Single Particle Tracking Analyses of Hyperspectral Fluorescent Images

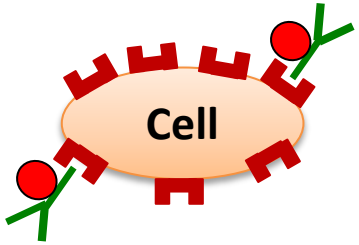
FACSS

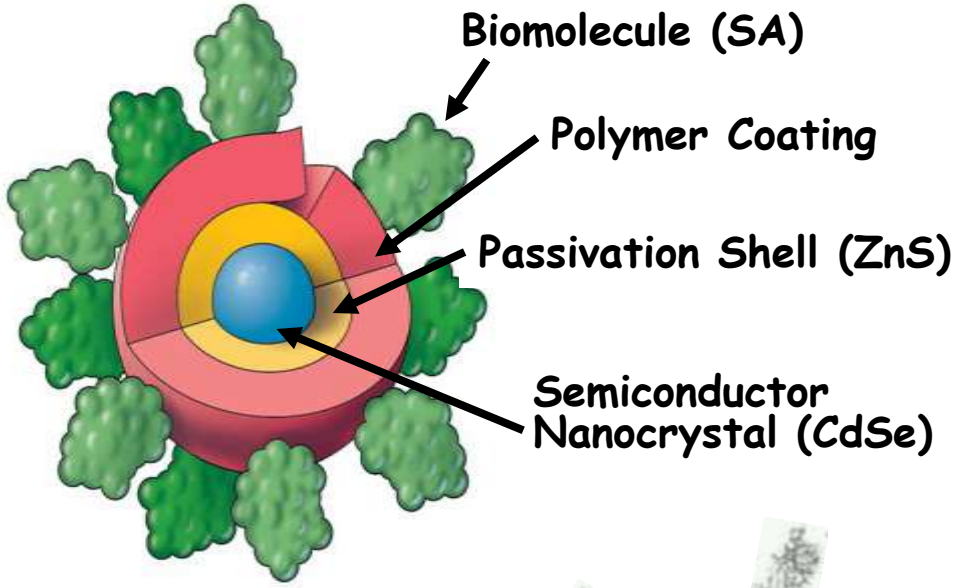
October 20th, 2010

Pat Cutler, University of New Mexico

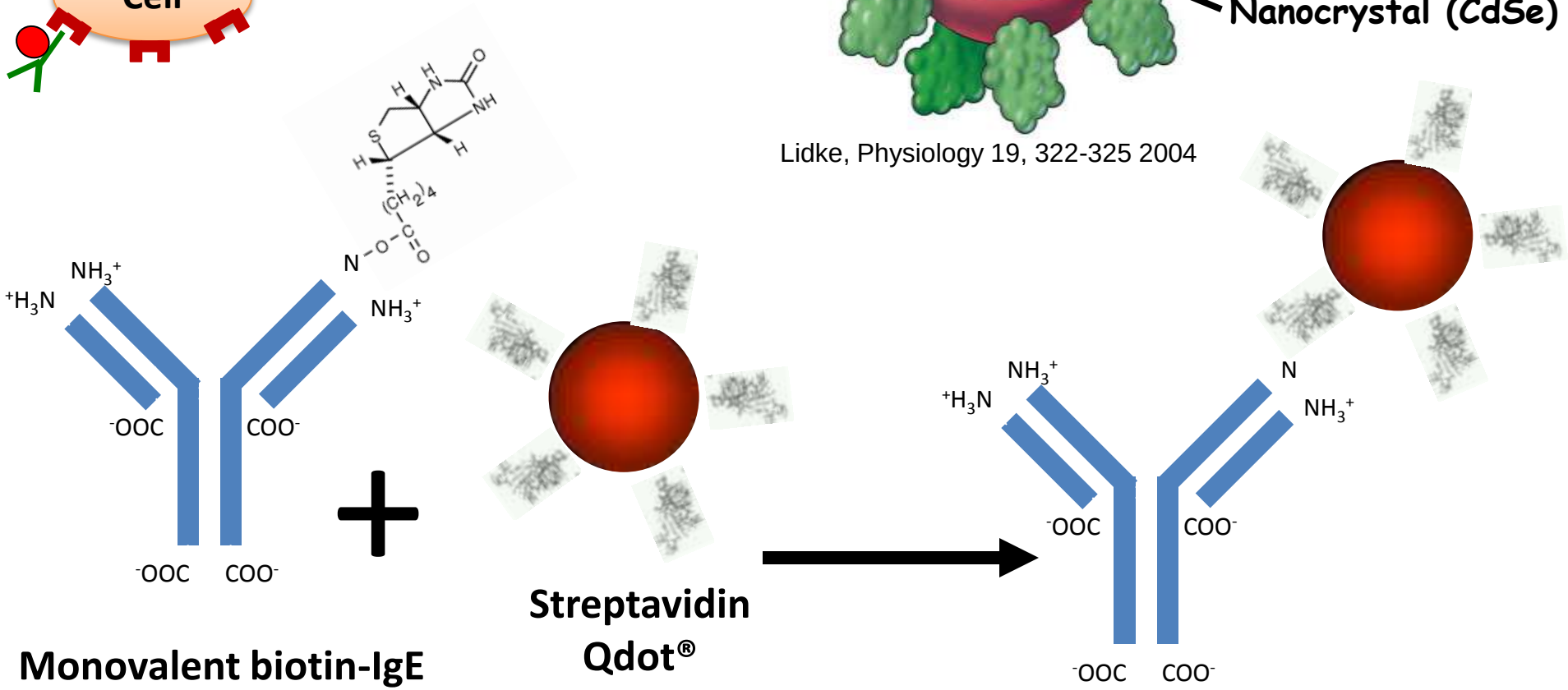
Labeling proteins with QDs

QD-IgE

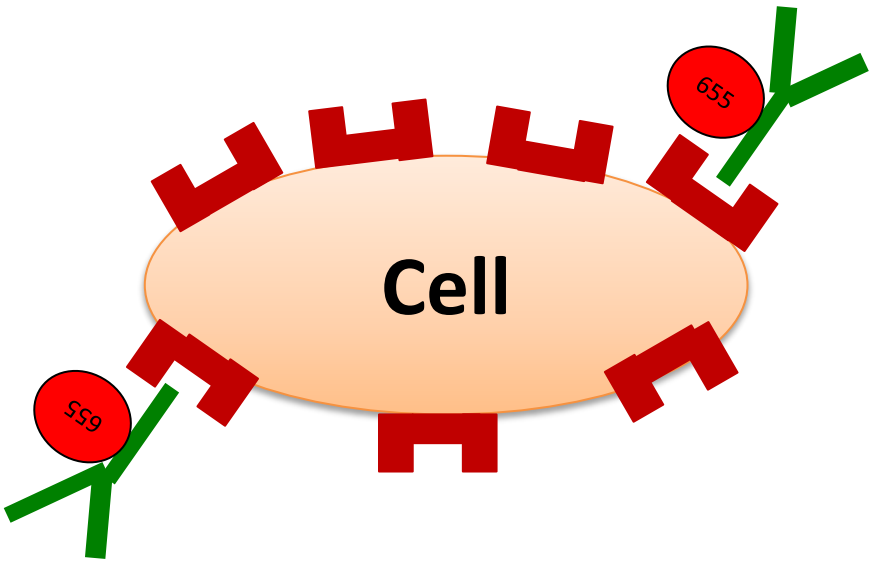


