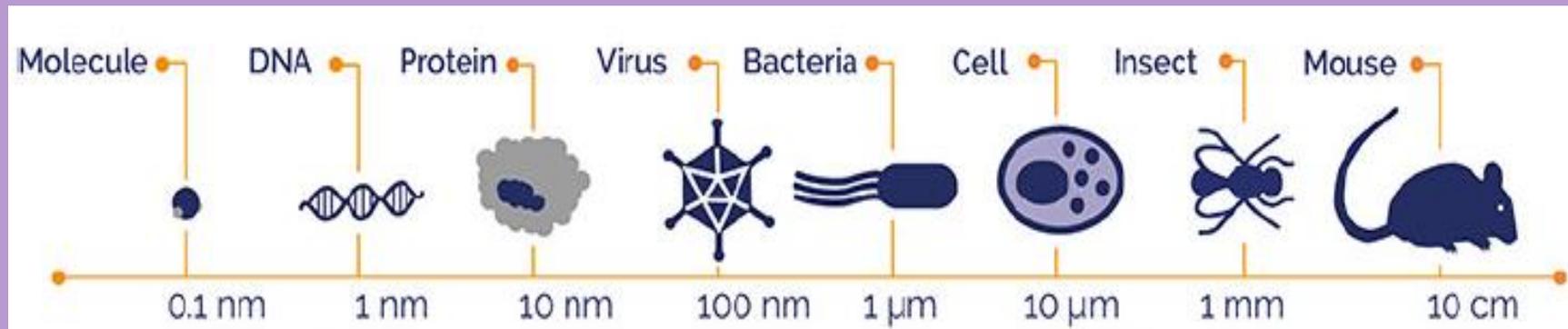




# Most Popular Microscopy Techniques

- Many microscopy techniques have been developed in life sciences to study biological organisms and their underlying biochemical and molecular processes. Therefore, life science research can span many orders of magnitude in scale, from studying single molecules through to whole model organisms. A wide range of microscopy techniques are available for life science research.



## TEM

In Transmission Electron Microscopy an electron beam is transmitted through a sample to form an image with improved resolution over light microscopy.

Samples for TEM are typically < 100 nm thick and require extensive preparation.

## FCS

Fluorescence Correlation Spectroscopy uses correlation analysis to determine changes to the intensity of fluorescence.

FCS can be used to study biochemical reactions and diffusion rates in living cells by determining the concentration of fluorescent molecules.<sup>1</sup>

## Super-Res

Super Resolution Microscopy techniques enhance resolution below the diffraction limit of light, which includes techniques such as PALM, STED and STORM.<sup>2</sup>

## TIRF

Total Internal Reflectance Fluorescence Microscopy uses an optical effect observed at the interface of two media with different refractive indices. Total internally reflected light at this interface extends ~100 nm into the sample.

TIRF is often used to study interactions at the plasma membrane.<sup>3</sup>

## Confocal

There are several different types of confocal microscopy including Confocal Laser Scanning Microscopy (CLSM) or Spinning Disk Confocal.

## SEM

Scanning Electron Microscopy, scans the topology of your sample with an electron beam.

SEM is challenging for biological samples as experimental conditions are under high vacuum which means samples must be dry.

## CLEM

Correlative Light and Electron Microscopy combines the resolution of electron microscopy with the ease of labelling of light microscopy.

CLEM is not a single, well-defined application. More information can be found in the review by Boer et al.<sup>4</sup>

## SRRF

Super-Resolution Radial Fluctuations is a combination of temporal fluctuation analysis and localization microscopy.

Andor's implementation of SRRF-Stream allows for real-time super-resolution microscopy.<sup>5</sup>

## SIM

Structured Illumination Microscopy uses a patterned light illumination technique to achieve enhanced resolution at low light intensities.

## Widefield

In widefield microscopy the entire sample is illuminated at once typically using a LED or lamp as an illumination source.

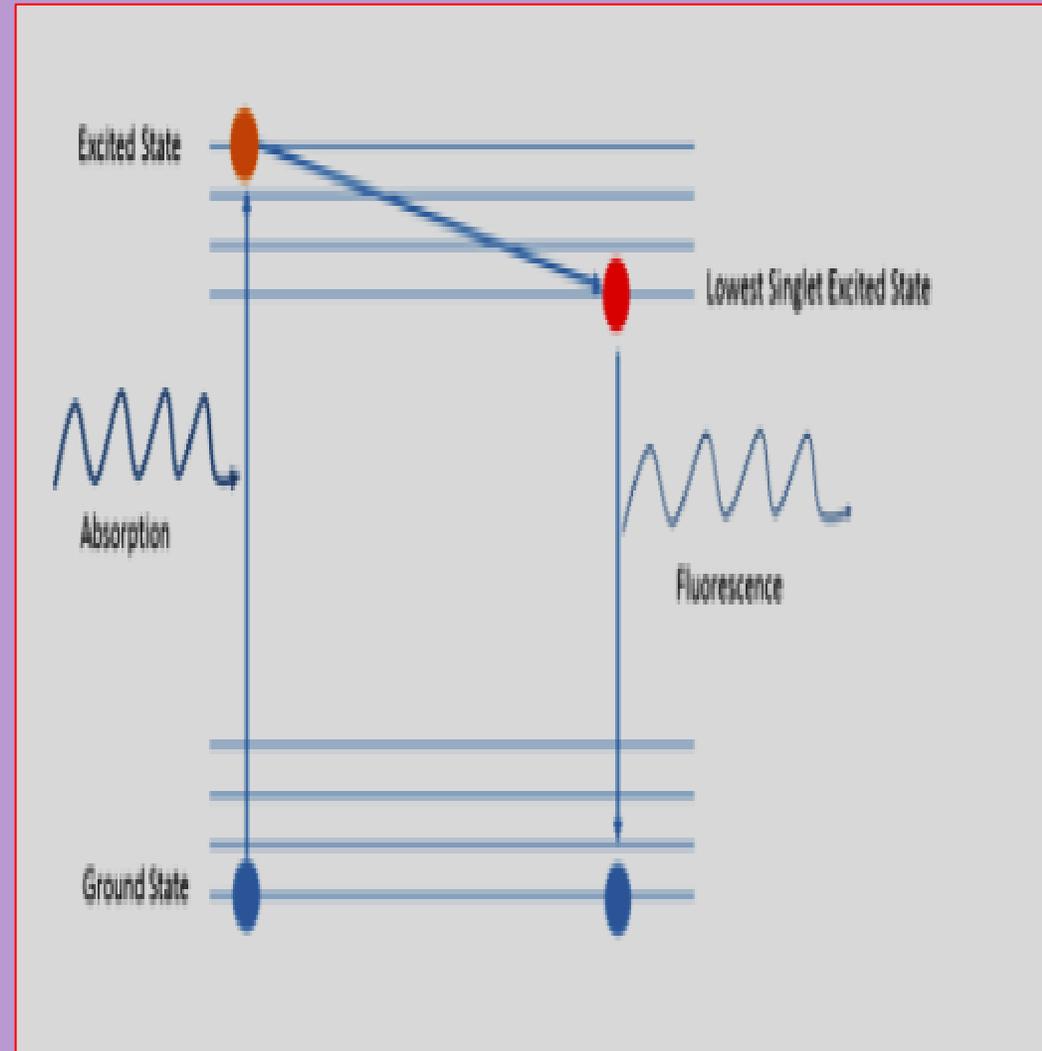
TIRF can be implemented using widefield microscopy.

