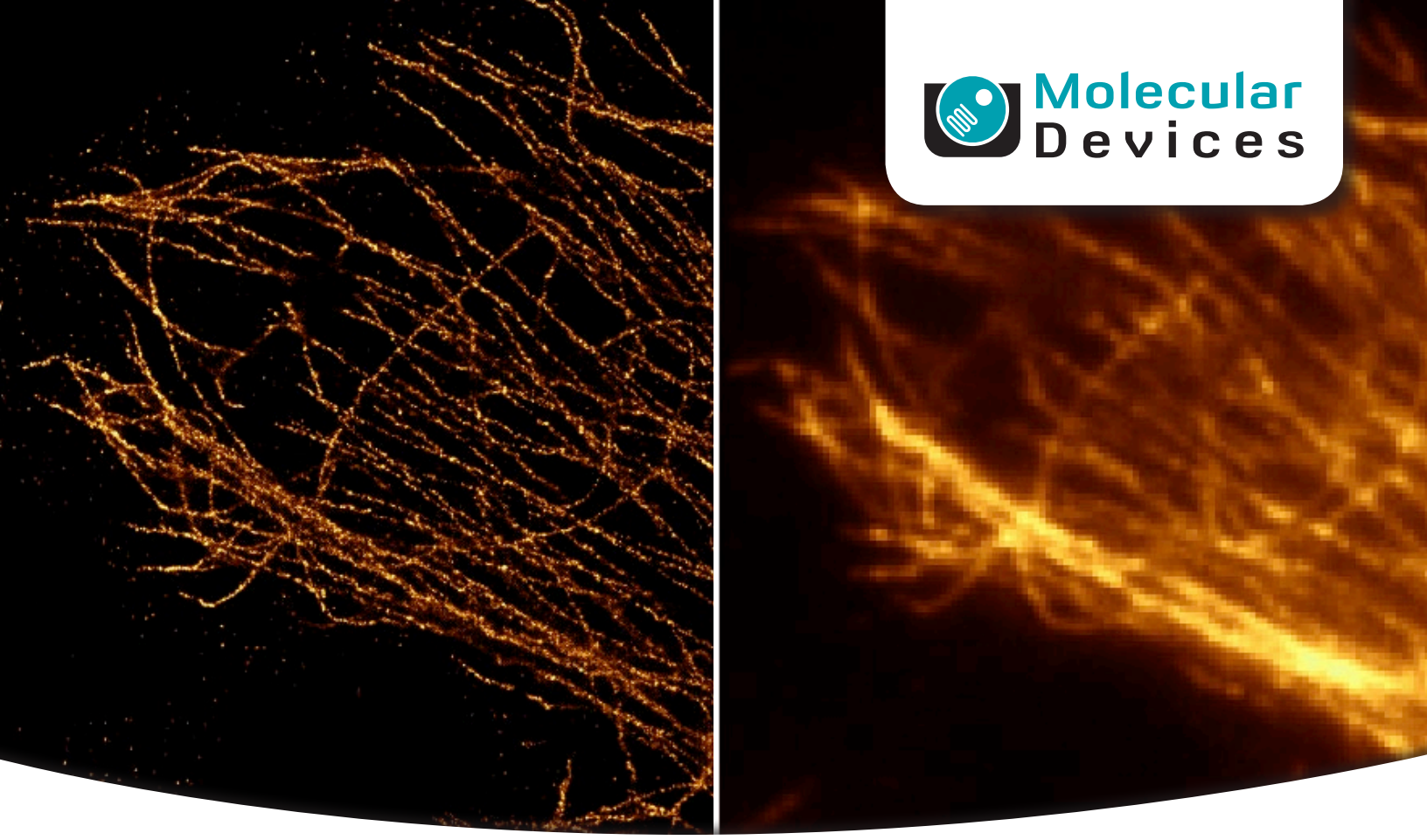


MetaMorph Super-Resolution System

Expands existing imaging capabilities

Key Features

- Lateral resolution 20 nm; axial resolution 40 nm
- Supports many single molecule localization super-resolution techniques
- Real-time super-resolution image construction at camera frame rate
- Offline analysis available

The resolution of widefield optical microscopy is limited by the diffraction of light according to the relationship discovered by Ernst Abbe. Object details smaller than 200 nm spatially are not readily discernible by light microscopy, posing a limit to the technique for studying many biological problems at a cellular level.

