High resolution extended depth of field microscopy using wavefront coding

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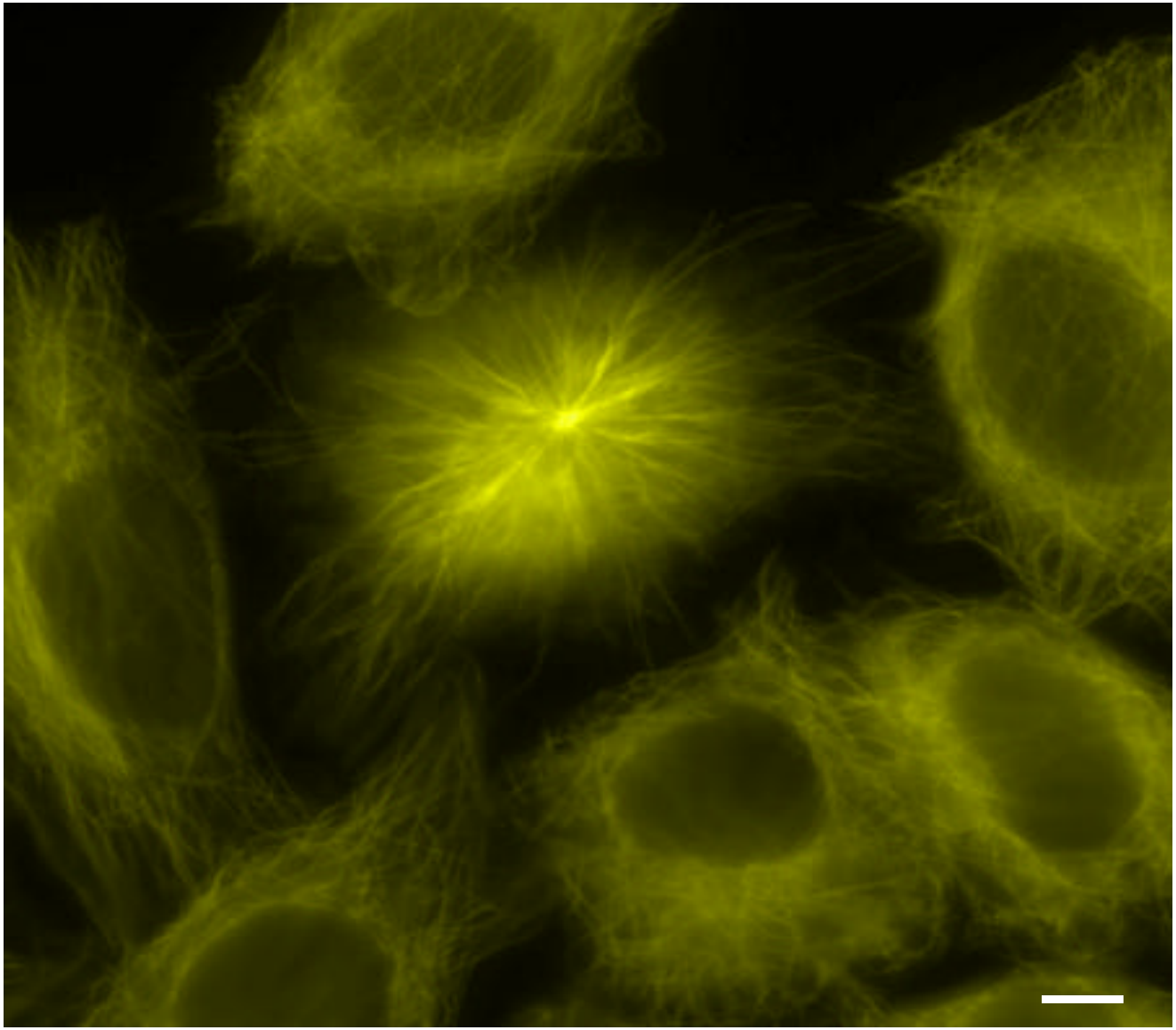
The Problem:

For 3D real-time fluorescence imaging of live-cell dynamics and *in vivo* processes,

confocal and widefield (deconvolution) microscopes are often too slow,

because they require sequential acquisition of many planes of focus to build up a 3D image.

