## MetaMorph Super-Resolution Software



#### Localization-based super-resolution microscopy

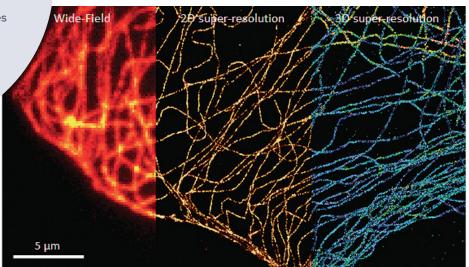




#### **KEY BENEFITS**

- Lateral Resolution up to 20 nm
   & Axial Resolution up to 40nm
- Real-time and offline superresolution image construction
- Automatic laser feedback and adjustments
- Supports many different localization microscopy techniques
- · Single molecule tracking
- Accelerated GPU processing
- Compatible with multiple hardware platforms





Comparison between widefield image (left) and super-resolution images in 2D (center) and 3D (right).  $\beta$ -tubulin labeled with Alexa647.

## Break through the diffraction limit

The MetaMorph Super-Resolution Software advances imaging by enabling a 10-fold improvement in resolution.

Our MetaMorph Microscopy and Imaging Software provides a means to control experimental hardware, capture images, perform localization calculations, and display the developing super-resolution image in real time.

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## Our company

Our innovative analytical solutions for cell and protein biology enable our customers to see more, do more, know more, and to answer life's most important questions.

#### Abbe's limit

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 are not readily discernible by conventional light microscopy, posing a limit for studying many biological problems at the cellular level.

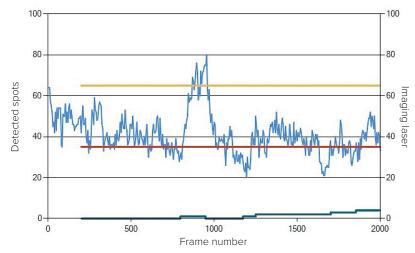
#### **Super-resolution image**

A random, sparse subset of fluorescent molecules are captured and localized while the rest of the molecules in the sample are temporarily non fluorescent. Then, the current group of fluorescent molecules are turned non fluorescent and a new pool of molecules are turned fluorescent.

Repeating this process a large number of times and pooling the localizations together allows reconstruction of super-resolution image from the individual molecule's coordinates

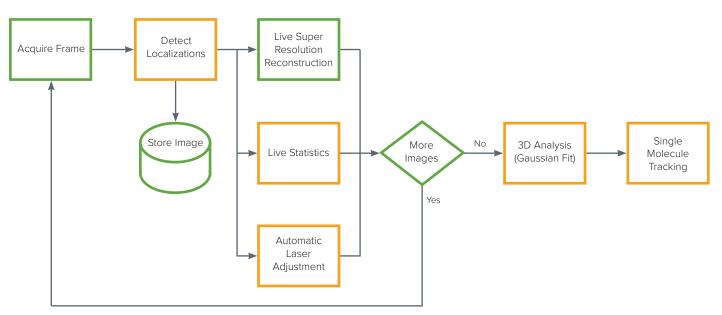
## Real-time localizations, saving, & feedback

Real-time capabilities allow reconstruction and visualization of the superresolution image during the acquisition. With real-time feedback, the system can automatically adjust activation laser power to compensate for a changing density of localizations while acquiring images.



The number of localizations per image and laser power with real-time adjustments. When the number of localizations is outside a baseline, the laser power automatically changes to increase or decrease the density of localizations.

#### **Optimized workflow**



Acquisition workflow for acquiring, analyzing and saving images. When your image acquisition completes, the localization analysis and storage is also complete.

### Integrates with your protocols and system

#### **Microscopy techniques**

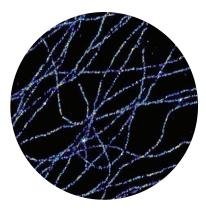
Common localization microcopy techniques using photo-switchable, photo-activatable, photo-convertible proteins, or standard fluorescent dyes are all supported.

#### **Hardware supported**

The MetaMorph Super-Resolution Software works with many commercially available microscopes, laser launches, TIRF optics, and can be enabled on previously-installed imaging systems compatible with MetaMorph Microscopy and Imaging Software.



Widefield image of microtubules



Super-resolution image of microtubules

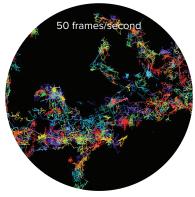
# Calculate diffusion coefficients to analyze motion

#### Single molecule tracking

In addition to being able to resolve small details in super-resolution images, you can also track and calculate dynamics of single molecules. After localizing all the fluorescent molecules from the image stack, single-molecule trajectories are computed. Mean squared distance (MSD) and diffusion coefficient (D) are calculated for each trajectory.



