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Optical imaging of CdSe quantum dot uptake and trafficking in live cells for studying toxicology

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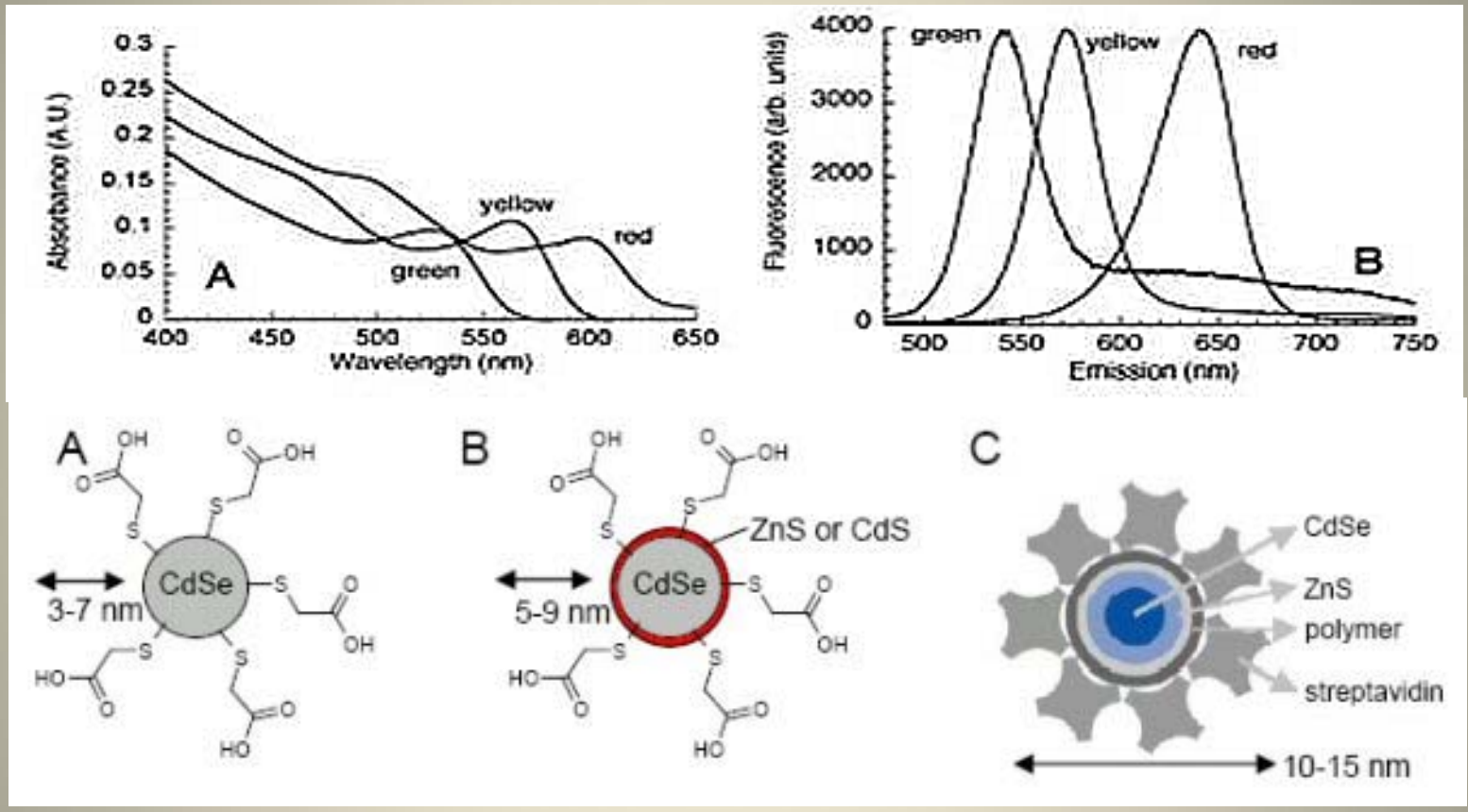
Sandia National Laboratories

Nanotoxicology

"Indeed, some doctors, scientists and consumer advocates are concerned that many industries are adopting nanotechnology ahead of studies that would establish whether regular ingestion, inhalation or dermal penetration of these particles constitute a health or environmental hazard."

-Singer, N. "New Products Bring Side Effect: Nanophobia" *New York Times,* Dec. 3, 2008

CdSe Quantum Dots



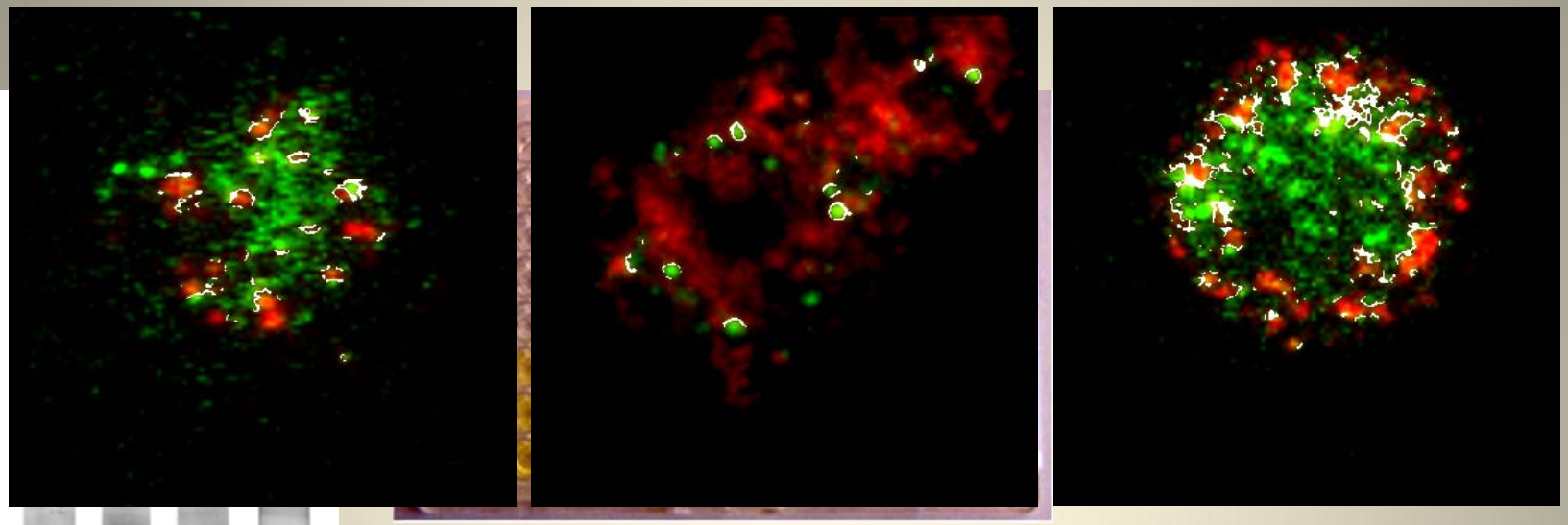
Kloepfer, J.A. et al. *Appl Environ Microbiol* 69, 4205-4213 (2003)

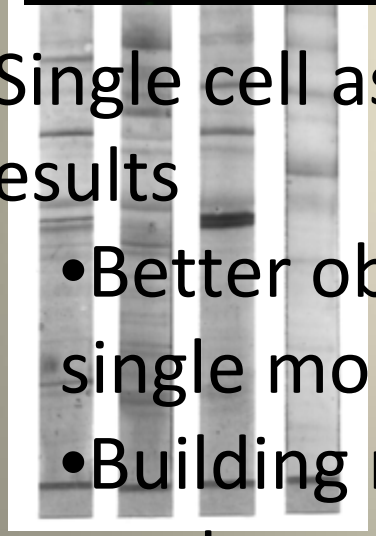
How to assess the toxicological effects of these materials at the mechanistic level??

