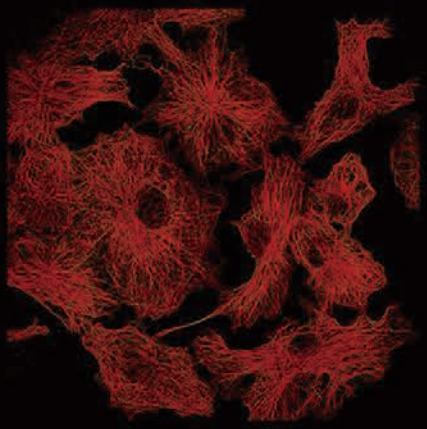
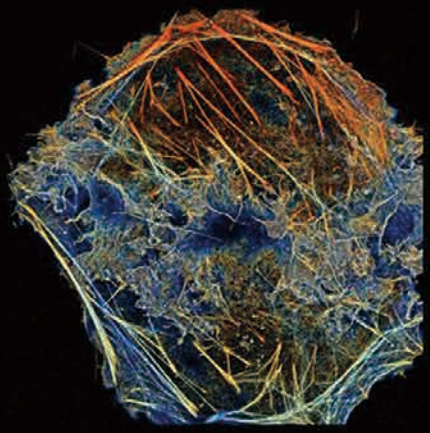
abbelight instrument

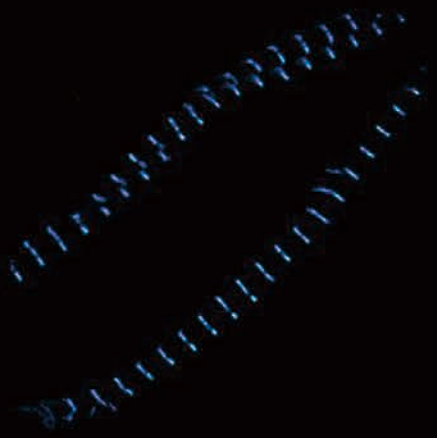
CAPTURE THE EVANESCENCE

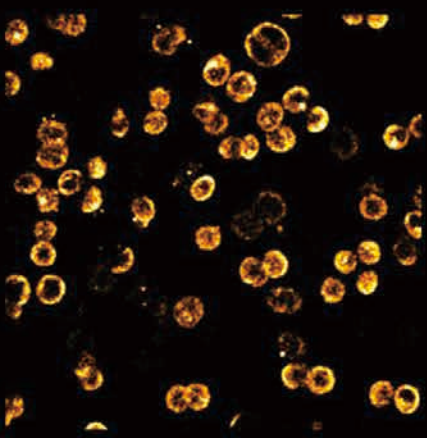
Your upgradable nanoscope for single-molecule imaging

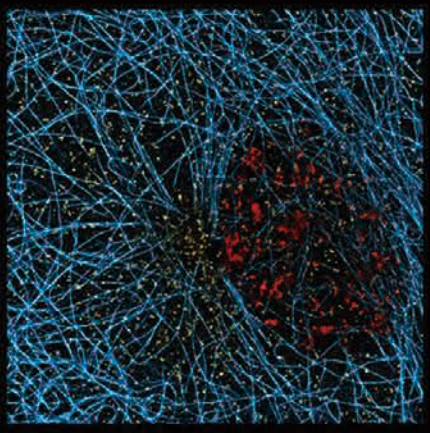
Abbelight
NOW, WE SEE

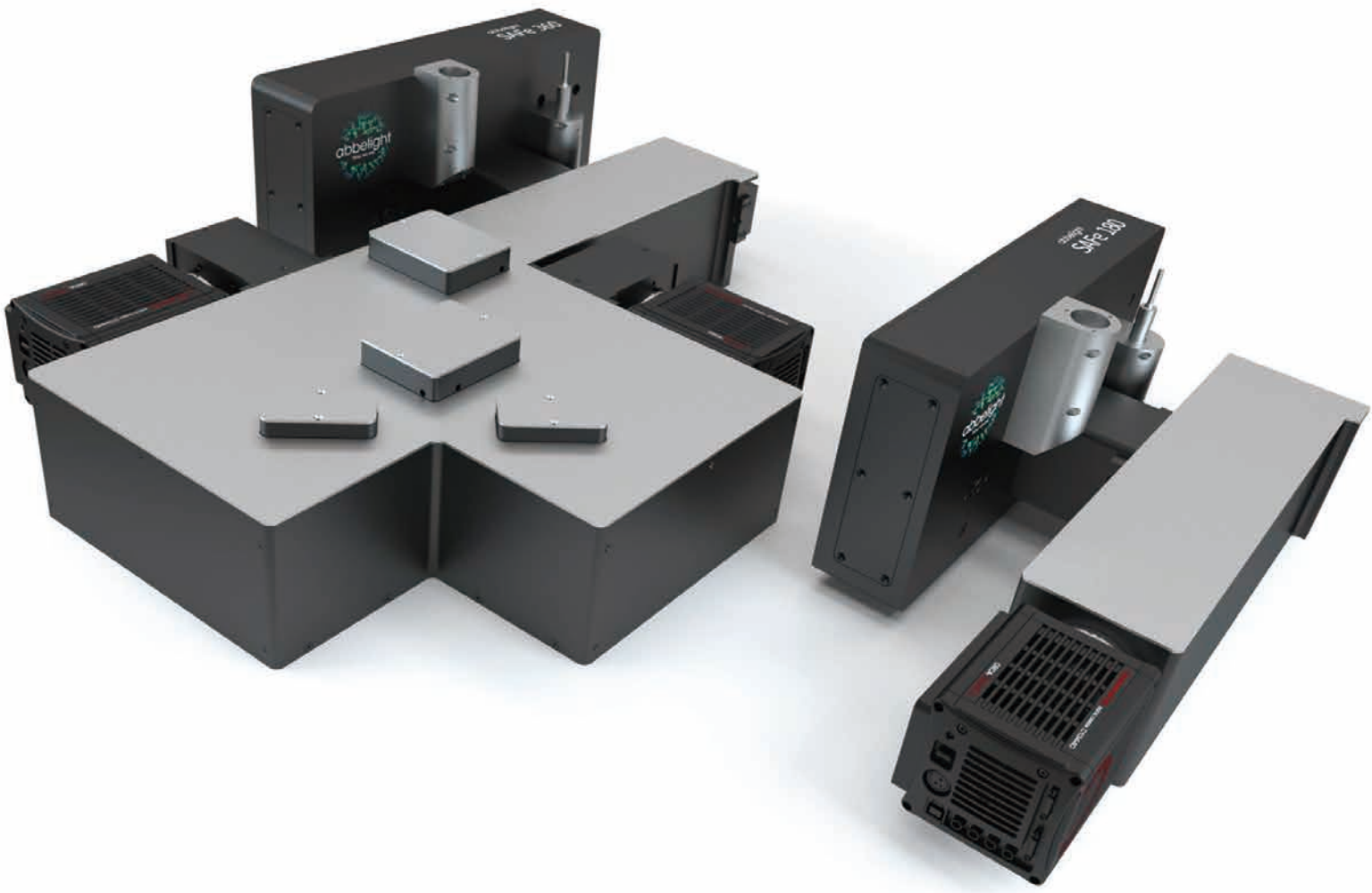












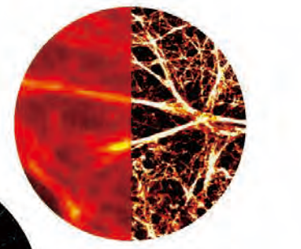
abbelight instrument

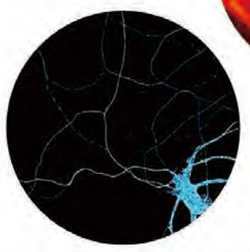
abbelight offers two modules adaptable to most inverted microscopes: **SAFe 180** and **SAFe 360**.

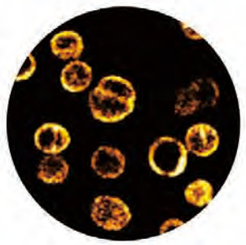
With these instruments, researchers can perform any type of single-molecule localization imaging.

SAFe 180

From microscopy to nanoscopy



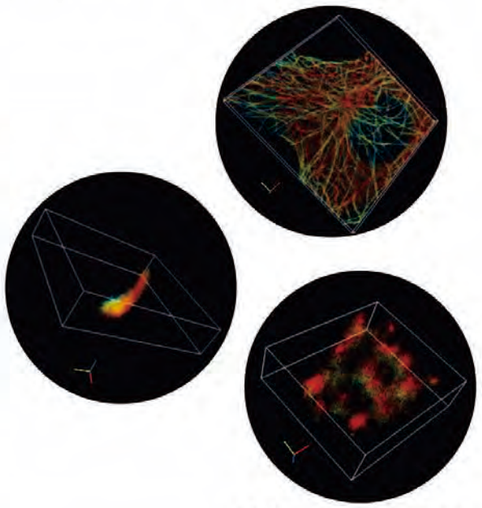




2D nanoscopy images

SAFe 360

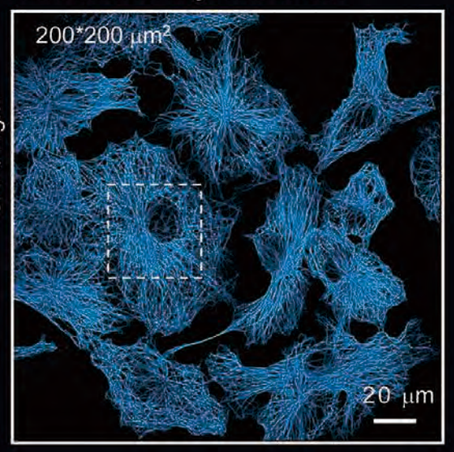
Nanoscopy in 3 dimensions



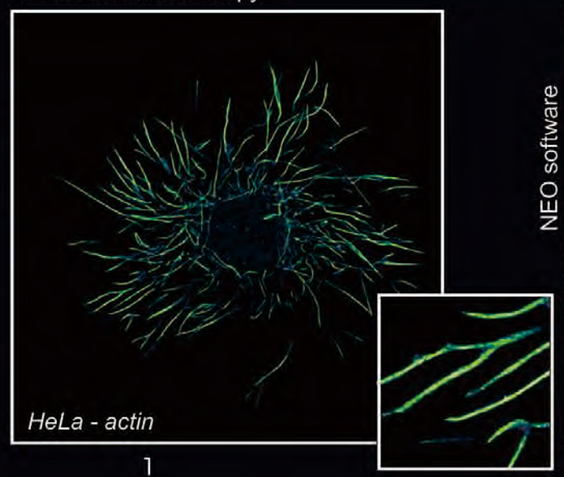
3D nanoscopy images

Feature	SAFe 180	SAFe 360	abbelight technology	More info
Large illumination TIRF/HiLo/EPI	✔	✔	SAFe Light	pages 13-14
2D Single-molecule localization	✔	✔		pages 5-8
Data analysis	✔	✔	NEO software	pages 17-18
3D Single-molecule localization	-	✔	DAISY technology	pages 10-13
Simultaneous multicolor	-	✔	Spectral demixing	Pages 15-16