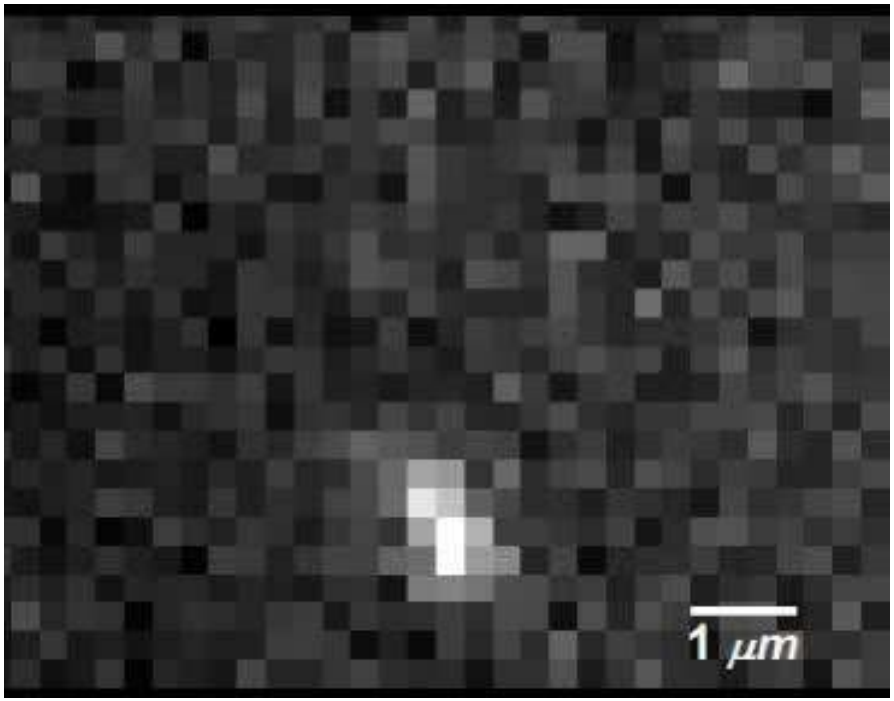


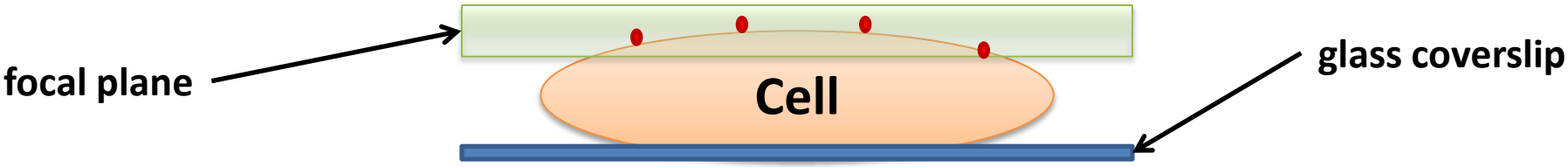
Lidke, Physiology 19, 322-325 2004



QD-IgE on live cells

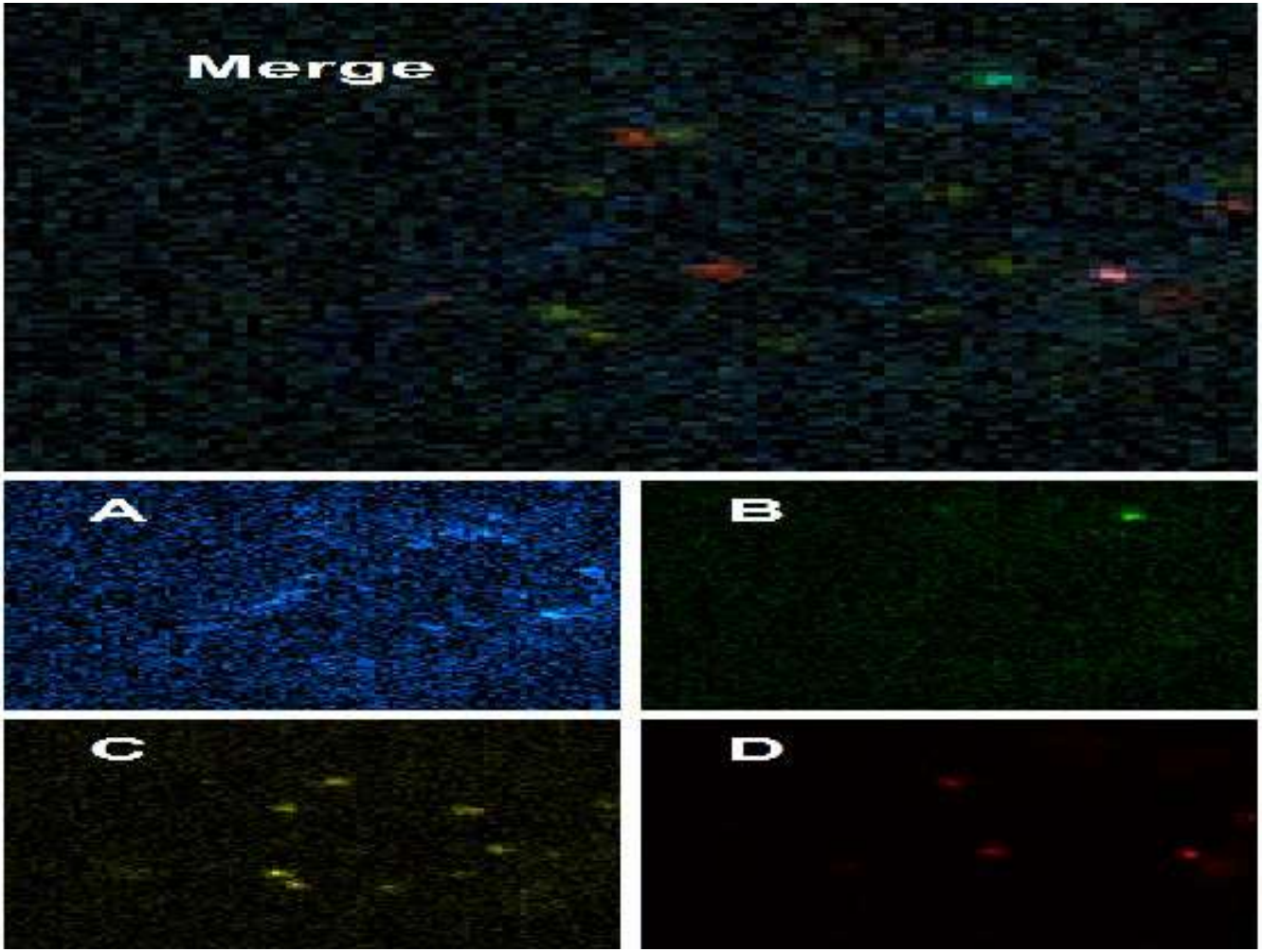


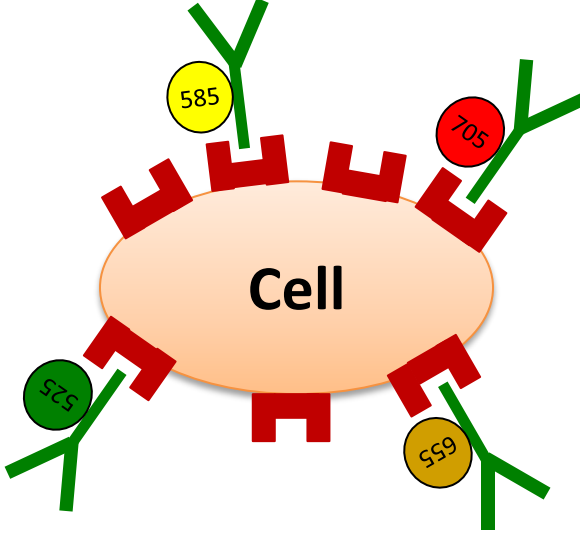




Observation of long lived oligomers

(4 second/frame 512 spectral channels)