Major Questions

- What are the mechanisms of cell-nanoparticle interaction?
- What is the behavior of QDs on the cell surface?
- How quickly does uptake occur?
- What is QD fate within the cell?
- Are there size dependencies to any of these behaviors?

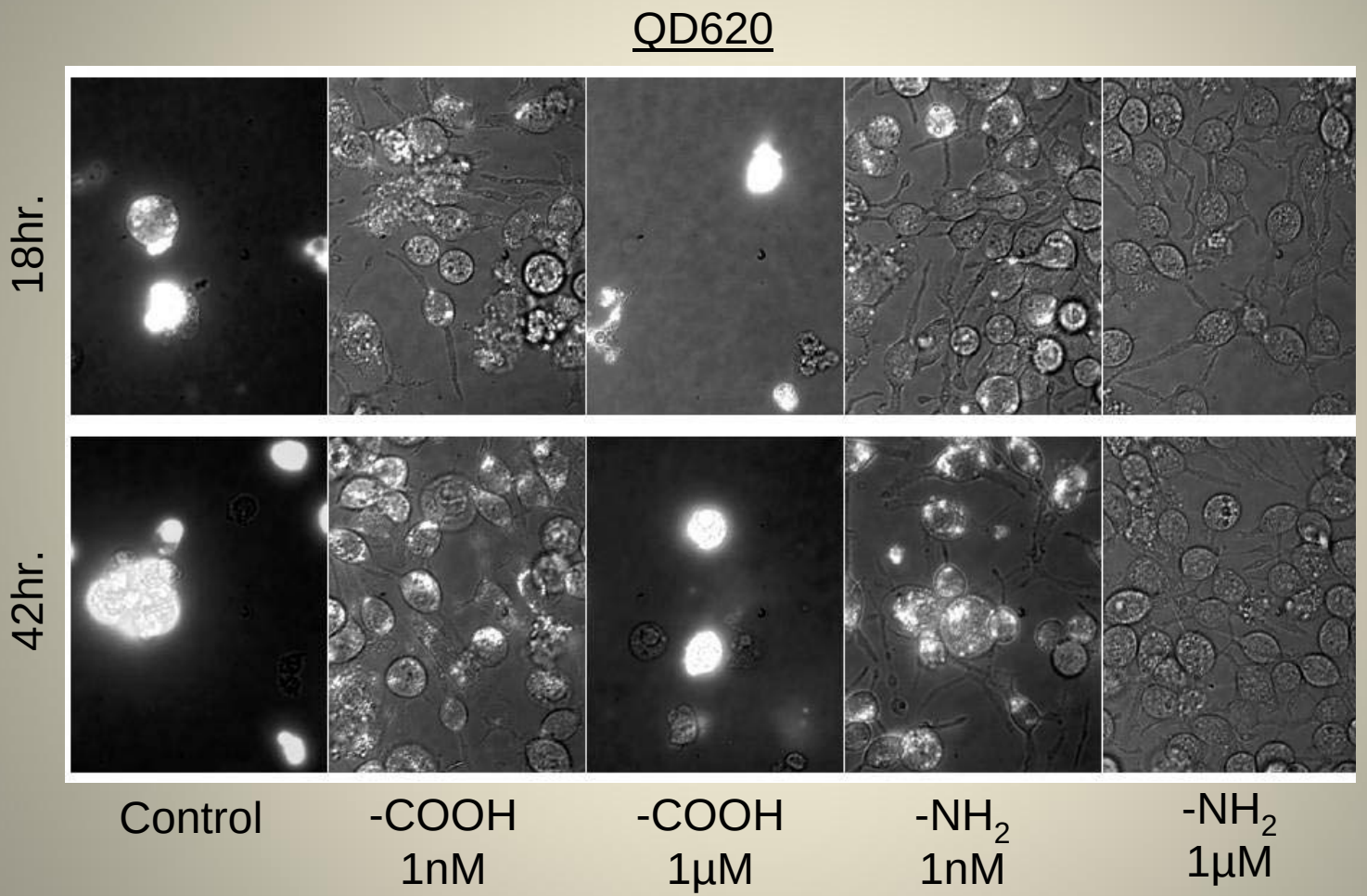
“Bulk” vs. Single Cell Assays



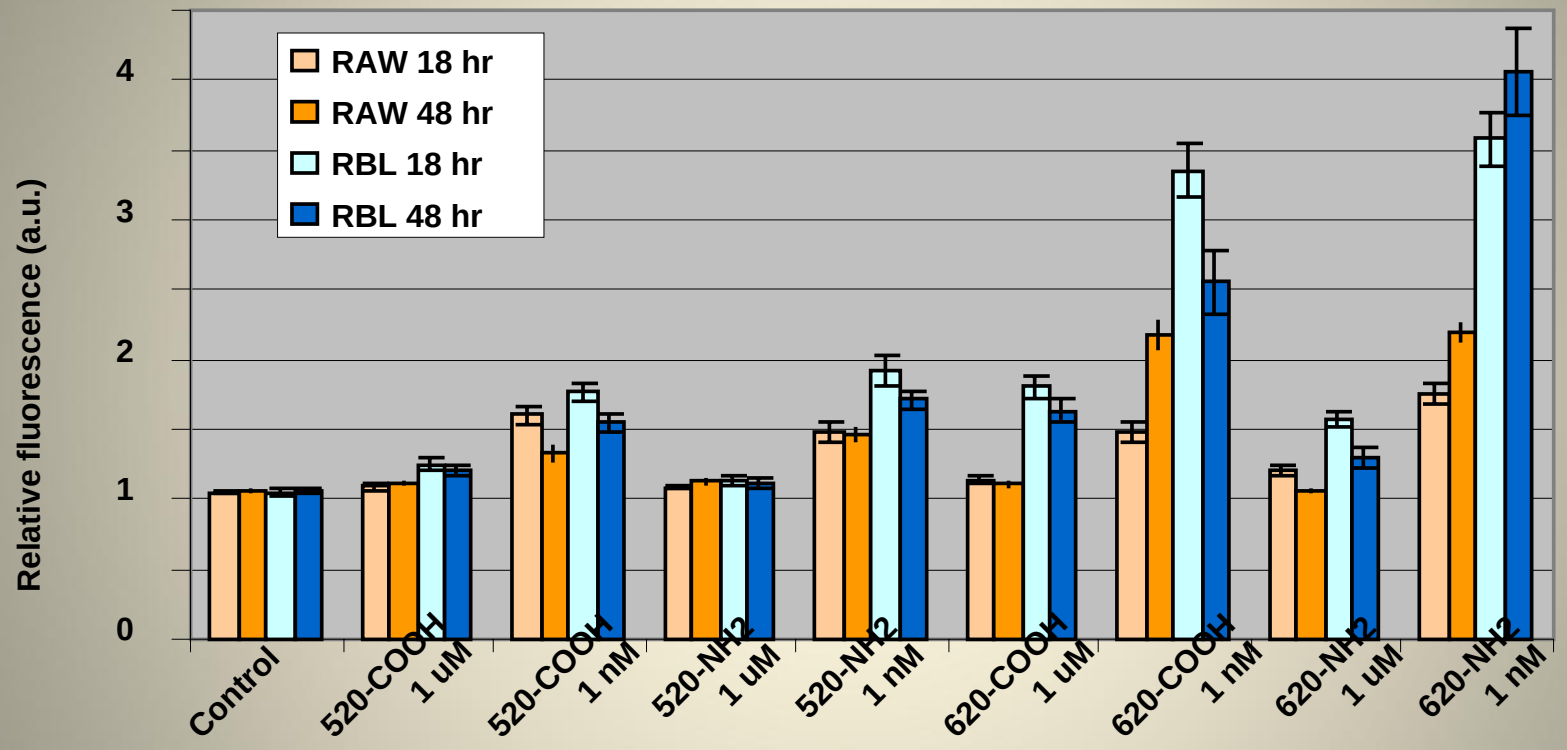


- Single cell assays can capture otherwise undetectable results
  - Better observation of “true” behavior (even at the single molecule level)
  - Building meaningful (i.e. statistical) picture is much more labor-intensive
- “Bulk” assays such as Western blots and ELISA give average indicators
  - Can easily indicate average response of a living system to a stressor
  - May mask certain stochastic behaviors