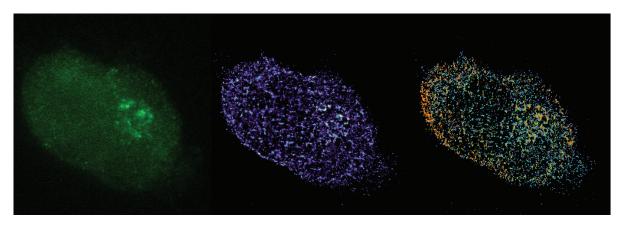
Super-Resolution image of GluA1-mEOS2



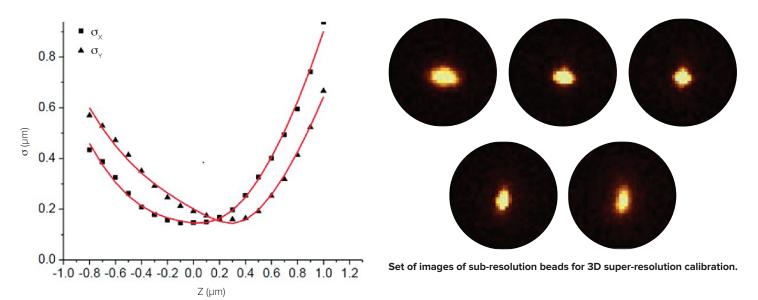
Single GluA1-mEOS2 trajectories of molecules localized and tracked more than 8 consecutive frames

## 3D super-resolution

Adding an optional cylindrical lens to the emission path of the microscope allows the software to precisely compute the axial position of the localized molecules. Localizations in the images are analyzed for the amount of asymmetry.



Visualization of widefield (left), super-resolution (center), and pseudocolor 3D super-resolution images (right).

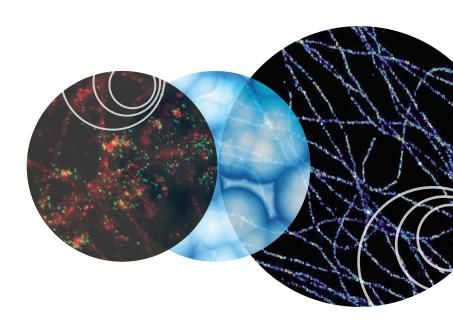


An example set of 3D calibration curves for 3D super-resolution.

Specifications	
Resolution <sup>1</sup>	• 20 nm lateral • 40 nm axial with 3D astigmatic lens
Speed <sup>2</sup>	<ul> <li>100,000/second single molecule localizations</li> <li>30,000 frames in under 5 minutes (256x256 pixels, 30-50 single molecules per frame)</li> <li>850 MB/sec to SSD RAID</li> </ul>
Computer recommendations	Windows 7x64 SP1     At least 32GB RAM     CUDA supported NVIDIA graphics card
Features	Wavelet filtering and Gaussian fitting  Beal-time super-resolution image display at any CCD frame rate  Drift correction using fiduciary markers  Automatic activation laser adjustment throughout image collection  Variable scaling of super-resolution image  Automatic thresholding and splitting of closely spaced molecules  Single molecule localization & tracking³  Arbitrary acquired image size  Image stack acquisition  Real time statistics on the number of localizations per frame  Text file generated for data exportation

I. Izeddin, J. Boulanger, V. Racine, C.G. Specht, A. Kechkar, D. Nair, A. Triller, D. Choquet, M. Dahan, and J.B. Sibarita, "Wavelet analysis for single molecule localization Microscopy", Optics Express, Vol. 20, No. 3, 2081-2095 (30 January 2012).

- 2. Kechkar A, Nair D, Heilemann M, Choquet D, Sibarita J-B (2013), "Real-Time Analysis and Visualization for Single-Molecule Based Super-Resolution Microscopy." *PLoS ONE* 8(4): e62918. doi:10.1371/journal.pone.0062918.
- 3. Nair D, Hosy E, Petersen JD, Constals A, Giannone G, Choquet D, Sibarita JB (2013) Super-Resolution Imaging Reveals That AMPA Receptors Inside Synapses Are Dynamically Organized in Nanodomains Regulated by PSD95. *J Neurosci* 33:13204-13224.



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



#### Contact Us

Phone: 800.635.5577

Web: www.moleculardevices.com

Email: info@moldev.com

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#### **Regional Offices**

USA and Canada +1.800.635.5577 United Kingdom

+44.118.944.8000 Europe\* 00800.665.32860 China (Beijing) +86.10.6410.8669 China (Shanghai) +86.21.3372.1088 Hong Kong

Japan (Osaka) +81.6.7174.8331 Japan (Tokyo) +81.3.6362.5260 South Korea +852.2248.6000 +82.2.3471.9531

Brazil

+55.11.3616.6607

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