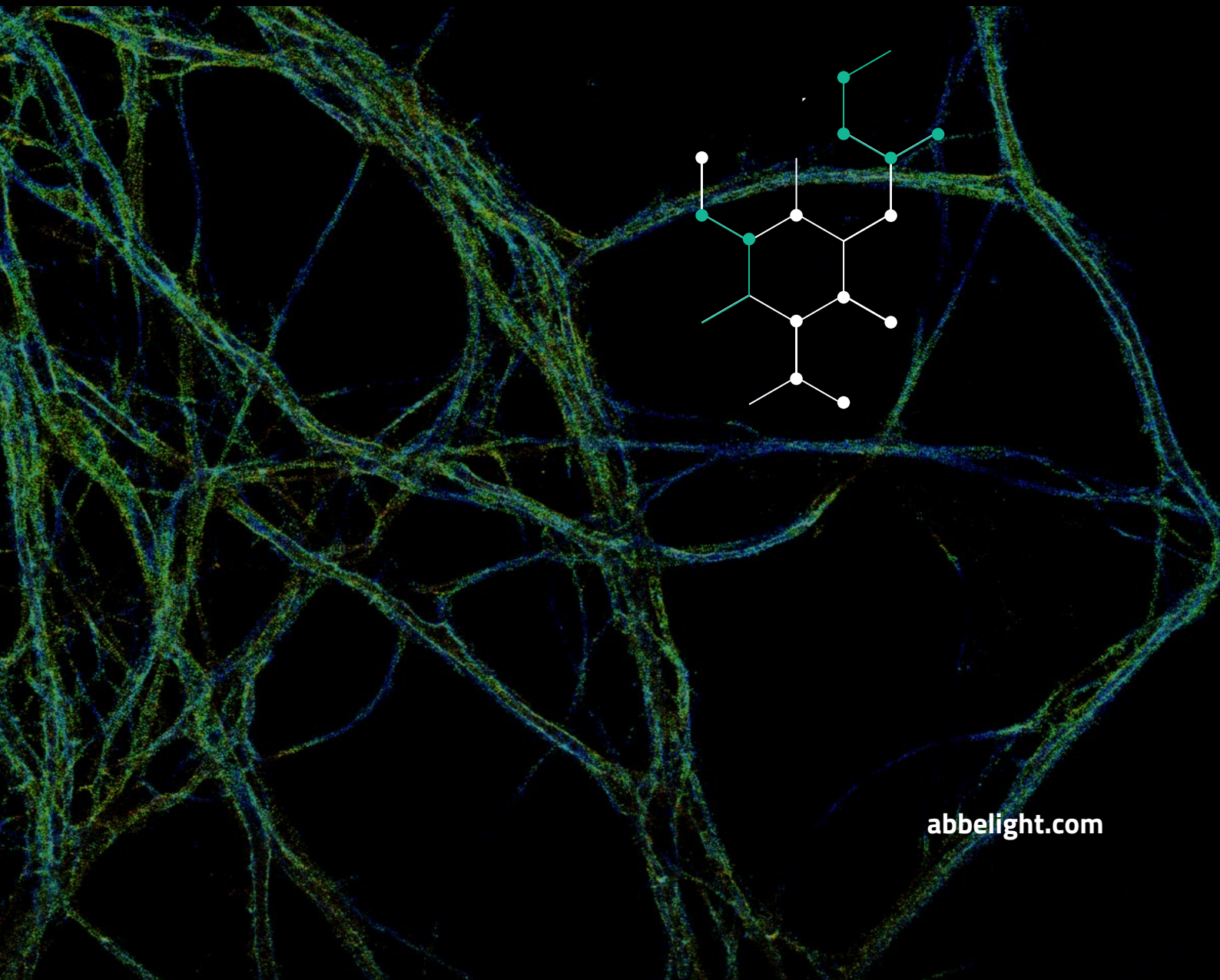
Abbelight
NOW, WE SEE

# MICROSCOPY & NANOSCOPY
## Technologies at a glance



abbelight.com

# A COMPLETE & INNOVATIVE PORTFOLIO

The ultimate multimodal bioimaging platforms

**Sample Preparation**

**SMART Products**

- Ready-to-use kits
- Reagents
- Automation devices

**Imaging**

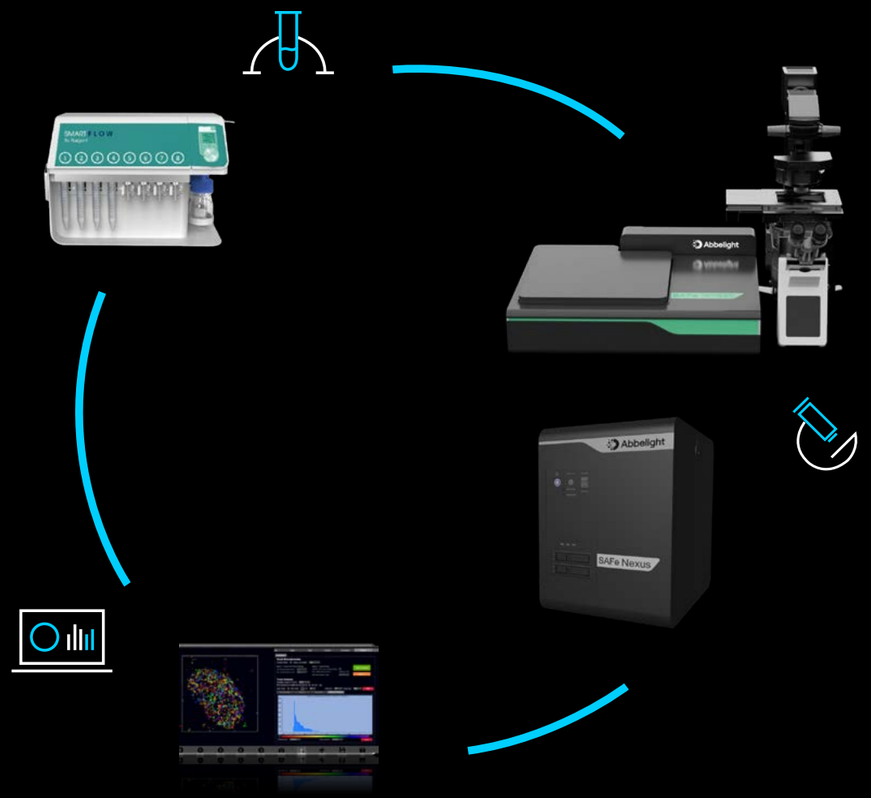
**SAFe Platforms**

- Upgradable
- Customizable
- Multimodal platforms

**Analysis**

**NEO Software Suite**

- For quantitative analysis
- For visualization at the nanoscale

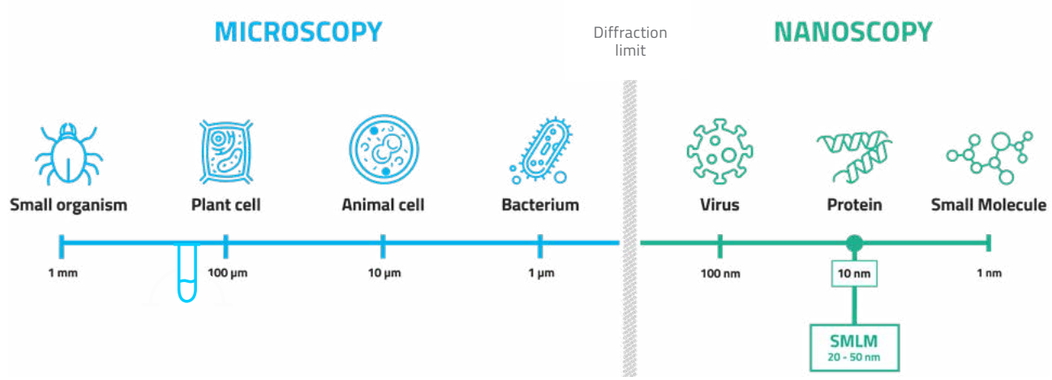



# FROM MICROSCOPY TECHNIQUE TO NANOSCOPY PRINCIPLE

## 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.

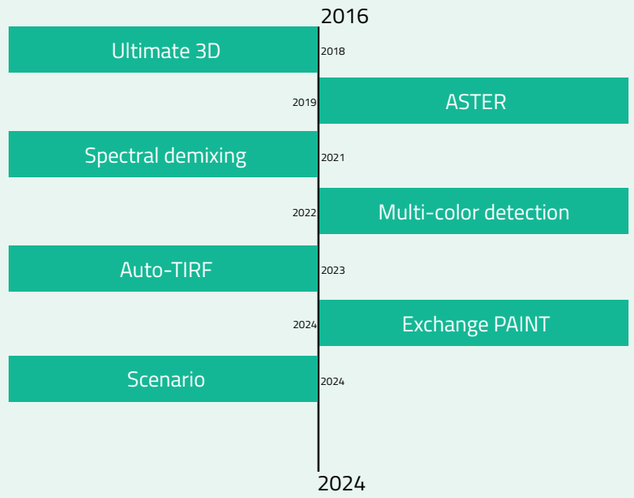
Among super-resolution techniques, Single-Molecule Localization Microscopy (SMLM) is one of the most widely used by scientists.




## Abbelight's technologies

**Pushing the boundaries of fluorescence**

Since 2016, Abbelight has been pushing the boundaries of fluorescence by creating innovative technologies to visualize and understand cell dynamics and structures, delivering powerful and user-friendly products.

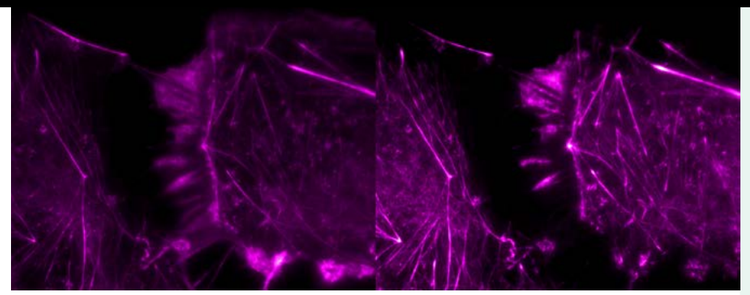


# FROM MICROSCOPY TECHNIQUE

# TO NANOSCOPY PRINCIPLE

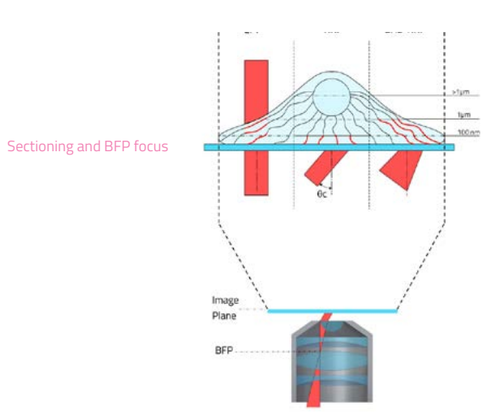
Total Internal Reflection Fluorescence microscopy (TIRF) (Axelrod, 2013) is the most suitable imaging method as it offers advantageous optical sectioning (up to a few hundred nanometers after the coverslip).

Nevertheless, in practice, the TIRF microscope remains quite difficult to preset for non-expert users. It requires perfect optical conjugation of the microscope and very precise positioning of the beam in the back focal plane (BFP) of the objective (Mattheyses et al., 2010), resulting in experiments that are not reproducible from day to day.



