Optimizing Spectral Differentiation in Super-Resolution Imaging via Chernoff Information

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THE UNIVERSITY of UTAH

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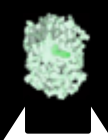
The Diffraction Limit

Green Fluorescent Protein (GFP) is small



~5 nm

Even smaller than the wavelength of light it emits (~500 nm)

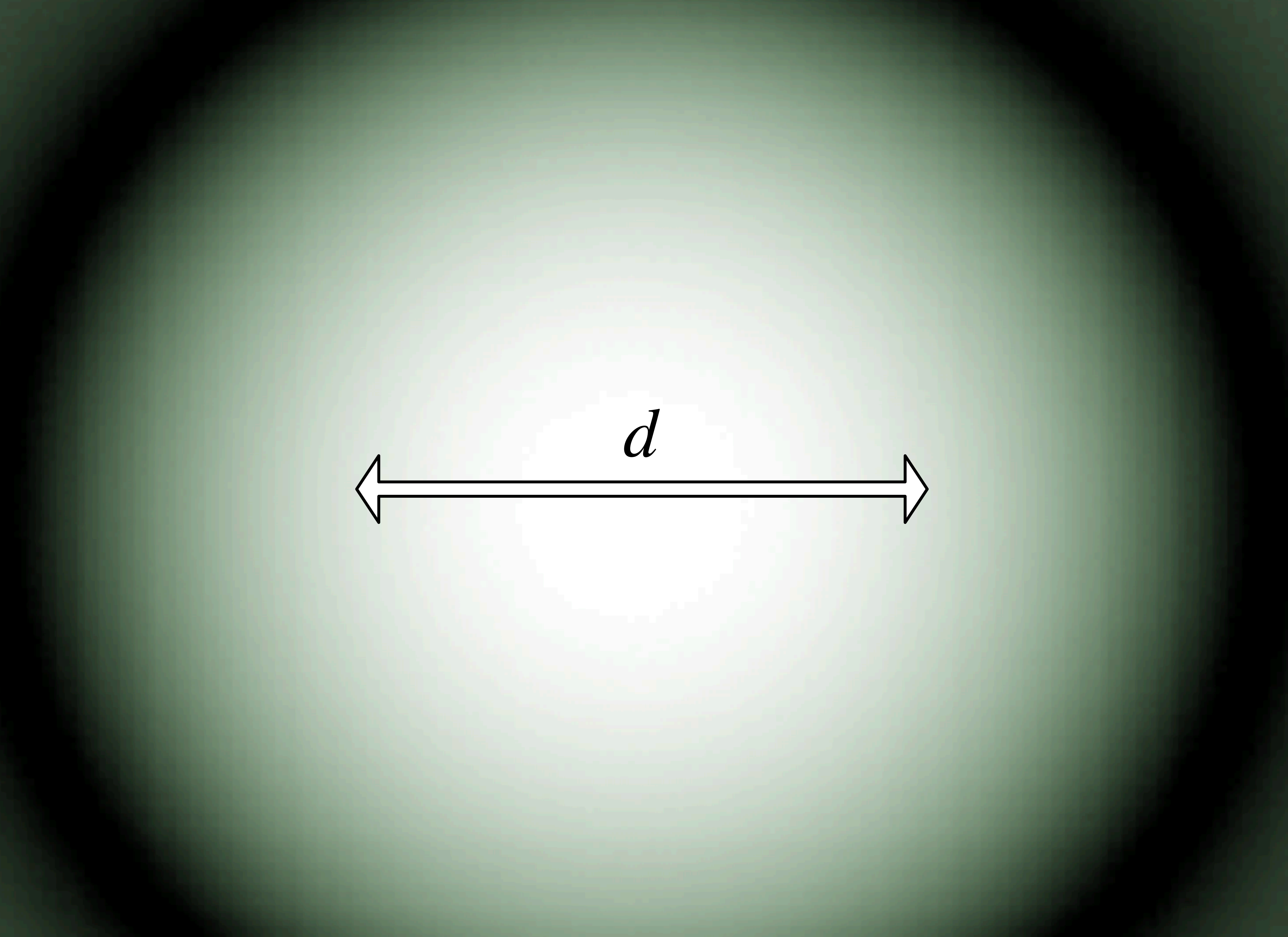
The Diffraction Limit

Interference results in a point-spread function (PSF)

For a perfectly circular aperture, this is known as the Airy disk

The Diffraction Limit

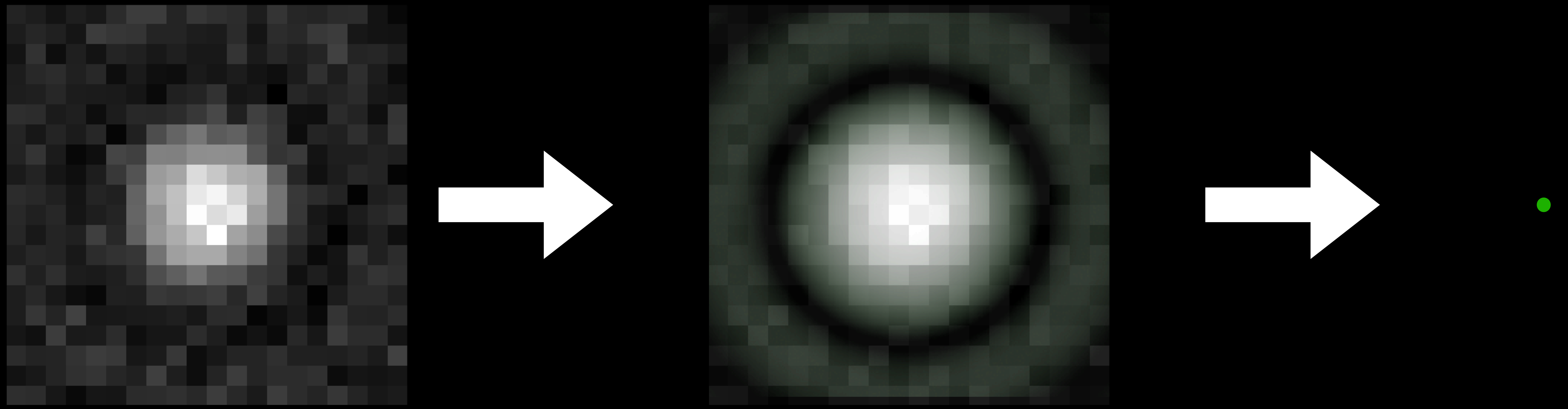
Structures smaller than the critical distance cannot be resolved by traditional light microscopes



