Physics of Nanotechnology

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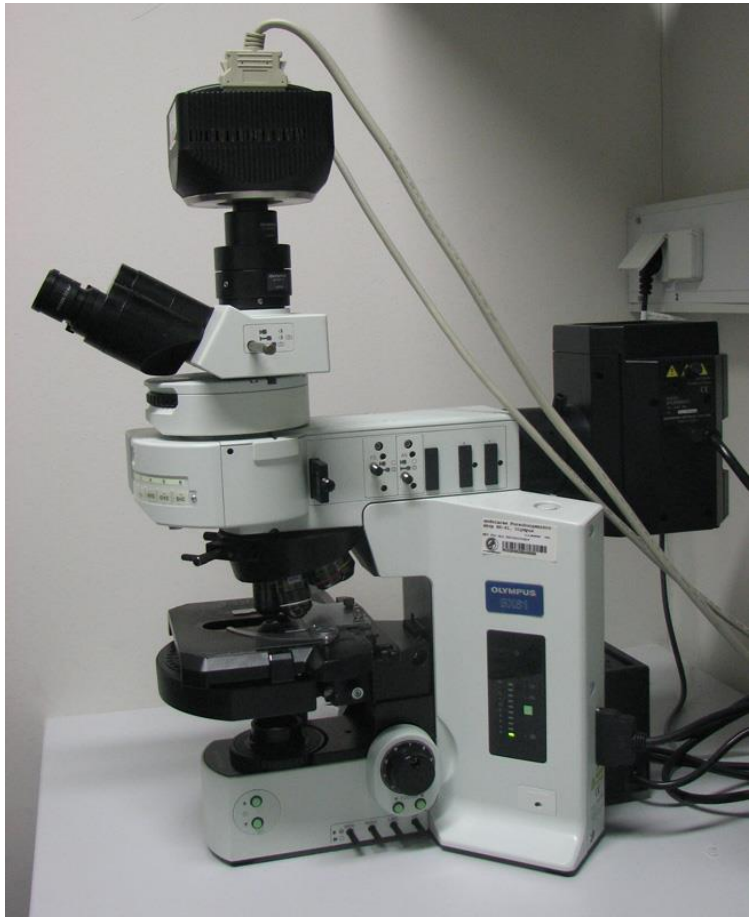
fluorescence microscope

"Fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescence image.

Fluorescence Microscope Image



What are **Fluorophores**

Fluorophores are **microscopic** molecules, which may be proteins, small organic compounds, or synthetic polymers that absorb light of specific wavelengths and emit light of longer wavelengths. ...
Different **fluorescence microscopy** instrumentation
requires **fluorophores** of varying excitation and emission wavelengths.