EPI illumination, no optical sectioning

TIRF illumination, nanoscale sectioning




## Abbelight's TIRF technology

This technology is a hardware and software solution that provides **optically perfect TIRF imaging**.

**Adaptable to any combination of microscopes, objectives, XYZ stages, and accessories**, our new product guarantees **automatically calibrated and reproducibly positioned TIRF angles**. Coupled with a detection optical module, it enables optimal, simultaneous multicolor imaging by leveraging **ASTER** technology. This technology provides **ultra-widefield and speckle-free illumination** of biological structures close to the coverslip, such as membranes, focal adhesions, and much more.

## Features and advantages

- **Adaptable** technology for any inverted microscope.
- **The largest, uniform and speckle-free field of view** on the market, thanks to Abbelight's ASTER illumination strategy.
- **Automatic, quantitative, and reproducible TIRF** to define the optimal position of TIRF angles and obtain reliable data over time, regardless of your sample.
- **Simultaneous multicolor TIRF** imaging that allows you to capture multiple structures in your samples simultaneously.
- **Multi-dimensional acquisition with Abbelight Scenario** offering options designed to best meet the needs of TIRF applications.

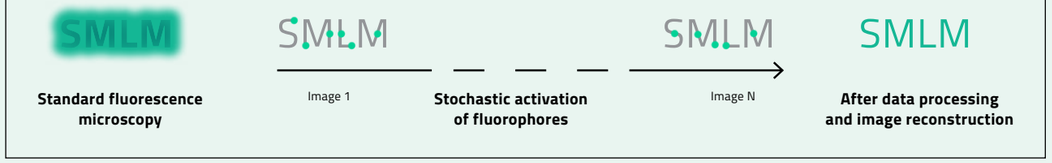
## SMLM principle

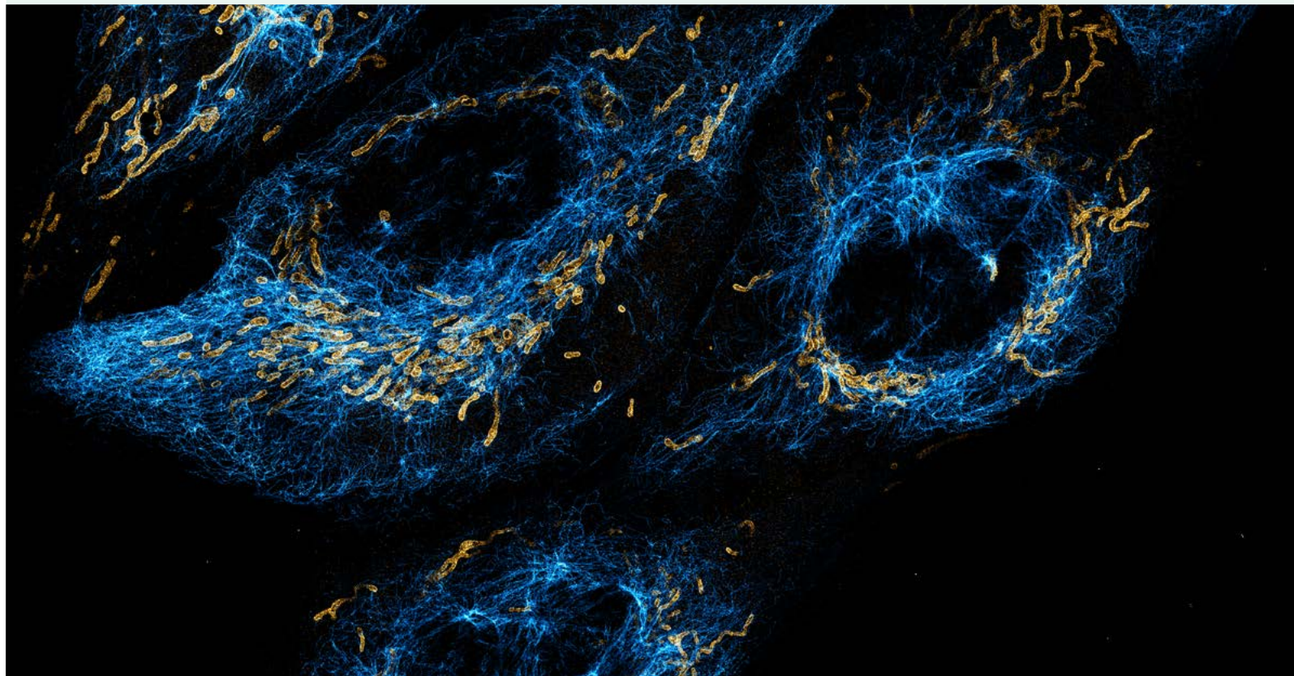
**SMLM relies on the ability to activate randomly only a subset of fluorescent molecules in order to distinguish them spatially.**

