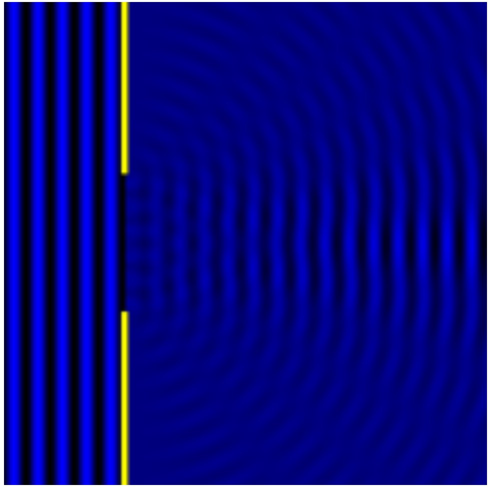
Superresolution fluorescence microscopy

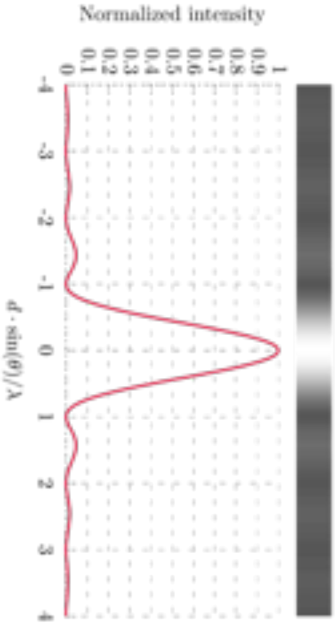
Leonid Keselman, Daniel Fernandes

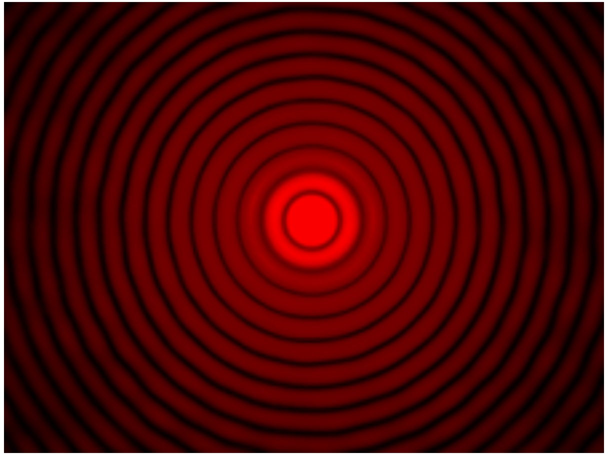
Overview

1. What is “super-resolution”
    a. Diffraction
    b. STORM
2. Compressed Sensing
    a. Applied to STORM
3. Light Sheet Imaging
    a. Lattice-Light Sheets

Natural Resolution Limits: Diffraction





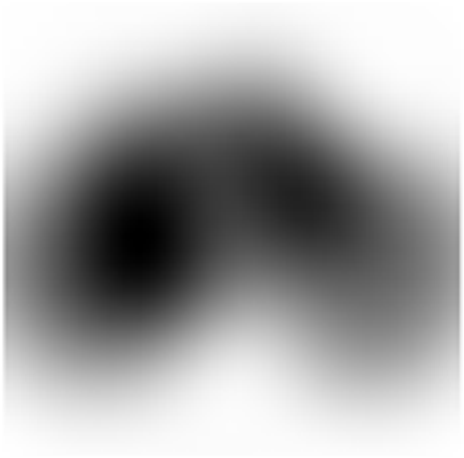


sources: Wikipedia (6wavelength=slitwidthblue.gif, Single_Slit_Diffraction_(english).svg, Beugungsscheibchen.k.720.jpg)

Natural Resolution Limits: Diffraction

*

=



sources: font-awesome fa-bicycle

Natural Resolution Limits: Diffraction

- For typical cameras

$$d = 1.22 * \lambda * f\#$$

Raleigh Criterion

iPhone 7:
=1.22 * 650nm * f/1.8
=1.4 μm
pixels are only 1.22 μm!

For microscopes

$$d = \frac{\lambda}{2n \sin \theta} = \frac{\lambda}{2\, NA}$$

Abbe diffraction limit

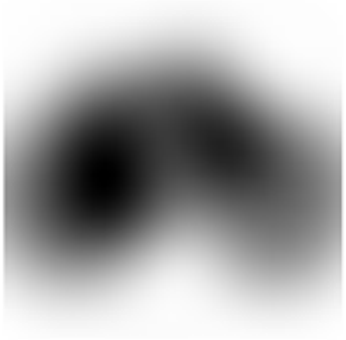
Typical Limit:
= 500nm/(2 * 1.25)
= 0.2 μm = 200nm
Microtubules are ~24nm

NA is typically 0.1-0.4 for common lenses in air, up to 1.0-1.5 for oil lenses.

STORM: Stochastic Optical Reconstruction Microscopy

Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature Methods* 3.10 (2006)

All pixels “on”:



Random 1% of pixels “on”

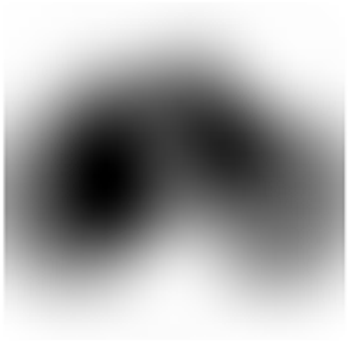
Random 1% of pixels “on”

Random 1% of pixels “on”

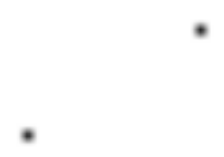
STORM: Stochastic Optical Reconstruction Microscopy

Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature Methods* 3.10 (2006)

All pixels “on”:



1 reading

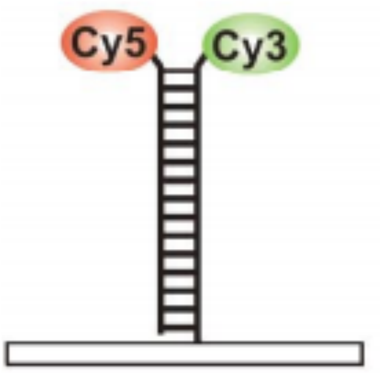


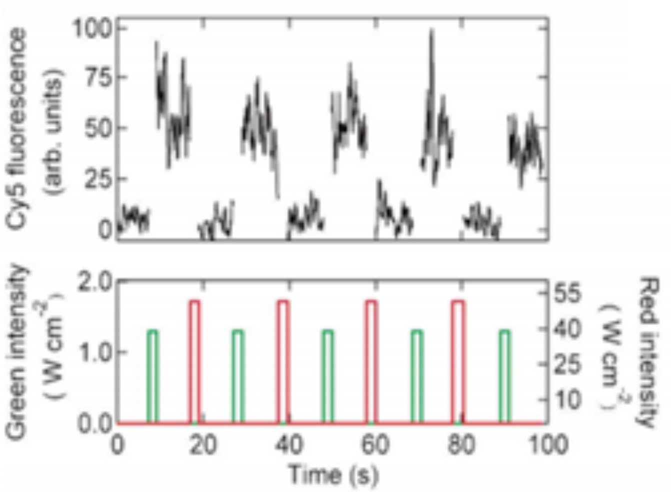
100 readings

1000 readings

STORM: Stochastic Optical Reconstruction Microscopy

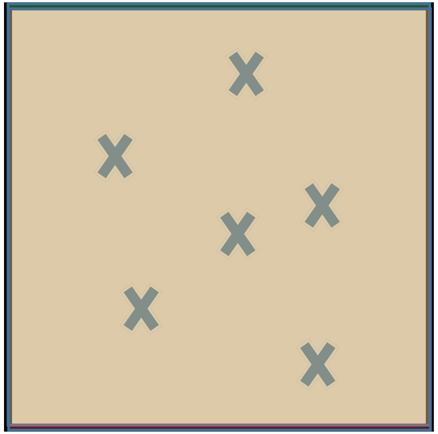
Bates, Blosser, Zhuang. "Short-range spectroscopic ruler based on a single-molecule optical switch." *Physical review letters* 94.10 (2005)





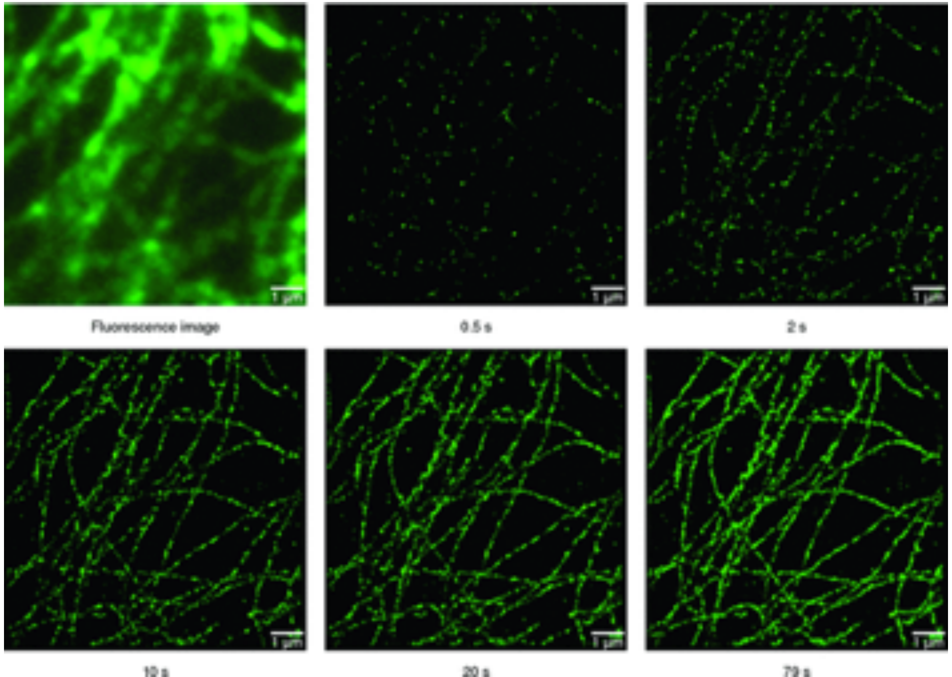
STORM: Stochastic Optical Reconstruction Microscopy

Bates, Blosser, Zhuang. "Short-range spectroscopic ruler based on a single-molecule optical switch." *Physical review letters* 94.10 (2005)



STORM: Stochastic Optical Reconstruction Microscopy

Wolter, Steve, et al. "Real-time computation of subdiffraction-resolution fluorescence images." *Journal of microscopy* 237.1 (2010)



Compressed Sensing (a.k.a. Sparse Sampling)

If your data is “compressible”, you can take just a handful of random measurements, and, using “simple” math, you can reconstruct your data (with minimal error and high probability)

Emmanuel Candes and Terence Tao. "Near-optimal signal recovery from random projections: Universal encoding strategies?." *arXiv:math/0410542* (2004)

$$\min \ \|x\|_{\ell_1} \quad \text{subject to} \quad \|Ax - y\|_{\ell_2} \leq \epsilon.$$

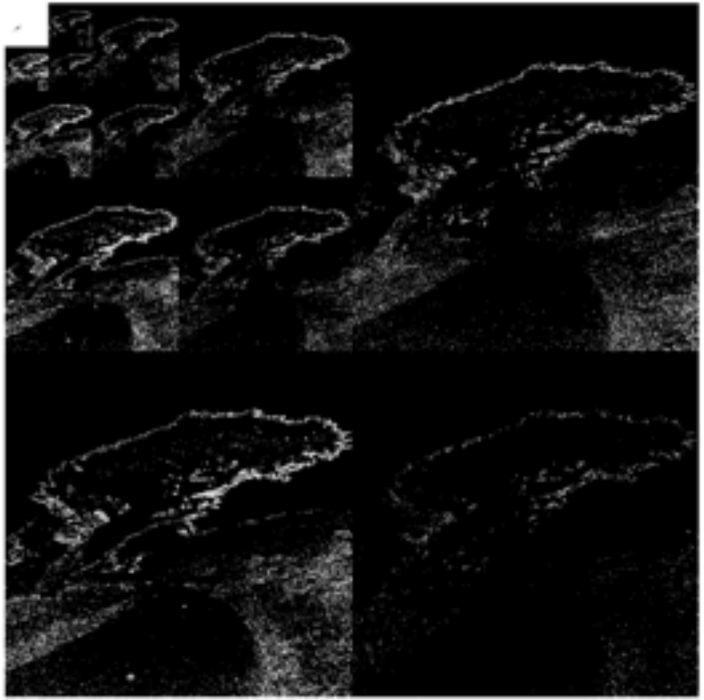
Compressed Sensing (a.k.a. Sparse Sampling)

