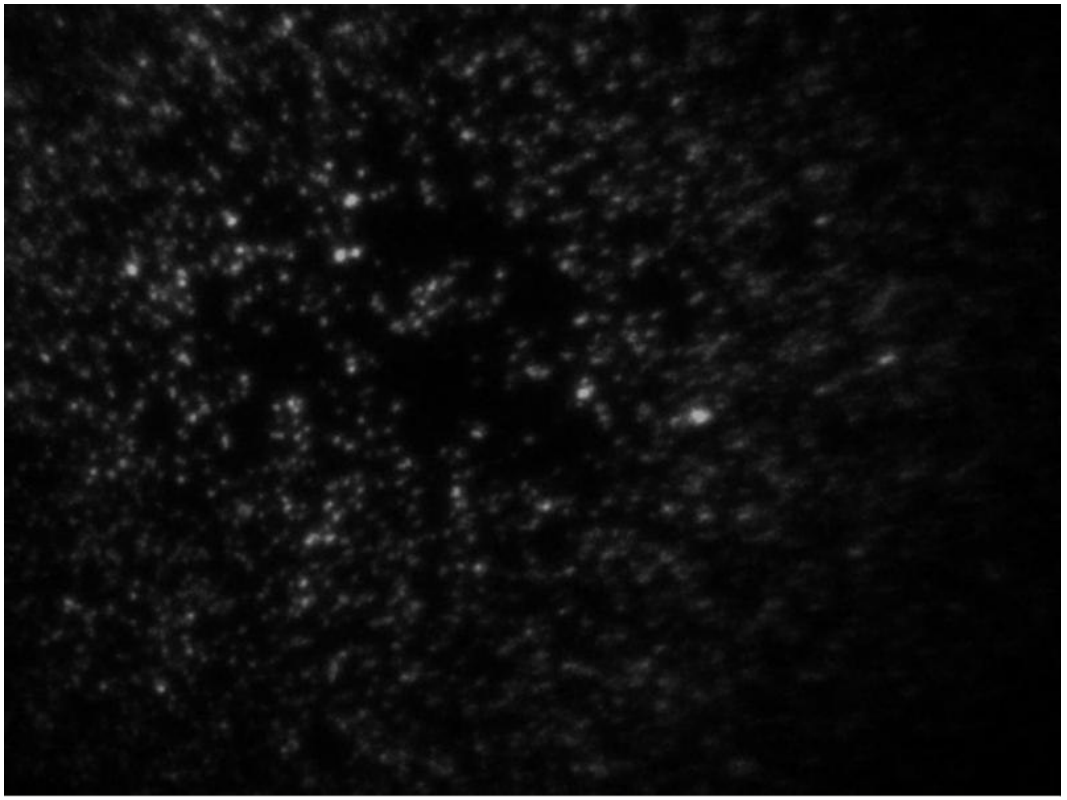
ACHIEVING SUPER RESOLUTION USING QUANTUM DOTS



