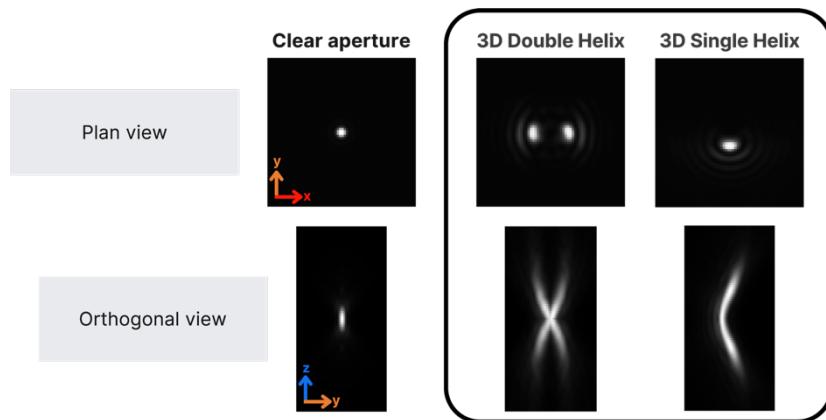


### ACHIEVE INSTANTANEOUS 3D WITH DHO ePSFs

Double Helix Optics (DHO) offers a library of engineered point spread functions (ePSFs) that enable instantaneous high-resolution 3D information capture. Our novel technique uses a phase mask to transform a microscope's native point spread function into an engineered point spread function, redistributing the available photons to both extend the depth of field and encode 3D positional information with high localization precision, eliminating the typical tradeoff between depth and resolution.

The depth extension can be matched to the application and system requirement to maximize signal efficiency and resolution, outperforming conventional imaging systems and those using astigmatic PSFs.

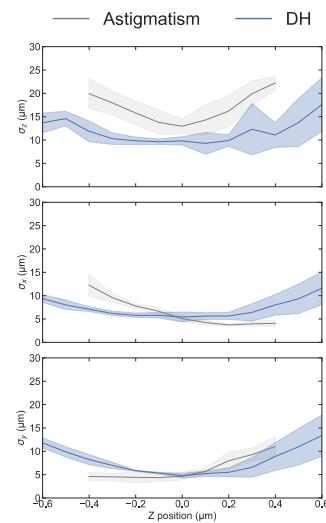


This figure shows the bi- and uni-lobed shapes of the Double Helix and Single Helix ePSFs, respectively, and how, compared with the clear aperture PSF, they stay in focus longer and rotate to provide depth sensitivity.

### COMPARISON WITH ASTIGMATIC PSFs

Cylindrical lenses are commonly used to induce an astigmatic aberration in microscope PSFs for the extraction of 3D information. However, rapid blurring limits depth capture to  $<1\text{ }\mu\text{m}$  with non-isotropic localization precision. In contrast, DHO ePSFs are carefully engineered to provide **tunable** depth extensions **with** isotropic localization precision.

	DHO	Astigmatic
Tunable depth extension - multiple of native depth	2-24x	<2x
PSF optimized for required depth extension	✓	✗
Isotropic nanoscale precision	✓	✗
Retain precision with depth	✓	✗
Best axial resolution & signal efficiency	✓	✗
Illumination adaptable to the sample	✓	✓



The figure on the right demonstrates the superior isotropic precision achieved by the Double Helix (DH) ePSF when compared with the astigmatic PSF (adapted from Nakatani et al. 2024, *The Journal of Physical Chemistry B*).

### TECHNICAL SPECIFICATIONS

PSF shape	Double Helix, Single Helix, Multicolor Double Helix
Depth-capture range	2-24x native depth of field of microscope
Localization precision	Isotropic in 3D: X = Y = Z = ~10-15 nm
Form factor	Fused silica phase mask (diffractive optical element)
Illumination compatibility	Epifluorescence/widefield   HILO   TIRF   light sheet Monochromatic fluorescence or label-free imaging Near-IR to near-UV
Transmission efficiency	>99% from 400 to 700 nm Anti-reflection (AR) coated
Objective lens compatibility	Wide compatibility for high and low magnifications and numerical apertures, and all immersion media
System integration	As standalone phase mask or via Double Helix Optics SPINDLE® modules (SPINDLE, SPINDLE <sup>2</sup> , μSPINDLE, InSPINDLE)
Compatible imaging techniques	<u>Life Sciences</u> 3D single-molecule localization microscopy (SMLM) 4D single-particle tracking Related techniques, including smFISH, smFRET, CLEM, and more For live cells or fixed samples <u>Inspection</u> 3D surface metrology

Select from our library of 3D ePSFs to match your application and system requirements.

Right: DHO phase masks and interchangeable SPINDLE<sup>2</sup> phase mask cartridges.