	1	2	3	4	5	6
1	X	X		X		
0		X	X	X		
2	X			X		X

Compressed Sensing (a.k.a. Sparse Sampling)

	1	2	3	4	5	6
1	X	O		O		
0		O	O	O		
2	X			O		X

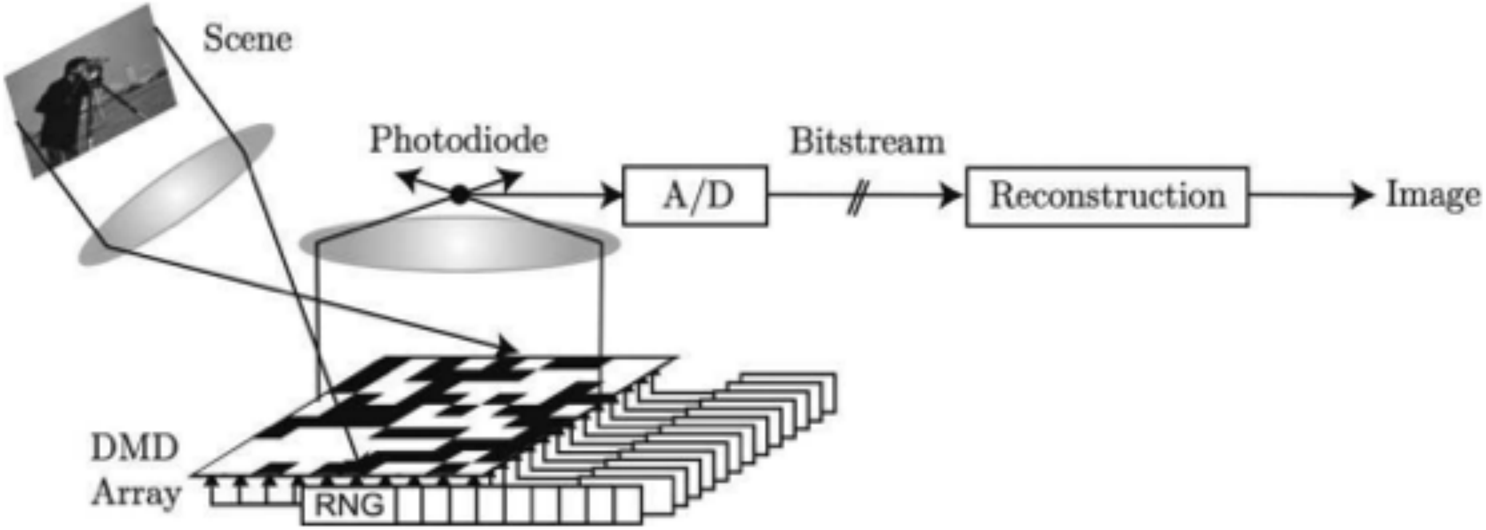
Compressed Sensing (a.k.a. Sparse Sampling)



Davenport, Duarte, Eldar, Kutynoik , *Introduction to Compressed Sensing*

Compressed Sensing

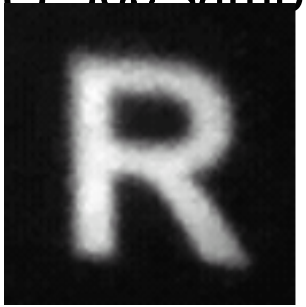
Duarte, et al. *Single-Pixel Imaging via Compressive Sampling*. (2008)



Compressed Sensing

Real Picture
(65,536

CS
Reconstruction
(3,300 samples)



CS
Reconstruction
(1,300 samples)

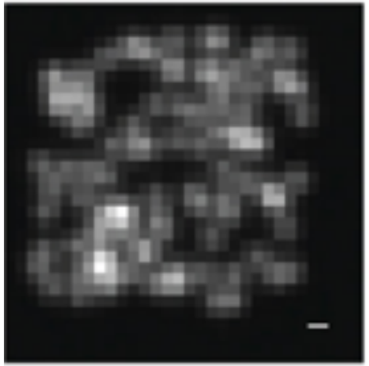
CS
Reconstruction
(6,500 samples)

Faster STORM using compressed sensing

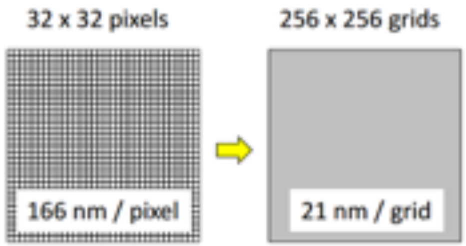
Zhu, et al."Faster STORM using compressed sensing." *Nature Methods* (2012)

1. Acquire PSF

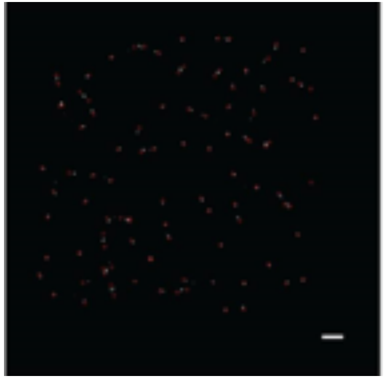
2. Get Image



3. Increase Grid



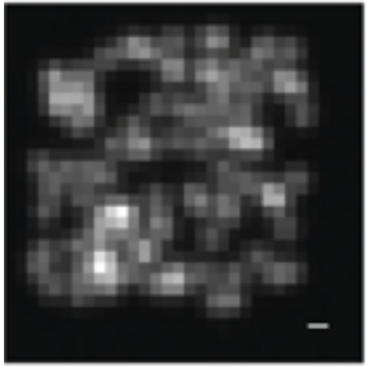
4. Solve CS problem



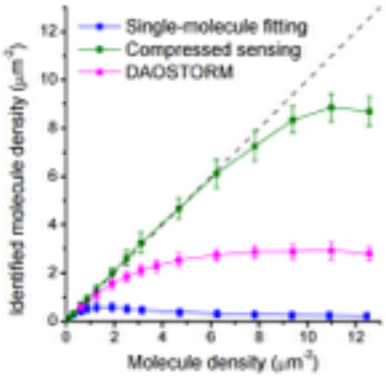
Faster STORM using compressed sensing

Zhu, et al."Faster STORM using compressed sensing." *Nature Methods* (2012)

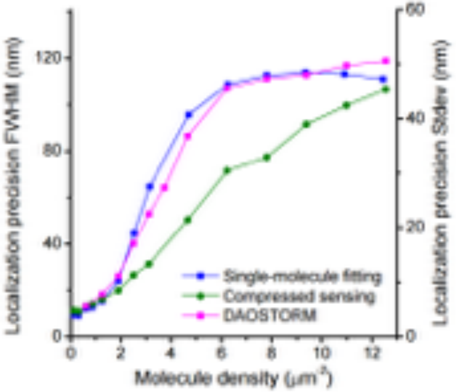
Denser Images!



