Precise Nanometer Localization Analysis for Individual Fluorescent Probes

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

Outline

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1. Motivation

- Minimal resolution of light microscopes
  \[ r \approx \frac{\lambda}{2} \approx 250\text{nm} \text{ (diffraction limit)} \]

- (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:
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:
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
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 $\sigma$ – known

$$p_G(i, j) = \frac{1}{2\pi\sqrt{\sigma}} \exp\left(-\frac{(i-x_0)^2}{2\sigma^2} - \frac{(j-y_0)^2}{2\sigma^2}\right)$$

We want: approximation of the center of $S_{ij}$ by fitting with a **pixelated** Gaussian, i.e.

$$G_{ij} = Np_G(i, j)$$
Least squares approach, i.e. we want to minimize \( \chi^2 = \sum_{i,j} \frac{(S_{ij} - G_{ij})^2}{\psi^2_{ij}} \) (here \( \psi^2_{ij} = \sigma^2 + b^2 \)).

For every minimum of \( \chi^2 \):

\[
\frac{d}{dx_0} \sum (S_{ij} - G_{ij})^2 = 0
\]

\[
\iff \sum 2(S_{ij} - G_{ij}) \frac{(i - x_0)}{\sigma^2} G_{ij} = 0
\]

\[
\iff \sum S_{ij} G_{ij} (i - x_0) + \sum G_{ij}^2 (i - x_0) = 0
\]

Full least squares

\[
x_0 = \frac{\sum i(S_{ij} - G_{ij})G_{ij}}{\sum (S_{ij} - G_{ij})G_{ij}}
\]
- 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 $\chi^2(x) = \sum \frac{(S_{ij} - G_{ij}(x))^2}{\psi_{ij}^2}$
- 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 $\Delta S_k = G_k(x_0) - S_k$
\[ 0 = \frac{d}{dx} \chi^2 = \sum 2 \frac{(S_k - G_k(x))}{b^2} G_k'(x) \]

\[ \iff 0 = \sum (-\Delta S_k - \Delta x G_k'(x_0)) G_k'(x_0) + O((\Delta x)^2) \]

\[ \iff 0 = \sum \Delta S_k G_k'(x_0) + \sum G_k'(x_0)^2 \Delta x + O((\Delta x)^2) \]

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

Now as \( G_k'(x_0) \) are constants

\[ \langle (\Delta S_k)^2 \rangle = \text{Var}(S_k) = b^2, \langle \Delta S_k \rangle = 0 \]

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

\[ \Rightarrow \langle (\Delta x)^2 \rangle = \frac{b^2}{\sum G_k'(x_0)^2} \]
\textbf{From} $G_k(x) = \frac{N}{\sqrt{2\pi\sigma}} \exp\left(-\frac{(k-x)^2}{2\sigma}\right)$ we derive

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

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

\textbf{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

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

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

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

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

\[
\langle (\Delta x)^2 \rangle = \frac{8b^2 \pi \sigma^4}{a^2 N^2} + \frac{\sigma^2 + \frac{a^2}{12}}{N}
\]
- Dependence on \( \frac{\text{spot size}}{\text{pixel size}} = \frac{s}{a} \)
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/