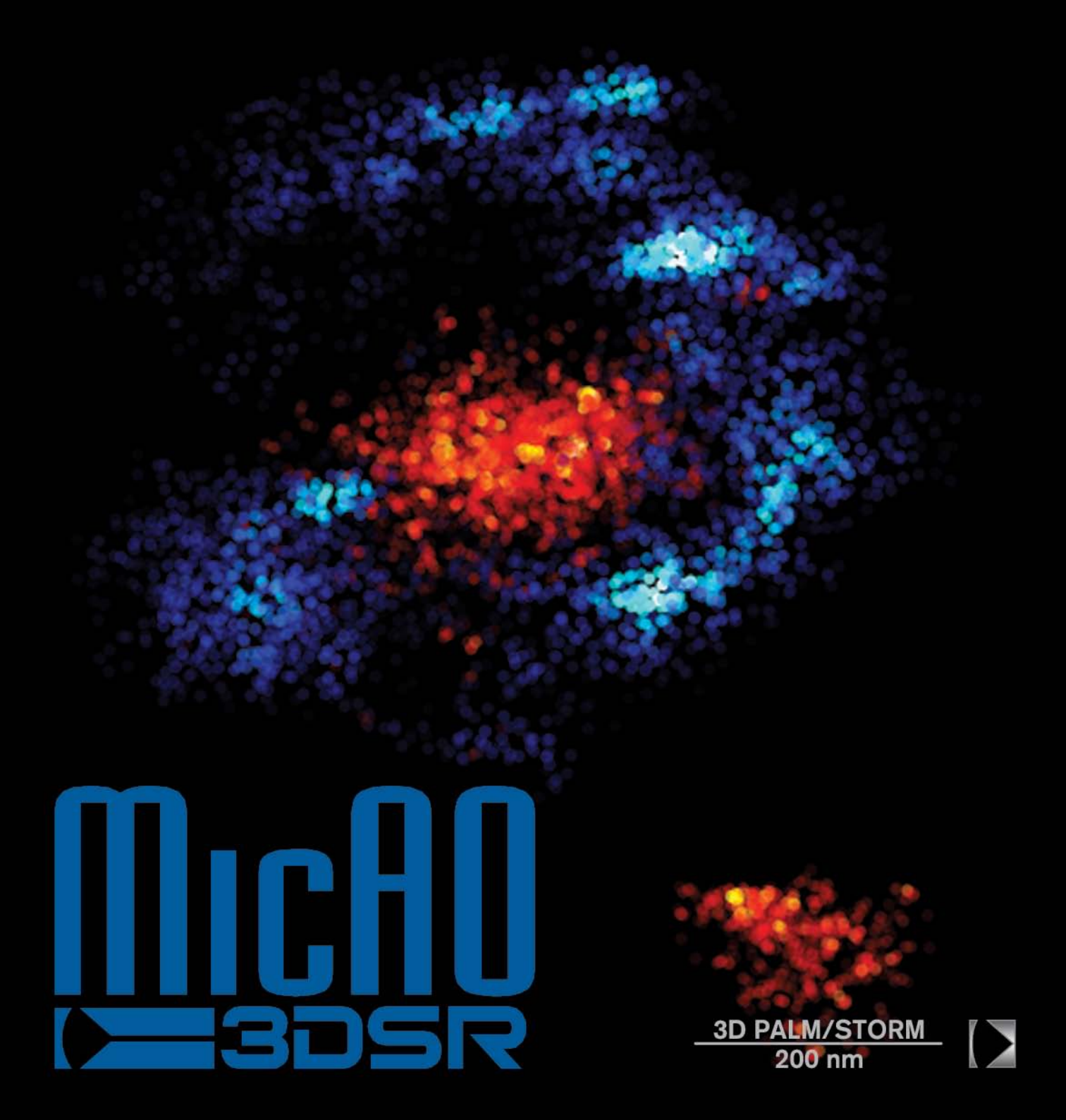