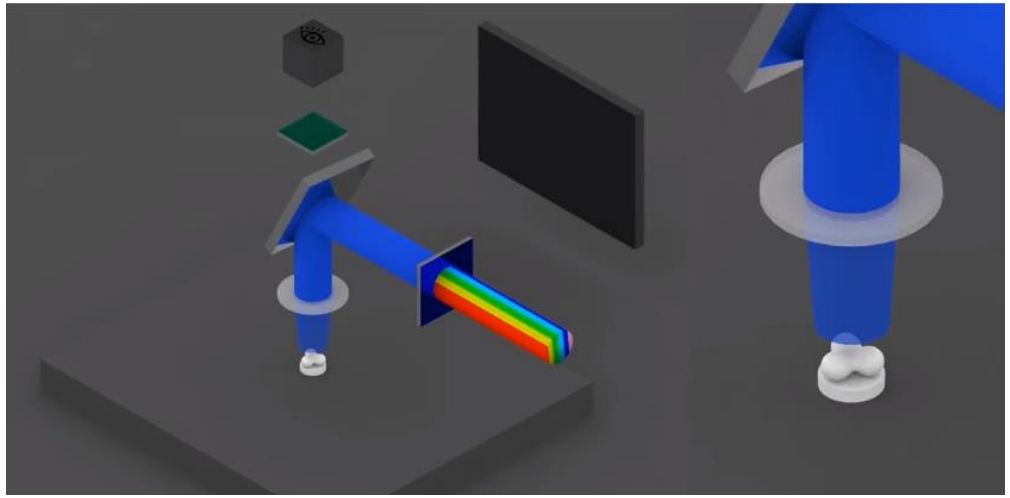
Principle

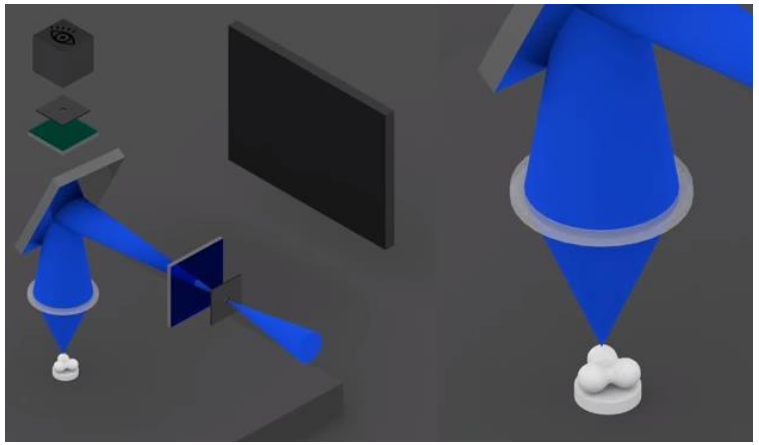
The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit light of longer wavelengths (i.e., of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of a spectral emission filter. Typical components of a fluorescence microscope are a light source (xenon arc lamp or mercury-vapor lamp are common; more advanced forms are high-power LEDs and lasers), the excitation filter, the dichroic mirror (or dichroic beamsplitter), and the emission filter (see figure below). The filters and the dichroic beamsplitter are chosen to match the spectral excitation and emission characteristics of the fluorophore used to label the specimen.[1] In this manner, the distribution of a single fluorophore (color) is imaged at a time. Multi-color images of several types of fluorophores must be composed by combining several single-color images.[1]