Ernst Abbe (1873): $d \geq \dfrac{\lambda}{2NA} \approx 200$ nm

Single Molecule Localization Microscopy (SMLM)

Measure | Fit | Reconstruct



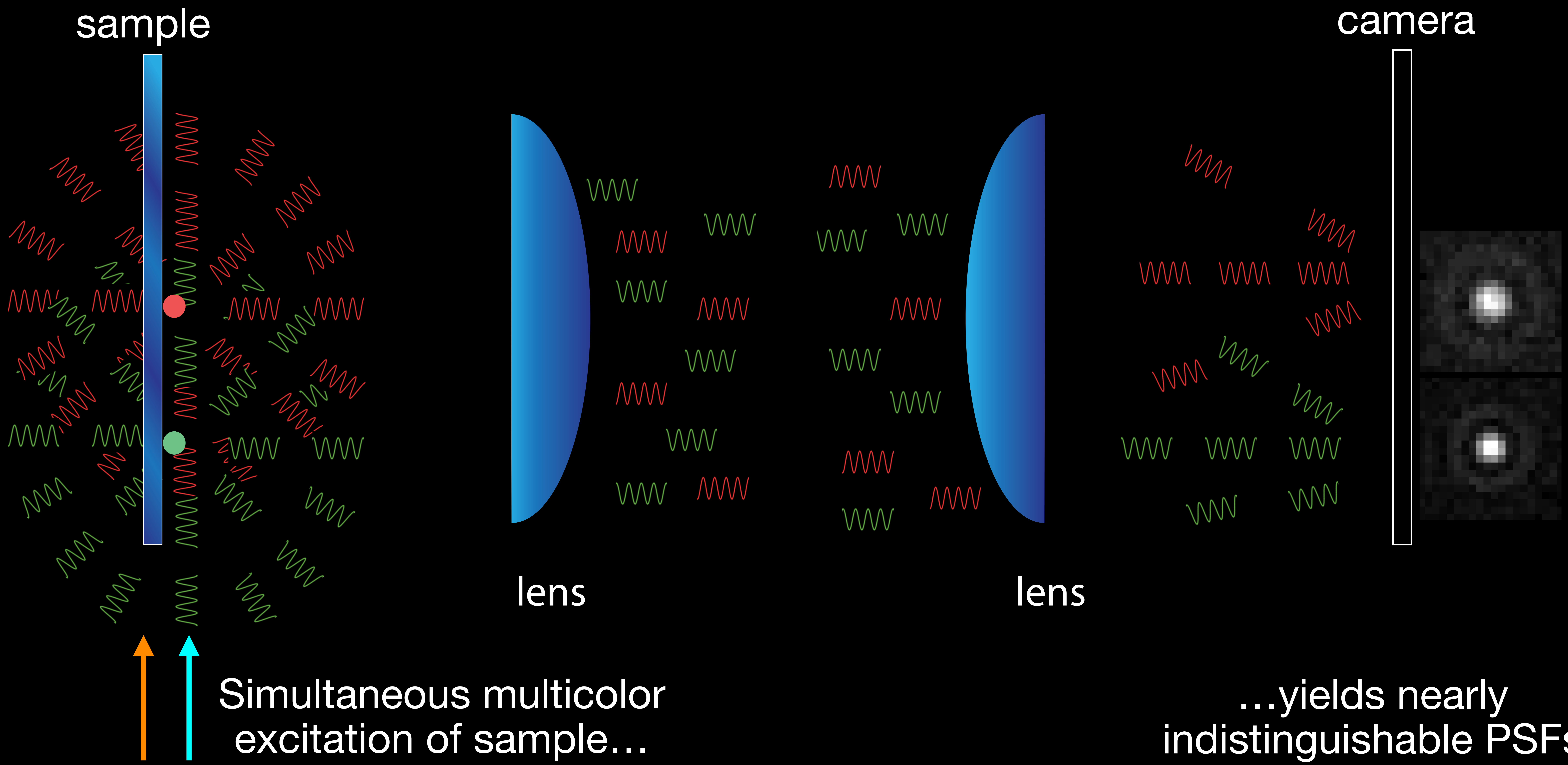
(Disclaimer: PSF is simulated)

STORM, PALM, dna-PAINT, etc

Spatial resolution of the reconstructed image is, at best, the *Cramer-Rao Bound* (~10 nm)

What about color information?