> 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.**



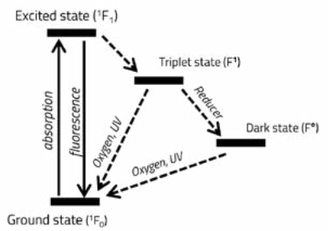
# SMLM approaches

**Current SMLM approaches only differ in how fluorophore activation and inactivation are 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

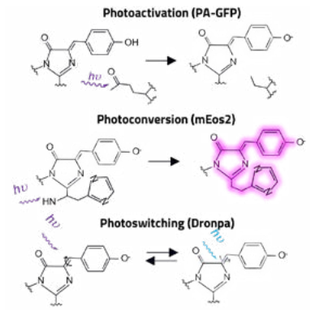
**STORM** (STochastic Optical Reconstruction Microscopy)

- 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)



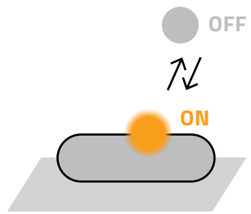

**PALM** (Photoactivated Localization Microscopy)

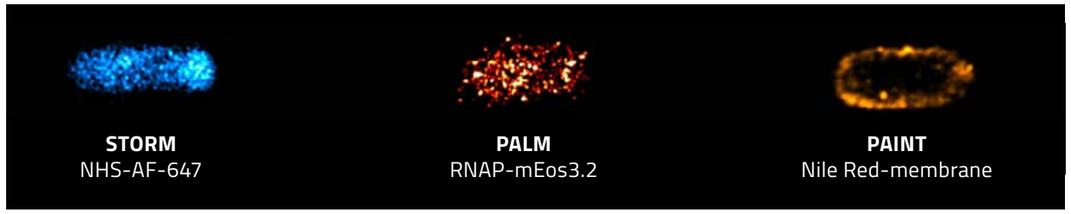
- Photo-activatable or -convertible fluorescent proteins (mEos3.2, Dendra2, PA-mCherry, mMaple,…);
- No specific buffer, live-cell compatible




**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



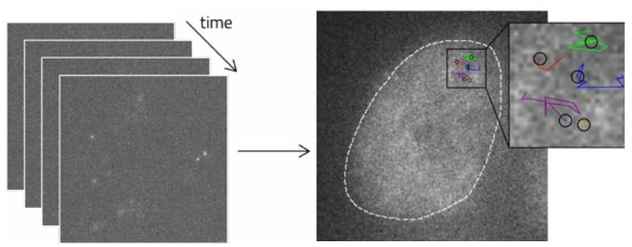
**STORM**
NHS-AF-647

**PALM**
RNAP-mEos3.2

**PAINT**
Nile Red-membrane

## DYNAMICS

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

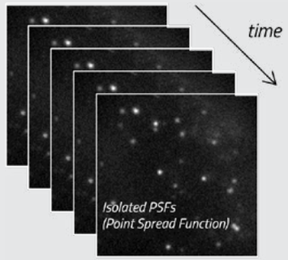



Raw data

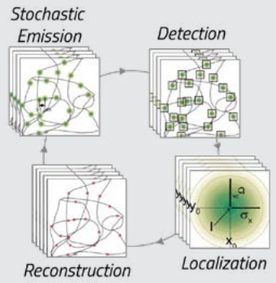
Single particle trajectories

# LOCALIZING MOLECULES IN 2D

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




Raw film




Data processing

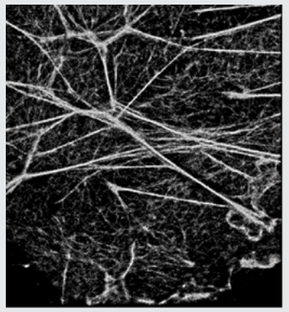
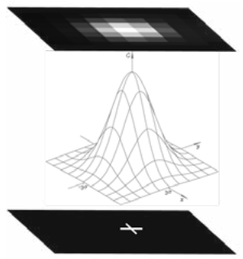


Image reconstruction

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

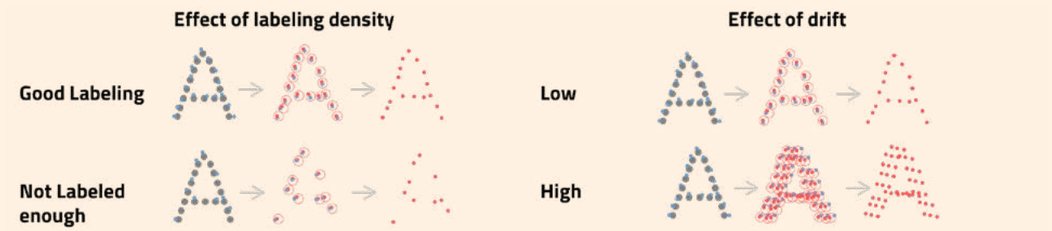



**Localization precision** $\approx \frac{\sigma}{\sqrt{N}}$

N = number of photons
σ = standard deviation

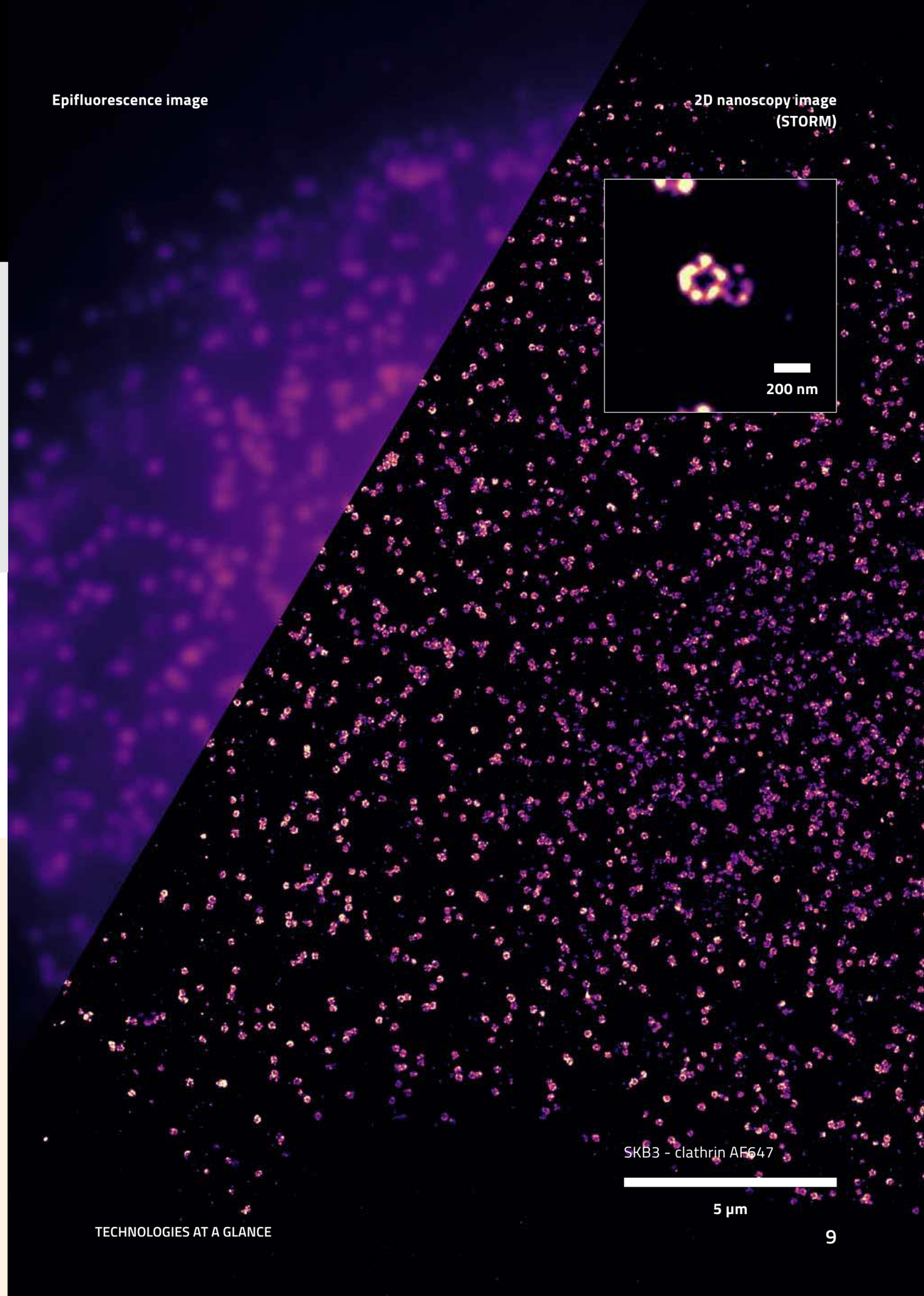
## THE LOCALIZATION PRECISION IS TYPICALLY 15 nm.

