A model for wavefront coding in high numerical aperture microscopy


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Outline

• Overview of how we use wavefront coding to extend the depth of field, showing previous high numerical aperture (NA) experimental results

• Why we might need a high NA model for wavefront coding

• Compare high NA point spread function model with the paraxial model

• Example of the impact of high NA results on imaging quality

• High NA transfer function theory for wavefront coding

• Conclusion
Why extend the depth of field?

For 3D real-time fluorescence imaging of live-cell dynamics

cnfocal and widefield microscopes are often too slow,

because they require sequential acquisition of many planes of focus to build up an image of a 3D object.
Solution: extend the depth of field

Our high-speed wavefront coding EDF fluorescence microscope:

* uses only a single exposure on a CCD

* followed by a single-step digital filter, which can run at video rates

* maintains high NA resolution, the tradeoff is a drop in signal to noise

* may also reduce photo-bleaching
Standard widefield fluorescence

Focal depth: 1µm

Specimen: human Hela cancer cells, imaged with 40x 1.3 NA oil lens.

scale = 6µm
EDF fluorescence

Specimen: human Hela cancer cells, imaged with 40x 1.3 NA oil lens.

Focal depth: 10µm

scale = 6µm
Diagram of wavefront coding system

- Object
- Objective Lens
- Phase Plate
- Encoder
- Dichroic Beam Splitter
- CCD
- Intermediated Image (blurred)
- Decoder
- Final Image
- Hg Arc Lamp
- Cubic Phase Plate w/ Square Aperture Mask

Cubic Phase Plate w/ Square Aperture Mask

Diagram shows the flow of light through the system, starting with the object, passing through the objective lens and phase plate, encoded by the dichroic beam splitter, captured by the CCD, subjected to deconvolution, and producing the final image.
The special *cubic phase plate* (CPP) has thickness corresponding to this 2-D function of spatial position:

\[ P(x, y) = C(x^3 + y^3) \]

The phase plate function “encodes” the wavefront, allowing for simple post-processing.
Point spread and modulation transfer functions: measured performance @ 40x 1.3 NA

EDF in a fluorescence microscope

Z = 0µm    Z = 5µm

Standard (a, b) vs. Cubic Phase Plate (c, d) PSFs

Standard vs. Cubic Phase Plate MTFs
High numerical aperture model

* Main tradeoff for wavefront coding is signal to noise (SNR).

* To maximise SNR we need accurate models of imaging performance.

* Wavefront coding was developed using the paraxial approximation.

* For NA higher than 0.5, paraxial optics becomes increasingly inaccurate.

* For best resolution in fluorescence microscopy, we use 1.3 NA, so high NA theory is essential for accurate modeling.
High NA point spread function (PSF) model

We simulate the system at high numerical aperture using the Rayleigh-Sommerfield diffraction formula.

The field $E$ is calculated by integrating across a square aperture.

$$E(x_p, y_p, z_p) = \int_{-a}^{a} \int_{-a}^{a} \exp(ik\varphi(x, y)) \frac{\exp(ik(R - r))}{rR} \frac{z_p + f}{R} dxdy$$

Where the cubic phase function is given by:

$$\varphi(x, y) = C(x^3 + y^3)$$
Theoretical point spread functions

Simulating a 40x 1.3 NA oil lens, as used for the experimental images.
High NA

Theoretical PSF: lateral intensity

Simulating a 40x 1.3 NA oil lens, as used for the experimental images.
3D transfer functions

- To see if we maintain high resolution we need to analyse the frequency response of the imaging system.

- Previous 3D transfer function calculations assume paraxial rays (Frieden, JOSA 57:1 p56 1967) or a radially symmetric pupil function (Sheppard, JOSA A, 11:2 p593 1994).

- Wavefront coding applies phase functions across the pupil which are not radially symmetric, e.g:

\[ \varphi(x, y) = C(x^3 + y^3) \]

- We therefore needed to generalise the 3D transfer function integrals to deal with arbitrary pupil functions.
Correlation of pupil functions: paraxial approximation

\[ K(m,n) \]
Correlation of 3D pupil functions

\[ \alpha = \pi/2 \]

\[ \alpha = \pi/3 \]
Correlation of 3D pupil functions

\[ \alpha = \frac{\pi}{2} \]
Correlation integral

\[ P(m_1, n_1, s_1) = \begin{cases} \frac{S(m_1, n_1)e^{2\pi\phi(m_1, n_1)}}{\sqrt{m_1^2 + n_1^2 + s_1^2}}, & \sqrt{m_1^2 + n_1^2 + s_1^2} = 1 \\ 0, & \text{otherwise} \end{cases} \]

\[ K = (m, n, s) \quad l = \sqrt{m^2 + n^2} \quad r_{ci} = \sqrt{1 - \frac{K^2}{4}} \]

\[ r_{ci}(K, \beta) = \begin{pmatrix} r_{ci} \left[ \frac{8}{K} \cos \beta \cos \left( \arctan(n/m) \right) - \sin \beta \sin \left( \arctan(n/m) \right) \right] \\ r_{ci} \left[ \frac{8}{K} \cos \beta \sin \left( \arctan(n/m) \right) + \sin \beta \cos \left( \arctan(n/m) \right) \right] \\ -r_{ci} \frac{1}{K} \cos \beta \end{pmatrix} \]

\[ C(K) = \int_{-\beta_1}^{\beta_1} P(r_{ci}(K, \beta) + \frac{K}{2})P^{*}(r_{ci}(K, \beta) - \frac{K}{2})d\beta \]
Defocussed optical transfer functions: early results

High NA ($\alpha=\pi/3$)
No cubic phase

Defocus added as an aberration function across pupil
Defocussed optical transfer functions: early results

High NA ($\alpha=\pi/3$)
No cubic phase

High NA ($\alpha=\pi/3$)
Strong cubic phase
(C=30)
Conclusion:

Wavefront coding is a new approach to microscopy. Instead of avoiding aberrations, we exploit them.

The behaviour of aberrations is significantly altered at high NA.

We have extended the wavefront coding model to high NA scalar theory for both point spread function and transfer function calculations.

This gives us more insight into potential noise sources, and gives us the tools to design second generation wavefront coding systems which further improve imaging performance.