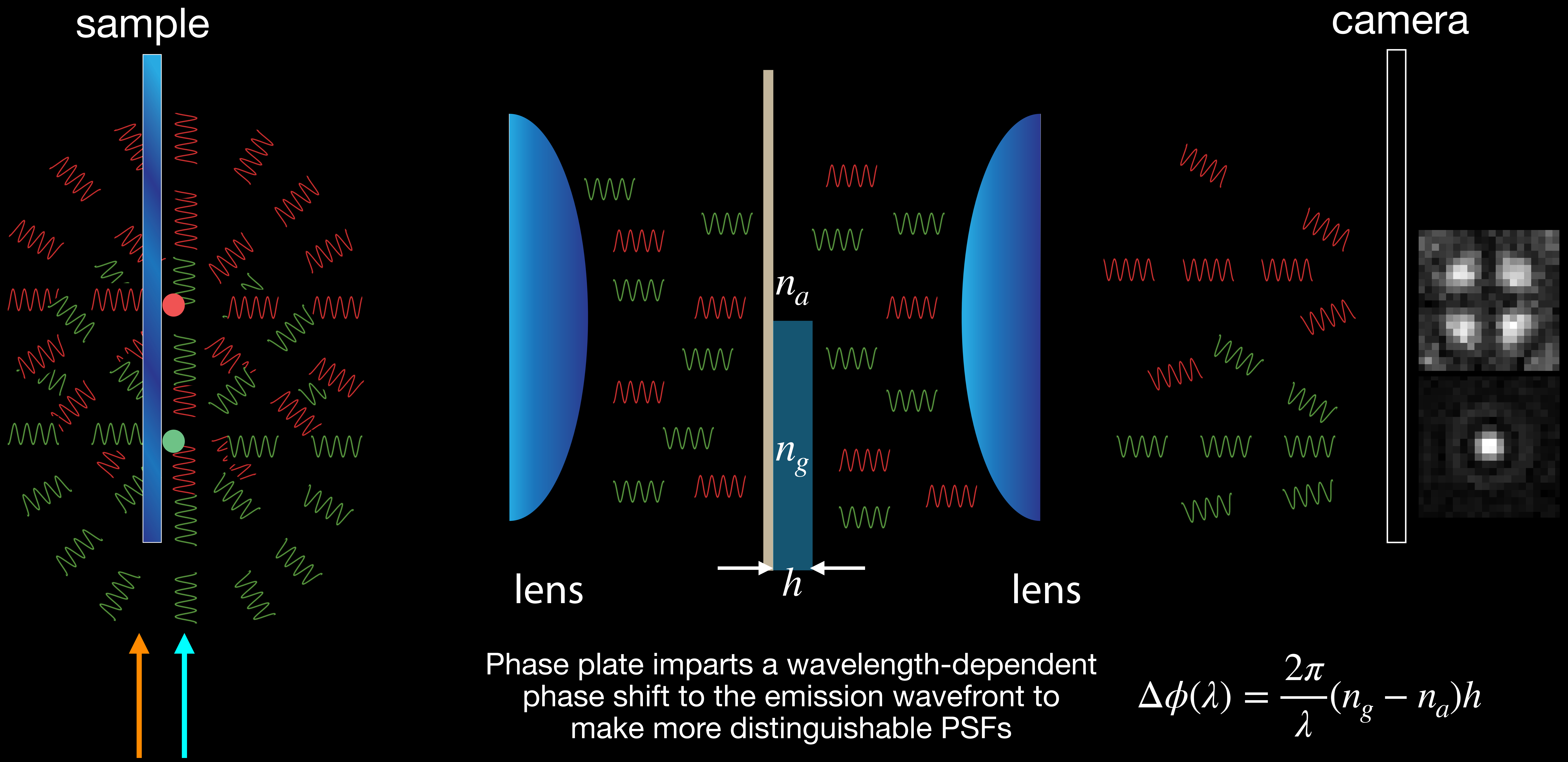
Simultaneous Multicolor SMLM?



Simultaneous multicolor excitation of sample…

…yields nearly indistinguishable PSFs

PSF Engineering with a Glass Phase Plate

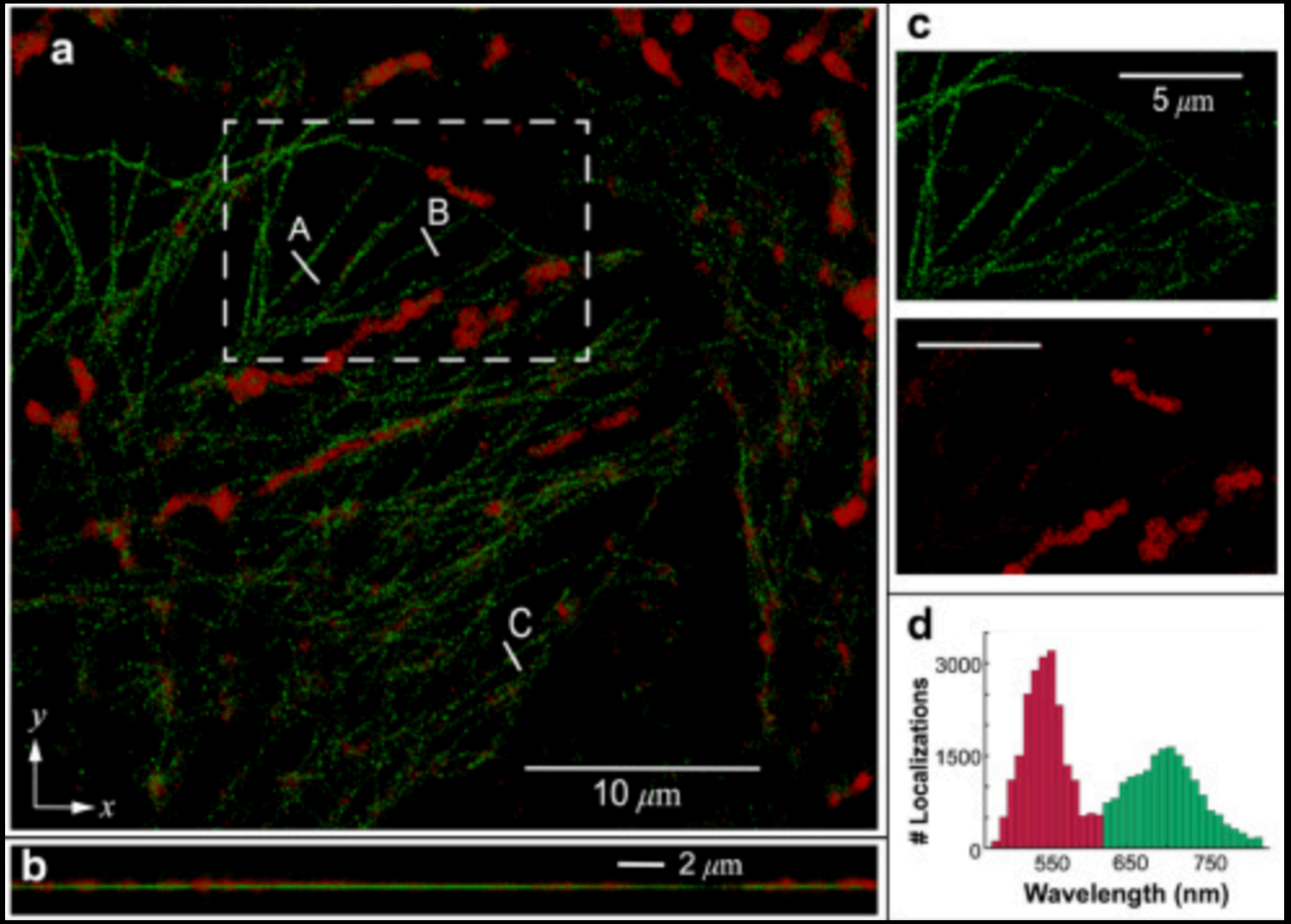


Phase plate imparts a wavelength-dependent phase shift to the emission wavefront to make more distinguishable PSFs