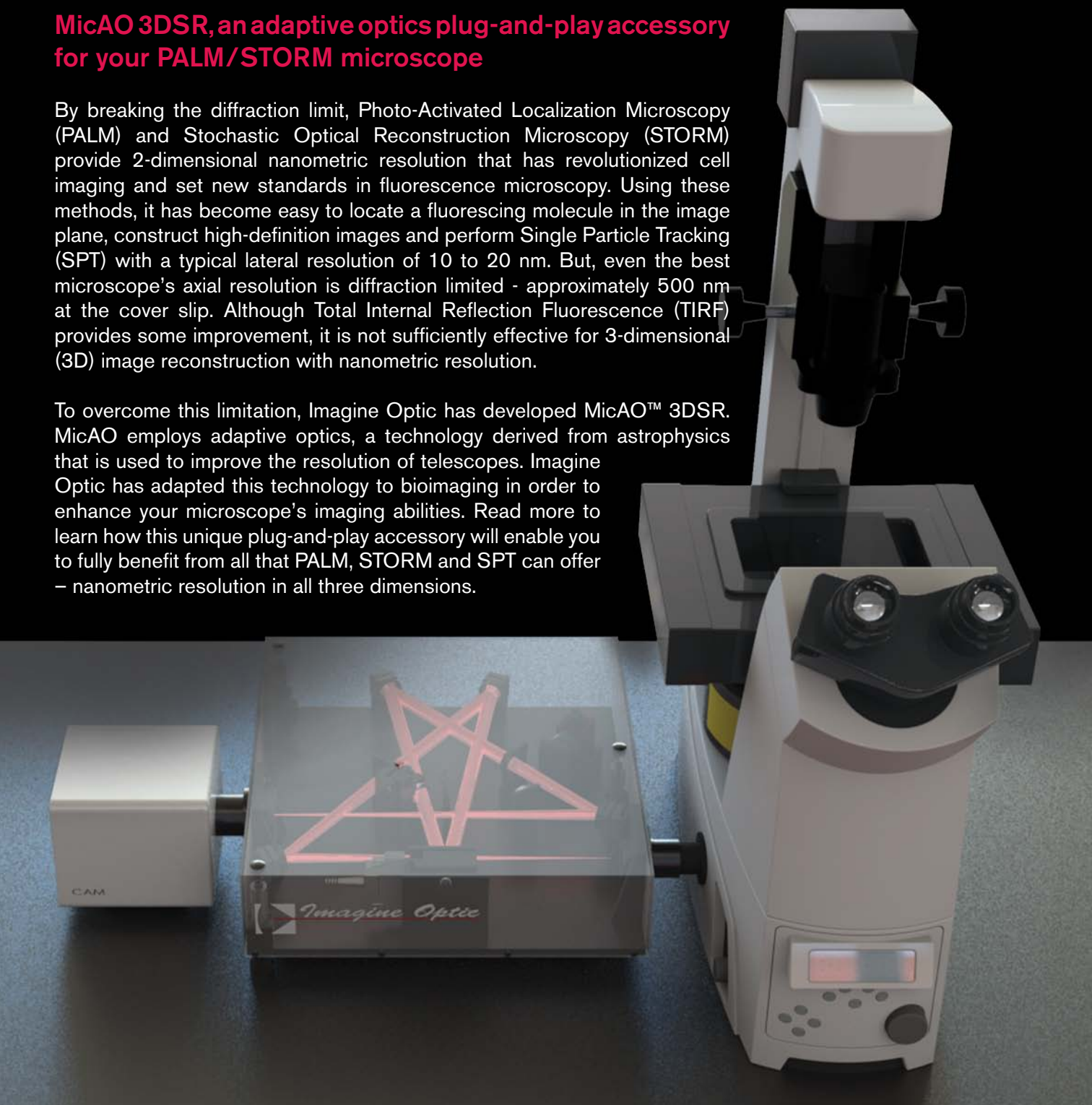

