Precise Nanometer Localization Analysis for Individual Fluorescent Probes

based on a paper by R.E. Thompson, D.R. Larson and W.W. Webb

Christoph Schaller, FU Berlin, 19.12.2012

Outline

1. Motivation
2. Algorithms
    a. Spot recognition
    b. Fitting algorithms
3. Error estimation
4. Monte Carlo sample generation
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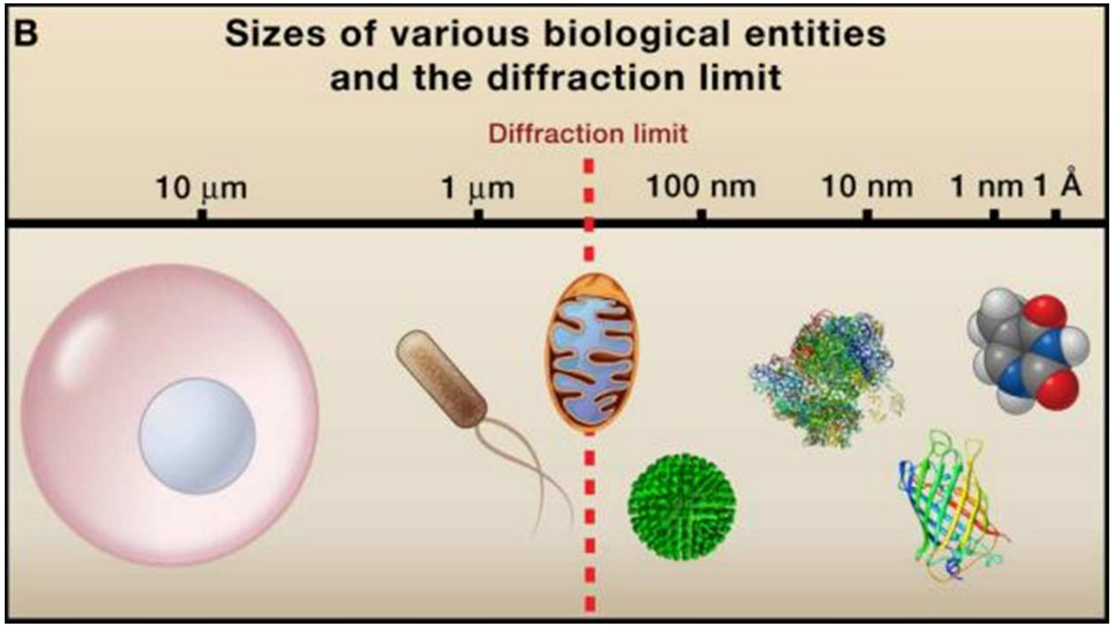
1. Motivation

- Minimal resolution of light microscopes

$$r \approx \frac{\lambda}{2} \approx 250\text{nm (diffraction limit)}$$

- (Left to right) A mammalian cell, a bacterial cell, a mitochondrion, an influenza virus, a ribosome, the green fluorescent protein, and a small molecule (thymine).