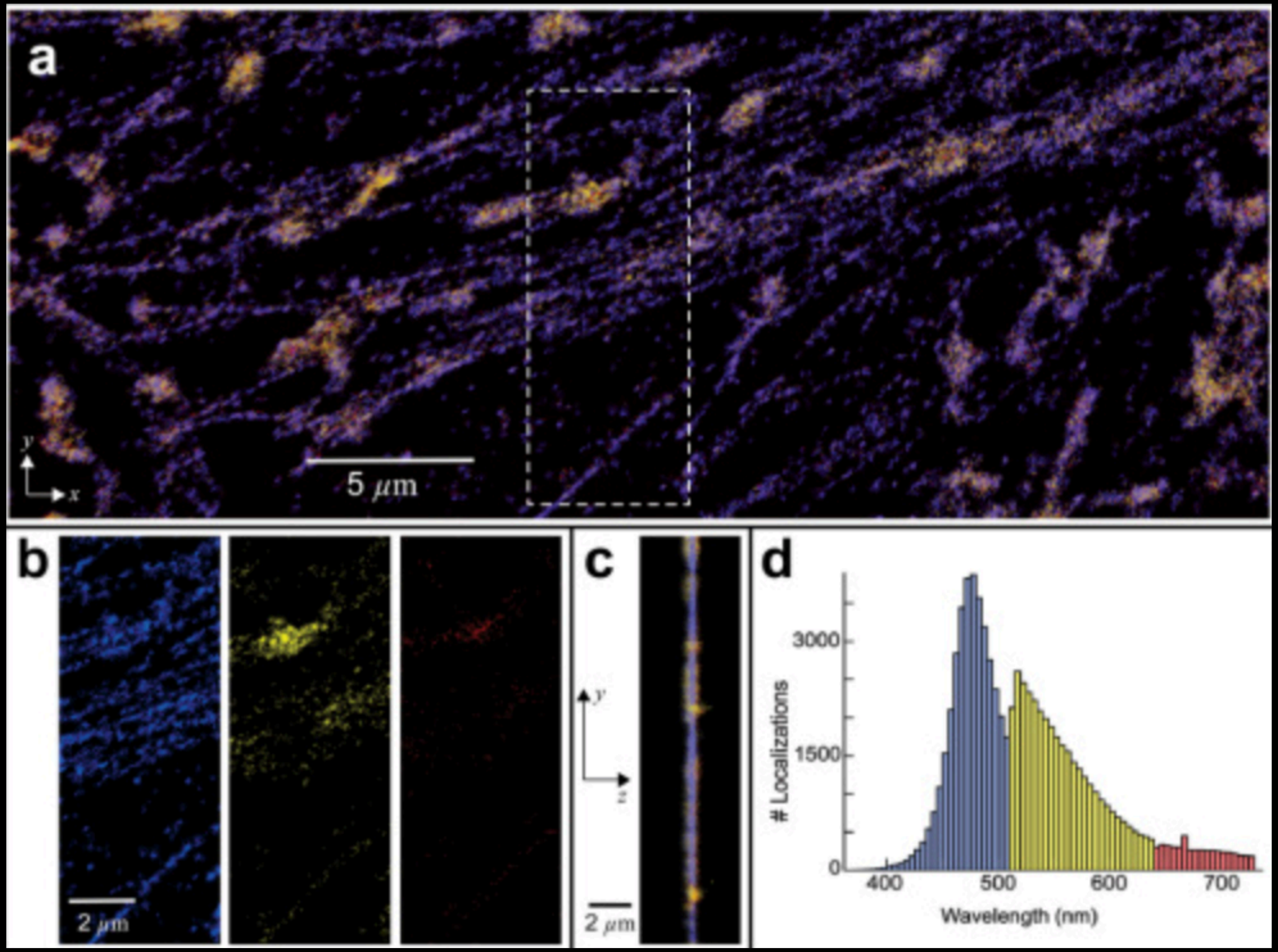
$$\Delta\phi(\lambda) = \frac{2\pi}{\lambda}(n_g - n_a)h$$

It Really Works!

Good distinguishability for 2 colors



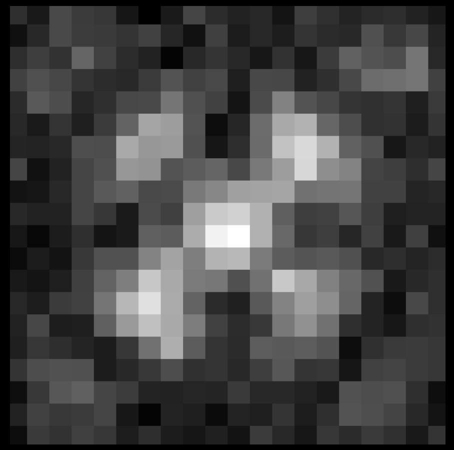
Worse distinguishability for 3 colors



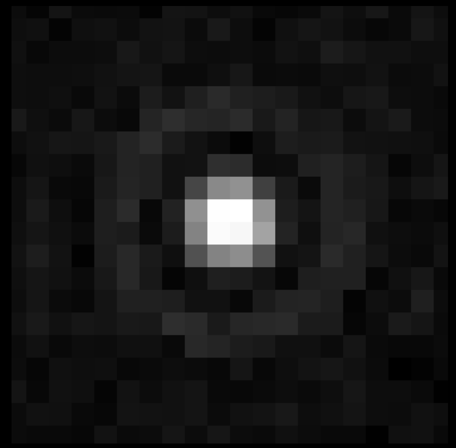
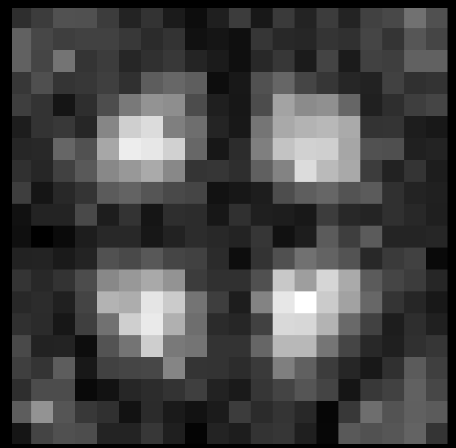
What's the best we could do?