MicAO
3DSR
3D PALM/STORM
200 nm

MicAO 3DSR, an adaptive optics plug-and-play accessory for your PALM/STORM microscope

By breaking the diffraction limit, Photo-Activated Localization Microscopy (PALM) and Stochastic Optical Reconstruction Microscopy (STORM) provide 2-dimensional nanometric resolution that has revolutionized cell imaging and set new standards in fluorescence microscopy. Using these methods, it has become easy to locate a fluorescing molecule in the image plane, construct high-definition images and perform Single Particle Tracking (SPT) with a typical lateral resolution of 10 to 20 nm. But, even the best microscope's axial resolution is diffraction limited - approximately 500 nm at the cover slip. Although Total Internal Reflection Fluorescence (TIRF) provides some improvement, it is not sufficiently effective for 3-dimensional (3D) image reconstruction with nanometric resolution.

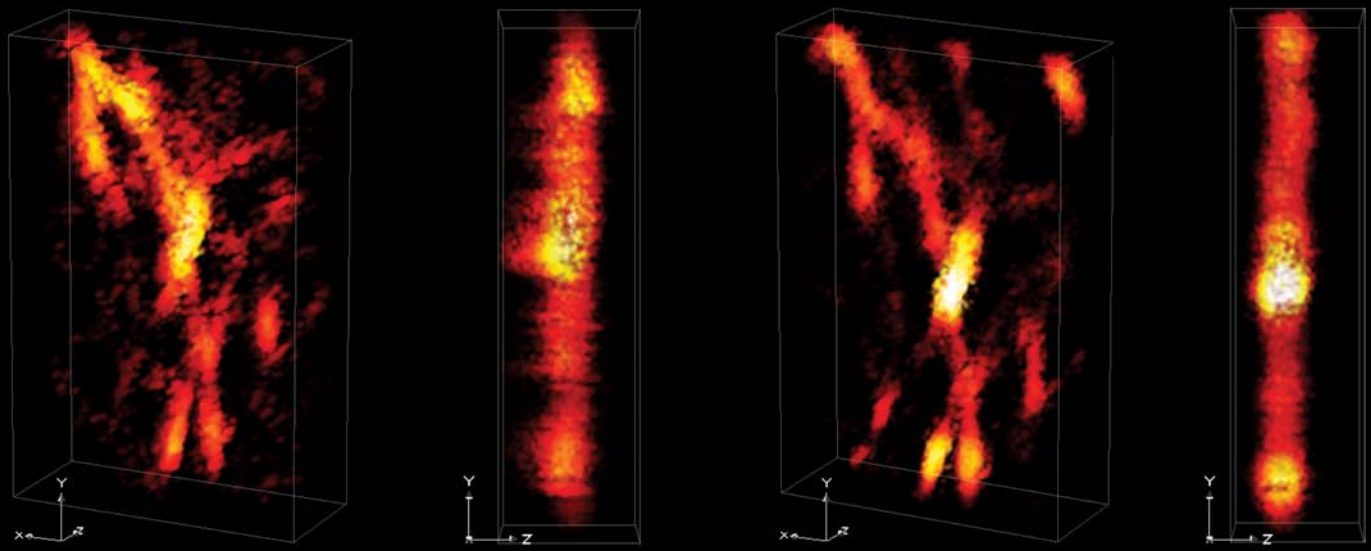
To overcome this limitation, Imagine Optic has developed MicAO™ 3DSR. MicAO employs adaptive optics, a technology derived from astrophysics that is used to improve the resolution of telescopes. Imagine Optic has adapted this technology to bioimaging in order to enhance your microscope's imaging abilities. Read more to learn how this unique plug-and-play accessory will enable you to fully benefit from all that PALM, STORM and SPT can offer – nanometric resolution in all three dimensions.



