

## Stabilization of focus in high resolution microscopy (P-1260)

### Key Facts

- monitoring and correcting relative position without disturbing the use of the microscope objective
- test beam directed onto a reflective surface connected to the sample/objective evaluated
- necessary for ultra-high resolution microscopy with increasing spatial resolution

### Background

The relative position of a microscope objective with regard to a sample is essential in imaging the sample using the microscope objective. If relative position varies unnoticed, the plane of the sample imaged using the microscope objective will also vary unnoticed. As a consequence, a series of images which are intended show the same sample location at consecutive points in time, for example, in fact show the sample at different focal planes or laterally shifted. Further, with any laser scanning microscopy requiring a longer period of time for even imaging one plane of the sample once, a distorted image of the sample will be generated, if the relative position of the microscope objective with regard to the sample varies.

With increasing spatial resolution achieved by ultra-high resolution microscopy techniques like STED the requirements to be fulfilled in keeping a fixed relative position of the microscope objective with regard to the sample increases further. The increased spatial resolution requires an equally increased stability of the relative position of the microscope objective with regard to the sample.

### Technology

The invention describes a method in which monitoring and correcting of a relative position of a microscope objective with regard to a sample is easily realized and does not disturb the primary use of the microscope objective. The procedure is carried out using a test beam directed onto a reflective surface connected to the sample and on the other hand using a test beam directed onto a reflective surface connected to the objective. Both results are registered, evaluated and used for correction.

### Development Stage

A prototype has been successfully tested in experiments, see figure.

### Applications and Commercial Opportunity

The invention can be used in high resolution microscopy such e.g. in a STED microscope.

### Inventor

The inventor is Johann Engelhardt from DKFZ.

### Intellectual Property

Priority [EP 3287829](#) dated Aug 25, 2016 and PCT application [WO 2018036877](#).

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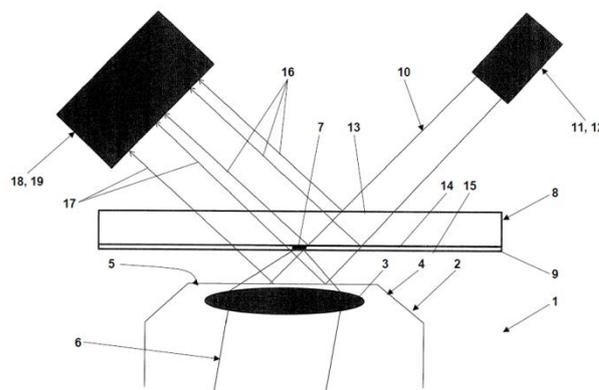


Fig. 1 depicts a microscope 1 including a microscope objective 2. The microscope objective 2 comprises a front lens 3 and a frame 4 holding the front lens 3 and having a metallic surface 5. In the operation of the microscope 1, a beam of excitation light 6 is focused into a sample 7 by means of the front lens 3. The focus of the beam of excitation light 6 is moved along a focal plane of the front lens 3 by means of a scanning device of the microscope 1, which is not depicted here. The focal plane of the microscope objective 2 only remains fixed with regard to the sample 7, if relative positions of the sample 7 and of a microscope slide 8 and a cover slip 9 between which the sample 7 is fixed remain fixed with regard to the microscope objective 2.