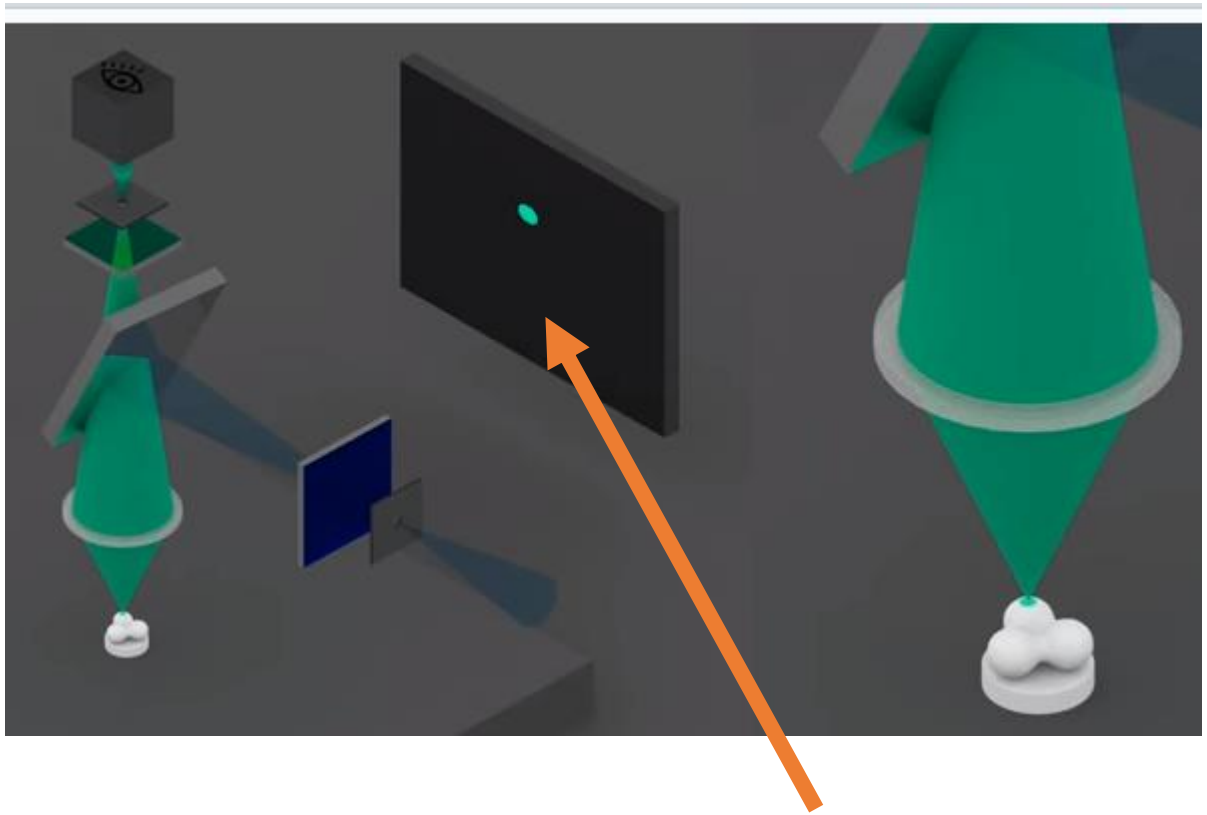
Principle

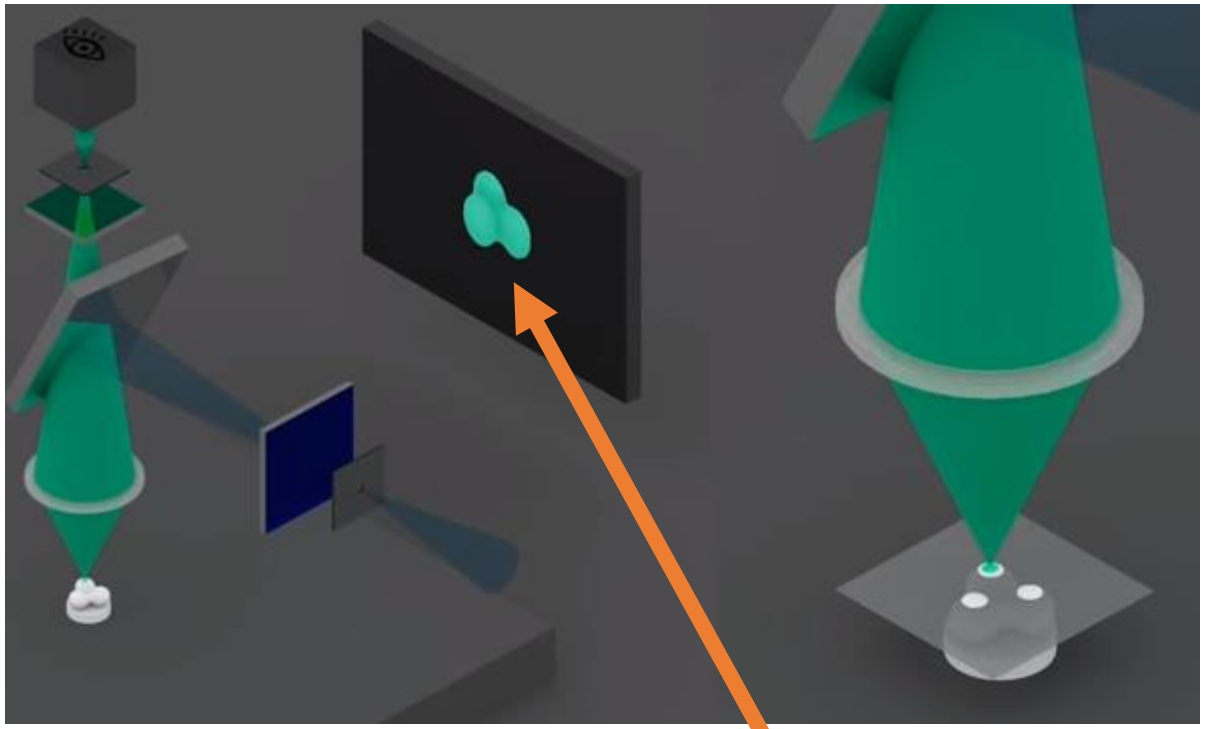
Most fluorescence microscopes in use are epifluorescence microscopes, where excitation of the fluorophore and detection of the fluorescence are done through the same light path (i.e. through the objective). These microscopes are widely used in biology and are the basis for more advanced microscope designs, such as the confocal microscope and the total internal reflection fluorescence microscope (TIRF).