Standard Fluorescence

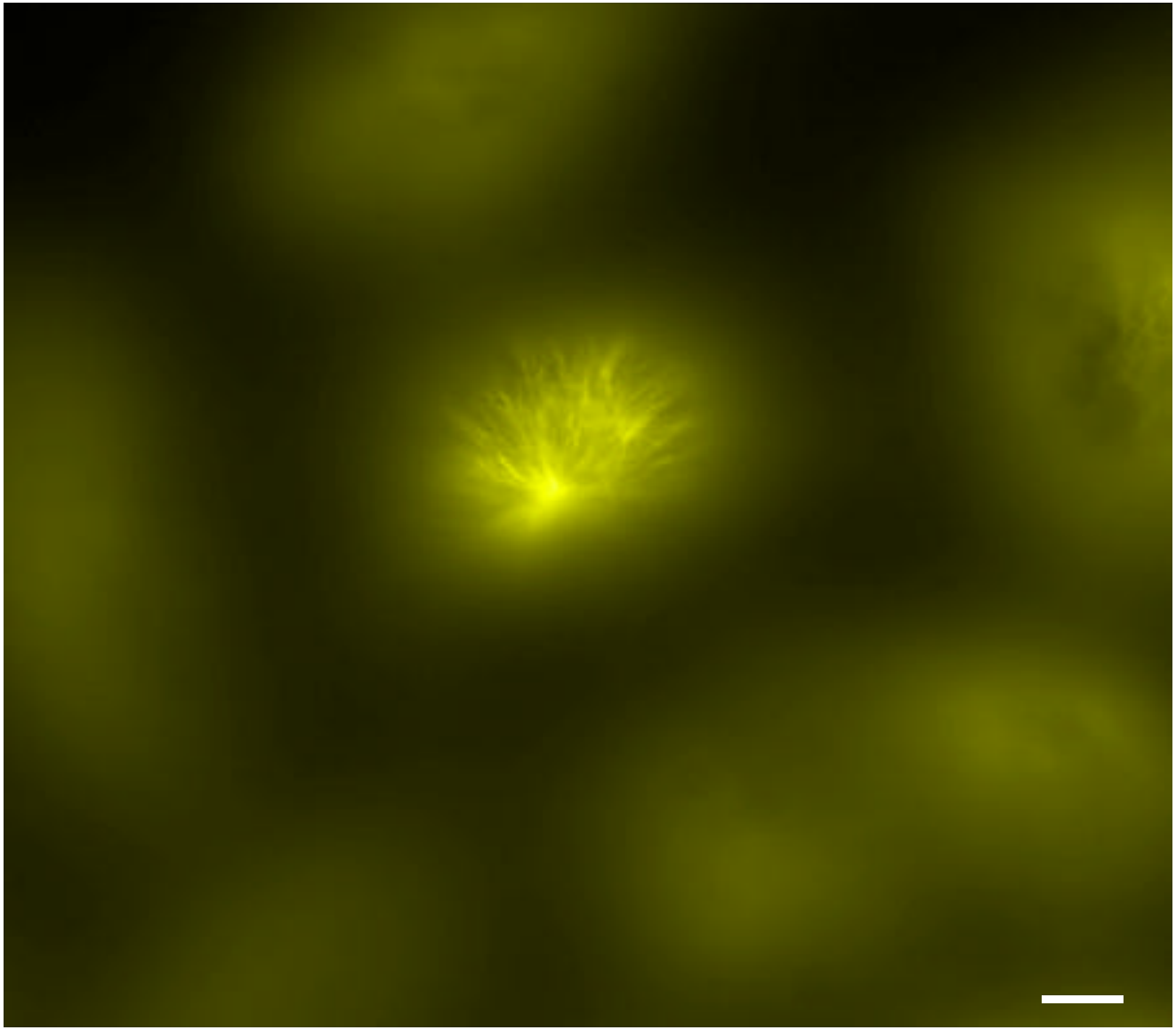


scale = 6µm

Focus at 1µm depth

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

Standard Fluorescence



scale = 6µm

Focus at 7µm depth

Solution: Extend the Depth of Field

Our high-speed EDF fluorescence microscope:

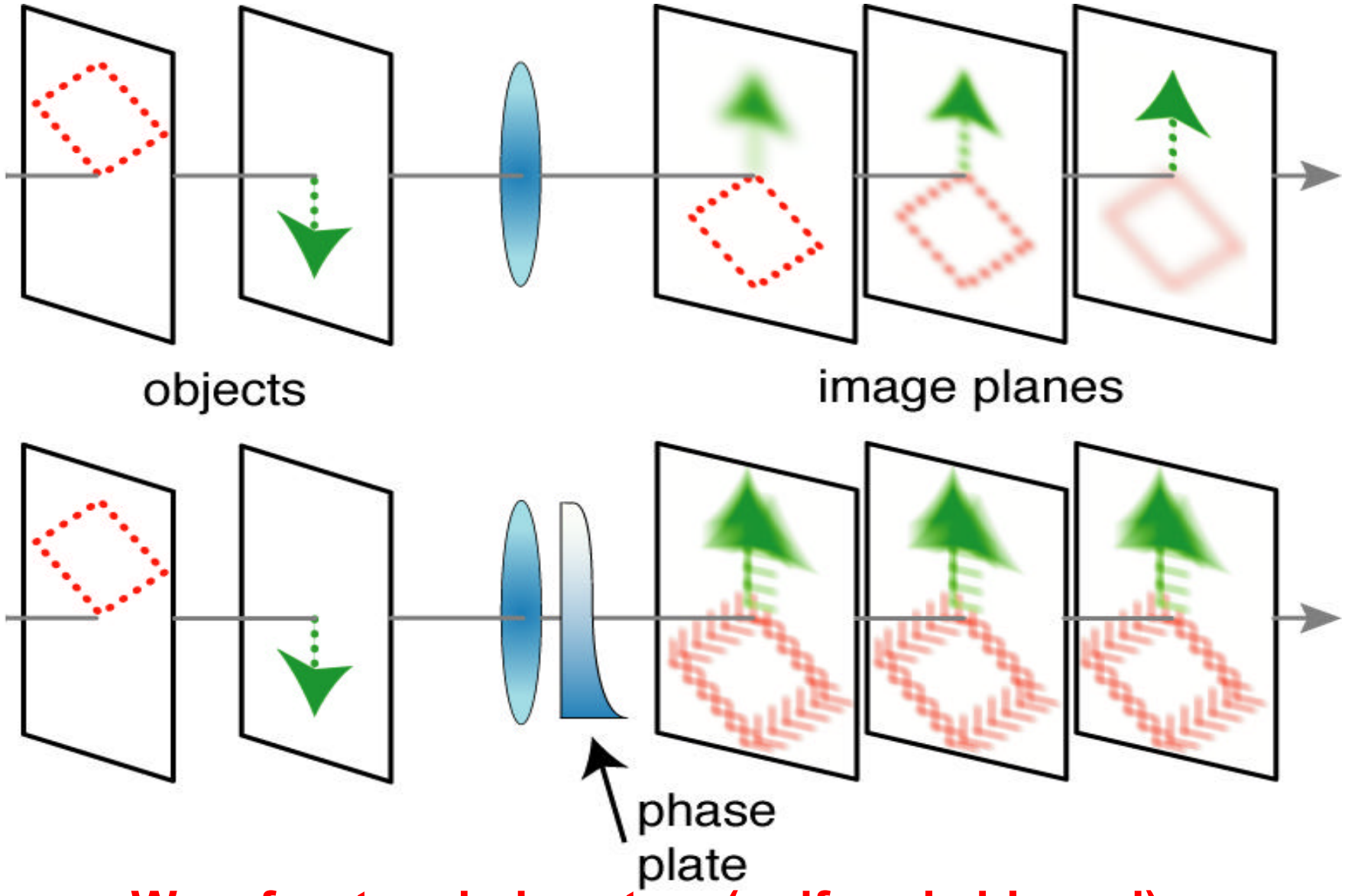
* uses only a single exposure on a CCD

* 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

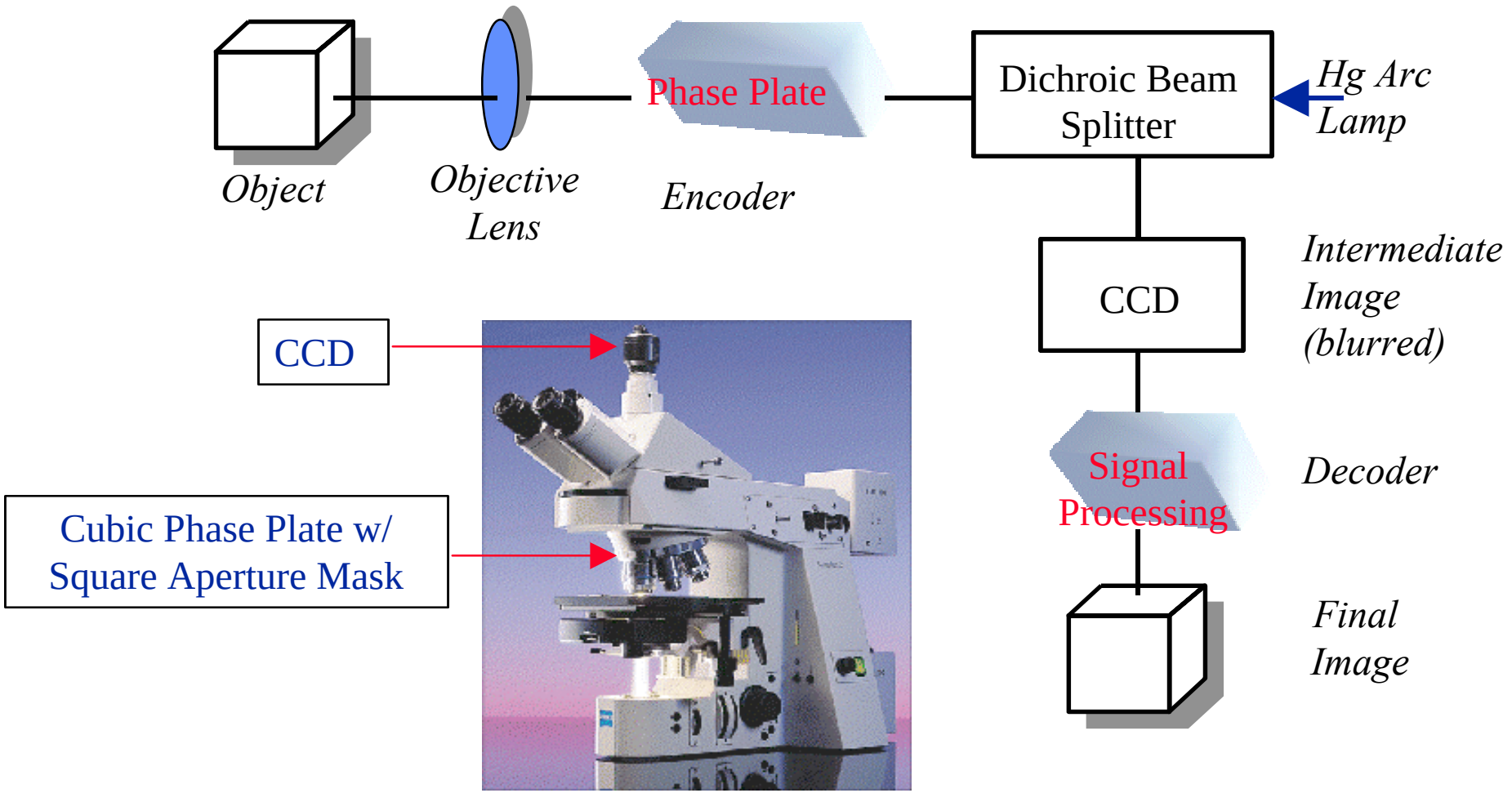
* may also reduce photo-bleaching

Normal optical system (limited depth-of-focus)



Wavefront coded system (uniformly blurred)

Diagram of EDF Optical/Digital System

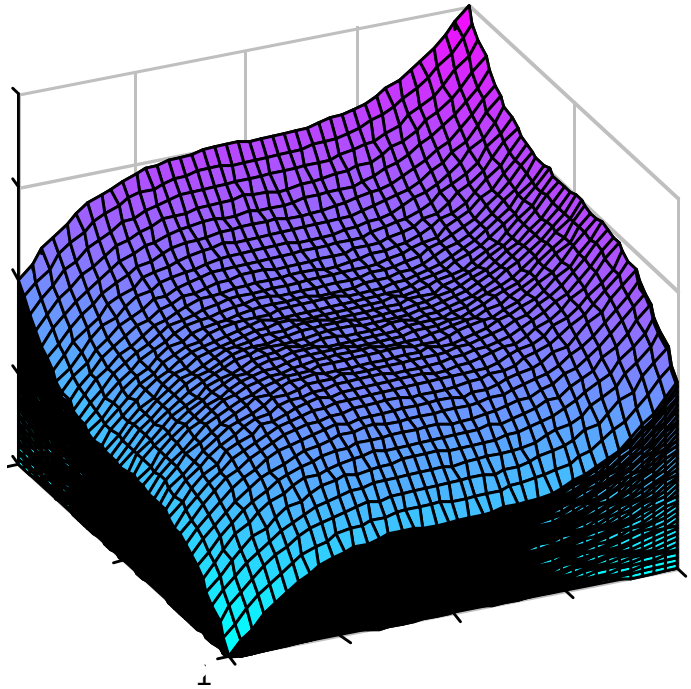


Cubic Phase Plate

The special *cubic phase plate* (CPP) has thickness corresponding to this 2-D function of spatial position:

$$P(x, y) = a(x^3 + y^3)$$

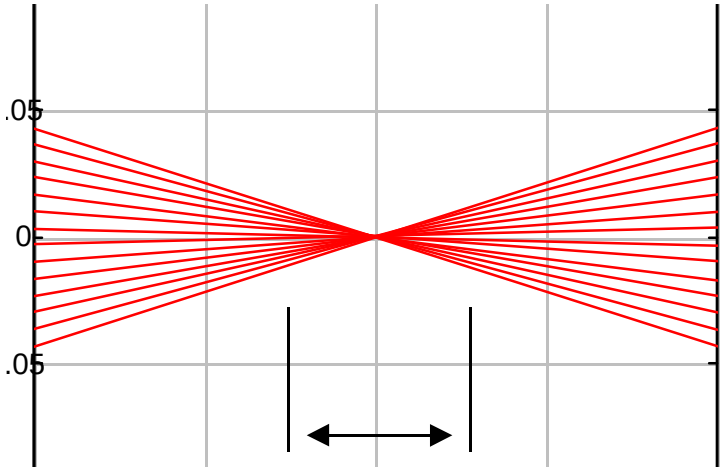
The phase plate function "encodes" the wavefront, allowing for simple post-processing.



Cubic Phase Plate
Phase Plot

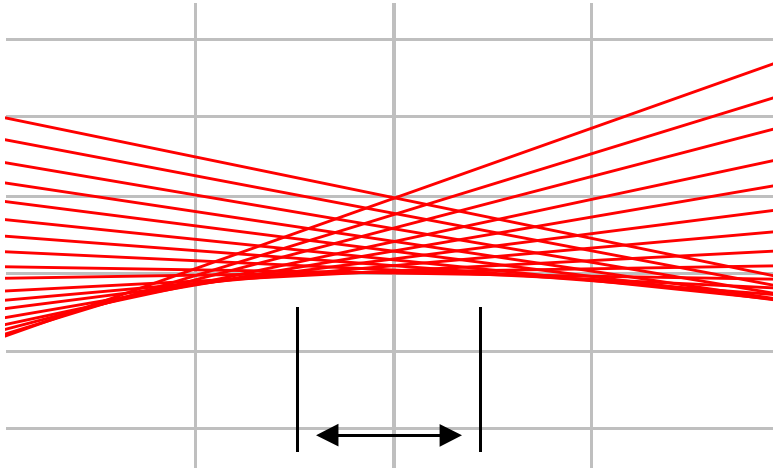
Conventional Lens vs Cubic Phase Plate (CPP) Ray Traces

