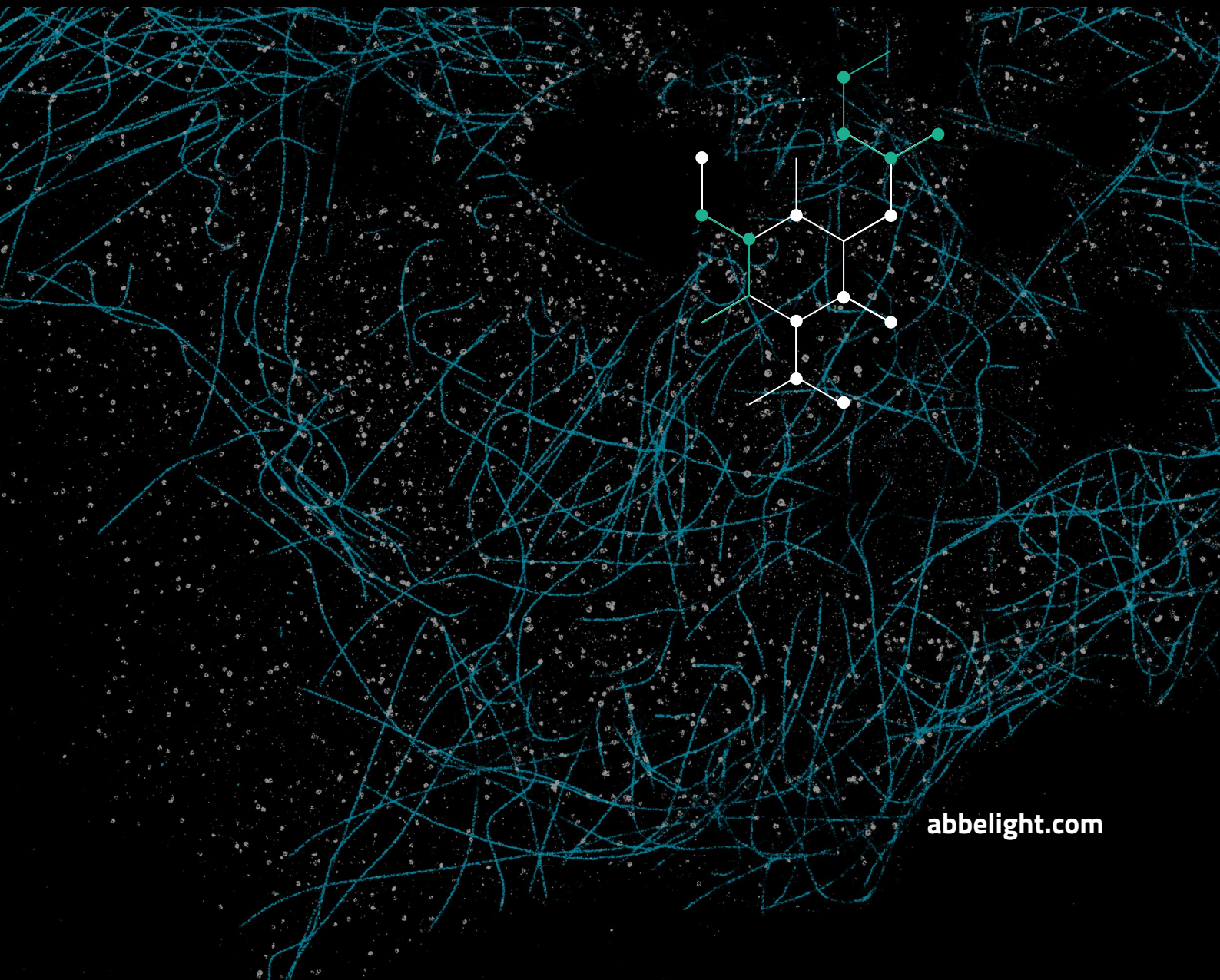
Abbelight
NOW, WE SEE

NANOSCOPY
Technologies at a glance



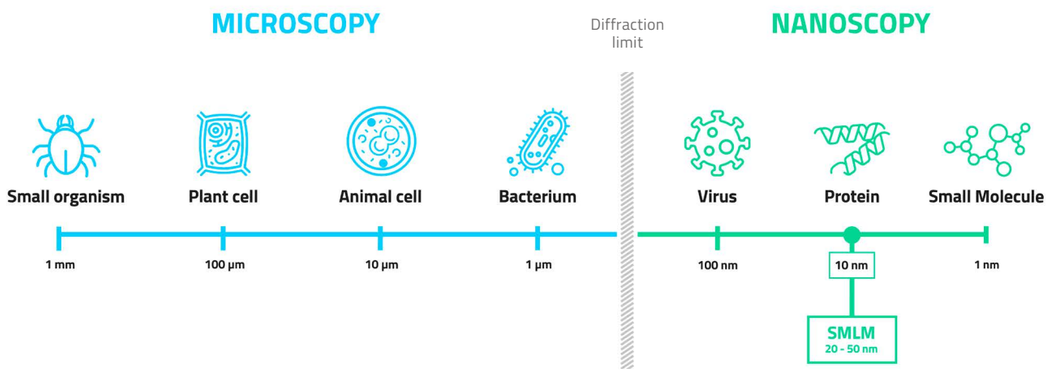
abbelight.com

FROM MICROSCOPY TECHNIQUE TO NANOSCOPY PRINCIPLE

Revealing structures and dynamics at the nanoscale

Standard **fluorescence microscopy** techniques (Widefield, confocal, etc.) operate in the resolution range of 200–300 nm laterally and 500–800 nm axially. However, biological structures and processes that occur at a lower scale require superior resolution.

Among super-resolution techniques, Single-Molecule Localization Microscopy (SMLM) is the most powerful one used by scientists.