fluorescence microscope working









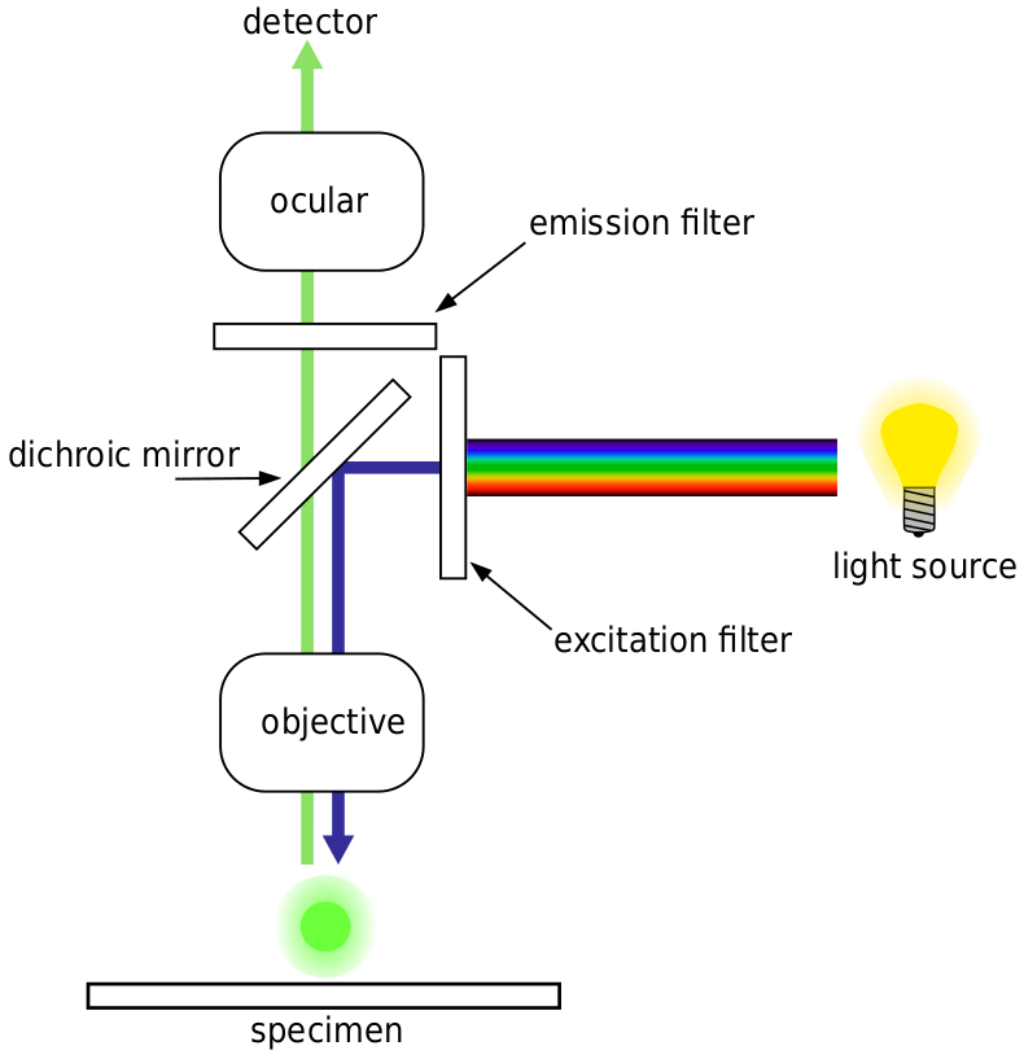
Epifluorescence microscopy

The majority of fluorescence microscopes, especially those used in the life sciences, are of the epifluorescence design shown in the diagram. Light of the excitation wavelength illuminates the specimen through the objective lens. The fluorescence emitted by the specimen is focused to the detector by the same objective that is used for the excitation which for greater resolution will need objective lens with higher numerical aperture. Since most of the excitation light is transmitted through the specimen, only reflected excitatory light reaches the objective together with the emitted light and the epifluorescence method therefore gives a high signal-to-noise ratio. The dichroic beamsplitter acts as a wavelength specific filter, transmitting fluoresced light through to the eyepiece or detector, but reflecting any remaining excitation light back towards the source.

Light sources

Fluorescence microscopy requires intense, near-monochromatic, illumination which some widespread light sources, like halogen lamps cannot provide. Four main types of light source are used, including xenon arc lamps or mercury-vapor lamps with an excitation filter, lasers, supercontinuum sources, and high-power LEDs. Lasers are most widely used for more complex fluorescence microscopy techniques like confocal microscopy and total internal reflection fluorescence microscopy while xenon lamps, and mercury lamps, and LEDs with a dichroic excitation filter are commonly used for widefield epifluorescence microscopes. By placing two microlens arrays into the illumination path of a widefield epifluorescence microscope,[4] highly uniform illumination with a coefficient of variation of 1-2% can be achieved.

Schematic of a fluorescence microscope.



What is fluorescent staining

A **staining** procedure that uses a **fluorescent** dye or substance that combines selectively with certain tissue components and then fluoresces on irradiation with ultraviolet or violet-blue light.

A **fluorophore** is a **fluorescent** chemical compound that can re-emit light upon light excitation. ... **Fluorophores** are notably used to **stain** tissues, cells, or materials in a variety of analytical methods, i.e., **fluorescent** imaging and spectroscopy.