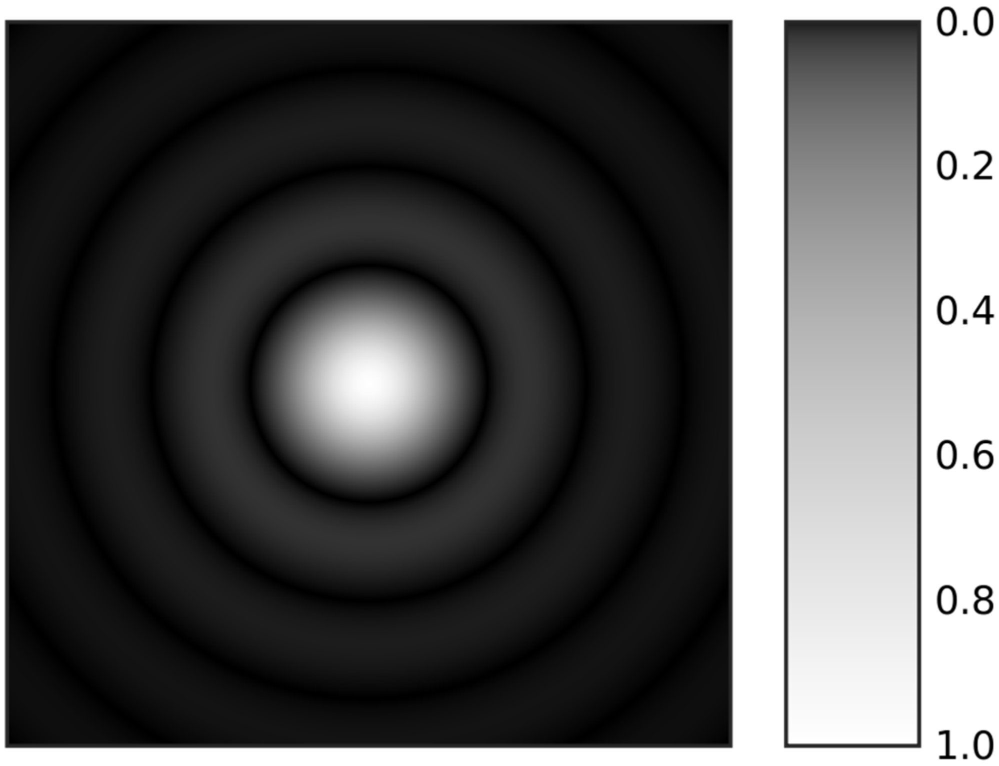


Source:
Huang et al., Breaking the Diffraction Barrier: Super-Resolution Imaging of Cells, Cell 143 (2010), p. 1047-1058