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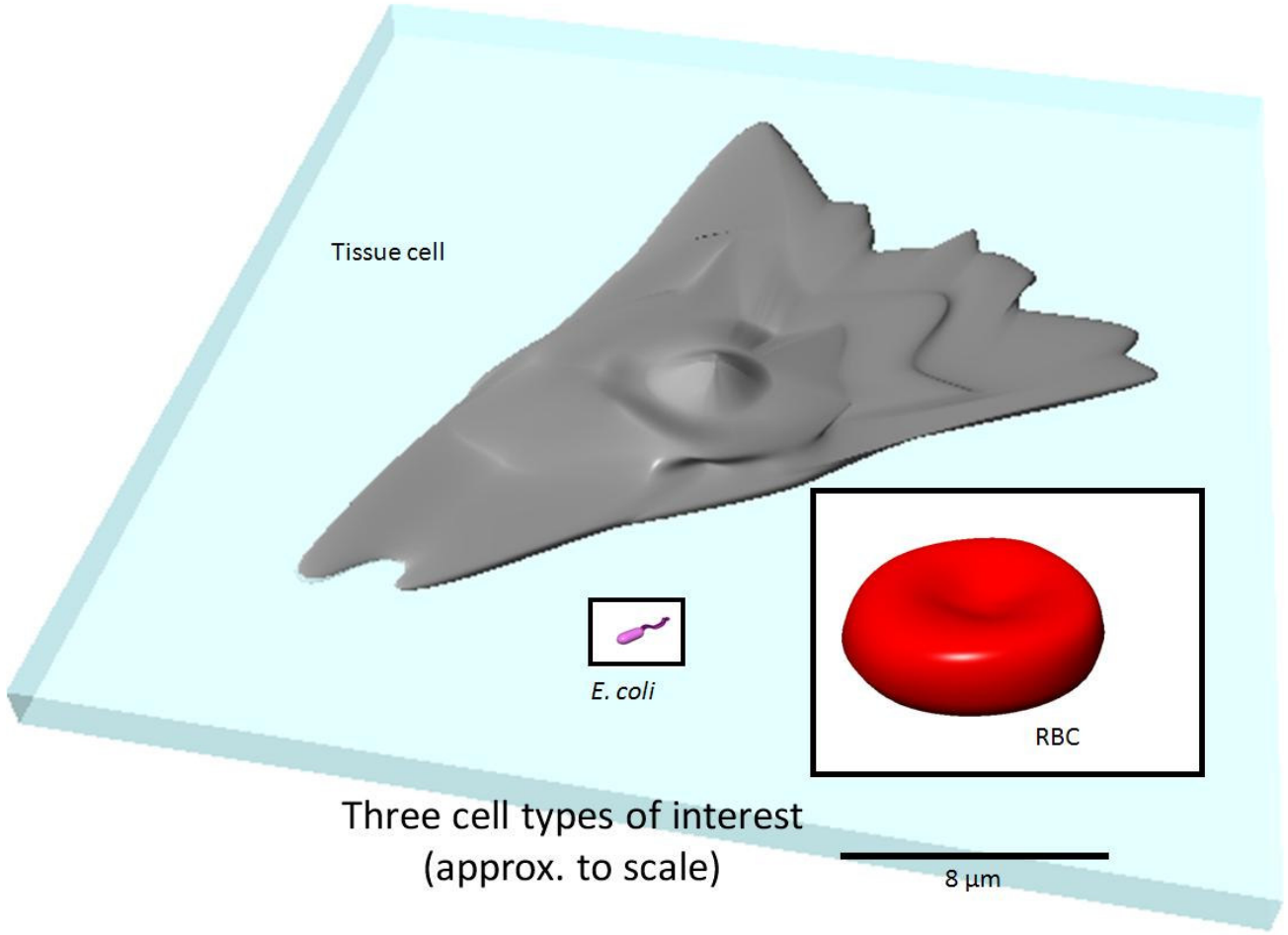
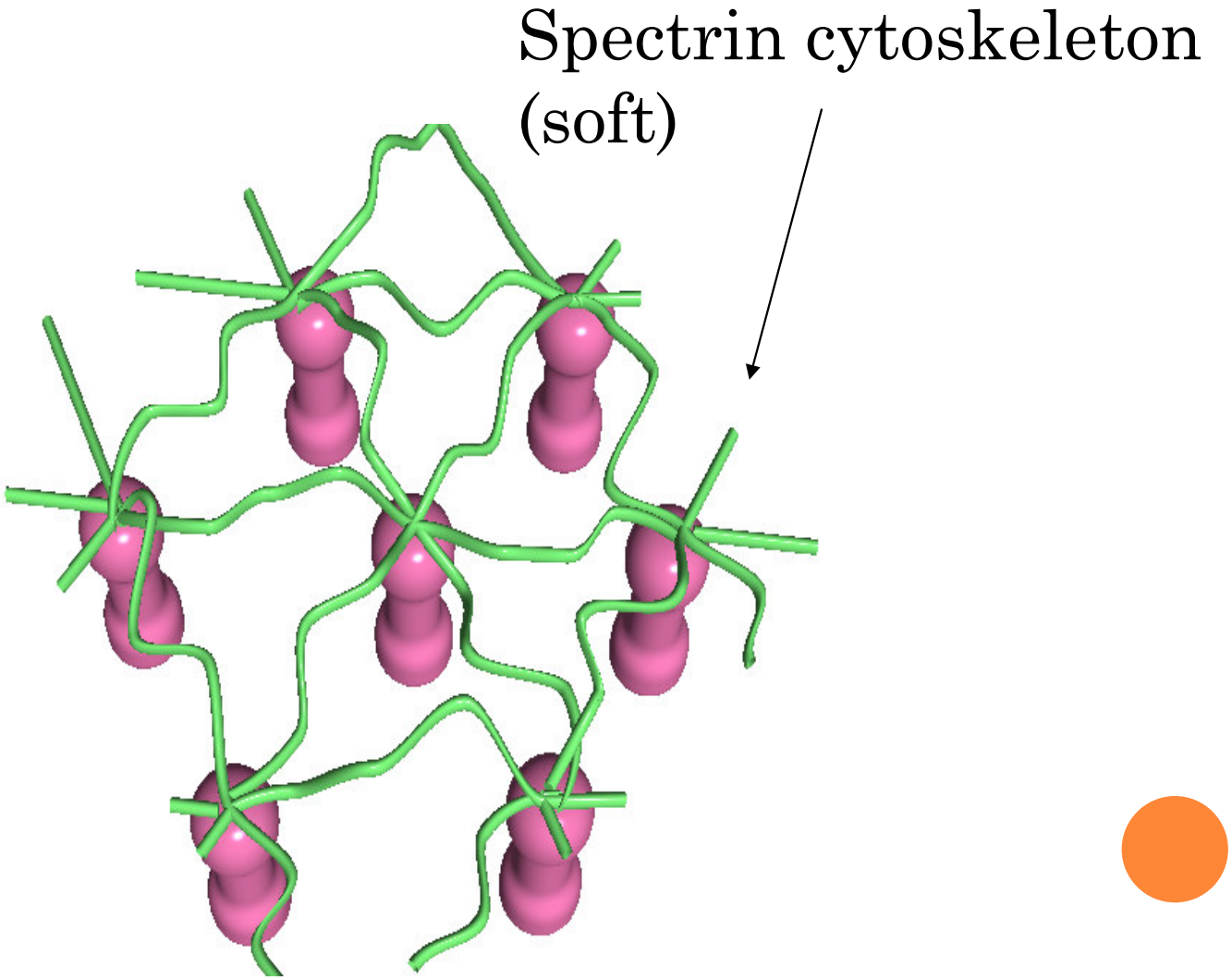
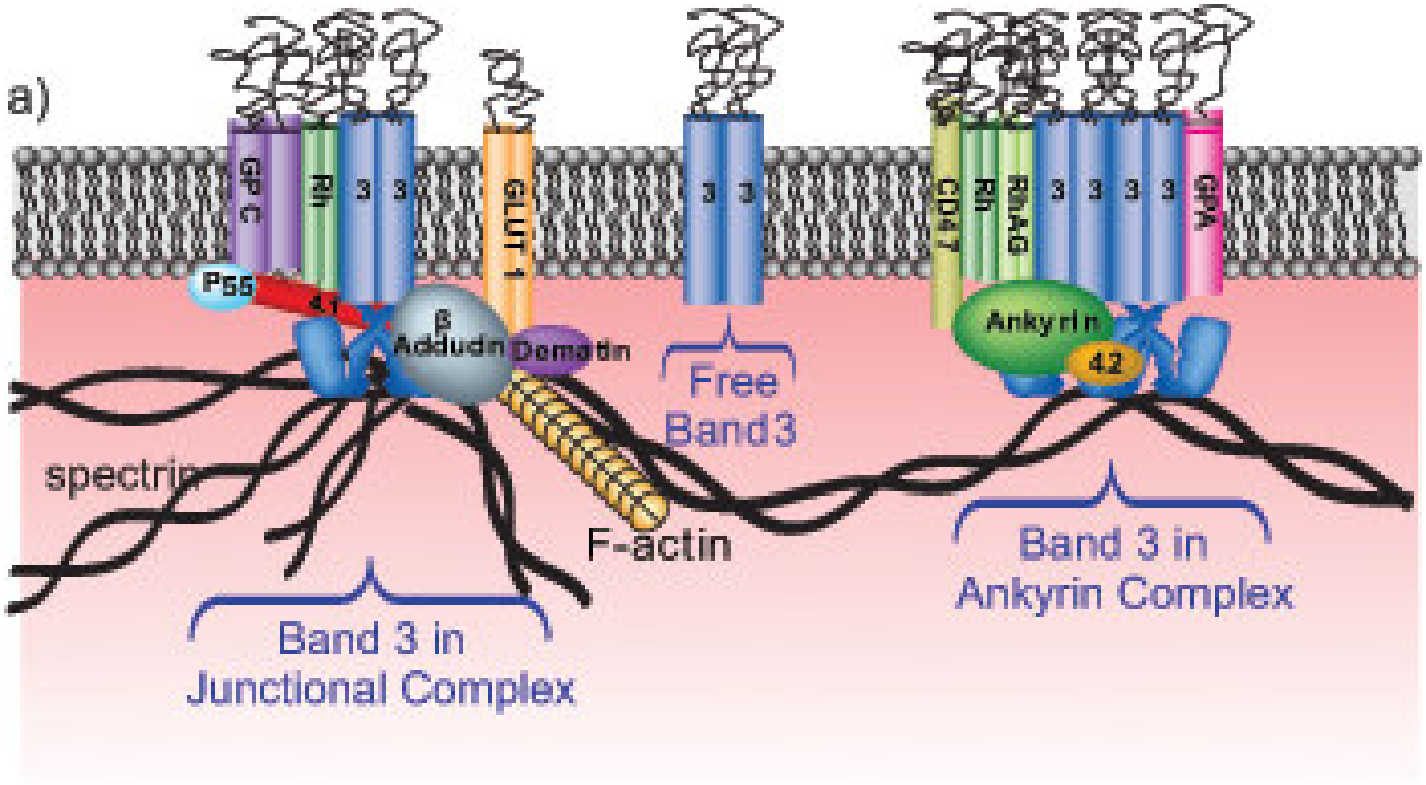
TYPES OF CELLS