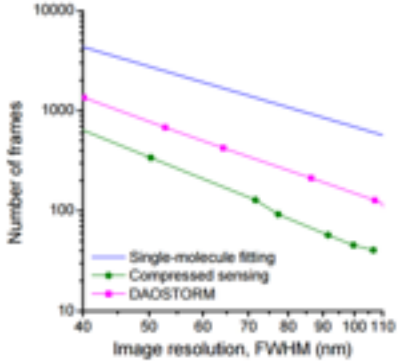
Many times denser



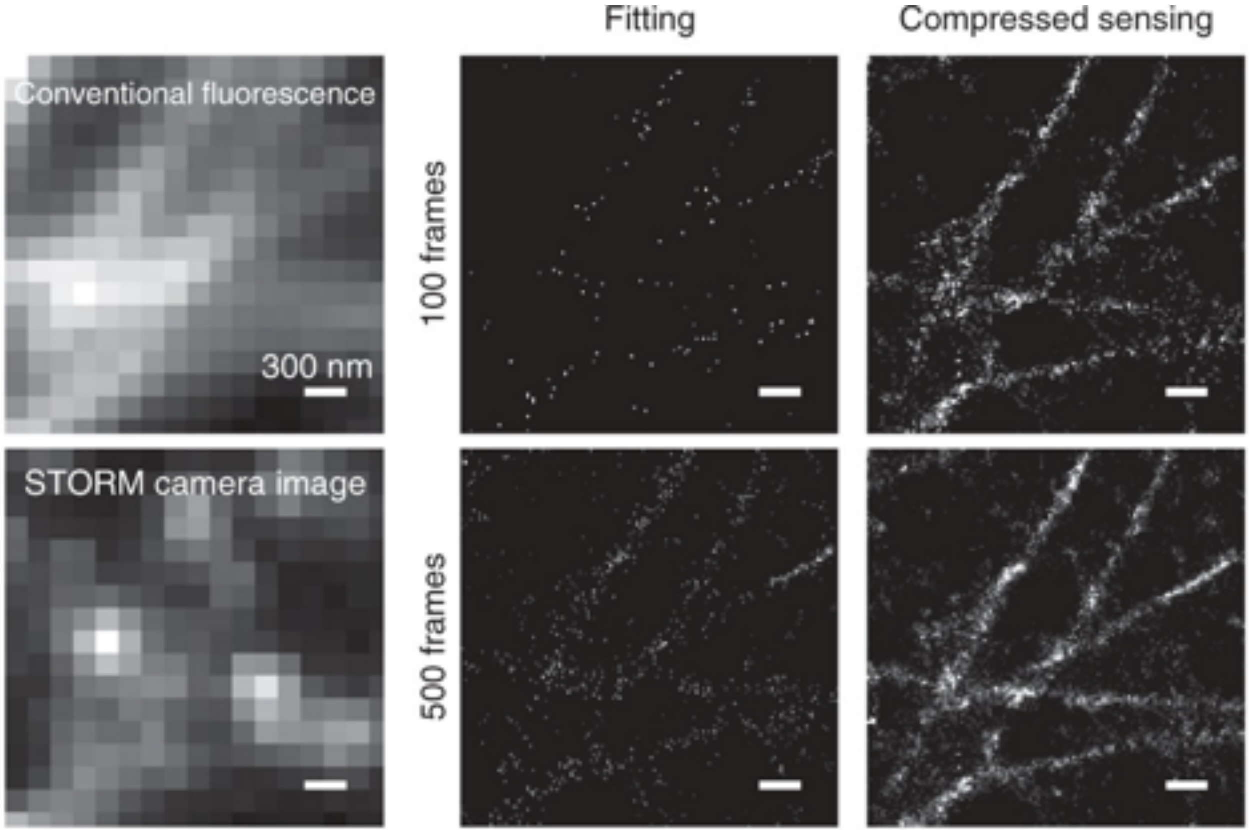
More precise

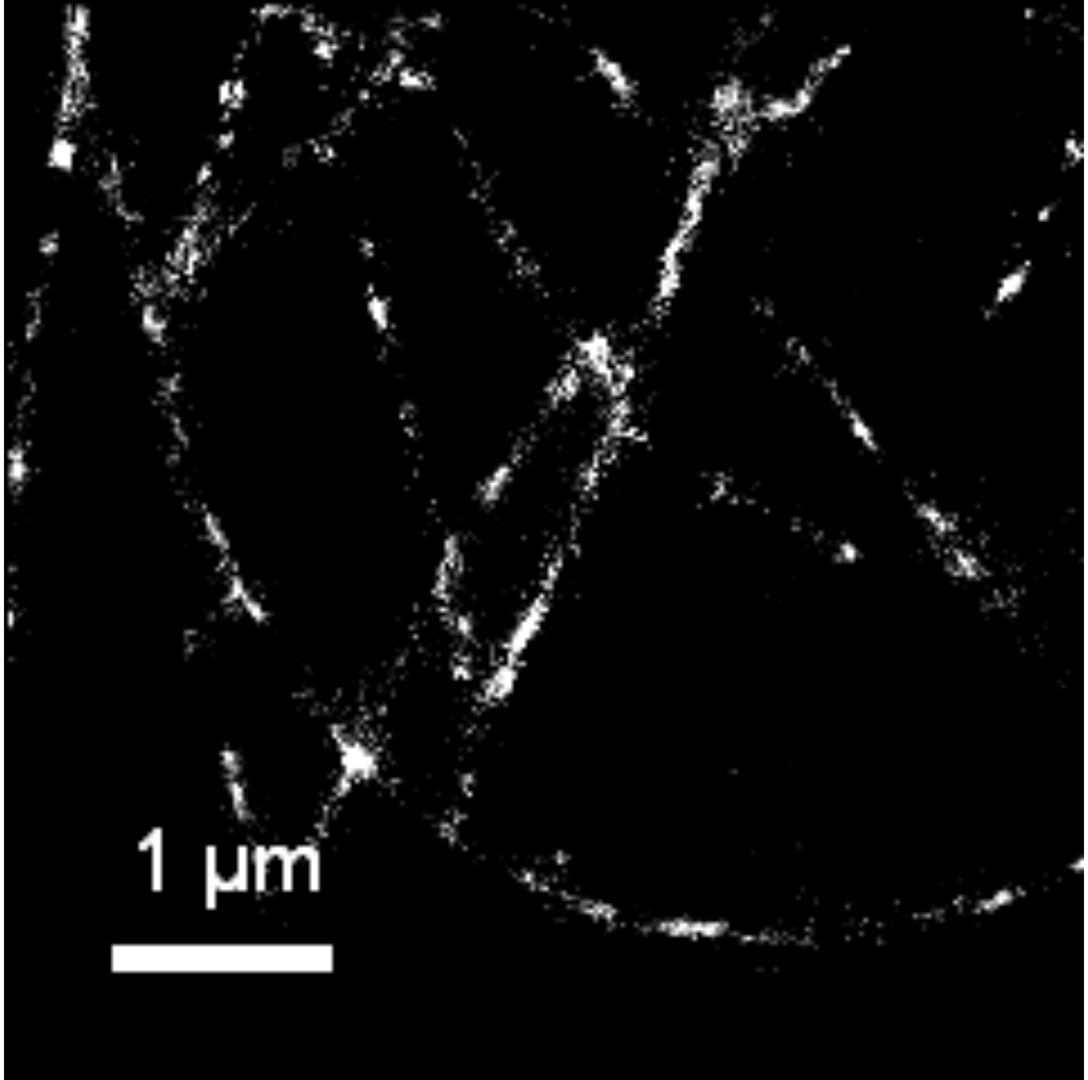


Faster imaging



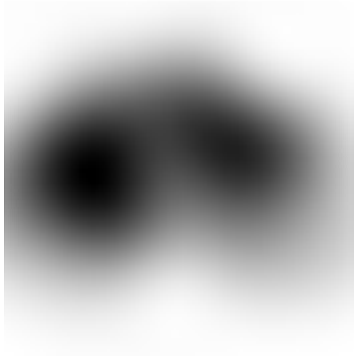
Faster STORM using compressed sensing



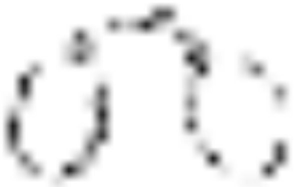

1 µm

Faster STORM using compressed sensing

40% pixels on



40% on, CS Solve



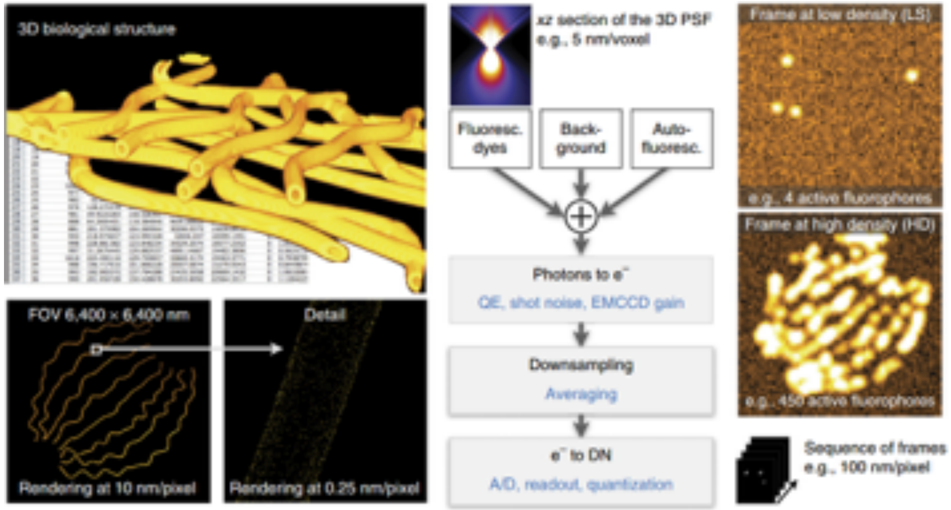
CS
50 readings
4% Density

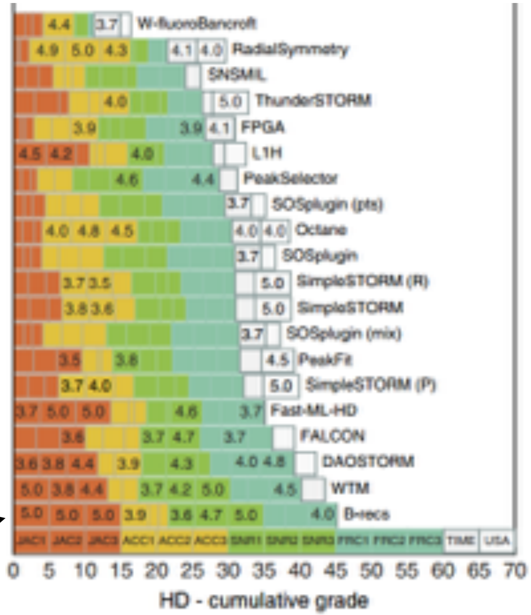
Classic
1000 readings
~0.8% Density

https://github.com/leonidk/cs371

Quantitative Comparison

Sage, Daniel, et al. "Quantitative evaluation of software packages for single-molecule localization microscopy." *Nature Methods* 12.8 (2015)



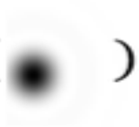


https://github.com/hrouault/Brecs

Extra Slides

Faster STORM using compressed sensing

Solve

$w_0($  $) + \cdots + w_{205}($  $) + \cdots + w_{819}($  $) + \cdots =$ 

With

$$\min \|\mathbf{w}\|_1$$

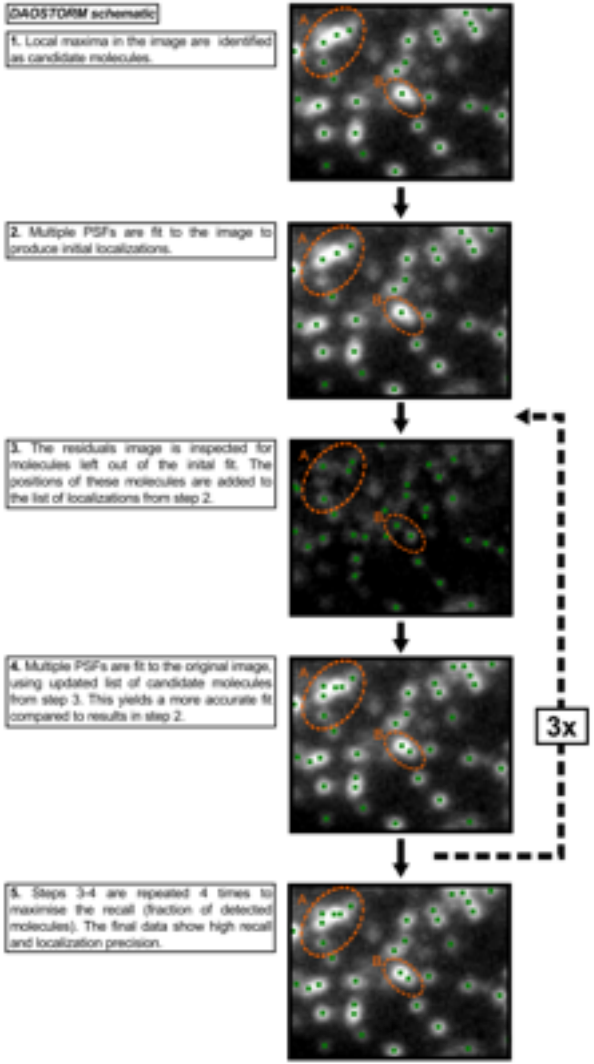
Gives

$\mathbf{w} \in \mathbb{R}^{1024} \;\Rightarrow\; \mathbf{w} \in \mathbb{R}^{32x32}$

$\mathbf{w} =$ 

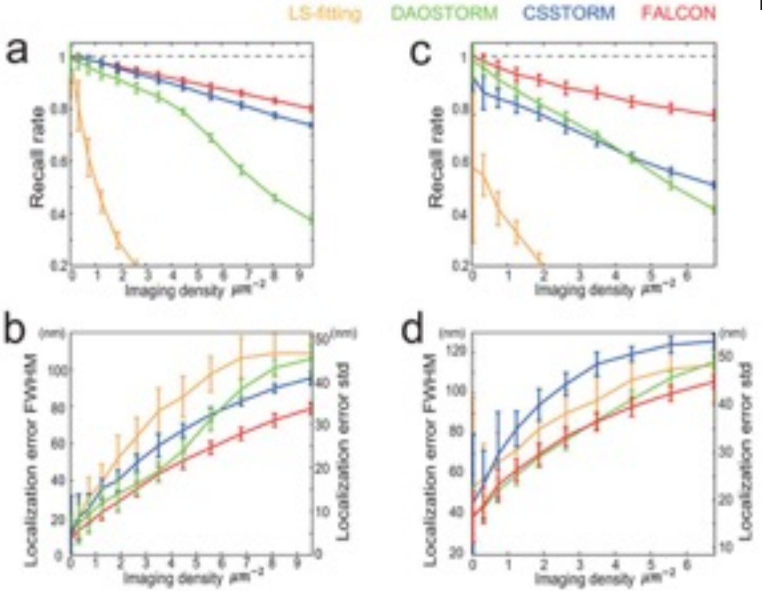
DAOSTORM

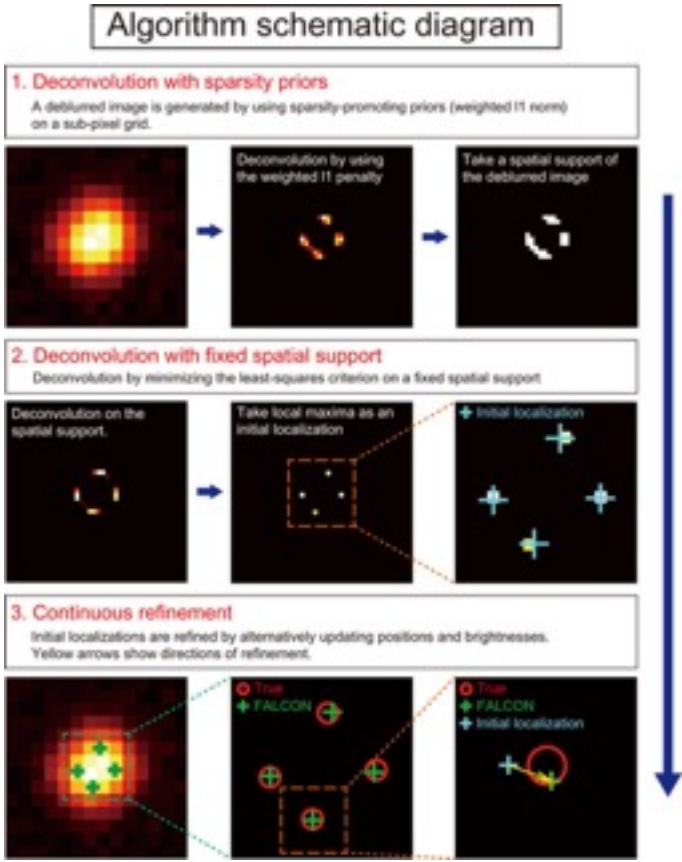
Stetson, Peter B. "DAOPHOT: A computer program for crowded-field stellar photometry." *Publications of the Astronomical Society of the Pacific* 99.613 (1987).