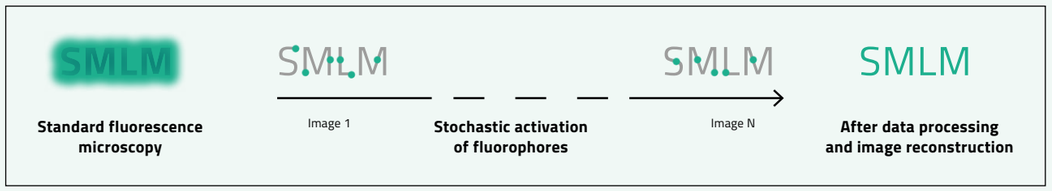
SMLM principle

SMLM relies on the ability to activate randomly only a subset of fluorescent molecules in order to distinguish them spatially.

> SMLM is the nanoscopy technique that retrieves quantitative structural and dynamic information with the highest precision achievable.

By repeating this process in consecutive image acquisitions, accumulated raw data are processed to detect single molecules with a nanometric precision (down to 15 nm).

Data quantification and analysis are then performed to resolve either structures or dynamics at **the nanoscale level**. The uniqueness of SMLM is that it gives rise to not only highly resolved images, but also to **single molecule 3D coordinates**, hence opening up new areas for **spatial and temporal quantitative analysis.**



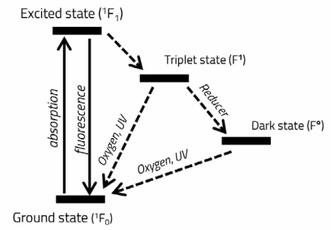
SMLM approaches

Current SMLM approaches only differ in how the fluorophore activation-inactivation is induced. Among them, STORM, PALM, and PAINT resolve spatial structures with nanometric precision, while SPT (Single Particle Tracking) reveals temporal dynamic processes in living cells.

STRUCTURES

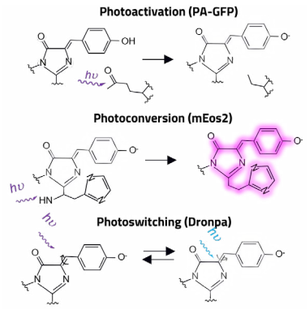
STORM (STochastic Optical Reconstruction Microscopy)

- Standard organic fluorescent dyes (cyanines, rhodamines, oxazines…)
- Specific imaging buffer (containing a reducer, which induces the transition to the dark state, and an oxygen scavenging system to stabilize this state before returning to the ground state)



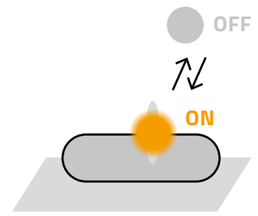
PALM (Photoactivated Localization Microscopy)

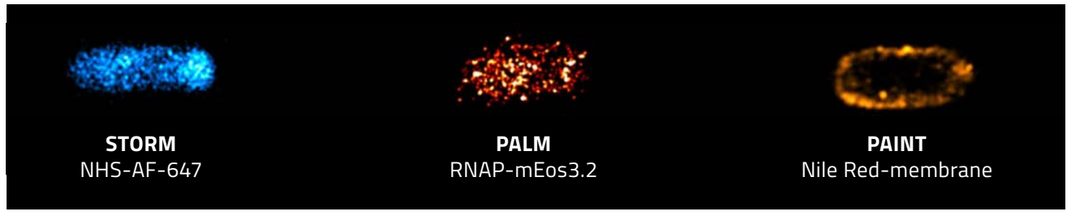
- Photo-activatable or -convertible fluorescent proteins (mEos3,2, Dendra2, PA-mCherry, mMaple,…);
- No specific buffer, live-cell compatible



PAINT (Point Accumulation for Imaging in Nanoscale Topography)

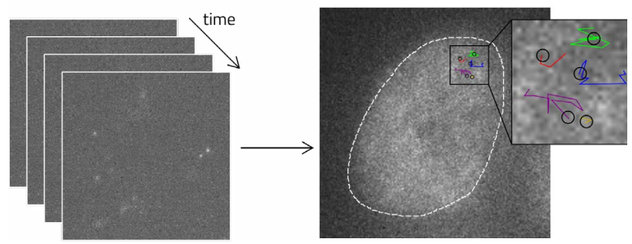
- Specific fluorophores that have the ability to emit fluorescence only upon binding to their biological target (e.g. Nile Red, which fluoresces only when interacting with membranes)
- No specific buffer, live-cell compatible





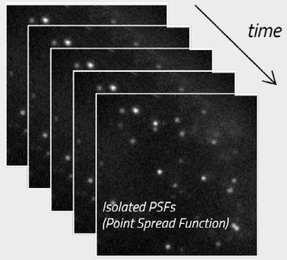
DYNAMICS

sptSMLM combines Single Particle Tracking with SMLM (PALM or STORM) to obtain spatially and temporally highly resolved diffusion maps of single molecules.

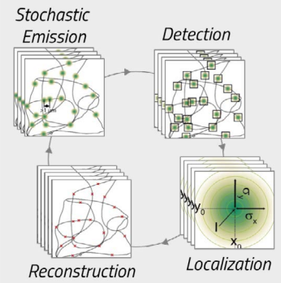


LOCALIZING MOLECULES IN 2D

In order to reconstruct a nanoscopy image, each molecule is detected and localized by specialized algorithms.



Raw film



Data processing

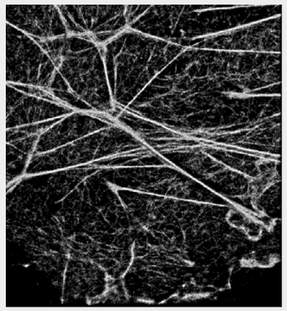
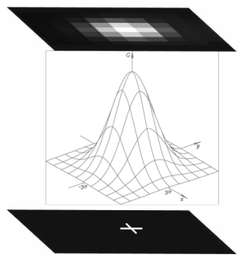


Image reconstruction

To determine the x and y positions of each molecule, a commonly used localization algorithm is Gaussian fitting.



