# nanoFLeye™

## Optical Microscopy beyond the Diffraction Limit


nanoFLeye

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## The Way to a Deeper Insight into the Cell

nanoFLeye™ (nanoFluorescenceEye) is the innovative reply on demands and needs in the field of super-resolving optical imaging based on the localization microscopy technique SPDM (Spectral Precision Distance Microscopy).

nanoFLeye™ excels with

- Super-Resolution Imaging based on SPDM
- High Stability
- High Flexibility
- Clear and User-Friendly Interface
- Possibility of Remote Control
- TIRF and Widefield Mode
- ✓ Choose up to Four Different Excitation Wavelengths Suitable to your Desired Dyes
- ✓ Choose between Different Microscope Objectives
- Easily Programmable Measurement Sequences and Automated Data Analysis

# Special Feature

nanoFLeye™ is equipped with the pioneering Recon*Flex*™ camera developed by Surface Concept GmbH making localization microscopy substantially easier and faster.
Up to now, Localization Microscopy in general has been characterized by recording an image stack comprising tens of thousands of images and time-consuming post-processing of the data to determine the position of each molecule (reconstruction).
Recon*Flex*™ facilitates an on-the-fly data analysis for localization microscopy providing super-resolved images in real-time.

It offers ultra-high flexibility in terms of its different modes:

- Normal Camera Mode: For Live Imaging, adjusting the Microscope and recording Image Stacks
- Reconstruction Mode: The Localization of the Molecules is being determined by the Camera itself

Both modes can also be applied simultaneously.

## Advantages

- ✓ Enormous Reduction in Data Transfer, Time and Disk Space
- ✓ Select Fiducial Markers prior to the Measurement to correct Possible Drifts in Real-Time
- ✓ The User retains Full Control over the Algorithms

Provided with Recon*Flex*™, nanoFLeye™ opens up a new door to real-time, easy and user-friendly super-resolving localization microscopy.

# Method

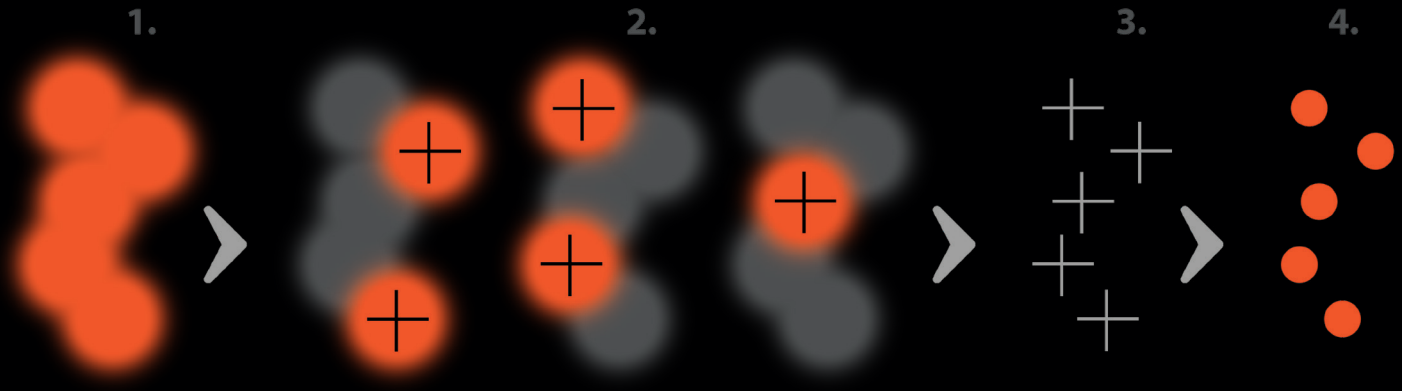
Conventional fluorescence microscopy is a versatile tool to perform functional cell biology analysis. Fluorophores are being coupled to antibodies which bind to their corresponding proteins in the cell. By analyzing the fluorescence signals in the microscopy image, one can get insight to the distribution of the chosen proteins inside the cell.

However, in terms of nano-science and detailed insight into biological processes on a molecular level, conventional fluorescence microscopy is stretched to its limit.

In an epifluorescence microscope the lateral resolution is determined by the diffraction limit, i.e. one cannot distinguish two molecules with a distance less than ~200nm from each other. In a confocal setup it is possible to improve the resolution slightly, but not sufficient to detect single molecules.

