

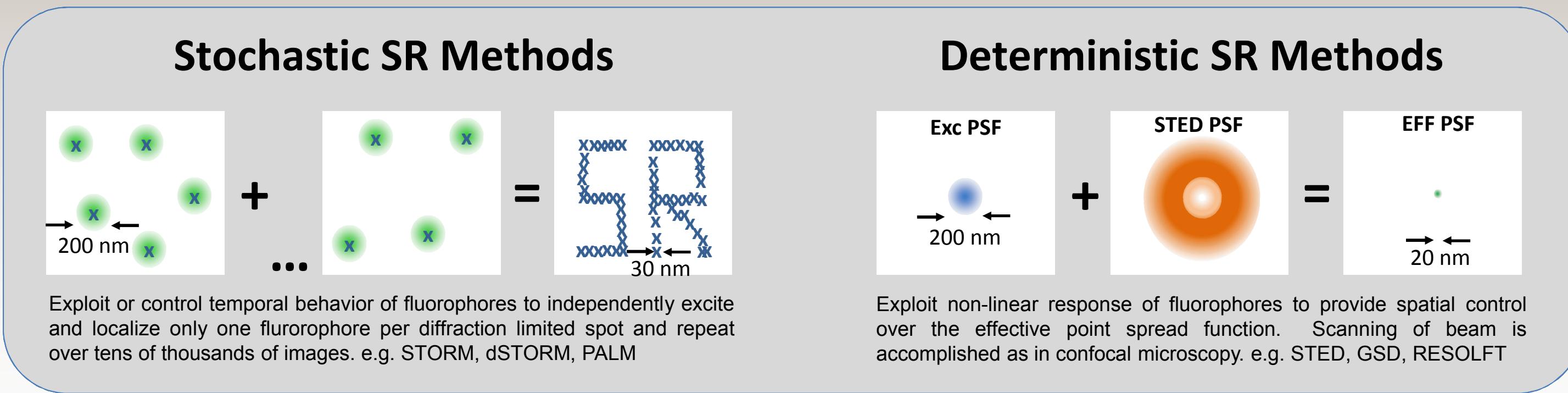
# Progress Towards Multiplexed Super-Resolution Microscopy



Jesse S. Aaron, Quinton B. Smith, Bryan D. Carson, Howland D.T. Jones, and Jerilyn A. Timlin  
 Bioenergy and Defense Technologies Department, Sandia National Laboratories, Albuquerque, NM 87185

