Super-resolution Fluorescence Microscopy

STORM
PALM
Stochastic Photoactivation

- If only one fluorophore is emitting light at a time within the diffraction volume, its position can be determined precisely.
- To be able to image closely separated identical probes, one needs to selectively activate and photobleach fluorophores.
Stochastic Optical Reconstruction Microscopy (STORM)

- Red light excites Cy5, which fluoresces and then photobleaches (dark state).
- Green light reactivates Cy5 if it is in close proximity to Cy3.
- This cycle can be repeated hundreds of times.
RecA-coated circular plasmid DNA
3D STORM

A

Objective

Cylindrical Lens

Imaging Lens

EMCCD

B

\[ w_y \]

\[ w_x \]

Width (nm)

z (nm)

C

Number of Points

z (nm)

x (nm)

y (nm)

0 100 200 300 400 500 600

x (nm)

y (nm)

z (nm)
Microtubule Imaging in 3D

[Caption: Images showing microtubules in 3D with scale bars at 5 μm and 200 nm. Graph showing distribution of points along the z-axis with a peak at 102 nm.]
Multicolor STORM

A

CyDye fluorescence (A.U.)

0  5  10  15  20

Activation pulses

Cy3 - Cy5

Cy3 - Cy5.5

Cy3 - Cy7

Time (s)

Cy5 fluorescence (A.U.)

0  5  10  15  20

Activation pulses

Cy3 - Cy5

Cy3 - Cy5

Cy2 - Cy5

Time (s)

A

Alexa405 / Cy5

Cy2 / Cy5

Cy3 / Cy5

250 nm

B

25 nm

C

25 nm
Photoactivated Light Microscopy (PALM)
A sparse subset of PA-FP molecules that are attached to proteins of interest and then fixed within a cell are activated (A and B) with a brief laser pulse at $\lambda_{\text{act}} = 405$ nm and then imaged at $\lambda_{\text{exc}} = 561$ nm until most are bleached (C).

This process is repeated many times (C and D).

Summing the molecular images across all frames (E and F).

If the location of each molecule is first determined, the molecule can be plotted as a Gaussian that has a standard deviation equal to the uncertainty $\sigma_{x,y}$.

Repeating with all molecules across all frames (A' through D') and summing the results yields a superresolution image (E' and F').