A BETTER 3D SMLM

Single-molecule localization microscopy (SMLM) has redefined optical microscopy by unlocking nanoscale investigation into cellular architecture and molecular dynamics. Recognized for its groundbreaking impact on scientific discovery with the 2014 Nobel Prize in Chemistry, SMLM enables the visualization of biological structures wsith localization precision as good as ~10 nm, more than an order of magnitude better than what can be achieved with conventional diffraction-limited fluorescence microscopy.

Increasingly, SMLM is the tool of choice for life scientists seeking to explore cellular and subcellular structures and processes at the highest resolution. Over the past decade, SMLM has seen advances ranging from more efficient fluorophores and labeling strategies to varied selective-illumination techniques. However, to date, this work has primarily been accomplished using 2D methods or astigmatic PSFs, both of which offer limited or biased insights and, as a result, poor clinical translatability.

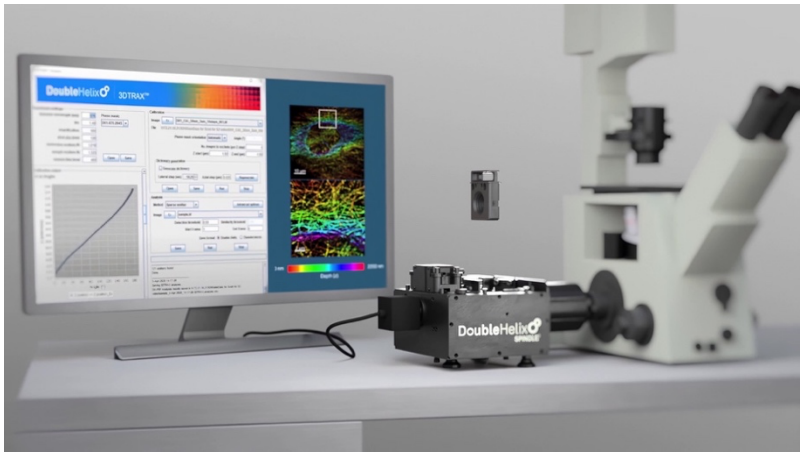
Double Helix Optics' innovative imaging platform overcomes many of these historical limitations to offer market-leading 3D SMLM capabilities. Implemented as phase masks in our SPINDLE microscope upgrade modules, DHO's engineered point spread functions (ePSFs) capture entire 3D structures in a single shot. In contrast, 2D SMLM systems require extensive axial scanning to approximate high-quality 3D reconstructions, and cost added time, photodamage, and photobleaching. Moreover, 3D SMLM using astigmatic point spread functions rapidly loses precision at depth >½ μm from focus and suffers from directionally varying precision. In contrast, DHO 3D SMLM offers spatially isotropic localization precision and the ability to select a phase mask that best matches the depth-capture requirements for a given application. For these reasons, DHO 3D SMLM is suitable for both live-cell imaging and tracking over extended volumes and time scales, and for fixed cell and tissue imaging.

Consisting of phase masks with different axial ranges to match application depth-capture demands, SPINDLE® microscope-upgrade modules, and 3DTRAX® software, DHO's 3D SMLM imaging platform gives users the ability to efficiently visualize nano and microscale 3D structures with molecular specificity. Key features of the platform include:

- Accelerated imaging, eliminating or decreasing requirements for axial scanning
- Isotropic nanoscale precision across the depth-capture range (to ~10 nm in X/Y/Z)
- Lessened photodamage to live samples
- Decreased photobleaching
- High signal efficiency, reduced background noise
- Sequential or simultaneous multicolor imaging for UV to NIR emission
- Localization and analysis algorithms optimized for DHO hardware, including AI-based options for increased localization accuracy and speed
- Wide compatibility with objective lenses (high NA, high magnification supported), microscopes (c-mount/f-mount connection), molecular labeling techniques (all forms of STORM, PALM, PAINT), and illumination modalities (epifluorescence/widefield, HILO, TIRF, light sheet)
- Small physical footprint

DHO 3D SMLM technology has been used as a vital tool across multiple areas of scientific study, including cancer biology, neuroscience, virology, bacteriology, genomics, botany, and chemical engineering, and has been validated by numerous conference presentations and publications.