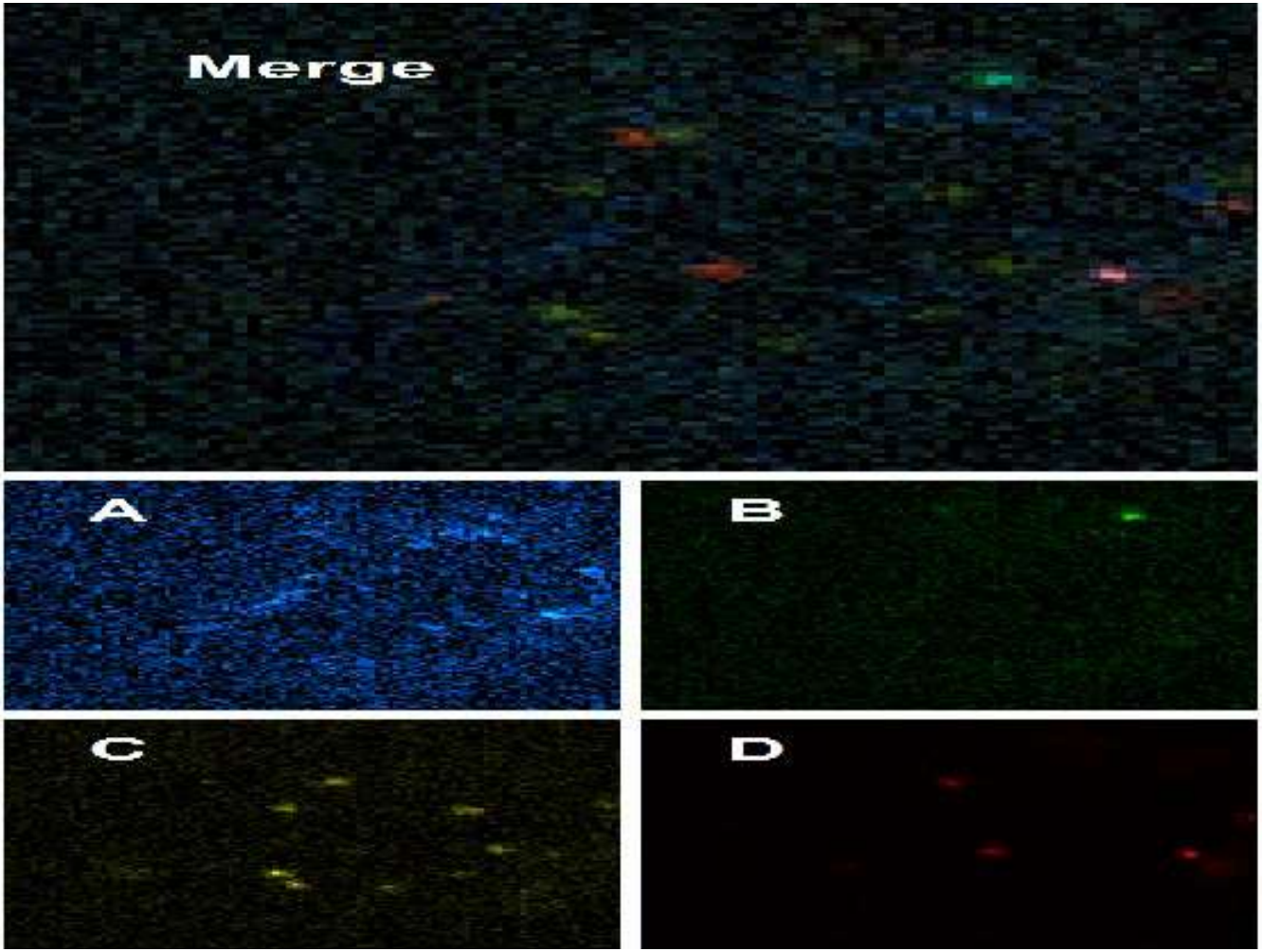


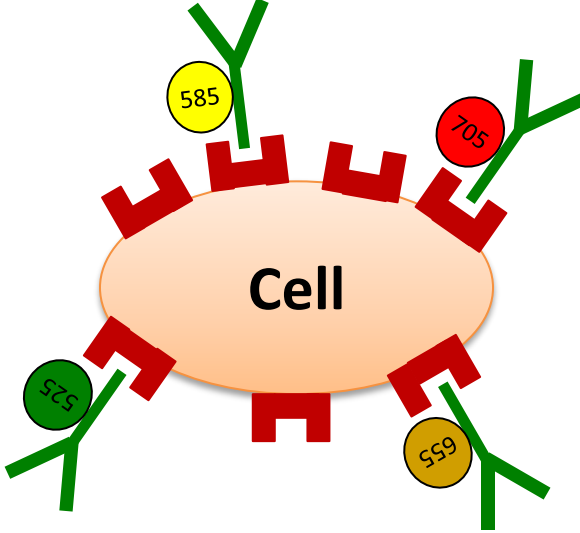
Nick Andrews
David Haaland

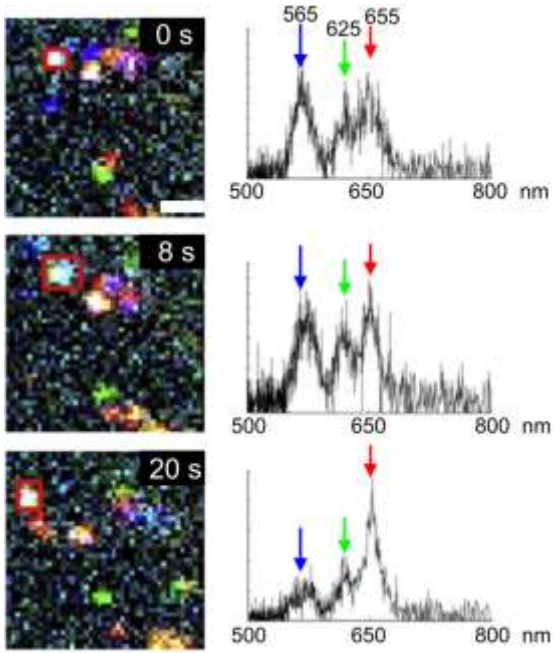
Andrews et. al. Immunity 31, 469-479 2009.

Observation of long lived oligomers

(4 second/frame 512 spectral channels)



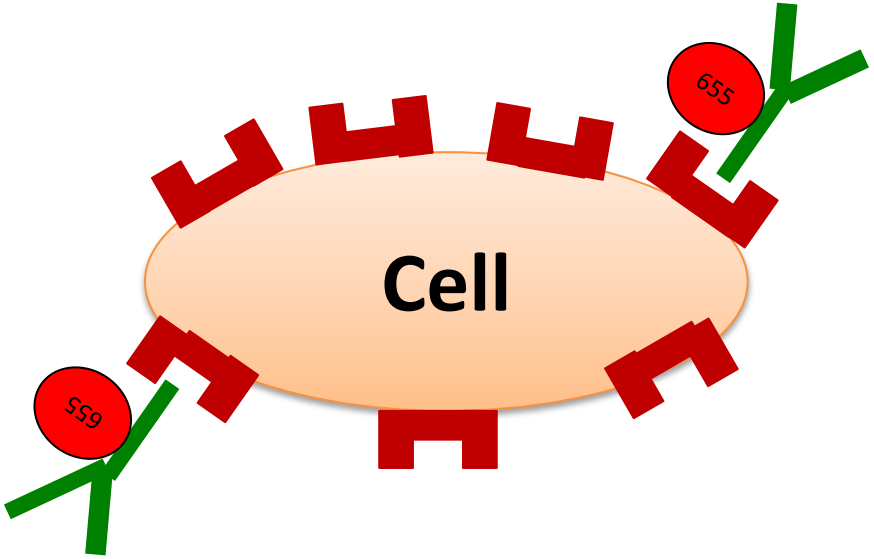


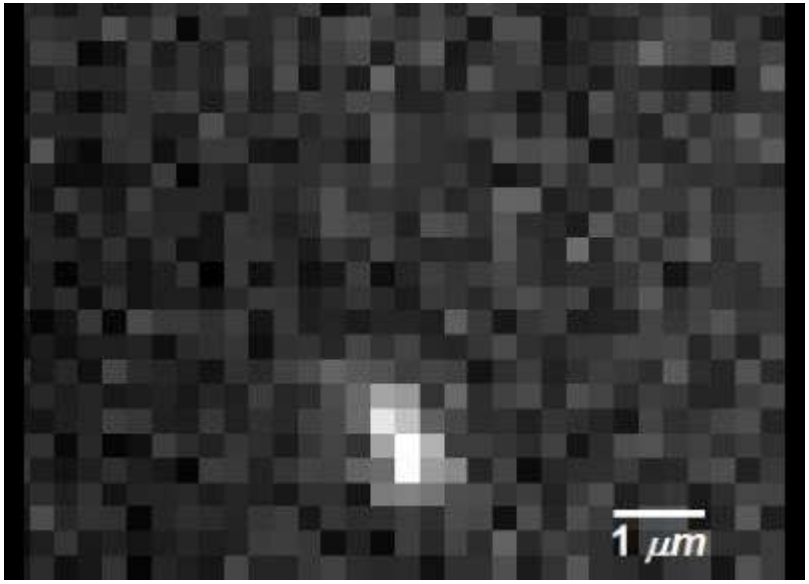


Nick Andrews
David Haaland

Andrews et. al. Immunity 31, 469-479 2009.