Chernoff Information

Suppose we perform hypothesis tests to classify

$\nu =$ 

as either

$\nu_1 =$  or $\nu_2 =$ 

Chernoff Information tightly bounds Bayes' probability of error when classifying via hypothesis testing (similar to CRB/Fisher Information)

The Chernoff Information between an observation ν and a known class ν_i (under some assumptions) is

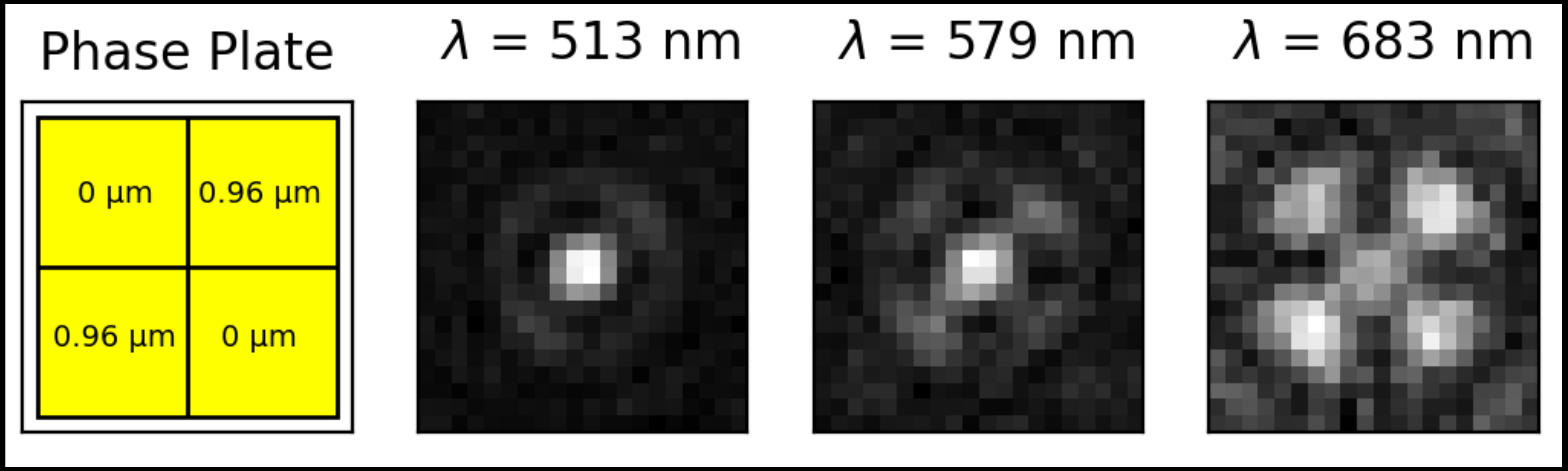
$$C_i(\beta) = \nu_i \frac{(\beta - 1)(\log(\frac{\beta - 1}{\log \beta}) - 1) + \log \beta}{\log \beta}; \quad \beta = \frac{\nu}{\nu_i}$$

If $C_1 < C_2 \implies \nu \to \nu_1$, else $\nu \to \nu_2$

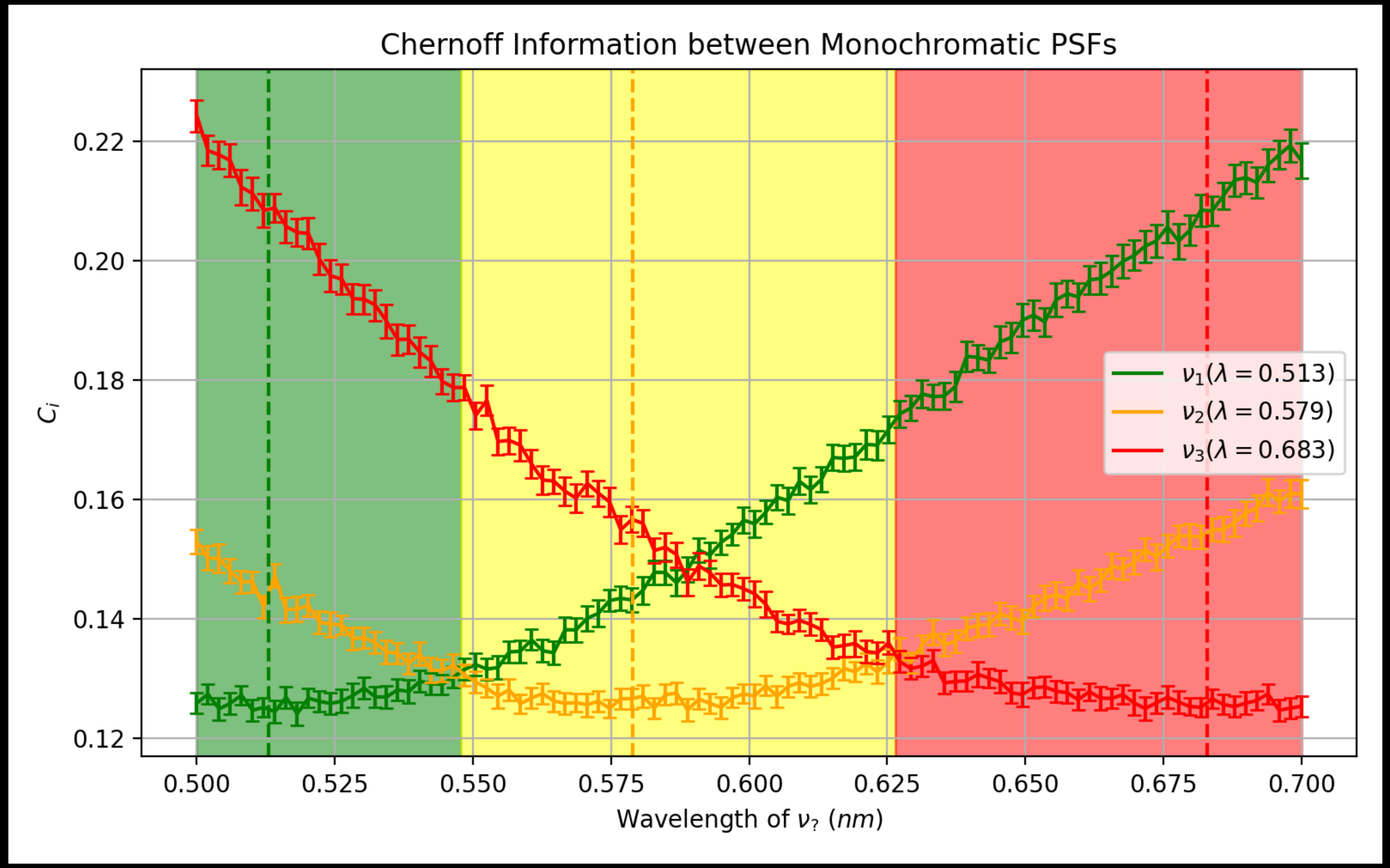
Tightest bound = best decision (least probability of error)