- Airy disk

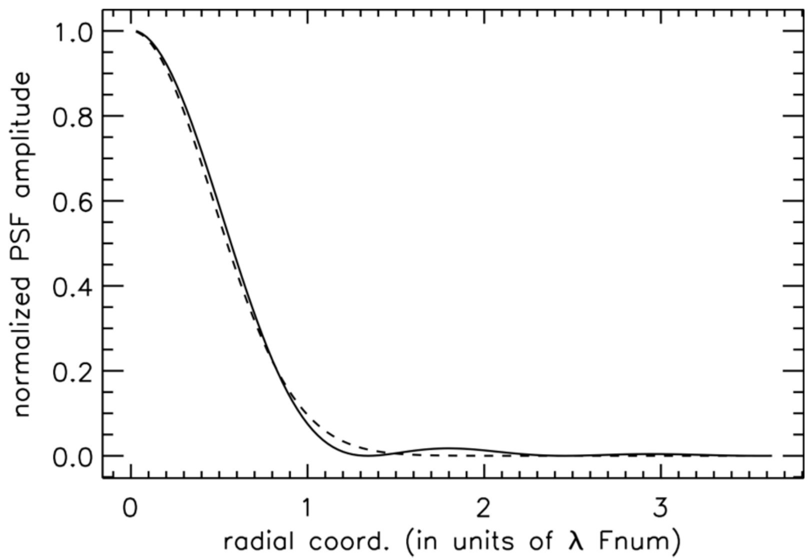
$$r \approx 0.61 \frac{\lambda}{NA}$$

$$(NA \leq 1.6)$$



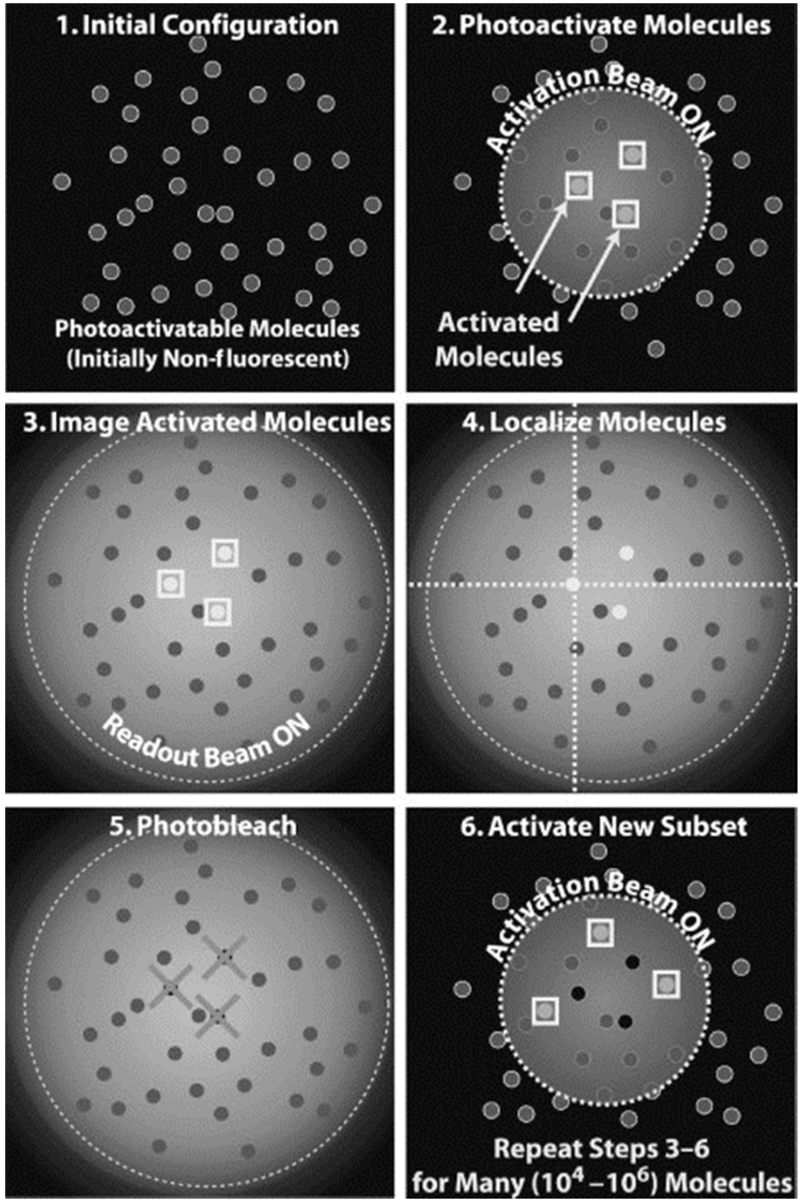
- Approximation by a Gaussian

$$\sigma \approx 0.42 \frac{\lambda}{NA}$$



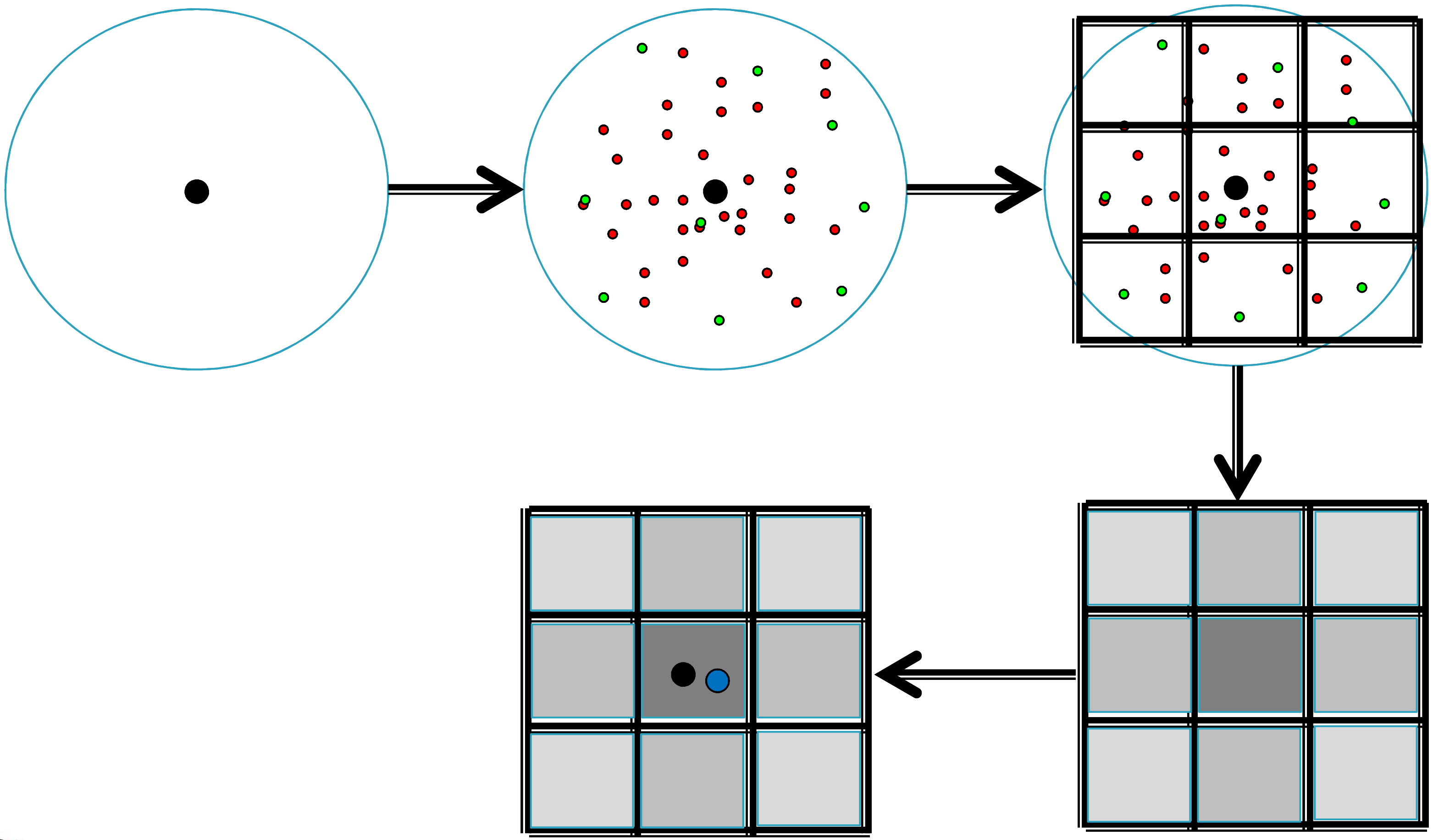
Source: en.wikipedia.org/wiki/Airy_disk

- Stochastical optical reconstruction microscopy (STORM)
- Reconstruction of spot centers
- Assumption: punctate and well-separated objects



Source:
T.J. Gould, S.T. Hess, Nanoscale Biological Fluorescence Imaging: Breaking the Diffraction Barrier, Methods in Cell Biology 89 (2008), p. 329-358

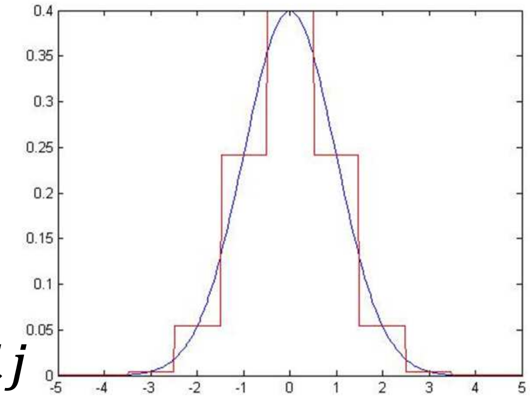
- Aggravated by noise
  - Background noise
  - Photon shot noise



2. Algorithms

- Given: matrix of photon counts for each pixel
- 2.a. Spot recognition
  - Find pixel values above some threshold (e.g. "8 standard deviations away from the mean")
  - Look for local maxima
  - Cut out a surrounding regions according to the potential spot
  - Mean remaining cells to obtain the background mean