QD Uptake – fluorescence Microscopy



QD Uptake Quantification

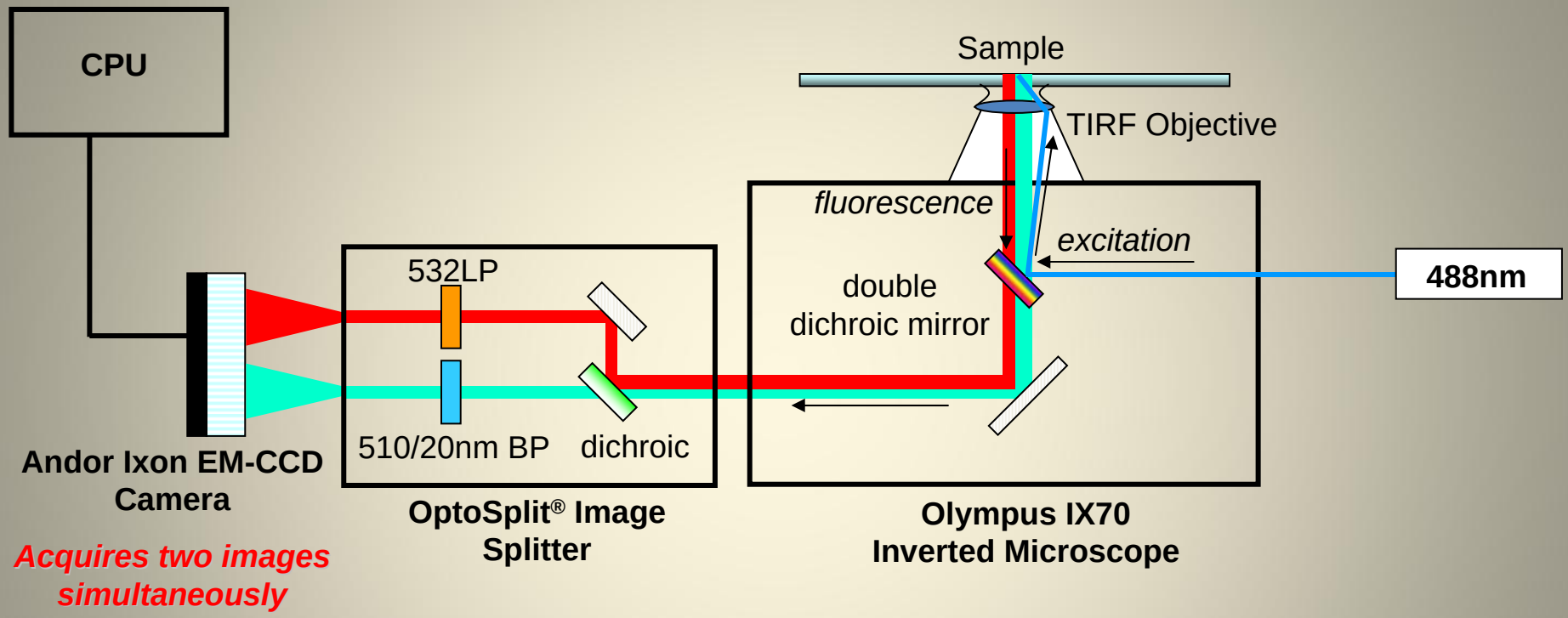


- General Trend of increased uptake with size

Initial cell interactions with QDs

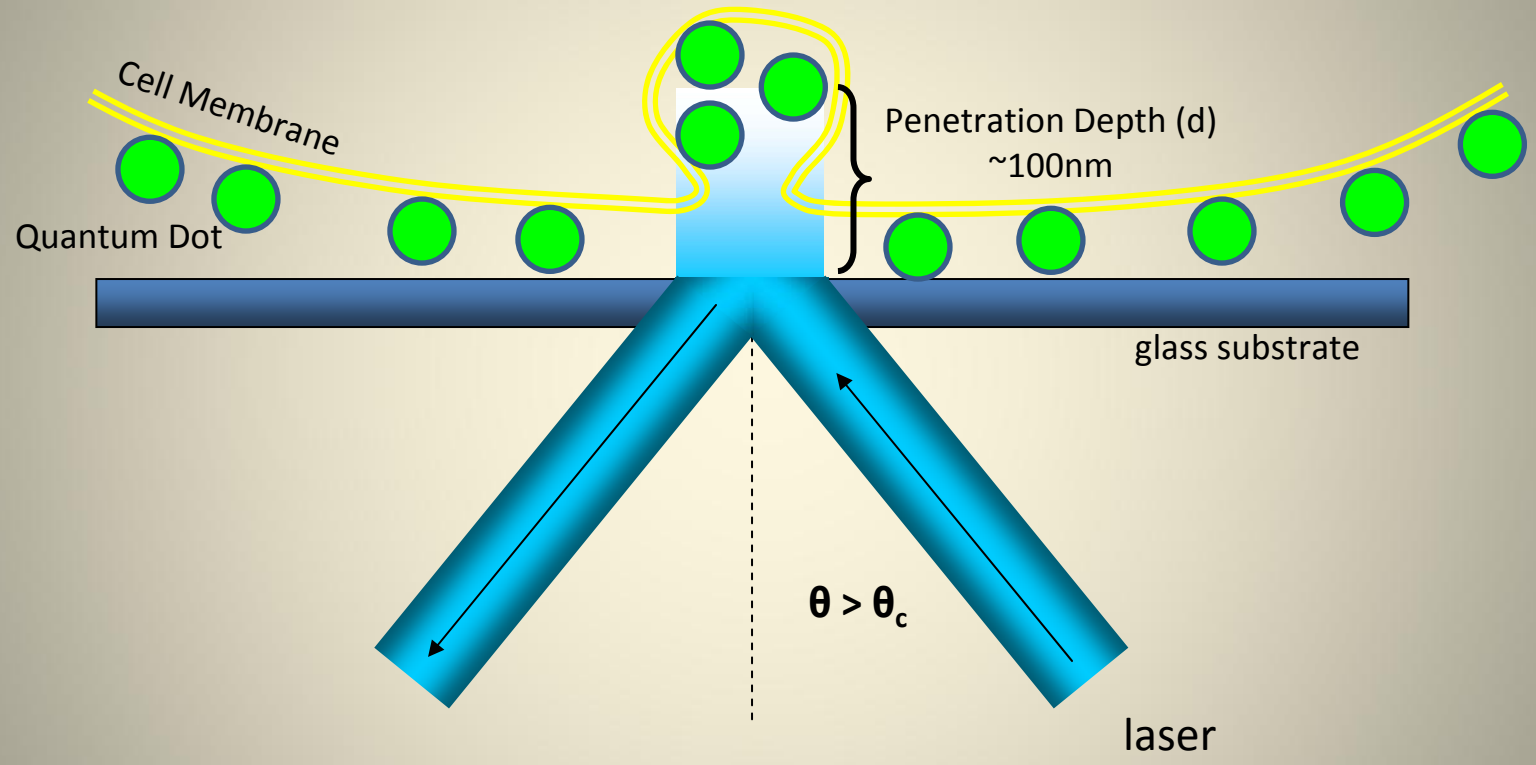
- What is the behavior of QDs once they bind to the surface?
- What are the uptake kinetics?

TIRF Microscope