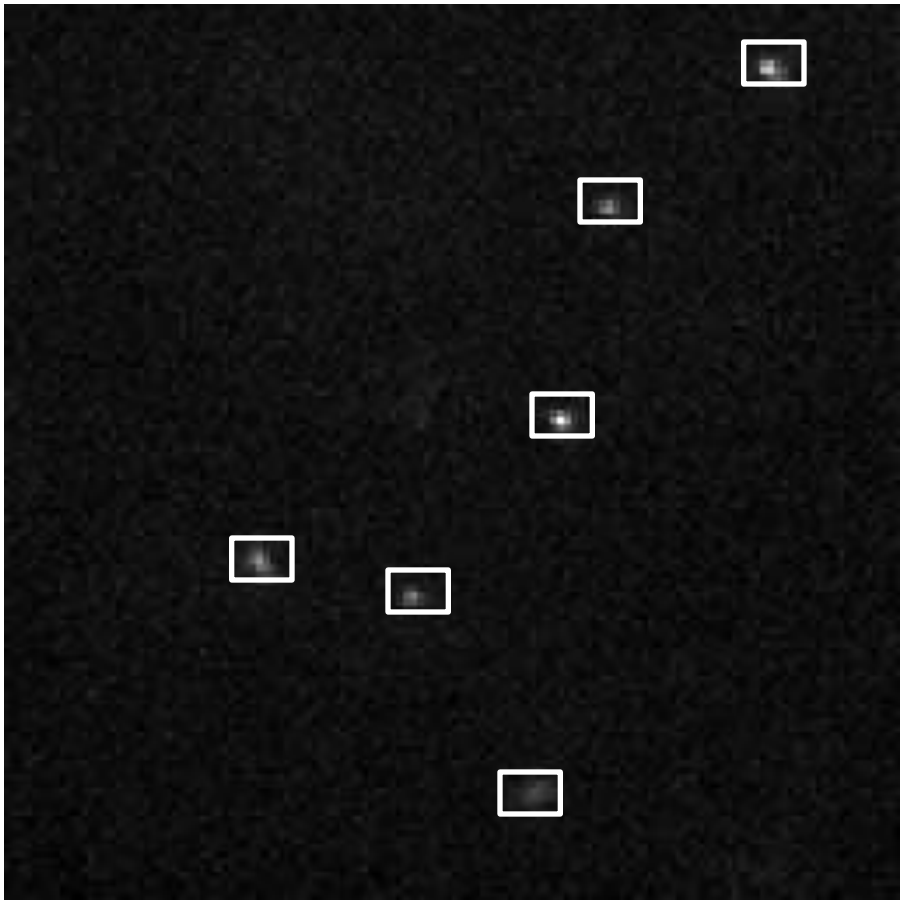
Single particle tracking on live cells

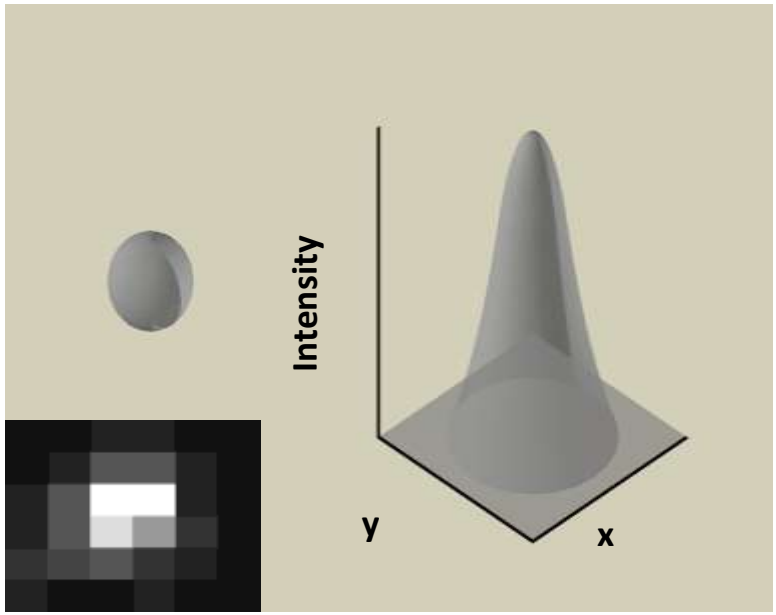


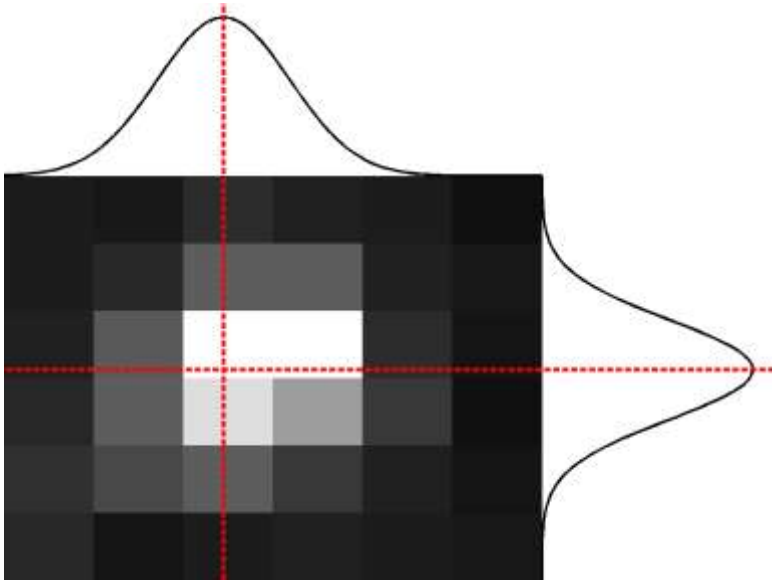


1. **Single particle localization**
2. **Build Trajectories**

Single particle tracking



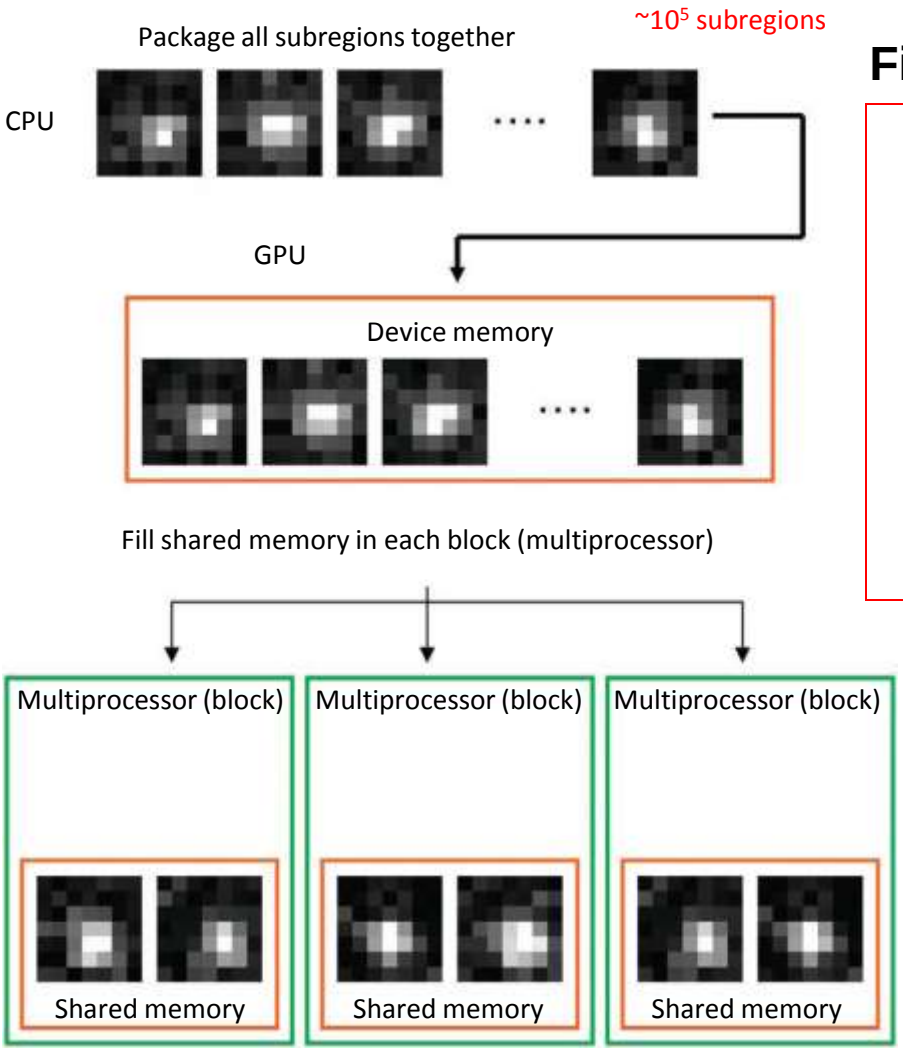




- Typically 1000 frames with 10-100 subregions per frame
- Total ~100,000 subregions

Subregion fitting

Compute Unified Device Architecture (CUDA) - Nvidia



Smith, Nat. Methods 7, 373–375 2010

Fitting Model: 2D finite pixel Gaussian PSF

$$\mathrm{PSF}(x,y) = \frac{1}{2\pi\sigma^2} e^{\frac{-(x-\theta_x)^2-(y-\theta_y)^2}{2\sigma^2}}$$

$$\mu_k(x,y) = \theta_{I_0} \iint_{A_k} \mathrm{PSF}(u,v)\,\mathrm{d}u\mathrm{d}v + \theta_{bg}$$

$$L(\vec{x}|\theta) = \Pi_k \frac{\mu_k(x,y)^{x_k} e^{-\mu_k(x,y)}}{x_k!}$$

θ_x: x-position

θ_y: y-position

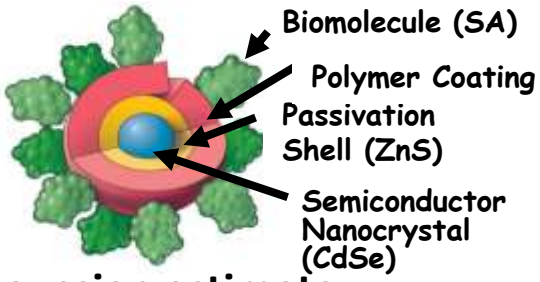
θ_{I0}: emission rate

θ_{bg}: background count rate

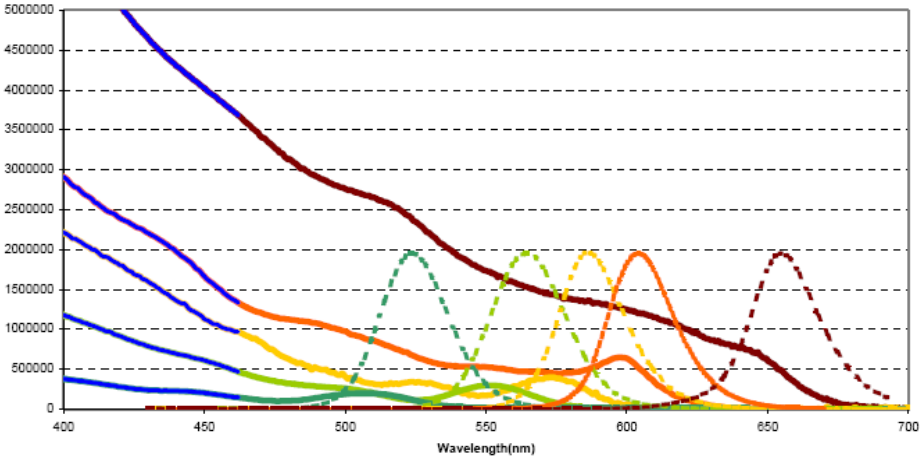
Newton's method iterative update: achieves CRLB

$$\theta_i \rightarrow \theta_i + \left[\sum_k \frac{\partial \mu_k(x,y)}{\partial \theta_i}\left(\frac{x_k}{\mu_k(x,y)} - 1\right)\right] \times \left[\sum_k \frac{\partial^2 \mu_k(x,y)}{\partial \theta_i^2}\left(\frac{x_k}{\mu_k(x,y)} - 1\right) - \frac{\partial \mu_k(x,y)}{\partial \theta_i}^2 \frac{x_k}{\mu_k(x,y)^2}\right]^{-1}$$

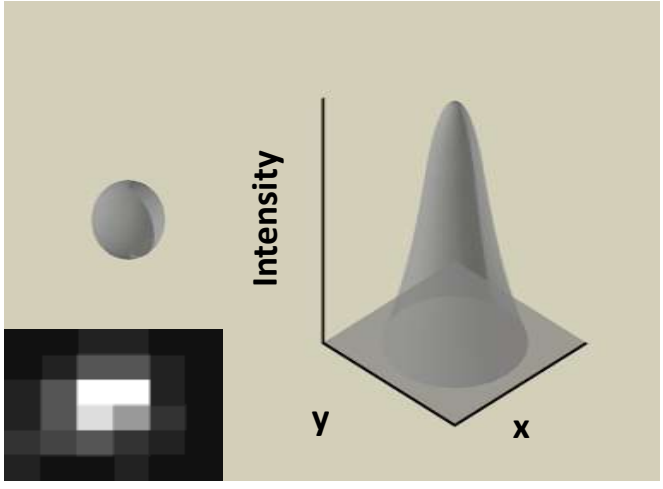
Single molecule localization in hyperspectral images