Symmetric X-PP is Optimal for 2 Colors

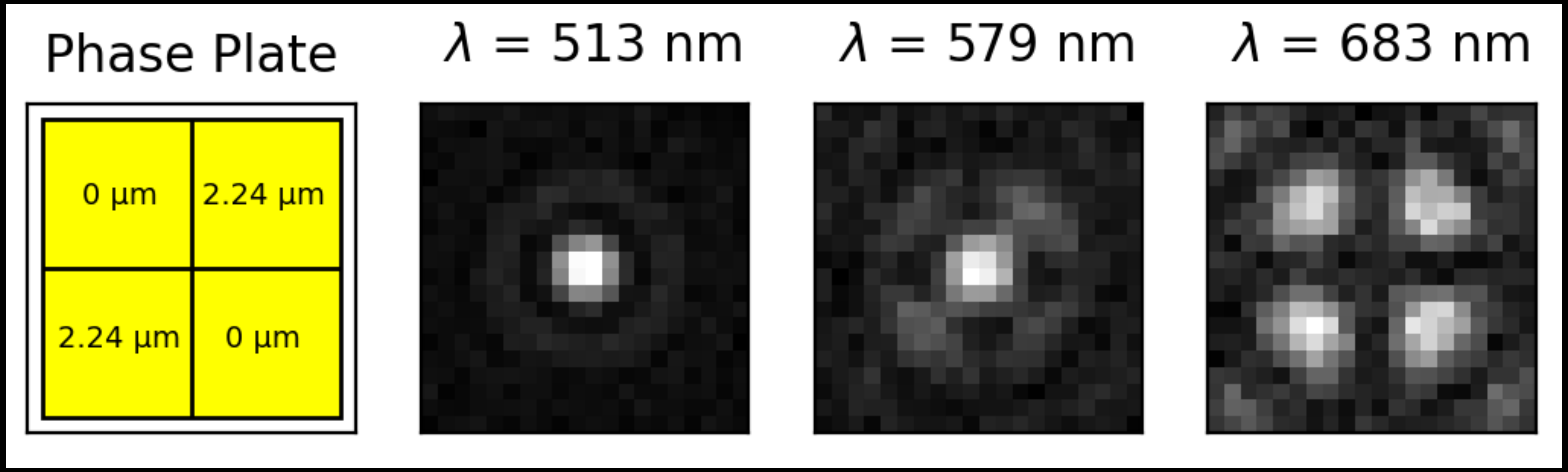
Original X-PP

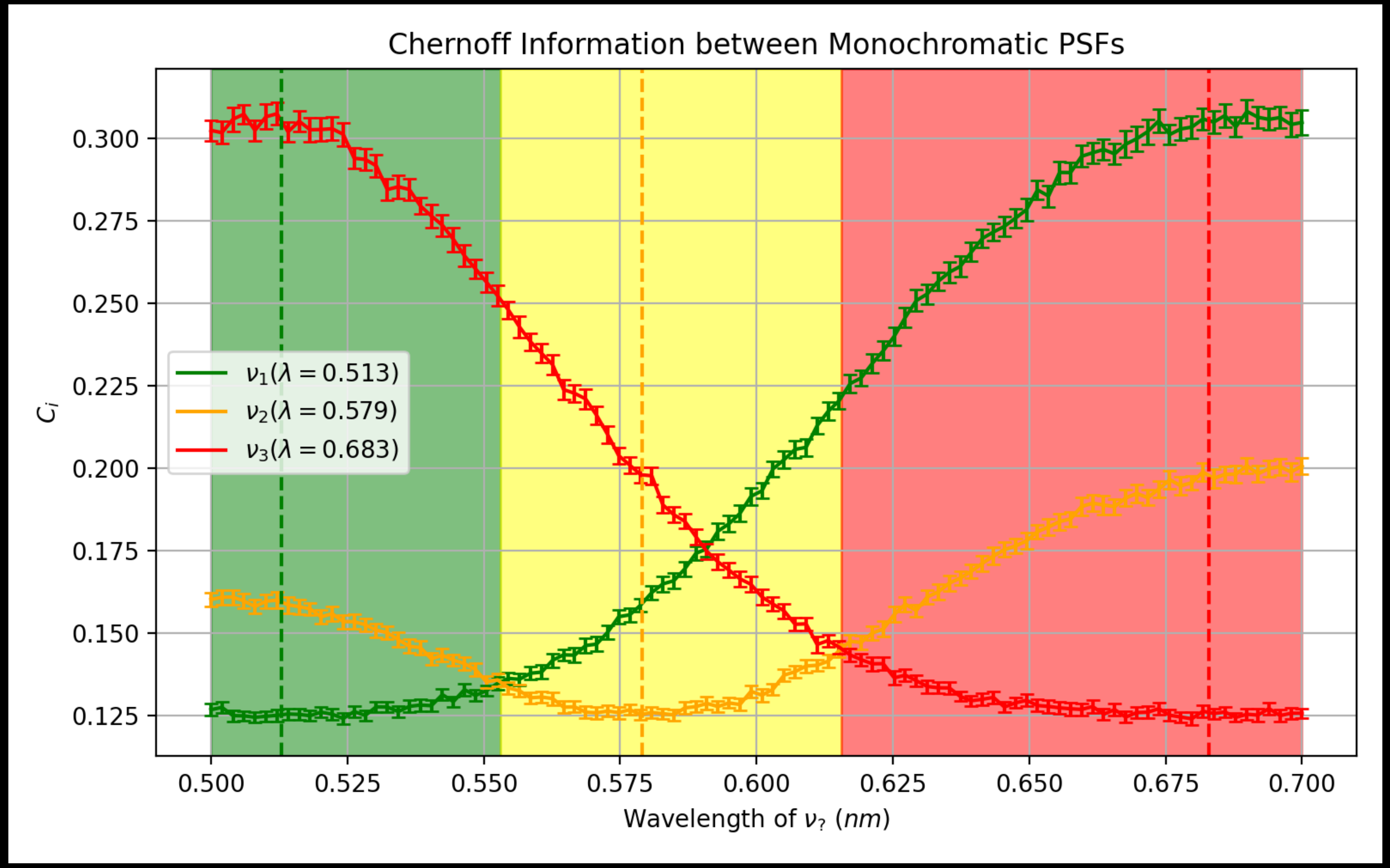


(Optimal for PSFs of wavelengths λ = 440 nm and λ = 880 nm)