Improve lateral resolution

PALM and STORM microscopy's resolution is primarily dependent on the number of photons reaching the Electron Multiplying Charged-Coupled Device's (EMCCD) detection array. This makes optimizing your photon budget, the total number of photons emitted by a fluorescent molecule bound to the structure of interest, essential to maintaining resolution. There are two main factors that can negatively impact lateral (x/y) resolution in PALM, STORM and SPT. Both are correctable by using MicAO 3DSR.

First, small defects are present in the optical elements of even the highest-quality microscopes. This is particularly true with regard to the high-magnification and high-numerical-aperture objectives commonly used in Super-Resolution (SR) microscopy. Together, they introduce aberrations that are largely due to technical limitations in the design and manufacturing processes. Second, the refractive index (n) mismatches induce aberrations when an oil (n=1.5) immersion objective is used to image biological samples (mostly water, n=1.33).



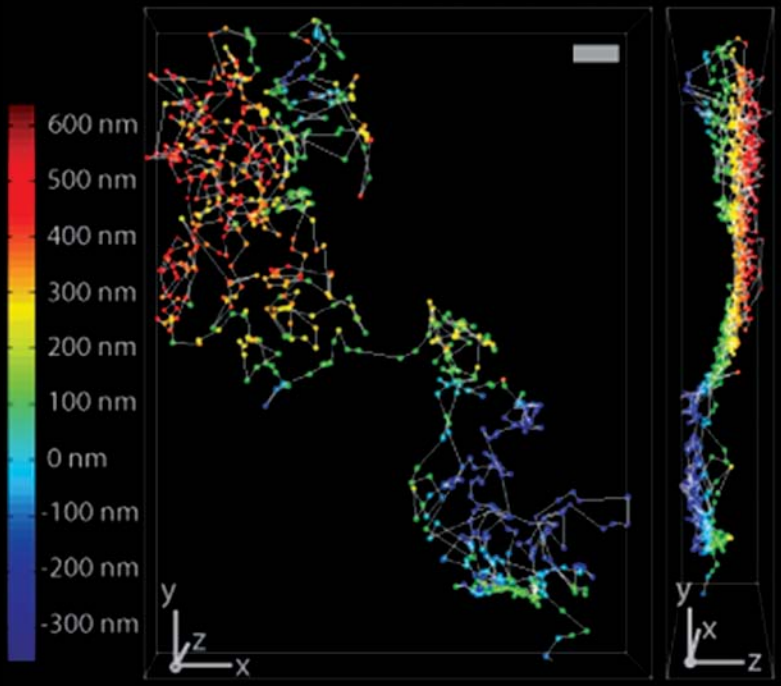
Three-dimensional PALM images of actin filament bundles in fibroblasts transfected with ABP-EOS. A comparison between cylindrical lens (left) and MicAO (right). Micao 3DSR axial resolution = 20 nm. Scale bar = 800nm. Color represents volume density. The quality of the image is improved when MicAO is used because all spherical aberrations are corrected and this drastically improves the precision of the point position determination.

To overcome these issues, MicAO employs a proprietary optical architecture whose primary components are a Shack-Hartmann (HASO™3) wavefront sensor that measures the aberrations and a deformable mirror (mirao™ 52-e) that reshapes its reflective surface's shape to the opposite of the measured aberrations. Sophisticated software that is completely transparent to the user calculates the difference between a perfect Point Spread Function (PSF) on the image plane versus the measured value in order to quantify all of the aberrations and send correction commands to mirao. This process cancels-out the effects of optical aberrations and recreates a perfect PSF at the focal plane which maximizes lateral resolution.

Achieve nanometric resolution in all 3 dimensions

As stated earlier, one of the biggest limitations of PALM and STORM is that these methods become diffraction limited (~500 nm) when imaging along the z-axis. Whereas some methods can partially overcome this limitation (e.g. bi-plane, double-helix, etc.), they all result in a depreciated photon budget. TIRF enables PALM and STORM users to break the diffraction limit, but its inherent constraints only provide a 5x improvement (resolution ≈ ± 100 nm) at the surface of the cover slip, hence it remains effectively 2-dimensional. Cylindrical lenses provide enhanced axial resolution but have distinct drawbacks: they are not commercially available for all microscopes and they can introduce additional aberrations into the light path, thereby reducing image quality.