  - Subtract the background mean from the spots
  - Fit the spot centers

2.b. Fitting algorithms



- Given: matrix of photon counts S_{ij}
- Notations
  - (i,j) location of pixel center in local coordinates
  - Spot center (x_0, y_0) – unknown
  - Number of photons in spot N – unknown
  - Standard deviation σ – known
  - $p_G(i,j) = \frac{1}{2\pi\sqrt{\sigma}} \exp(-\frac{(i-x_0)^2}{2\sigma^2} - \frac{(j-y_0)^2}{2\sigma^2})$
- We want: approximation of the center of S_{ij} by fitting with a pixelated Gaussian, i.e. $G_{ij} = N p_G(i,j)$

- Least squares approach, i.e. we want to minimize $\chi^2 = \sum_{i,j} \frac{\left(S_{ij}-G_{ij}\right)^2}{\psi_{ij}^2}$ (here $\psi_{ij}^2 = \sigma^2 + b^2$)
- For every minimum of χ^2:

$$\frac{d}{dx_0}\sum\left(S_{ij} - G_{ij}\right)^2 = 0$$

$$\Leftrightarrow \sum 2\left(S_{ij} - G_{ij}\right)\frac{(i - x_0)}{\sigma^2}G_{ij} = 0$$

$$\Leftrightarrow \sum S_{ij}G_{ij}(i - x_0) + \sum G_{ij}^2(i - x_0) = 0$$

- Full least squares

$$x_0 = \frac{\sum i(S_{ij} - G_{ij})G_{ij}}{\sum\left(S_{ij} - G_{ij}\right)G_{ij}}$$