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...)**





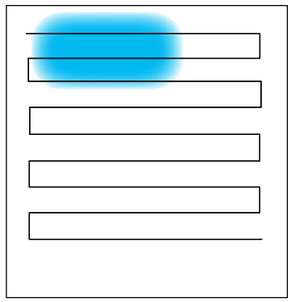
# EXCITATION TECHNIQUES

## ASTER

**Our patented 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 excitation module and NEO software suite.

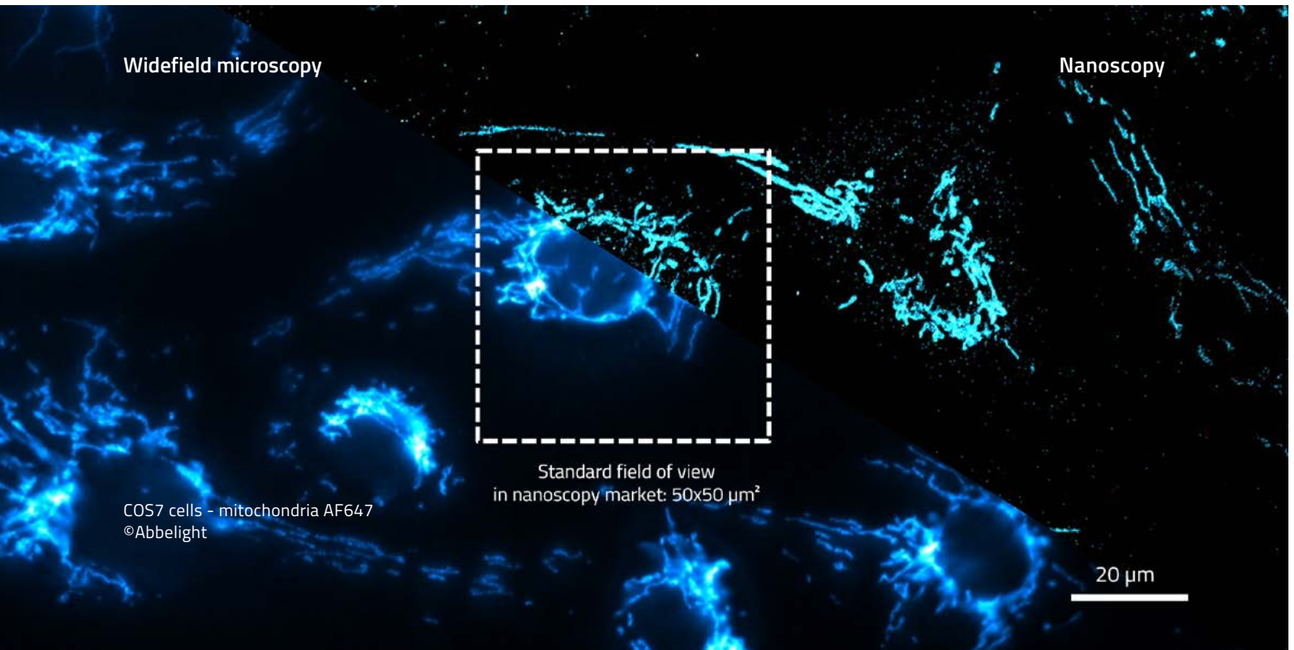
Mau,A et al.2021



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

**This unique innovation offers many advantages**

- Up to 230 x 230 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




## Very large and uniform FOV

**Abbelight's patented ASTER technology offers homogeneous TIRF illumination of the sample over the largest FOV on the market.**

| | Simultaneous 2C | Simultaneous 4C |
|---|---|---|
| 60X | 230 x 230 µm² | 150 x 150 µm² |
| 100x | 150 x 150 µm² | 80 x 80 µm² |

Blue F-Actin-AF488 - Green Tub-AF568 - Red Clathrin-AF647

## Abbelight's ASTER illumination

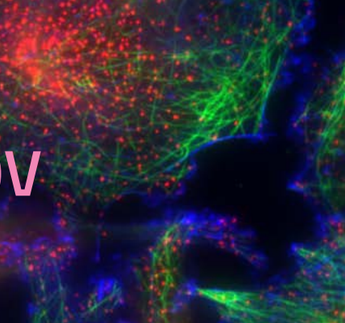
ASTER TIRF illumination, co-developed with Sandrine Lévêque-Fort and published in Nature Communications (A. Mau et al., 2021), outperforms the standard TIRF strategy because it breaks classical interference fringes like those seen in the Ring/Azimuthal TIRF method.

ASTER TIRF illumination also surpasses the Ring/Azimuthal method because it offers >95% uniform excitation over the largest FOV, while Ring/Azimuthal TIRF is strongly limited by the Gaussian shape of the laser irradiance.

**Standard TIRF**

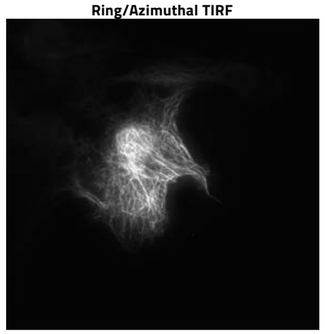
Interference fringe issues

**ASTER TIRF**



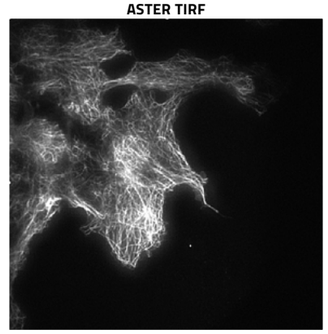
No fringes offering reliable image

**Ring/Azimuthal TIRF**



Gaussian illumination leading to non-uniform excitation

**ASTER TIRF**

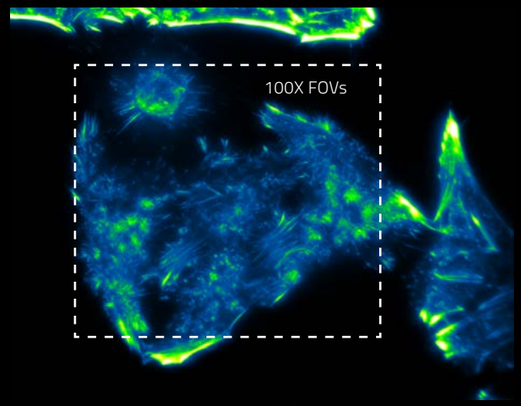


> 95% excitation uniformity over the whole FOV

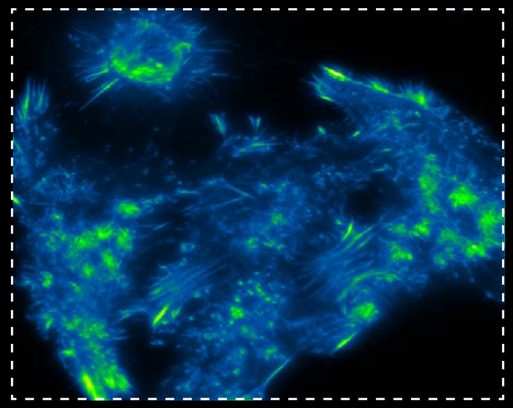
# Adaptable

**The optical system installed in our excitation module is designed to obtain the optimal TIRF angle position and get the perfect TIRF illumination compatible with any combination of:**