Illumination on large field of view



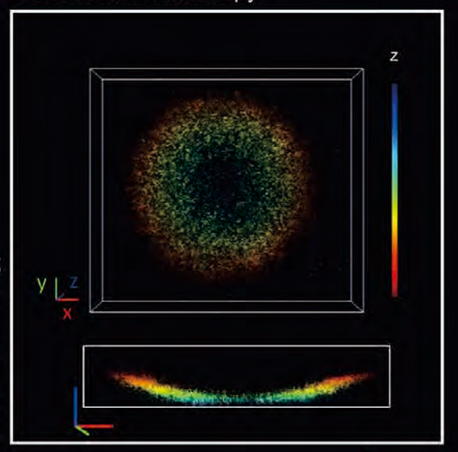
2D STORM nanoscopy



1

Data analysis

3D STORM nanoscopy



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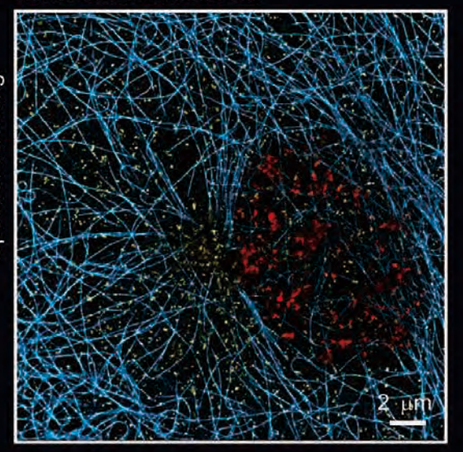
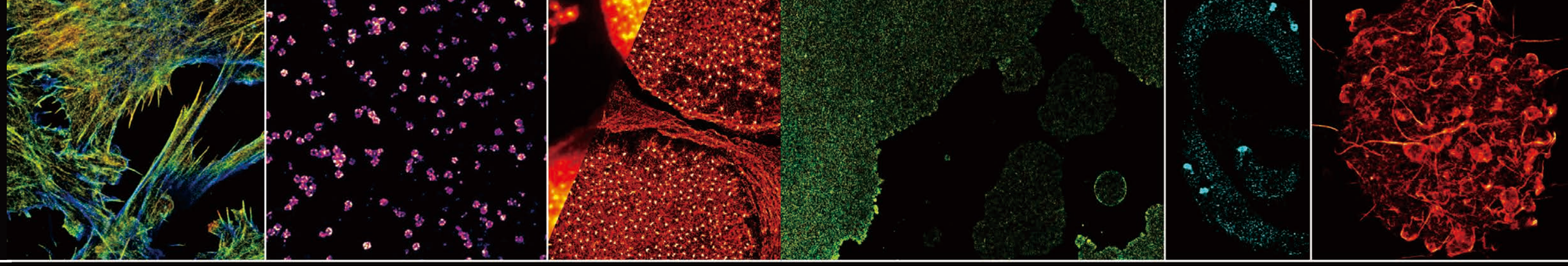
Simultaneous multicolor





samples

research

reagents

expertise

instruments

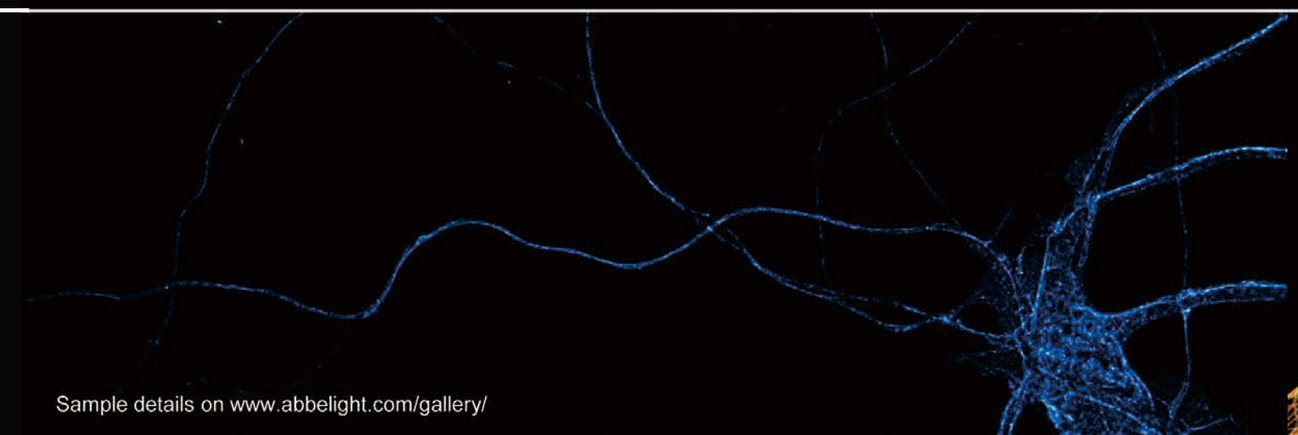
technology

software

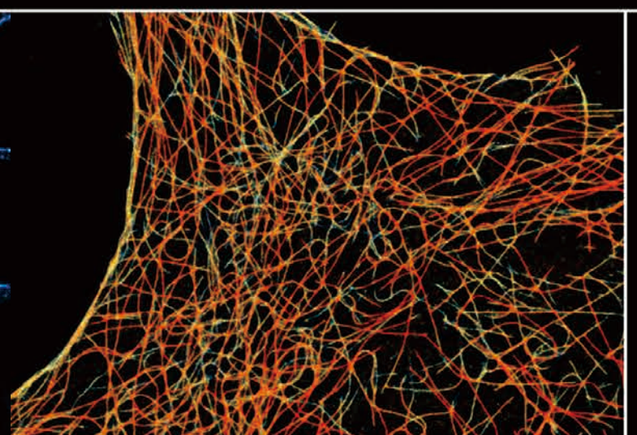
analysis

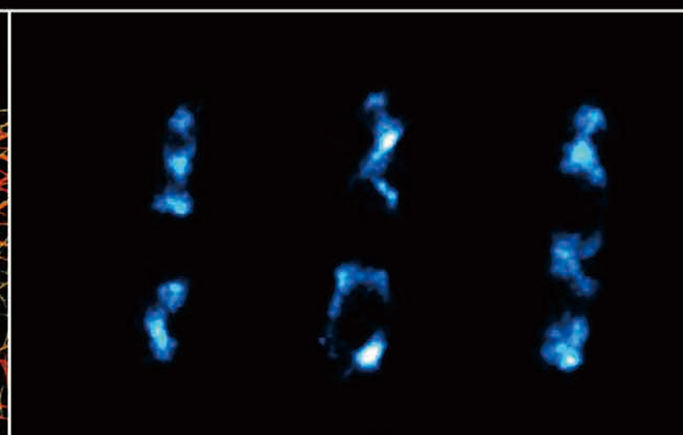
support

visualization



Sample details on www.abbelight.com/gallery/



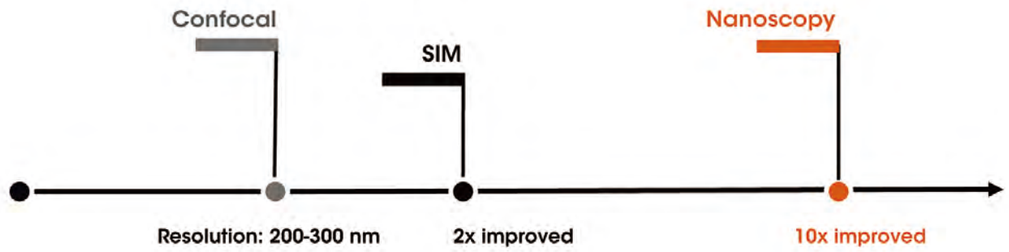


From microscopy to nanoscopy

Revealing structures and dynamics at the nanoscale

Standard **fluorescence microscopy** techniques (widefield, confocal,…) 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 recent techniques that break the diffraction limit, *i.e.* super-resolution techniques, **nanoscopy** retrieves structural or dynamic quantitative information with the highest resolution achievable in light microscopy.



Stefan Hell, Eric Betzig, and William Moerner were awarded the chemistry Nobel Prize in 2014 for their work on nanoscopy techniques.

Single-molecule Localization Microscopy (SMLM) is the nanoscopy technique that retrieves structural or dynamic quantitative information with the highest precision achievable.