RED BLOOD CELL PLASMA MEMBRANE



POTENTIAL USE

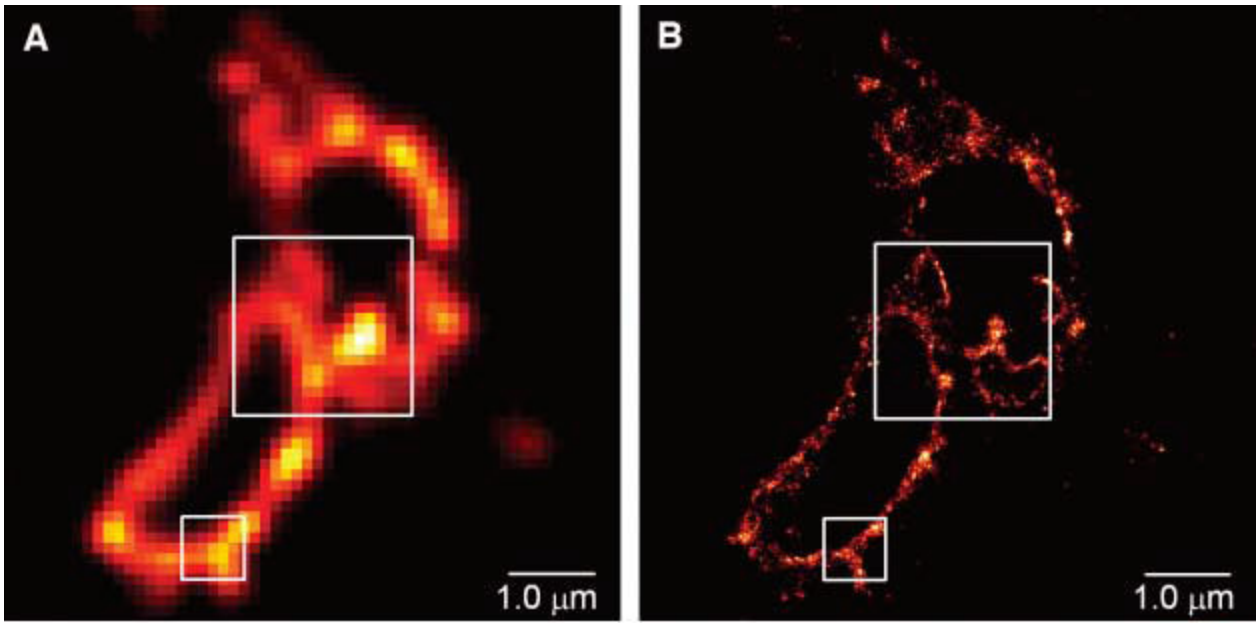


PURPOSE OF STUDY

- Cells are made of many small proteins
- High energy photons denature proteins
- High energy photons are necessary to see finer structures but will kill the cell!
- Using super resolution one can still see these small structures

WHAT IS SUPER RESOLUTION?