FALCON

Min, Junhong, et al. "FALCON: fast and unbiased reconstruction of high-density super-resolution microscopy data." *Scientific reports* 4 (2014)





Supplementary Figure: Switching kinetics

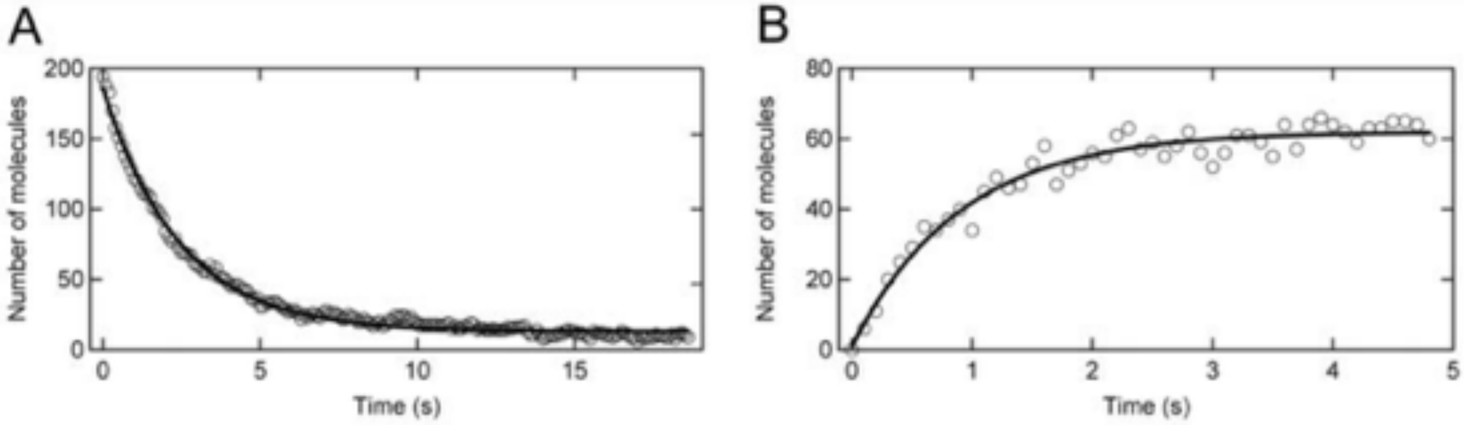


Figure S1. The first-order switching kinetics of the molecular switch. **A**, The number of molecules remaining fluorescent as a function of time after the green laser was turned off. A single exponential fit of the data (solid line) gives $k_{off} = 0.4\ s^{-1}$. **B**, The number of molecules that were converted back to the fluorescent state as a function of time after the green laser was turned on. A single exponential fit (solid line) gives the observed rate constant for switching Cy5 on ($k_{on\_obs} = 1.1\ s^{-1}$). Considering the competing actions of the red and green lasers, the actual rate constant k_{on} for switching the dye on by the green laser is equal to $k_{on\_obs} - k_{off}$. Data in **A** and **B** are not from the same experiment.

Abstract

Suppose we are given a vector f in a class $\mathcal{F} \subset \mathbb{R}^N$, e.g. a class of digital signals or digital images. How many linear measurements do we need to make about f to be able to recover f to within precision ϵ in the Euclidean (ℓ_2) metric?

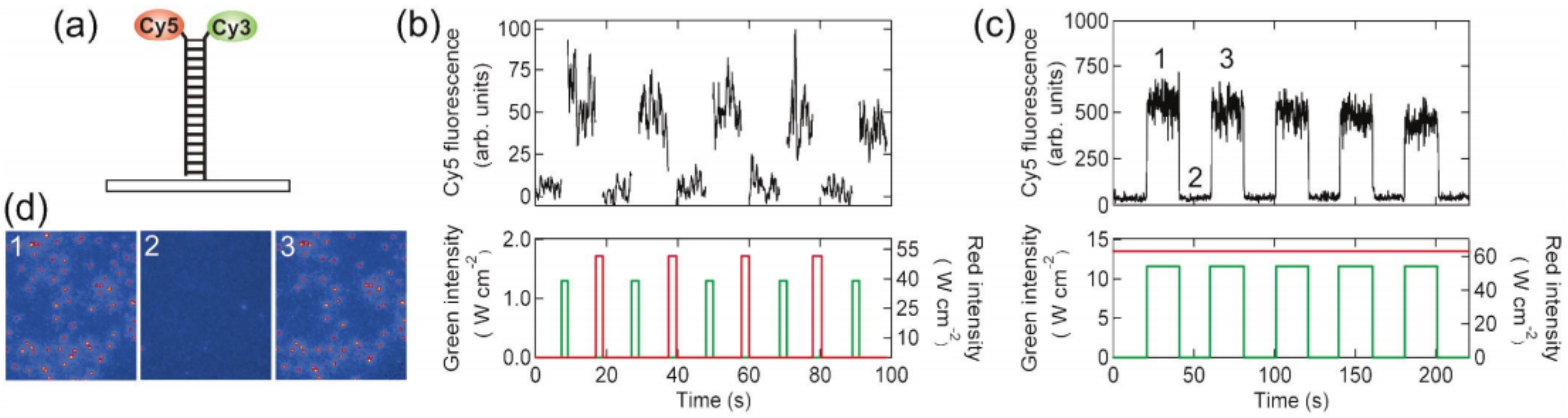
This paper shows that if the objects of interest are sparse in a fixed basis or compressible, then it is possible to reconstruct f to within very high accuracy from a small number of random measurements by solving a simple linear program. More precisely, suppose that the nth largest entry of the vector $|f|$ (or of its coefficients in a fixed basis) obeys $|f|_{(n)} \leq R \cdot n^{-1/p}$, where $R > 0$ and $p > 0$. Suppose that we take measurements $y_k = \langle f, X_k \rangle$, $k = 1, \ldots, K$, where the X_k are N-dimensional Gaussian vectors with independent standard normal entries. Then for each f obeying the decay estimate above for some $0 < p < 1$ and with overwhelming probability, our reconstruction $f^\sharp$, defined as the solution to the constraints $y_k = \langle f^\sharp, X_k \rangle$ with minimal ℓ_1 norm, obeys

$$\|f - f^\sharp\|_{\ell_2} \leq C_p \cdot R \cdot (K/\log N)^{-r}, \quad r = 1/p - 1/2.$$

There is a sense in which this result is optimal; it is generally impossible to obtain a higher accuracy from any set of K measurements whatsoever. The methodology extends to various other random measurement ensembles; for example, we show that similar results hold if one observes few randomly sampled Fourier coefficients of f. In fact, the results are quite general and require only two hypotheses on the measurement ensemble which are detailed.

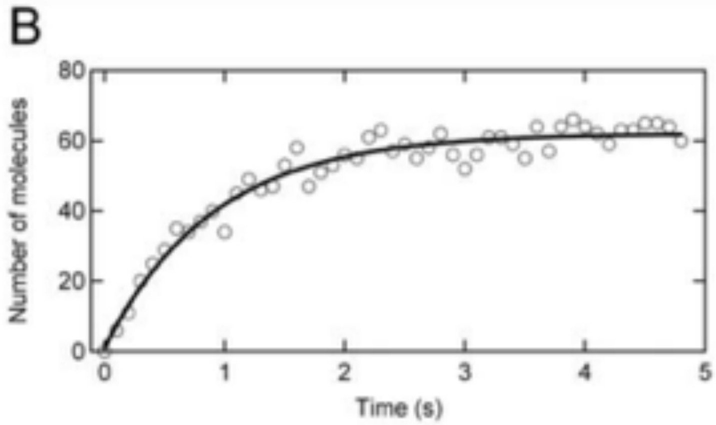
STORM: Stochastic Optical Reconstruction Microscopy

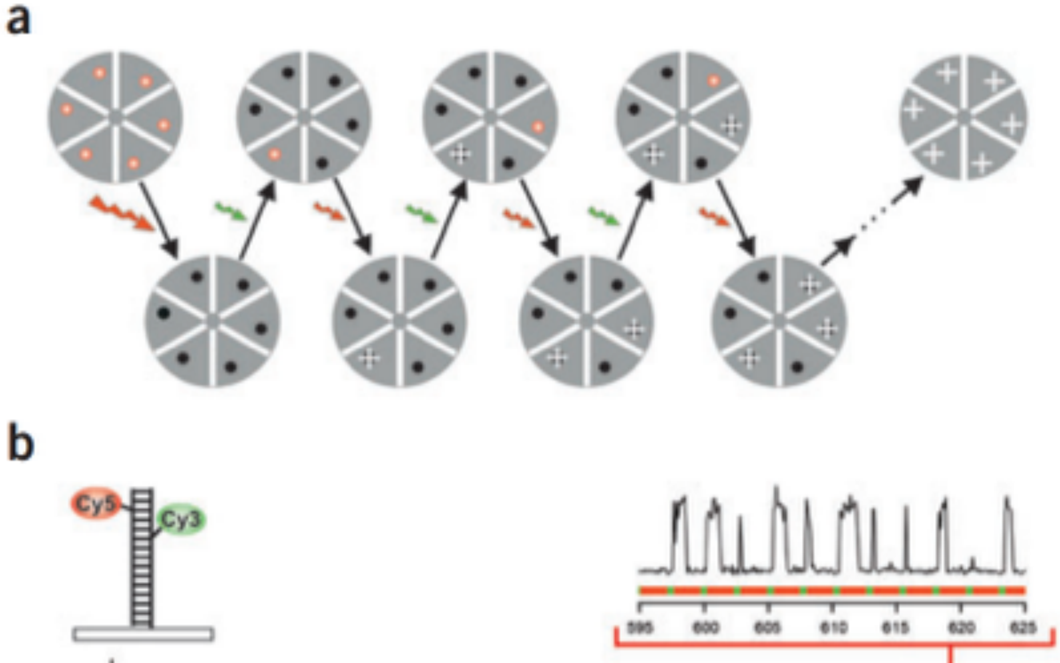
Bates, Blosser, Zhuang. "Short-range spectroscopic ruler based on a single-molecule optical switch." *Physical review letters* 94.10 (2005)



STORM: Stochastic Optical Reconstruction Microscopy

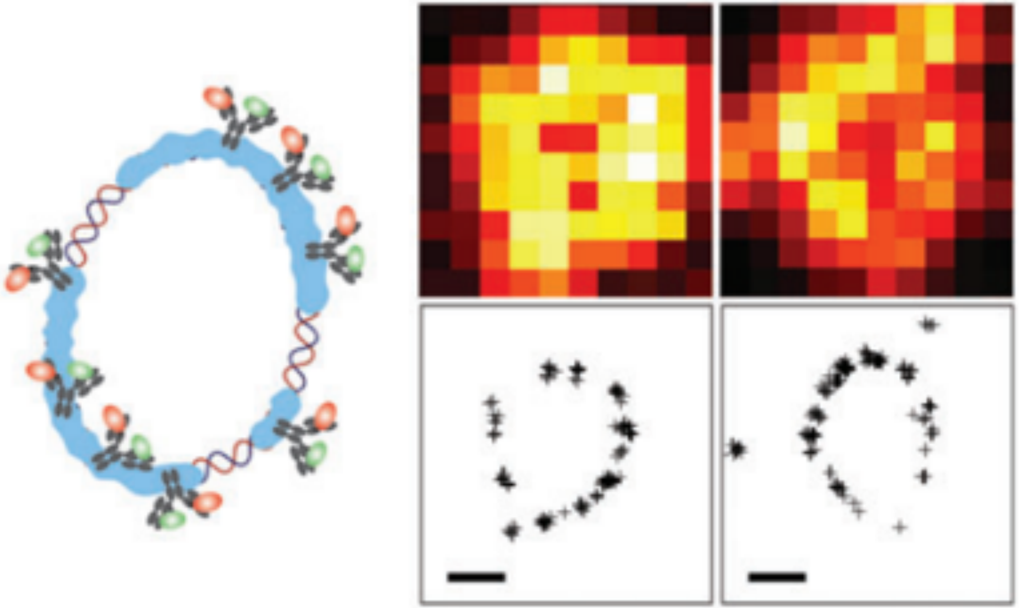
Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature methods* 3.10 (2006)