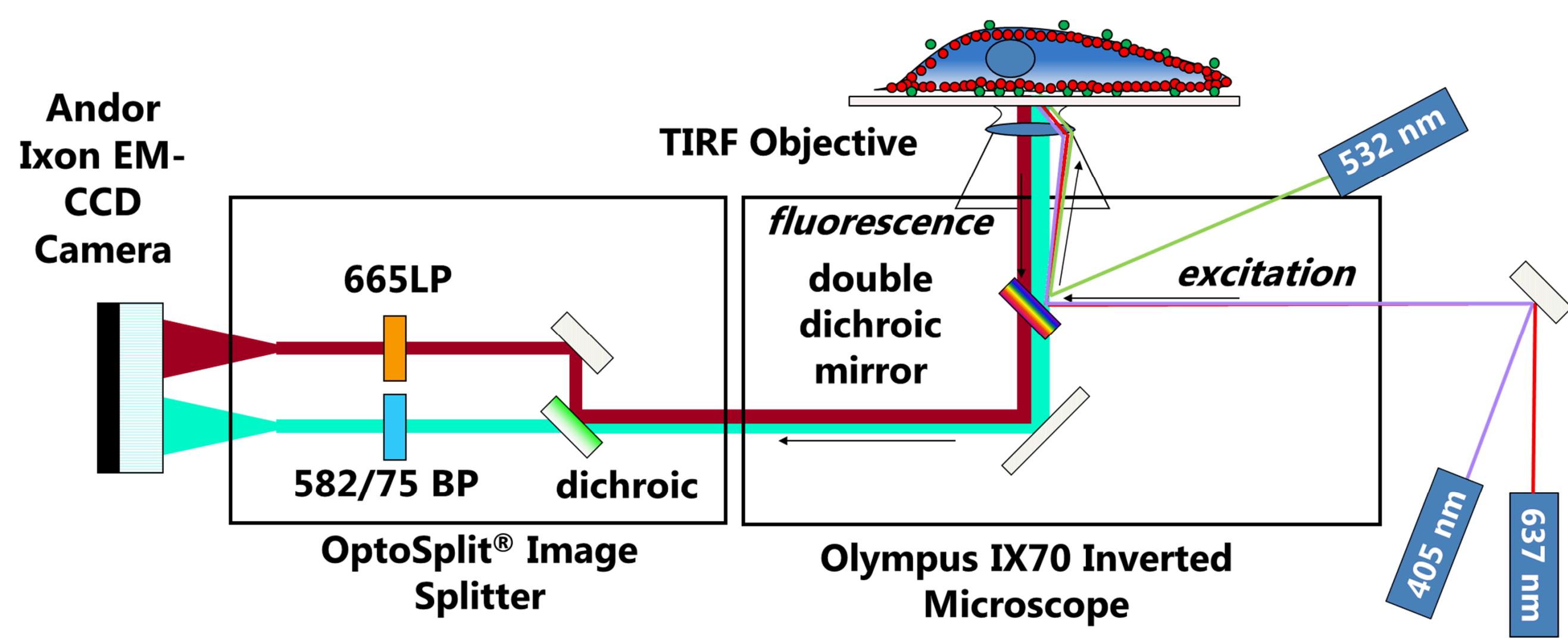
## Introduction & Motivation

- Optical diffraction limits imaging resolution to 200-400nm
- Biological processes operate at much smaller scales
  - Protein-protein interactions
  - Protein complex formation
  - Biomolecule distributions and dynamics
- Optical super-resolution techniques can achieve nanoscale resolutions

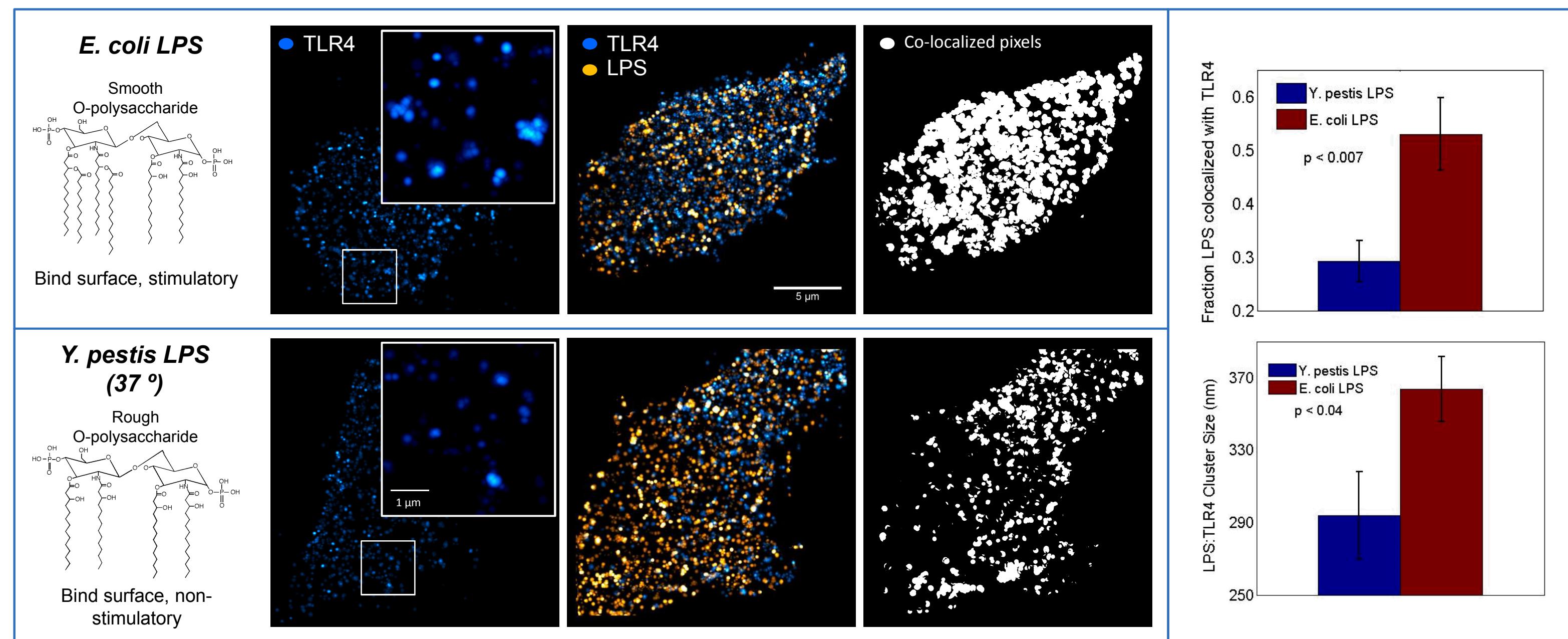


- But, current SR methods are limited in multiplexing & speed
  - We address those limitations through two independent approaches