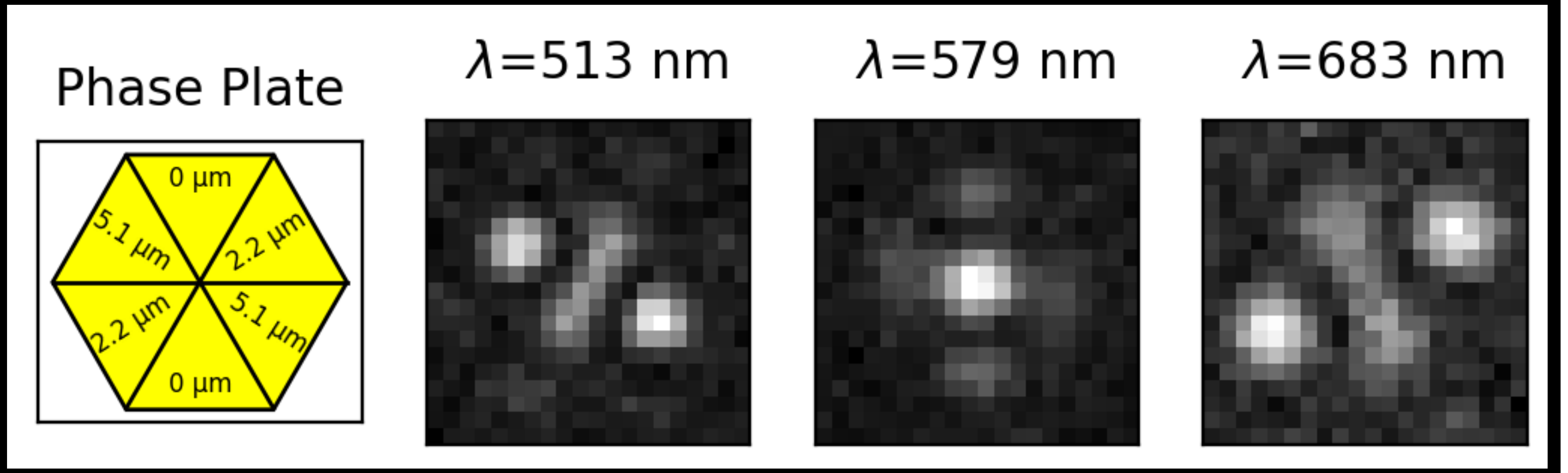


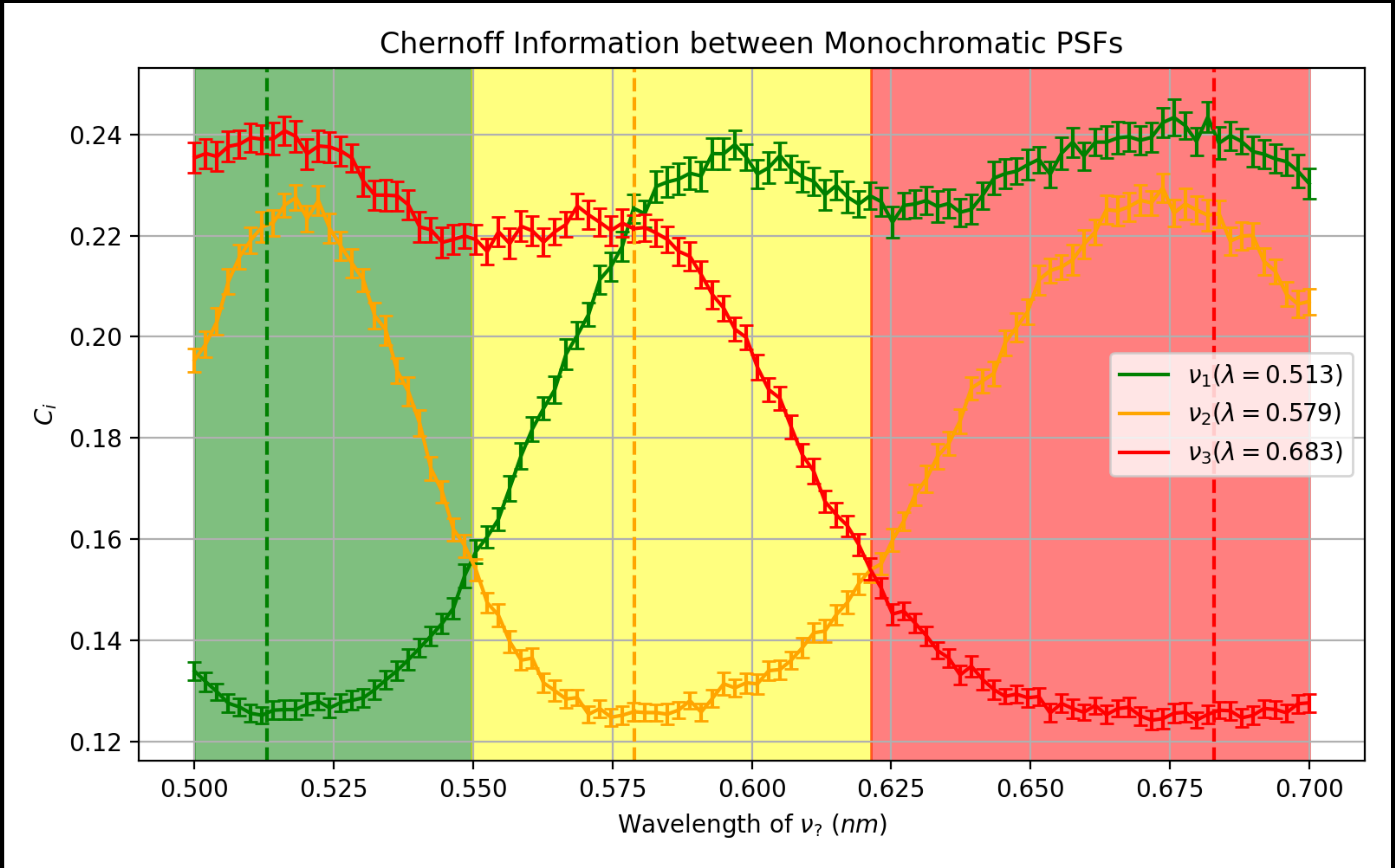
Chernoff-Information-Maximized X-PP





Symmetric Hex-PP is Optimal for 3 Colors





Diffraction-Limited Fluorescent Microspheres

The **0.1 µm TetraSpeck™ microspheres** are stained throughout with four different fluorescent dyes, yielding beads that each