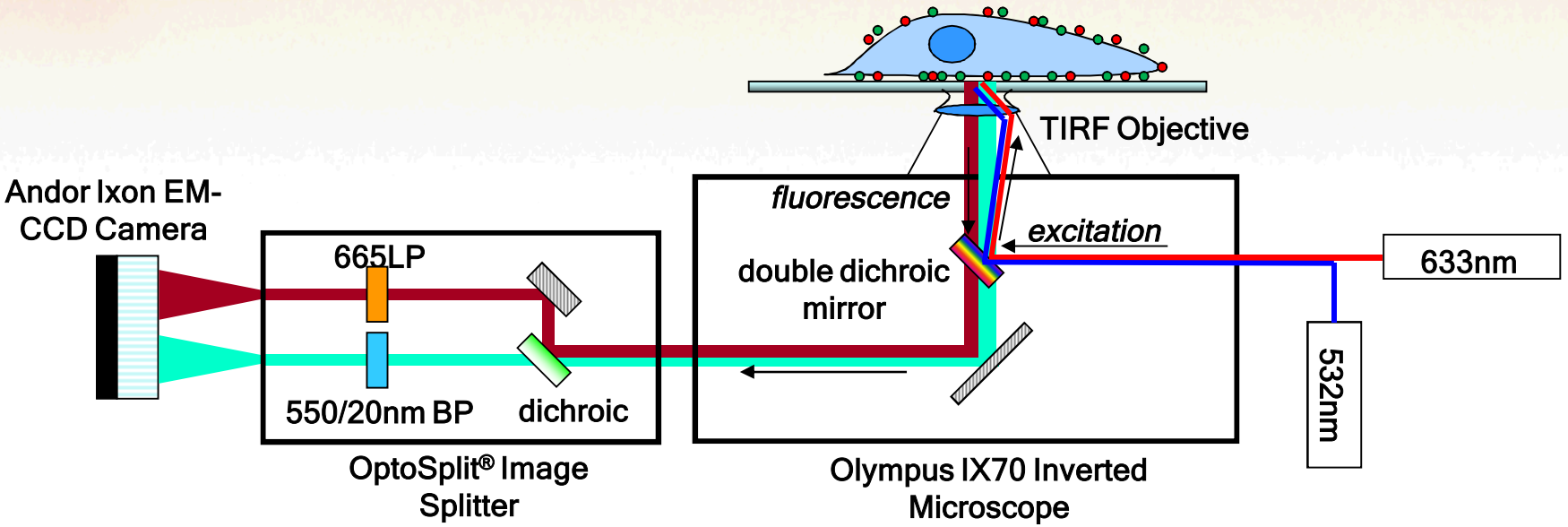
- Future applications
  - High-throughput, flow assay
  - Cell-sorting based on multiplexed information



Sinclair, et. al. "Hyperspectral confocal microscope," Applied Optics, 45, 6283-6291 (2006).

Sandia National Laboratories

Multicolor TIRF/STORM Setup



Unique capabilities:

- Four excitation λ's (405, 488, 532, 633nm), variable angle
- Simultaneous dual-color emission
- Capable of >50fps over 30µm x 30µm FOV

Advantageous in:

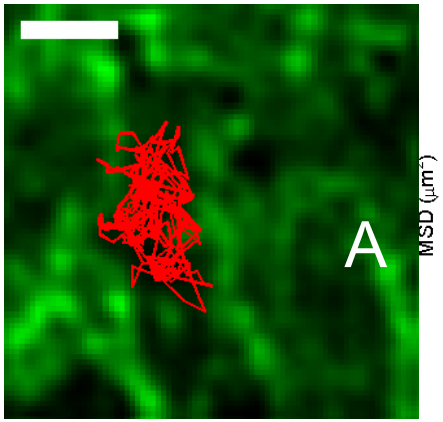
- Receptor reorganization
- Nanoparticle-membrane interactions, uptake
  - Engineered NPs
  - Natural NPs - Viral trafficking

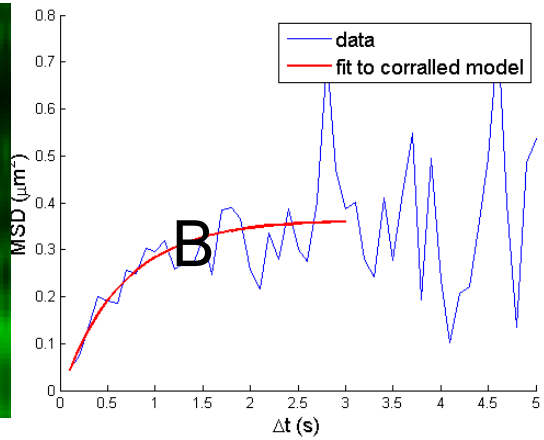
Sandia National Laboratories

Resolving Dynamics of Cell Signaling via Real-Time Imaging of the Immunological Synapse

Approach

Cellular response is determined by dynamic, stepwise interactions of receptor proteins w/ key membrane proteins. Unfortunately, the details are vague. We apply advanced dual-color TIRF microscopy to resolve spatio-temporal dynamics in immune response.