It is when one resolves past the diffraction limit of an image.



An example of super resolution achieved by the PALM method.

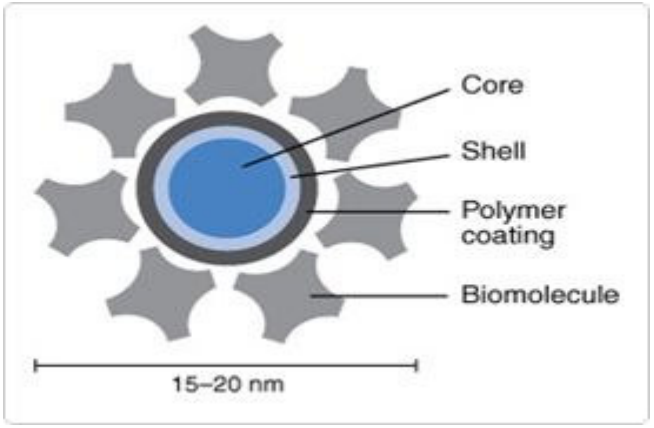
HOW DOES ONE GET SUPER RESOLUTION?

- Requires fluorescent material and a series of the same image
- The material must “photobleach”
- Perform some statistical analysis and recreate the image

WHAT ARE QUANTUM DOTS?

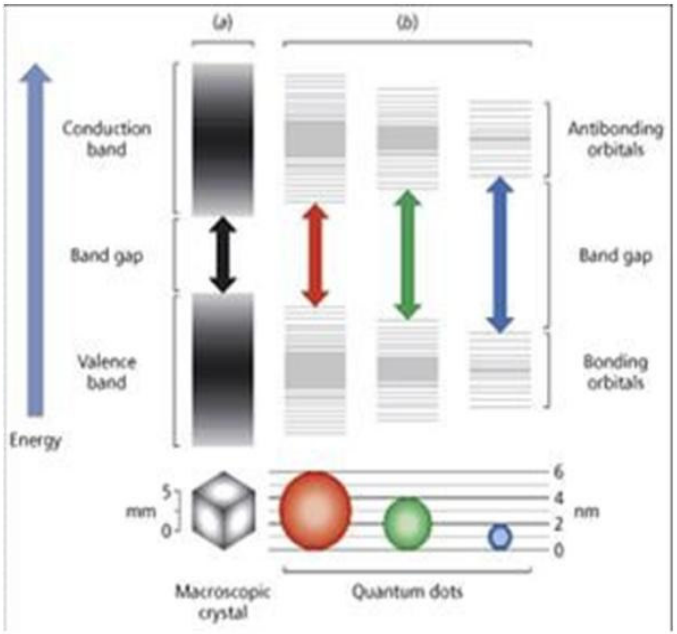
Quantum Dots (QD's) are generally made of four shells:

1. A semiconductor core usually made of CdSe
2. A semiconductor shell usually made of ZnS
3. A polymer wrapper
4. A biologically important molecule



WHY DO QD'S FLUORESCE?

- The cores are semiconductors!
- When the electron falls back to the valence band the loss in energy results in a photon emission.
- Note that the band gap is dependent on the size of the semiconductor. This allows for the quantum dot to be tuned to emit in certain wavelengths.