display four well-separated excitation/emission peaks - 360/430 nm (blue), 505/515 nm (green), 560/580 nm (orange) and 660/680 (dark red).

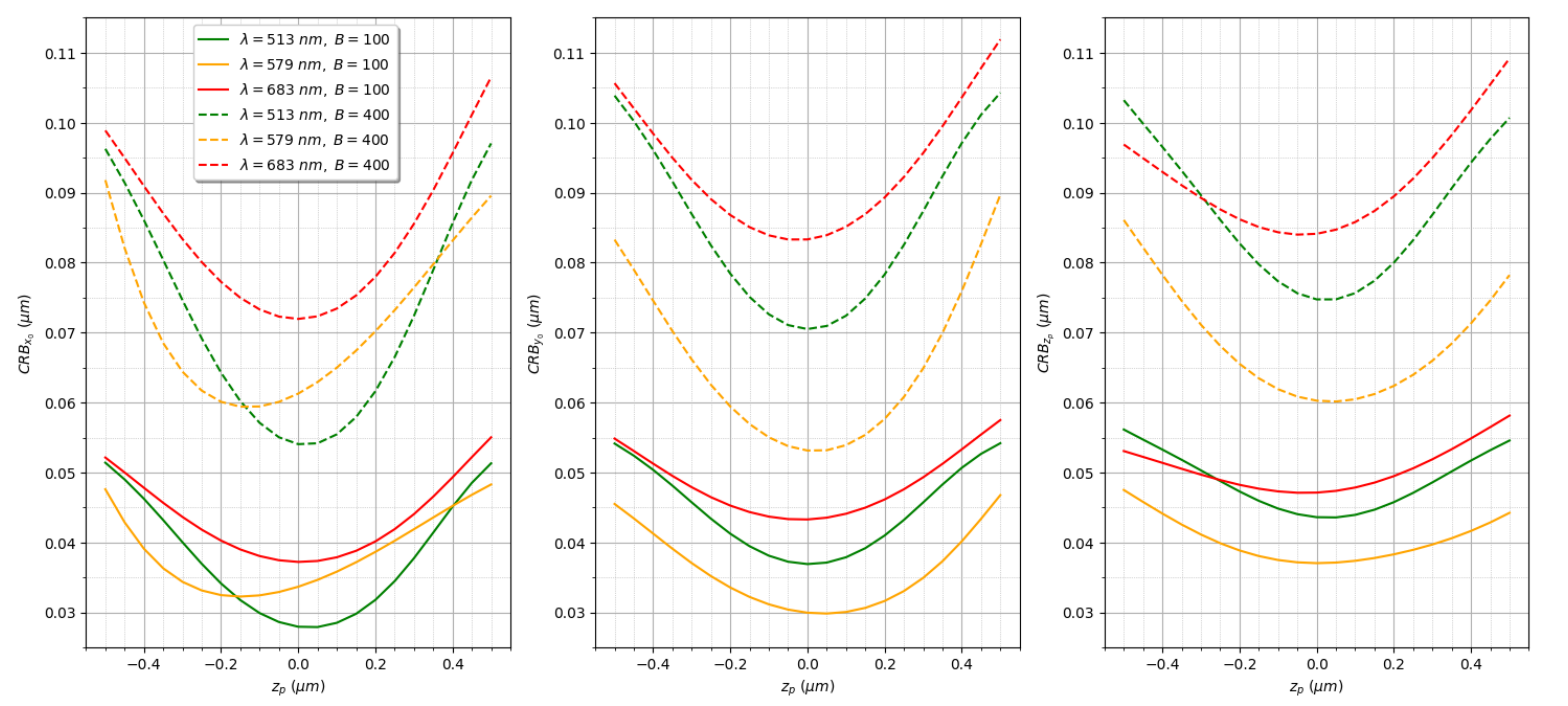
$\lambda_{exc} = 488$ nm

$\lambda_{exc} = 568$ nm

$\lambda_{exc} = 647$ nm

Improved Spectral Classification…

… at the Cost of Spatial Resolution



Thank You!