Device for stabilizing images in high resolution microscopes (P-743)

Keywords

- Image stabilization against externally induced oscillations and drift effects
- Application in high resolution microscopes (fluorescence, Raman, STED, localization)

Abstract
The technology specifies a novel device which allows to stabilize images of optical microscopes with high resolutions against externally induced oscillations and drift effects. In particular for optical microscopes, which achieve a higher resolution, it is important to ensure a high mechanical stability of the structure of microscope for a good image quality. It improves the performance of instruments such as laser scanners, fluorescence microscopes, Raman systems, STED- or localization-microscopes.

Background
The mechanical properties of optical microscopes are usually constructed for a diffraction limited resolution of 200-500 nm. Therefore a position stability of several 10 nm against thermal drifts stability and externally induced oscillations for reproducible images are sufficient. However, for microscopes achieving a higher resolution, the requirements for mechanical stability of the microscope constructions increase significantly. This applies in particular to STED microscopy, where the object has to be held/guided stably in nanometer range within the object focus. At other microscope types, such as PALM, STORM etc., using localisation of individual fluorescence molecules, long recording times are required. So there especially techniques for drift reduction are necessary.
At present the sample mounting and guidance of optical microscopes are relatively soft and flexible resulting with external excitation in unwanted oscillation amplitudes in the order of 10-100 nm. The object of interest is usually coupled over distance of many centimetres by various materials to the microscope and thus to the focus of the micro-objective. The whole arrangement is susceptible to thermal drift, which is typically in the order of 1nm/sec. This drift may be even stronger shortly after switching on and off devices of the microscope.

Technology
In order to assure a high images quality without oscillation and drift effects a rigorous reduction of the distance between object and micro-objective head is proposed. One basic exemplary embodiment proposes to directly attach the object to the objective head instead to the microscope body. In fact this is an option in high resolution microscopy since the objects usually are small and lightweight and the micro-objectives are rarely changed during an experiment. The guidance of the object may be done by small, rigid and short-stroke piezo actuators.

Advantages
The novel construction of the object directly attached to the objective head instead of to the microscope body improves the following critical issues present in microscopes:

1. Less impact of external oscillations to the focussing unit: At an inverse microscope the focussing unit usually is at the object revolver attachment and at an upright microscope it is in the stage attachment. Both, object and revolver attachment as well as table construction typically have massive designs and are therefore susceptible to external oscillations.
2. Protection against collision of the objective: Micro-objectives typically exhibit a collision protection mechanism which causes mechanical tolerances between the focus and the objective turret.
3. Reduction of the distance between object and focal plane: Due to a shorter distance between object and focal plane the mechanical pathways

are minimized decreasing the thermal impact and drift effects. The result can be even more improved by carefully selecting the particular material involved. The proposed device for image stabilization in microscopes can be mounted to all available standard microscopes.
Due to the new and simple object holder technique the high-resolution microscopes can obtain almost oscillation- and drift-free images under regular laboratory conditions. This fact is in particular of advantage for long time observations very often disturbed by drift effects or oscillations. The improved image recording spares the requirement of time consuming and tedious post processing of image data.

Commercial Opportunity
This technology is able to improve in particular
(1) Fluorescence microscopes, Laserscanners, Raman Systems, STED and Localization Microscopes
(2) Optical Accessories and Devices: Flowzytometer, Mikroarrayreader, Spectrometer

Development Stage
The device for stabilisation of images in high resolution microscopes is successfully applied in one of the research STED microscopes as well as in a GSDim microscope at the DKFZ. It is also already in use at GSDim microscopes at the MAX-Planck-Institute in Göttingen.

Intellectual Property
We filed August 11, 2009 a German utility patent application “Anordnung zur Abbildungsstabilisierung”, which was issued as Gebrauchsmuster DE202009010772.

Inventors
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Further Information
No other public information is currently available (publication in preparation), but further information (speaking with the inventor) is available under a signed Confidential Disclosure Agreement (CDA).

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Figure 1:

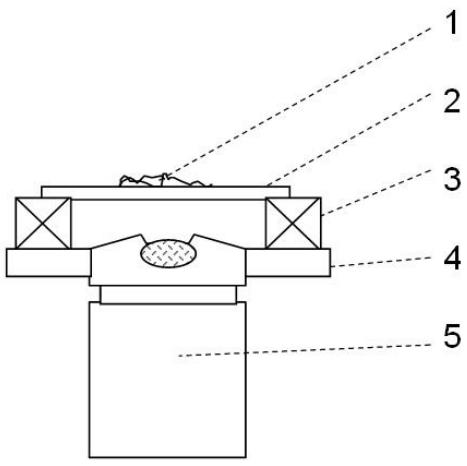

Figure legend 1: Schematic design of a device for image stabilisation. A base plate (4) is mounted and fixed at the head of a microscope objective (5). Onto the base plate (4) an adjustable object holder (2) for positioning of the object (1) mounted. For focussing of the object (1) screws and/or piezos (3) are installed in order to position the object holding plate axial relatively to the base plate (4).

Figure 2:

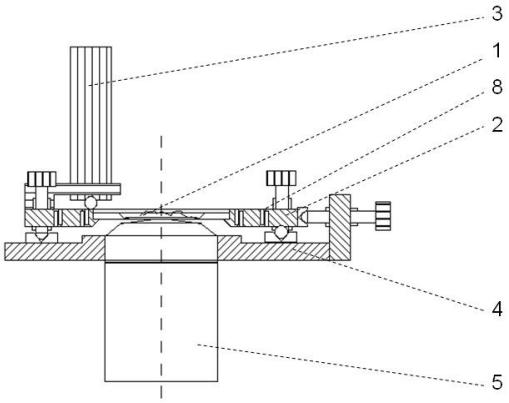

Figure legend 2: Schematic design of an execution example of a device for image stabilisation
The object holder consists out of a base plate (4), which is bind/clamp to the objective head and a objective holding plate (objective leader). It is possible to adjust the objective leader via fine screws in height above the objective for gross focussing. Using these fine screws the object can also be tilt over in addition relatively to the focal plane. For lateral adjustment the object leader is mounted with holding screws and sliding components on the base plate. Springs and magnets are used for prestressing of the screws (here not shown).