STORM: Stochastic Optical Reconstruction Microscopy

Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature methods* 3.10 (2006)



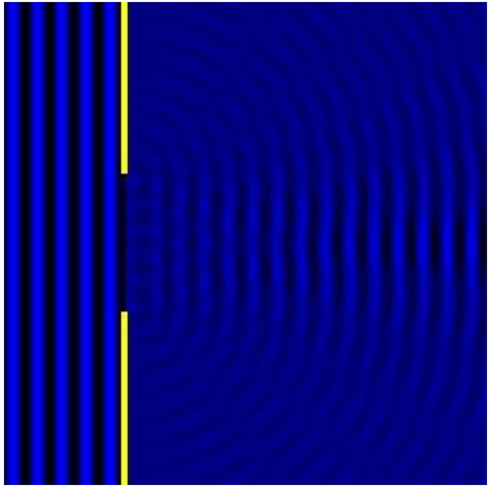
Superresolution fluorescence microscopy

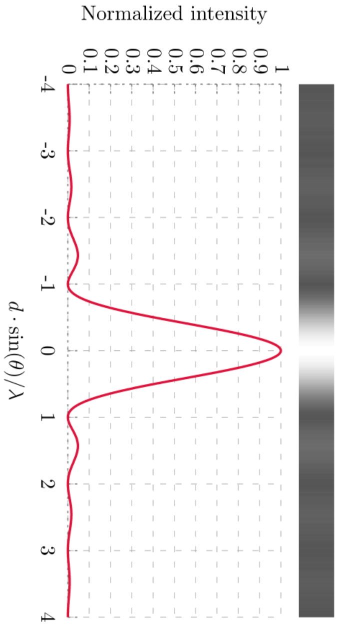
Leonid Keselman, Daniel Fernandes

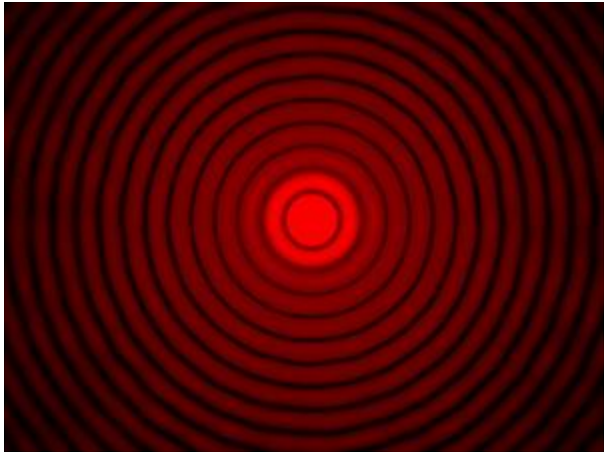
Overview

1. What is “super-resolution”
   a. Diffraction
   b. STORM
2. Compressed Sensing
   a. Applied to STORM
3. Light Sheet Imaging
   a. Lattice-Light Sheets

Natural Resolution Limits: Diffraction

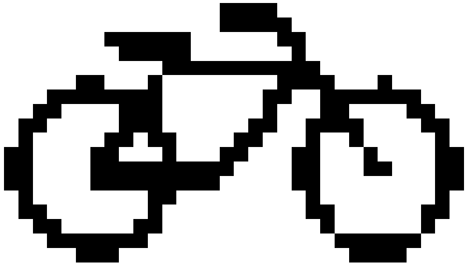






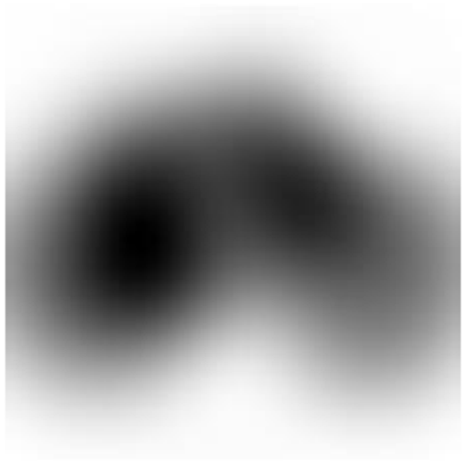
sources: Wikipedia (6wavelength=slitwidthblue.gif, Single_Slit_Diffraction_(english).svg, Beugungsscheibchen.k.720.jpg)

Natural Resolution Limits: Diffraction



*

=



sources: font-awesome fa-bicycle

Natural Resolution Limits: Diffraction

For typical cameras

$$d = 1.22 \; * \; \lambda \; * f\#$$

Raleigh Criterion

iPhone 7:
=1.22 * 650nm * f/1.8
=1.4 μm
pixels are only 1.22 μm!

For microscopes

$$d = \frac{\lambda}{2n \sin \theta} = \frac{\lambda}{2 \, NA}$$

Abbe diffraction limit

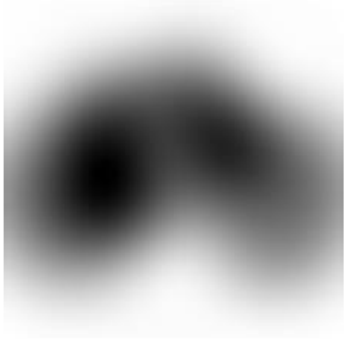
Typical Limit:
= 500nm/(2 * 1.25)
= 0.2 μm = 200nm
Microtubules are ~24nm

NA is typically 0.1-0.4 for common lenses in air, up to 1.0-1.5 for oil lenses.

STORM: Stochastic Optical Reconstruction Microscopy

Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature Methods* 3.10 (2006)

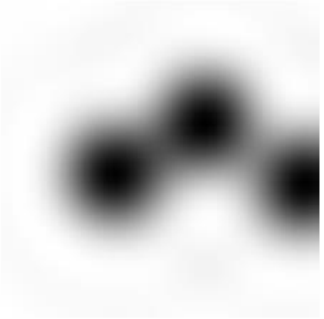
All pixels "on":



Random 1% of pixels "on"

Random 1% of pixels "on"

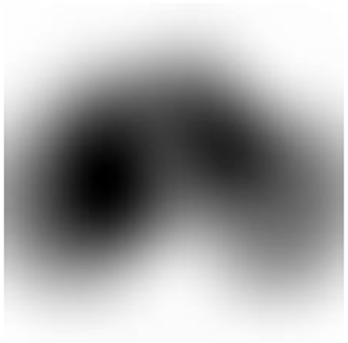
Random 1% of pixels "on"



STORM: Stochastic Optical Reconstruction Microscopy

Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature Methods* 3.10 (2006)

All pixels “on”:



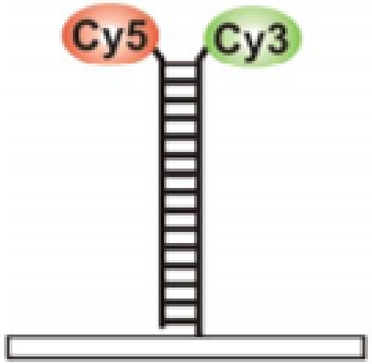
1 reading

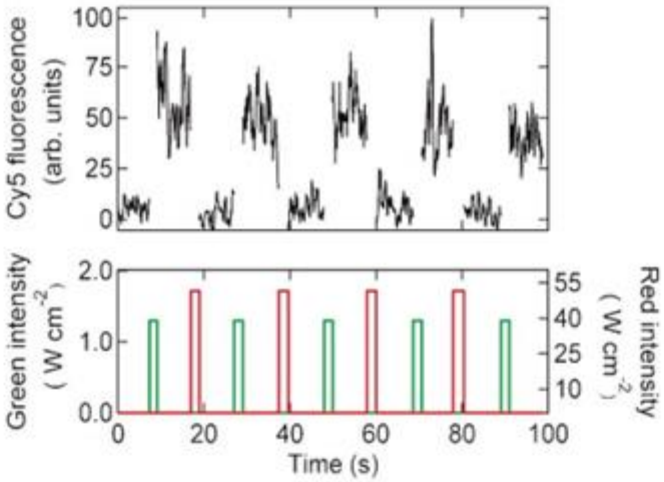
100 readings

1000 readings

STORM: Stochastic Optical Reconstruction Microscopy

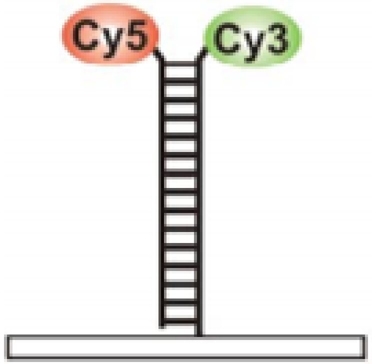
Bates, Blosser, Zhuang. "Short-range spectroscopic ruler based on a single-molecule optical switch." *Physical review letters* 94.10 (2005)

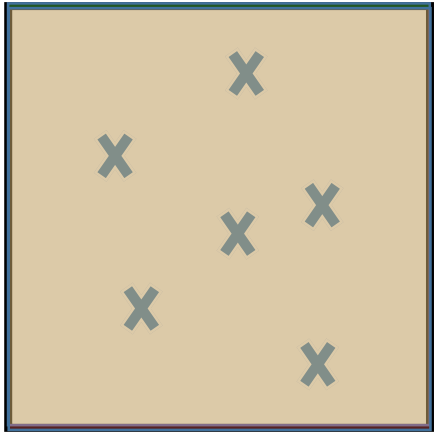




STORM: Stochastic Optical Reconstruction Microscopy

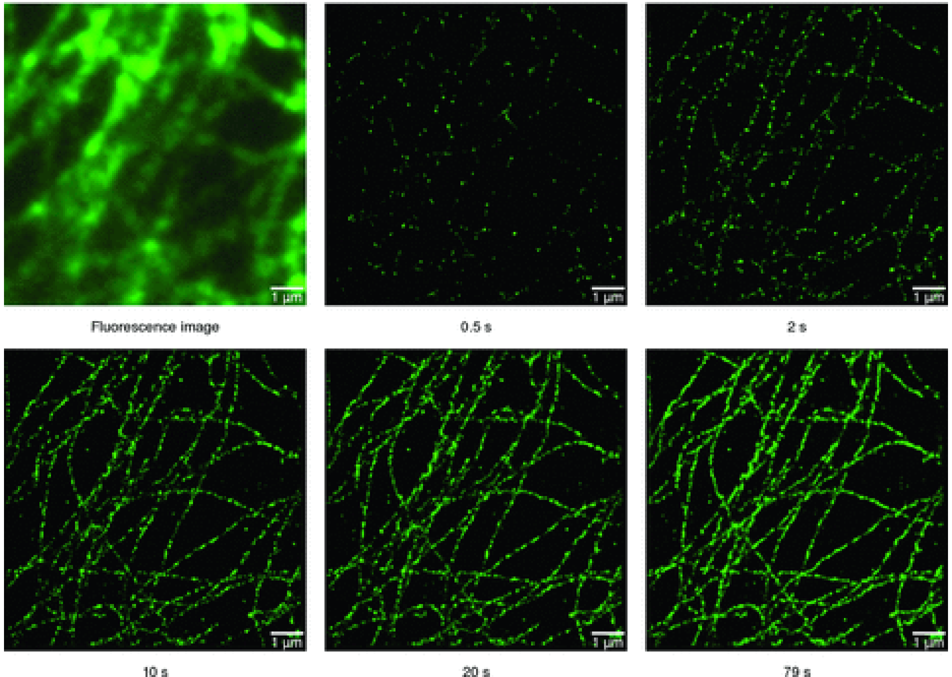
Bates, Blosser, Zhuang. "Short-range spectroscopic ruler based on a single-molecule optical switch." *Physical review letters* 94.10 (2005)





STORM: Stochastic Optical Reconstruction Microscopy

Wolter, Steve, et al. "Real-time computation of subdiffraction-resolution fluorescence images." *Journal of microscopy* 237.1 (2010)



Compressed Sensing (a.k.a. Sparse Sampling)

If your data is “compressible”, you can take just a handful of random measurements, and, using “simple” math, you can reconstruct your data (with minimal error and high probability)