Conventional microscope (no CPP)



No extended range of focus

EDF microscope (with CPP)



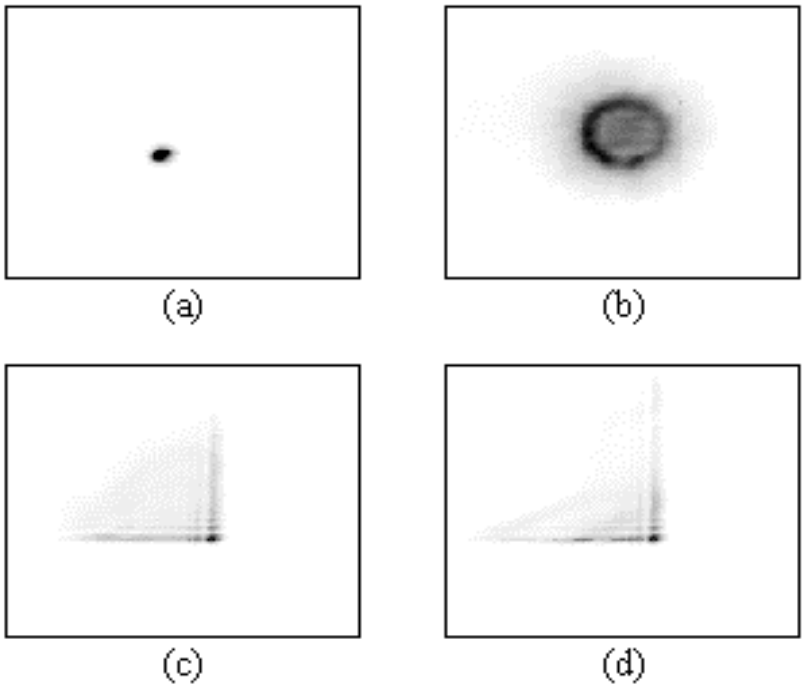
Extended range of focus

With the addition of a CPP, focus invariance is extended along the z axis by an amount determined by the properties of the CPP and the lens numerical aperture.

Focus Invariance:

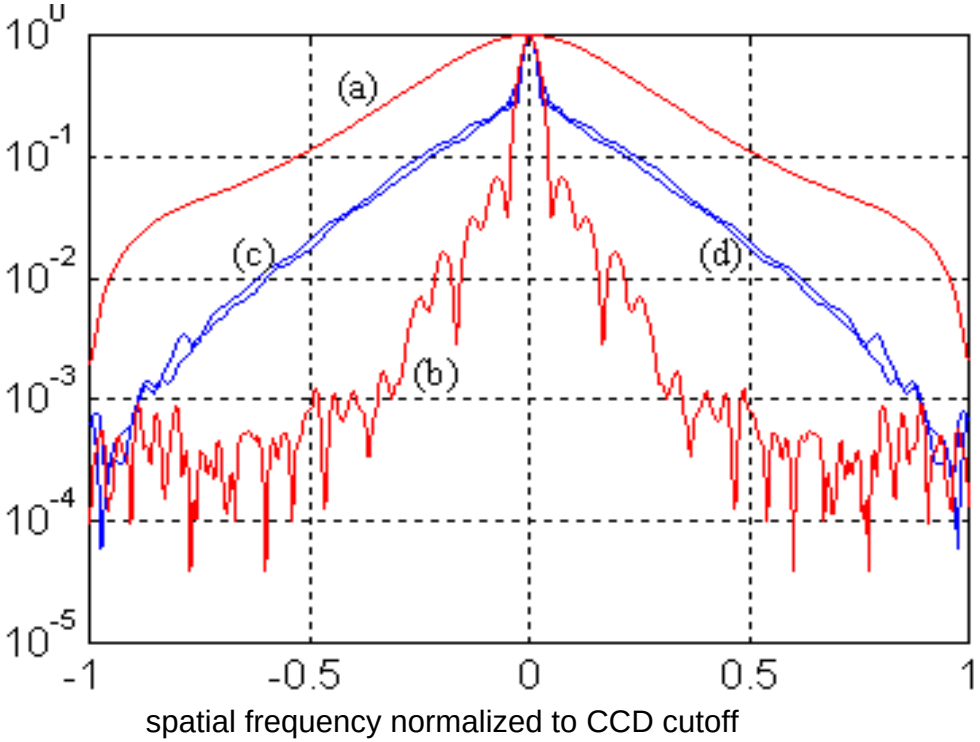
Point Spread and Modulation Transfer Functions