Microscopes Objectives XYZ stages Accessories



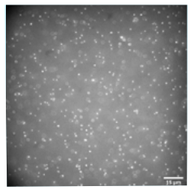

60X 1.42-1.49 objectives

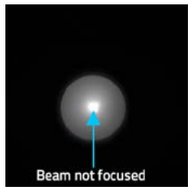


100X 1.4-1.5 NA objective

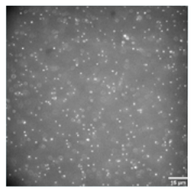
## EPI Illumination

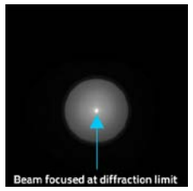
IMAGE PLANE BACK FOCAL PLANE






Beam NOT perfectly focused on BFP
**No difference in EPI**

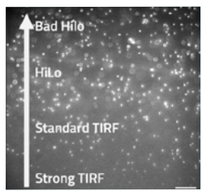


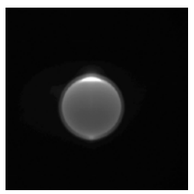



Beam perfectly focused on BFP
**No difference in EPI**

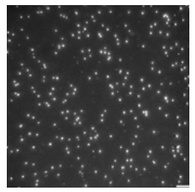
## TIRF Illumination

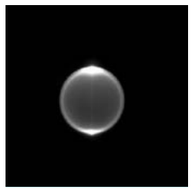
IMAGE PLANE BACK FOCAL PLANE



Beam NOT perfectly focused on BFP
**TIRF not available**



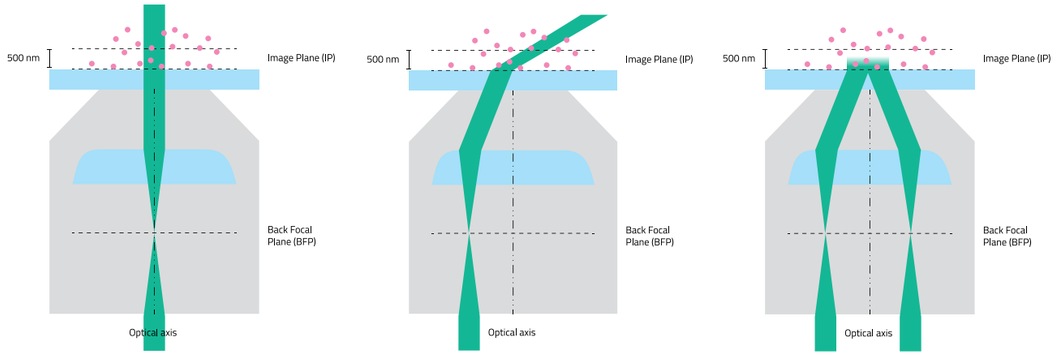


Beam perfectly focused on BFP
**Perfect TIRF**



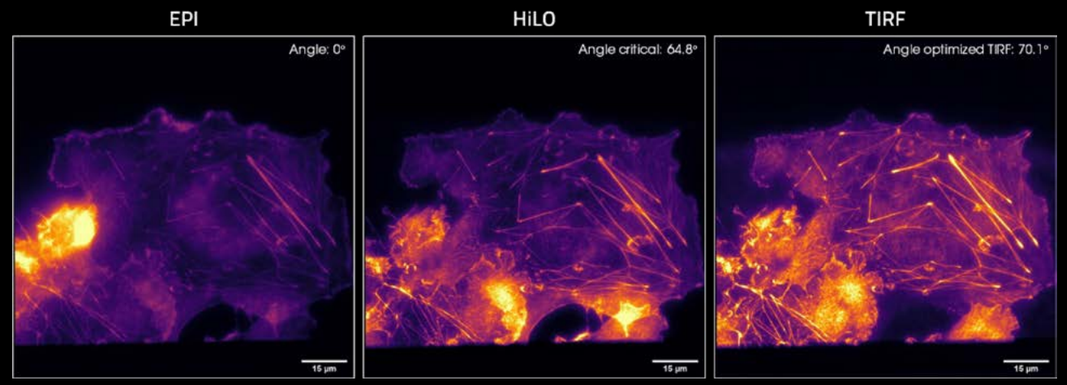
# Illumination modes

Abbelight's bioimaging platform guarantees automatically calibrated and reproducible positioning of different illumination angles.



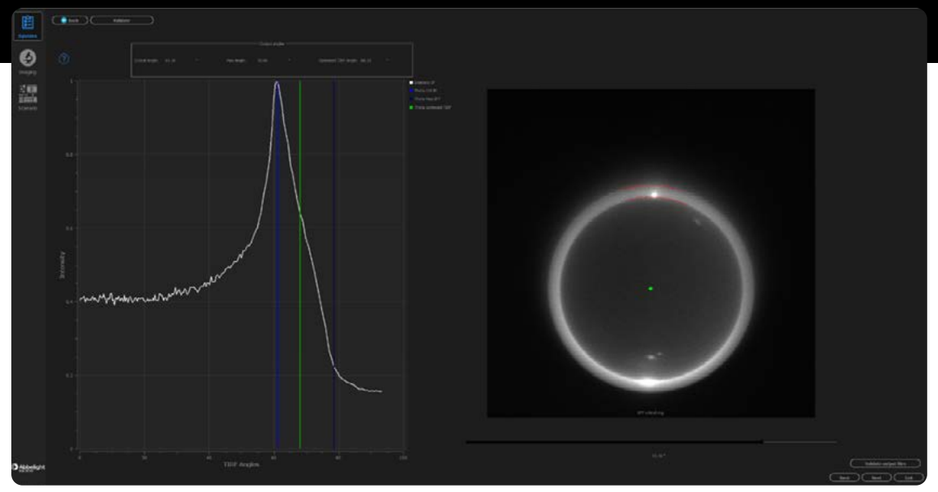

| EPI | HILO | TIRF |
|---|---|---|
| **Epifluorescence** | **Highly Inclined and Laminated Optical sheet** | **Total Internal Reflection Fluorescence** |
| In-depth illumination, higher background | Limited background, not restricted to coverslip up to 10µm in depth | Illumination close to the coverslip, 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



## Automatic, quantitative and reproducible

Our technology combined with a software application and a calibration sample allows automatic definition of the TIRF angles with <1.2% error, leading to reliable data over time.



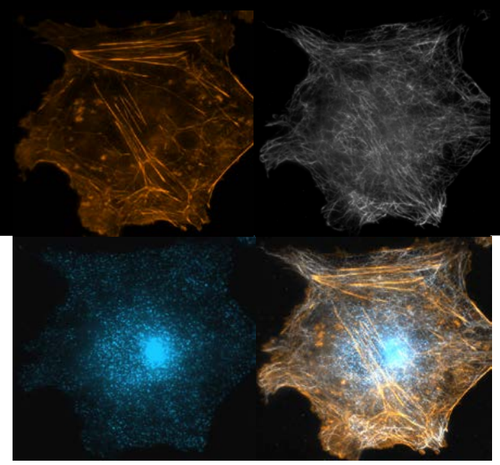
Visualization of the results of calibration: The algorithm uses the two planes (BFP/Image plane) to calculate the **critical angle**, **maximum angle** and an **optimized TIRF**

- **Software application integrated into Abbelight's NEO** aids in calculation and backup of TIRF angles based on image processing algorithm, that exploits image plane intensity as well as distribution and segmentation of your objective's BFP
- **Calibration sample** based on nanometric beads embedded into a highly controlled and long life-time gel with refractive index of water. The calibration sample is supplied with our Support Program Abbelight Care 1
- **Ultra stable TIRF stage** based on a closed-loop long range piezo stage offering nanoscale repositionning of the beam at BFP




## Reliable multicolor imaging

A robust optical design and software architecture leads to highly stable, multicolor imaging without chromatic aberration, optical artefact or misalignment over time.