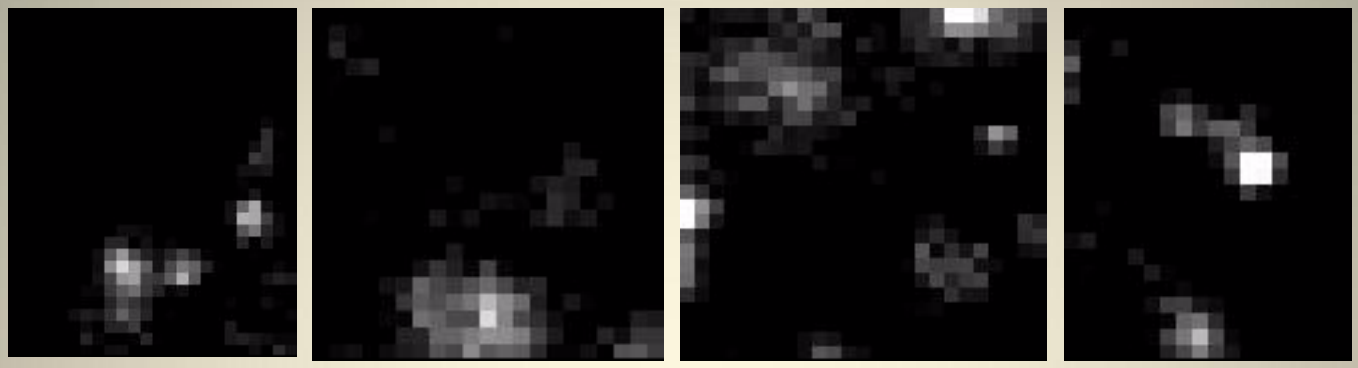
- Our system can image in two fluorescent channels simultaneously via the OptoSplit
- Detection with EMCCD – capable of imaging 60fps

Imaging QDs with TIRF Microscopy



- Allows selective imaging of QD behavior on cell membrane
- Signal decays as QDs are internalized
- Higher axial resolution than confocal (~100nm)