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4. P. de Boer et al., 2015, Nature Methods, 12, 503-513. <https://doi.org/10.1038/nmeth.3400>
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# 1) FLUORESCENCE MICROSCOPY

- Fluorescence microscopy is an imaging technique that relies on the use of fluorophores to detect target molecules.
- Fluorophores emit fluorescent light upon excitation with electromagnetic radiation of a shorter wavelength.
- Fluorescence is produced when light excites an electron to a higher energy state.
- When the molecule returns to the ground state, the energy absorbed is reemitted in the form of light of a longer wavelength and lower energy than the original light absorbed.
- The difference between the wavelength of the incident and emitted radiation is known as the Stokes shift and it is an important characteristic of fluorophores.
- The larger this value, the easier it is to discriminate between the incident and emitted light.

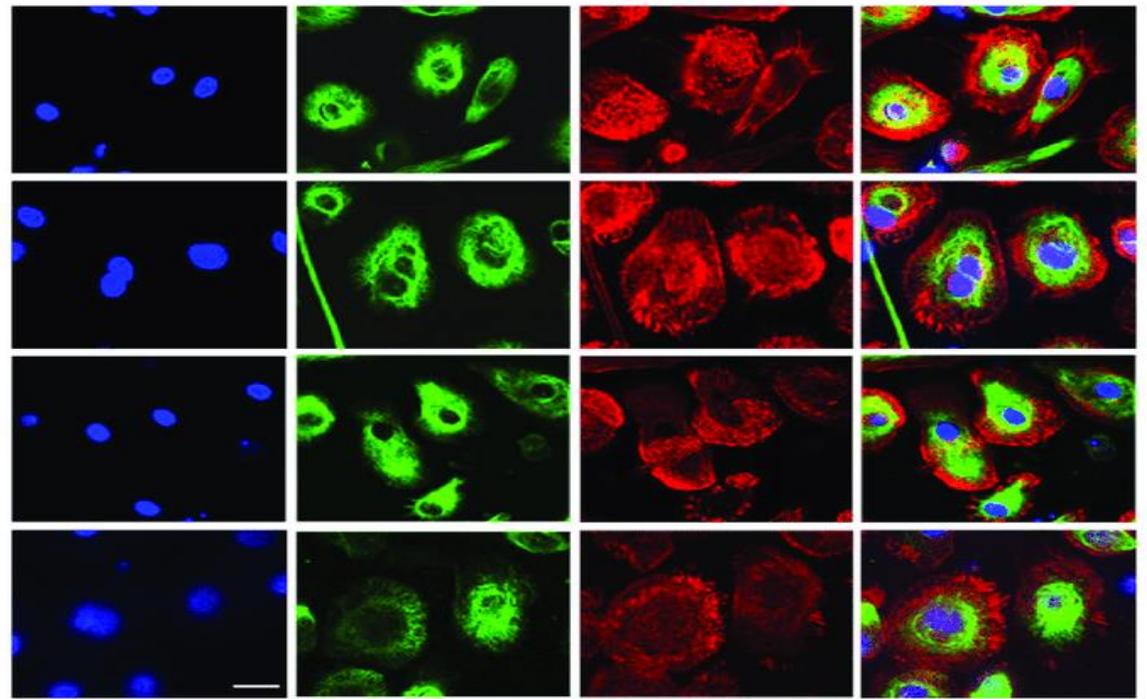
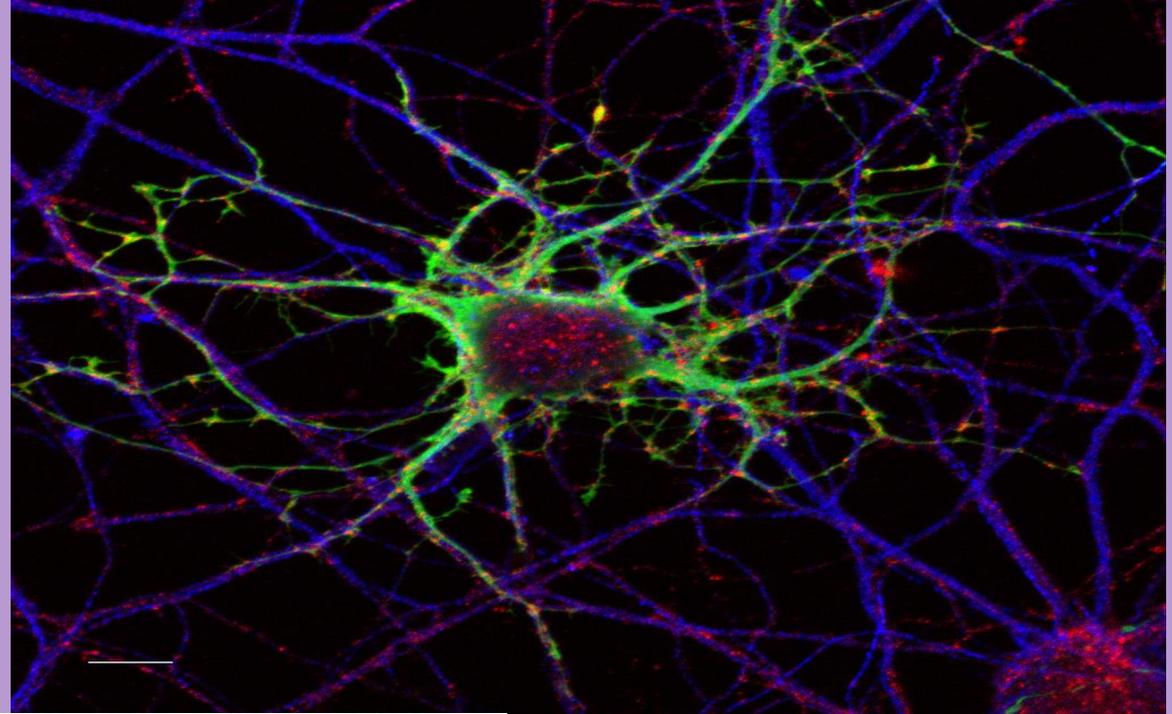
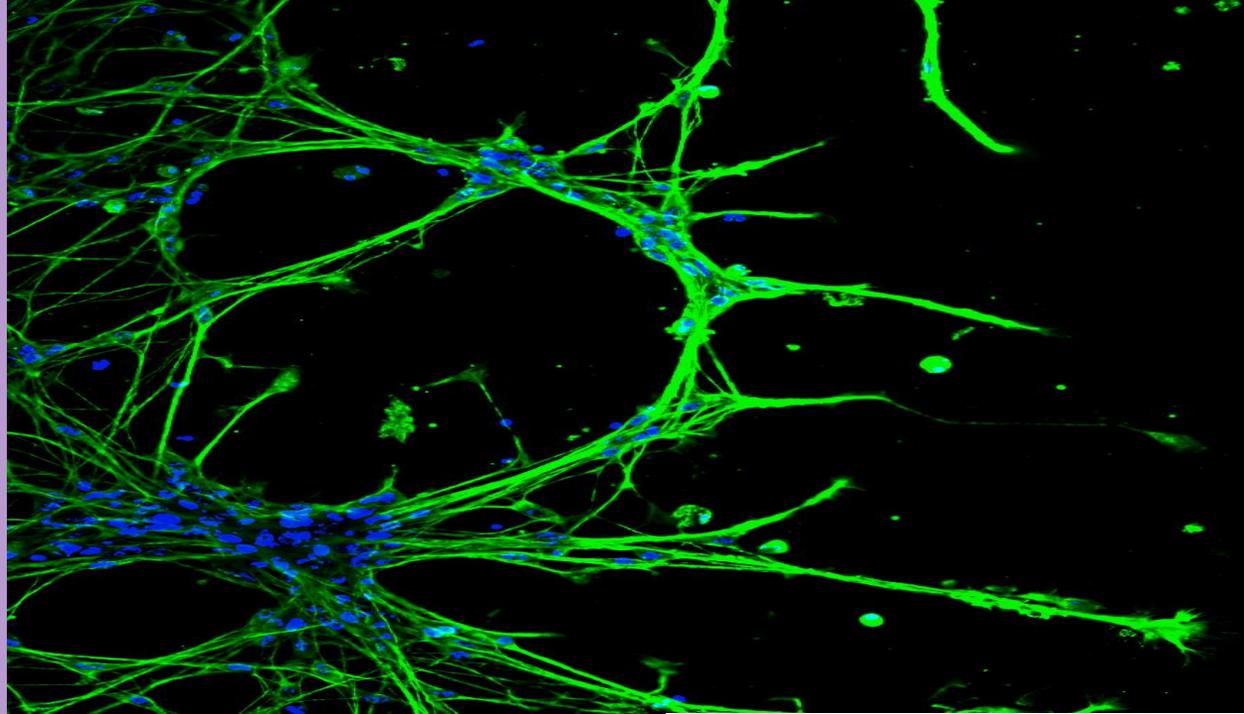