QD spectra



2-D gaussian estimate



Fitting Model: 3D finite pixel Gaussian PSF

$$PSF(x,y,\lambda) = \frac{1}{(2\pi)^{2/3}\theta_{\sigma_x}\theta_{\sigma_y}\theta_{\sigma_\lambda}} e^{-\frac{(x-\theta_x)^2}{2\theta_{\sigma_x}{}^2} - \frac{(y-\theta_y)^2}{2\theta_{\sigma_y}{}^2} - \frac{(\lambda-\theta_\lambda)^2}{2\theta_{\sigma_\lambda}{}^2}}$$

$$\mu_k(x,y,\lambda) = \theta_{I_0} \int\int\int_{A_k} PSF(u,v,w)\,du\,dv\,dw + \theta_{bg_k}$$

$$L(\vec{x}|\theta) = \prod_k \frac{\mu_k(x,y,\lambda)^{x_k} e^{-\mu_k(x,y,\lambda)}}{x_k!}$$

θ_x: x-position

θ_y: y-position

θ_λ: λ-position

θ_{σ_x}: x-sigma

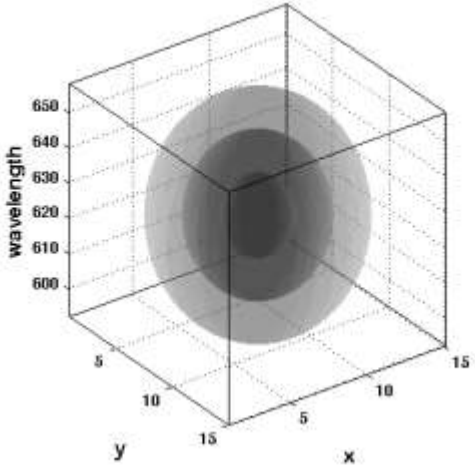
θ_{σ_y}: y-sigma

θ_{σ_λ}: λ-sigma

θ_{I0}: emission rate

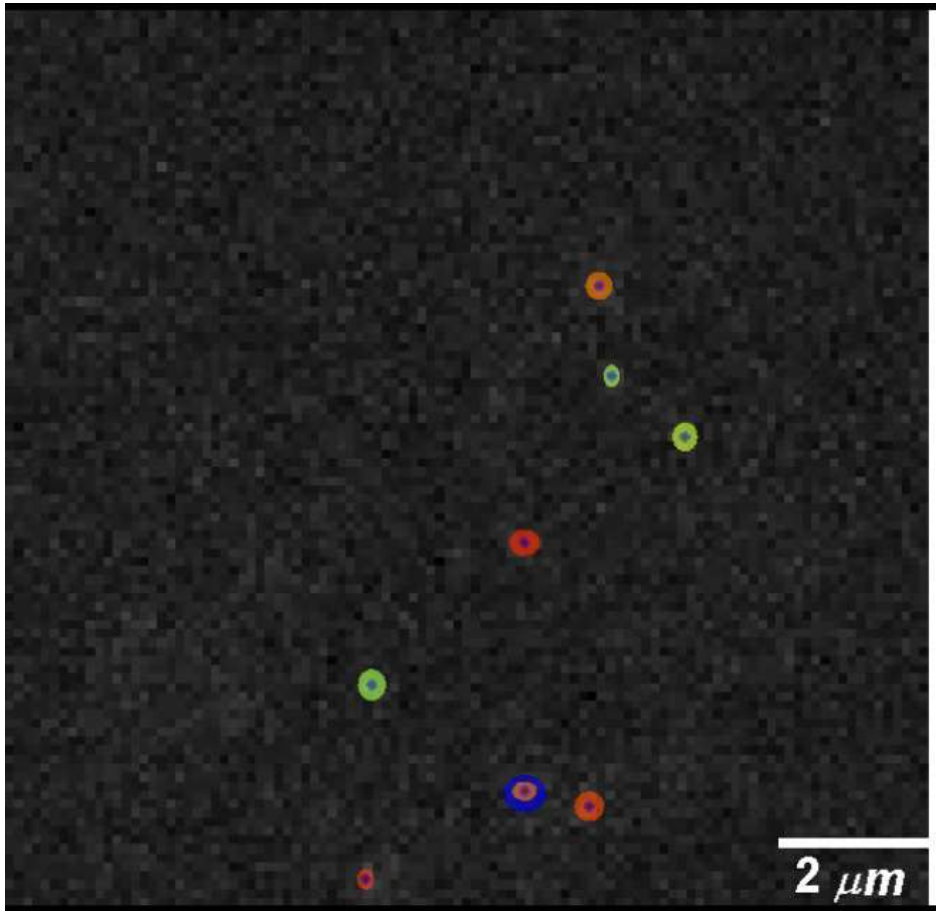
θ_{bg}: background count rate

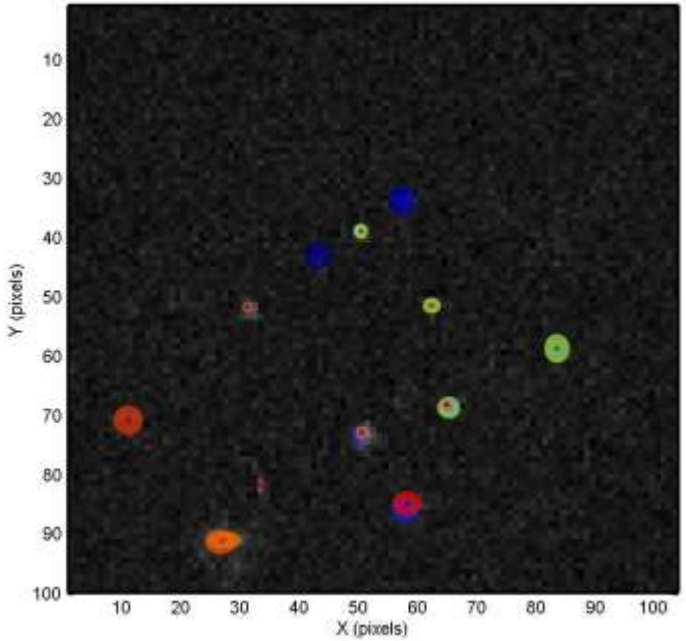
3-D gaussian estimate

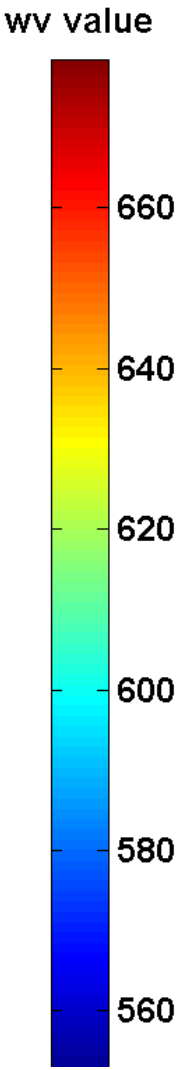


Single particle localization in of QD-IgE in hyperspectral images

(4 seconds/frame 512 spectral channels)