EDF in a fluorescence microscope



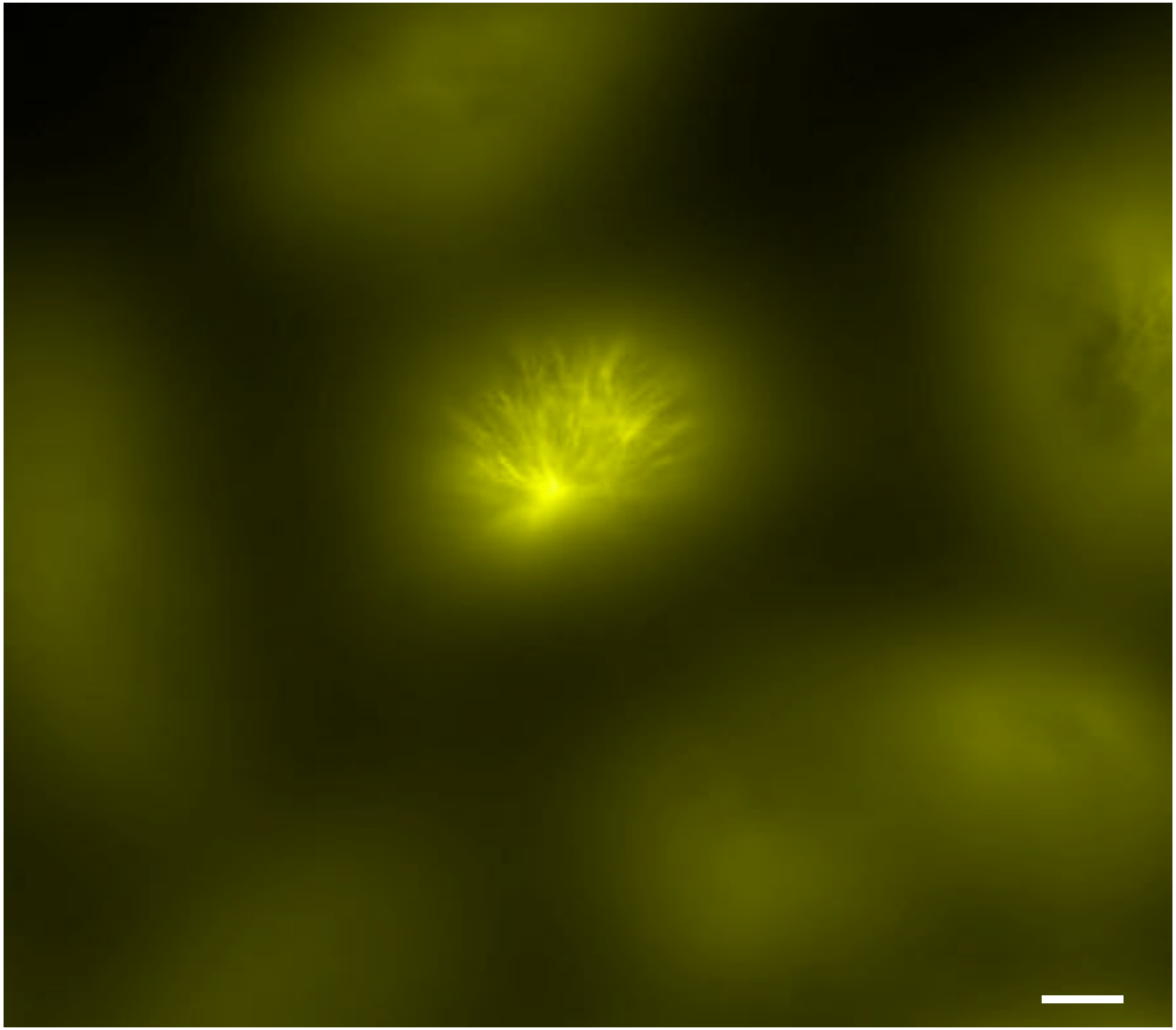
Z = 0µm Z = 5µm

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



Standard vs. Cubic Phase
Plate MTFs

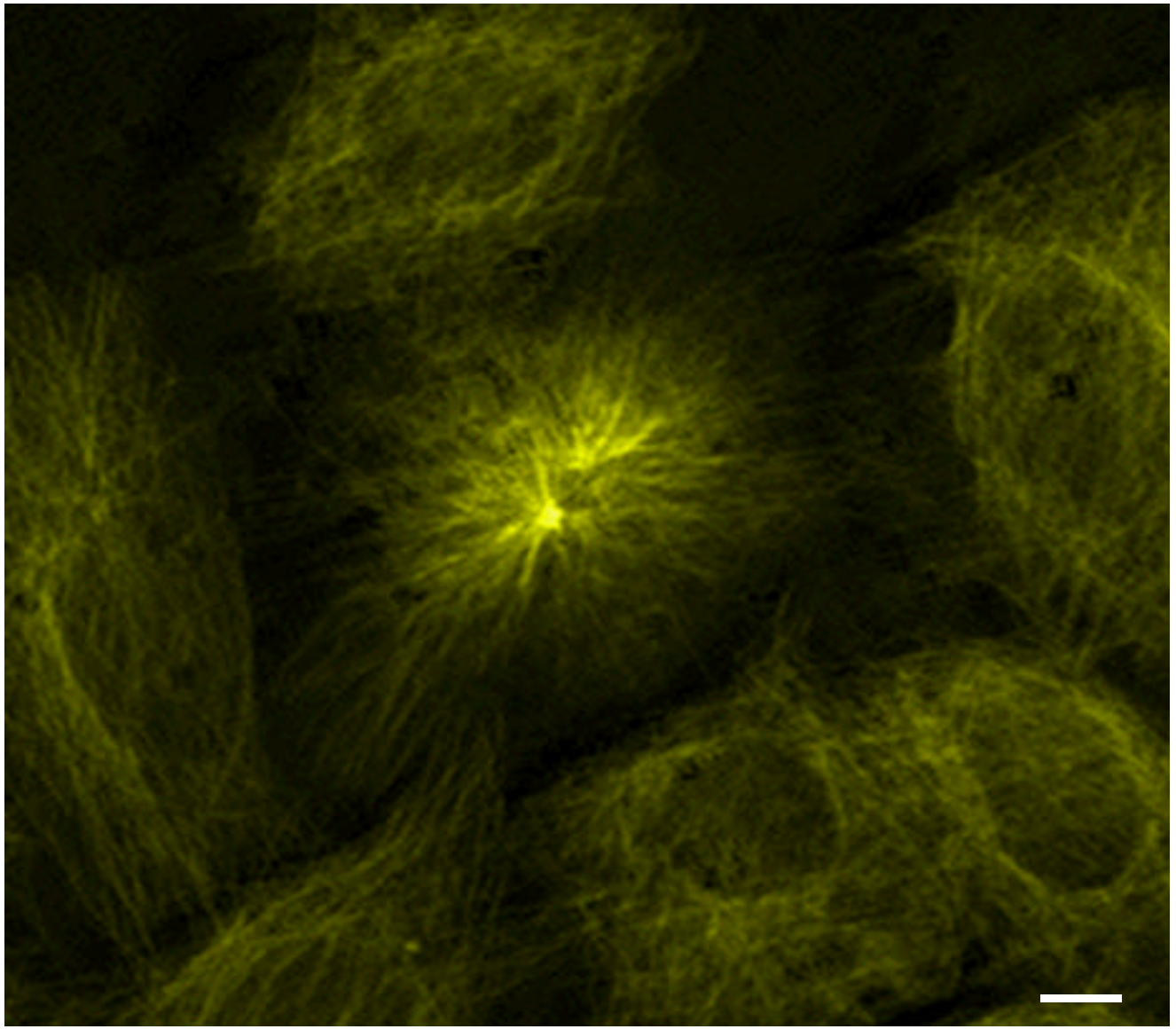
Standard Fluorescence



scale = 6µm

Focus at 7µm depth

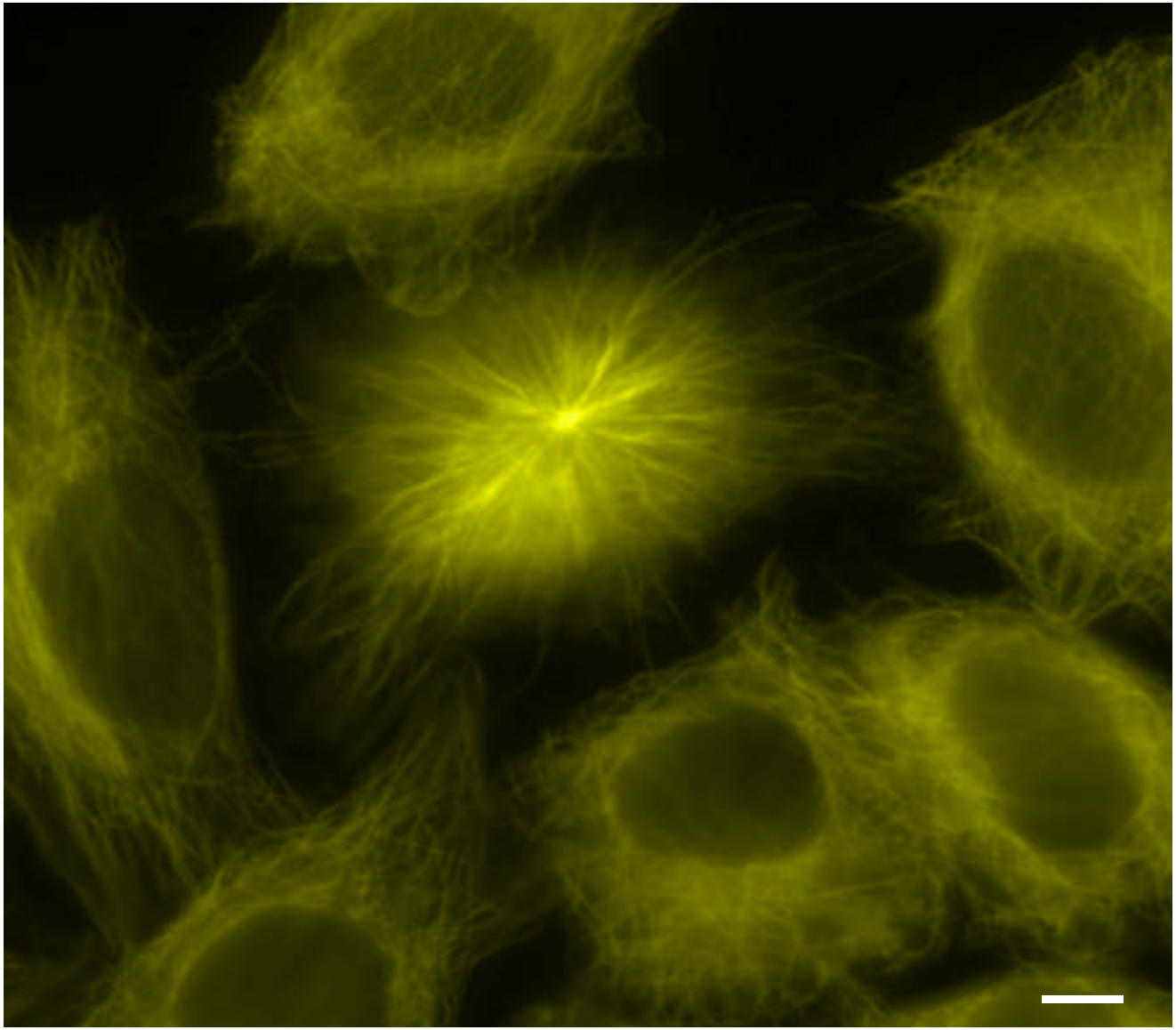