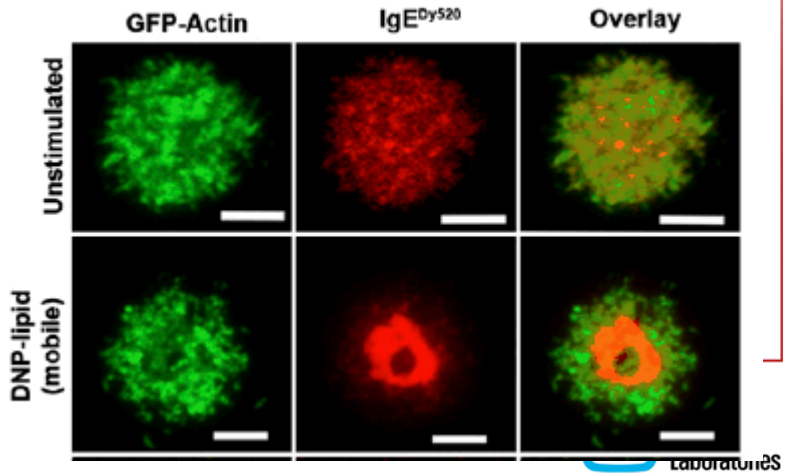


Single nanoparticle tracking demonstrated in RBL cells. The actin cytoskeleton (GFP) acts as a corral. Scale bar = 1μm

Formation and dynamics of a mast cell synapse on mobile antigen presenting bilayer.

Key Accomplishments

- Spendier K, et al. (2010) Distribution and dynamics of RBL IgE receptors (FcεRI) observed on planar ligand-presenting surfaces. Biophys J 99: 388-397.
- Carroll-Portillo A, S, et al. (2010) Formation of a Mast Cell Synapse: FcεRI Membrane Dynamics upon Binding Mobile or Immobilized Ligands on Surfaces. J Immun 184: 1328-1338.
- Andrews NL, et al. (2008) Actin restricts Fc[epsiv]RI diffusion and facilitates antigen-induced receptor immobilization. Nature Cell Biology 10: 955-963.



Engineered Nanoparticle Interactions with Cells

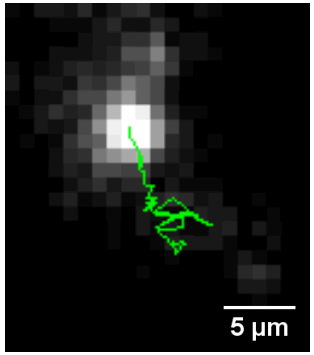
Project Purpose and Approach

- To characterize size and shape dependencies of QD interactions with live cells
- Using TIRF imaging and Hyperspectral Microscopy, acquire dynamic, high resolution data describing particle diffusion, uptake, partitioning in the membrane, and ultimate fate within lysosomal vesicles

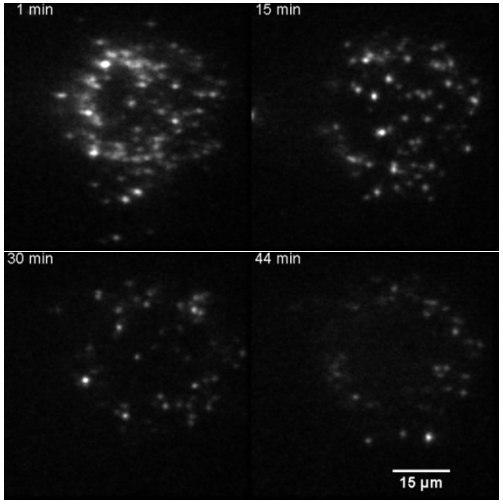
Key Accomplishments

- Discovered clear shape dependencies in particle diffusion in cell membrane, and uptake kinetics
- Different QDs bind distinct regions in the membrane, producing particle "patchwork" on cell periphery
- Despite this, particle uptake and ultimate sorting into lysosomes is size dependent
- JS Aaron, et. al. (2011) *Small*. 7: 334-341