- Odd symmetry: $\sum G_{ij}^2(i - x_0) \approx 0$
- Gaussian mask fitting

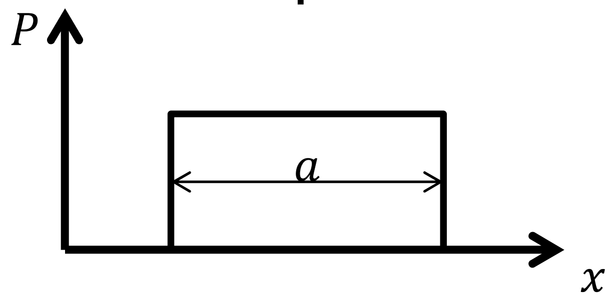
$$x_0 = \frac{\sum i S_{ij} G_{ij}}{\sum S_{ij} G_{ij}} = \frac{\sum i S_{ij} p_G(i,j)}{\sum S_{ij} p_G(i,j)}$$

- Number of photons within spot

$$N = \frac{\sum S_{ij} p_G(i,j)}{\sum p_G(i,j)^2}$$

3. Error estimation

- Split into two cases
  - Photon shot noise-limited (a)
  - Background noise-limited (b)
- Case (a)
  - No pixelation: $\langle (\Delta x)^2 \rangle = \frac{Var(x)}{N} = \frac{\sigma^2}{N}$
  - Pixelation noise: $\langle (\Delta x)^2 \rangle = \frac{\sigma^2 + \frac{a^2}{12}}{N}$
  - pixel size a, $\frac{a^2}{12}$ is the variance of a top-hat distribution of size a



- Case (b)
  - We minimized $\chi^2(x) = \sum \frac{\left(S_{ij} - G_{ij}(\mathrm{x})\right)^2}{\psi_{ij}^2}$
  - 1-dimensional $\chi^2(x) = \sum \frac{(S_k - G_k(x))^2}{\psi_k^2}$
  - Background noise only $\psi_k = b \ \ (b \in R)$
  - Taylor expansion

    $$G_k(x) = G_k(x_0) + \underbrace{(x - x_0)}_{\Delta x} G_k'(x_0) + O((\Delta x)^2)$$
  - Denote $\Delta S_k = G_k(x_0) - S_k$

$$0 = \frac{d}{dx}\chi^2 = \sum 2\frac{(\mathrm{S_k} - \mathrm{G_k(x)})}{b^2}G_k'(x)$$

$$\underset{TE}{\Leftrightarrow} 0 = \sum(-\Delta S_k - \Delta x G_k'(x_0))G_k'(x_0) + O((\Delta x)^2)$$

$$\Leftrightarrow 0 = \sum \Delta S_k G_k'(x_0) + \sum G_k'(x_0)^2 \Delta x + O((\Delta x)^2)$$

$$\Rightarrow \Delta x \approx -\frac{\sum \Delta S_k G_k'(x_0)}{\sum G_k'(x_0)^2}$$

- Now as $G_k'(x_0)$ are constants

$$\langle(\Delta S_k)^2\rangle = Var(S_k) = b^2, \langle \Delta S_k\rangle = 0$$

$$\Rightarrow \langle\left(\sum \Delta S_k G_k'(x_0)\right)^2\rangle = (\sum\langle(\Delta S_k)^2\rangle G_k'(x_0)^2) = \sum b^2 G_k'(x_0)^2$$

$$\Rightarrow \langle(\Delta x)^2\rangle = \frac{b^2}{\sum G_k'(x_0)^2}$$

- From $G_k(x) = \frac{N}{\sqrt{2\pi}\sigma}\exp(-\frac{(k-x)^2}{2\sigma})$ we derive

$$G_k'(x) = \frac{N}{\sqrt{2\pi}\sigma^2}(k-x)\exp\left(-\frac{(k-x)^2}{2\sigma}\right)$$

$$\Rightarrow G_k'(x)^2 = \frac{N^2}{2\pi\sigma^4}(k-x)^2\exp\left(-\frac{(k-x)^2}{\sigma}\right)$$

- Finally we use
  - $\sum G_k'(x_0)^2 \approx a\int G_k'(x_0)^2 dk = \frac{aN^2}{2\pi\sigma^4}\int(k-x)^2\exp\left(-\frac{(k-x)^2}{\sigma^2}\right)dk = \frac{aN^2}{4\sqrt{\pi}\sigma^3}$

  to obtain

$$\langle(\Delta x)^2\rangle = \frac{4b^2\sqrt{\pi}\sigma^3}{aN^2}$$

- Similarly in two dimensions

$$\langle(\Delta x)^2\rangle = \frac{8b^2\pi\sigma^4}{a^2N^2}$$

- Altogether the sum of (a) and (b) yields

$$\langle(\Delta x)^2\rangle \approx \frac{8b^2\pi\sigma^4}{a^2N^2} + \frac{\sigma^2 + \frac{a^2}{12}}{N}$$