New EDF Fluorescence



scale = 6µm

Focus at 7µm depth

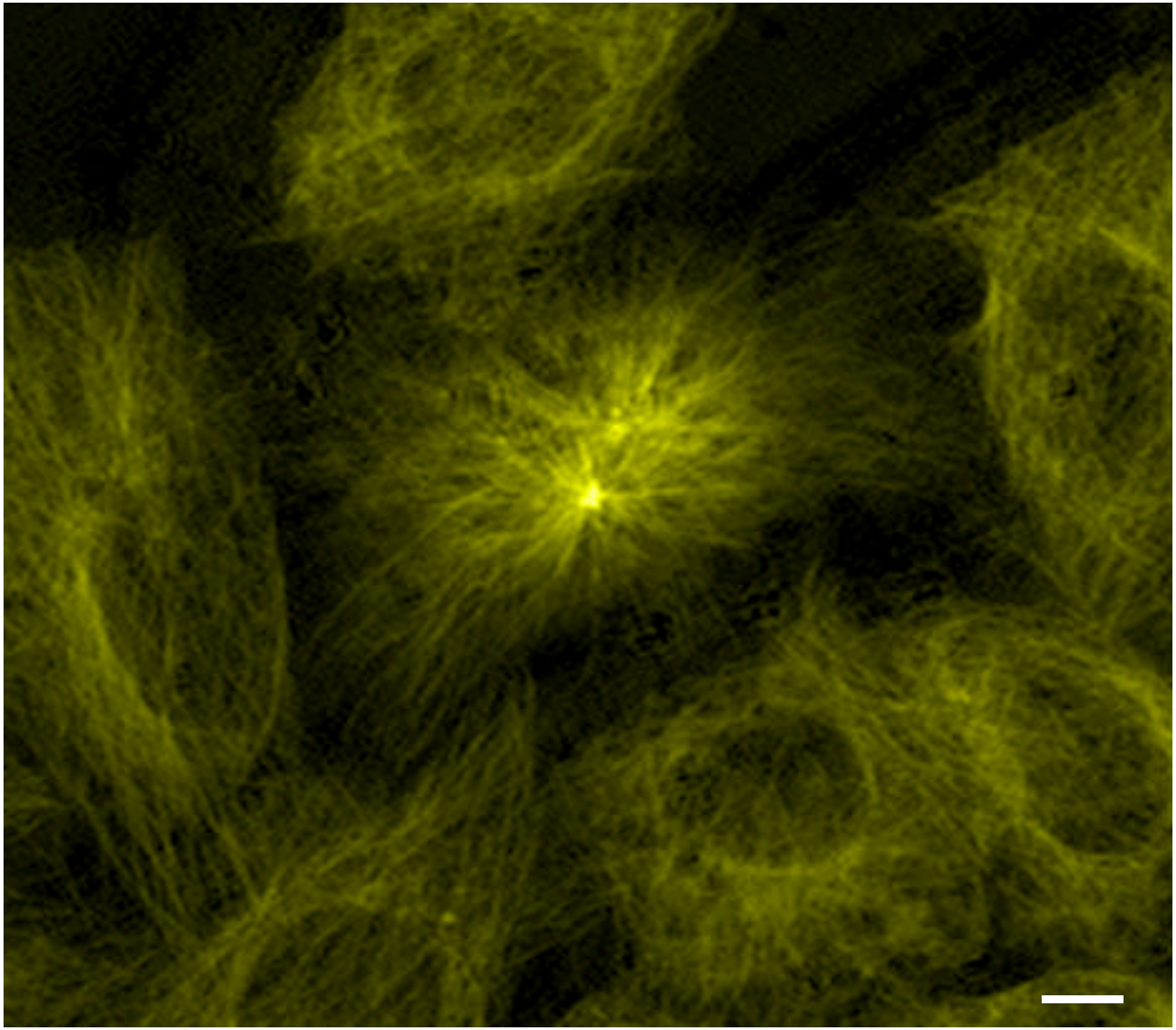
Standard Fluorescence



scale = 6µm

Focus at 1µm depth

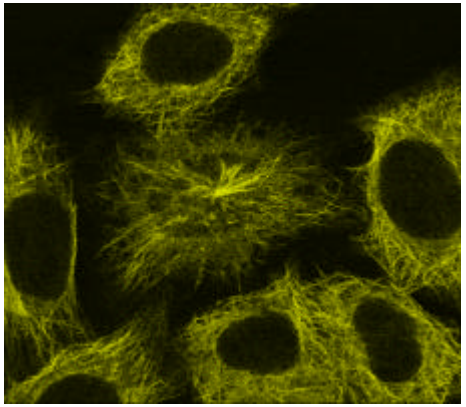
New EDF Fluorescence



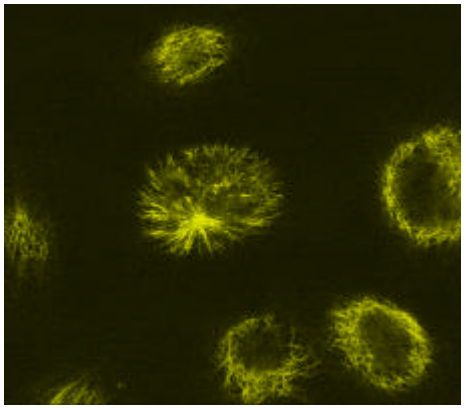
scale = 6µm

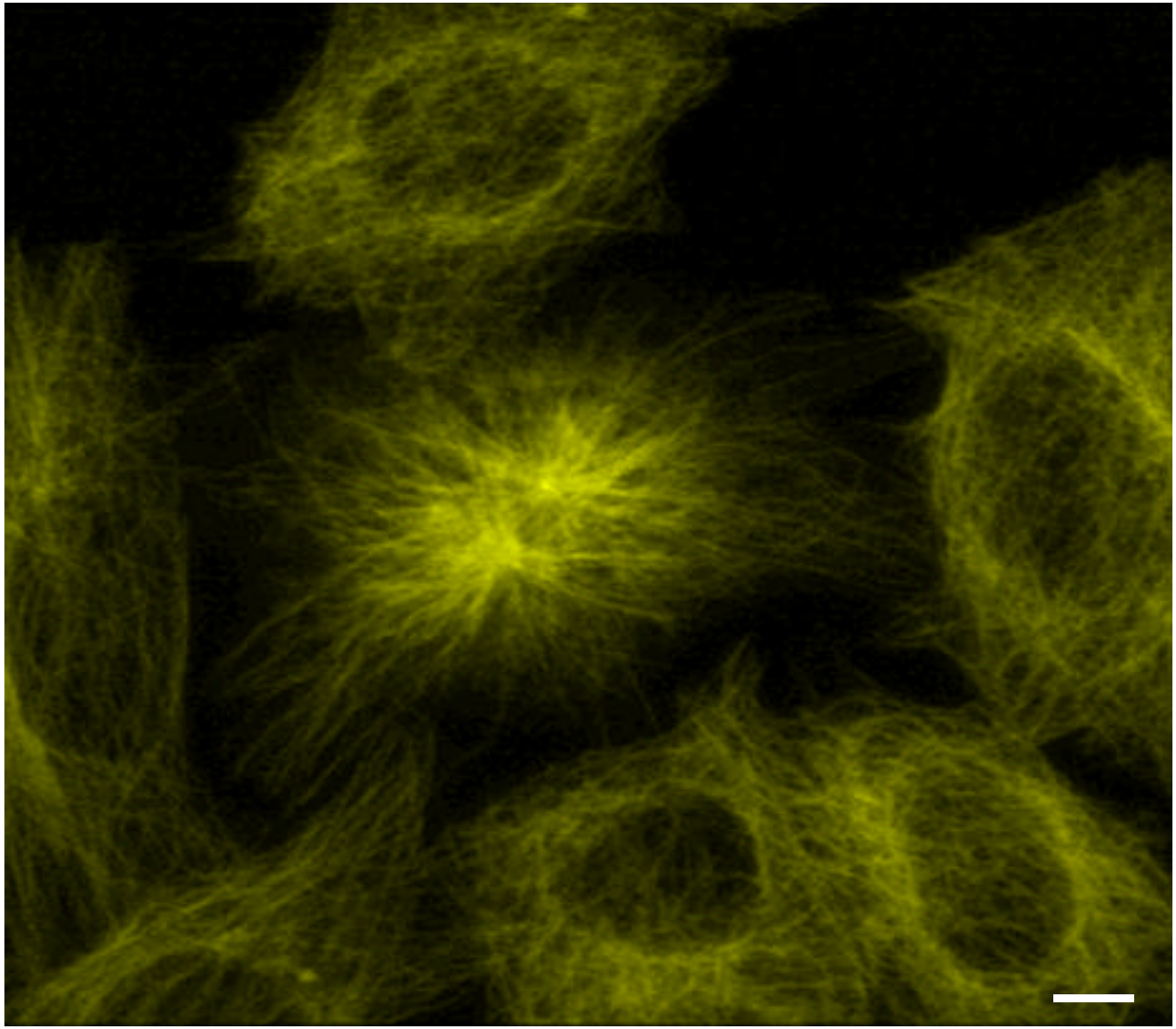
Focus at 1µm depth

