Single-Molecule Color Sensing From PSF Fitting

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Single-Molecule Color Sensing Methods

Goal

Sensing spectral mean of a single emitter along with its position

- Available options
  - Optical filter bank & lambda stack construction
  - PSF engineering based on chromatic dispersion (gratings[1], prism[2], programmable phase mask with 4f setup[3], etc.)
- **What is desired?**

[1] Ma, Y. *et al. Analytical Chemistry* **72,** 4640–4645 (2000).
[2] Zhang, Z. *et al. Nat Meth* **12,** 935–938 (2015).
[3] Shechtman, Y. *et al. Nat Photon* **10,** 590–594 (2016).

Color Sensing Methods: Criteria

- Minimum overhead in the optical setup
  - Less aberration & insertion loss, easy to apply, avoid complicated calibration
- Minimum impact on lateral/axial localization accuracy
  - Localization accuracy degrades by $\sqrt{N}$ for $N\times$ less photons due to beam-split
- Avoid PSF footprint enlargement
  - In SMLM context, minimize the probability of overlap of PSFs (affects temporal resolution)
- Flexibility (no need for a priori wavelength info)
- Good λ estimation accuracy for given photon budget
- Avoid possibility of bias from non-idealities

SM Color Sensing From PSF Fitting

- Diffraction-limited image of a single molecule already contains color information

$$\text{First minimum of Airy pattern} = 0.61\frac{\lambda}{NA}$$

- In principle, it should be possible to estimate emitter color from the *scale* of the fitted PSF
- Spectrally resolved SMLM: just installation of a ImageJ plugin!
  - Literally zero impact on the optical setup/xyz accuracy
  - No PSF modification (more than whats required for 3D)
  - No a priori information

Questions to be answered

- Good enough λ estimation accuracy in realistic scenario?
  - Axial position dependency in 2D situation
- Possibility of bias from non-idealities (not limited to)
  - Chromatic/spherical aberration
  - Spectral profile of the fluorophore (bandwidth, skewness)
  - Non-uniform background
  - Deconvolution artifacts
  - Motion blur
  - Fluorophore orientation

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Performance of Measurement Method

Task

Estimation of x, y, z, $\overline{\lambda}$ of a single emitter from values of sensor array (EMCCD, sCMOS) within certain ROI

- A "measurement method" includes:
  - PSF design (how you encode the information to actual image: prism/grating, astigmatism, double helix, etc.)
  - Corresponding optical setting (affecting SNR, bias)
  - Reconstruction algorithm (LS/MLE, ROI selection, b/g correction, etc.)
- Performance metrics
  - Highest accuracy (smallest σ) for given number of photons & noise profile
  - Unbiased
  - PSF footprint

Design of Measurement Method

- Major design decisions in our context
  - PSF design/PSF model to use (optical setting is determined by this)
  - Deconvolution algorithm

- Today's question: what's the theoretical bound for wavelength estimation accuracy and the impact of PSF choice?
  - Fix everything other than PSF
    - Magnification, noise, pixel size, ROI, NA, illumination
  - Consider only major physical implication of PSF design choice
    - e.g. consider beam split, but not the insertion loss from additional optical elements

Problem Setting

- PSF options
  - *Plain-vanilla* circular aperture
  - Multifocus (biplane)
  - Astigmatism
  - Double-helix
- Assumptions
  - NA=1.4, effective pixel size=100nm, ROI=20x20 pixels
  - Only shot noise, uniform background of 10 photons/pixel
  - Ignore aberration
  - Model a fluorophore as an monochromatic/isotropic emitter
- Procedure
  - Compute Cramér-Rao lower bound (CRLB) for different z location & number of photons

Circular Aperture Case

- 3D PSF model for simple circular aperture: Born & Wolf model

$$q_{z_0}(\vec{r}-\vec{r}_0\,) = \left\| A\int_0^1 J_0\left(\frac{2\pi}{\lambda}\mathrm{NA}\|\vec{r}-\vec{r}_0\|\rho\right) e^{-j\frac{\pi}{\lambda}\rho^2 z_0\left(\frac{\mathrm{NA}^2}{n_i}\right)}\rho d\rho \right\|^2$$

A: normalization constant, n_i: refractive index of the immersion oil

J_0: zeroth order Bessel function of the first kind

- Fisher information matrix with pixelation & shot noise[4]

$$I_{ij}(\boldsymbol{\theta}) = \sum_{k=1}^{N_{pixel}} \frac{1}{\mu_k(\boldsymbol{\theta})+\beta}\left(\frac{\partial \mu_k(\boldsymbol{\theta})}{\partial \theta_i}\right)\left(\frac{\partial \mu_k(\boldsymbol{\theta})}{\partial \theta_j}\right)$$