# 1) FLUORESCENCE MICROSCOPY

- Fluorescence microscopy is routinely used in research to study complex biochemical pathways and in diagnosis to detect markers indicative of disease states and progression.
- A plethora of fluorescent molecules, comprising organic dyes and fluorescent proteins, are available for fluorescence microscopy, and new, improved ones are continuously added to researchers' arsenal.

# 1) FLUORESCENCE MICROSCOPY

- Fluorescent microscopy is used to detect target molecules in tissues or cells in order to evaluate their spatial resolution and expression levels in different tissues and cellular compartments.
- Imaging and analysis of samples labeled with fluorescent probes that bind to specific cell markers can be achieved with different fluorescent detection methods.



## 2) FLUORESCENCE CORRELATION SPECTROSCOPY (FCS)

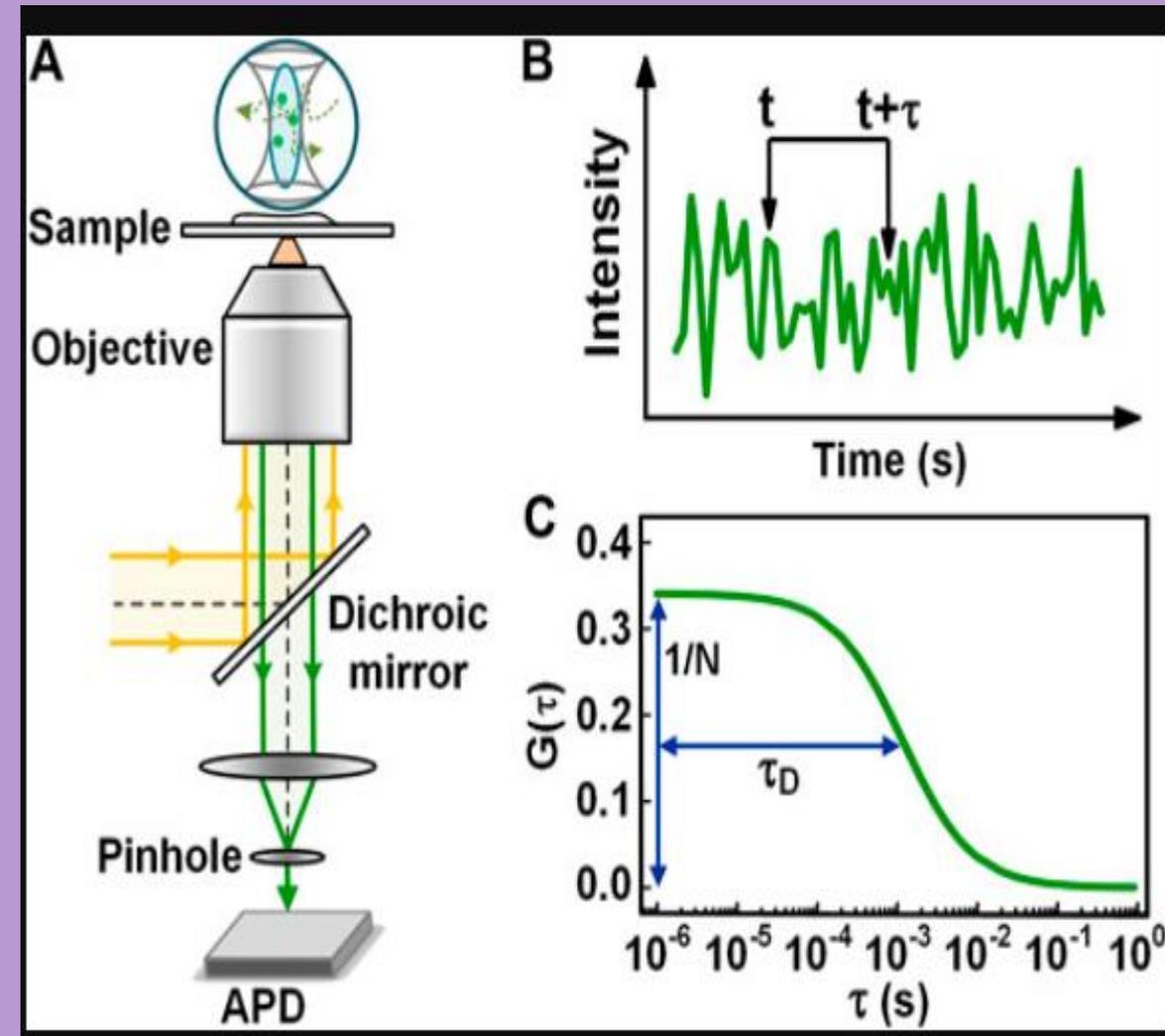
- Molecular dynamics in biological systems are the foundation of life events.
- Fluorescence correlation spectroscopy (FCS) is a powerful tool for detecting molecular dynamics through analyzing the intensity fluctuation emitted by biomolecules diffusing in and out of a focused light.
- The local concentration, hydrodynamic radius, diffusion coefficient, and the interaction of different proteins, etc. can be accurately measured with FCS.
- Compared with other dynamics orientated approaches, FCS has a broader measurable time range.

