# From a Microscope to a Nanoscope

**Piezoelectric solutions for super-resolution microscopy**

**Theresa Kuntze and Rudyard Urtecho**

**In conventional confocal microscopy, the resolution is limited to half of the wavelength of light. For blue light, this is 200 nm. This resolution limit was postulated in 1873 by Ernst Abbe, and for decades it was considered that this limit could not be overcome. The reason lies in the diffraction which occurs at two clearly differentiated objects and makes both objects appear blurred together as one. Using the stimulated emission depletion method (STED), developed by Stefan Hell, a higher resolution could be achieved which was far below the previously mentioned limit. The microscope became a nanoscope and altered long-standing conceptions about the resolving powers in light microscopy.**

**Theresa Kuntze**, Business Administration (M.A.), **Rudyard Urtecho**, Engineer, piezosystem jena GmbH

In the STED method, a specimen is excited by a diffraction-limited pulsed ultra-fast laser source (10 to 300 picoseconds) reaching a fluorescent state. The specimen is phase delayed being overlaid by a depleting laser source. This second laser pulse (STED-beam) takes advantage of the subsequently molecular relaxation and of the interferences of coherent light. This produces radial depletion zones and gives the beam a doughnut-shape. As a result the specimen is not excited, except in the focal spot, which is in the central area of the doughnut-shaped beam. The remaining spot can be reduced in circumferences to achieve a higher resolution. The remaining fluorizing spot is being scanned over the complete sample to obtain the final image. Molecular processes can be inspected *in vivo* and can also be investigated and tracked in real time.

In contrary to other microscopy methods, the specimen is not being destroyed, and its molecular constitution remains preserved because the microscope uses harmless visible light. Studying living cells is an interesting and demanding subject in the nanoscopy (**Fig. 1**). The non-invasiveness is one of the biggest advantages of STED microscopy. 3D-images can be obtained of samples like proteins down to a resolution of nanometers, almost the real size of the proteins themselves. Another big advantage that contributes to the increasing popularity of the STED method is its speed. With 80 fps for a range of 60 to 80 nanometres, STED is the fastest microscopy method. The method works with every fluorescent molecule, and the image processing is not complicated.

The scanning processes in STED microscopy set-ups have to be very fast to meet those special requirements. Piezoelectric stages, tilting systems and objective positioners from piezosystem jena GmbH are suitable instruments which are indispensable in these STED set-ups. The functionality of these systems is based on the inverse piezoelectrical effect, first discovered by the Curie brothers in 1880. The inverse piezoelectrical effect states that an

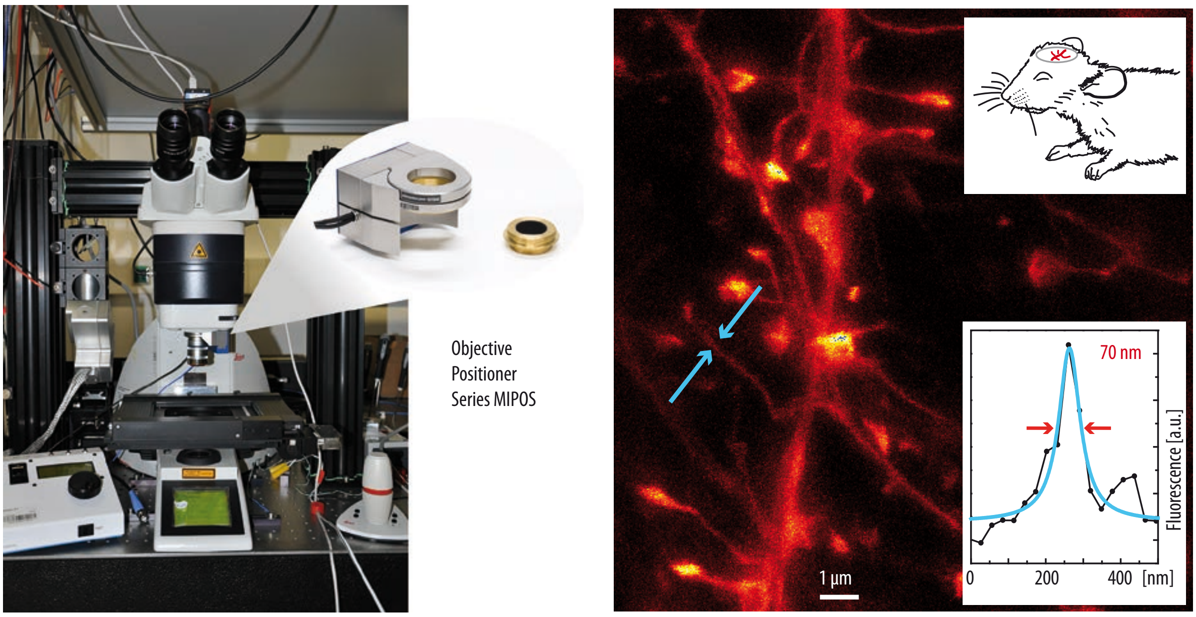




**Fig. 1** Super-resolution imaging of a neuronal cell in a living mouse brain: Microscope positioner MIPOS 100 CAP (left) and piezo amplifier NV120CLE (right).

 

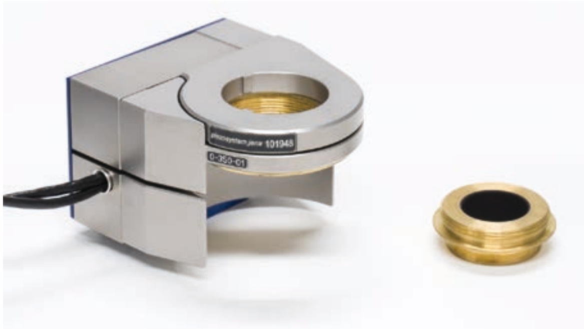


**Fig. 2** MIPOS 500: Microscope objective positioner


**Fig. 3** d-Drivepro: New digital piezo controller, with new features like vector and arbitrary generator, storable functions and a voltage noise RMS ≤ 0.15 mV @500 Hz

applied voltage to a piezoelectrical material causes a change in the dimensions of the material. This change in dimension generates a motion. Actuators from piezosystem jena GmbH use a special ceramic consisting of lead zirconate titanate (PZT) to achieve motions down to the nanometer range. The company also offers actuators for applications such as laser technologies, semiconductor industry or life science.

The objective positioners of the MIPOS series, the mirror tilting system series PSH and the *z*-axis scanner PZ300AP are examples for products that can be used for STED microscopy. These elements can move to pre-defined positions very fast with a high precision in the nanometer range (**Fig. 2**).

The MIPOS series offers microscope objective positioners for standard and special objectives for all major microscope brands, e.g., Carl Zeiss, Nikon, Leica, Mitutoyo, just to mention a few. They can be used for single objectives or to move the complete objective revolver. The PSH series includes large tilting stages for mirrors and other optics that can be tilted and positioned with frequencies up to the kilohertz range.

With the PZ300 AP piezosystem jena GmbH developed a special product for microscopy applications. With a very small overall height and a very big aperture, the PZ300 AP accommodates standard microscopy accessories like incubators and multiwells sample holders. The stage has been designed to fit into Märzhauser and Prior XY-microscope standard stages.

All systems can be controlled by proprietary amplifiers. You can easily find one that meets your requirements within a wide range of products and a variety of features such as: high current, integrated function generators with storage function, filters (notch, low pass), triggering and interfaces (USB, RS232, Ethernet; **Fig. 3**).