$\mu_k(\boldsymbol{\theta})$: the value of the model PSF in pixel k

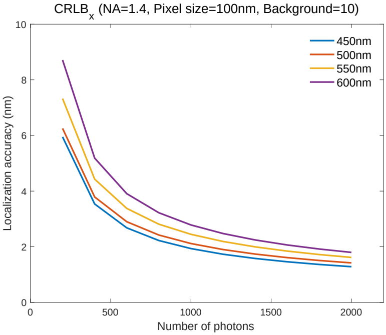
N_{pixel}: the number of pixels in the ROI

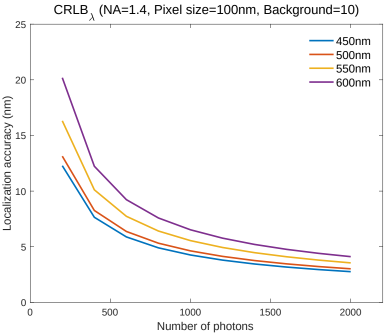
β: background photons per pixel

[4] Ober, R. J. *et al. Biophysical Journal* **86,** 1185–1200 (2004).

Circular Aperture Case

- For $z = 0$





Impact of Defocusing

- Defocusing introduces biasing to wavelength estimation
  - Accuracy bound should take into account both bias & CRLB
- Generalized CRLB in presence of bias

$$\mathrm{Var}(\hat{\lambda}) \geq \frac{[1 + b'(\lambda)]^2}{I(\lambda)}$$

$b(\lambda)$: bias, $I(\lambda)$: Fisher information

- CRLB is z dependent

$$b(\lambda) = \lambda(M(z) - 1), \quad b'(\lambda) = M(z) - 1$$

$$\mathrm{Var}(\hat{\lambda})|_z \geq \frac{M(z)^2}{I(\lambda)|_z}$$

$M(z)$: magnification factor of the PSF at z

Impact of Defocusing

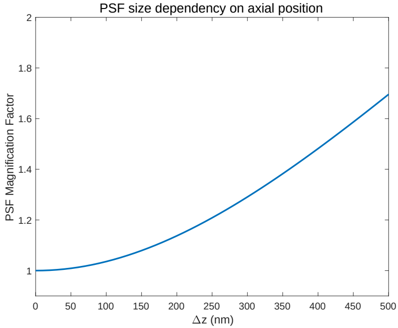
- Assuming certain distribution for z, performance bound in presence of z variation can be calculated
  - Mean squared error at z: $E[(\hat{\lambda} - \lambda)^2]|_z = \mathrm{Var}(\hat{\lambda})|_z + b(z)^2$
  - Final CRLB is calculated as a weighted sum over z

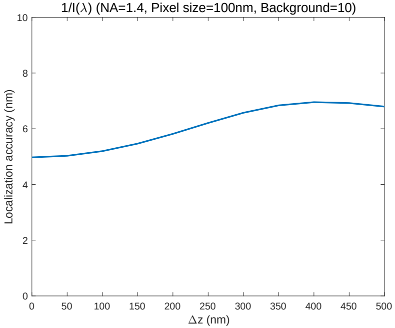
$$
\begin{aligned}
CRLB_\lambda &= \int_{z_{min}}^{z_{max}} E[(\hat{\lambda} - \lambda)^2] f(z) dz \\
&= \int_{z_{min}}^{z_{max}} \left(\mathrm{Var}(\hat{\lambda})|_z + b(\lambda)^2 \right) f(z) dz \\
&\geq 2 \int_{0}^{\Delta z_{max}} \left(\frac{M(z)^2}{I(\lambda)|_z} + \lambda^2 (M(z) - 1)^2 \right) f(z) dz
\end{aligned}
$$

  - PSF Symmetry over z, $f(z)$: probability distribution of z
  - For $M(z) = 1$, converges to the CRLB at $z = 0$

Impact of Defocusing

- Magnification factor & Fisher information vs. z
  - N_{photon}=1500, λ=600nm, n_i=1.5, Born & Wolf model





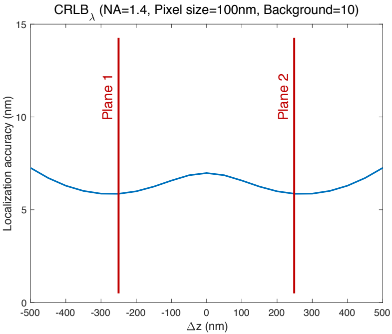
- For Δz_{max}=100nm & $f(z)$=uniform, CRLB=9.86nm (originally 4.97nm)
- For Δz_{max}=500nm, CRLB=177nm!