## Localization Microscopy

Spectral Precision Distance Microscopy (SPDM):
Spectral features are used to achieve optical isolation




1. In conventional Fluorescence Microscopy the Full-Width-at-Half-Maximum (FWHM) of the Point-Spread-Function (PSF) is >200nm. Signals of adjacent dyes overlap, therefore single molecules cannot be resolved

2. Using SPDM, randomly activated dyes are „optically isolated", i.e. no overlap of the signal of adjacent molecules can occur

3. The locations of the optically isolated fluorophores are determined by the localization algorithm with a precision down to 20nm

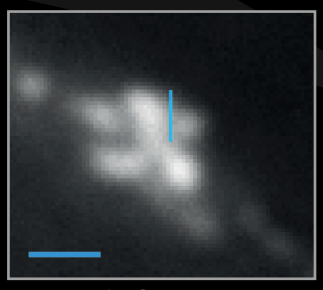
4. All localizations found in a stack of usually ten thousands of images are displayed in a single reconstructed super-resolved image

# Application Examples

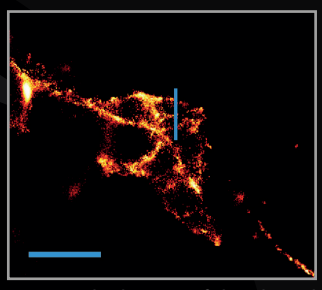
nanoFLeye™ allows to reveal structures well below the Abbe limit being of interest for biomedical as well as material science applications. The inverted setup of nanoFLeye™ and its flexible sample holder allows for the investigation of a huge variety of sample configurations.

## ✓ Protein-Polymers in a Cell Culture Specimen

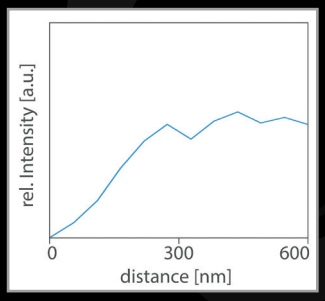
- The images show a segment of fluorescence-labeled microtubules in a cell culture (HeLa-cells)
- nanoFLeye™ uncovers sub-structures within the polymer configuration
- The corresponding linescans show the intensity distribution along the implied line in the images



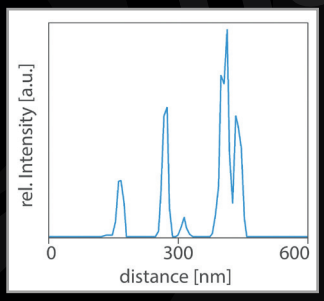
Conventional epifluorescent microscopy image of Alexa647-labeled microtubules of HeLa-cells; scale bar 1µm.



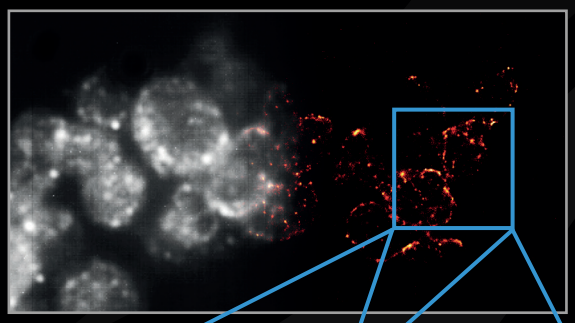
Super-resolved image of the identical sample position recorded by nanoFleye™; scale bar 1µm.




Linescan (vertical line in the upper image) of the epifluorescent image.



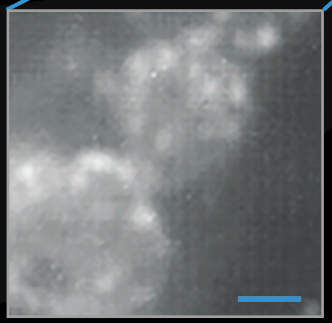

Linescan (vertical line in the upper image) of the super-resolved image.



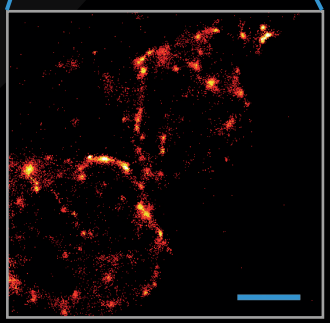
Alexa680-labeled human platelets (HuPLTs, PF4, A680, native)
sample preparation courtesy of: Dr. M. Schmitt, LMU München
left: conventional epifluorescent microscopy image
right: super-resolved image recorded by nanoFLeye™

## ✓ Human Thrombocytes

- The images show the PF4 distribution inside the platelets
- nanoFLeye™ uncovers the number of the labeled cytokines as well as their formation in the platelets