- Transition point

$$N_t = \frac{8b^2\pi\sigma^4}{a^2(\sigma^2 + \frac{a^2}{12})}$$

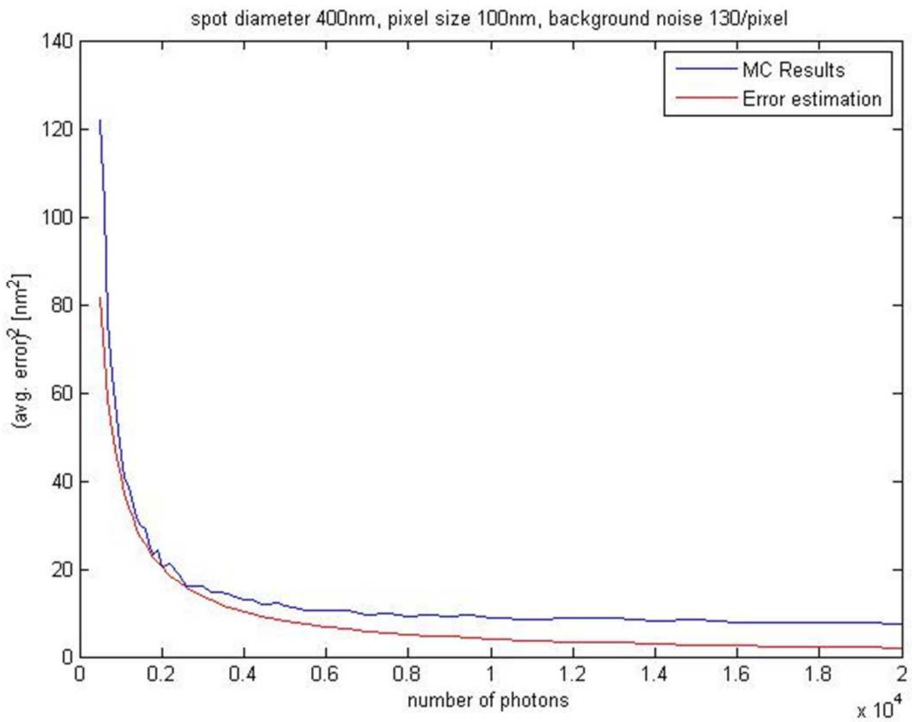
- Analogously we obtain

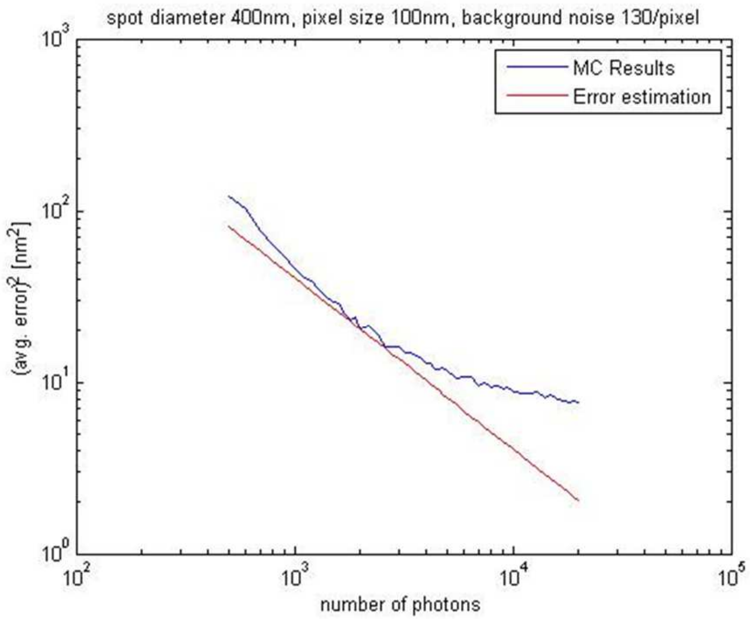
$$\langle(\Delta N)^2\rangle = N + \frac{4\pi\sigma^2 b^2}{a^2}$$

4. Monte Carlo sample generation

- Basic algorithm
  - Fix some (arbitrary) spot center
  - Generate random photon positions (Gaussian distributed)
  - Collect photons on coarse grid
  - Add (Poisson distributed) background for every pixel
- Parameters
  - Spot diameter, pixel size
  - Number of photons in spot
  - Background noise level