MicAO 3DSR breaks the diffraction limit along the optical axis by using its deformable mirror to introduce subtle changes in astigmatism to effectively beam-shape the axial PSF. Via this process, MicAO 3DSR optimizes the photon budget and allows images to be acquired with an axial resolution of 10-20 nm over approximately a 1 µm depth of focus and up to several of microns into the sample. Because the shape of the PSF along all axes in perfectly calibrated and aberration free, MicAO 3DSR provides superior results and the information necessary to precisely locate a fluorescing emitter's position along the z axis to reconstruct 3D volumetric images.



Three-dimensional trajectory of quantum dot bound to a transmembrane protein diffusing in the plasma membrane of a cultured HeLa cell. The color bar corresponds to the z position. Scale bar = 500 nm. Axial Resolution = 40nm

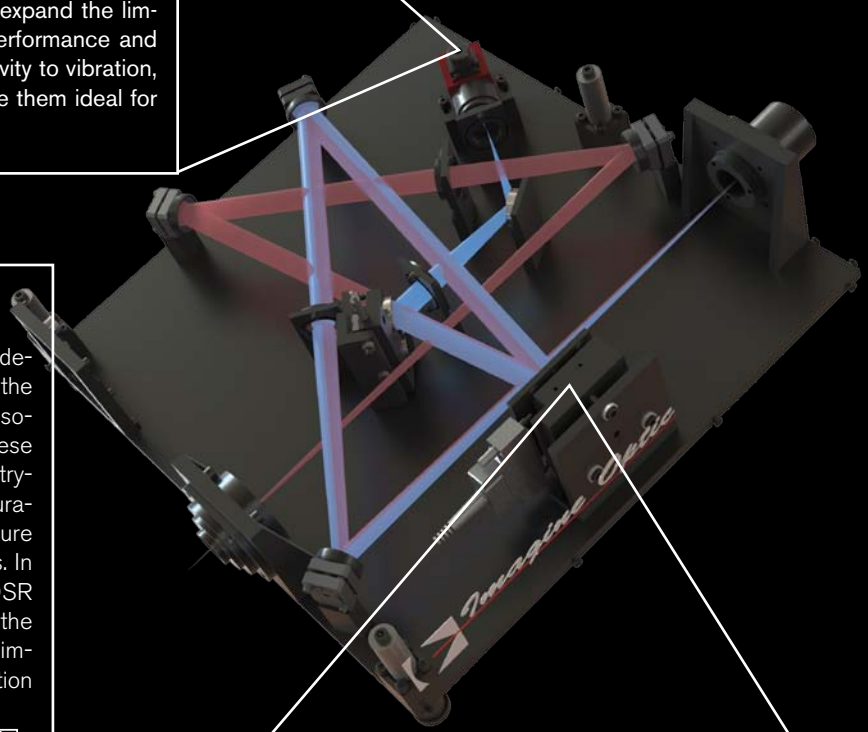
Easy to use

MicAO is a plug-and-play device which is simply placed between your microscope's camera port and the EMCCD camera itself. Its high optical quality, based on Imagine Optic's patented adaptive-optics technology, ensures 95% optical transmission so that every photon counts. The software drivers fully integrate MicAO's functionality into MetaMorph or µ-Manager via plug-in, making it user-friendly and easy to master especially for people not explicitly trained in adaptive optics.

To find out more, please see the contact information on the back cover or visit www.imagine-optic.com/find.