Detected PSF

Gaussian fit

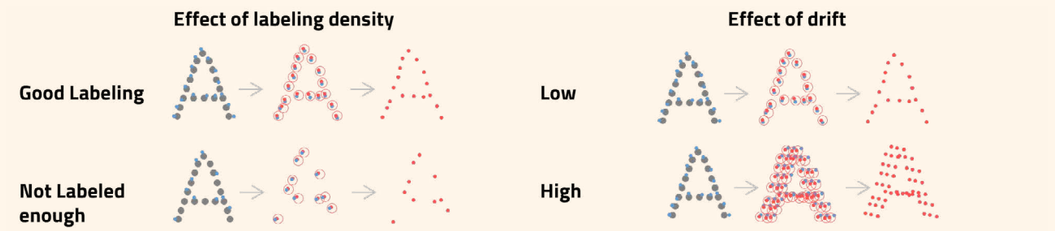
Localization

Localization precision $\approx \frac{\sigma}{\sqrt{N}}$

N = number of photons
σ = standard deviation

THE LOCALIZATION PRECISION IS TYPICALLY 15 nm.

Because images are now obtained at the nanoscale level, new challenges arise. Effects that were negligible at the microscopy level now need to be considered.



Resolution = 2,35 × (Localization precision) ⊗ (labeling density, drift...)

Epifluoresecence image

2D nanoscopy image (STORM)

200 nm

SKB3 - clathrin AF647

5 µm

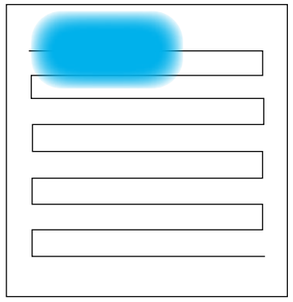
EXCITATION TECHNIQUES

ASTER

The patented Abbelight Adaptative Scanning for Tunable Excitation Rendering (ASTER) technology is a unique, versatile illumination scheme that creates the largest uniform field of view (FOV) of a sample on a scalable platform.

The principle of ASTER technology is to scan with a very thin Gaussian beam on the sample in order to create a homogeneous illumination over any field of view. This can be achieved thanks to 2D galvanometers controlled with our Abbelight SAFe Excitation and Abbelight software suite.

Mau,A et al.2021



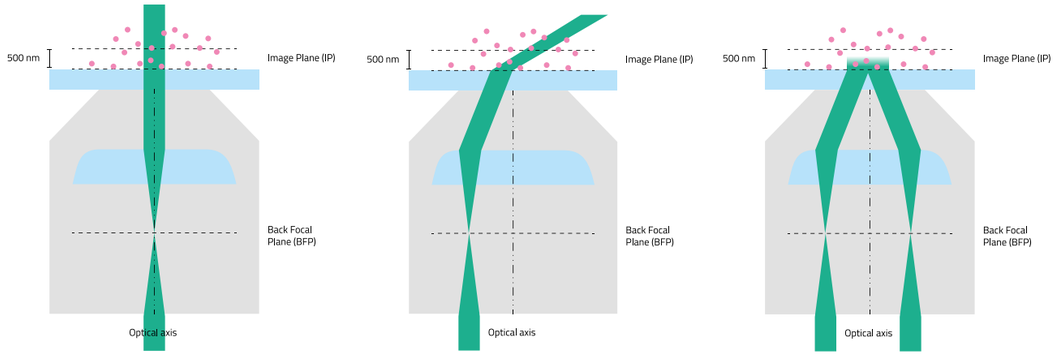
Scanning for homogenous illumination faster than the camera acquisition time (200 fps for the largest field of view)

This unique innovation offers many advantages

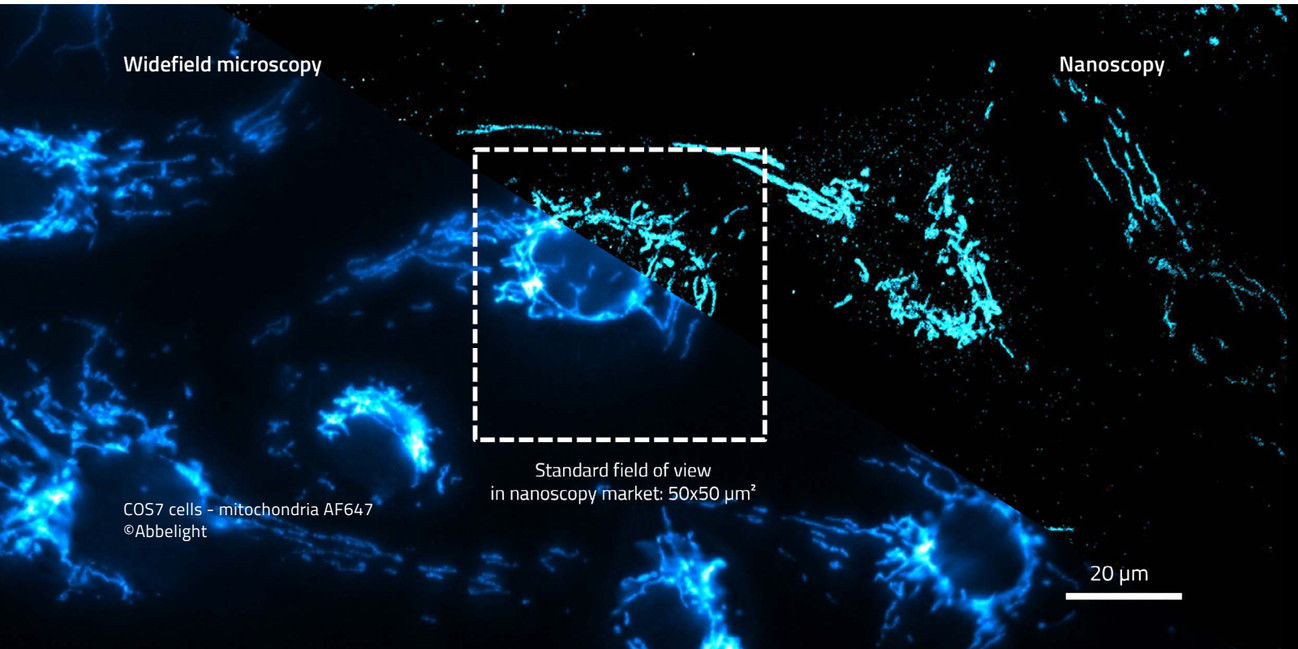
- 150x150 µm² field of view
- More intensity on the sample with lower laser power
- Illumination adaptable to the sample
- TIRF, HiLo or EPI illumination modes
- More quantitative data
- Homogeneous illumination, no interference patterns in the image