Emmanuel Candes and Terence Tao. "Near-optimal signal recovery from random projections: Universal encoding strategies?." *arXiv:math/0410542* (2004)

$$\min \ \|x\|_{\ell_1} \quad \text{subject to} \quad \|Ax - y\|_{\ell_2} \leq \epsilon.$$

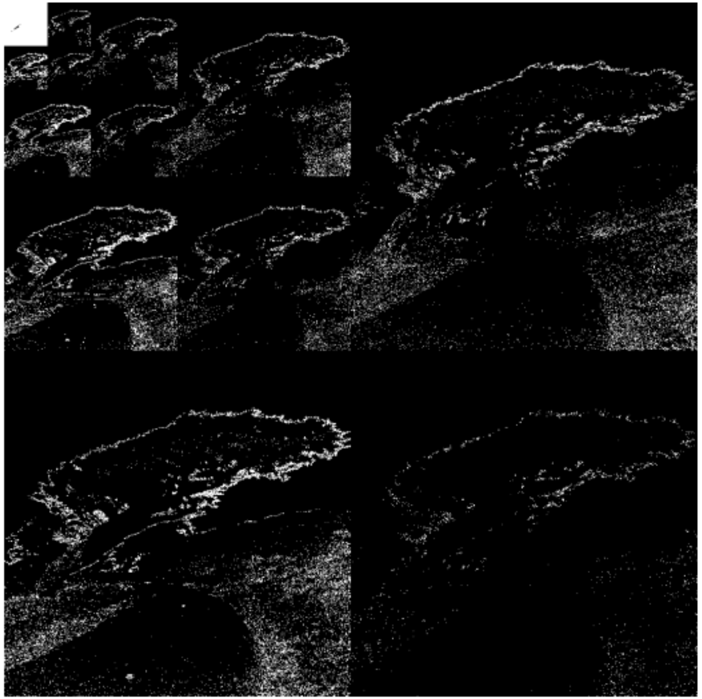
Compressed Sensing (a.k.a. Sparse Sampling)

	1	2	3	4	5	6
1	X	X		X		
0		X	X	X		
2	X			X		X

Compressed Sensing (a.k.a. Sparse Sampling)

	1	2	3	4	5	6
1	X	O		O		
0		O	O	O		
2	X			O		X

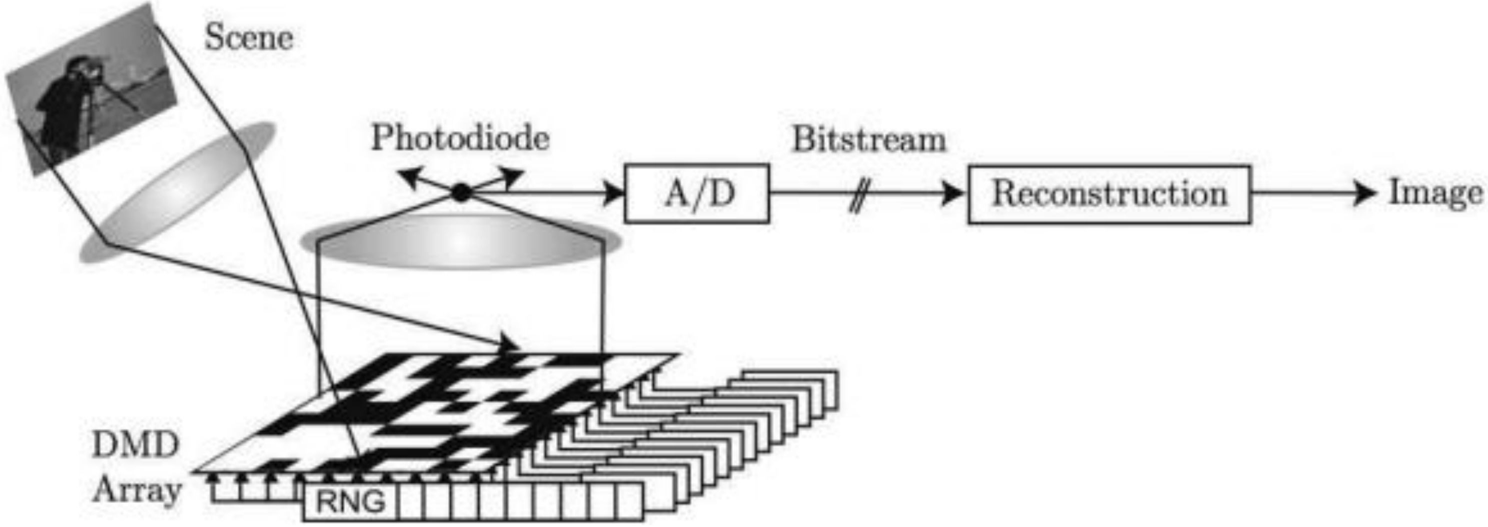
Compressed Sensing (a.k.a. Sparse Sampling)



Davenport, Duarte, Eldar, Kutynoik , *Introduction to Compressed Sensing*

Compressed Sensing

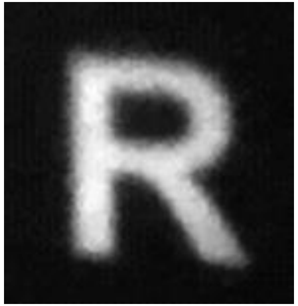
Duarte, et al. *Single-Pixel Imaging via Compressive Sampling.* (2008)



Compressed Sensing

Real Picture
(65,536 pixels)

CS Reconstruction
(3,300 samples)



CS Reconstruction
(1,300 samples)

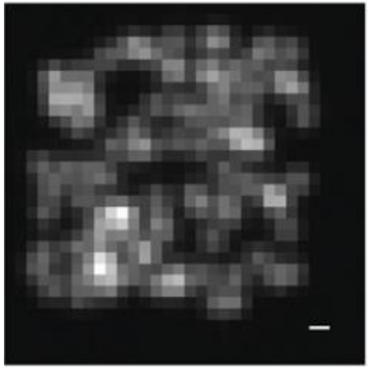
CS Reconstruction
(6,500 samples)

Faster STORM using compressed sensing

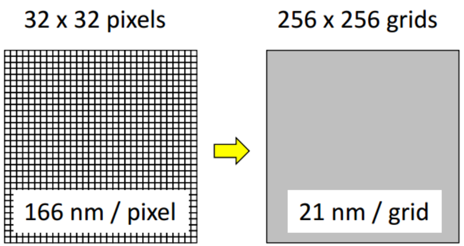
Zhu, et al."Faster STORM using compressed sensing." *Nature Methods* (2012)

1. Acquire PSF

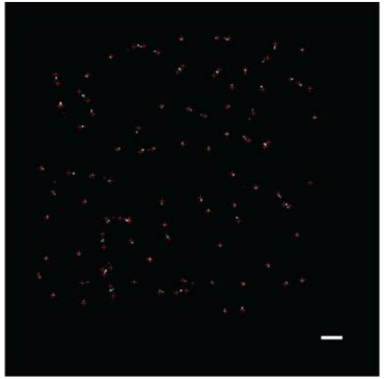
2. Get Image



3. Increase Grid



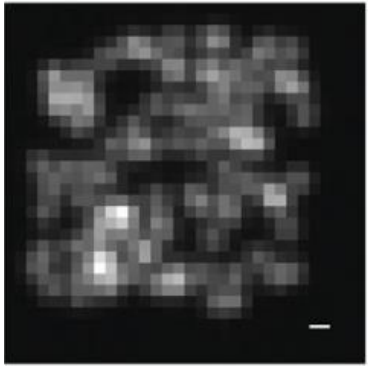
4. Solve CS problem



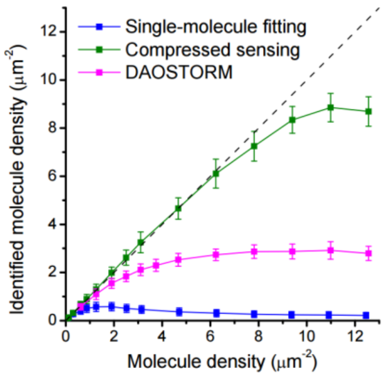
Faster STORM using compressed sensing

Zhu, et al."Faster STORM using compressed sensing." *Nature Methods* (2012)

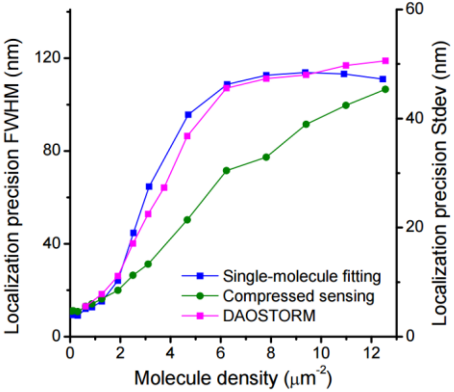
Denser Images!