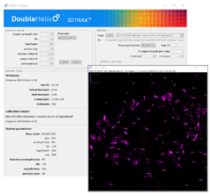
imaging@doublehelixoptics.com
www.doublehelixoptics.com



DHO OFFERS A COMPLETE 3D SMLM IMAGING PLATFORM

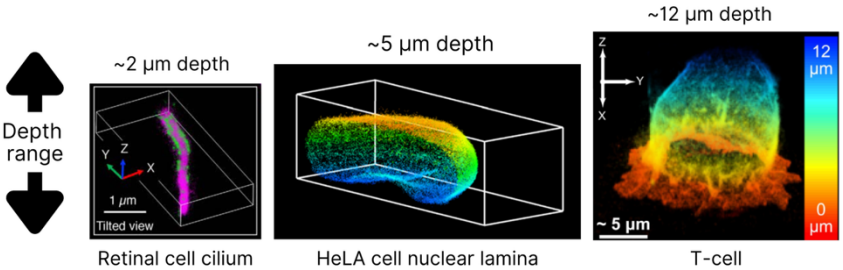
SPINDLE®

SPINDLE²



3DTRAX® & 3DTRAX.AI

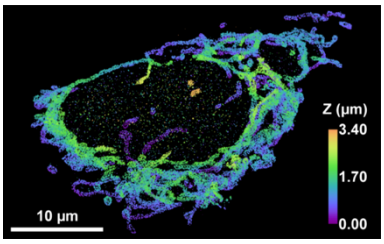
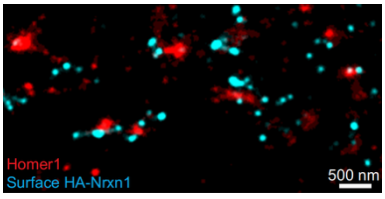
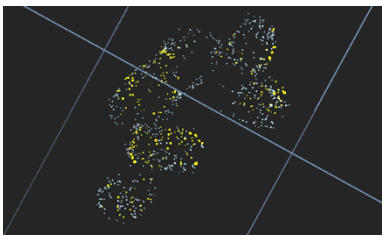
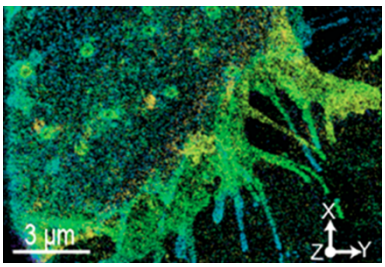
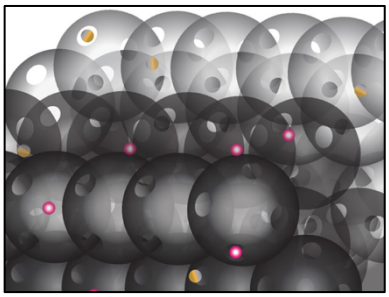
Top: Example DHO 3D SMLM setup, including inverted microscope, SPINDLE² module, phase mask, and 3DTRAX image processing and analysis software. Bottom: Single-channel SPINDLE, Dual-channel SPINDLE², and 3DTRAX GUI. SPINDLE modules feature robust interchangeability of phase masks, bypass mode for conventional imaging, and broad compatibility with sCMOS and EMCCD cameras. 3DTRAX leverages advanced AI models to increase 3D localization accuracy and speed.



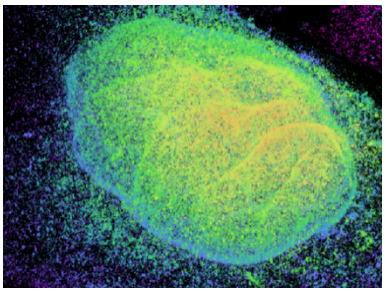
DHO phase masks, selected to match different axial depth-capture ranges, enable efficient 3D SMLM imaging of biological structures spanning a wide range of sizes. Images from H.W. Bennet et al., MBoC (2020), A.K. Gustavsson et al., Nat. Commun. (2018), E.W. Sanders et al., Angew. Chem., Int. Ed. (2022).

imaging@doublehelixoptics.com
www.doublehelixoptics.com

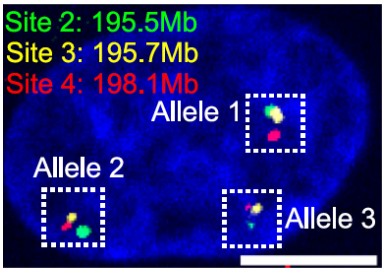
DEMONSTRATED RESULTS ACROSS APPLICATIONS