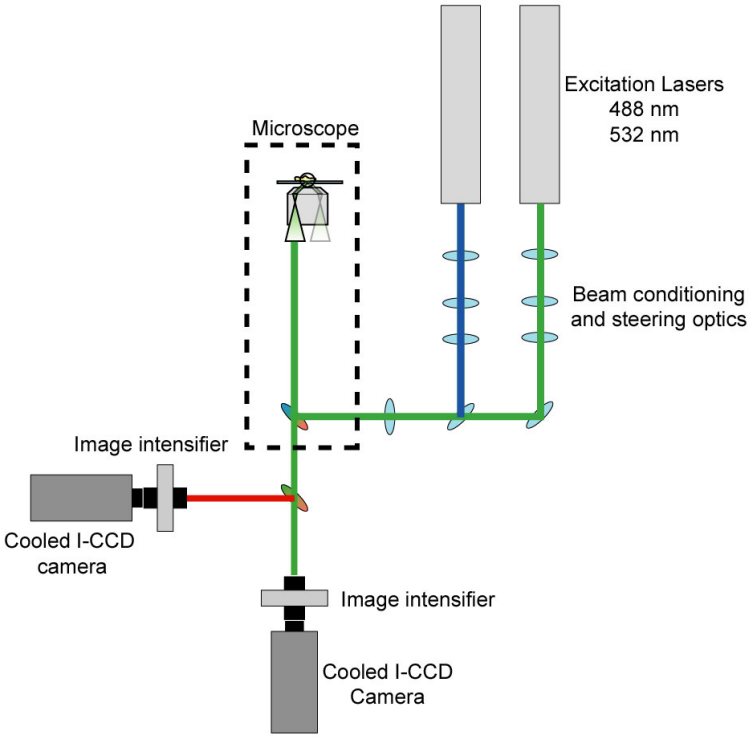


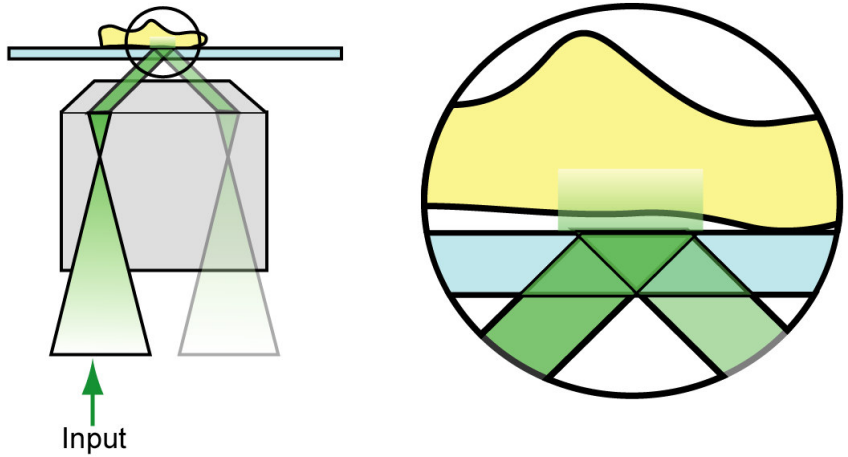
WHY DO QD'S BLINK?

- Normally the electrons will fall back to the valence band and emit a photon
- Sometimes the electrons become trapped in a surface potential and cannot fall back to the valence shell

DATA GATHERING

Objective-type total internal reflection microscope





Evanescent field illuminates only about 100 nm beyond the coverslip
- to reduce background due to excess fluorophores in solution and auto-fluorescence from the cell

HOW TO ACHIEVE SUPER RESOLUTION

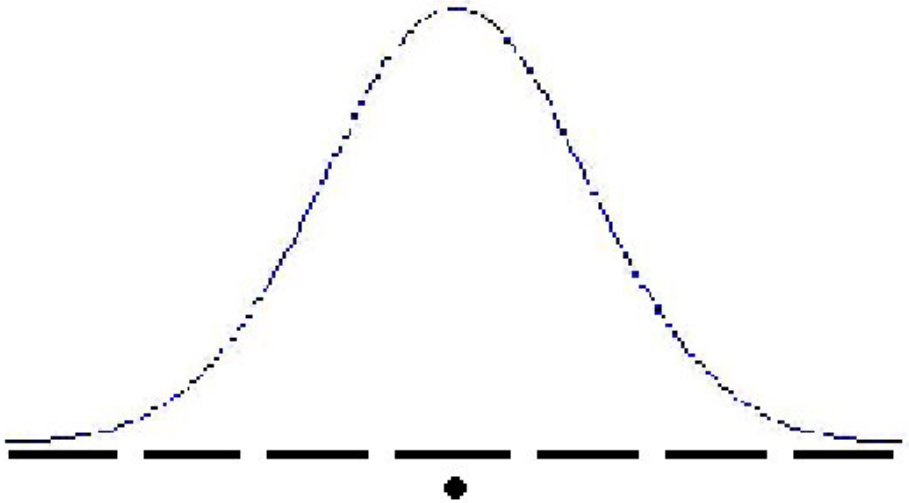
- Statistical analysis
- Independent Component Analysis
  - Not a viable method
  - Did not give any more information

STATISTICS FOR SUCCESS

- A single quantum dot will have an intensity between 0 and 1
- Two quantum dots will have an intensity between 0 and 2
- Since the dots blink independently one should see three discrete states at 0, 1, and 2

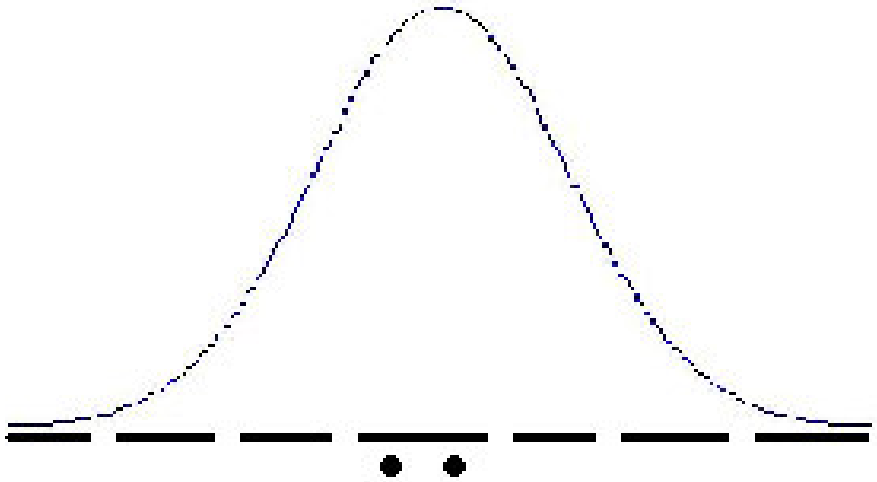
STATISTICS OF INSTENSITIES

- Like a flashlight a quantum dot will have a higher intensity at the center of the spot than at the edges