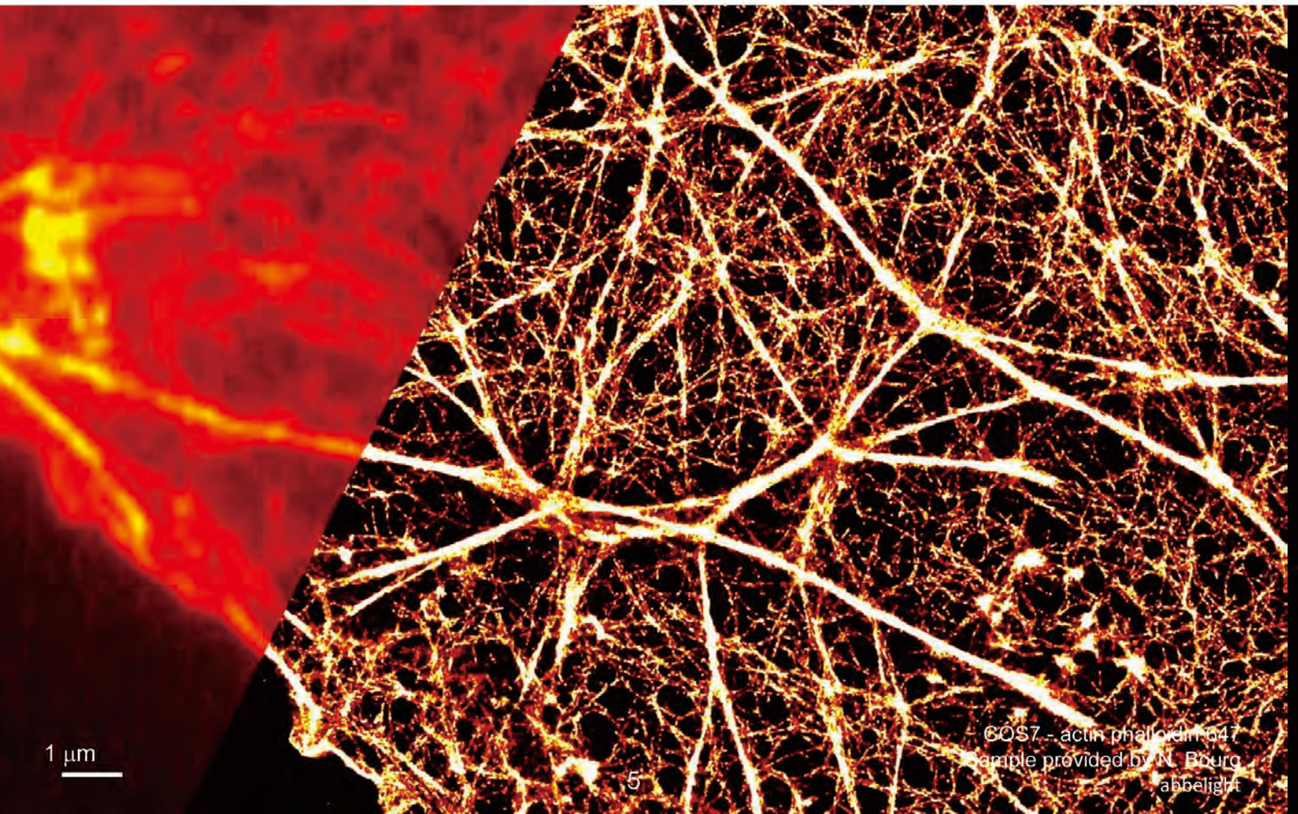
SMLM principle

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

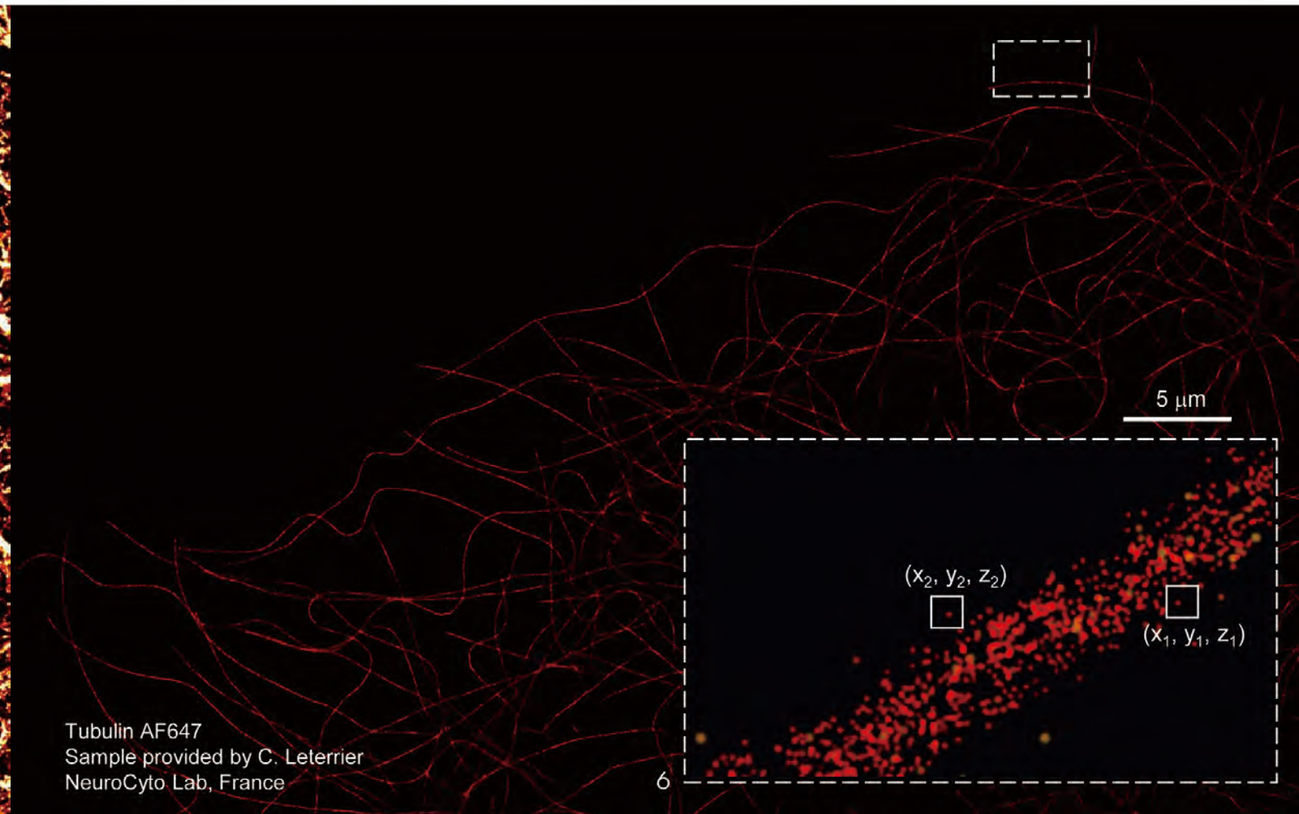
By repeating the process in consecutive image acquisitions, accumulated raw data are processed to detect single molecules with a nanometric precision (down to 10 nm).

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 not only to highly resolved images, but also to the **3D coordinates of single molecules**, opening up new avenues for **spatial and temporal quantitative analysis**.



5 COS7 - actin phalloidin 647
Sample provided by N. Bourg
abbelight

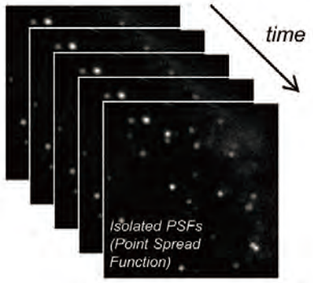


Tubulin AF647
Sample provided by C. Leterrier
NeuroCyto Lab, France

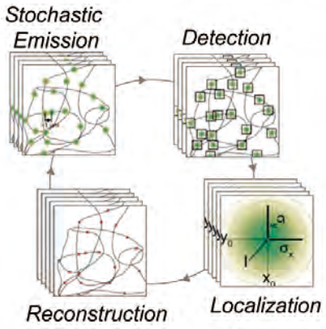
6

Localizing molecules in 2D

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



Raw film



Data processing

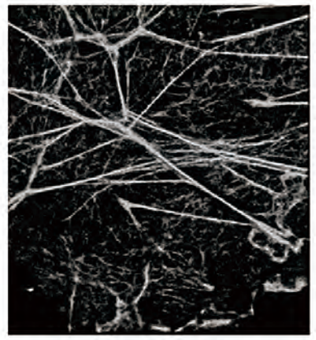
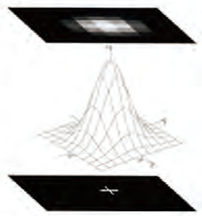


Image reconstruction

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

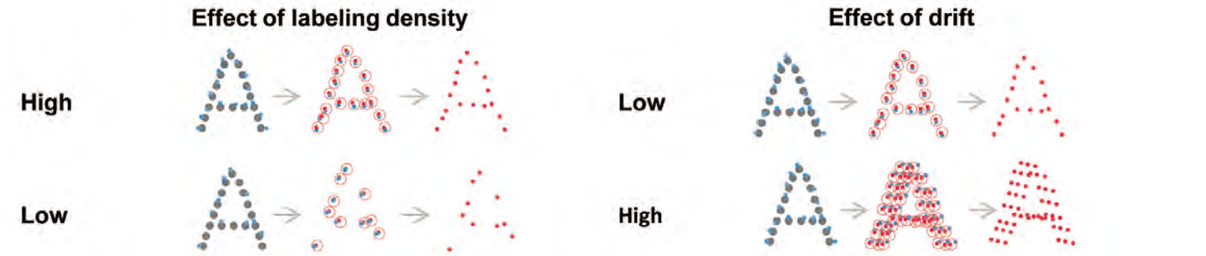
$$\text{Localization precision} \approx \frac{\sigma}{\sqrt{N}}$$

N=number of photons
σ =standard deviation

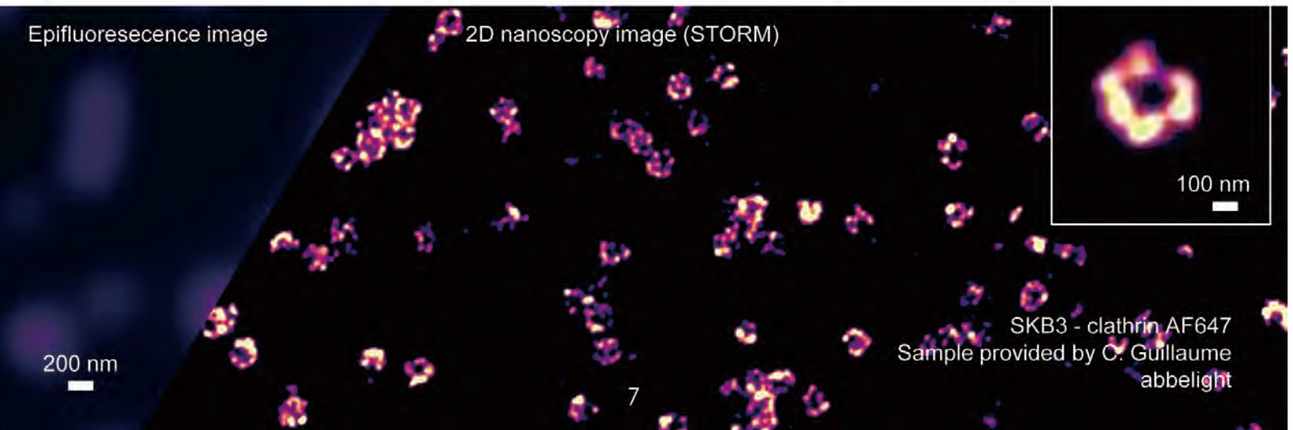


The localization precision is typically 10 nm.

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



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



SMLM approaches… from structure to dynamics

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

Structures

❖ **STORM** (STochastic Optical Reconstruction Microscopy)

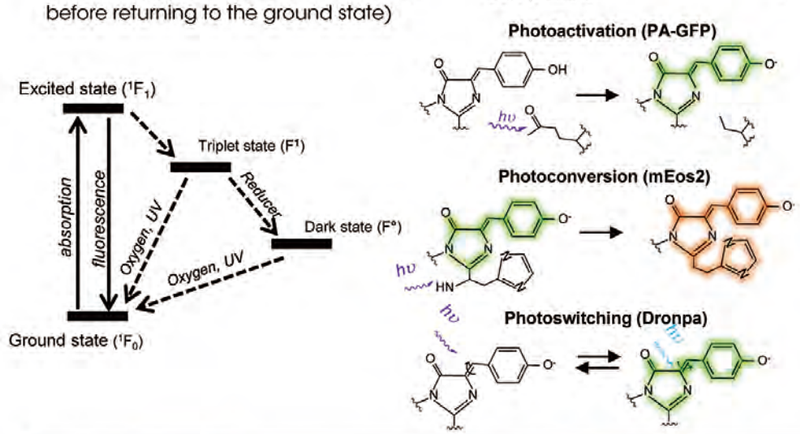
- ✓ Standard organic fluorescent dyes (cyanines, rhodamines, oxazines…)
- ✓ Specific imaging buffer (containing a reducer, which induces the transition to a dark state, and an oxygen scavenging system to stabilize this state before returning to the ground state)

❖ **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)

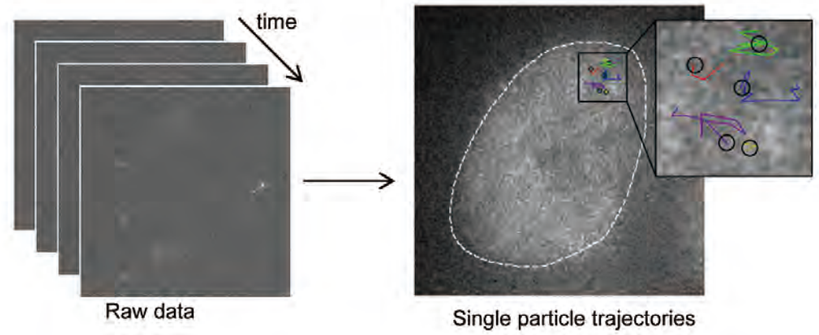
- ✓ Specific fluorophores that have the ability to emit fluorescence only upon binding to their biological target (ex: Nile Red, which fluoresces only when interacting with membranes)
- ✓ No specific buffer, live-cell compatible