3 color simultaneous TIRF images of Actin, Tubulin and Clathrin

### Abbelight's multicolor strategy

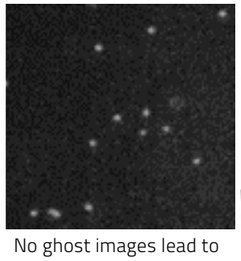
Optics inside the illumination device are designed to r**educe drastically chromatic aberration** for the range **488-640 nm,** thus offering **TIRF excitation over a wide spectrum.** Special **custom dichroic CUBES** have been designed to **remove ghost images and also to reduce crosstalk between channels**, thus offering up to **4 colors simultaneous imaging.**

**Highly stable optomechanical components** holding dichroic cubes have been carefully chosen to **simplify the alignment of the 4 colors images**. A **software digital alignment tool** has also been developed to offer < 0,7 pixel precision of alignment.

**STANDARD DICHROIC**

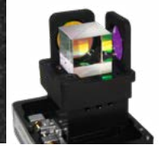
Ghost images

**ABBELIGHT CUBE**

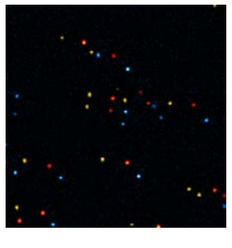


No ghost images lead to reliable data

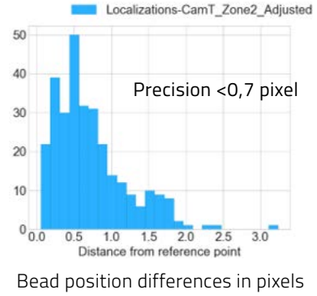
30 x 30 $mm^3$ dichroic cube designed to reduce aberration and crosstalk.



**BAD ALIGNMENT**



**AFTER OPTICAL AND SOFTWARE ALIGNMENT**




Bead position differences in pixels

# SMLM multicolor imaging

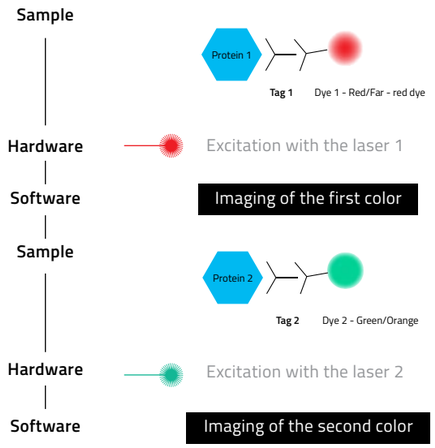
**Multicolor imaging is a powerful way to assess spatial relation between different biological structures.**

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

## METHOD 1: ACQUISITION OF DIFFERENT COLORS SEQUENTIALLY

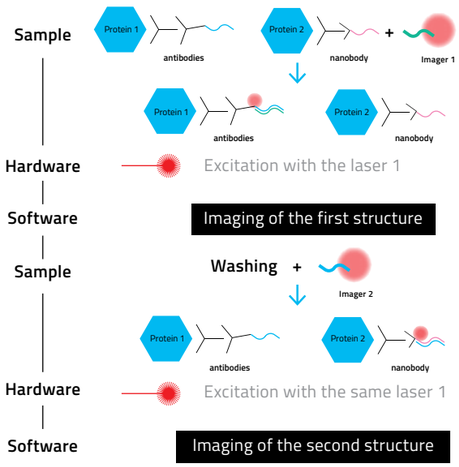
### With multiple lasers:

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:

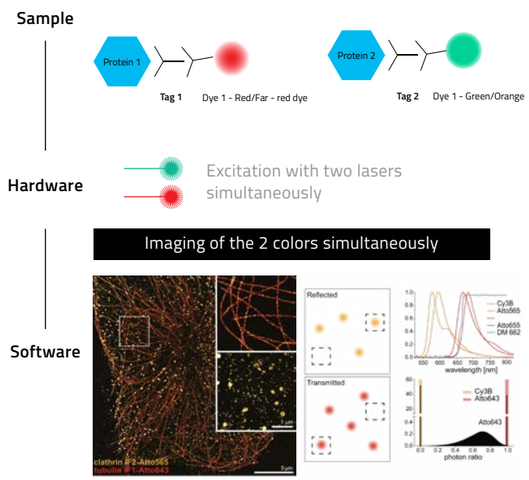



## METHOD 2: DICHROIC CUBES TO SPLIT EMISSION LIGHT AND FILTERS FOR SIMULTANEOUS MULTI-COLOR NANOSCOPY

Friedl,K et al.2023

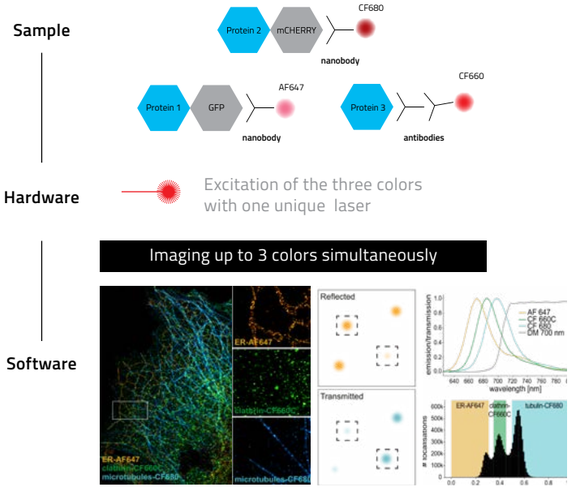
### With multiple lasers:

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

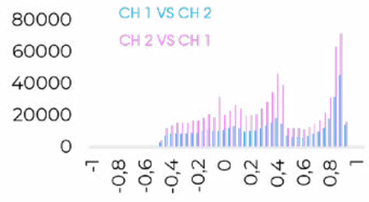


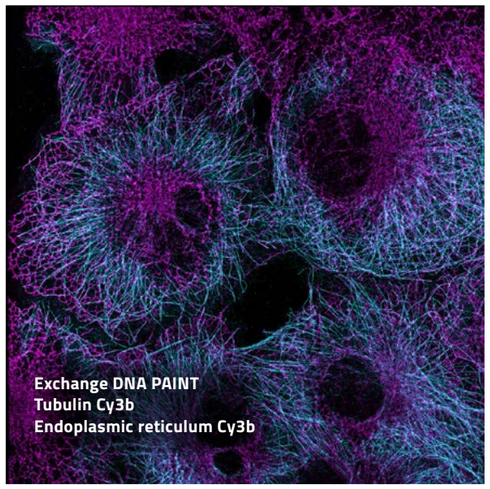


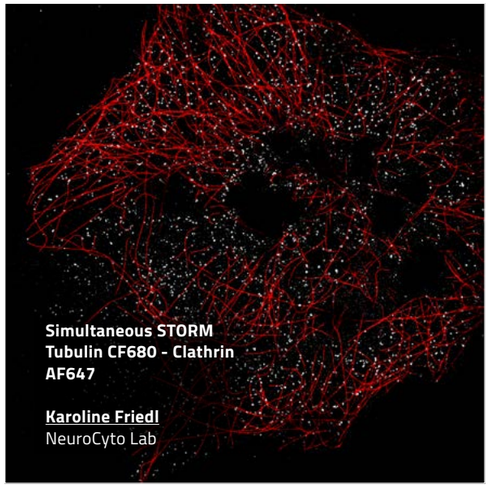

# Revolutionize imaging with Abbelight's automated multi-color Spectral Demixing

This advanced technology delivers precise quantitative crosstalk measurement, ensuring clearer, more reliable imaging for complex multi-color analyses. Unlock the full potential of your data with efficient spectral separation.