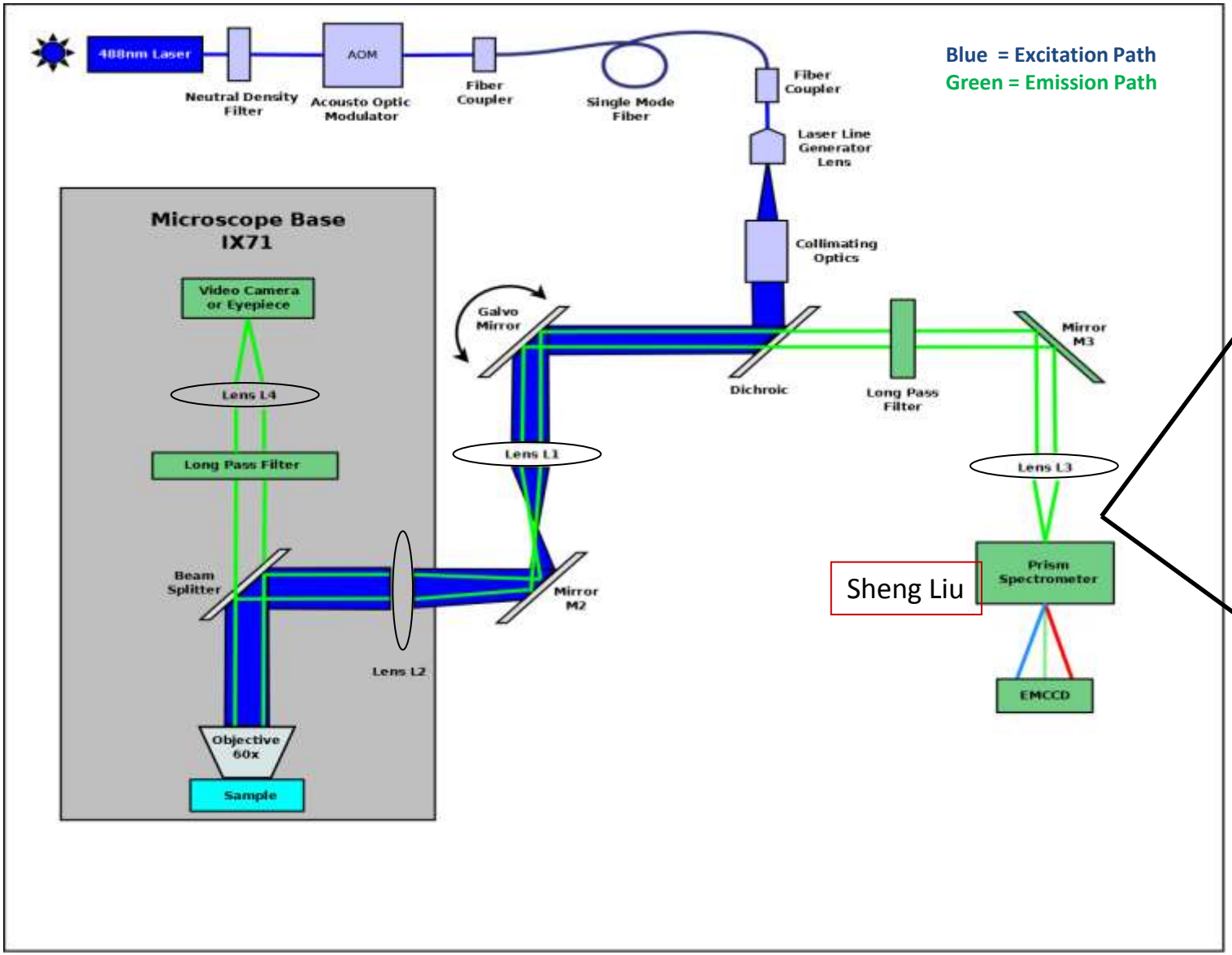






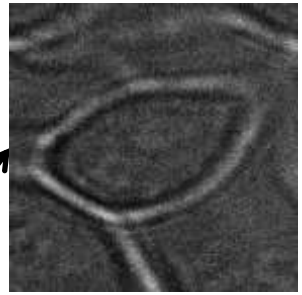
UNM hyperspectral line scanning microscope

(30 frames/second (fps) 128 spectral channels)

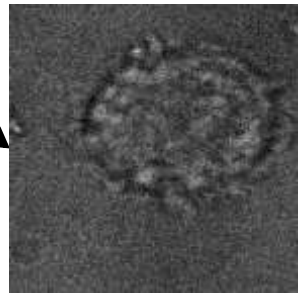


Sheng Liu

Resting



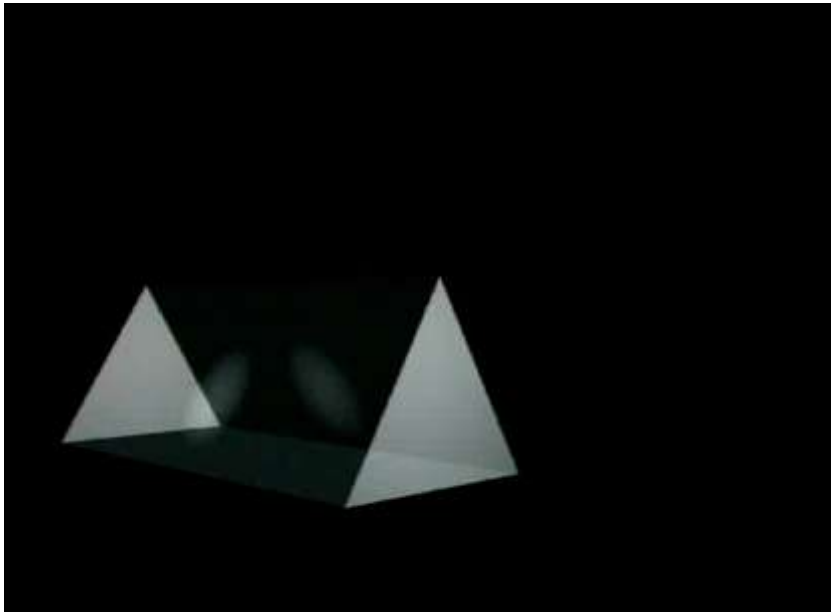
Activated



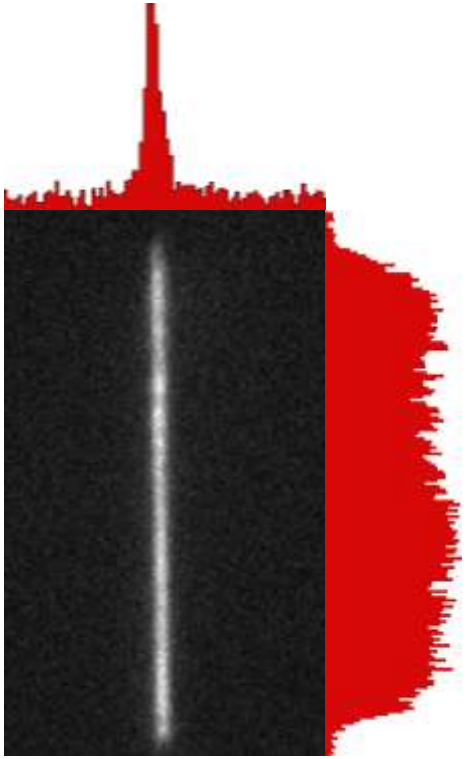
Michael Malik

Michael Malik

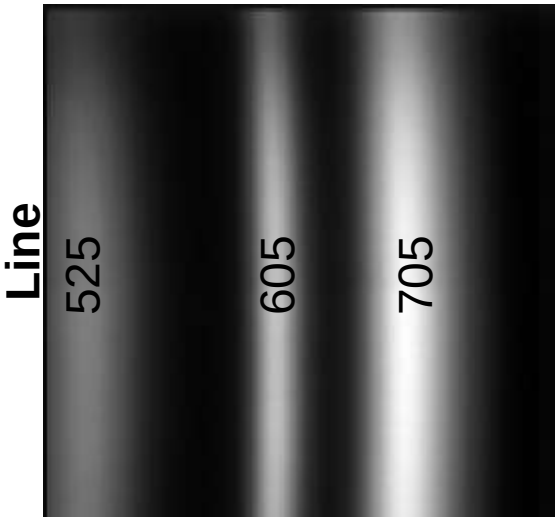
Microscope characterization

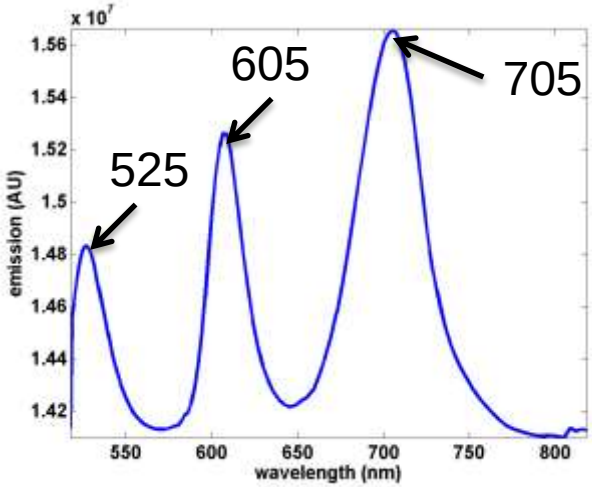


Characterization of line on sample



Spectral characterization of QDs (ensemble)





128 spectral channels

Hyperspectral data flow

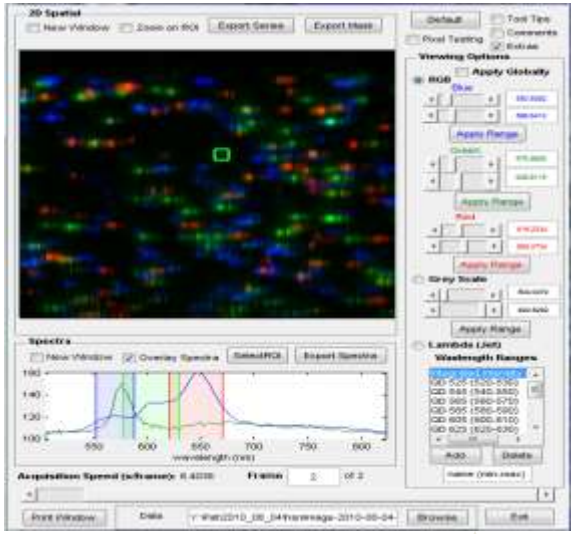
line-scanning hyperspectral microscope

(data acquisition)

data, params

RGB conversion

real-time display



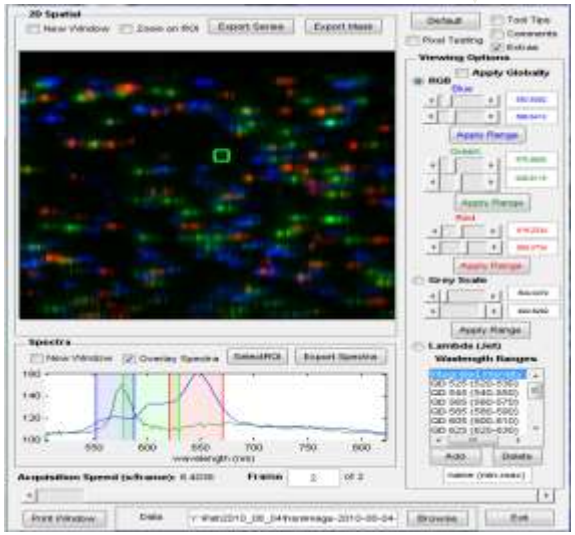
async

save data & acquistion parameters

data structure ('.hsi', '.ics')