Confocal Fluorescence



24 planes of focus at 0.5µm steps, averaged



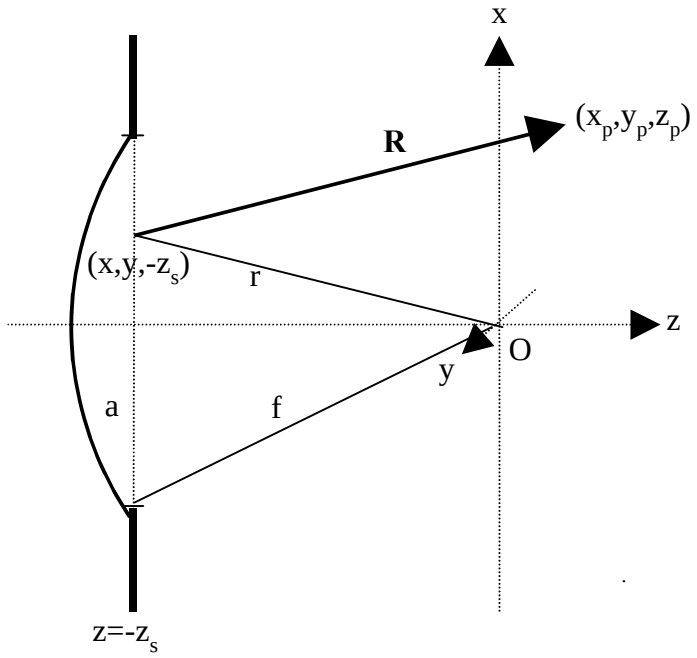


This took 20 times longer to acquire than our EDF images.

High Numerical Aperture Model

Previous work on wavefront coding has used the paraxial approximation. Here 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.