- Automatic localization assignment for up to three populations using monocolor calibration models.
- Adaptable demixing by tuning crosstalk levels for better separation of biological structures.
- Guidance and time-saving features improve data quality and enhance repeatability.




| 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's NEO Software Suite includes an optimal live drift correction

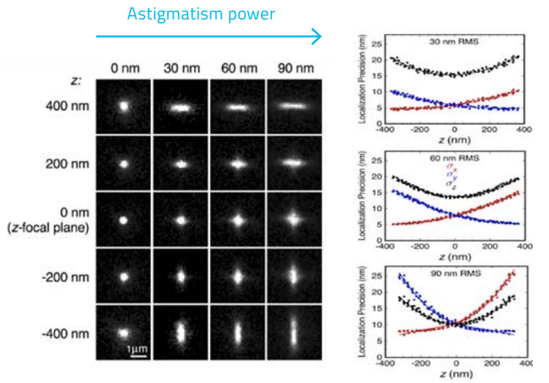
# ULTIMATE 3D

**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.

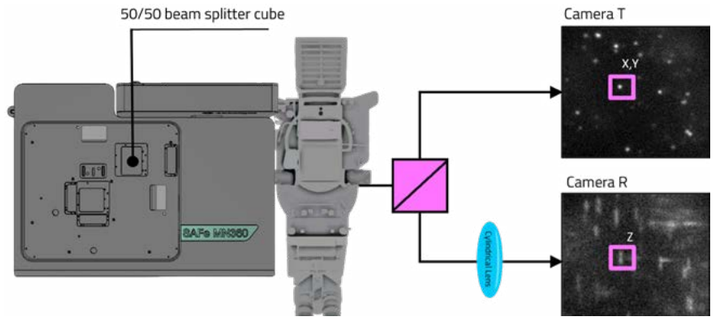
The second channel is therefore used to preserve the best lateral resolution, enabling uncompromised access to the most accurate information possible in all three axes.

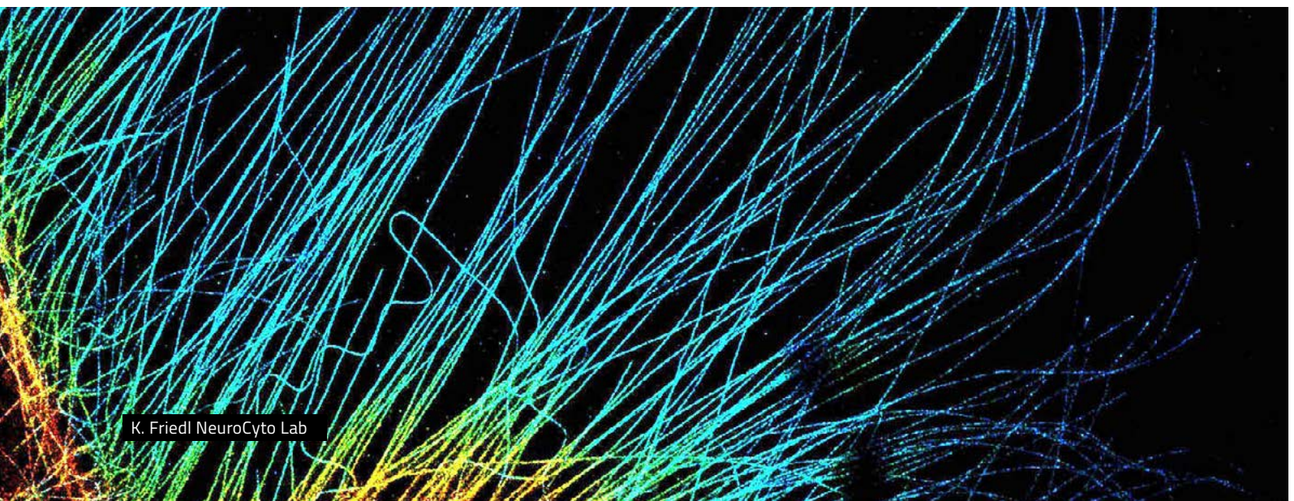



Chowdhury, R. et al. (2022)

**Abbelight's Ultimate 3D gives access to :**

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




# A COMPLETE SOFTWARE SOLUTION

## with NEO Software Suite

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

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

| Feature | The NEO Software Suite |
|---|---|
| **Control of instrument** | Laser power and illumination angle (EPI, HiLo, TIRF)<br>Cameras (up to 230 x 230 µm² FOV) |
| **Control of acquisition parameters** | Region of interest size, Field of excitation size<br>Exposure time (down to 10 ms per frame)<br>Frame number |
| **Project Management** | Smart Digital Notebook |
| **Live reconstruction of nanoscopy data** | Choice of localization parameters:<br>▪ Intensity threshold<br>▪ Background subtraction method |
| **Live visualization** | 2D and 3D vizualisation |
| **Live drift correction** | Cross-correlation |
| **Multi Dimensional acquisition** | Acquisition scenario (spatial, axial, time point and illumination angles) |
| **Visualization** | 3D visualization<br>Multicolor visualization<br>Possibility to export images in TIFF format compatible with commonly used software |
| **Descriptive spatial statistics** | Localization distribution<br>Measuring tools |
| **Automatic settings** | Automatic multi-view alignment for multicolor modalities<br>Automatic Hilo and TIRF illumination settings |
| **Post acquisition processing** | Adjustment of parameters and batch processing |
| **Clustering analysis** | K-Ripley function, DBSCAN, Voronoi tessellation<br>Centroid, density and volume measurements |
| **Single-particle tracking** | Track reconstruction, quantification of the number of tracks, track duration, diffusion coefficient, MSD etc. |
| **Spectral demixing** | Separation of far-red dyes for multicolor imaging |
| **Co-localization** | CBC Coordinate-Based Colocalization |

# Filtering

**Abbelight's 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.

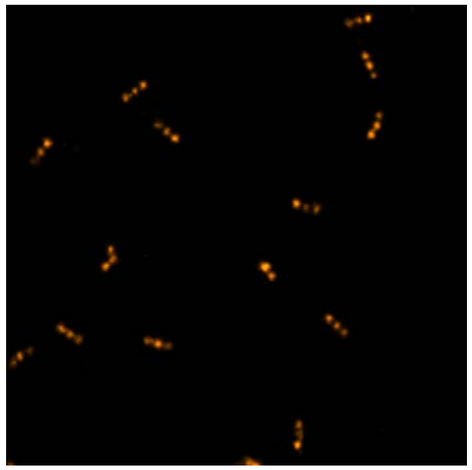
## INTENSITY

Number of photons per localization

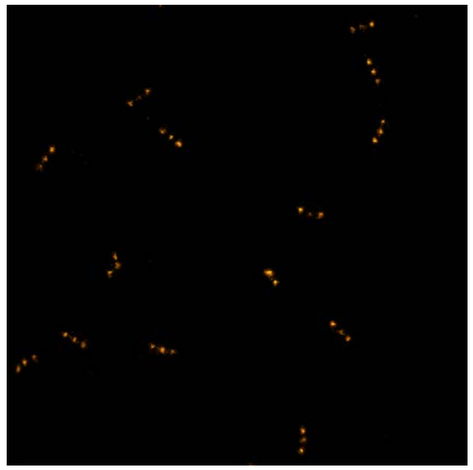
## BLINKING

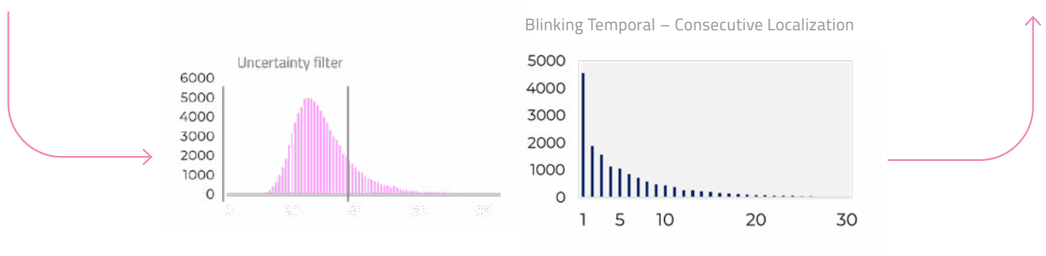
Indicates how many locations appear in several consecutive images and allows to the merge.