Clone

Post processing



saved data

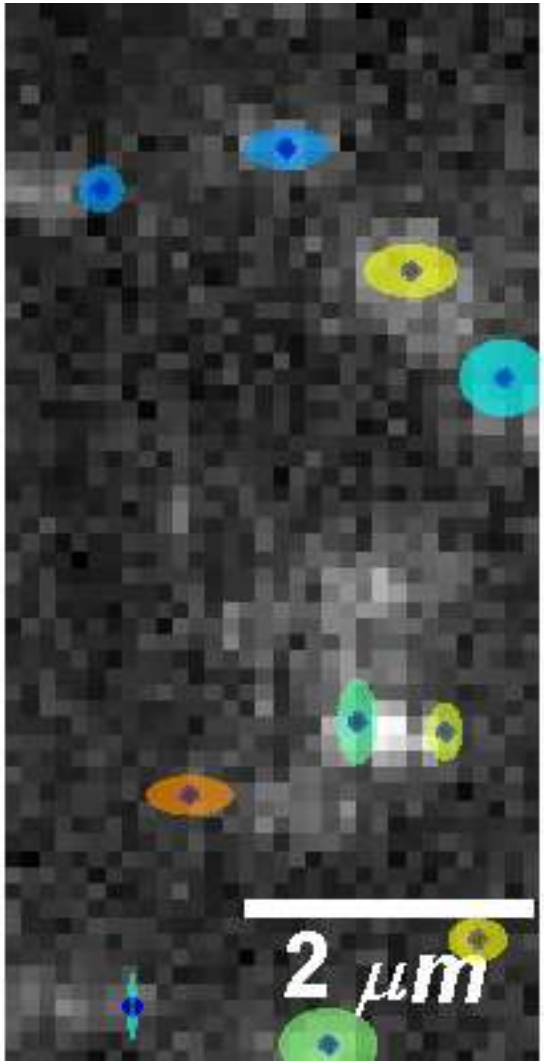
Analysis

Single molecule localization (x,y,λ)

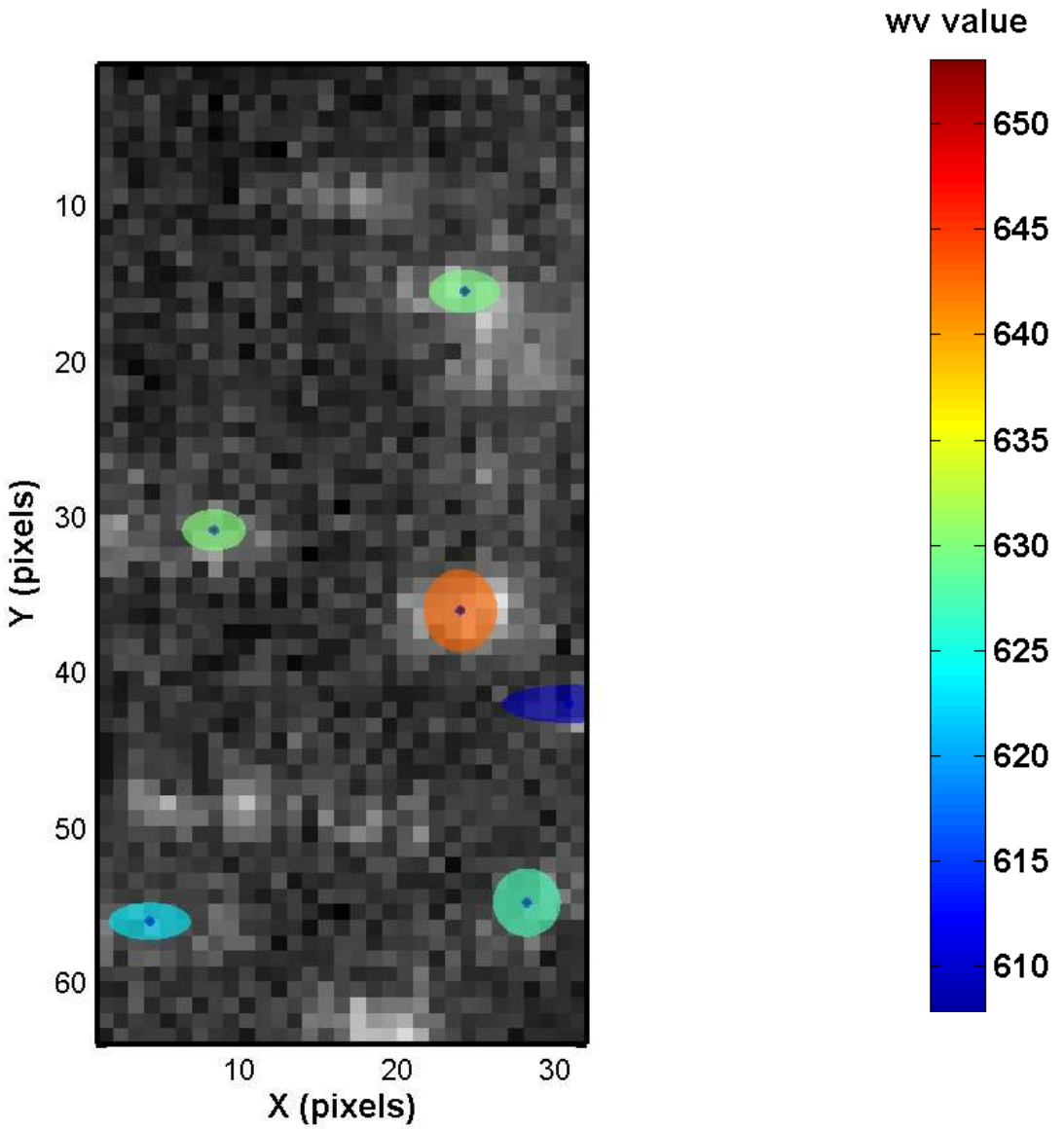
Estimation of background/fluorophore concentration map

QD625 on glass

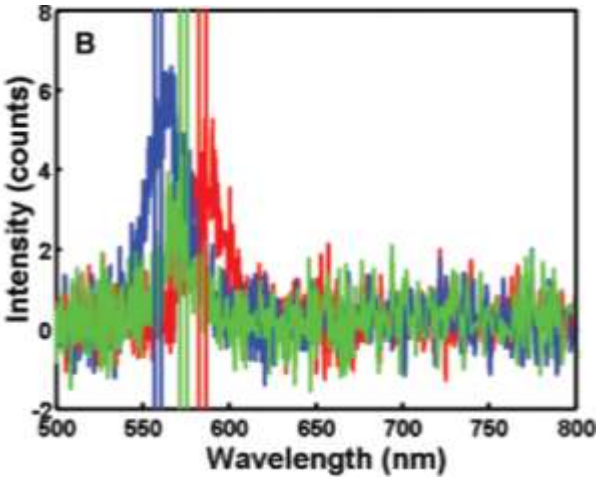
(15 fps 128 spectral channels)

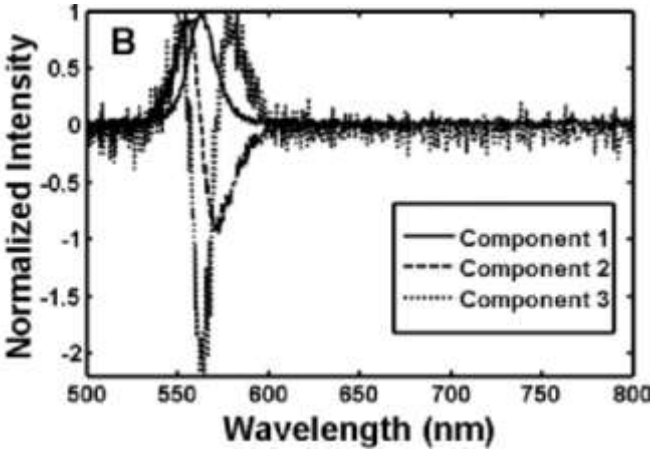


Playback at 2x speed



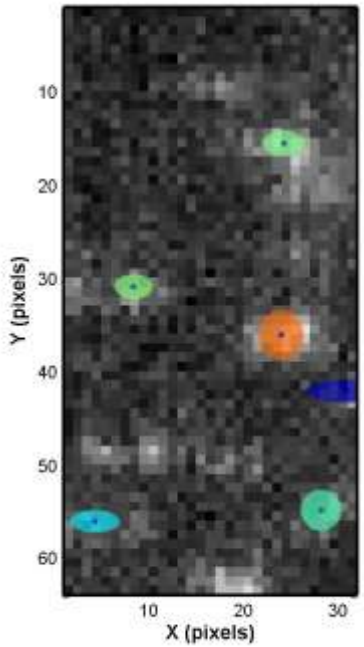
Single molecule localization of 625 QDs on glass