Illumination modes

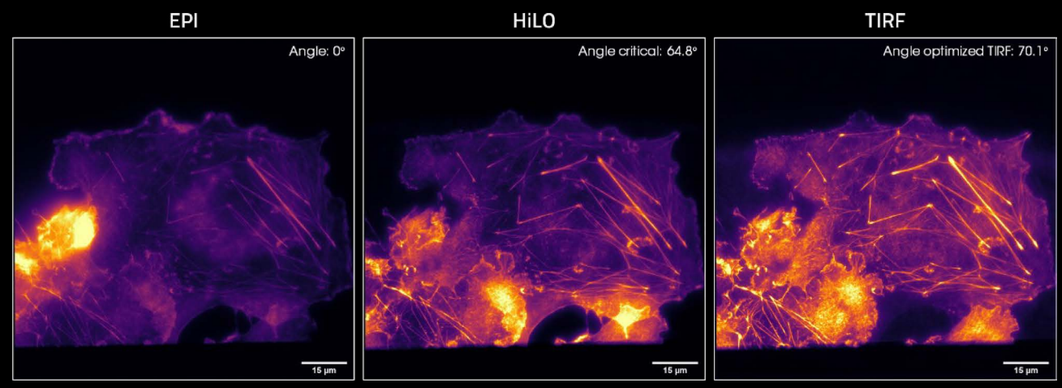
Abbelight bioimaging platform enables the adjustment of different illumination modes. Adaptable to any combination of microscopes, objectives, XYZ stages and accessories, this solution guarantees automatically calibrated and reproducible positioning of different illumination angles.



EPI	HILO	TIRF
Epifluorescence	**Highly Inclined and Laminated Optical sheet**	**Total internal reflection fluorescence**
In-depth illumination, higher background	Limited background, not restricted to coverslip up to 10µm depth	Illumination close to the coverslip, removal of in-depth background
To image Structures far from the coverslip: nuclei, thick cells, tissues…	To image slightly in-depth samples Perfect for SMLM imaging	To image Structures close to the coverslip (e.g. membranes, cytoskeleton)



EASILY SWITCH BETWEEN DIFFERENT MODES



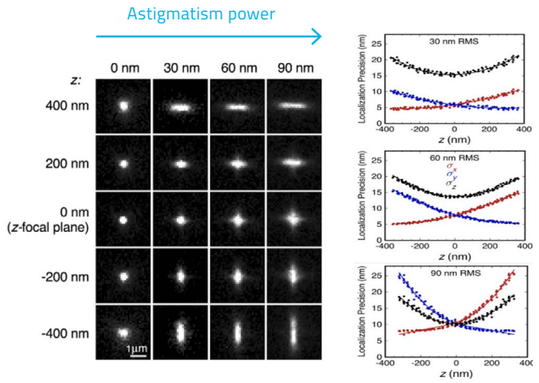
ULTIMATE 3D

To extract the 3D position of molecules with an ultimate precision and isotropy, Abbelights's enhanced 3D technology relies on a unique two-channel principle.

Cabriel, C. al (2019, Abbelight), Bourg, N. et al (2015, Abbelight)

On one of the two channels, a controlled astigmatism aberration is induced by a custom cylindrical lens. This astigmatism is then measured and related to the distance between the focal plane of the objective lens and the emitting particle. The greater the astigmatism power, the better the axial Z precision and the lower lateral XY precision. Consequently, for conventional single-camera setups, a compromise has to be found between the power of astigmatism and x,y localization precision.

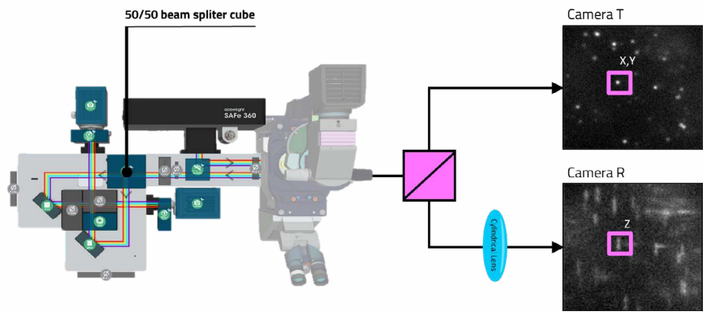
The second channel is therefore used to preserve the best lateral resolution, enabling uncompromised access to the most accurate information possible in all three axes.