3D+Color: Multifocal Plane

- Total Fisher information matrix is the sum of FI from each imaging plane[5]

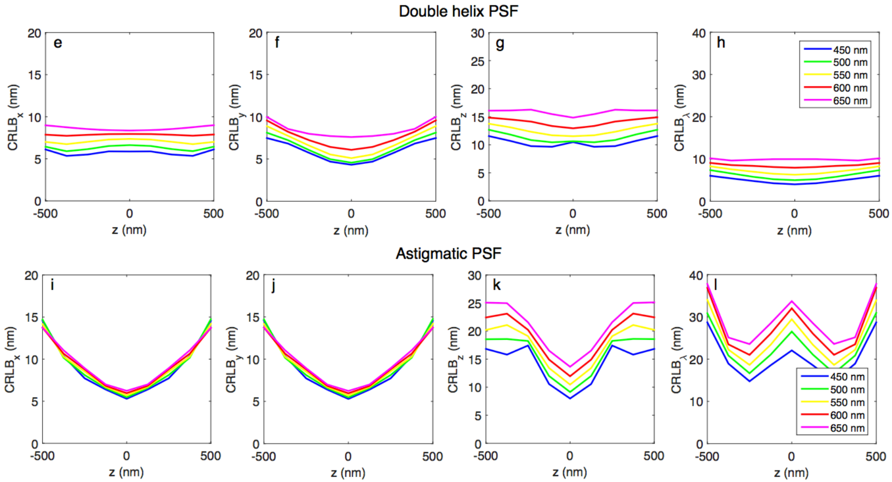
$$I_{tot}(\boldsymbol{\theta}) = \sum_{i=1}^{N_{plane}} I_i(\boldsymbol{\theta})$$

- Biplane setting
  - N_{photon}=1500, λ=600nm
  - 50/50 beam split (750 photons/plane)
  - Distance between planes: 500nm



[5] Ram, S. *et al. Biophysical Journal* **95,** 6025–6043 (2008).

3D+Color: Double Helix/Astigmatic[6]



[6]Smith, C. *et al. Optics Express* **24** (2016).

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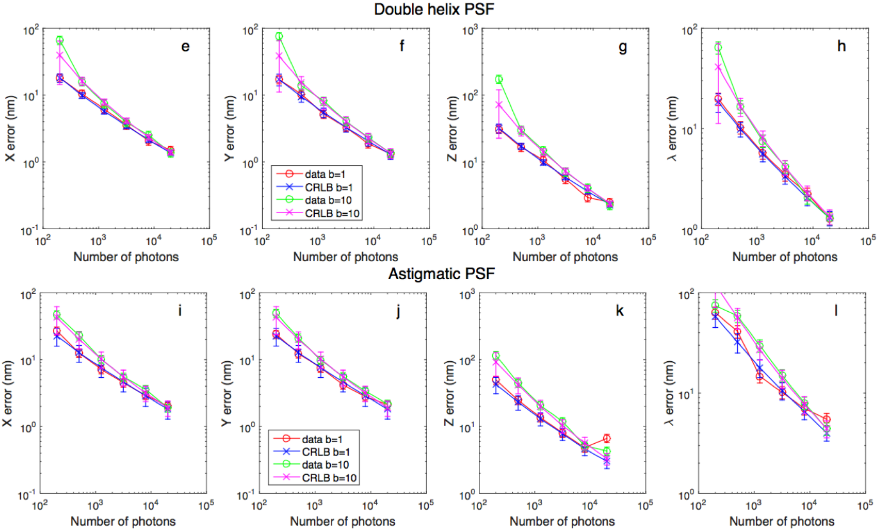

[6] Smith, C. *et al. Optics Express* **24** (2016).

Conclusions

- Takeaways from CRLB calculation
  - Can work with useful accuracy (∼10nm) only when z & λ are disentangled
  - Looks promising for biplane & dual helix PSF
- Next steps:
  - Fluorophore spectrum/dipole moment dependence
    - If there's a performance difference between beads and single molecule, this could be the reason
  - Quantitative comparison with chromatic dispersion-based methods
  - Characterize readout noise
  - Actual PSF & chromatic aberration analysis for microscopes in the lab
    - Tools such as PSFj[7] can be applied
  - Compare to the fitted simulation dataset or experimental data
  - Right fitting algorithm to achieve close-CRLB performance

[7] Theer, P. *et al.* *Nat Meth* **11,** 981–982 (2014).