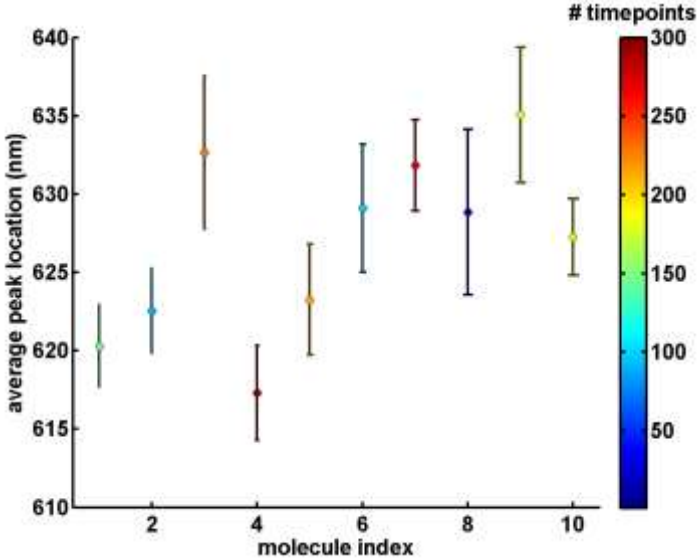


UNM HSM

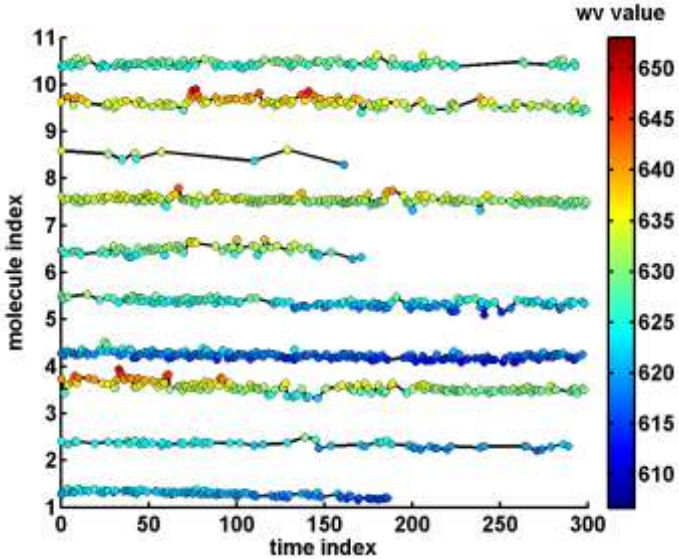
(15 fps 128 spectral channels)



Peak variability



Peak variability over time



Haaland et.al., Applied Spectroscopy 63(3), 271-279 2009.

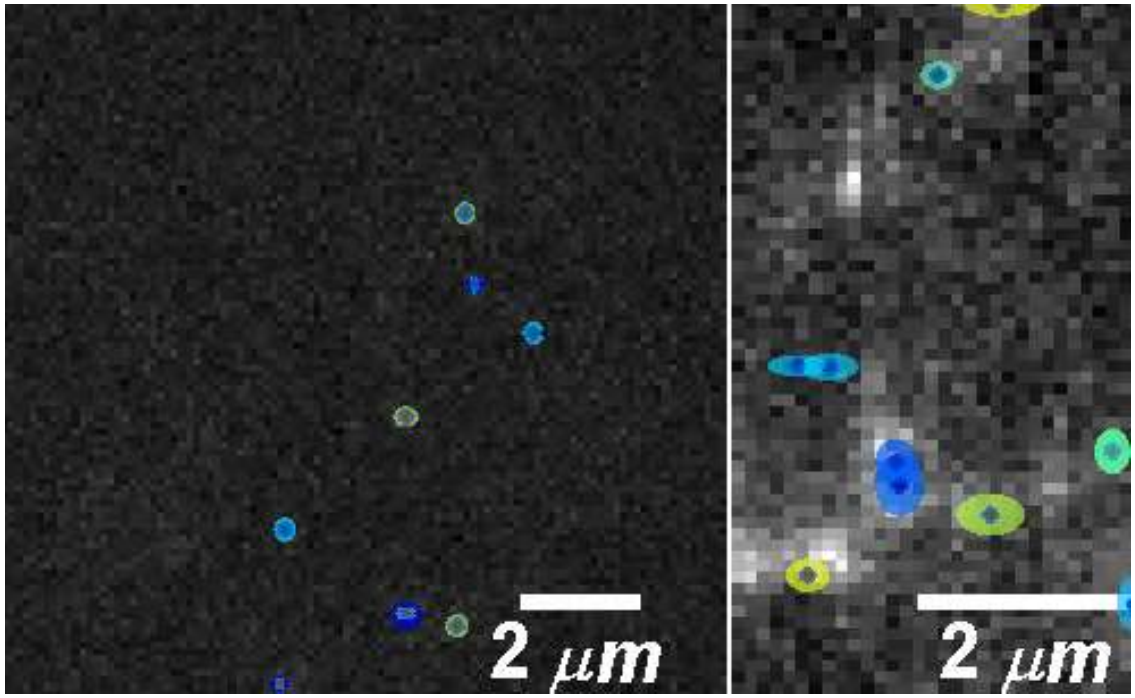
Observing high speed membrane protein dynamics

Sandia HSM

(0.25 fps 512 spectral channels)

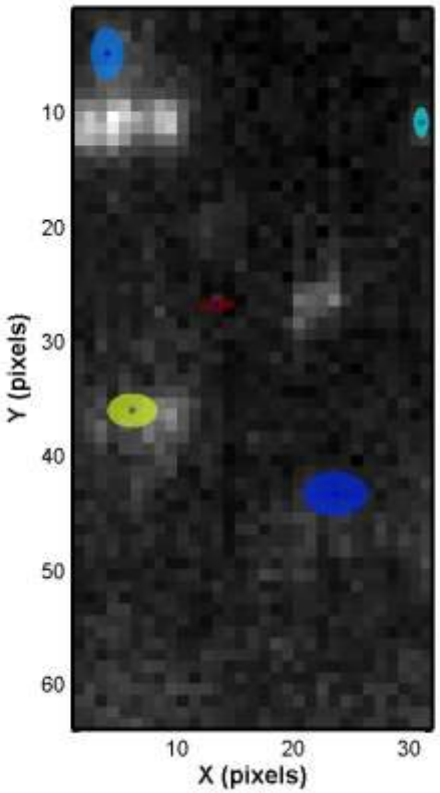
UNM HSM

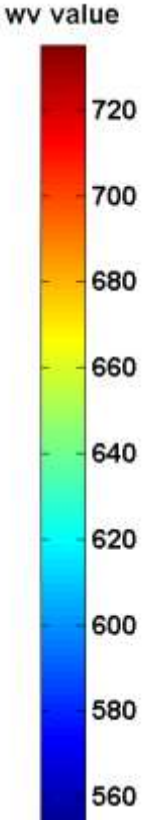
(15 fps 128 spectral channels)



Playback at 2x speed

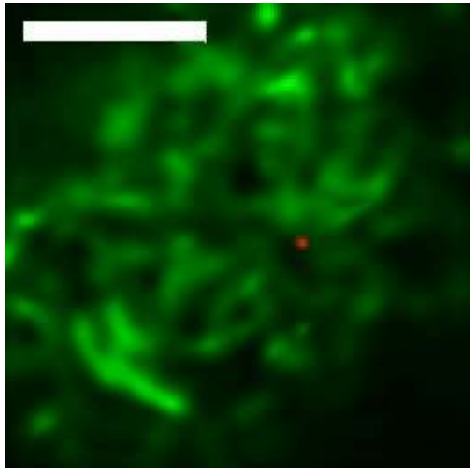
Dynamic spectral range





Conclusions

- Developed hyperspectral line scanning microscope with capability of acquiring 30fps with 128 spectral channels (approximately 20 μm^2 on sample FOV)
- Successfully performed single particle localization on hyperspectral images
  - CUDA implementation gives speed up but needs to be further optimized for architecture
- Future work
  - Utilize multi-fluorophore model fitting for localization
  - Utilize spectral information to help build trajectories
  - Combine with multivariate curve resolution techniques
    - Autofluorescence
    - other structures (actin, cholesterol, etc.)



Acknowledgements

UNM Cell Pathology

Bridget Wilson

Diane Lidke
Advisor

Janet Oliver

Shalini Low-Nam

Sam Schwartz

Nick Andrews

- **UNM Physics**
  - Keith Lidke - Advisor
  - Michael Malik - Microscope
  - Sheng Liu - Spectrometer
  - Fang Huang
  - Jason Byars – CUDA
- **Sandia National Laboratories**
  - David Haaland

Funding

- NIH
  - A1051575
  - R21RR024438
  - P20 GM067594
- INCBN-IGERT Fellowship
  - Integrating Nanotechnology with Cell Biology and Neuroscience
  - NSF grant number DGE-0549500
- STMC
  - SpatioTemporal Modeling Center
  - P50 GM085273

Variance in estimated parameters

Cramér-Rao Lower Bound (CRLB)

$$I(\theta)_{i,j} = E[\frac{\partial \ln(L(\vec{x}|\theta))}{\partial \theta_i} \frac{\partial \ln(L(\vec{x}|\theta))}{\partial \theta_j}]$$

$$I(\theta)_{i,j} = \sum_k \frac{1}{\mu_k(x,y)} \frac{\partial \mu_k(x,y)}{\partial \theta_i} \frac{\partial \mu_k(x,y)}{\partial \theta_j}$$

$$var(\hat{\theta}) \geq I(\theta)^{-1}$$

Use CRLB to estimate localization accuracy

CRLB: Minimum variance of an estimated parameter

Overview

- System of interest (QD-IgE)
- Single particle tracking
- Single particle localization in conventional and hyperspectral images
- Development of UNM hyperspectral line scanning microscope and analysis of hyperspectral images