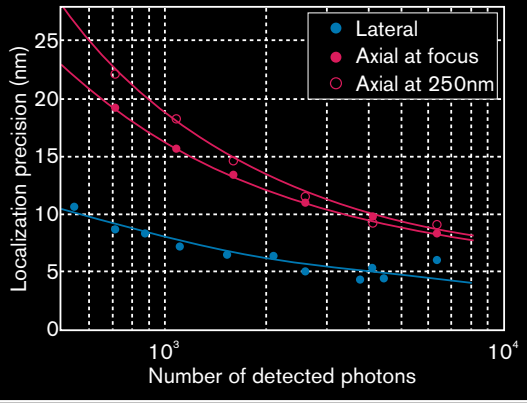
About HASO™3 - First

HASO3, based on patented Shack-Hartmann technology, is the only line of wavefront sensors that provides absolute measurement of both phase and intensity independently, simultaneously and in real-time. With hundreds of satisfied customers around the world, HASO sensors continue to expand the limits of wavefront metrology with their speed, performance and ease of integration. What's more, their insensitivity to vibration, compact design and Firewire connectivity make them ideal for integration in MicAO.



Optimizing the photon budget

The resolution of PALM microscopy primarily depends on the amount of photons detected by the EMCCD camera. The graph below shows the resolution's dependence on thus for the z-axis. These measurements were acquired using an industry-standard inverted microscope in PALM configuration that was fitted with a high-numerical aperture 100x objective and MicAO to obtain 3D images. In addition to introducing 3D capability, MicAO 3DSR corrects for the various aberrations induced by the microscope and the sample itself. In this way, it improves the photon budget and, hence, the resolution in all three dimensions.



About mirao™ 52-e

mirao is based on patented electromagnetic technology that overcomes many of the barriers that limited deformable mirror performance in the past, enabling it to deform its reflective surface up to ±50µm. Additionally, its compact size, low-voltage requirements and USB 2 connectivity make it ideally suited for integration into biomedical devices.

MicAO 3DSR specifications	
Microscope compatibility	Standard inverted frames
Objective compatibility	Optimized for 100x | n/a >1.3
Optical transmission	95%
Lateral localization precision	8 nm @ 1000 photons 5 nm @ 4000 photons
Axial (z axis) localization precision	16 nm @ 1000 photons 10 nm @ 4000 photons
Operating wavelength range	400 - 700 nm
Software	MicAO v1
Camera interface	I/O camera port
Dimensions / weight	410 x 390 x 100 mm / 17 kg
Working environment	Room temperature w/NCRH
Power supply	110 - 220 V / 50 - 60 Hz

Front cover image: Combined two-color 3 dimensional PALM and dSTORM imaging of mEos2-Centrin1 (orange) and Alexa 647 labeled Cep164 (light blue). For the visualization of results we used the PALMview program developed by Mohamed El Beheiry (Institute Curie). Images taken by J. Sillibourne and I. Izeddin, collaboration between the labs of M. Bornens (Institute Curie), M. Dahan (Institute Curie) and X. Darzacq (ENS, Paris).

Imagine Optic SA (main office)

18 rue Charles de Gaulle
91400 Orsay France
Telephone: +33 (0)1 64 86 15 60
Fax: +33 (0)1 64 86 15 61
E-mail: contact@imagine-optic.com

Imagine Optic, Inc.

Boston Office (Headquarters)
Cambridge Innovation Center
One Broadway, 14th floor
Cambridge, MA 02142 - USA
Telephone: +1 (617) 401-2198
Fax: +1 (425) 930-9818

San Francisco Office
2415 3rd Street, Suite 231
San Francisco, CA 94107 - USA
Telephone: +1 (415) 525-5557
Fax: +1 (415) 525-5558

COSINGO (Imagine Optic Spain SL)

Mediterranean Technology Park
Av. del Canal Olimpic s/n
08860 Castelldefels (Barcelona) Spain
Telephone: +34 935 534 148
Fax: +34 935 534 000
E-mail: info@cosingo.com

Imagine Optic China

Pythagore Optical Business Center
323 Guo Ding Rd, Bld 3 - 9th
Yangpu District
200 433 Shanghai
Telephone: +86 021 6299 6274
E-mail: china@imagine-optic.com

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