* Sample provided by U. Endesfelder, Max Planck Institute, Marburg

Dynamics

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



DAISY technology

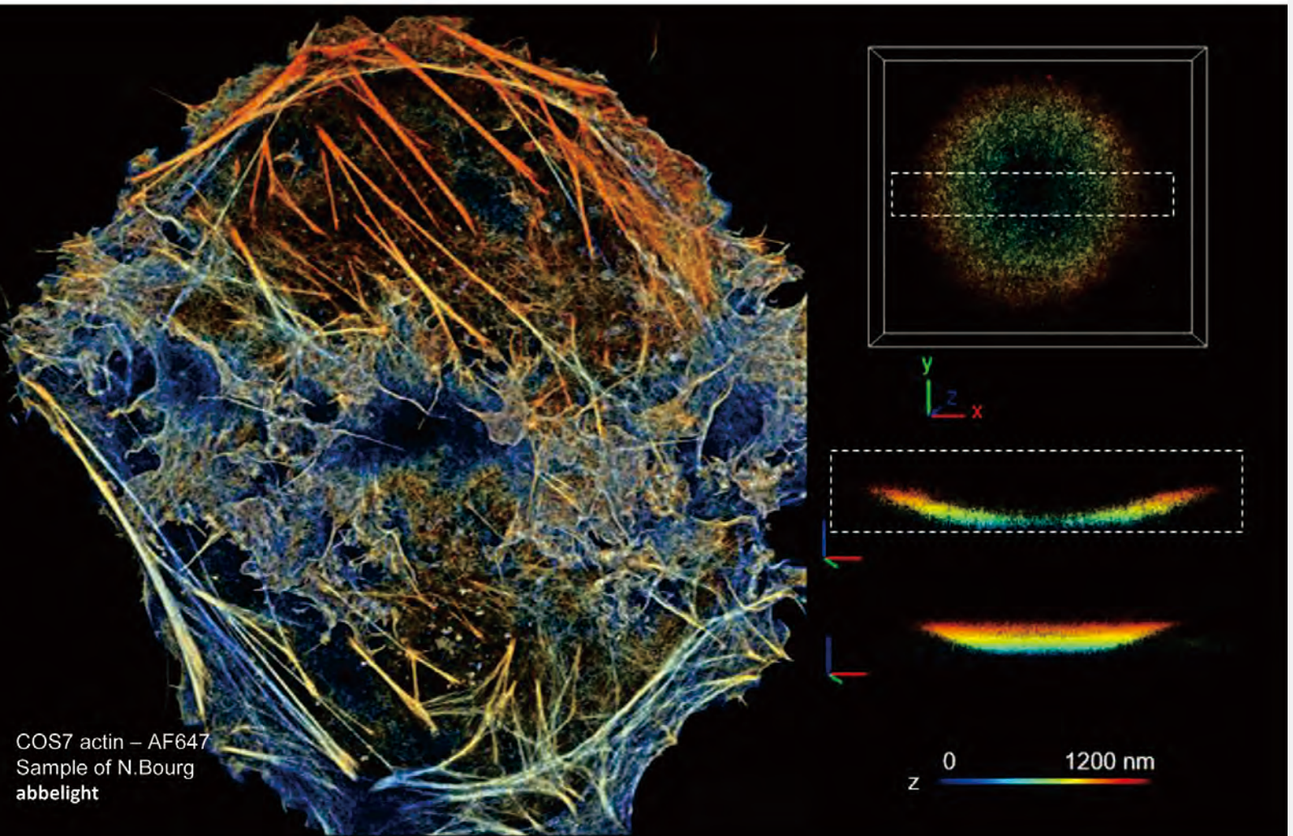
DAISY is the combination of two complementary strategies developed by abbelight and used to extract the "Z" position of a particle. (Cabriel et al. *BioRxiv 2018*)

The first approach is called **Magnified Astigmatism** and is inspired by the astigmatic PSF shaping published by Huang et al. (*Science 2008*) and commonly used in commercial systems.

The second approach exploits the near field information encoded in every single emitter, developed and published under the name **DONALD** by Bourg et al. (*Nature Photonics 2015*)

	abbelight instrument			Nanoscopy market
3D method	Magnified astigmatism *(in dual-view system)*	DONALD	DAISY	Standard astigmatism
Imaging depth	5-10 µm	0,5 µm	5-10 µm	5-10 µm
Capture range	1 µm	0,5 µm	1 µm	600 nm
Lateral loc. Precision*	10 nm	10 nm *	10 nm	10 nm
Lateral resolution**	23 nm	23 nm	23 nm	23 nm
Axial loc. Precision*	13 nm	13 nm *	13 nm	22 nm
Axial resolution**	> 30 nm + focus & drift dependence	30 nm	30 nm	> 50 nm +focus & drift dependence
Axial drift	Degrade axial resolution above	Not sensitive	Not sensitive	Degrade axial resolution above

* Mean value, for dSTORM imaging using AF647 and abbelight buffer
** Mean value, resolution = 2,35 × (Localization precision) ⊗ (labeling density, drift…)



COS7 actin – AF647
Sample of N.Bourg
abbelight

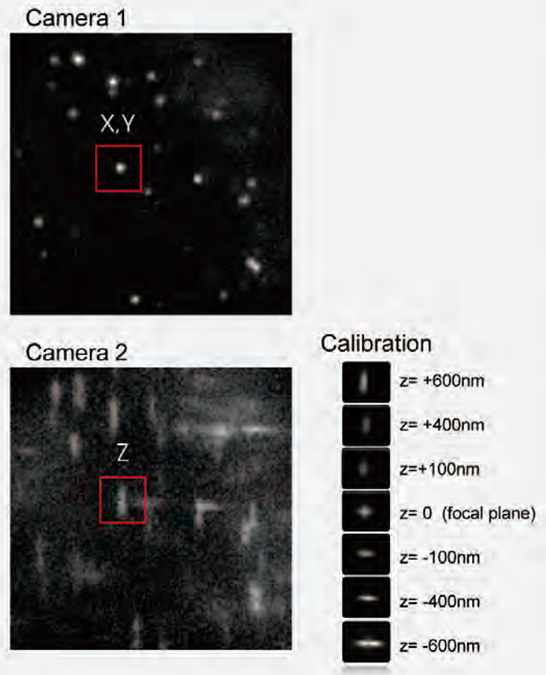
Magnified Astigmatism

ASTIGMATIC PSF SHAPING is a very efficient way to extract the relative position of a single particle regarding the focus plane of the objective. Using an astigmatic lens, a controlled aberration can be induced, measured, and related to the distance between the objective's focal plane and the emitting particle.
The stronger the aberration is, the better the axial precision is. However, the lateral resolution is also degraded. Therefore, for conventional setups using a single camera, a compromise has to be found between astigmatism strength and X,Y localization precision.
Using a dual camera system, the astigmatic deformation can be enhanced while preserving the best lateral resolution. This is what we call **Magnified Astigmatism**.

Key features

+ Capture range of 1200 nm
+ Enhanced astigmatic lens for better Z precision
+ No loss of lateral resolution
+ Two controls for false positive detections

- Relative to the focal plane: sensitive to axial drift
- Amplified chromatic aberrations



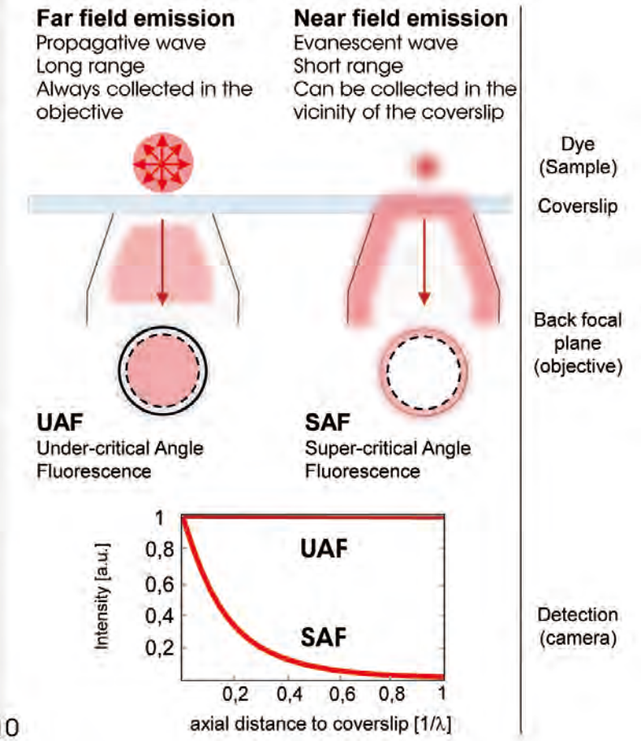
DONALD

Any single emitter is a dipole, emitting two components of fluorescence:
The **far-field** emission is a propagative wave, always collected in the low angles of the objective (UAF) and commonly used for any fluorescence microscopy technique.
Although it is often forgotten, dyes also have a **near-field** emission, which can also be collected in the objective - if the dye is close enough to the coverslip - but in the high angles of the objective (SAF).
Since UAF is constant and SAF decays exponentially, a simple ratio of intensities, for each dye, determines its absolute distance to the coverslip.
Besides its simplicity, the strength of this photophysical measurement is its insensitivity to axial drift or aberration.

Key features

+ Absolute measurement: insensitive to drift
+ No loss of lateral resolution
+ No chromatic aberration
+ Compatible with PSF shaping methods

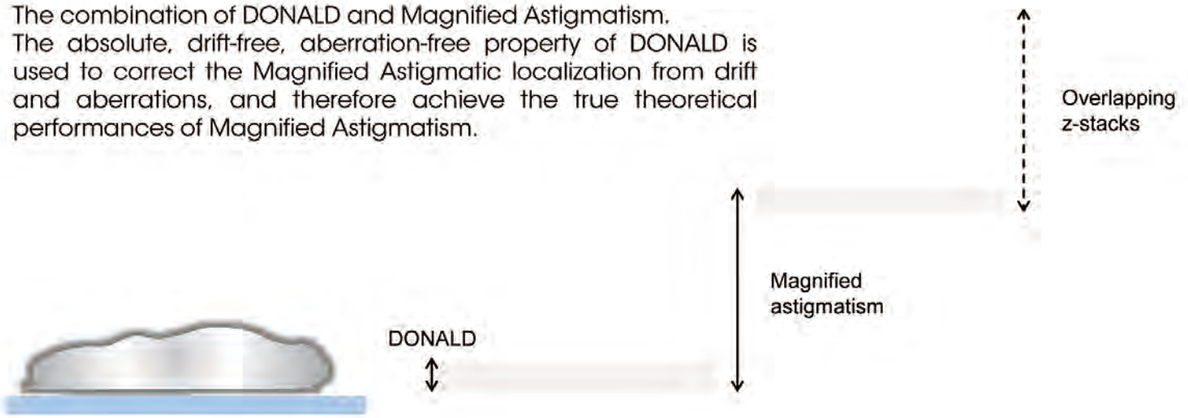
- Loss of axial precision above 300 nm
- Capture range limited to 600 nm above coverslip



DAISY technology

DAISY

The combination of DONALD and Magnified Astigmatism.
The absolute, drift-free, aberration-free property of DONALD is used to correct the Magnified Astigmatic localization from drift and aberrations, and therefore achieve the true theoretical performances of Magnified Astigmatism.

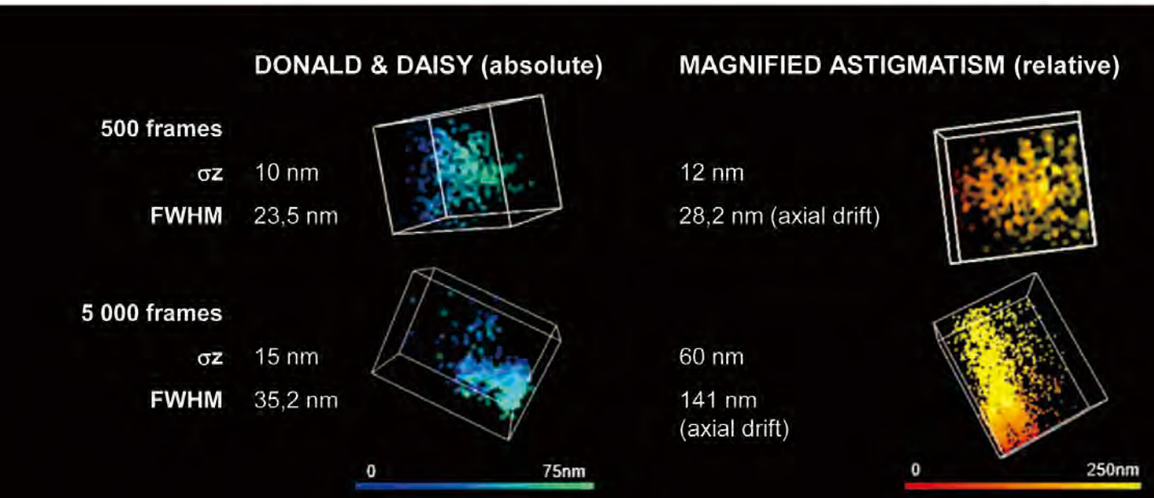


Absolute VS Relative measurement

DRIFT-FREE
Since the theoretical localization precision is almost the same between DONALD and Magnified Astigmatism, the final resolution of the image will be different because of the axial drift.
Even with the best focus control system, small oscillations and large drift of the focal plane position can occur, directly impacting the resolution of the final image.