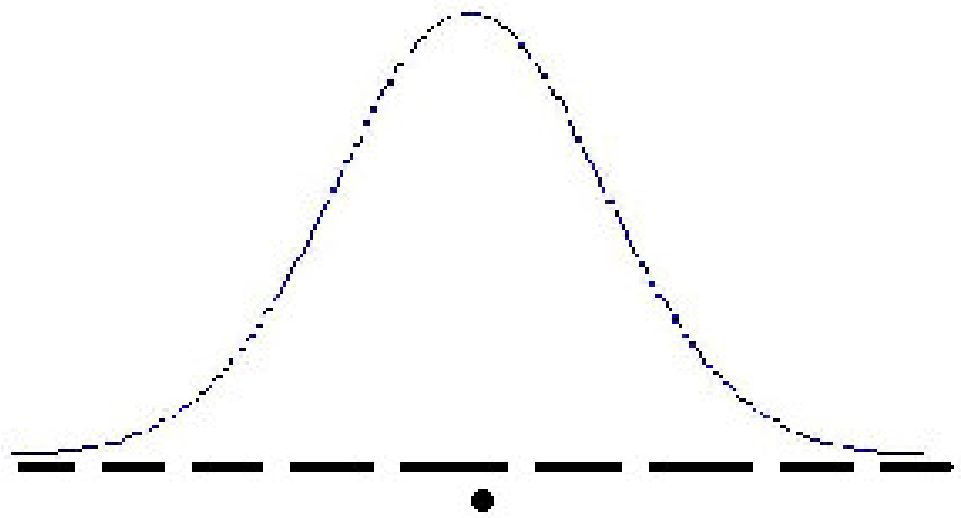
INTENSITIES OF MULTIPLE SOURCES

- If two dots occupy the same pixel one can approximate with a single dot that has double the intensity



INTENSITIES OF BLINKING SOURCES

- Since the QDs blink the intensity curve will be shifted slightly to the side of the “on” dot



GAUSSIAN FITS TO THE RESCUE

- Fitting Gaussians to the intensity profile of a spot will yield the location of the quantum dot
- This is done independently for the x and y coordinates of the image
- Also done independently for every frame

MAJOR OBSTACLE

- What does a single QD look like?
- Any group of multiple QDs can exhibit the three state intensity curve
- How can one be sure that the image is of two quantum dots

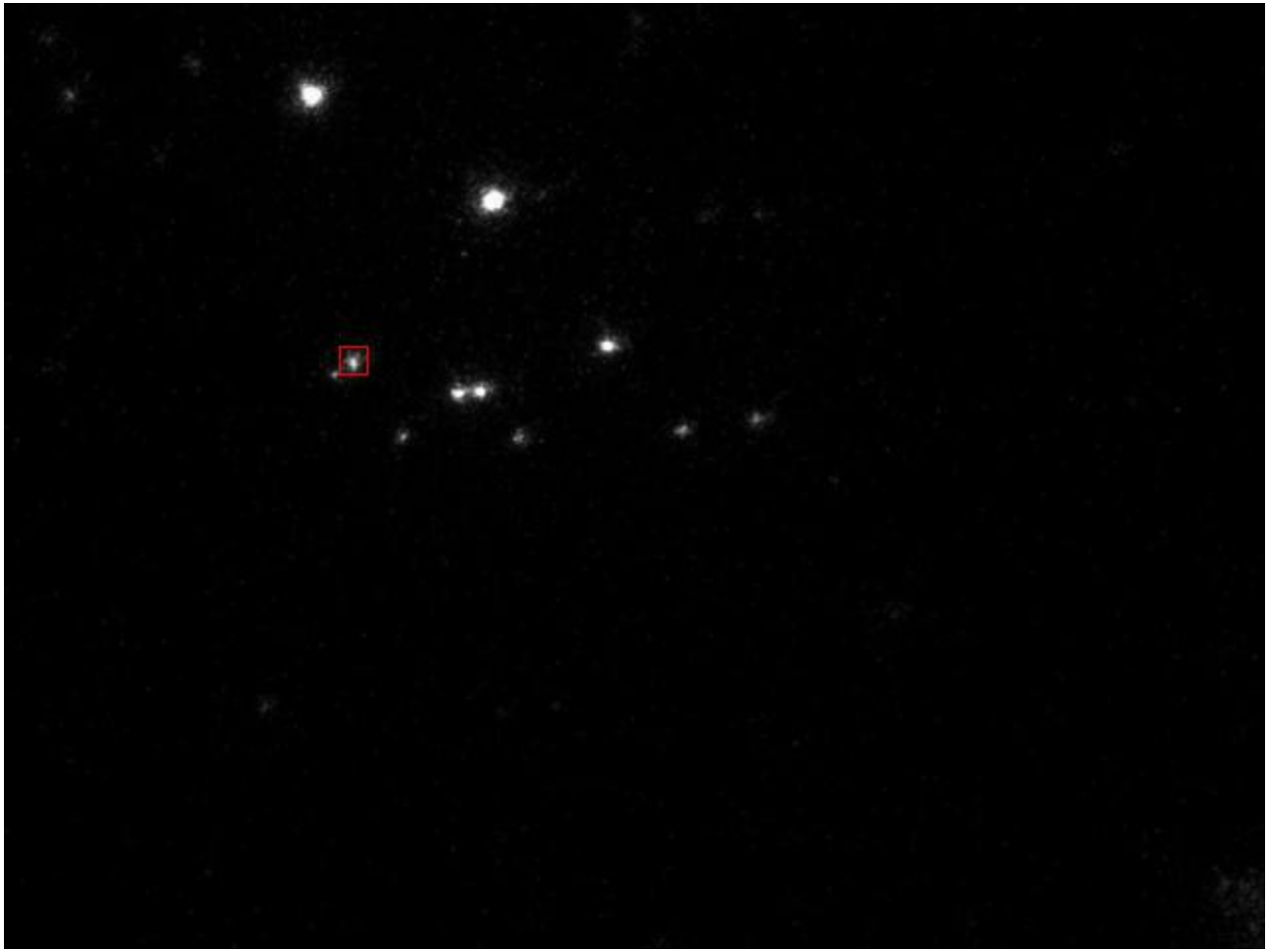
SOLUTION – STUDY THE SINGLE QD

- Take several videos of just QDs at a certain gain
- Put all of the intensity values into a single histogram
- Fit two Gaussians to the histograms to get the approximate QD intensity
- Filter the original set for:
  - Dots that are not on very much
  - Dots that are generally brighter than the approximate one QD
- Result: At a gain of 1.552 the single dot should be about 60 (according to the analysis program)