**Nanorulers before filtering**



**Nanorulers after filtering**







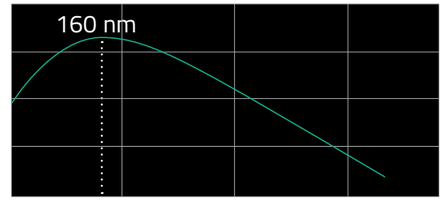

# Clustering

**The NEO Software suite 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.

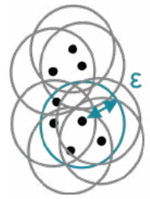
The bell-shaped curve indicates the presence of aggregated data points and provides an estimate of the size of these aggregates.




## 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.

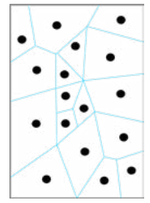
Ester et al. 1996




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.

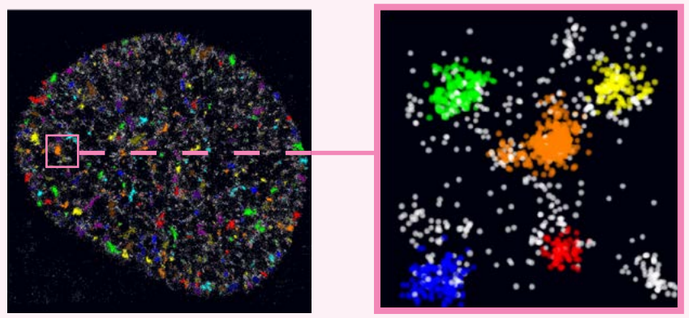
Levet et al. 2015



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.



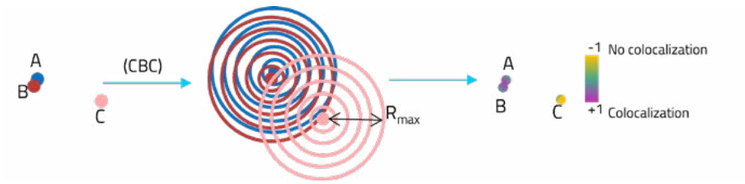
COS7 cells – DNA replication sites
EdU-AlexaFluor647
©Abbelight

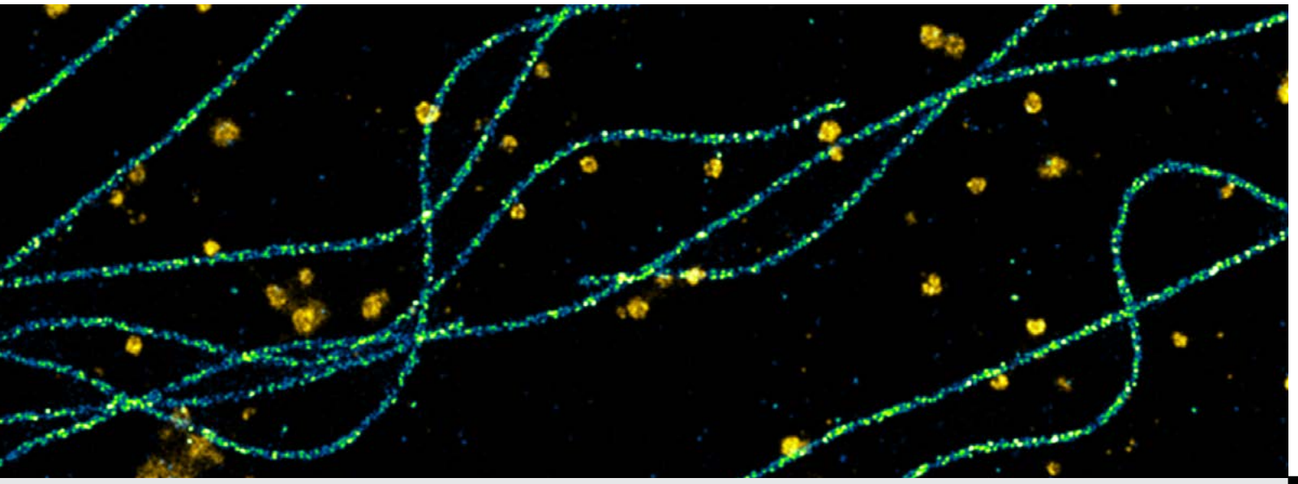
# Colocalization

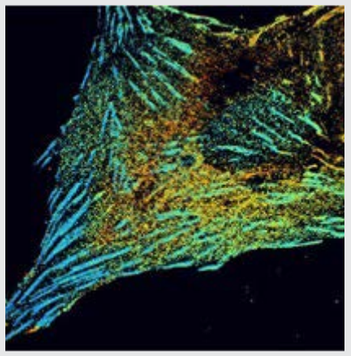
## COORDINATE-BASED COLOCALIZATION

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

Malkusch et al 2012

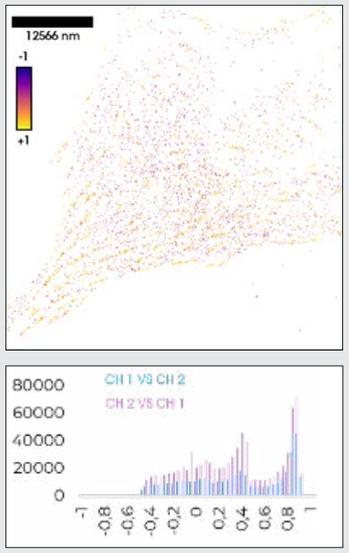




Paxilin – Vinculin 3D STORM

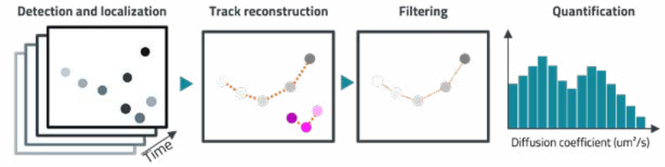
NEO Software analysis with CBC algorithm






# Single-Particle Tracking (SPT) analysis

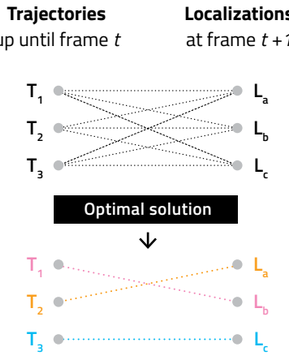
**To study the dynamics of single particles, Abbelight's NEO Software Suite can reconstruct trajectories from raw SPT data.**




## RECONSTRUCTING TRAJECTORIES

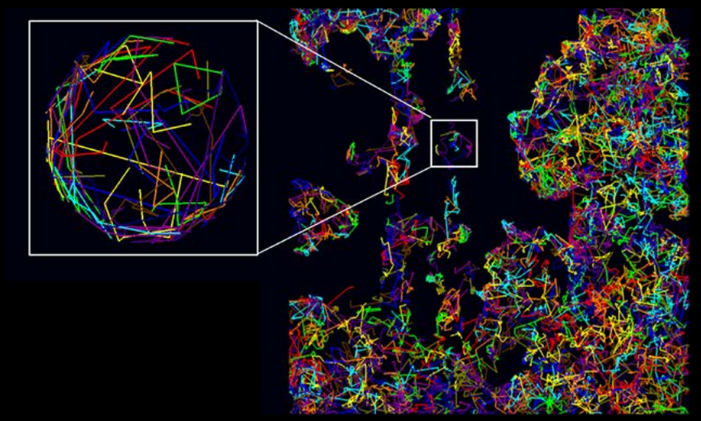
The goal of an SPT algorithm is to connect the localizations from frame to frame. The algorithm takes all the tracks at frame t and all the dots at frame t+1, and calculates the probability of assigning each track to each localization. Afterward, it chooses the solution that maximizes the probability. 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 expertise in chemistry, optics, and computer science to provide a comprehensive solution. It covers everything from sample preparation to data analysis, including a flexible bio-imaging platform adaptable to the needs of researchers, biotech labs, and medical facilities.

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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