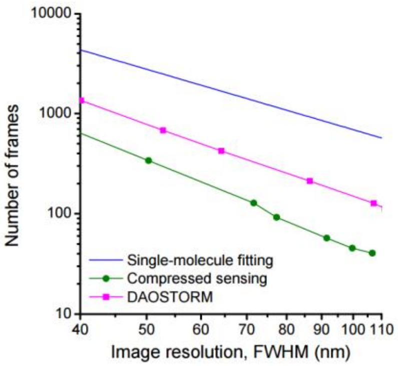
Many times denser



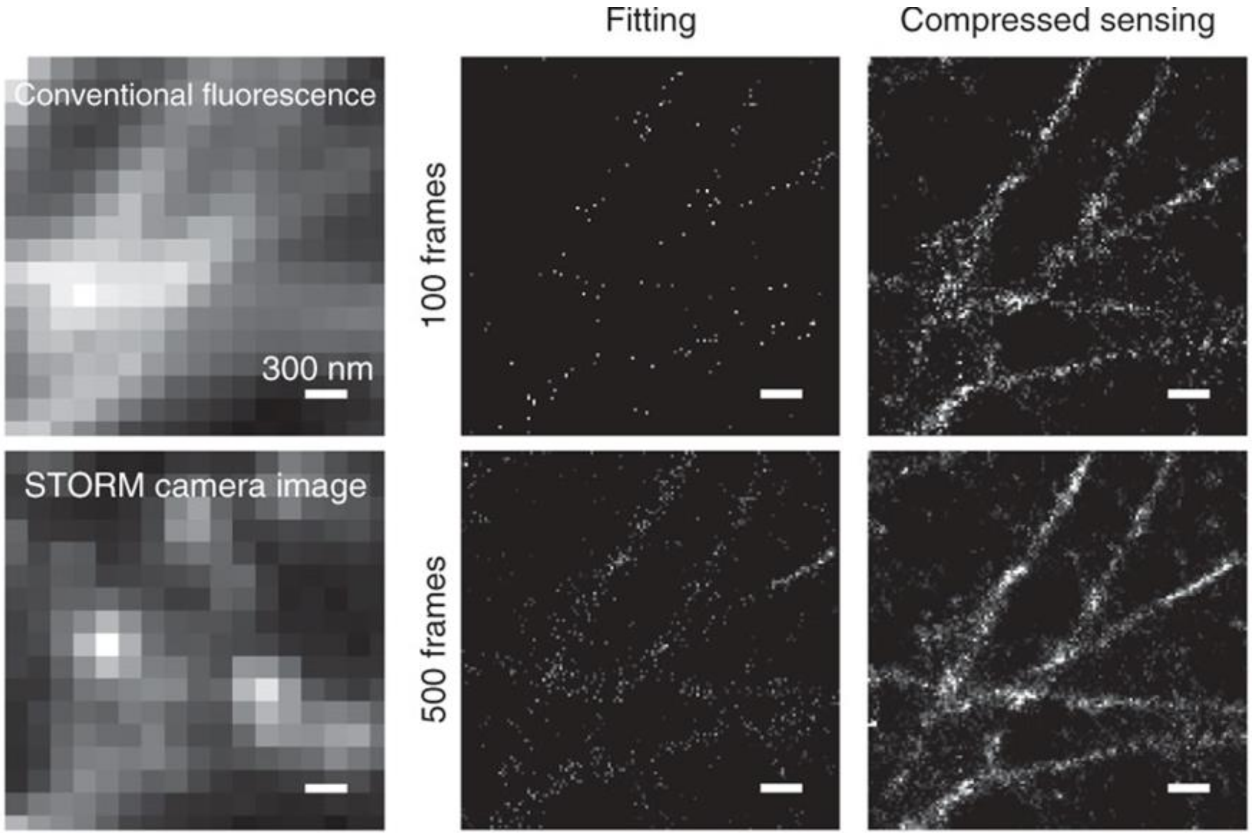
More precise

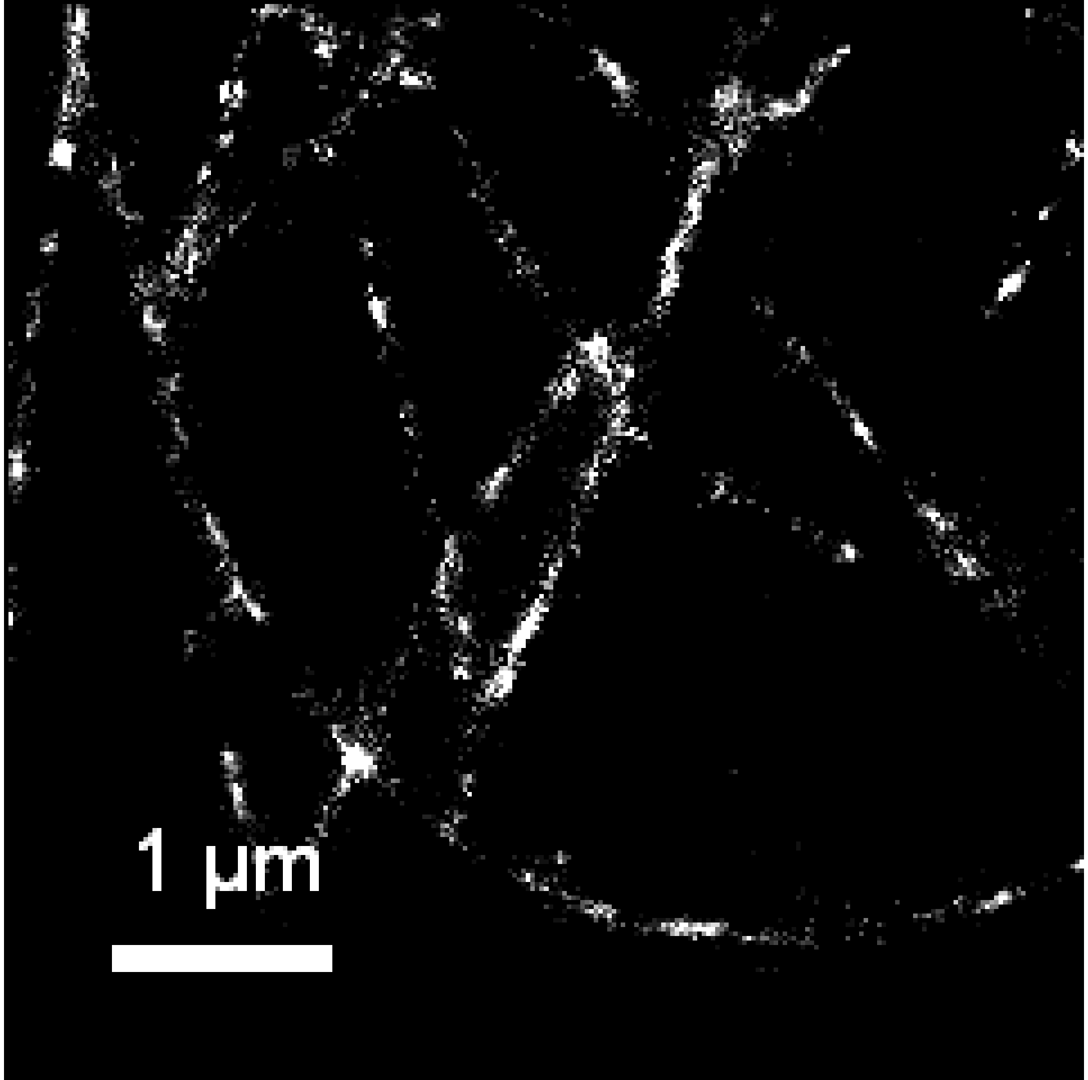


Faster imaging



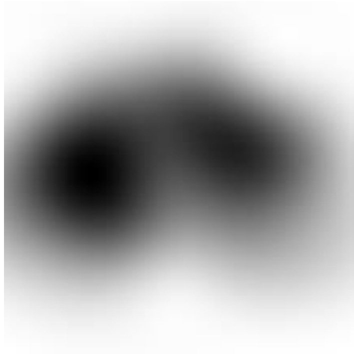
Faster STORM using compressed sensing




1 µm

Faster STORM using compressed sensing

40% pixels on



40% on, CS Solve

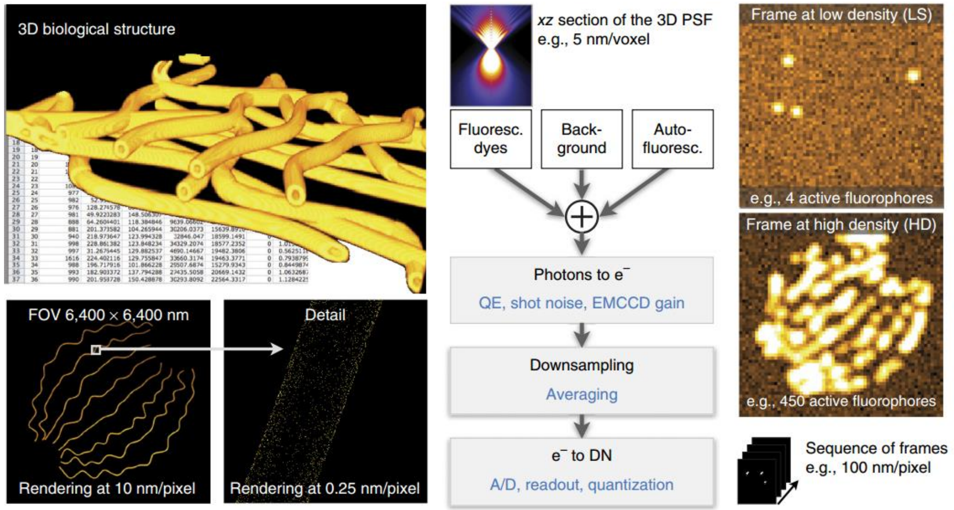
CS
50 readings
4% Density

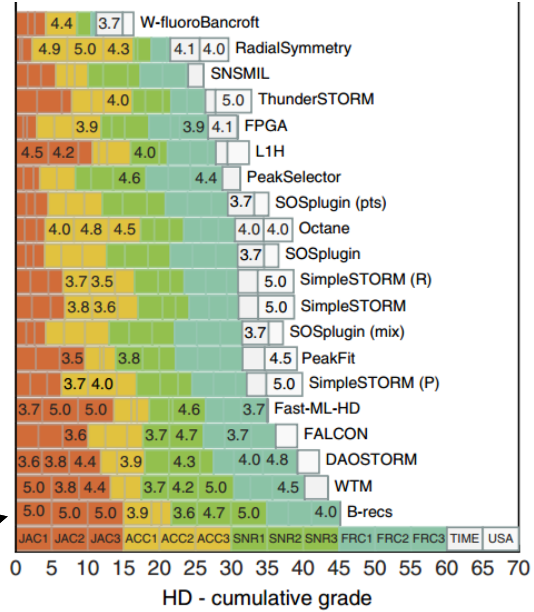
Classic
1000 readings
~0.8% Density

https://github.com/leonidk/cs371

Quantitative Comparison

Sage, Daniel, et al. "Quantitative evaluation of software packages for single-molecule localization microscopy." *Nature Methods* 12.8 (2015)



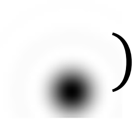
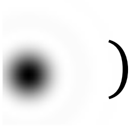
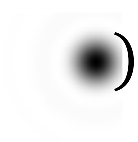


https://github.com/hrouault/Brecs

Extra Slides

Faster STORM using compressed sensing

Solve

$w_0($  $) + \cdots + w_{205}($  $) + \cdots + w_{819}($  $) + \cdots =$ 

With

$$\min \|\mathbf{w}\|_1$$

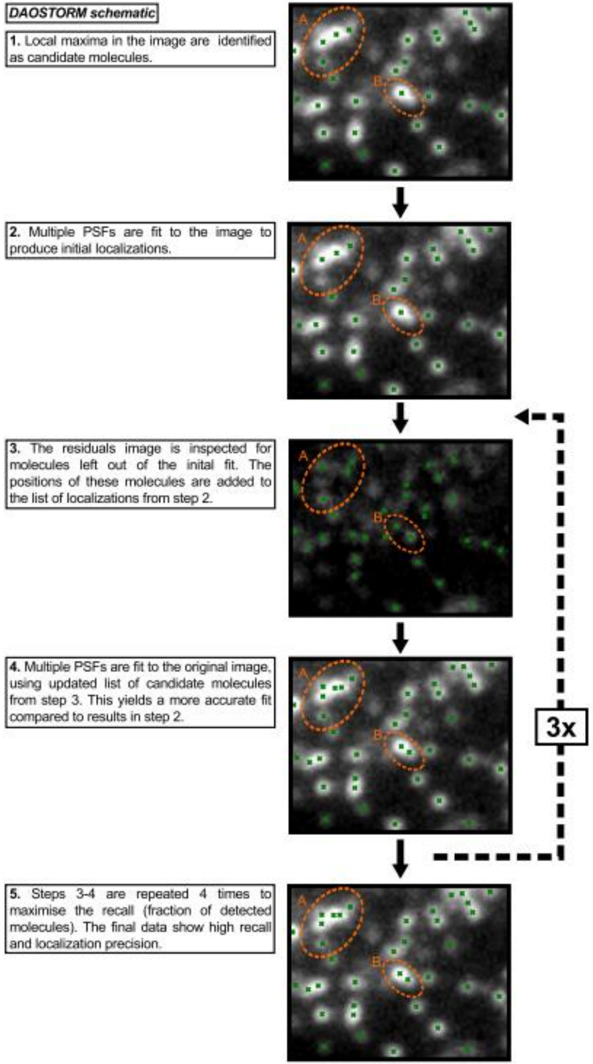
Gives

$\mathbf{w} \in \mathbb{R}^{1024} \;\Rightarrow\; \mathbf{w} \in \mathbb{R}^{32\text{x}32}$

$\mathbf{w} =$ 

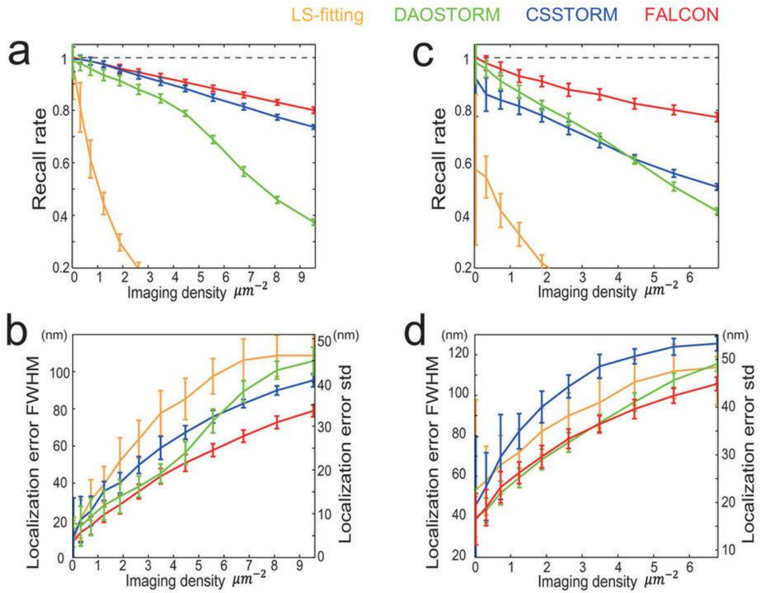
DAOSTORM

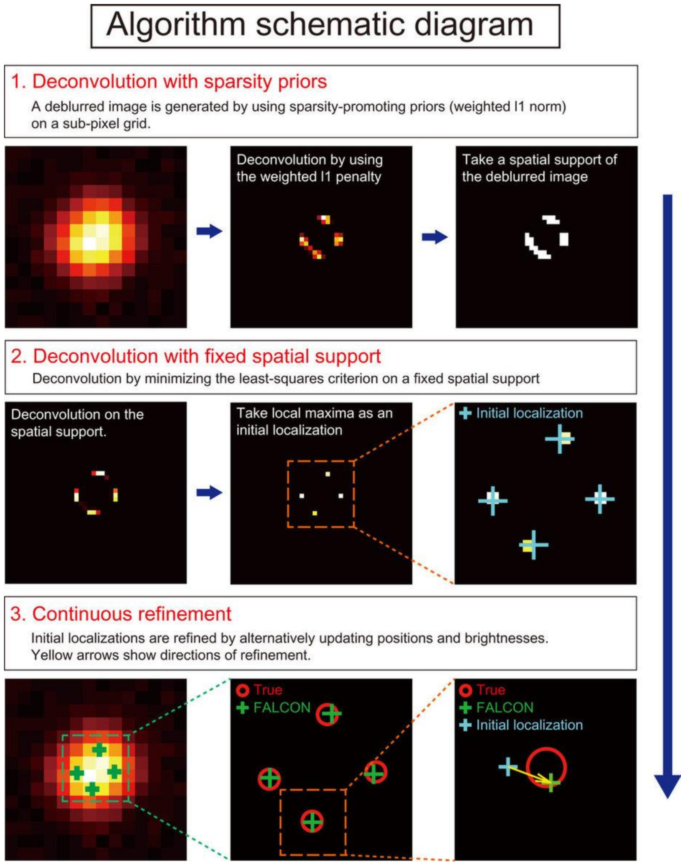
Stetson, Peter B. "DAOPHOT: A computer program for crowded-field stellar photometry." *Publications of the Astronomical Society of the Pacific* 99.613 (1987).