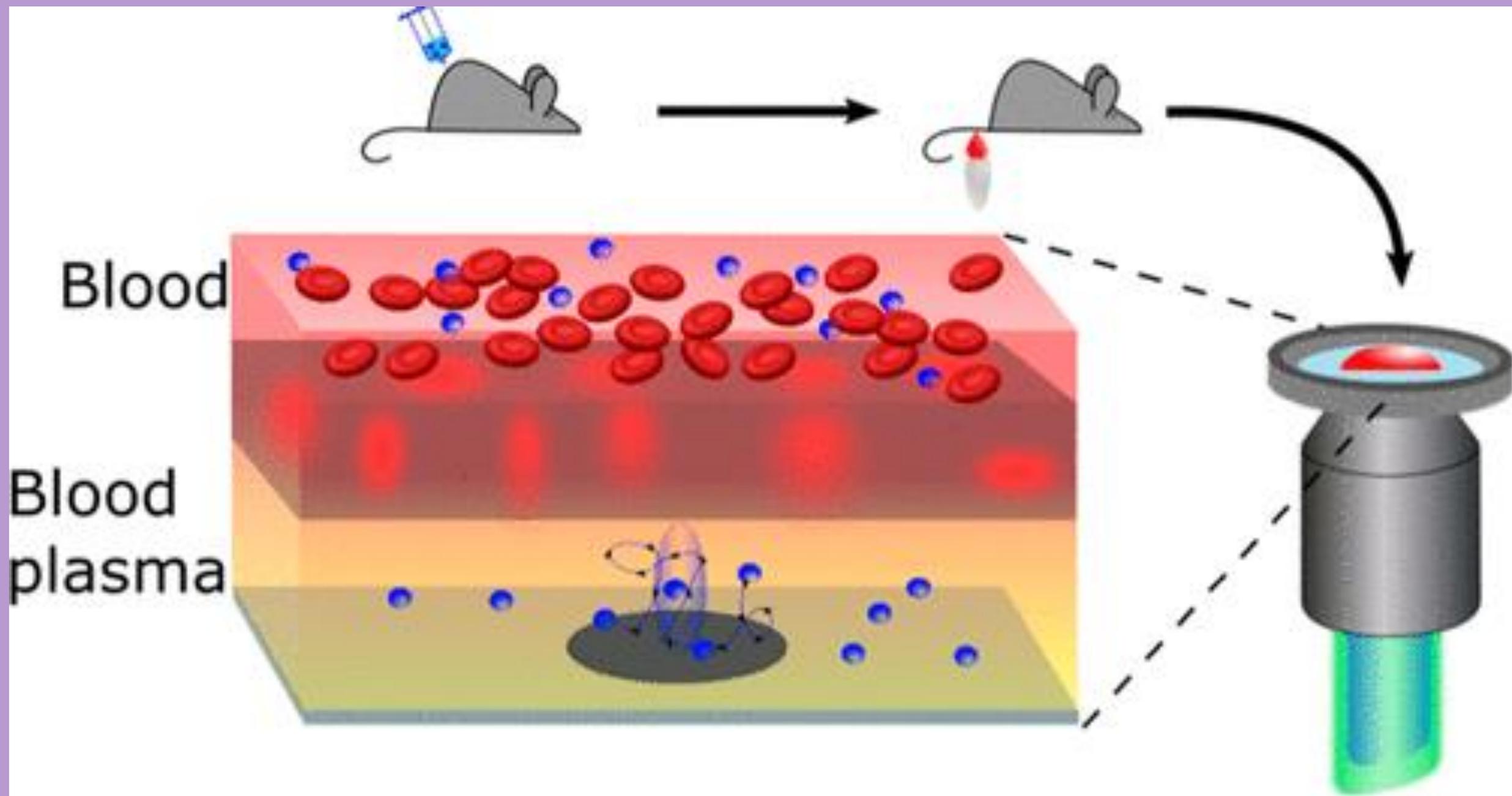
## 2) FLUORESCENCE CORRELATION SPECTROSCOPY (FCS)

- FCS was first proposed in 1972, which was designed for the measurement of the binding of the fluorescent dye EtBr and DNA.
- It has become a practical tool for the investigation of molecular dynamics since the 1990s when FCS was firstly implemented with confocal microscopy.
- Confocal microscopy provides a much-confined observation volume, which enhances the signal-to-noise ratio (SNR) of FCS significantly.

## 2) FLUORESCENCE CORRELATION SPECTROSCOPY (FCS)

- FCS is based on the analysis of time correlations in fluorescence fluctuation emitted when fluorescently labeled molecules are diffusing in and out of a tiny observation volume.
- In the implementation, FCS is often performed in a confocal system, as illustrated in the figure.
- The fluorescence emitted from the fluorescently-labeled molecules in the observation volume is collected by the same objective, and propagate along the opposite direction to that of the excitation/depletion light.
- After passing through a long-pass dichroic mirror, the fluorescence is focused through a pinhole onto an avalanche photodiode (APD).





# 3) SUPER-RESOLUTION MICROSCOPY (SRM)

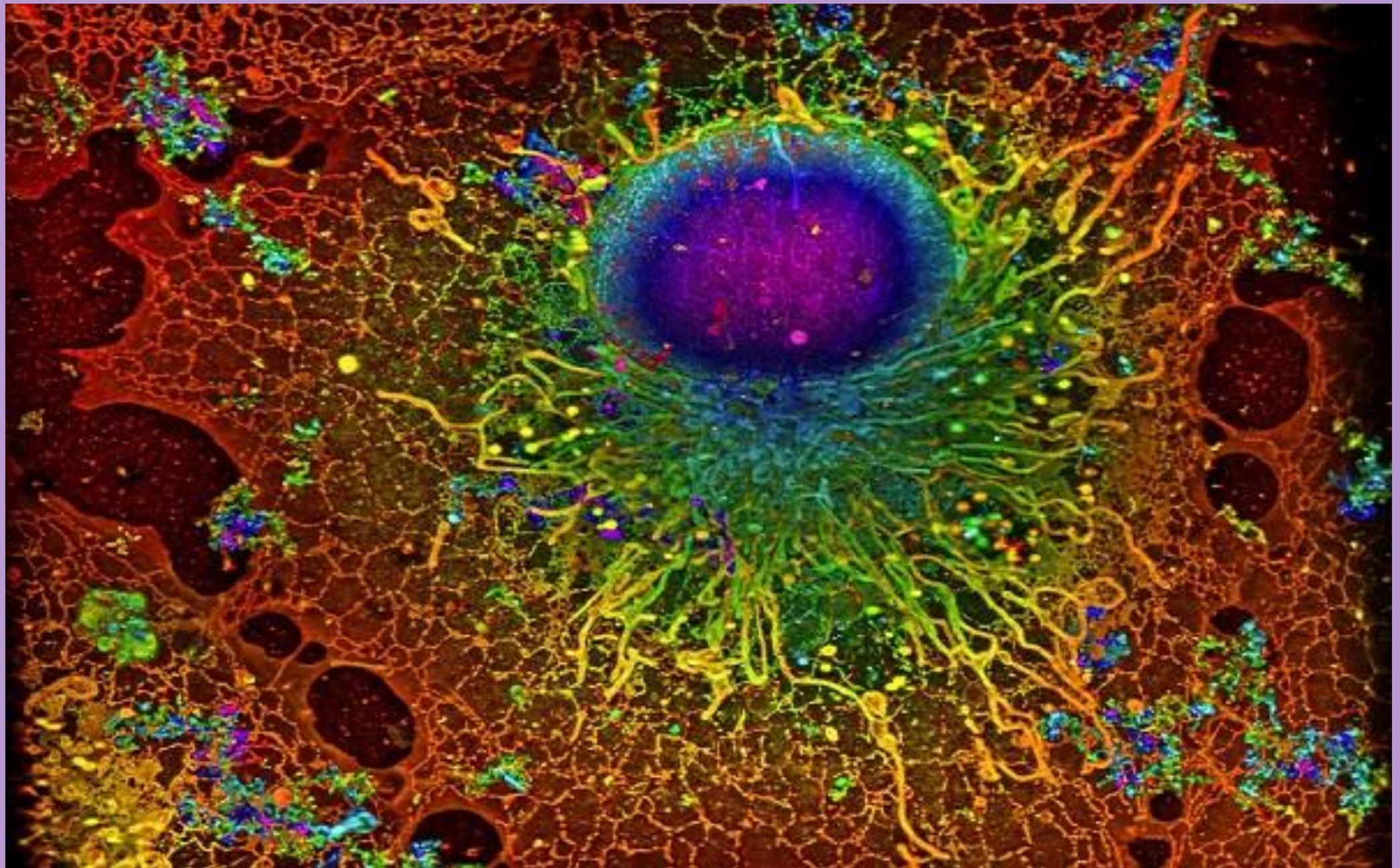
- Super-resolution microscopy (SRM) is a fast developing field that encompasses fluorescence imaging techniques with the capability to resolve objects below the classical diffraction limit of optical resolution.
- Acknowledged with the Nobel prize in 2014, numerous SRM methods have meanwhile evolved and are being widely applied in biomedical research, all with specific strengths and shortcomings.
- While some techniques are capable of nanometre scale molecular resolution, others are geared towards volumetric three-dimensional multi-colour or fast live-cell imaging.