Total Internal Reflectance Fluorescence (TIRF) Microscopy

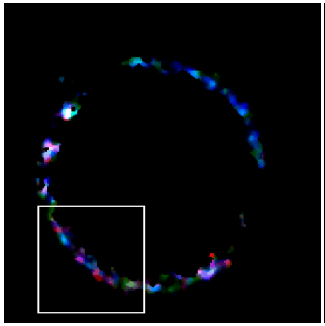


Single particle tracking in membrane

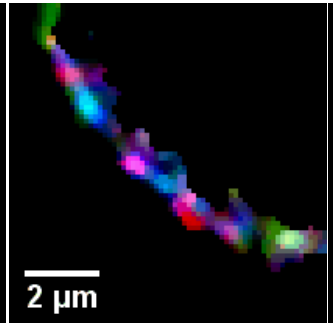


Particle uptake kinetics

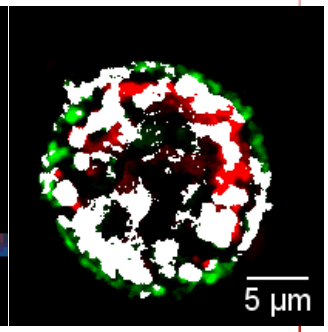
Hyperspectral Confocal Fluorescence (HCF) Microscopy



Simultaneous detection of multiple QDs in live cells



membrane partitioning of QDs



Lysosomal co-localization

Sandia National Laboratories

Imaging Cellular Processes Below the Limits of Optical Diffraction

Background & Motivation:

- Optical super-resolution (SR) uses stochastic or specific photoswitching of fluorescent molecules to obtain spatial resolution of ~50 nm.
- SNL has unique capabilities in SR
  → Simultaneous, dual color detection of membrane receptors, proteins
  → Multiplexed (>4) detection of intracellular proteins in living cells

Current & Future Applications:

Innate immune responses

- *TLR4/LPS/MD2 interactions as a function of LPS type*

Bacterial pathogenesis/virulence mechanisms

- *F. novicida* iglA/iglB protein interactions in live host cells

Viral entry and fusion

- *Dengue virus E-protein interactions with endosomal membrane*

Nanoparticle toxicity

- *Engineered or natural nanoparticle uptake mechanisms, intracellular trafficking*

Enzyme kinetics

- *Dynamic tracking of cellulose enzymes*

Impact in Biological Sciences:
Unprecedented view of protein organization, interactions, translocation, complex formation.

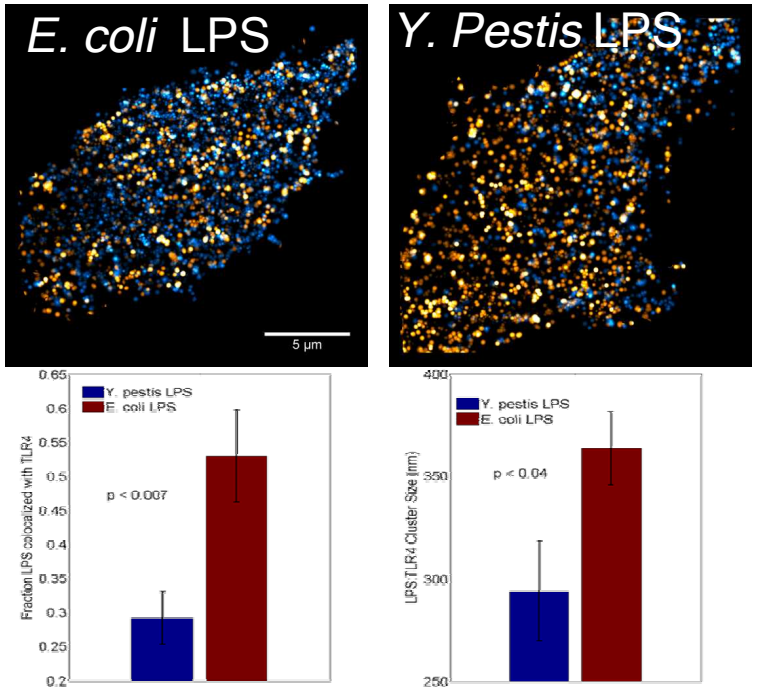
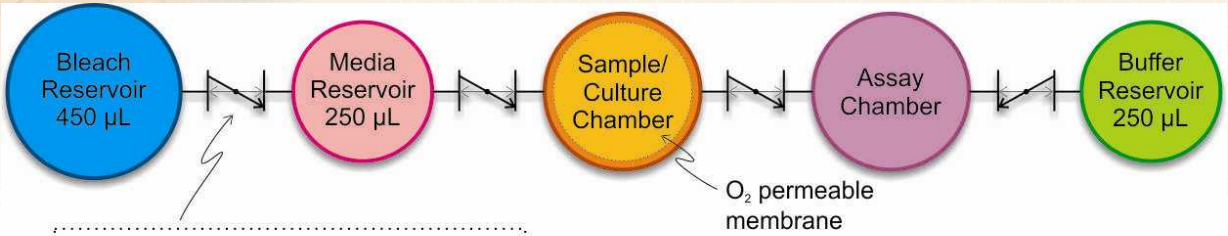
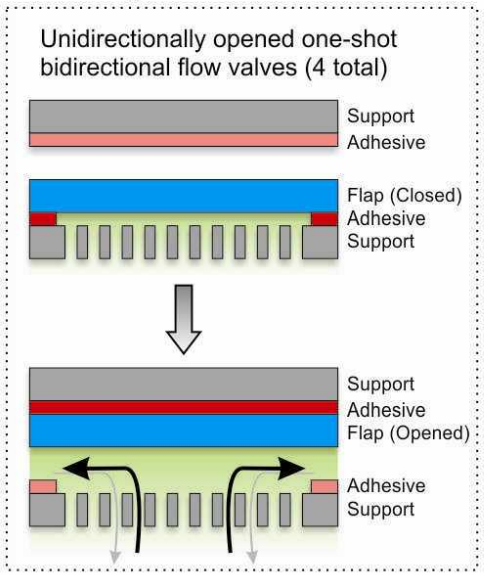