FALCON

Min, Junhong, et al. "FALCON: fast and unbiased reconstruction of high-density super-resolution microscopy data." *Scientific reports* 4 (2014)





Supplementary Figure: Switching kinetics

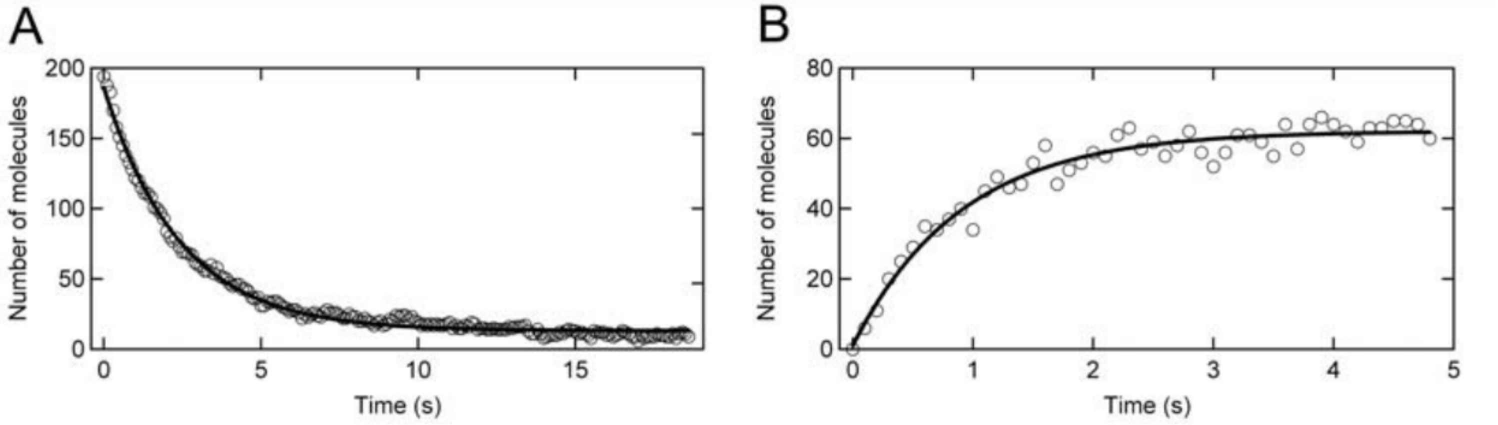


Figure S1. The first-order switching kinetics of the molecular switch. **A**, The number of molecules remaining fluorescent as a function of time after the green laser was turned off. A single exponential fit of the data (solid line) gives $k_{off} = 0.4\ s^{-1}$. **B**, The number of molecules that were converted back to the fluorescent state as a function of time after the green laser was turned on. A single exponential fit (solid line) gives the observed rate constant for switching Cy5 on ($k_{on\_obs} = 1.1\ s^{-1}$). Considering the competing actions of the red and green lasers, the actual rate constant k_{on} for switching the dye on by the green laser is equal to k_{on_obs} - k_{off}. Data in **A** and **B** are not from the same experiment.

Abstract

Suppose we are given a vector f in a class $\mathcal{F} \subset \mathbb{R}^N$, e.g. a class of digital signals or digital images. How many linear measurements do we need to make about f to be able to recover f to within precision ϵ in the Euclidean (ℓ_2) metric?

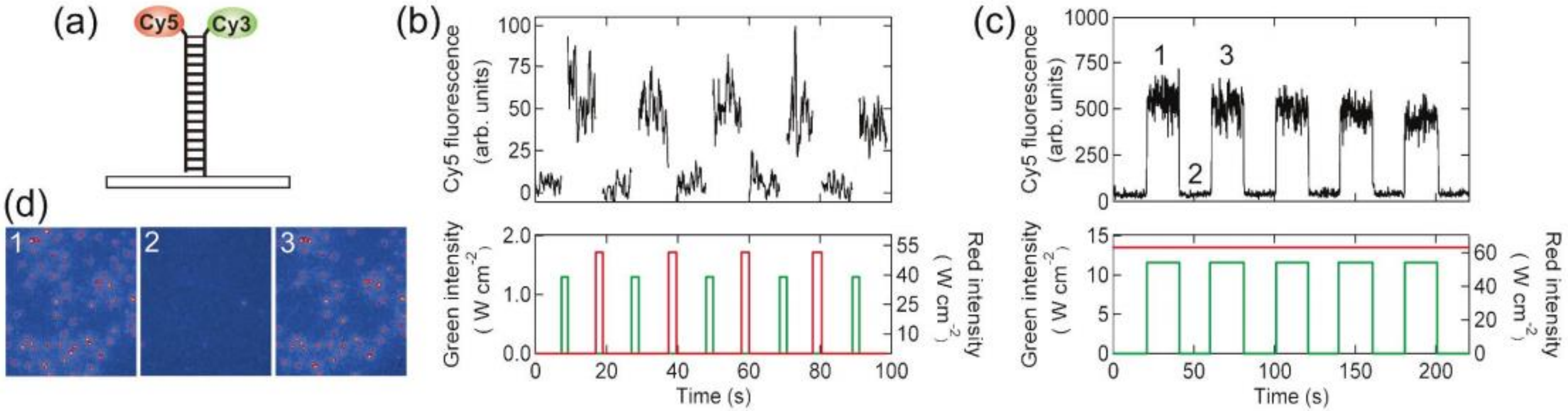
This paper shows that if the objects of interest are sparse in a fixed basis or compressible, then it is possible to reconstruct f to within very high accuracy from a small number of random measurements by solving a simple linear program. More precisely, suppose that the nth largest entry of the vector $|f|$ (or of its coefficients in a fixed basis) obeys $|f|_{(n)} \le R \cdot n^{-1/p}$, where $R > 0$ and $p > 0$. Suppose that we take measurements $y_k = \langle f, X_k \rangle$, $k = 1, \ldots, K$, where the X_k are N-dimensional Gaussian vectors with independent standard normal entries. Then for each f obeying the decay estimate above for some $0 < p < 1$ and with overwhelming probability, our reconstruction $f^\sharp$, defined as the solution to the constraints $y_k = \langle f^\sharp, X_k \rangle$ with minimal ℓ_1 norm, obeys

$$\|f - f^\sharp\|_{\ell_2} \le C_p \cdot R \cdot (K/\log N)^{-r}, \quad r = 1/p - 1/2.$$

There is a sense in which this result is optimal; it is generally impossible to obtain a higher accuracy from any set of K measurements whatsoever. The methodology extends to various other random measurement ensembles; for example, we show that similar results hold if one observes few randomly sampled Fourier coefficients of f. In fact, the results are quite general and require only two hypotheses on the measurement ensemble which are detailed.

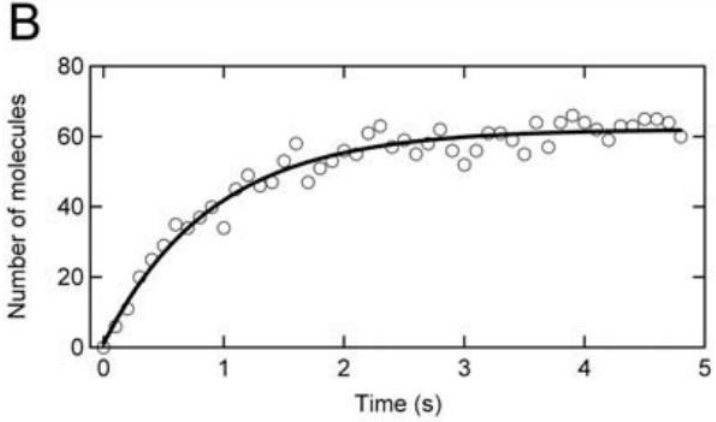
STORM: Stochastic Optical Reconstruction Microscopy

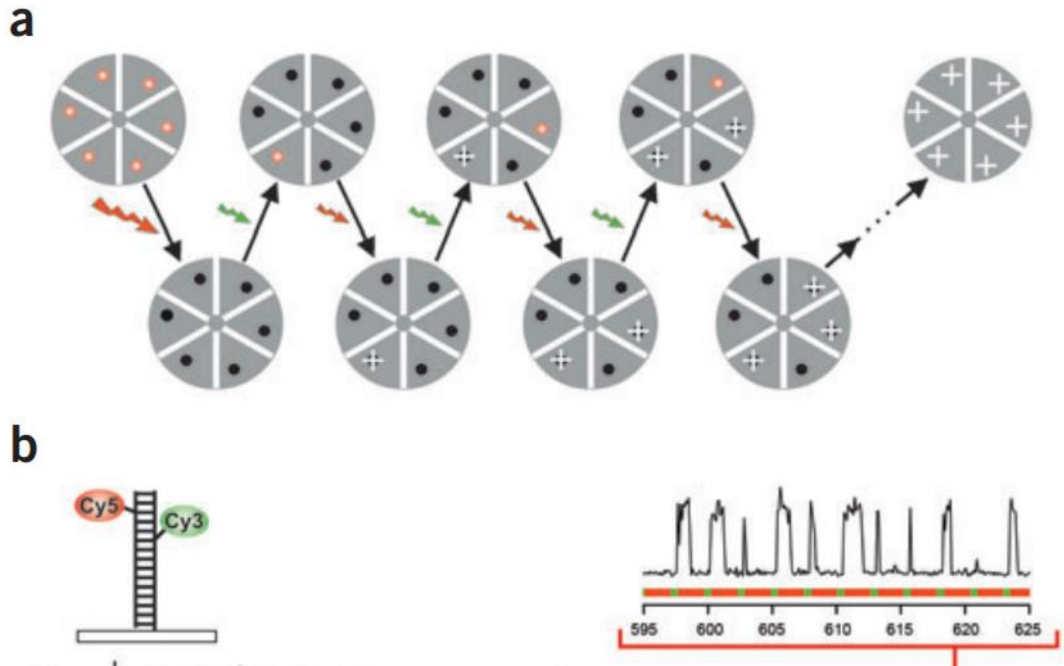
Bates, Blosser, Zhuang. "Short-range spectroscopic ruler based on a single-molecule optical switch." *Physical review letters* 94.10 (2005)



STORM: Stochastic Optical Reconstruction Microscopy

Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature methods* 3.10 (2006)





STORM: Stochastic Optical Reconstruction Microscopy

Rust, Bates, Zhuang. "Stochastic optical reconstruction microscopy (STORM) provides sub-diffraction-limit image resolution." *Nature methods* 3.10 (2006)