## 3) SUPER-RESOLUTION MICROSCOPY (SRM)

- Super-resolution microscopy (SRM) encompasses multiple techniques that achieve higher resolution than traditional light microscopy.
- The resolution of conventional light microscopy is limited to around 200 nm due to the diffraction of light.
- As light passes through the surrounding medium in a light microscope, a single point of light (called a fluorophore) will appear blurry. The size of the blur is known as the point-spread function.
- When two structures are closer than the diffraction limit, they will appear as a single blur rather than two separate structures.

# 3) SUPER-RESOLUTION MICROSCOPY (SRM)

- Current SRM approaches can achieve resolution of around 200 times greater than that of conventional light microscopy.
- In practice, that means two fluorophores separated by 10 nm are visible as separate entities with certain SRM methods, whereas they would need to be around 200 nm apart to appear separate in conventional light microscopy.



## 4) STRUCTURED ILLUMINATION MICROSCOPY (SIM)

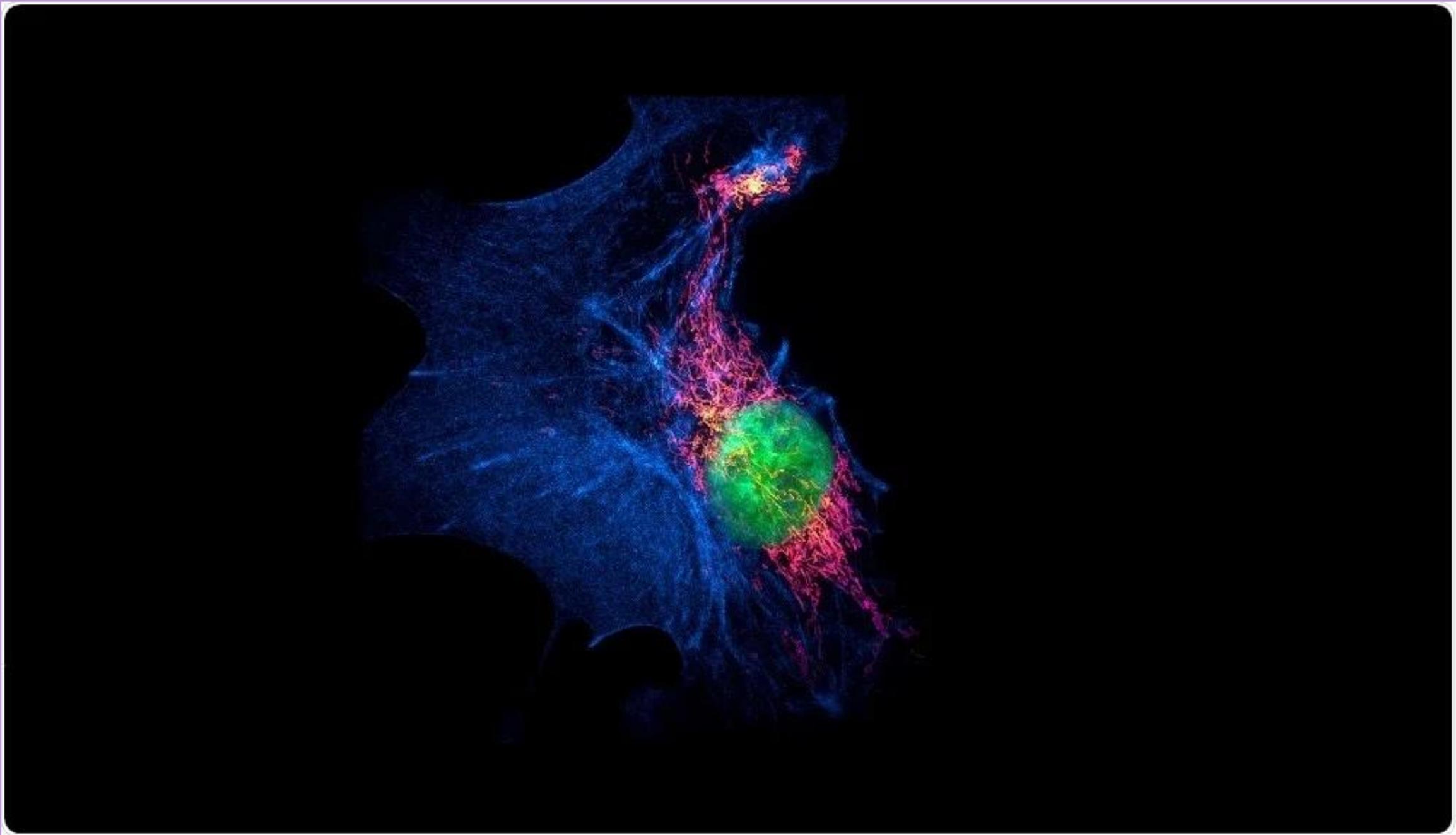
- Structured Illumination Microscopy (SIM) is used to increase the spatial resolution of light microscopy.
- A fluorescent sample is excited multiple times using striped illumination patterns.
- Each time the orientation and position of the stripes are changed.

## 4) STRUCTURED ILLUMINATION MICROSCOPY (SIM)

- These images are analyzed using computer software. The stripes fired at the sample interact with high frequency light produced from the sample. This interaction produces a third pattern that can be more easily analyzed.
- Using multiple images, further detail is obtained, and an image is reconstructed with around twice the resolution as traditional light microscopy.

The benefits of SIM include:

- Live cell imaging
  - 3D imaging
- Thick section imaging



# 5) TOTAL INTERNAL REFLECTANCE FLUORESCENCE MICROSCOPY (TIRFM)

- TIRFM is a powerful technique for selectively imaging fluorescent molecules (e.g., GFP, membrane dyes, fluorochromes attached to antibodies, etc.) in an aqueous environment that are very near a solid substance with a high refractive index (e.g. coverglass).
- Depending on the excitation wavelength and objective numerical aperture, the thickness of the excitation depth, which is called the evanescent field, can be less than 100 nm from the solid surface.
- In comparison, the thickness of a confocal image section is approximately 500 nm.