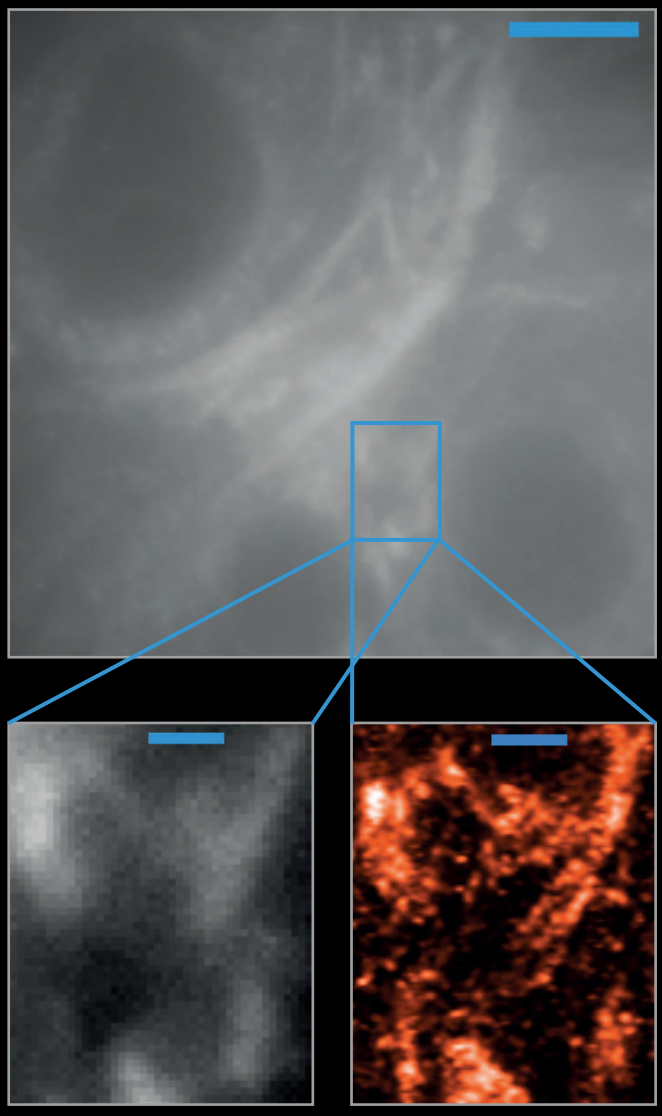
Conventional epifluorescent microscopy image of Alexa680-labeled HuPLTs; scale bar 1µm.



Super-resolved image of the identical sample position recorded by nanoFLeye™; scale bar 1µm.

# Protein-Polymers in a Tissue Sample

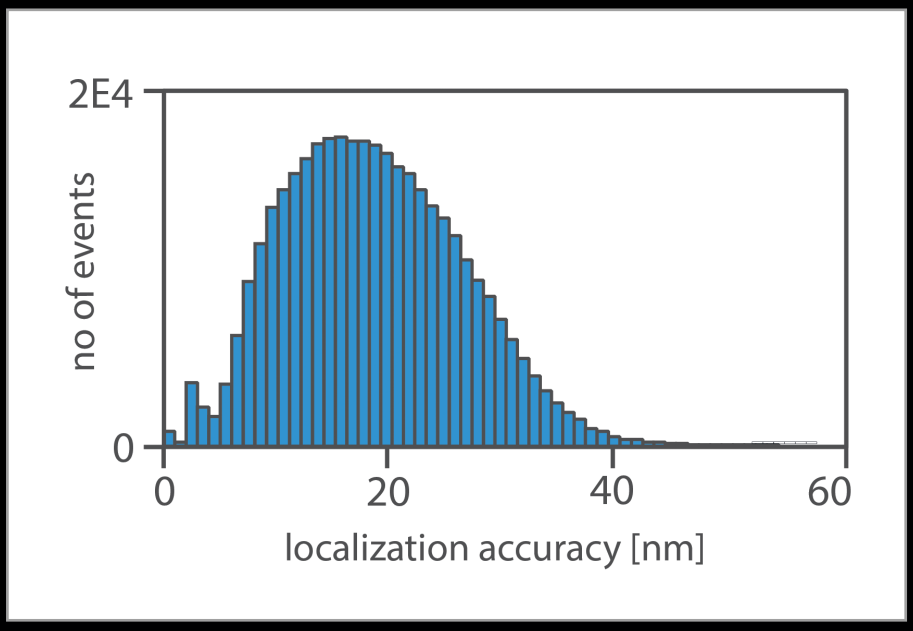
- The images show a segment of fluorescence-labeled microtubules in 30µm thick rat brain tissue
- The histogram shows the distribution of the localization accuracy of the detected events. A mean localization accuracy of 18nm could be achieved



Epifluorescence (30µm x 30µm); scale bar 5µm
Immunofluorescence micrographs of a 30µm thick rat brain tissue
ICV 10 Hippocampus (DG)
Staining: microtubules (Alexa647)

Epifluorecence; scale bar 1µm.

Super-resolved image of the identical section; scale bar 1µm.




Mean localization accuracy: 18nm

Sample preparation courtesy of:
Dr. Sebastian Bauer
Leiter AG Translationale Epileptologie
Epilepsiezentrum Frankfurt Rhein-Main
Klinik für Neurologie
Goethe Universität Frankfurt

www.nanofleye.com


Surface Concept GmbH

Am Sägewerk 23a
55124 Mainz
Germany

phone: +49 6131 62716 0
fax: +49 6131 62716 29
email: info@nanofleye.com
web: www.nanofleye.com

email:

SURFACE CONCEPT