QD tracking on cell membrane

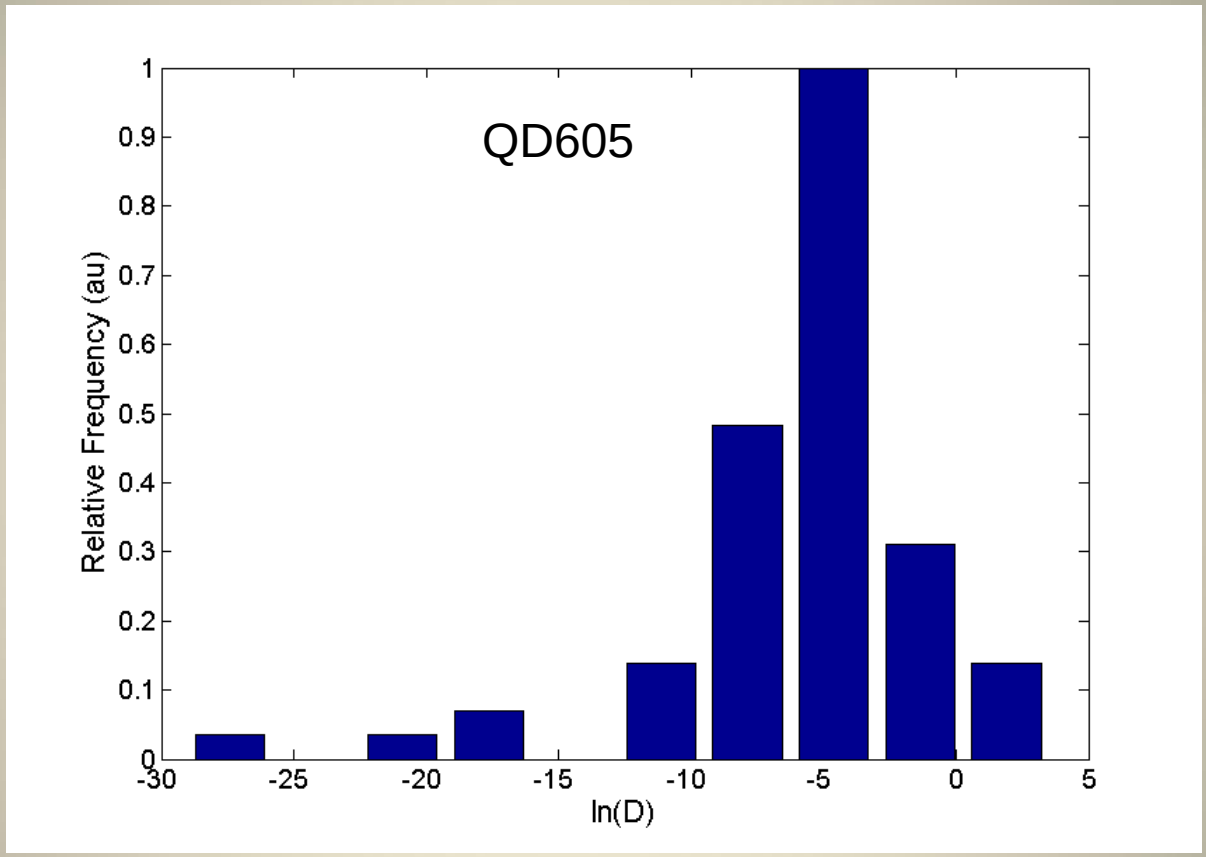


QD605

- Cell membrane is a multi-domain system
- We use particle tracking algorithms to gain insight into diffusion behavior
- We do not expect “pure” Brownian motion to occur
- We model particle behavior as anomalous diffusion, i.e.

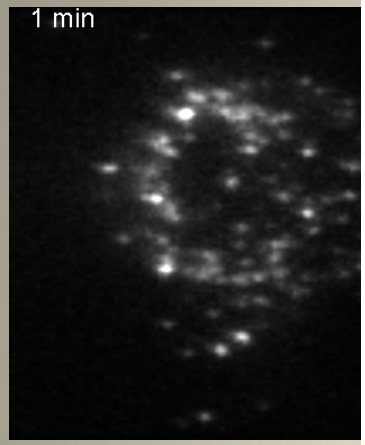
$$MSD(t) \sim Dt^{\alpha}$$

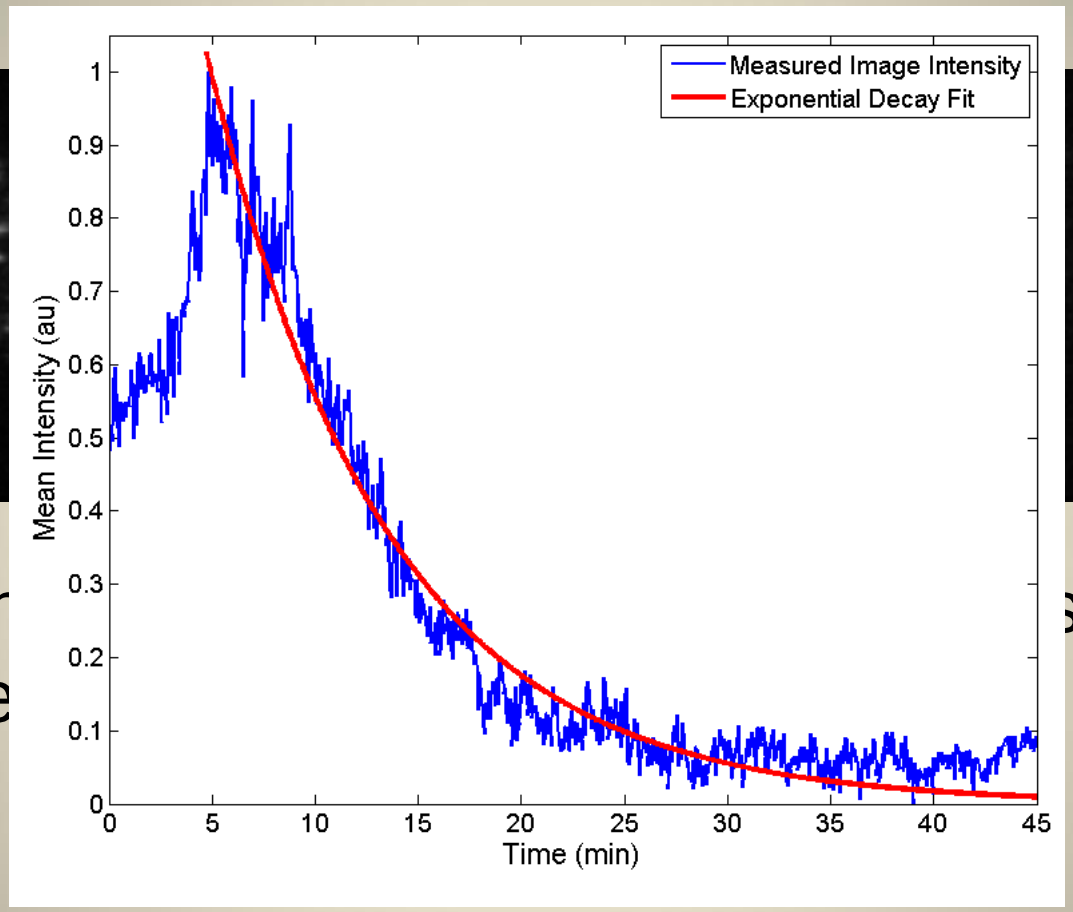
2D Diffusion Coefficients

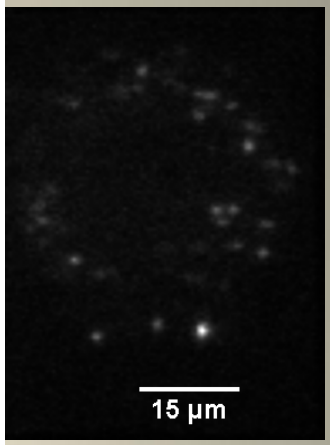


- Large range of diffusion constants, indicative of a highly heterogeneous cell membrane