## Results: Simultaneous, Dual-color STORM Reveals Nanoscale Receptor Dynamics



### Reorganization of TLR4 During Innate Immune Response



- E. coli* LPS produces a significant increase in TLR4 cluster size within 30 minutes, as compared to a non-specific ligand and non-stimulatory *Y. pestis*.
- Y. pestis* LPS and *E. coli* LPS exhibit similar association with plasma membrane
- Y. pestis* LPS exhibits less co-localization with TLR4 and is less able to recruit TLR4 into clusters as compared to *E. coli* LPS

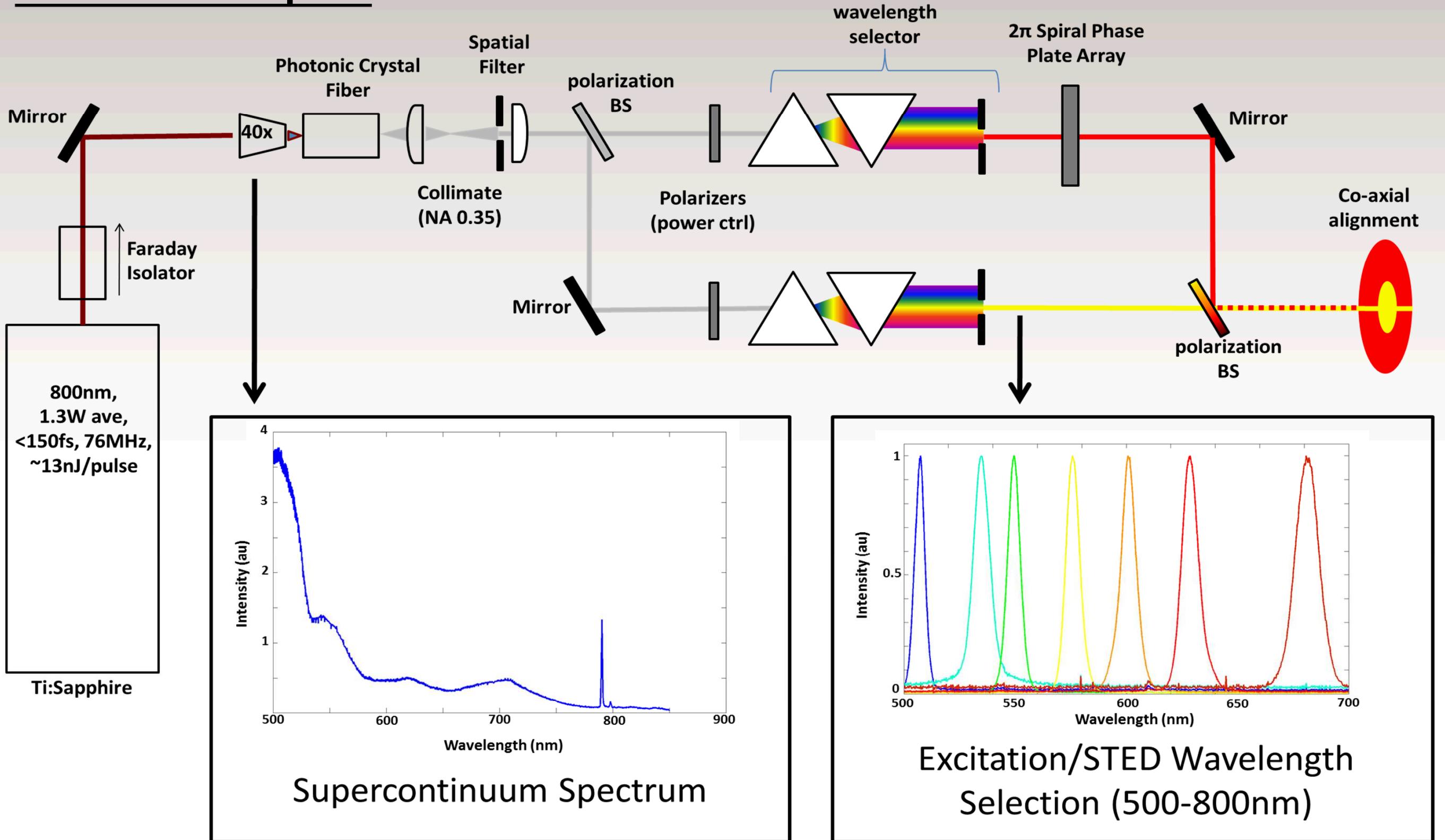
For more details see:

Aaron JS, Carson BD, Timlin JA, "Characterization of Differential Toll-Like Receptor Responses below the Optical Diffraction Limit". *Small* 2012; in press.