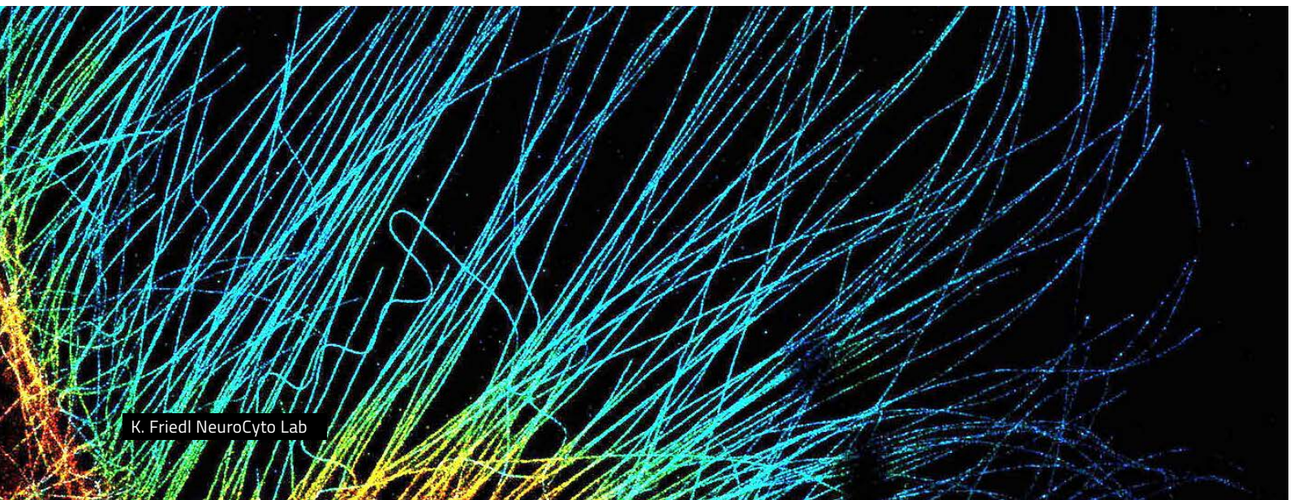


Chowdhury, R. et al. (2022)

Abbelight enhanced 3D gives access to :

- Capture range of 1200 nm
- Enhanced astigmatic lens for better z precision
- No loss of lateral resolution





K. Friedl NeuroCyto Lab

A COMPLETE SOFTWARE SOLUTION

Abbelight™ SAFe Neo Software Suite

Provides a user-friendly all-in-one workspace for acquisition, processing, and analysis of nanoscopy data.

Voronoi segmentation

Nanoscopy data, unlike standard microscopy images, are coordinate-based rather than pixel-based, opening up new areas for in-depth data analysis. SAFe Neo software offers a variety of tools for nanoscopy data visualization and analysis.

Feature	SAFe Neo Software Suite
Control of instrument	Laser power and illumination angle (EPI, HiLo, TIRF) Cameras (up to 150x150 µm² FOV)
Control of acquisition parameters	Region of interest size, Field of excitation size Exposure time (down to 10 ms per frame) Frame number
Project Management	Smart Digital Notebook
Live reconstruction of nanoscopy data	Choice of localization parameters: ▪ Intensity threshold ▪ Background subtraction method
Live vizualisation	2D and 3D vizualisation
Live drift correction	Cross-correlation
Multi Dimensional acquisition	Acquisition scenario (spatial, axial, time point and illumination angles)
Visualization	3D visualization Mutlicolor visualization Possibility to export images in TIFF format compatible with commonly used software
Descriptive spatial statistics	Localization distribution Measuring tools
Automatic settings	Automatic multi-view alignment for multicolor modalities Automatic Hilo and TIRF illumination settings
Post acquisition processing	Adjustment of parameters and batch processing
Clustering analysis	K-Ripley function, DBSCAN, Voronoi tessellation Centroid, density and volume measurements
Single-particle tracking	Track reconstruction, quantification of the number of tracks, track duration, diffusion coefficient, etc.
Spectral demixing	Separation of far-red dyes for multicolor imaging
Co-localization	CBC Coordinate-Based Colocalization

SMLM Multicolor imaging

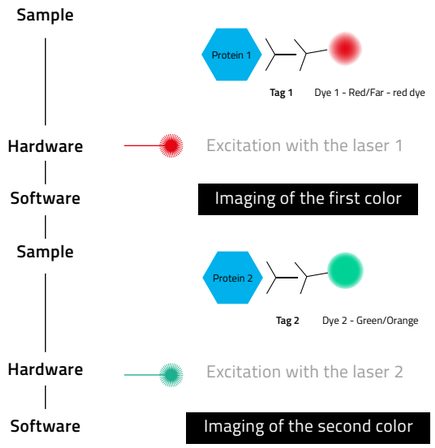
Multicolor imaging is a powerful way to assess spatial relation between different biological structures.

Abbelight bioimaging platform offers the possibility to access different methods of multicolor SMLM modalities:

METHOD 1: ACQUISITION OF DIFFERENT COLORS SEQUENTIALLY

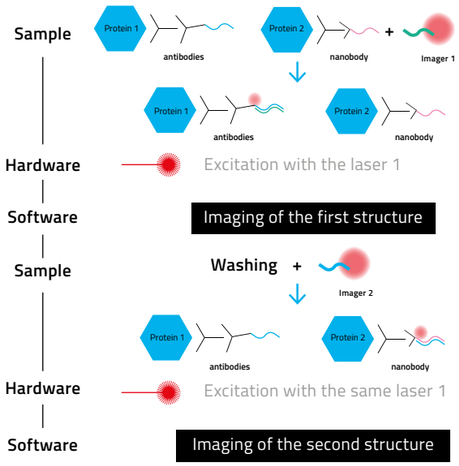
With multiple lasers:

Imaging sequence of spectrally different fluorophores with their respective excitation sources



With same Laser, with exchange DNA PAINT method:

Exchange of the labelling species between acquisition rounds:

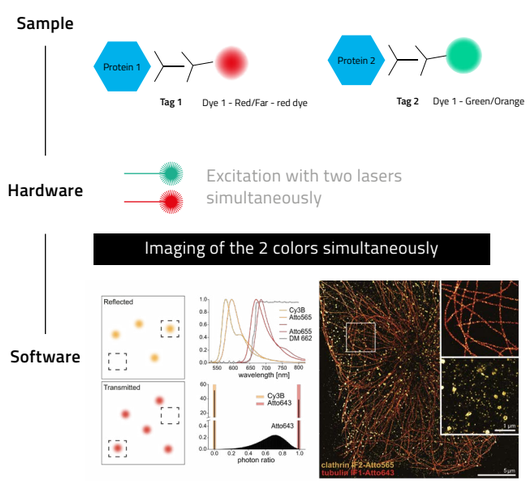


METHOD 2: DICHROIC CUBES TO SPLIT EMISSION LIGHT AND FILTERS FOR SIMULTANEOUS MULTI-COLOR NANOSCOPY

Friedl,K et al.2023

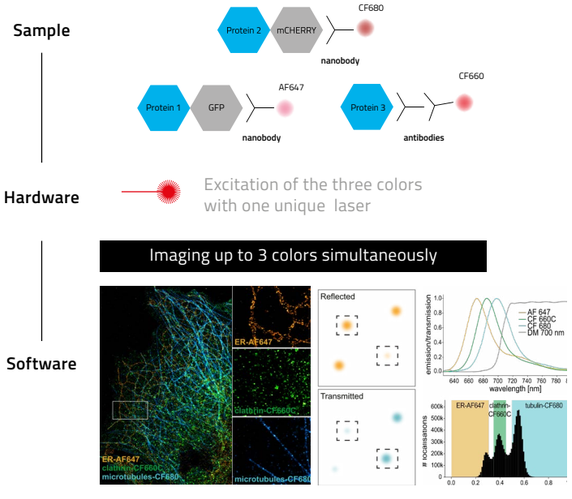
With multiple lasers:

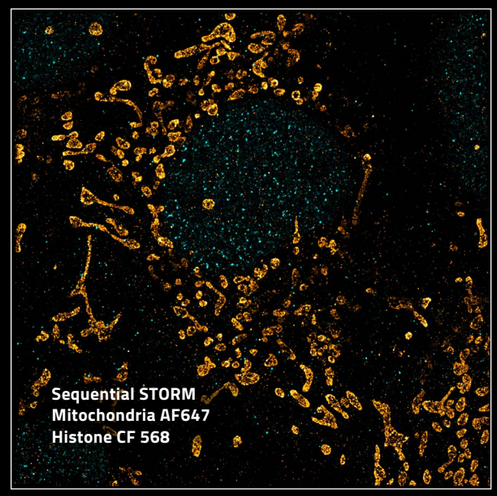
Sequence of simultaneous imaging of spectrally different fluorophores with their respective excitation sources

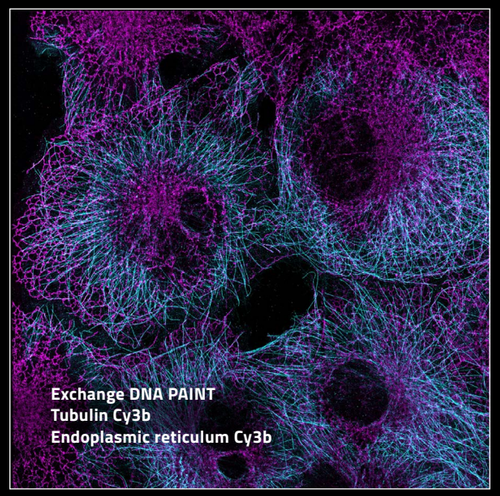


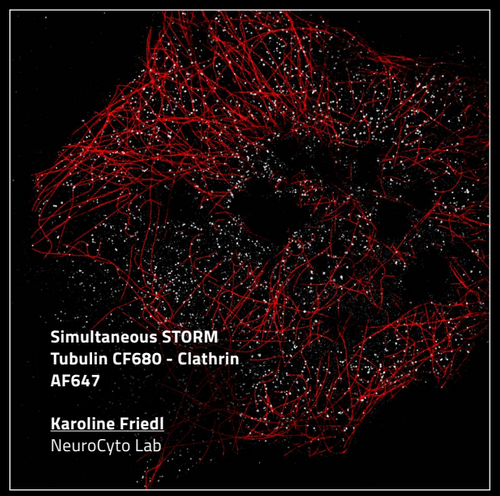
With same Laser:

Spectral demixing technology, sequence of simultaneous imaging of spectrally different fluorophores that can be excited with the same laser









Multicolor modality		Acquisition duration	Drift duration between channels	Chromatic aberrations between channels	Highly recommended SMLM techniques
Sequential	with multiple lasers	Standard	Yes drift to be corrected *	Yes, to be calibrated	PAINT, PALM
	with one laser	Standard	Yes drift to be corrected *	No	PAINT
Simultaneous	with multiple lasers	2 times faster	No drift	Yes, to be calibrated	PAINT, PALM
	with one laser	2 times faster	No drift	No	STORM, PAINT

*Abbelight SAFe Neo Software Suite includes an optimal live drift correction

Filtering

Abbelight™ SAFe Neo Software Suite and the filtering tools can provide access to quantitative information about the quality of SMLM data: frame, uncertainty, intensity, and blinking.

FRAME

Remove a selected number of frames on a dataset (at the beginning or the end)

UNCERTAINTY

This value is calculated using the Webb/Mortensen formula taking into account the number of photons, the size of the PSF and the background.