QD internalization



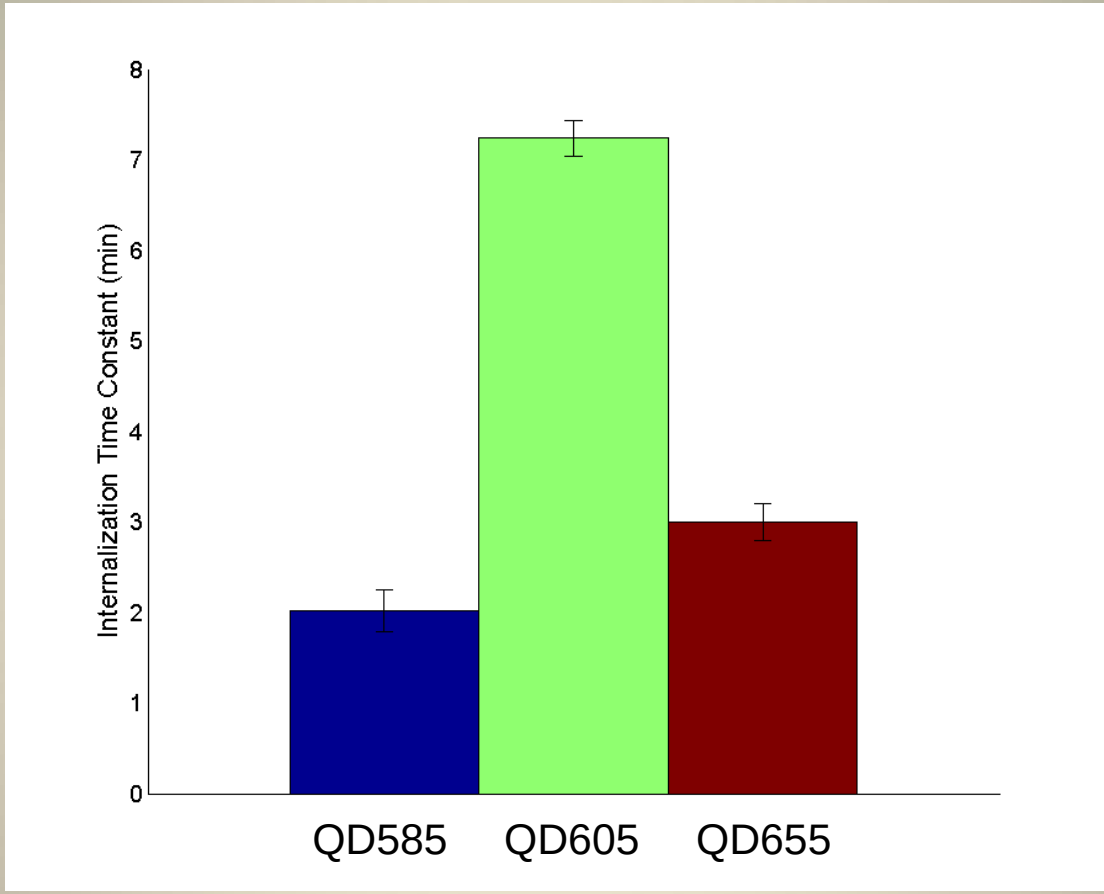




•TIRF sign s are internalize

•Modeling this as an exponential decay gives a characteristic time constant for each QD type

Internalization Kinetics

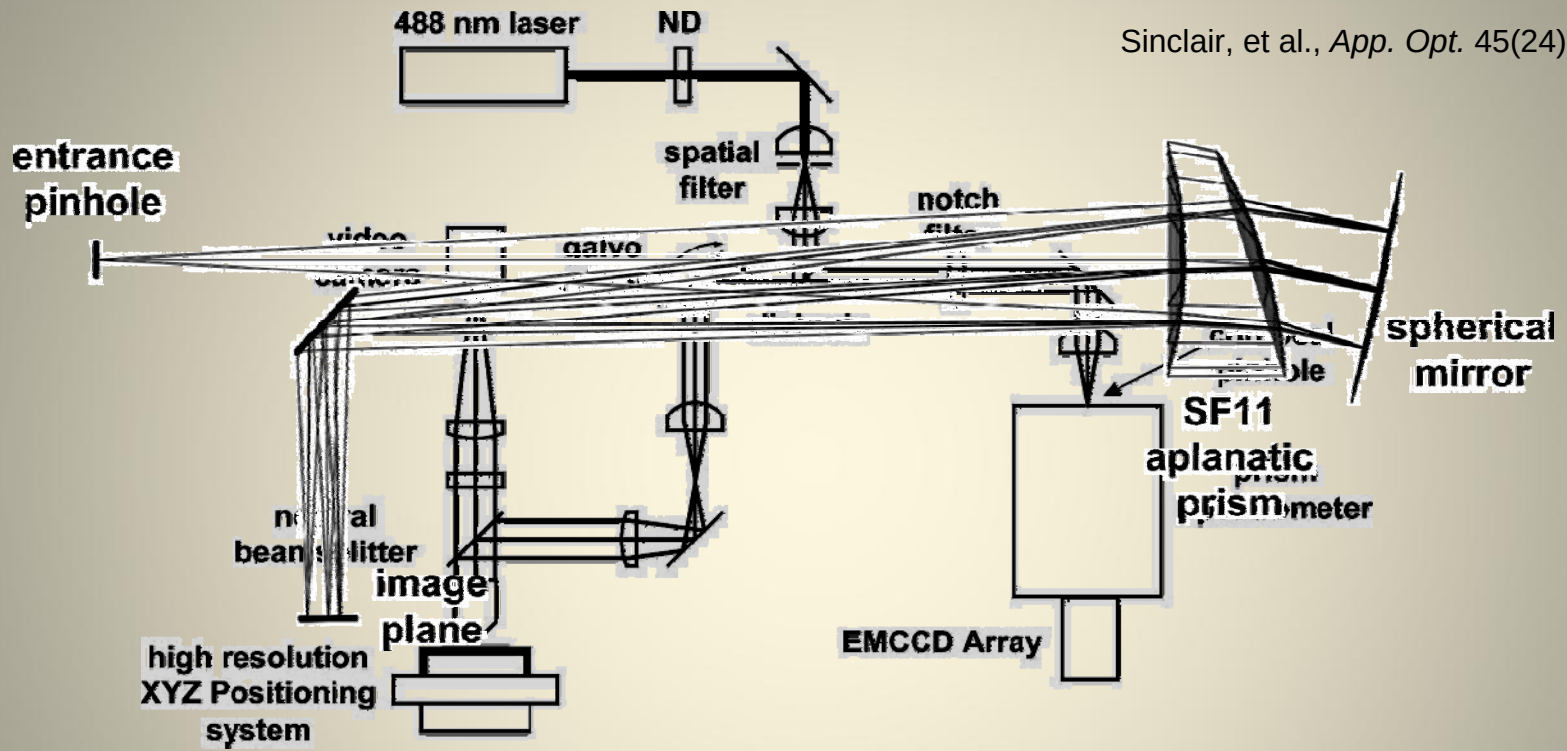


- Surprisingly, no clear size dependence on uptake time

QD Sorting within the Cell?

- What is the localization of QDs in the cell once they are internalized?