	**WHOLE-CELL IMAGING:** Nakatani et al. (2024, J. Phys. Chem. B) benchmarked the localization precision of DHO's Double Helix engineered point spread functions (DH-PSFs) under varying imaging conditions. They demonstrated that DH-PSFs surpass 2D imaging and limited-range astigmatic PSFs by providing isotropic, consistently high localization precision across an axial range up to 12x the standard depth of field of traditional systems.
	**NEUROSCIENCE:** Lloyd et al. (2023, Nat. Commun.) used multicolor 3D dSTORM to study the nanoscale organization of key trans-synaptic proteins and identify how these molecules coordinate brain signaling. Synapses are 3D structures with associated chemical and electrical processes that cannot be fully understood with 2D imaging.
	**LIVE-CELL IMAGING & TRACKING:** Upton et al. (2023, Biophys. J./ASCB 2023 presentation) used multicolor 3D single-molecule imaging and tracking to reveal how two key proteins behave inside living *E. coli* cells, offering new insight into fundamental cellular mechanisms that could be targeted by next-generation antibiotics. Several varieties of bacteria have evolved in recent decades to quickly bypass traditional antibiotic mechanisms.
	**INTERFACIAL SCIENCE:** Roy et al. (2022, ACS Nano) used 3D super-resolution microscopy to explore how cells interact with nanoscale surface topographies. Understanding reactive changes in cellular morphology at the 3D nano-bio interface is critical for designing improved biomedical implants and tissue engineering scaffolds, where controlling cell behavior is a primary goal.
	**DRUG DELIVERY:** Shi et al. (2023, Sci. Adv.) used 3D single-nanoparticle tracking to discover and characterize hydrodynamic coupling between passive particles and dilute nanomotors in an interconnected confined environment. This emergent behavior has to the potential to open new avenues for future drug delivery systems.

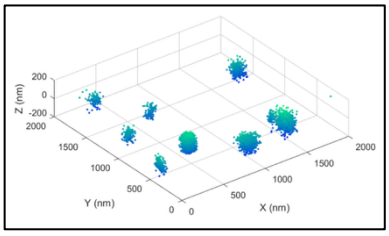
imaging@doublehelixoptics.com
www.doublehelixoptics.com



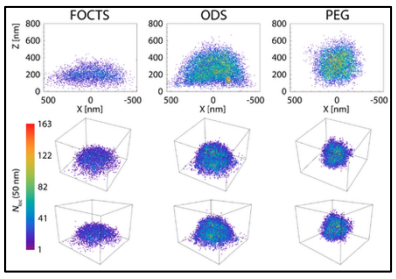
BIOPHYSICS METHOD DEVELOPMENT: Saliba et al. (2024, Nat. Commun.) developed a 3D SMLM imaging platform that integrates a single-objective lens tilted light sheet with custom microfluidics to improve speed and localization precision in whole-cell imaging. Overcoming these technical hurdles is critical to understanding the complex distributions and interplay between multiple subcellular structures at the nanoscale throughout entire cells.



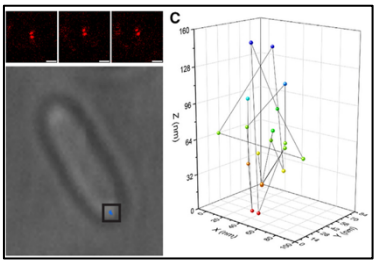
GENOMICS: To address the challenge of visualizing real-time DNA interactions, Zhu et al. (2025, Cell) introduced Oligo-LiveFISH, a super-resolved, high-temporal-resolution 3D imaging approach for tracking previously difficult-to-image non-repetitive genomic regions in living cells. Oligo-LiveFISH enables the direct observation of dynamic processes like enhancer-promoter communication, providing new insights into how the genome's 3D architecture governs gene transcription and other cellular functions.



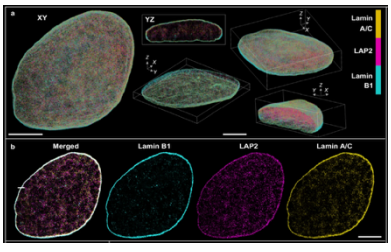
EXTRACELLULAR VESICLES: M.L.P. Bailey et al. (presented at ISEVtech 2024, see application note) used DHO's technology platform to show that 2D imaging of extracellular vesicles (EVs) provides an incomplete and potentially misleading picture of biological reality. This type of more realistic SMLM imaging of EVs – 3D with <10 nm spatially isotropic precision – provides an enhanced quantitative baseline for applications ranging from basic science to diagnostics and drug delivery.



STORM



PALM



PAINT

DHO's 3D SMLM platform is compatible with all fluorescent labeling chemistries, including the many variants of STORM, PALM, and PAINT. *Images from L.H. Alvarez et al., Nano Lett. (2019), S.L. Upton et al., Biophys. J. (2023), and N. Saliba et al., Nat. Commun. (2024).*

imaging@doublehelixoptics.com
www.doublehelixoptics.com