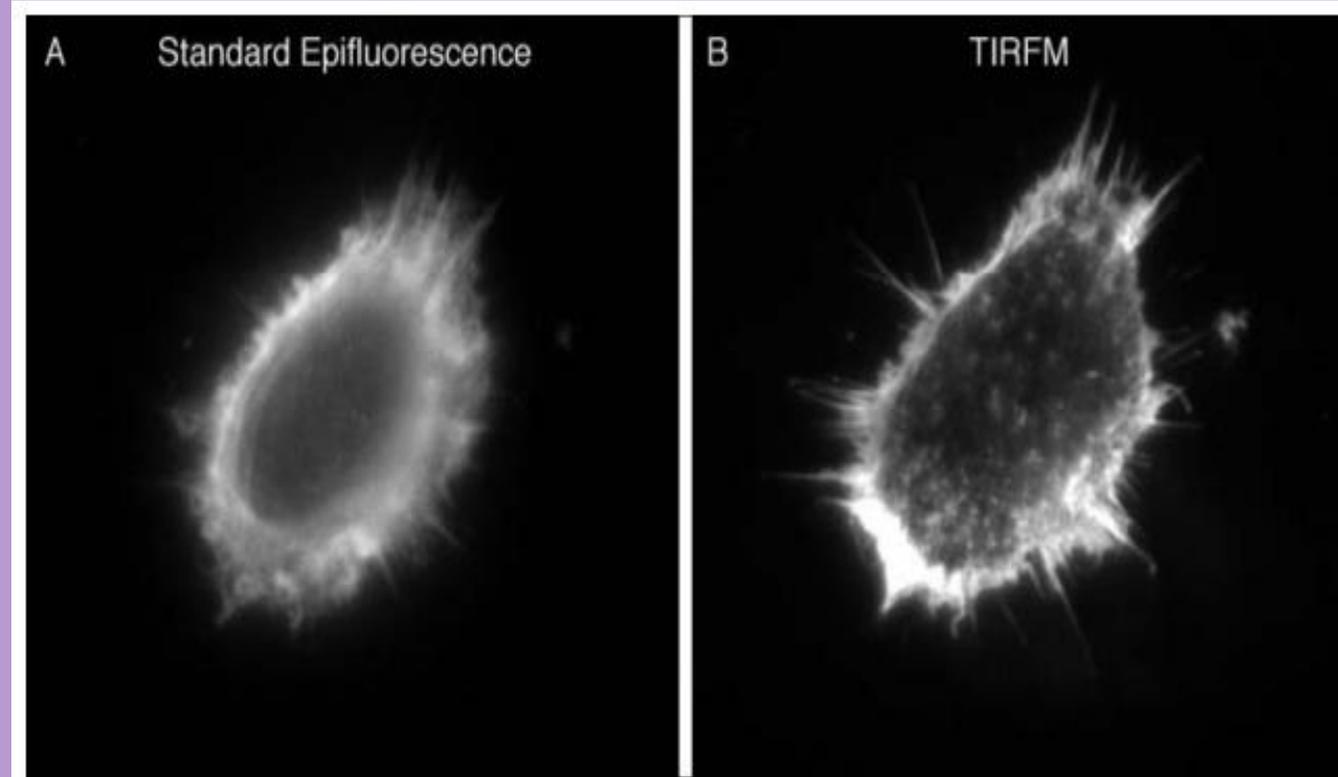
# 5) TOTAL INTERNAL REFLECTANCE FLUORESCENCE MICROSCOPY (TIRFM)

- The advantage of such a small illumination volume is three fold:

- (1) The background is greater than 2,000-fold lower than when imaging by normal epifluorescence microscopy (Funatsu et al., 1995), which results in a high signal-to-background ratio;

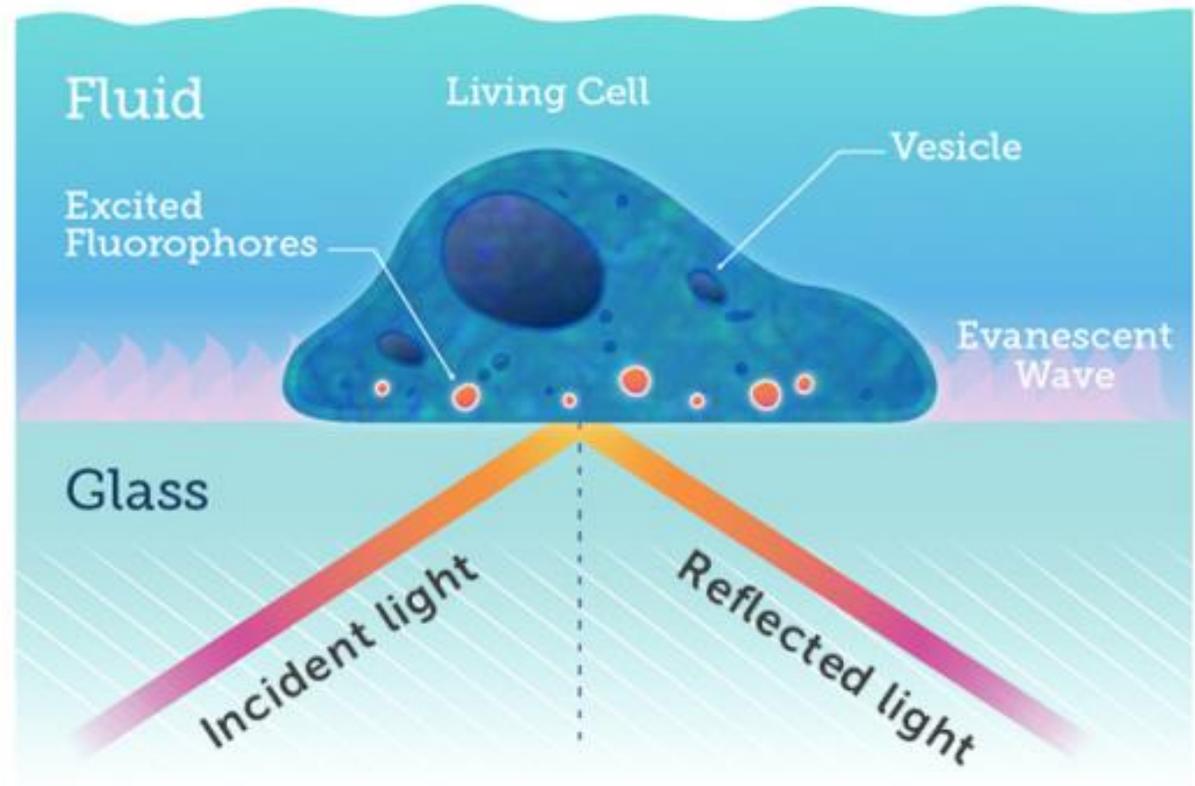
- (2) There is virtually no out-of focus fluorescence collected

- (3) Cells are exposed to a significantly smaller amount of light.



**Figure 1.**

Information revealed by TIRF microscopy. (A-B) HeLa cells were cultured on 18 mm round 1.78 RI coverslips. Post fixation with 4% paraformaldehyde, rhodamine-conjugated phalloidin was used to visualize F-actin. The cell was imaged in HBSS using a 100X 1.65 NA objective and 1.78 RI immersion liquid. Both images were taken at the same plane with the same exposure settings.