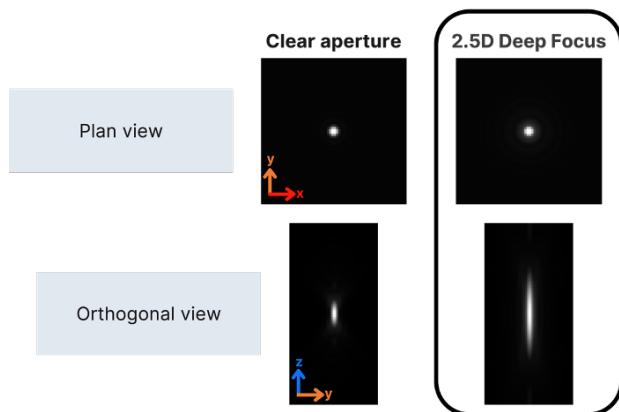


### ACHIEVE INSTANTANEOUS DEPTH EXTENSION WITH DHO DEEP FOCUS

Double Helix Optics (DHO) offers a library of Deep Focus™ engineered point spread functions (ePSFs) that enable instantaneous high-resolution 2.5D information capture with extended depth of field.

Our novel technique uses a phase mask to transform a microscope's native point spread function into an engineered point spread function, redistributing the available photons to both extend the in-focus depth of field and retain high spatial resolution, eliminating the typical tradeoff between these parameters.

The depth extension can be matched to the application and system requirement to maximize signal efficiency and resolution, outperforming at comparable depth ranges conventional imaging systems and those using cubic phase or Bessel beam PSFs.



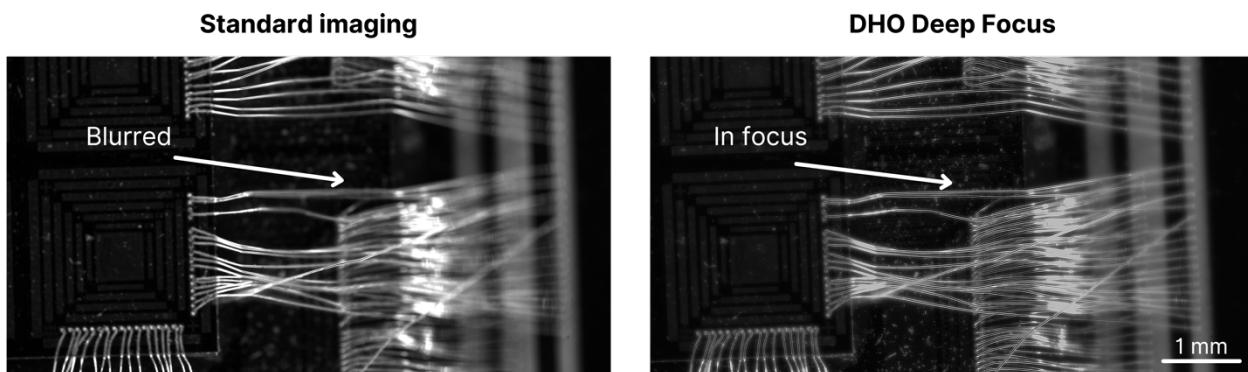
*This figure shows how the Deep Focus ePSF stays in focus longer than the clear aperture PSF while retaining a similar lateral resolution.*

### COMPARISON WITH OTHER PSFs

Historically, phase masks that induce cubic phase or Bessel beam PSFs have been used to extend a microscope's depth of field for 2.5D imaging. However, in comparison with DHO Deep Focus, these PSFs are not signal efficient and require postprocessing to be useable.

	Deep Focus	Cubic Phase	Bessel Beam
Tunable depth extension - multiple of native depth	2-6x	✓	✓
Operates in real time	✓	✗	✗
Deconvolution not required	✓	✗	✗
Signal efficiency	High	Low	Low
Near-diffraction-limited resolution*	✓	✗	✗

*\*DHO Deep Focus exceeds clear-aperture resolution when combined with computational recovery*



As shown in the table, DHO Deep Focus ePSFs have several advantages compared with cubic phase and Bessel beam PSFs. The images of wire-bonded chips demonstrate the superior depth of field achieved by the Deep Focus ePSF when compared with the clear aperture PSF.

### TECHNICAL SPECIFICATIONS

PSF shape	Deep Focus
Depth-capture range	2-6x native depth of field of microscope
Lateral resolution	~7-10% < clear-aperture resolution in real time > Clear-aperture resolution with postprocessing
Form factor	Fused silica phase mask
Illumination compatibility	Epifluorescence, transmitted, ring illumination, and more Monochromatic fluorescence or white light
Transmission efficiency	>97% from 400 to 700 nm
Objective lens compatibility	Wide compatibility for high and low magnifications and numerical apertures, and all immersion media
System integration	As standalone phase mask or via Double Helix Optics SPINDLE® modules (SPINDLE, SPINDLE <sup>2</sup> , μSPINDLE, InSPINDLE)
Example application areas	<u>Life Sciences</u> <u>Spatial omics</u> <u>High-throughput cell or tissue screening</u> <u>Live cell imaging and tracking</u> <u>Inspection</u> <u>High-throughput inline or offline inspection</u> <u>Inspecting curved or out-of-plane samples</u>