STATISTICAL ANALYSIS
An absolute axial measurement enables straightforward statistical 3D multicolor analysis, since the reference is always the coverslip surface. It is now possible to compare thousands of acquisitions, on different samples, for different proteins, and easily compile the data*.

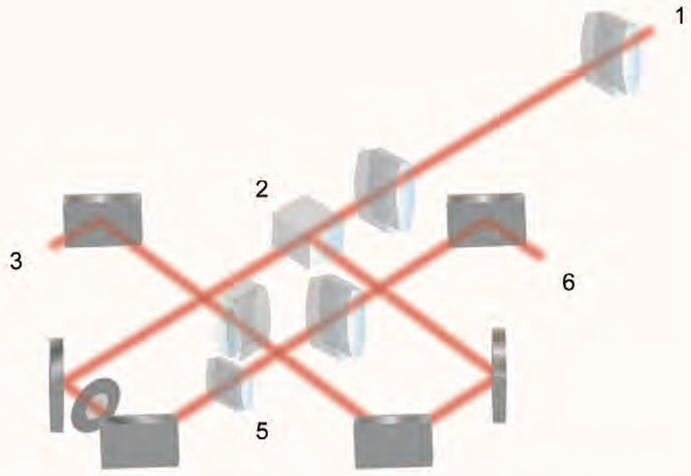
*Bouissou et al. **ACSnano** 2017

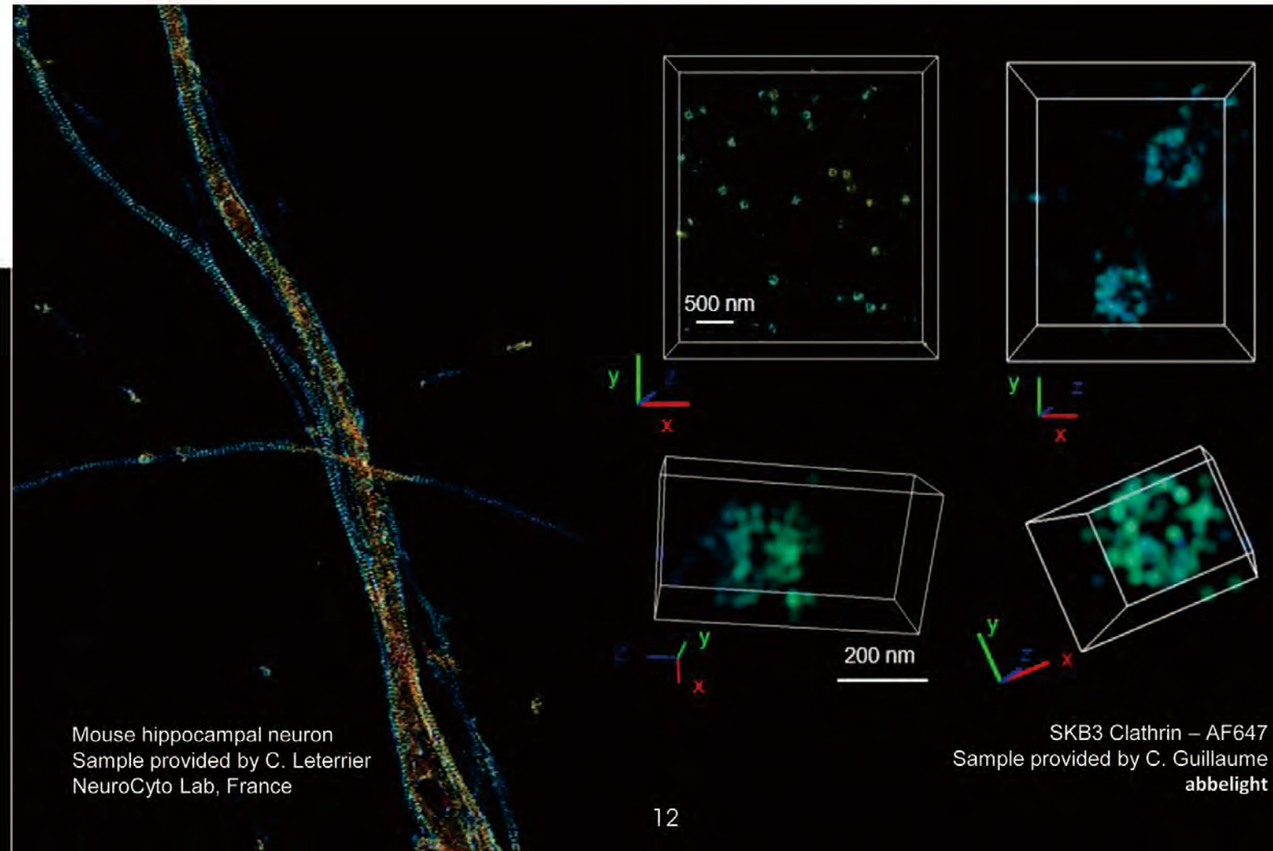


SAFe 360 optical path

The SAFe 360 module has been designed to integrate and use both **Magnified Astigmatism** and **DONALD** at the same time, and therefore DAISY.
The optical cubes and specific lenses can be easily replaced to tune the system depending on the user's needs.

1. Fluorescence from the microscope
2. Beam splitter 50/50
3. Camera 1 for 2D and SAF detection
4. SAF physical filtering for DONALD
5. Strong cylindrical lens to induce magnified astigmatism
6. Camera 2 for 3D astigmatic detection





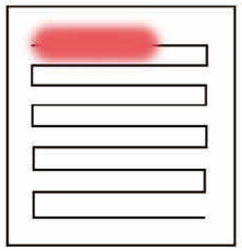
Mouse hippocampal neuron
Sample provided by C. Leterrier
NeuroCyto Lab, France

SKB3 Clathrin – AF647
Sample provided by C. Guillaume
abbelight

SAFe Light

abbelight **SAFe Light** technology offers the **largest field of view** in nanoscopy and requires **lower laser power.**

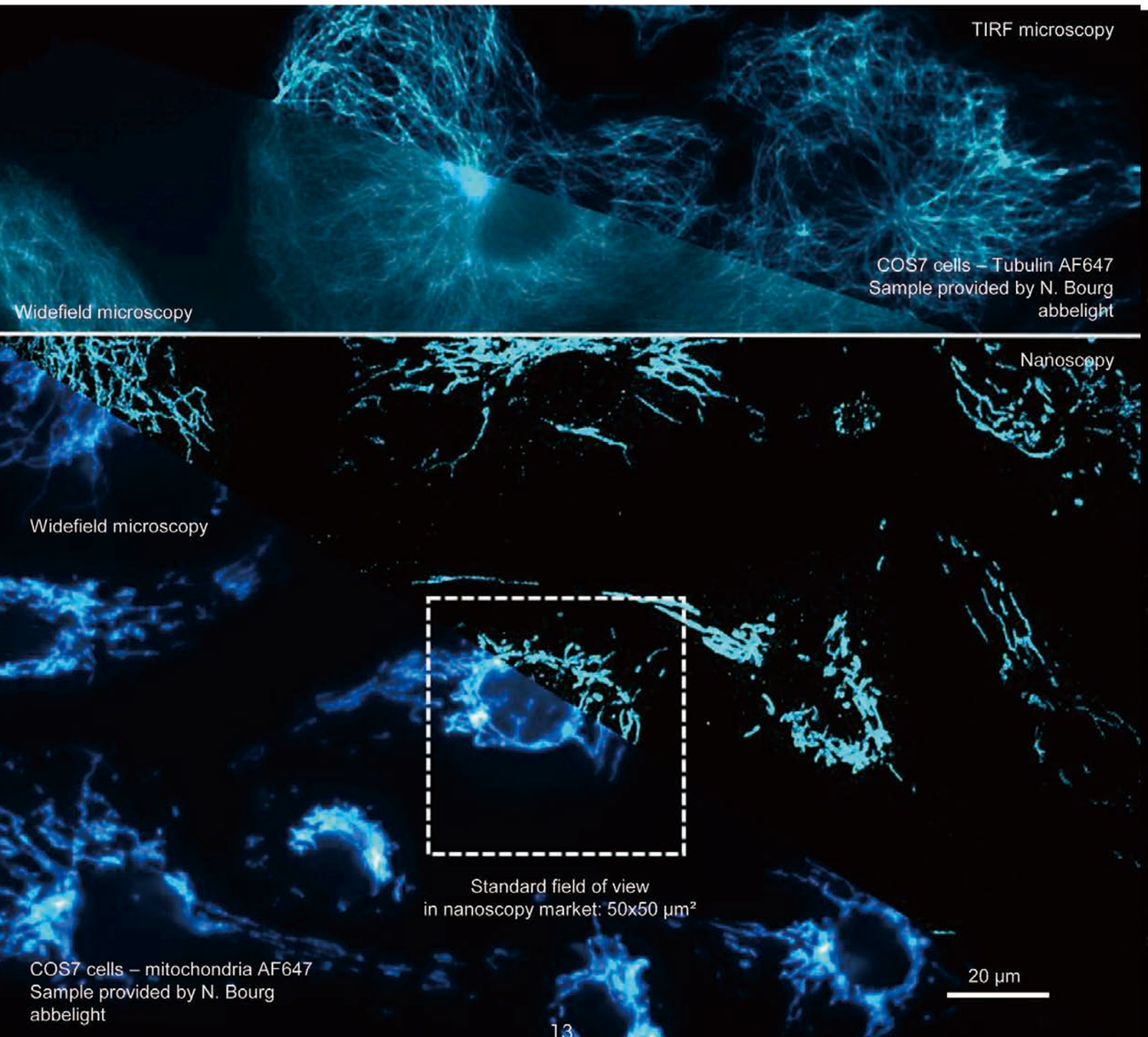
- 150x150 µm² field of view with 300 mW laser power
- More intensity on the sample with lower laser power
- Illumination adaptable to the sample
- TIRF, HiLo or EPI illumination modes
- 16-times more quantitative data
- Homogeneous illumination, no interference patterns in the image

