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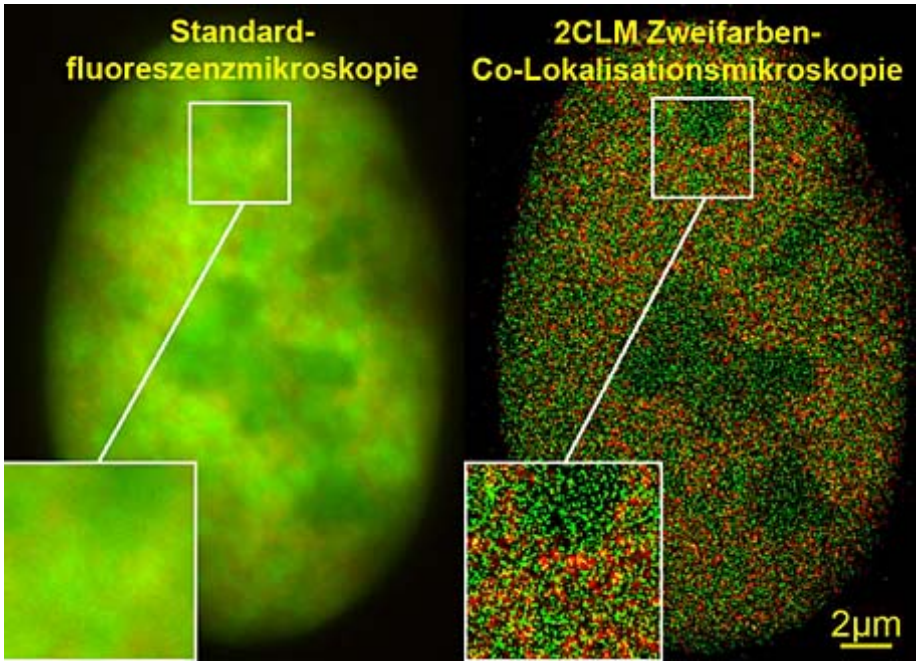
Investigation of living cellular complexes with the fastest nano light microscope in the world

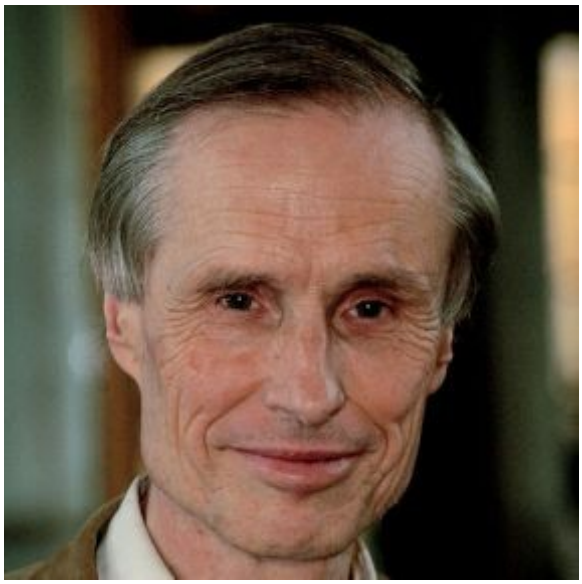
Prof. Dr. Dr. Christoph Cremer of the Kirchhoff Institute of Physics at the University of Heidelberg and his team have developed a high-performance microscope. This cutting edge microscope enables scientists to investigate molecular details of several cells simultaneously using standard fluorescent dyes such as green fluorescent protein (GFP). The world's fastest nano light microscope uses a new localisation microscope method known as spectral precision distance microscopy (SPDM) for the three-dimensional analysis of cells.



A bone cancer cell visualized by normal fluorescence microscopy (left) and by 2-colour co-localisation microscopy using the spectral presicion distance microscope: 120.000 molecules can be identified.

Fluorescent dyes are the key to high-resolution nano light microscopy. To localise adjacent molecules in the "twilight" of the cell and make them independently visible, a temporally convertable light signal is required. So far, signals like this have been supplied by special fluorescent molecules that can be switched on and off by means of light. Many "conventional" dyes can be switched in this way, as long as certain photophysical conditions are fulfilled. These conditions can be achieved via the so-called "reversible photobleaching" of the fluorescent dyes. According to Professor Cremer there are millions of gene constructs with dyes from the GFP group available in biomedical laboratories all over

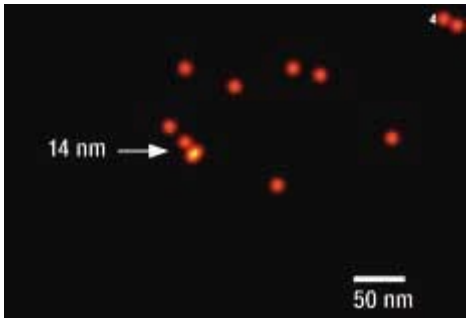
the world. They could be put into immediate use for this new kind of localisation microscopy.



Prof. Dr. Dr. Christoph Cremer, Kirchhoff Institute of Physics, University of Heidelberg

With their SPDM localisation microscopy Christoph Cremer and his team have enhanced the potential of their nanoscope Vertico SMI, which combines far-field light microscopy with extraordinary nanometric accuracy. With the use of visible laser light, this means that not only large cell areas but also cell complexes can be studied two-dimensionally with a spatial resolution of anything down to the range of 10 nanometres. High data acquisition speed makes it possible for the first time to achieve nanoscale 3D recordings of entire, living cells with a resolution of up to 40 nanometres in real time.

High-density molecule visibility is important in recognising agglomerations of molecules as sites of increased activity. With the nanoscopic technique developed by Professor Cremer, several million individual molecules can be recorded within 30 seconds combining up to 2,000 individual images, which is sufficient for a total picture. This high data-acquisition speed even makes it possible to observe adjacent molecules in nanostructures in living cells. With the extension of SPDM to multicolour co-localisation microscopy, two different protein types can be labelled by conventional fluorescent molecules, for example from the GFP group, and detected with different light wavelengths. This enables the researchers to obtain more precise information on potential interactions of individual localised protein molecules in nanostructures than is the case with the conventional FRET method (fluorescence resonance energy transfer).



Optical resolution of Spectral Precision Distance Microscopy

The combination of the nanoscope with localisation microscopy can be used in pharmaceutical, cell-biological, medical and biophysical research, i.e., wherever molecular imaging is required on a cellular level. At present, the combination is being employed for cooperative research projects in pharmacology, cardiology and adult stem-cell research. Further potential uses are studies on the interaction of viruses and cells where the relevant structures are too small for detection by conventional light microscopes, research on age-related neurological degeneration and cancer research. The determination of the position of individual molecules can furnish new insights on the regulation and activities of genes and proteins or on changes in cellular nanostructures. Other potential uses are in materials research, the quality control of nanocoatings, the assessment of damage resulting from cracks or the detection of infinitesimal amounts of material in ecological and environmental research.

The commercialisation of the patent folio for the nanoscopy technology and its applications is managed by the Technology Licence Office (Technologie-Lizenz-Büro, TLB) in Karlsruhe.

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