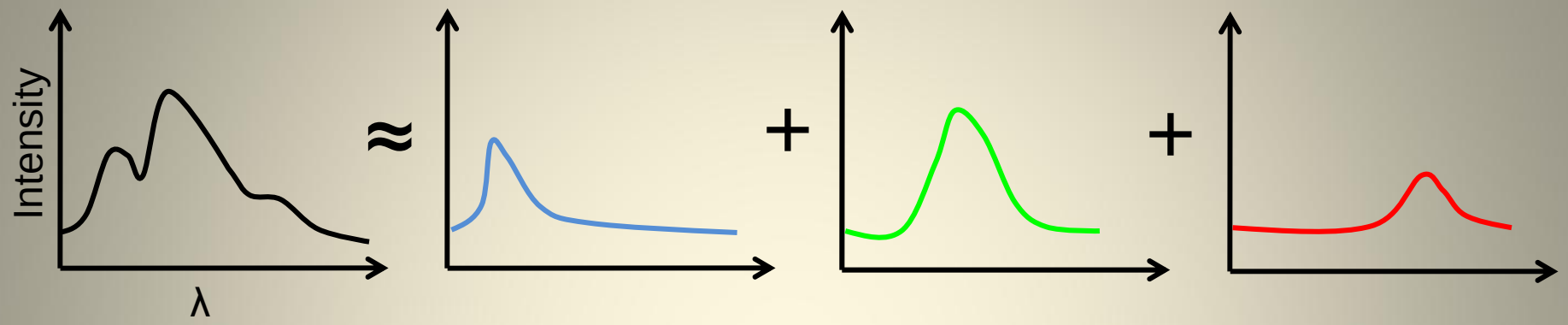
Hyperspectral Microscopy



Sinclair, et al., *App. Opt.* 45(24), 6283-6291

- Illumination path is typical confocal configuration
- Light is dispersed via a custom prism-based spectrometer with EMCCD detector
- Capable of ~8300 spectra per second

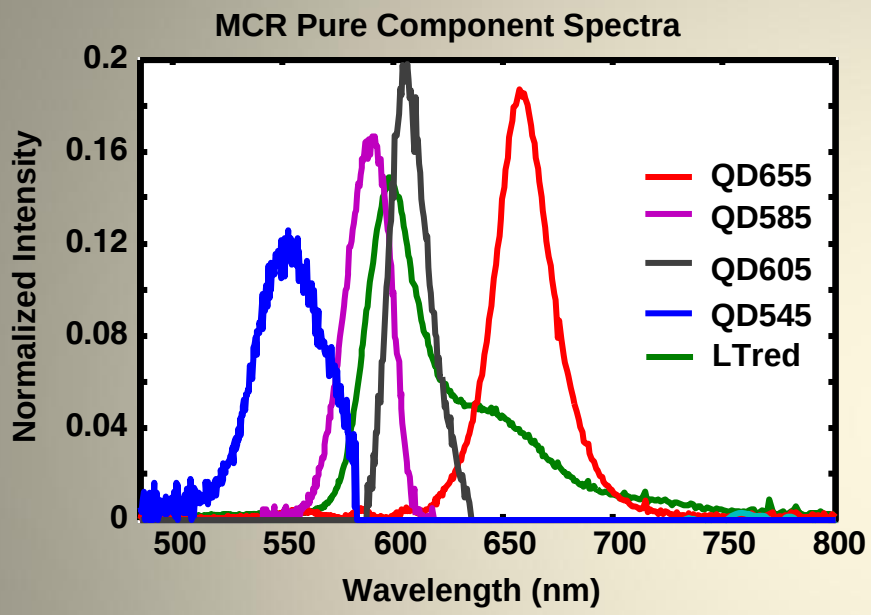
Multivariate Curve Resolution (MCR)



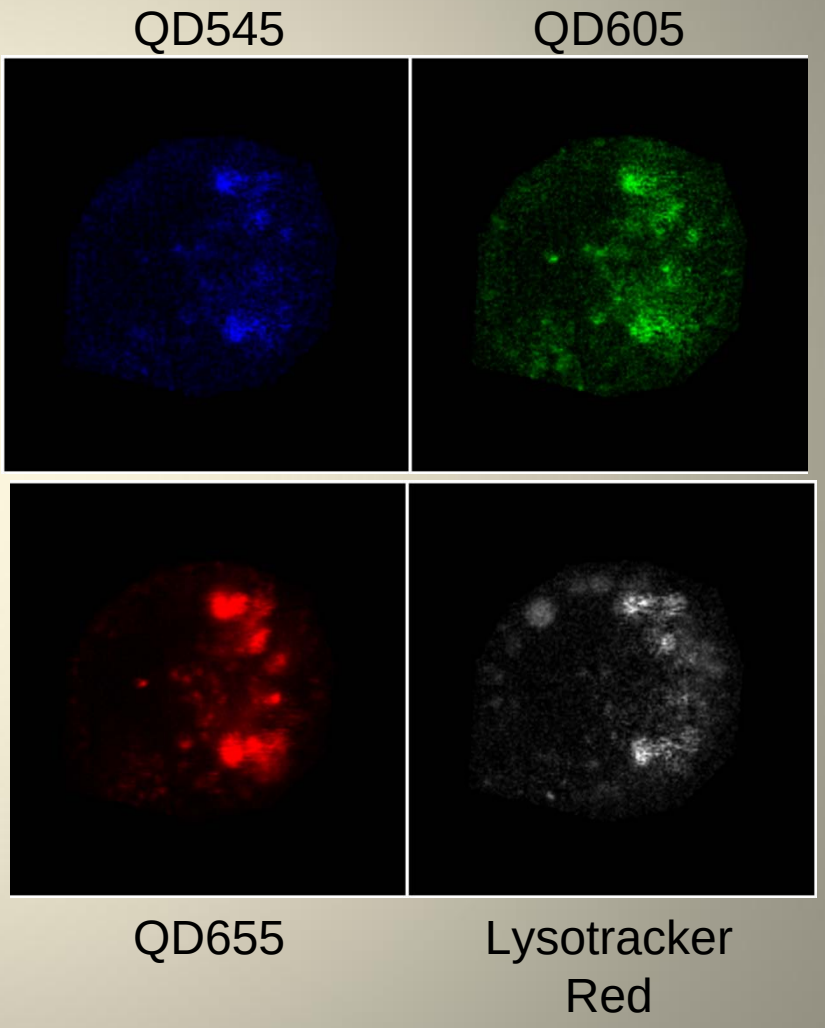
- Requires no *a priori* information about spectral components
- Operates via an initial PCA analysis to determine number of components
- Utilizes a constrained least-squares fit

Haaland, et al., *Proc. SPIE*, Vol. 4959, 55 (2003)

Imaging multiple QDs simultaneously

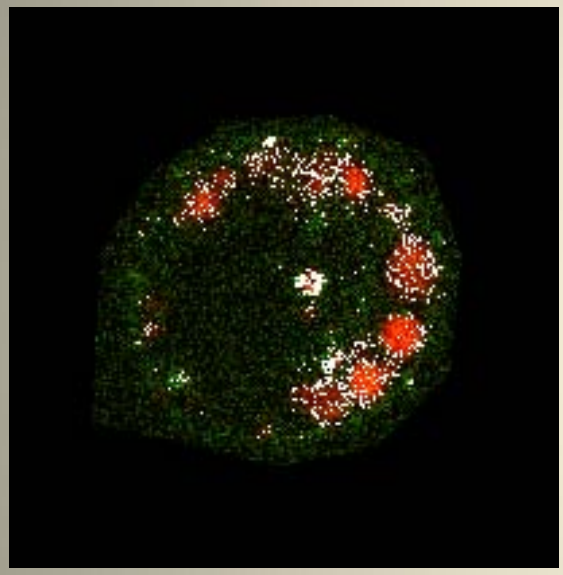


- MCR is able to resolve 5 spectral components from the confocal images
- These conform well with the known emission of the QDs and lysosome stain