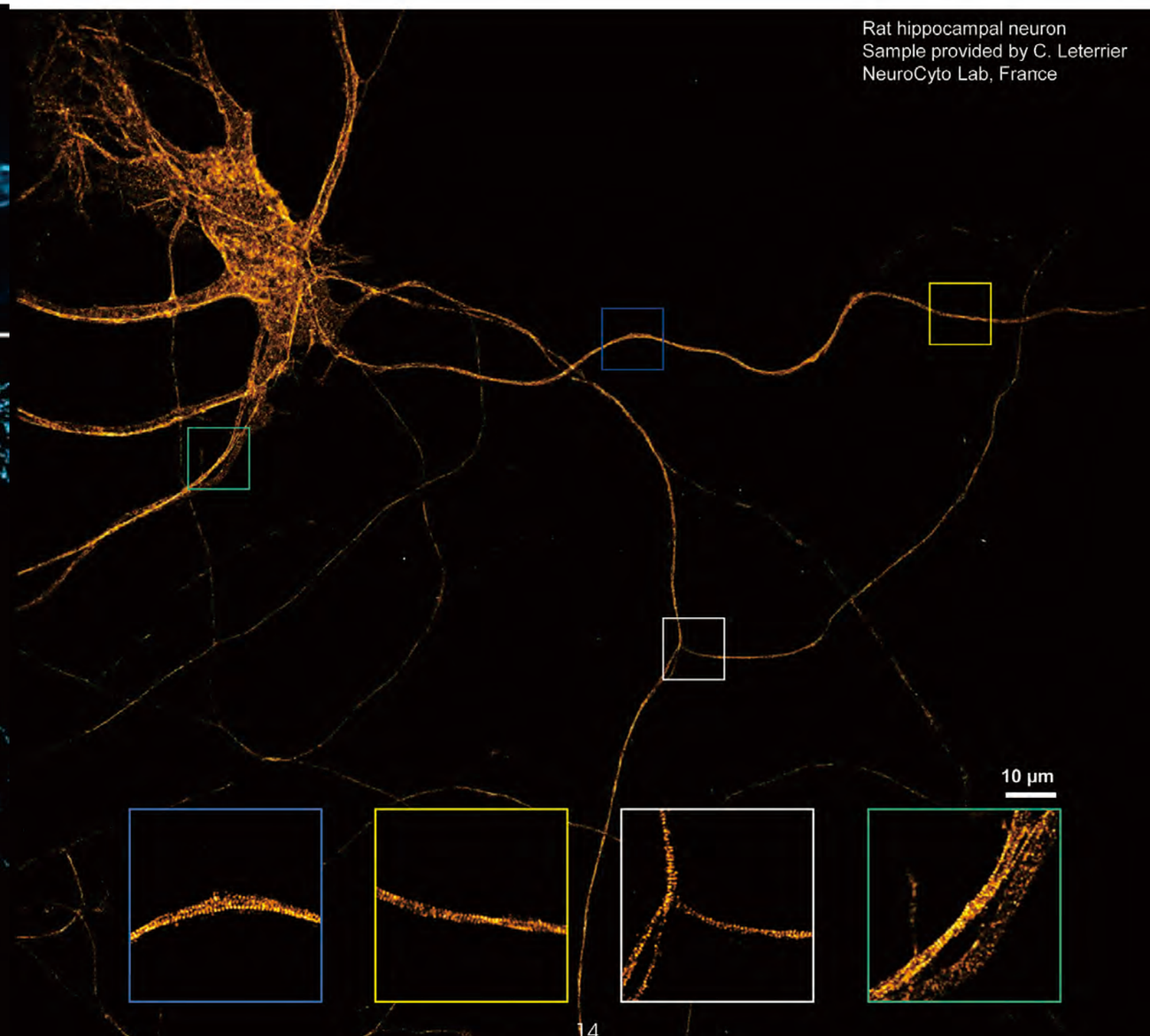


Scanning for homogenous illumination faster than the camera acquisition time (200 fps for the largest field of view)

Illumination adapted to the sample

	EPI	HiLo	TIRF
Name	Epifluorescence	Highly inclined and laminated optical sheet	Total internal reflection fluorescence
Type of illumination	In-depth illumination, higher background	Limited background, not restricted to coverslip	Illumination close to the coverslip, removal of in-depth background
Examples of biological structures	Structures far from the coverslip: nucleus, thick cells, tissues…	Slightly in-depth samples	Structures close to the coverslip: membranes, cytoskeleton, in vitro surfaces…



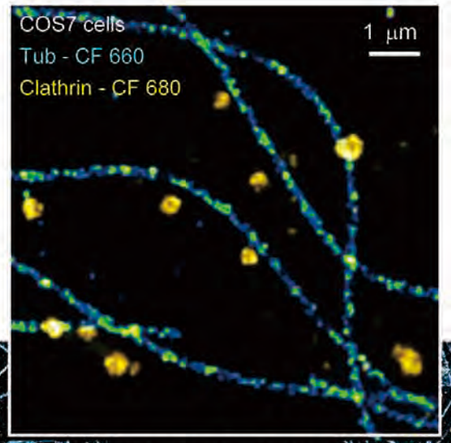


Simultaneous multicolor

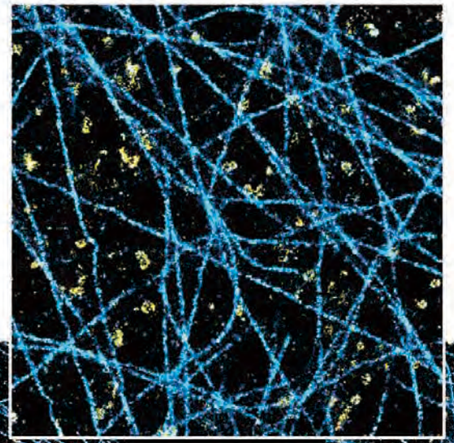
Multicolor imaging is a powerful way to assess colocalization between different biological structures.

- Method 1: acquire different colors **sequentially**
- Method 2: use dichroic cubes to allow **simultaneous multicolor nanoscopy**.

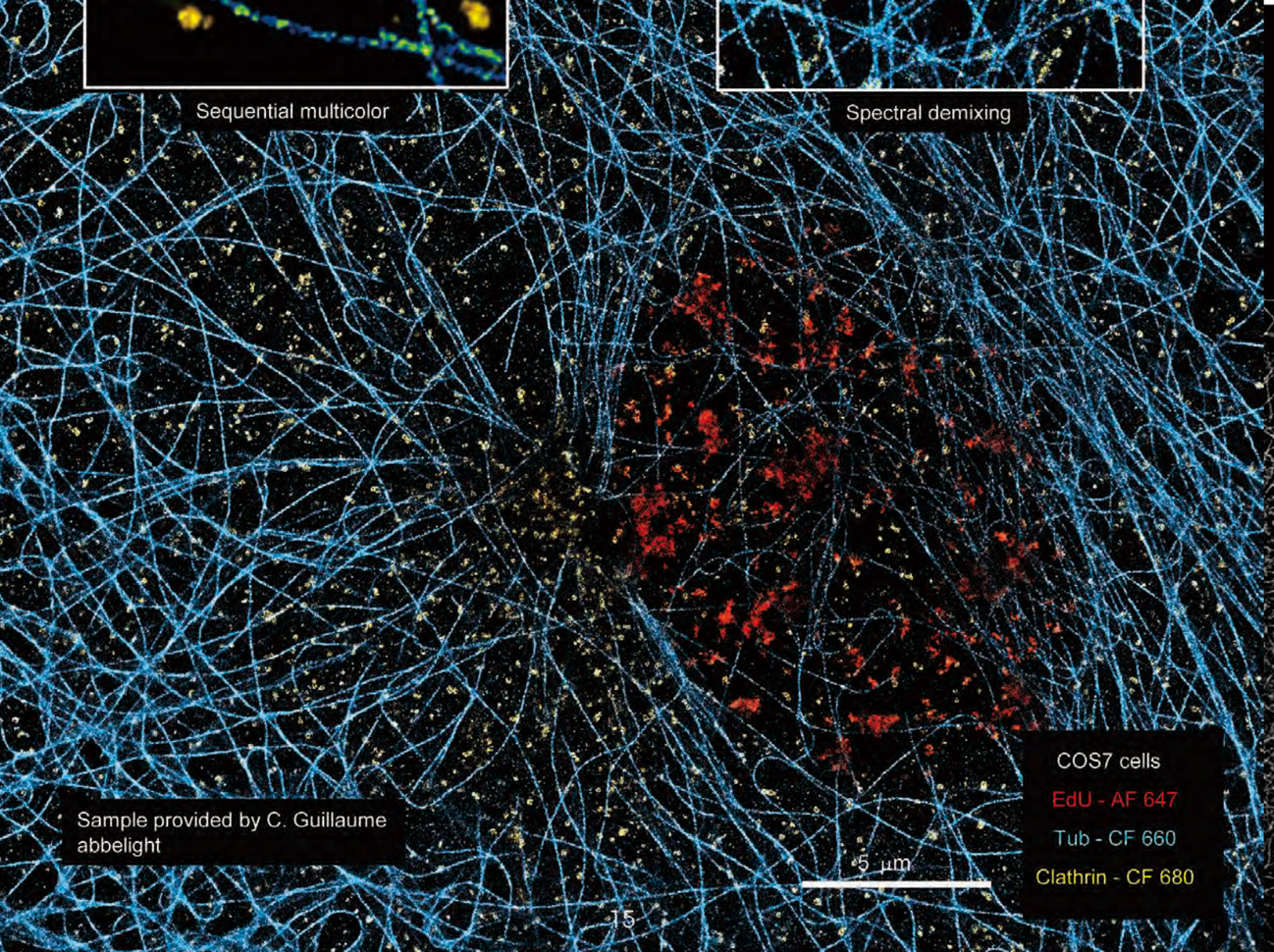
Both these methods are possible with SAFe 360.
However, they both require excitation with several lasers and compatibility of imaging buffers, and they can lead to chromatic aberrations.



Sequential multicolor

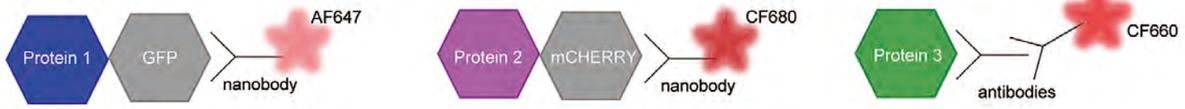


Spectral demixing



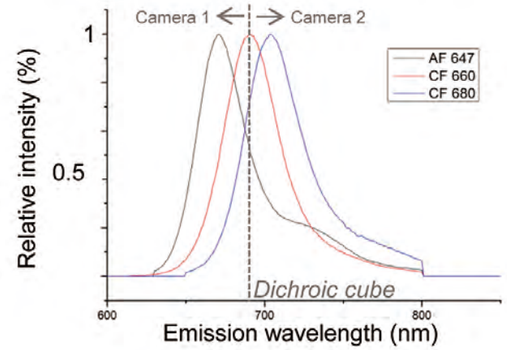
abbelight implemented a technology to perform simultaneous multicolor single-molecule imaging with only one excitation laser: **spectral demixing** *(Winterflood et al. Bophys 2015)*.

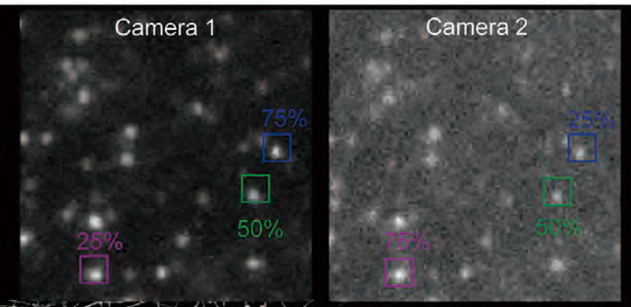
This method is **compatible** with a vast array of cell lines and standard commercial fluorophores.
For example:



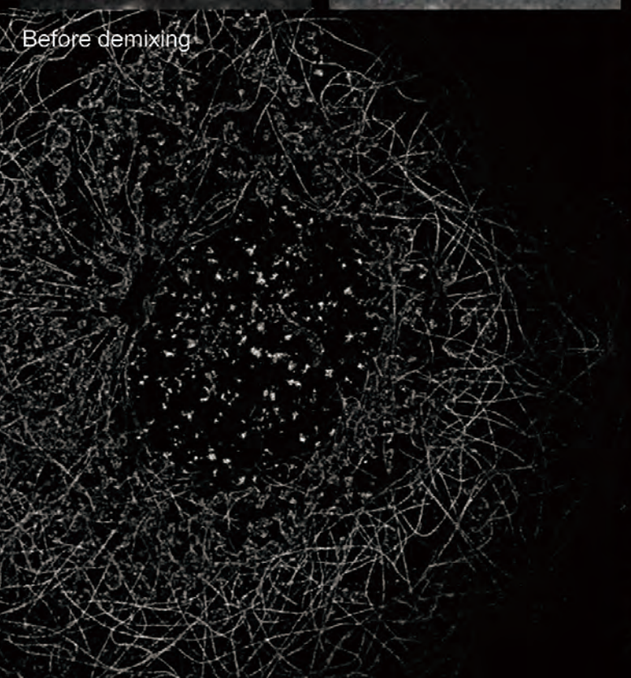
Spectral demixing principle

- Excitation with only 1 laser (640 nm)
- Same blinking efficiency
- Detection **in 2D or 3D** on the 2 cameras
- For each localization:
  - Measurement of the **intensity ratio** between the 2 cameras
  - Determination of the wavelength