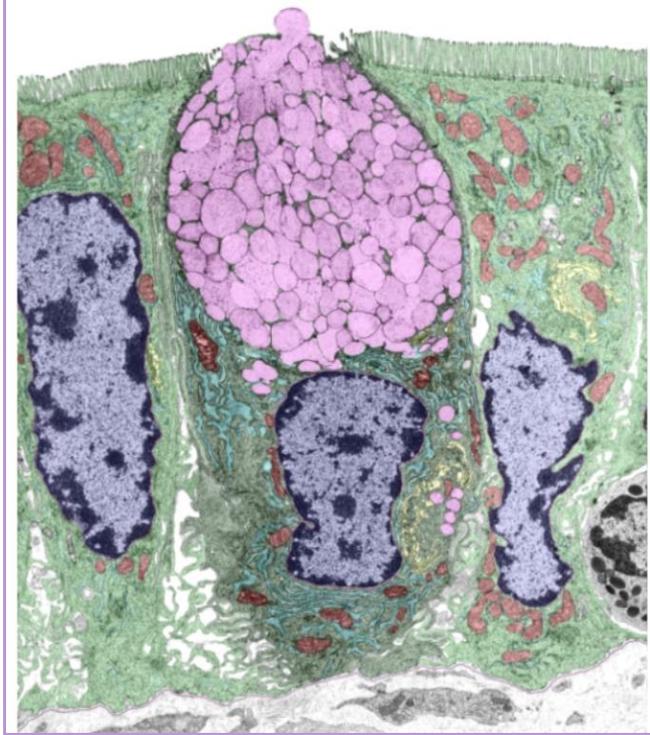
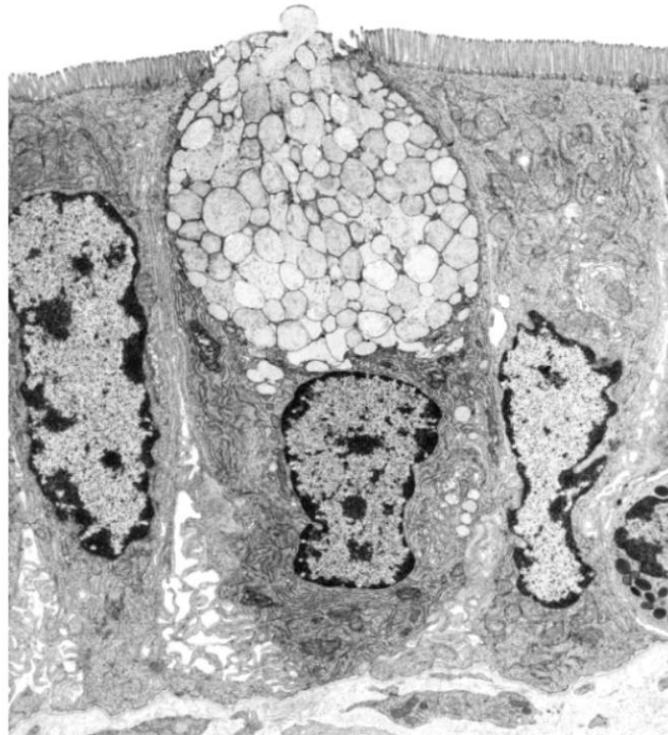
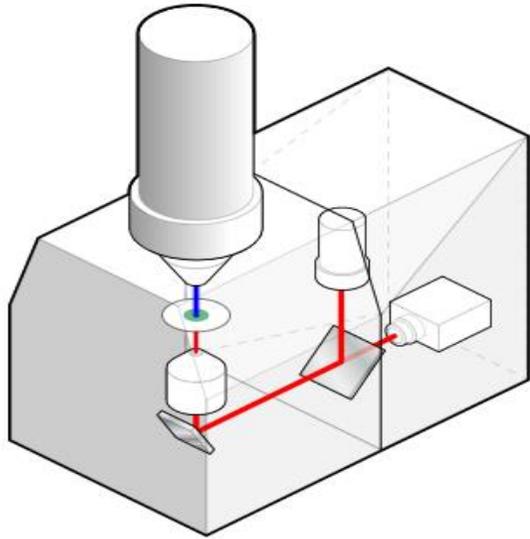


## 6) CORRELATIVE LIGHT AND ELECTRON MICROSCOPY (CLEM)

- Correlative light and electron microscopy (CLEM) is a combination of fluorescence microscopy (FM) with high-resolution electron microscopy (EM).
- In order to analyze various aspects of the complex organization of cells, there is increasing demand to study the same sample at different length scales in biology.
- The great potential of CLEM lies in the combination of two modalities: multi-color labelling together with high resolution contextual information.
- Therefore, CLEM enables you to correlate the two different types of information on the exact same area of interest: cellular function (from FM) and ultrastructure (from EM).

## 6) CORRELATIVE LIGHT AND ELECTRON MICROSCOPY (CLEM)

- This combined power makes it a powerful technique, finding application in several fields in the life sciences, such as neuroscience, cell biology and tissue research, to name just a few.
- In recent times, advancements in super-resolution optical techniques have further enhanced the value that CLEM can bring to research in biology, as a result of sub-diffraction limited optical microscopy.



A diagram showing Integrated CLEM, with the SECOM platform. The electron beam of the scanning electron microscope is shown in blue. The added optical light path of the SECOM platform is shown in red and yellow.