Sample preparation

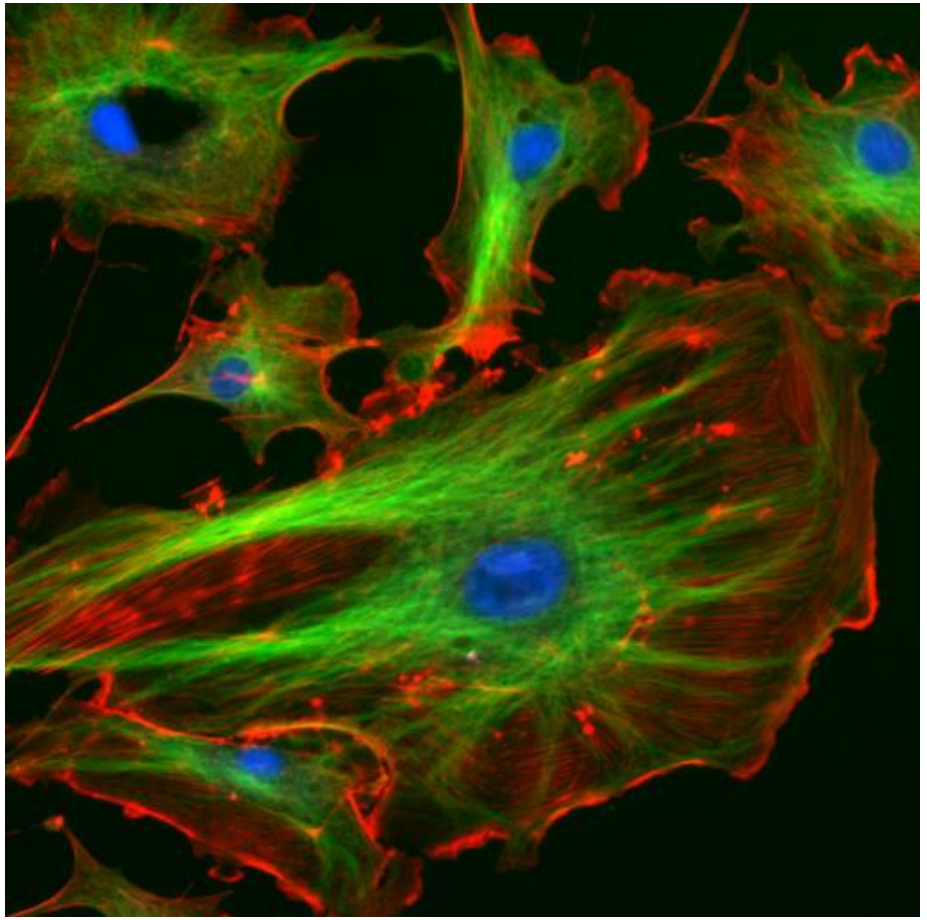
- In order for a sample to be suitable for fluorescence microscopy it must be fluorescent. There are several methods of creating a fluorescent sample; the main techniques are labelling with fluorescent stains or, in the case of biological samples, expression of a fluorescent protein. Alternatively the intrinsic fluorescence of a sample (i.e., autofluorescence) can be used.[1] In the life sciences fluorescence microscopy is a powerful tool which allows the specific and sensitive staining of a specimen in order to detect the distribution of proteins or other molecules of interest. As a result, there is a diverse range of techniques for fluorescent staining of biological samples.