Before demixing



Sample provided by C. Guillaume
abbelight

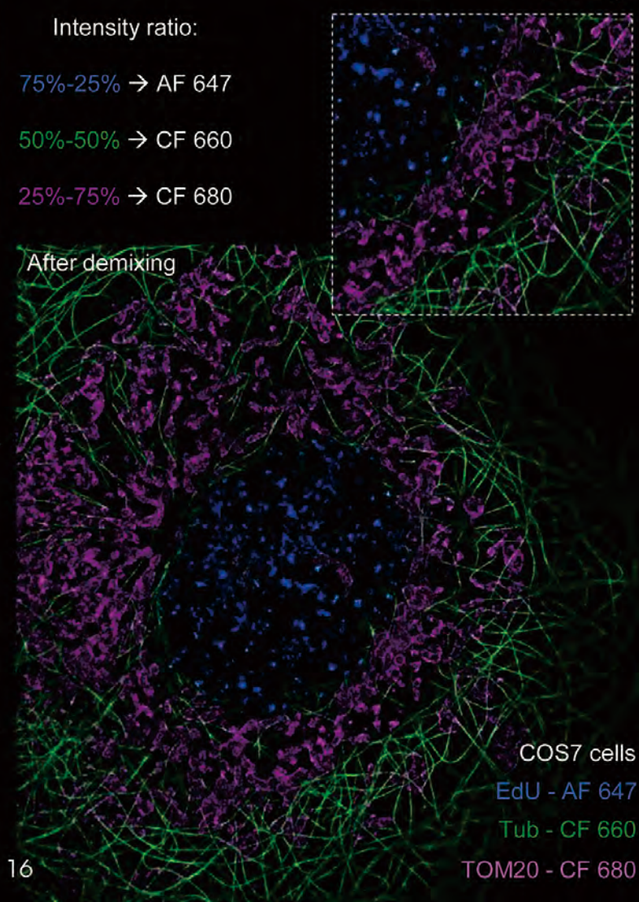
Intensity ratio:

75%-25% → AF 647

50%-50% → CF 660

25%-75% → CF 680

After demixing



NEO software

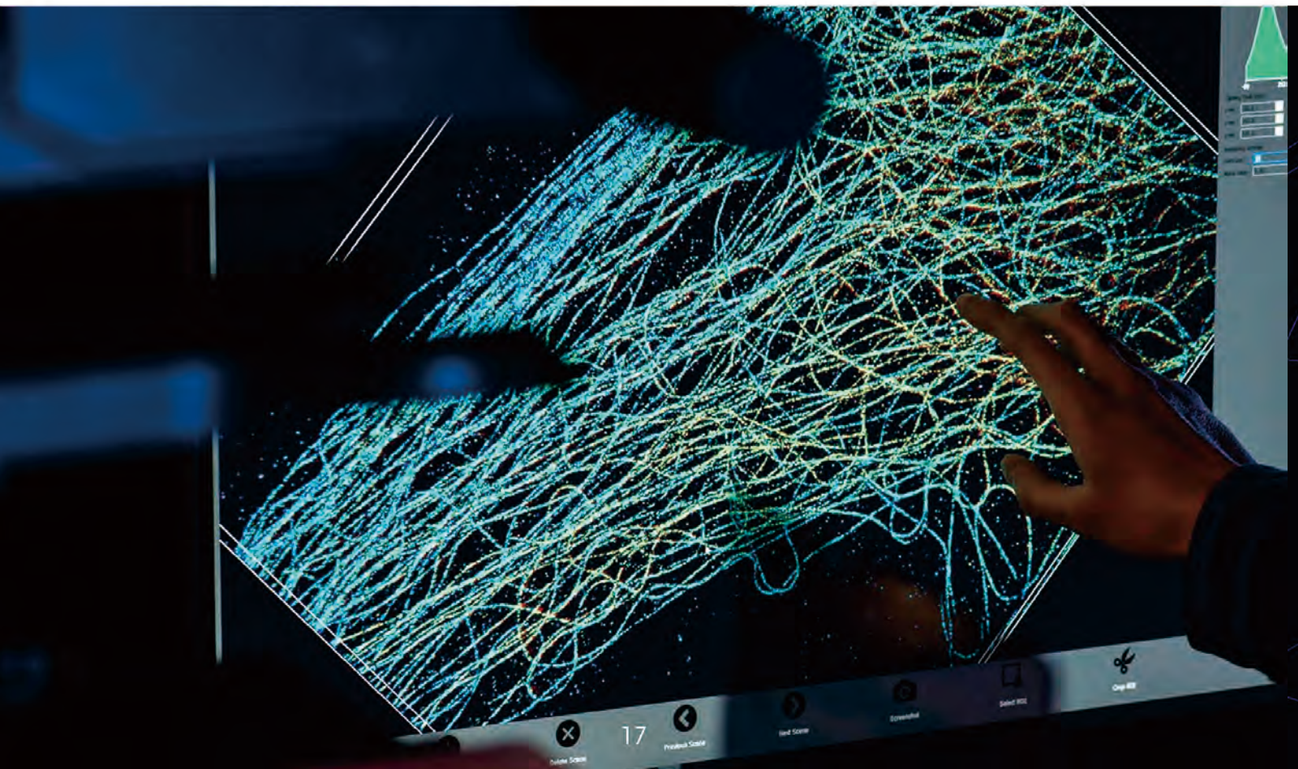
Abbelight's NEO SAFe software provides **a user-friendly all-in-one workspace** for acquisition, processing, and analysis of nanoscopy data.

Feature	NEO SAFe software
Control of instrument	Laser power and illumination angle (EPI, HiLo, TIRF)
	Cameras
Control of acquisition parameters	Region of interest size
	Exposure time (down to 10 ms per frame)
	Frame number
Live reconstruction of nanoscopy data	Choice of localization parameters: Localization algorithms (center of mass, Gaussian fitting, phasor) Intensity threshold Background subtraction method
	Live visualization and reporting
Live drift correction	Cross-correlation
Decision-making tools to guide acquisition	Real-time SQUIRREL algorithm, Culley et al. 2017

Nanoscopy data, unlike standard microscopy images, are **coordinate-based** rather than pixel-based, opening up new avenues for in-depth data analysis.

NEO offers a variety of tools for nanoscopy data visualization and analysis.

Feature	NEO SAFe software
Visualization	3D visualization
	Mutlicolor visualization
	Possibility to export images in TIFF format compatible with commonly used softwares
Descriptive spatial statistics	Localization distribution
	Measuring tools
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…
Spectral demixing	Separation of far-red dyes for multicolor imaging



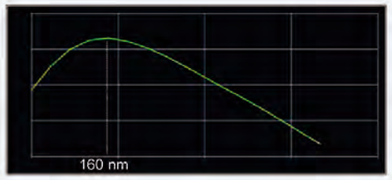
Voronoi segmentation

Clustering analysis

Among several analysis tools, Abbelight's NEO SAFe software 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 datapoints 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) requires two input parameters: a distance ε and a minimum number of neighbors MinPts. For each localization in the dataset, the algorithm searches whether it has enough neighbors MinPts within the distance ε. If yes, it considers the localization as part of a cluster, etc.
Ester et al. 1996

DBSCAN

Number of neighbors within a distance of ε indicates density

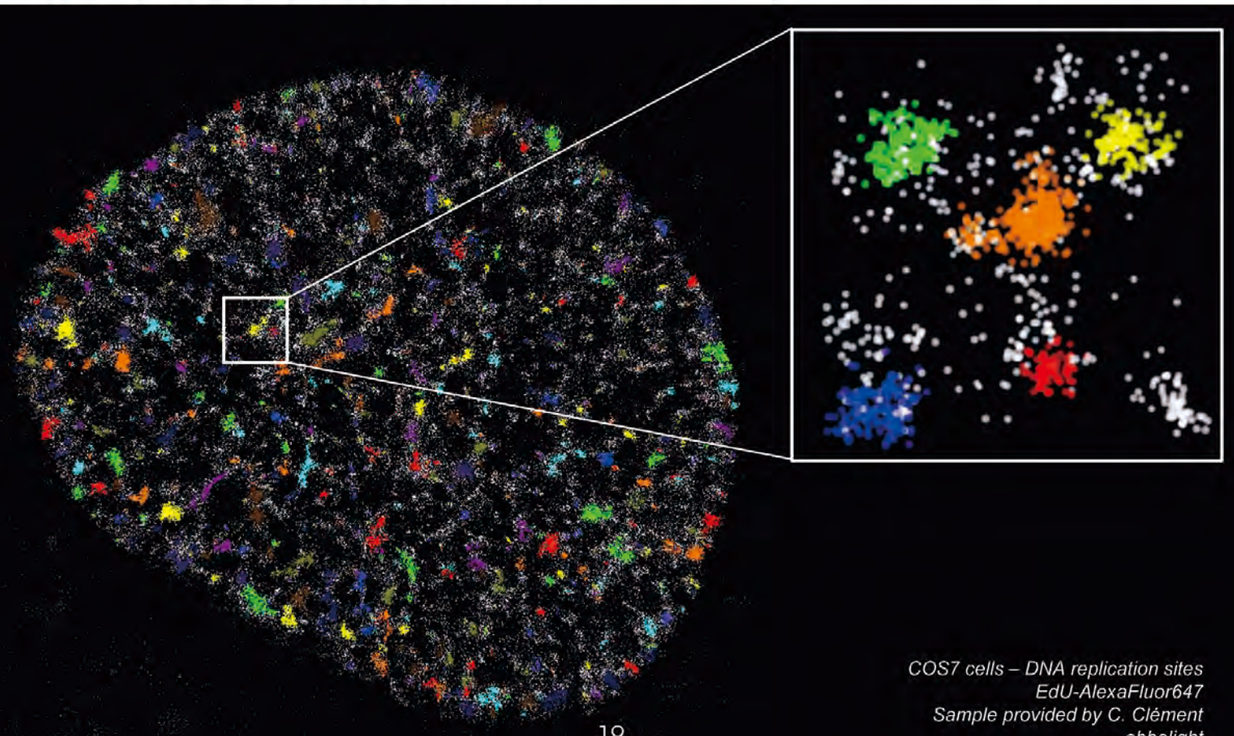
Voronoi partitions the image into polygons, where each polygon contains one, and only one, localization. The area of the polygon is indicative of the density of localizations: a dense region will have small polygons while a low-density region will have big polygons. The user can choose a density threshold, above which localizations are considered as part of a cluster.
Levet et al. 2015

Voronoi

Polygon size indicates density

Quantification

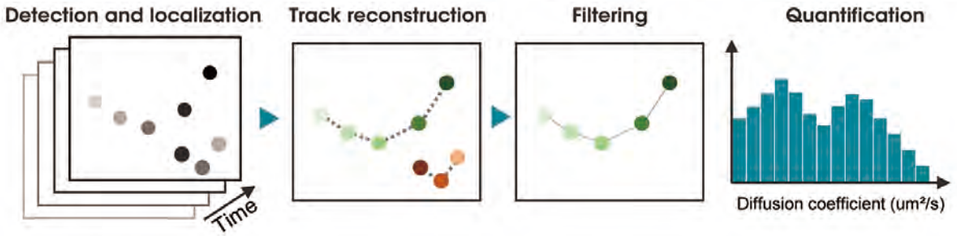
Once clusters are identified, the software can quantify: the number of clusters, their localization, their volume, their density, their radius of gyration…



COS7 cells – DNA replication sites
EdU-AlexaFluor647
Sample provided by C. Clément
abbelight

Single-particle tracking analysis (SPT)

To study the dynamics of single particles, NEO software can reconstruct trajectories from SPT raw data.