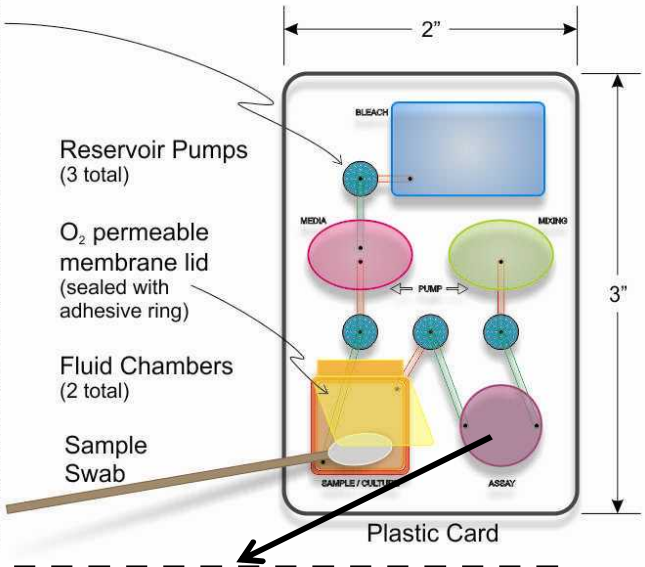
Figure 1: Optical SR reveals pathogen specific TLR4 reorganization upon binding of lipopolysaccharide (LPS). *Y. pestis* LPS co-localizes significantly less with TLR4 compared to *E. coli* LPS. In addition LPS-TLR4 clusters formed with *Y. pestis* LPS are significantly smaller than *E. coli* LPS-TLR4 clusters

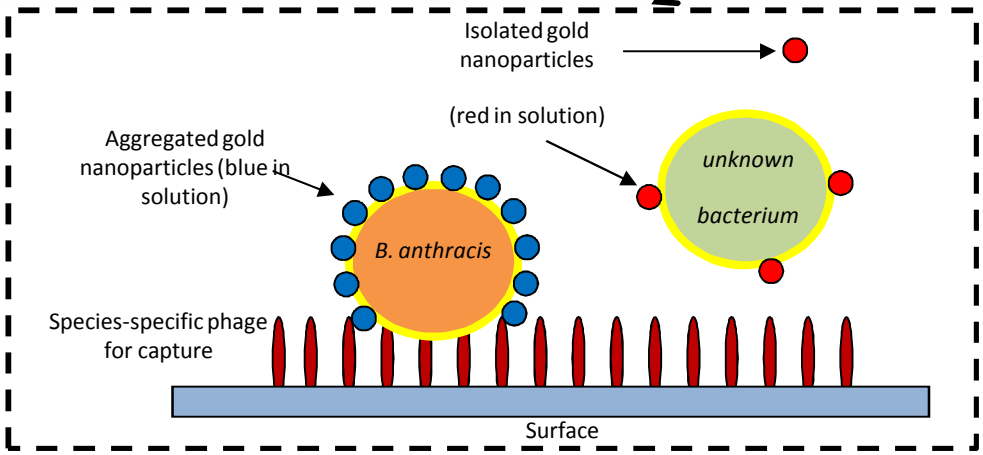
Sandia National Laboratories

Robust B. anthracis monitoring in developing countries using micro-culture chip and plasmonics-based reporting









Contact: Melissa Finley (mfinley@sandia.gov) or Jesse Aaron (jsaaron@sandia.gov) for more information

Key Features:

- Bacterial amplification in sealed/secure micro-culture device
- Species-specific phage capture, that will also infect & lyse bacteria after readout
- Simple nanoparticle reporter containing confirmatory species specific antibodies
- colorimetric plasmon resonance coupling detection scheme
- High sensitivity & specificity with no external instrumentation, power requirements, little expertise needed

Sandia National Laboratories