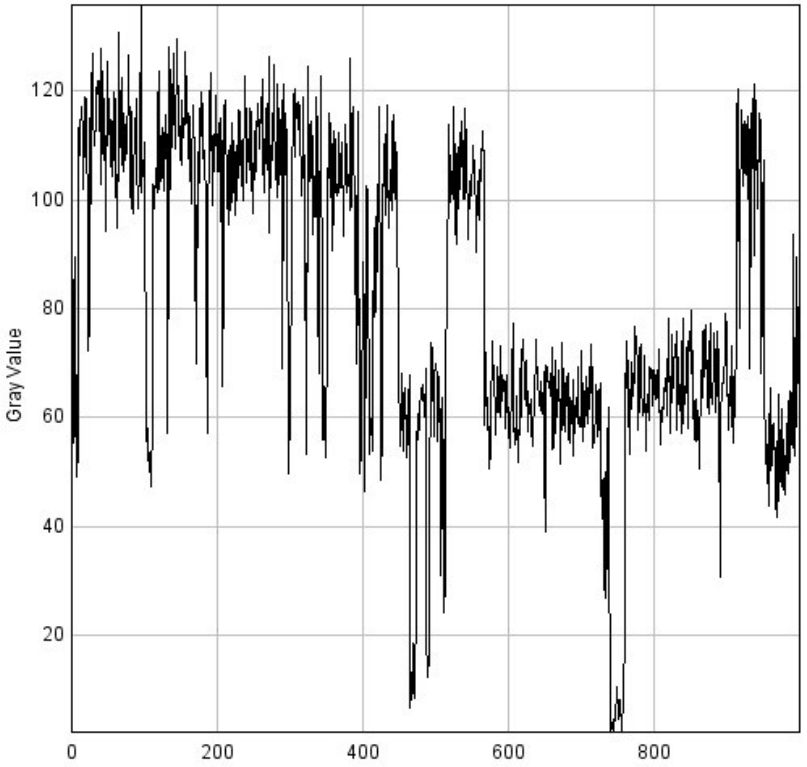
TESTING FOR SUPER RESOLUTION

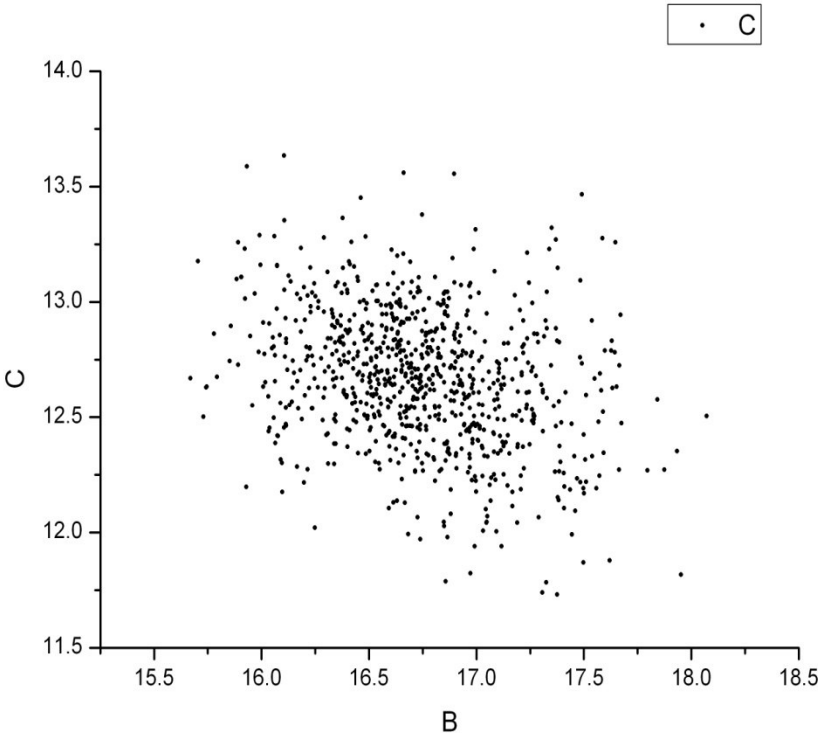
- A single pixel is about 75nm with the set-up
- 70nm DNA was cut and QDs were attached to the ends