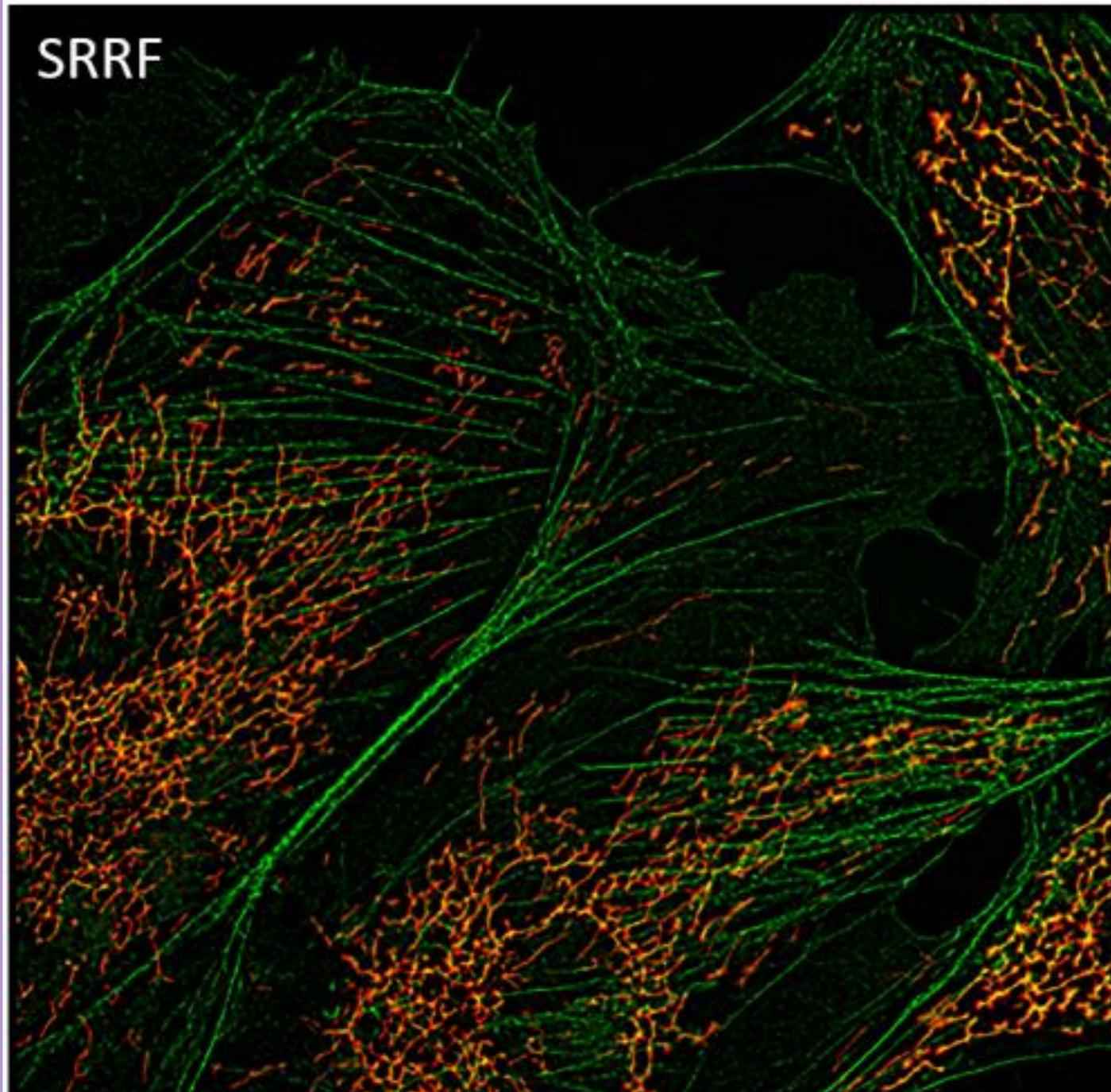
# 7) SUPER-RESOLUTION RADIAL FLUCTUATIONS (SRRF)

- Super-resolution Radial Fluctuations (SRRF) imaging is a computational approach to fixed and live-cell super-resolution microscopy that is highly accessible to life science researchers since it uses common microscopes.
- This allows users to generate super-resolution images using the same equipment, fluorophores, fluorescent proteins and methods they routinely employ for their studies without specialized sample preparations or reagents.

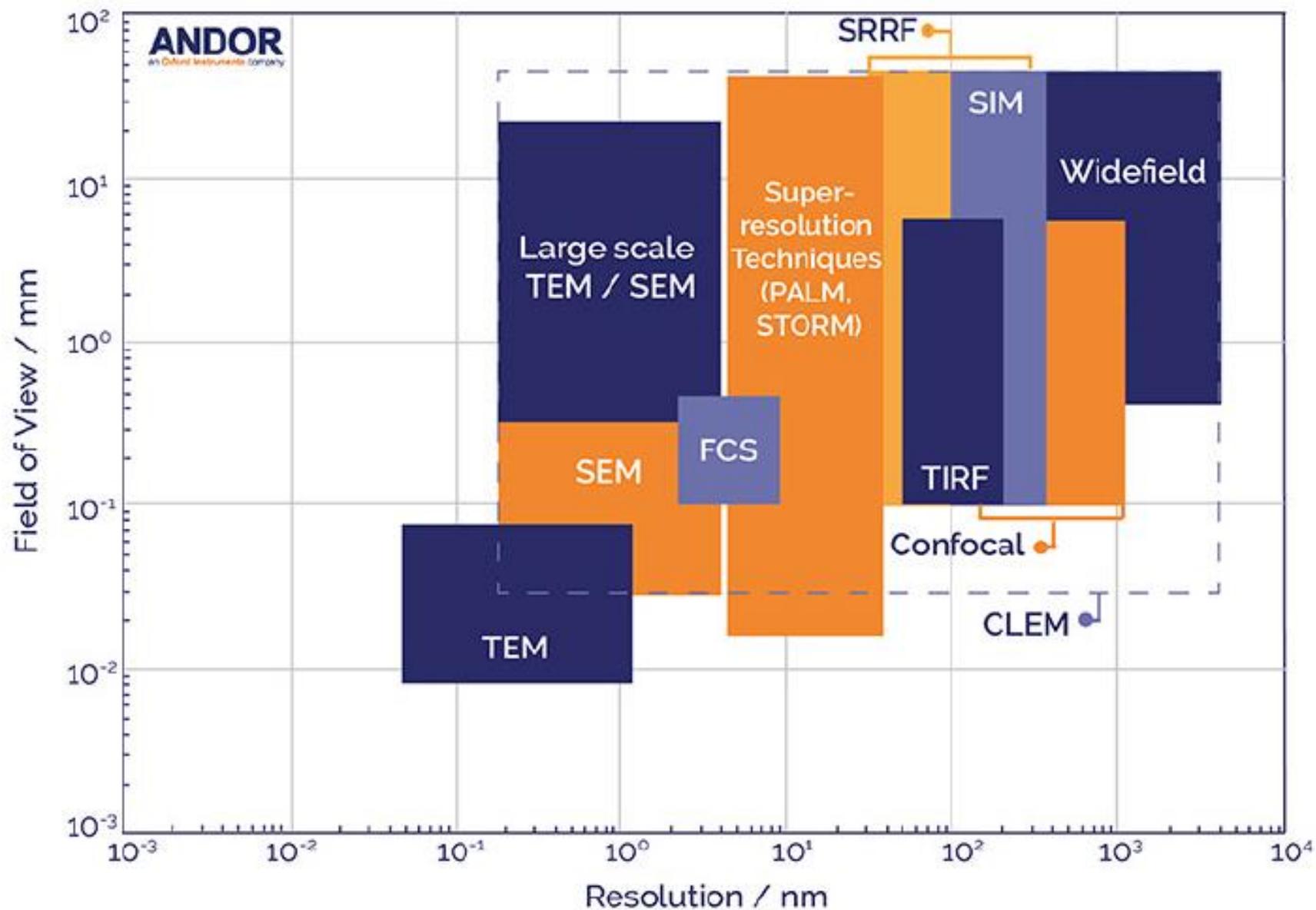
# 7) SUPER-RESOLUTION RADIAL FLUCTUATIONS (SRRF)

- Super-resolution with standard microscopes: SRRF is capable of super-resolving cellular structures imaged with widefield, TIRF or confocal modern microscopes without the need for specialized optics. Additionally, SRRF has sample illumination intensity requirements orders of magnitude lower than other super-resolution methods.
- Super-resolution with conventional fluorophores such as GFP: SRRF is able to produce super-resolution images from samples labelled with a wide range of conventional fluorophores, such as GFP.
- Live-cell super-resolution with minimal phototoxicity: as SRRF is able to extract high-fidelity super-resolution information from low signal-to-noise ratio samples, it requires lower sample illumination than most other super-resolution methods. For this reason, SRRF enables live-cell imaging over timescales ranging from minutes to hours. Imaged cells generally remain capable of undergoing mitosis, mitochondrial motility and cytoskeletal reorganisation as expected in normal healthy conditions.

SRRF



# Most Popular Microscopy Techniques



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