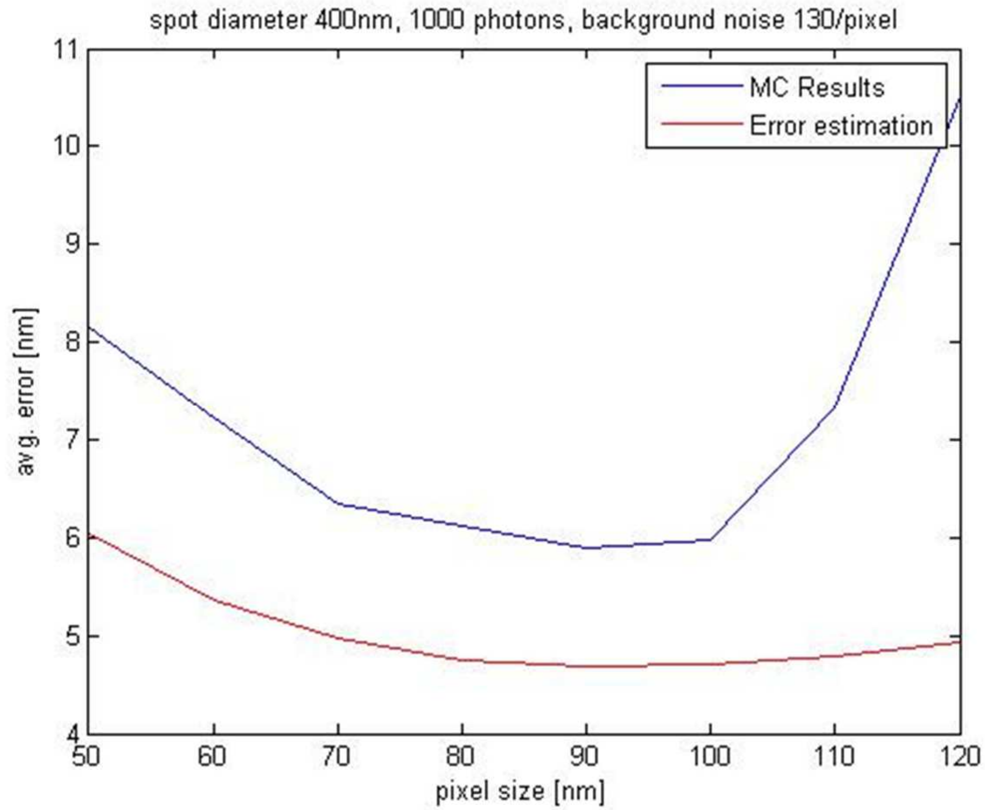
- Dependence on the photon number
- $N_t \approx 1317$ photons





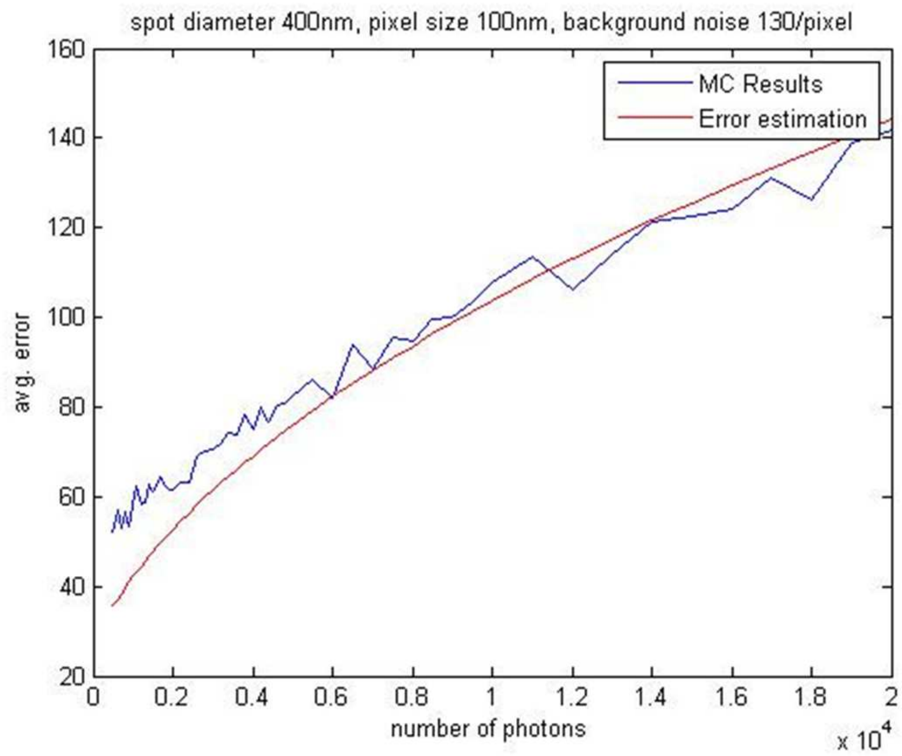
$$\langle(\Delta x)^2\rangle = \frac{8b^2\pi\sigma^4}{a^2N^2} + \frac{\sigma^2 + \frac{a^2}{12}}{N}$$