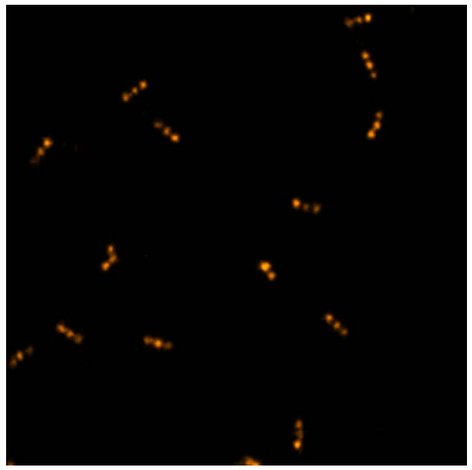
INTENSITY

Number of photons per localization

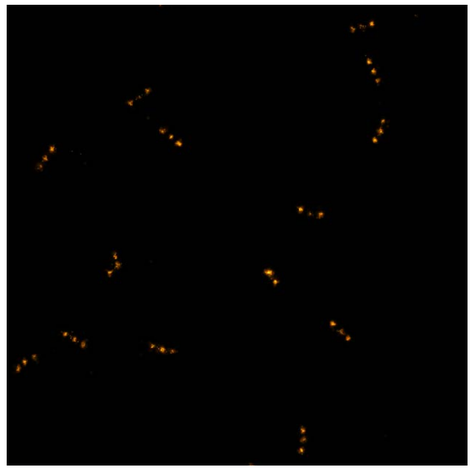
BLINKING

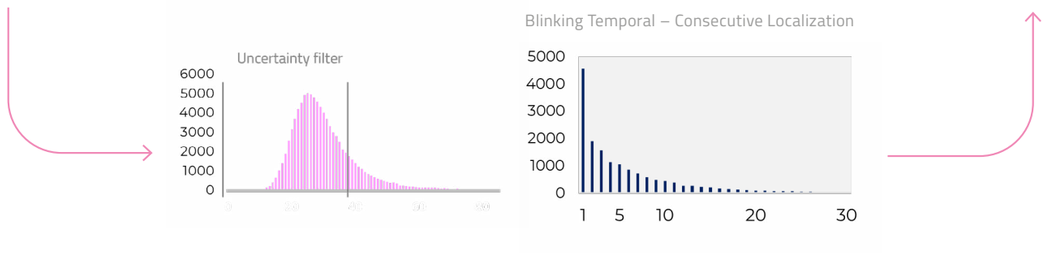
Indicates how many locations appear in several consecutive images and allows to the merge.

Nanorulers before filtering



Nanorulers after filtering





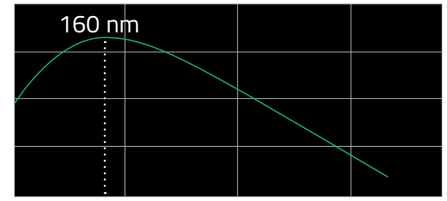
Clustering

SAFe Neo provides several methods to analyze clusters in a dataset.

DETERMINING IF A DATASET IS CLUSTERED

The K Ripley function evaluates whether a population of localizations is aggregated or not based on a neighborhood analysis.

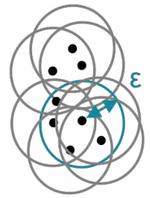
The bell-shaped curve indicates the presence of aggregated data points and provides an estimate of the size of these aggregates.



ISOLATING CLUSTERS IN A DATASET: TWO METHODS

DBSCAN Density-Based Spatial Clustering of Applications with Noise. For each localization in the dataset, the algorithm searches whether it has enough neighboring MinPts within the distance Σ. If yes, it considers the localization part of a cluster.

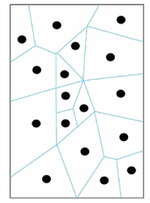
Ester et al. 1996



Number of neighbors within a distance of Σ indicates density

Voronoi partitions the image into polygons, where each polygon contains one and only one localization. The area of the polygon is indicative of the density of localizations: a dense region will have small polygons, while a low-density region will have big polygons. The user can choose a density threshold, above which localizations are considered part of a cluster.

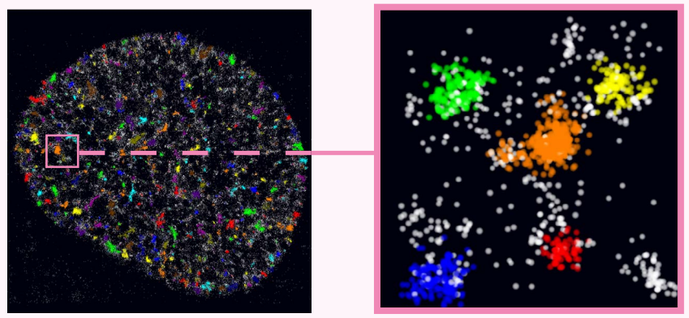
Levet et al. 2015



Polygon size indicates density

QUANTIFICATION

Once clusters are identified, the software can quantify the number of clusters, their localization, their volume, their density, their radius of gyration, among others.