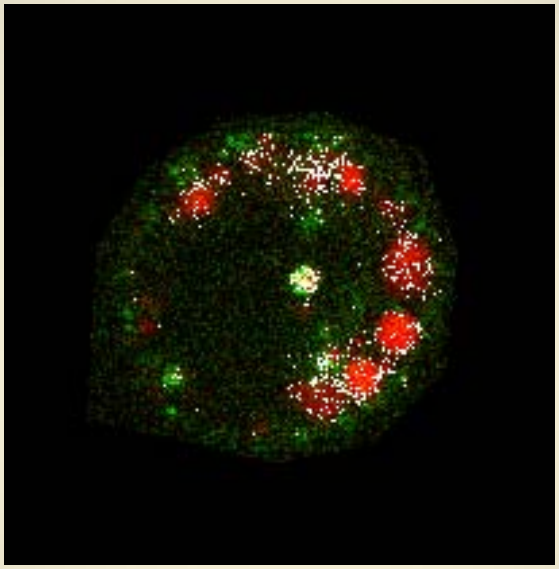


Lysosomal Co-localization

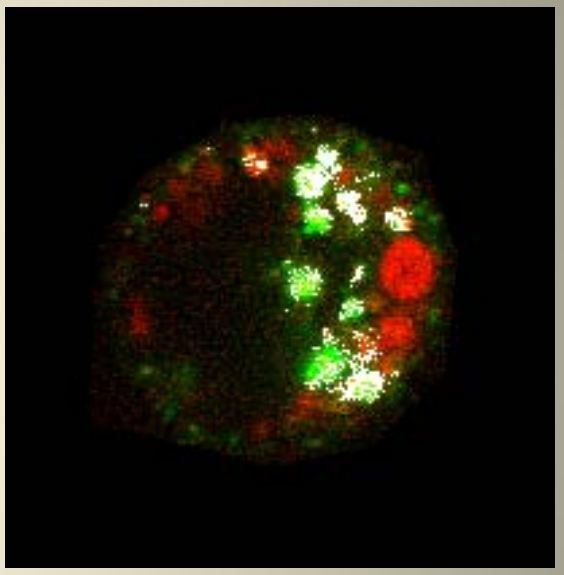
QD545



QD605



QD655

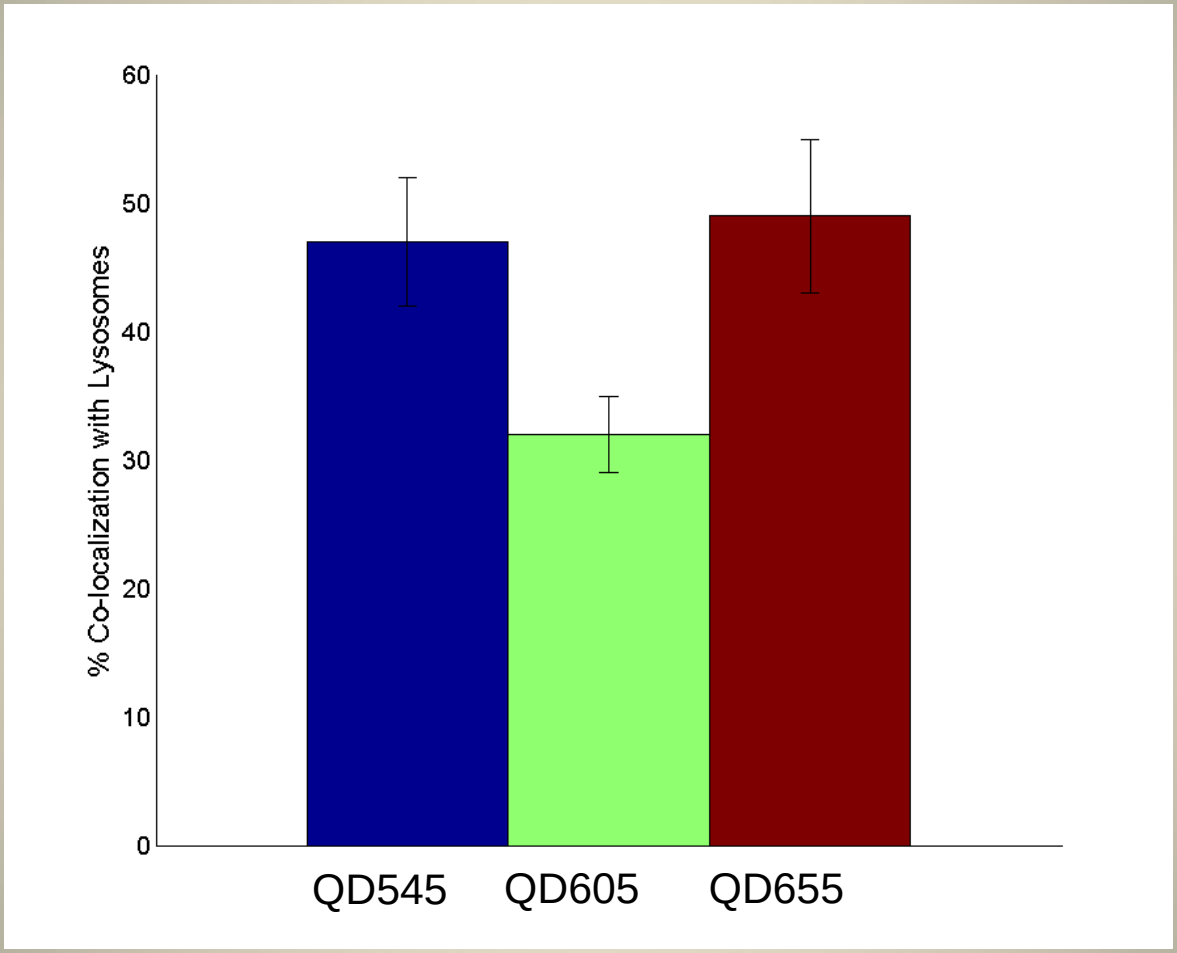


Green = Quantum Dots

Red = Lysotracker Red Dye

White = co-localization

Lysosomal Co-localization



•Again, no clear relationship between particle size and lysosomal sorting

Conclusions

- Advanced imaging strategies allow for stochastic evaluation of nanoparticle-cell interactions
  - Appears to be an inverse relationship between particle uptake speed and its localization to endosomes
  - No clear relationship between particle uptake/sorting and quantum dot size
  - Particle shape?

Acknowledgements

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Questions?