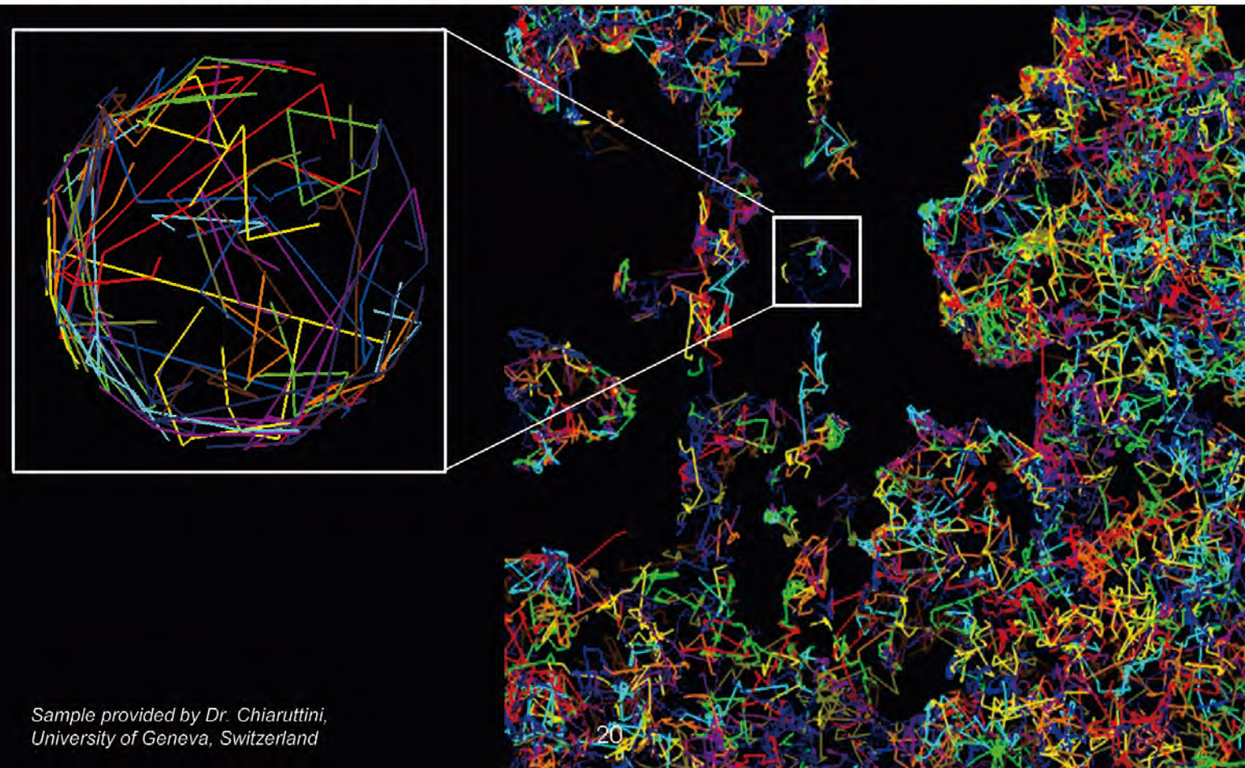


Reconstructing trajectories

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. Afterwards, it chooses the solution that maximizes the probability. These probabilities can be calculated based on a number of factors, including distance and motion speed.
(Jaqaman et al. 2008, Sergé et al. 2008)

Quantification

After reconstruction of the tracks, the software can quantify: the number of tracks, their duration, their average intensity, their diffusion coefficient (based on Mean Square Displacement analysis)…



Sample provided by Dr. Chiaruttini,
University of Geneva, Switzerland

Examples of biological applications

Abbelight technologies have been applied to a number of biological fields - ranging from cytoskeleton architecture to chromatin dynamics - and in a great number of organisms – human cell lines, bacteria or yeast, drosophila tissue…

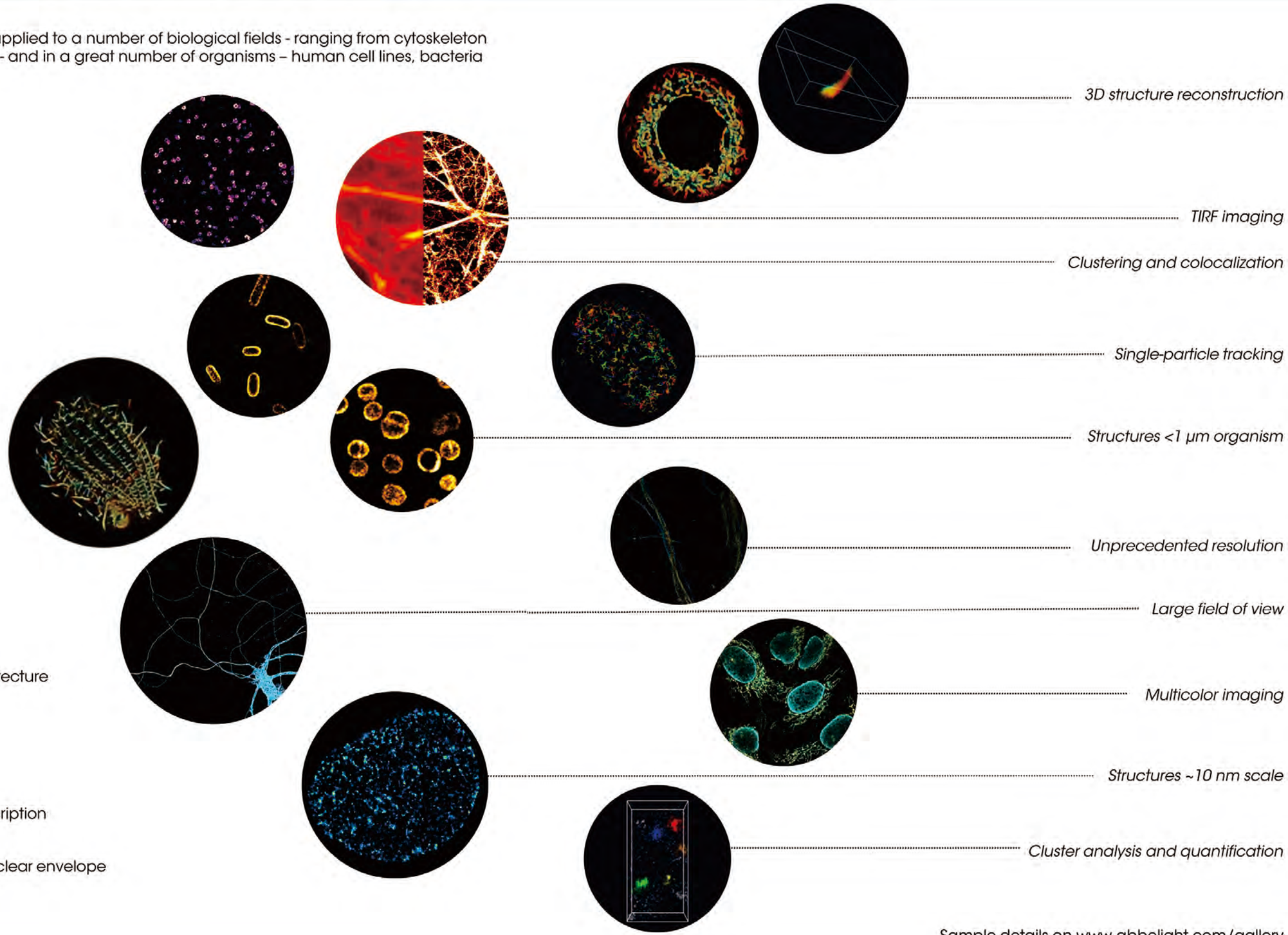
Cell Biology
- Cytoskeleton
- Metabolism
- Membrane proteins
- Transport
- Signaling

Bacteriology
- Antibiotic resistance
- Replication
- Membranes

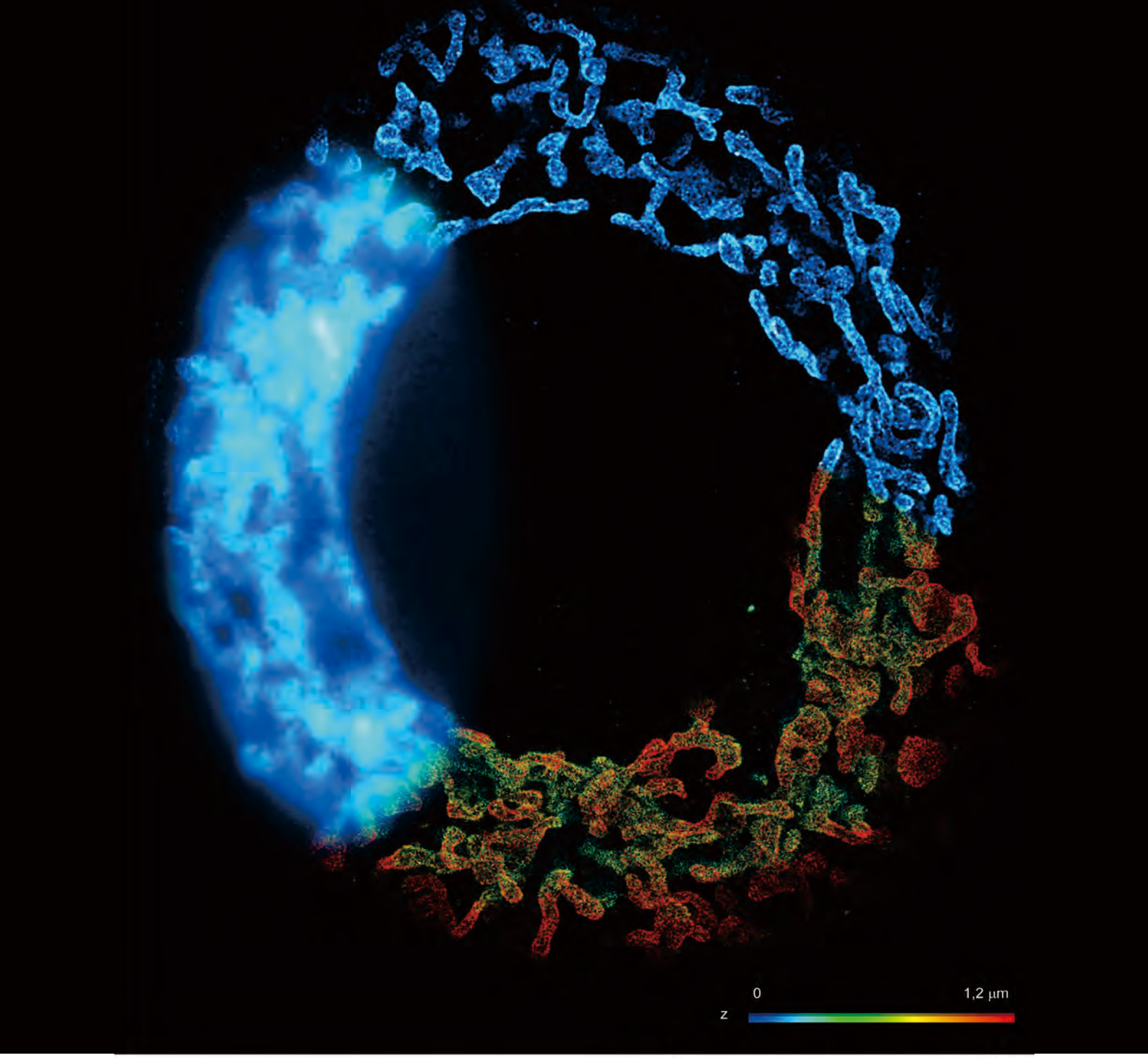
Immunology
- Immune receptors

Neuroscience
- Axon nanoscale architecture

Nucleus
- Replication and transcription
- Chromatin
- Nuclear pores and nuclear envelope



Sample details on www.abbelight.com/gallery



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