However, recent imaging advances have enabled 10-fold improvement in resolution and allow researchers to see into the world beyond the diffraction limit. These advances involve capture of emission from a random limited subset of fluorescent molecules and repeating the process thousands of times very quickly to form the entire image. Common techniques like photo-activation localization microscopy (PALM), [direct] stochastic optical reconstruction microscopy ([d]STORM), and ground state depletion (GSD) all depend on experimental procedures to induce only a handful of molecules in the sample to fluoresce during a typical camera exposure time of 5–20 ms while the rest are temporarily turned off. Subsequent exposures turn on a different subset of fluorescent molecules and allow their coordinates to be calculated. The whole sequence of such exposures constructs a super-resolution image from the coordinates of individually localized fluorescent molecules.

The MetaMorph® Super-Resolution System from Molecular Devices® provides a means to control experimental hardware, capture image sequences, perform localization calculations and display the developing super-resolution image in real time. The MetaMorph Super-Resolution System currently works with many commercially available laser launches as well as TIRF optics, and can be enabled on previously installed imaging systems compatible with MetaMorph Software. Molecular Devices has partnered with key hardware suppliers to facilitate a complete super-resolution system tailored to customer needs.

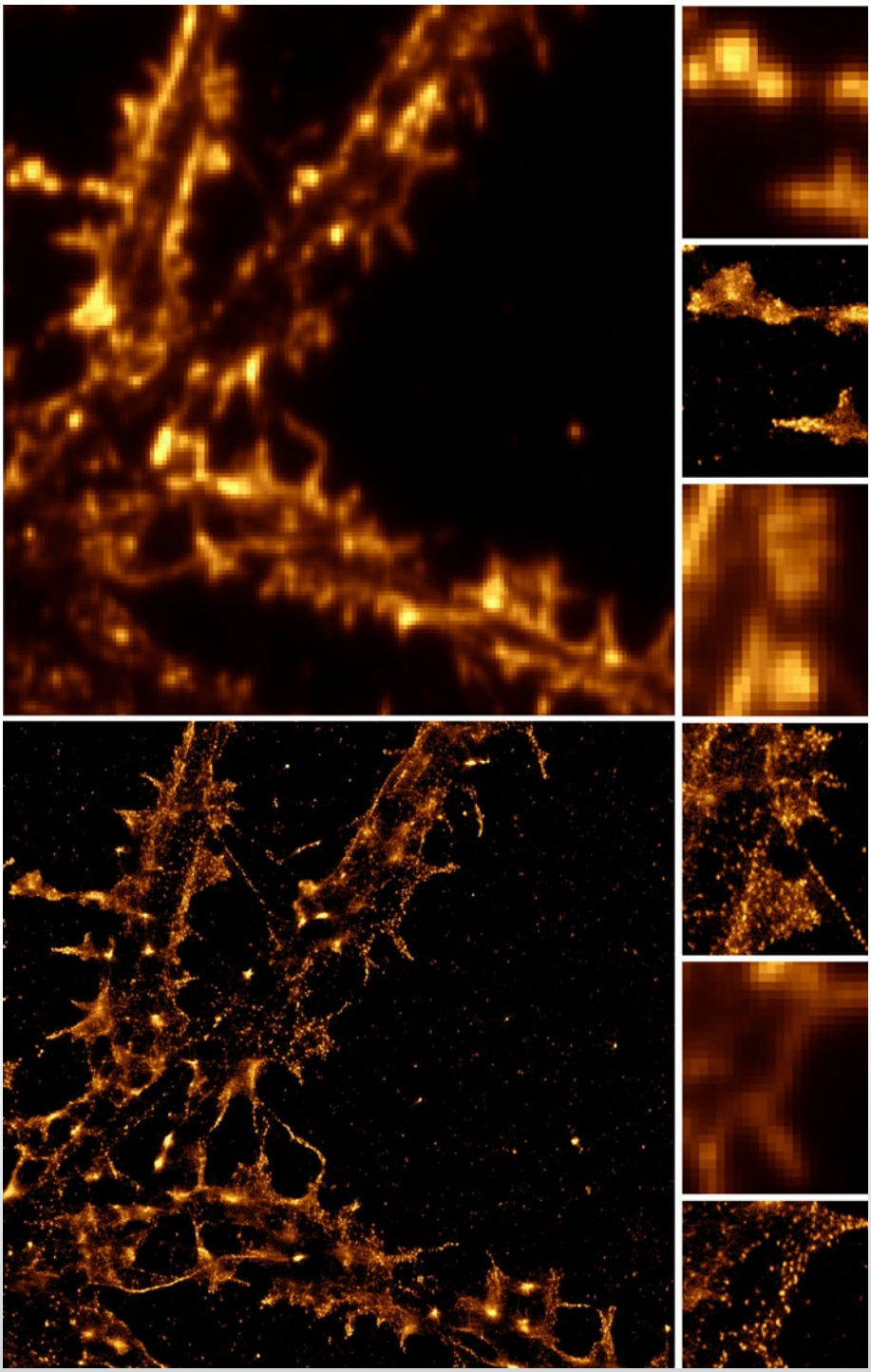
Break through the diffraction limit

The MetaMorph Super-Resolution System lets researchers bypass the diffraction limit with patent-pending image processing techniques. The system automatically adjusts laser excitation power to keep the number of activated fluorescent probes per frame optimal and constant throughout image collection. Users can observe the construction of a high-resolution image as a series of single-molecule images are acquired.

The MetaMorph Super-Resolution System allows:

- Wavelet filtering and Gaussian fitting
- 3-D localization using astigmatism
- Real-time super-resolution image display at any CCD frame rate
- Offline processing
- Drift correction using fiduciary markers
- Variable scaling of super-resolution image
- Automatic thresholding and splitting of closely spaced molecules
- Single molecule localization text file generation for data exportation
- Image stack acquisition
- Arbitrary acquired image size