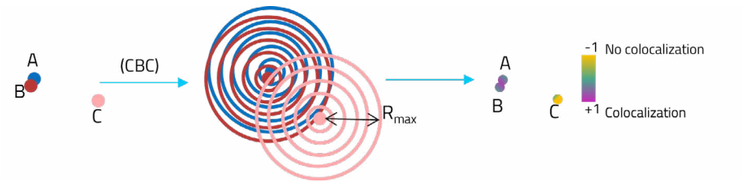


COS7 cells – DNA replication sites
EdU-AlexaFluor647
©Abbelight

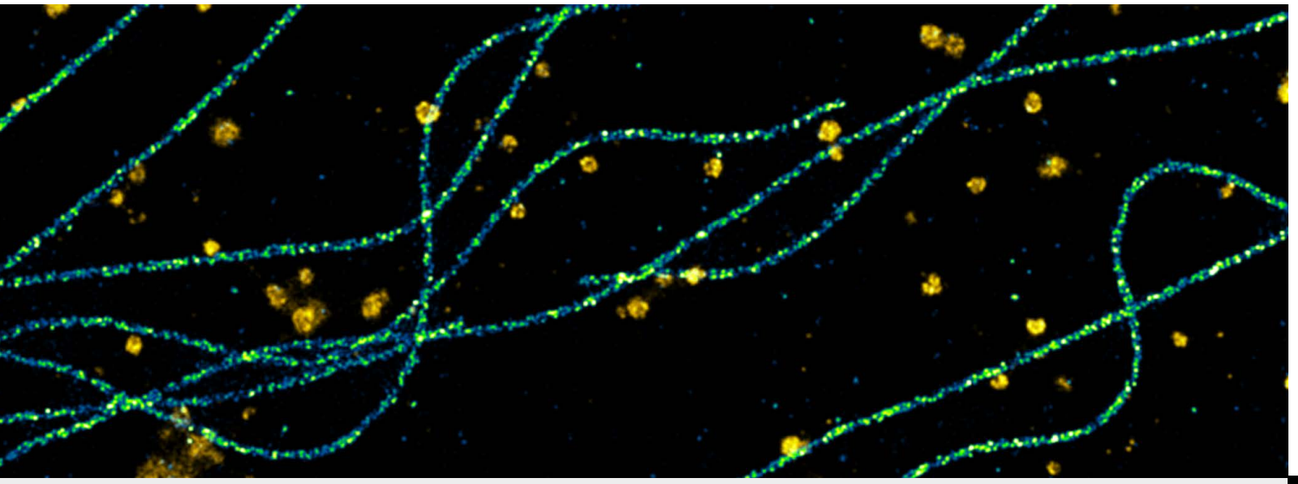
Colocalization

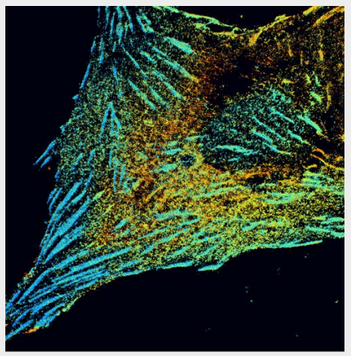
COORDINATE-BASED COLOCALIZATION

CBC algorithm takes into account the spatial distribution of biomolecules and provides a colocalization value for each single-molecule localization (A, B, C). Each species is assigned an individual colocalization value [-1;1] in the distance Rmax.



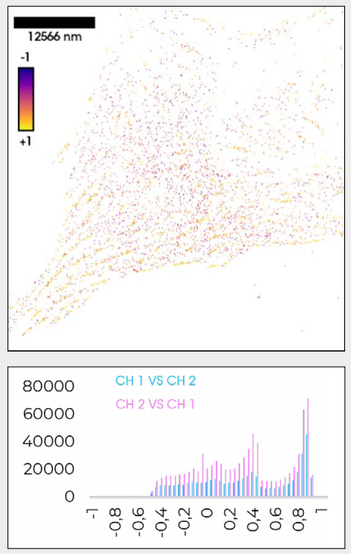
Malkusch et al 2012





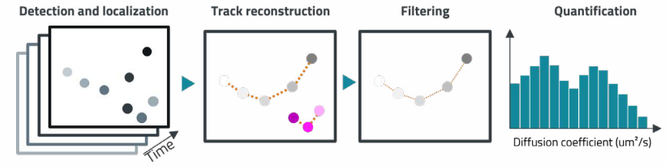
Paxilin – Vinculin 3D STORM

NEO Software analysis with CBC algorithm



Single-Particle Tracking (SPT) analysis

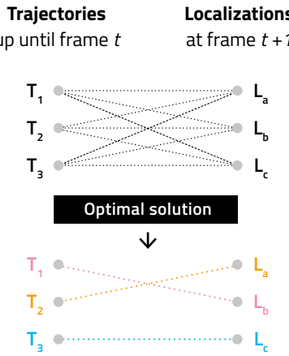
To study the dynamics of single particles, Abbelight™ SAFe Neo Software Suite can reconstruct trajectories from raw SPT data.



RECONSTRUCTING TRAJECTORIES

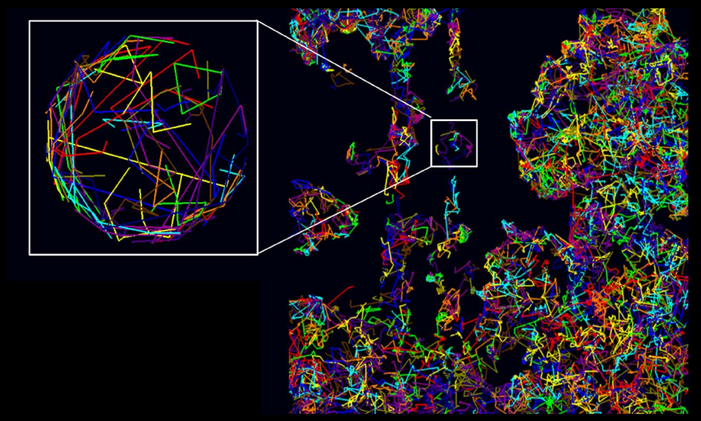
The goal of an SPT algorithm is to connect the localizations from frame to frame. The algorithm takes all the tracks at frame t and all the dots at frame t+1, and calculates the probability of assigning each track to each localization. Afterward, it chooses the solution that maximizes the probability. These probabilities can be calculated based on several factors, including distance and motion speed.

Jaqaman et al. 2008, Sergé et al. 2008



QUANTIFICATION

After the track reconstruction, the software can quantify parameters like the number of tracks, track duration, average intensity, and diffusion coefficient (based on Mean Square Displacement analysis).



Sample provided by Dr. Chiaruttini
University of Geneva, Switzerland

Abbelight

Founded in 2016, Abbelight is a fast-growing company specialized in imaging solutions focusing on microscopy and unique single molecule detection (super-resolution).

The portfolio integrates a constantly evolving know-how on chemistry, optics, and computer science to offer a complete solution, from sample preparation to data management, including an optimal bio-imaging platform that can be adapted to all researchers', biotech labs' and medical facilities' needs.

Abbelight is a French company developed by four passionate researchers who aim to help improve human health in various areas such as **bacteriology, extracellular vesicles, neurosciences, structural biology...**

Today, Abbelight employs over 60 people who are all driven by the goal of providing the best solutions and support to our customers all around the world.

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