RESULTS – THE RAW DATA

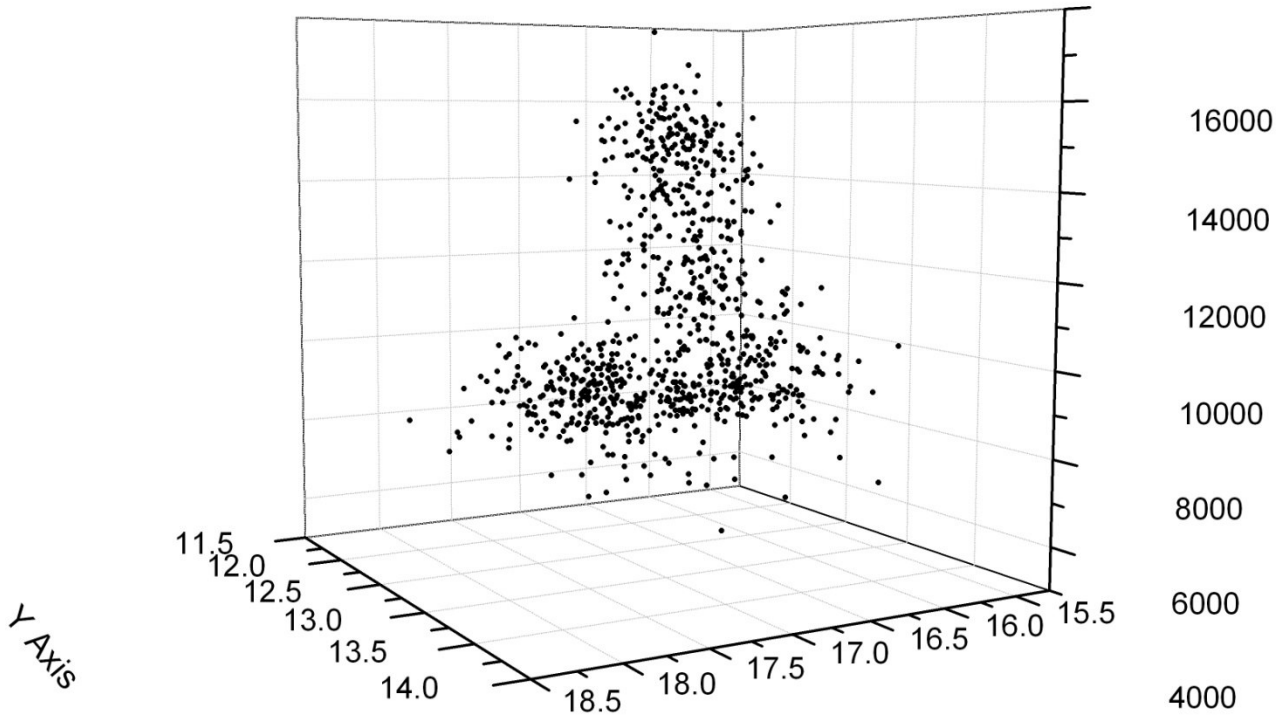


RESULTS – ANALYSIS I





RESULTS – ANALYSIS II

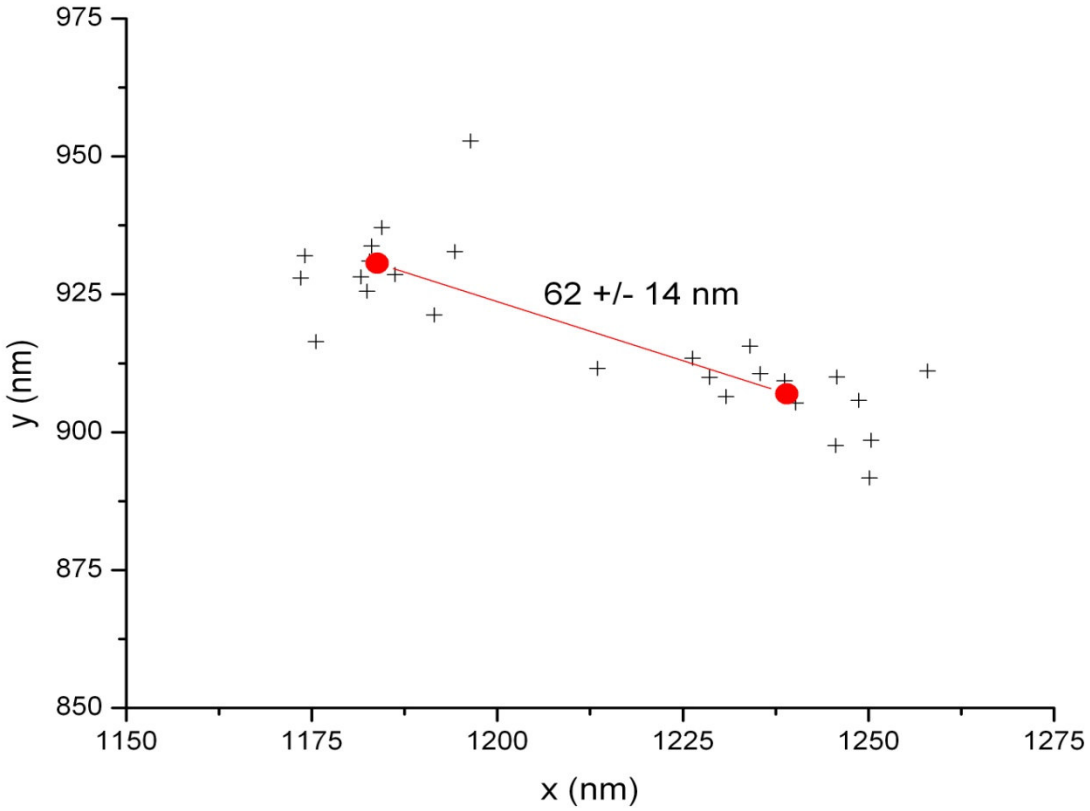


HOW TO FILTER DOUBLES

- Use a tracking program on just the double
- Fit two Gaussians to the intensity data from it
- Find all slices within one standard deviation of the lower Gaussian
- Average the X and Y centers of the remaining contiguous pieces
- Plot the new list of centers

FINAL RESULTS

Success!!



FINAL THOUGHTS

- QDs for super resolution is better suited for large scale structures like the spectrin cytoskeleton

ACKNOWLEDGEMENTS

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- Dr. Jeff Spector
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