2-D super-resolution acquisition in real time



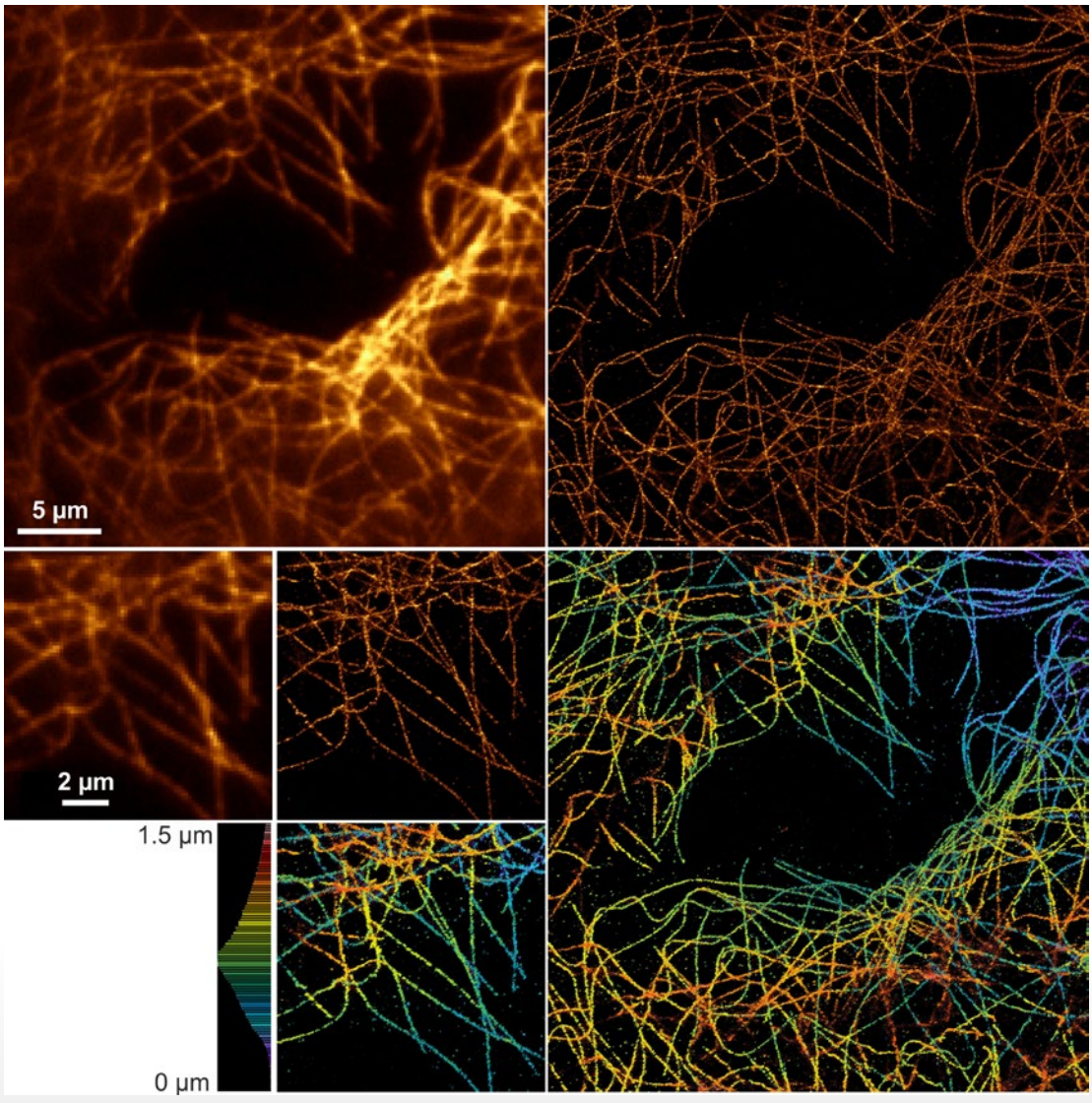
Super resolution images of the actin cytoskeleton of rat hippocampal neurons expressing ABP-tdEosFP. Photo-activation was performed with a 405 nm laser and excitation with a 561 nm laser.

Discern object details less than 200 nm

The MetaMorph Super-Resolution System is compatible with many single molecule localization techniques using photo-switchable, photo-activatable , photo-convertible proteins, or standard fluorescent dyes. In a benchmark study, the system was able to obtain 30,000 frames in under 5 minutes (256x256 pixels, 30-50 single molecules per frame and 1,200,000 molecules total).

Images courtesy of Adel Kachar, Deepak Nair, Daniel Choquet, Jean-Baptiste Sibarita. Interdisciplinary Institute for Neuroscience, CNRS UMR 5297, F-33000. Bordeaux, France.

3-D super-resolution imaging with GPU acceleration



COS7 cell tubulin labelled with Alexa Fluor 647 and imaged using a standard microscopy technique. Alexa Fluor 647 was first converted in to dark state using a 640 nm laser. The number of single molecules detected per frame was controlled by using a 405 nm laser.

Technical specifications and compatibility

Resolution	• 20 nm lateral minimum spec (15 nm possible) • 40 nm axial with 3D astigmatic lens
Speed	• 100,000/second single molecule localizations • 30,000 frames in under 5 minutes (256x256 pixels, 30–50 single molecules per frame) • 850 MB /sec. to SSD RAID
Automated microscopes	• Leica • Nikon • Olympus • Zeiss
Laser launch systems	• Spectral Applied Research • Roper SARL
Certified cameras	• Andor iXON Ultra • Photometrics Evolve Delta

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