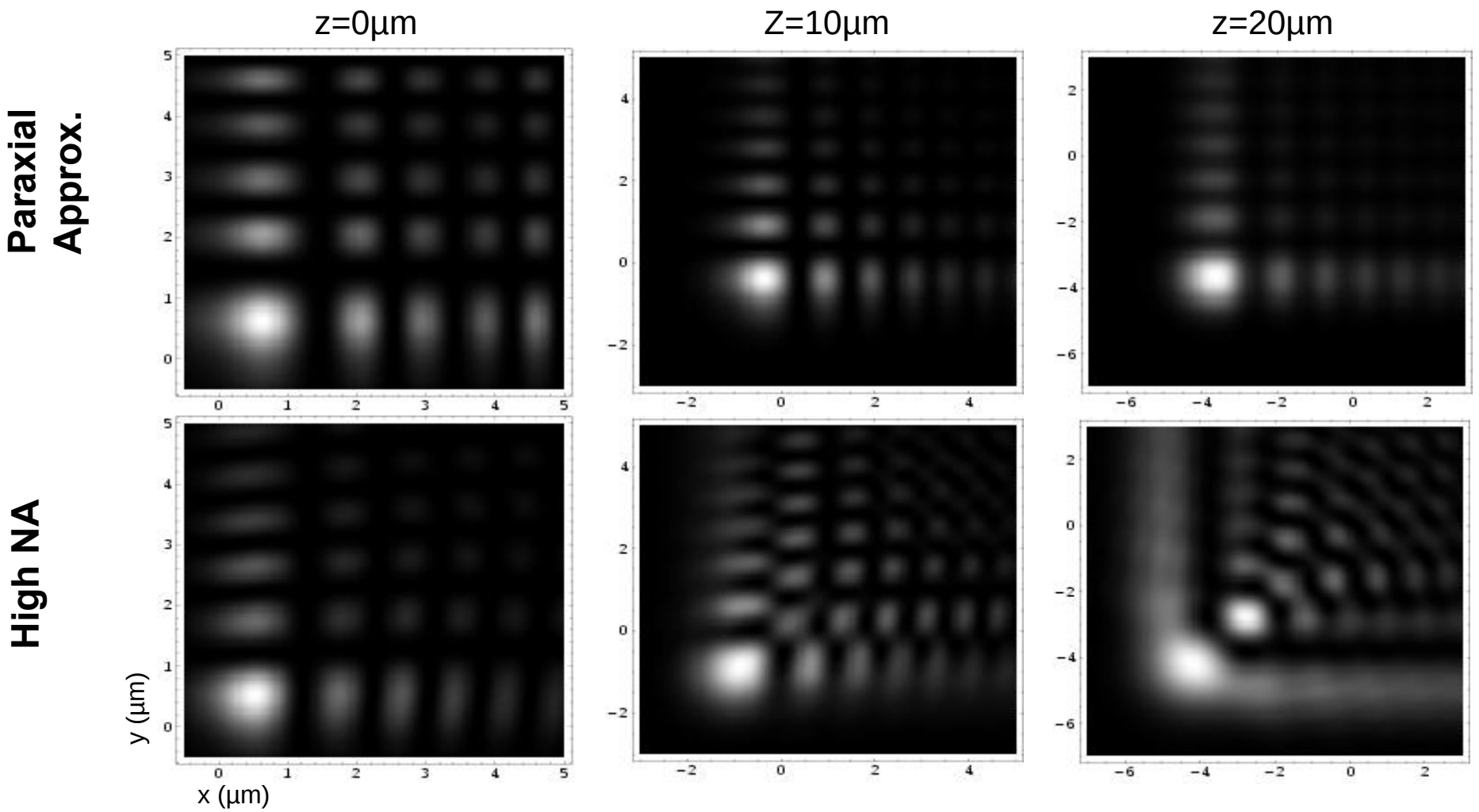


$$E(x_p, y_p, z_p) = \int_{-a}^{a}\int_{-a}^{a} \exp(ik\varphi(x,y)) \frac{\exp(ik(R-r))}{rR} \frac{z_p + f}{R} dxdy$$

Where the cubic phase function is given by:

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

Theoretical Point Spread Functions



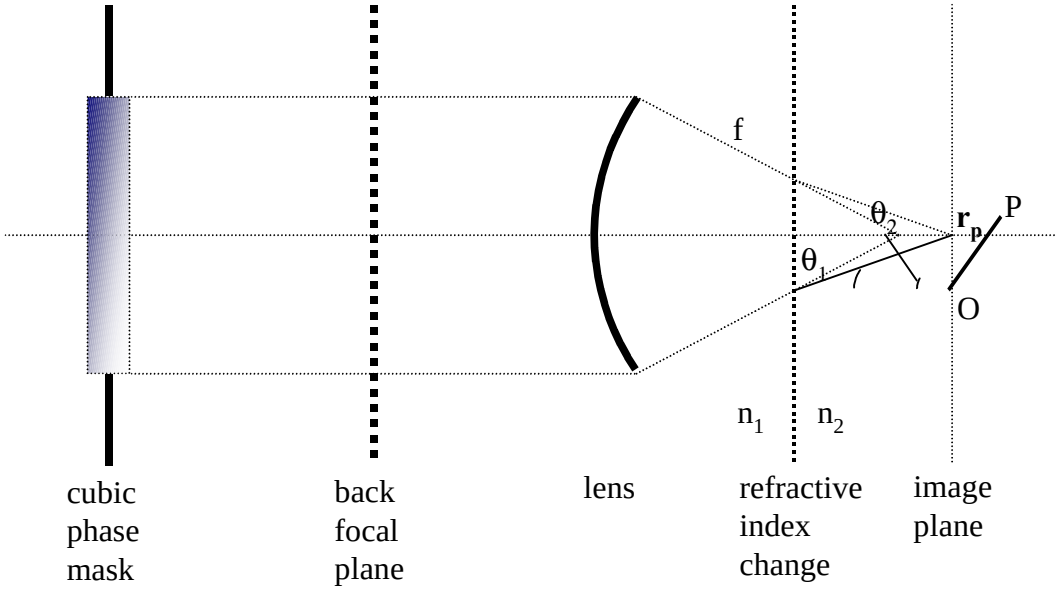
Simulating a 40x 1.3 NA oil lens, as used for the experimental images.

What Next for the Model?

* Take better measurements of the high NA PSF to compare with theory.

* Simulate the effects of other useful phase mask functions.

* Add a change in refractive index - typically producing spherical aberration.



Conclusion:

Wavefront coding is a new approach to 3D fluorescence microscopy and to optical design in general. Instead of avoiding aberrations, we exploit them.

The system is inexpensive because it requires only small modifications to a standard fluorescence microscope.

This opens the way for new studies of a wide range of live-cell dynamics.