- Dependence on $\frac{spot\ size}{pixel\ size} = \frac{s}{a}$



- Underestimation due to
  - First order Taylor approximation
  - Interpolation between two limited cases
  - Transformation of a discrete sum into an integral
  - Assumption of $\frac{s}{a} \gg 1$
  - Simplification of Least Squares

- Error in N

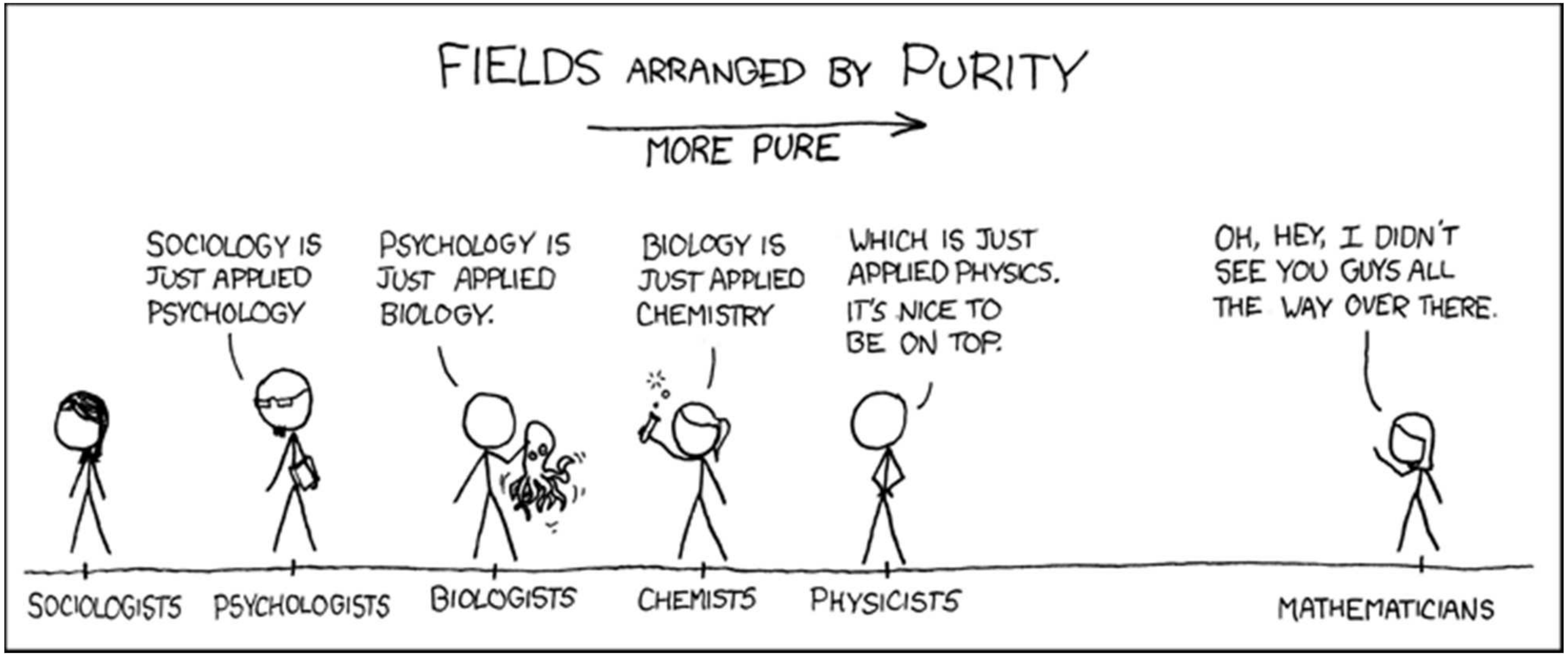


5. Summary & Outlook

- Basic idea of achieving nanometer resolution in microscopy
- Construction of fitting Algorithms
- Simple, but rough estimates for the errors

- Application to experimental data, comparison with common software
- Check for potential improvements
- …

THANK YOU FOR YOUR ATTENTION!



Source: xkcd.com/435/