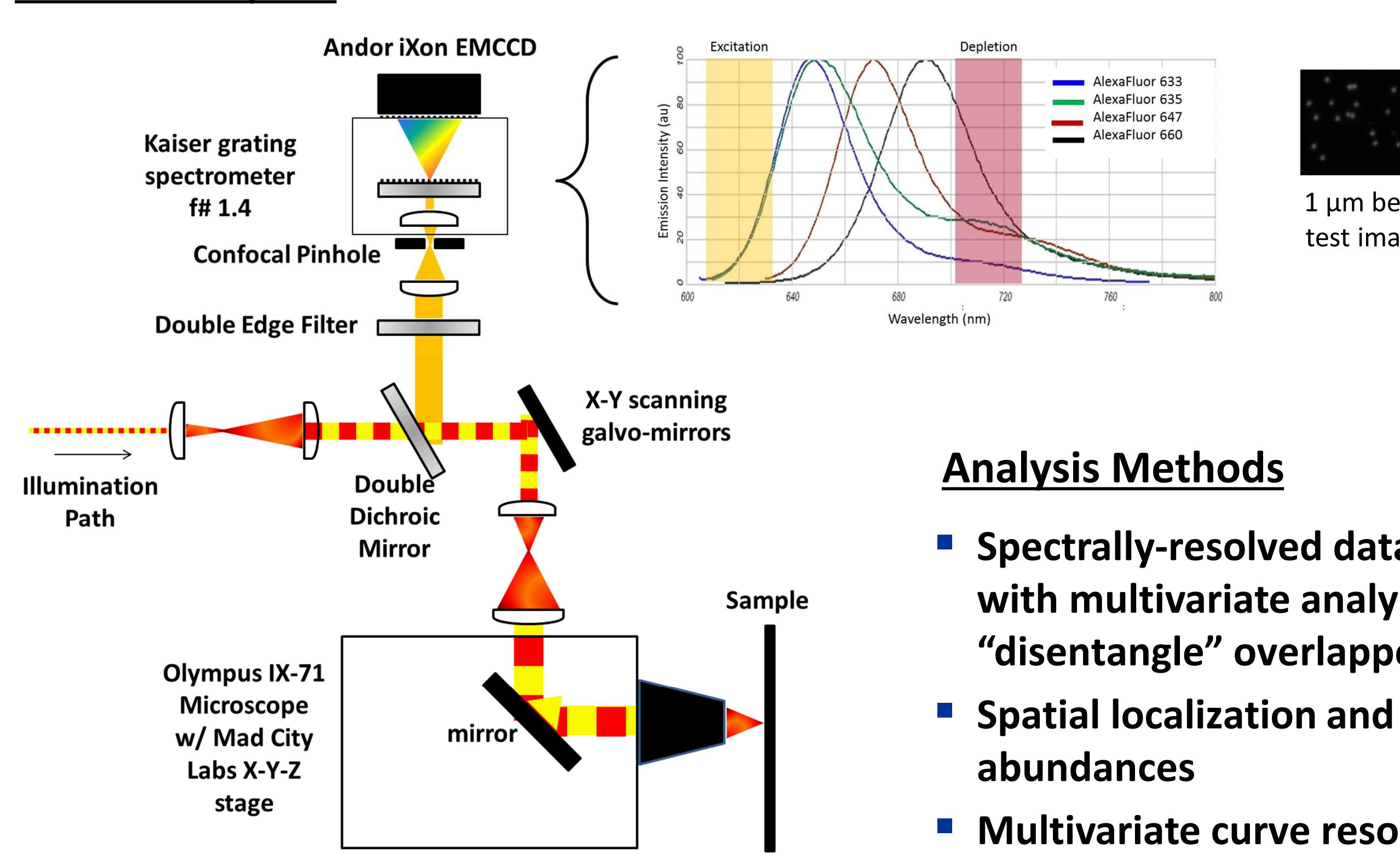
## Results: Development of Spectrally-Resolved STED Microscope

- Microscope design and construction completed
- Alignment and testing underway
- Co-development of hardware and analysis software

### Illumination Optics



### Detection Optics



### Analysis Methods

- Spectrally-resolved data combined with multivariate analysis tools can "disentangle" overlapped signals
- Spatial localization and relative abundances
- Multivariate curve resolution (MCR)
- Simulations improve understanding of FRET

## Ongoing & Future Projects

### Innate immune responses

- TLR4 reorganization as a function of LPS structure, membrane characteristics

### Bacterial pathogenesis/virulence mechanisms

- F. novicida* IgA/IgB protein interactions in living host cells

### Viral entry and fusion

- Dengue virus e-protein interactions with endosomal membrane

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