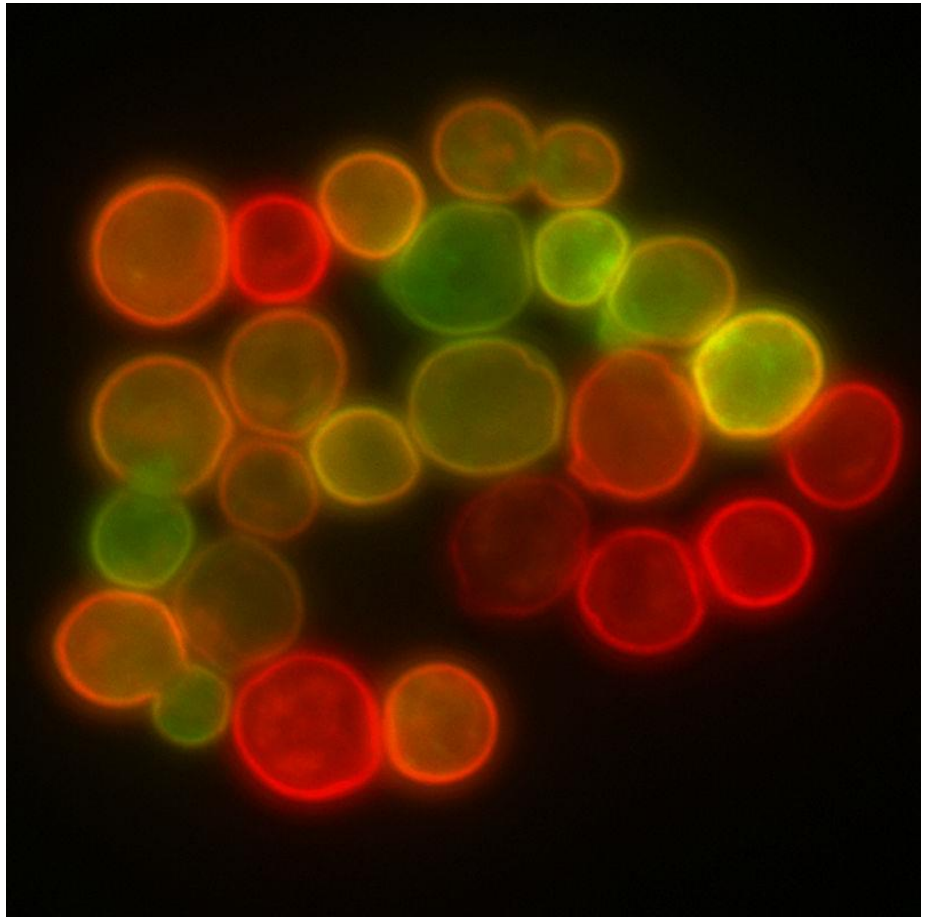
Sub-diffraction techniques

- Integrated correlative microscopy combines a fluorescence microscope with an electron microscope. This allows one to visualize ultrastructure and contextual information with the electron microscope while using the data from the fluorescence microscope as a labelling tool.[7]
- The first technique to really achieve a sub-diffraction resolution was STED microscopy, proposed in 1994. This method and all techniques following the RESOLFT concept rely on a strong non-linear interaction between light and fluorescing molecules. The molecules are driven strongly between distinguishable molecular states at each specific location, so that finally light can be emitted at only a small fraction of space, hence an increased resolution.
- As well in the 1990s another super resolution microscopy method based on wide field microscopy has been developed. Substantially improved size resolution of cellular nanostructures stained with a fluorescent marker was achieved by development of SPDM (special precise distance microscopy) localization microscopy and the structured laser illumination (spatially modulated illumination, SMI). Combining the principle of SPDM with SMI resulted in the development of the Vertico SMI microscope.[9][10] Single molecule detection of normal blinking fluorescent dyes like green fluorescent protein (GFP) can be achieved by using a further development of SPDM the so-called SPDMphymod technology which makes it possible to detect and count two different fluorescent molecule types at the molecular level (this technology is referred to as two-color localization microscopy or 2CLM).

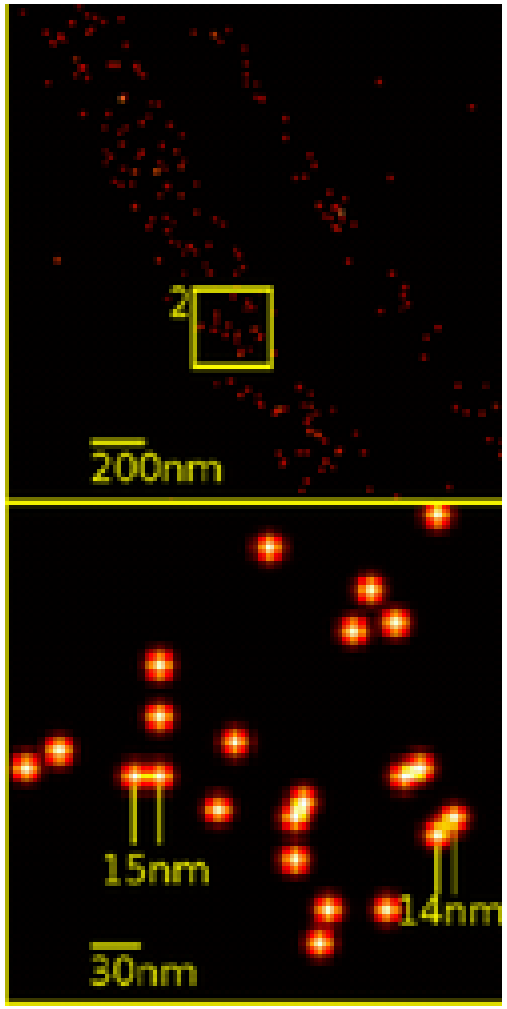
Fluorescence micrograph gallery

Endothelial cells under the microscope. Nuclei are stained blue with DAPI, microtubules are marked green by an antibody bound to FITC





Yeast Cell membrane visualization



Cancer cell detection in human