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A Versatile 2π Tilting Device for Conventional Light – and Confocal Laser Scanning Microscopy

J. Bradl[1], B. Schneider[1], B. Rinke[1], E. H. K. Stelzer[2], M. Durm[1], M. Hausmann[1] and C. Cremer[1]
[1]Institute of Applied Physics, University of Heidelberg, D-69120 Heidelberg, F.R.G.
[2]Light Microscopy Group, European Molecular Biology Laboratory, D-69117, Heidelberg, F.R.G.

A tilting device for full rotation (0-360°) of biological specimens has been built on the basis of the 3D-system described by Bradl et al. 1992 and Bradl et al. 1994. The main feature of this device is a quartz glass capillary (diameter 0.2mm, length 75mm) containing cells or cell nuclei of interest. The axis of rotation is perpendicular to the optical axis, and can be oriented on the microscope stage parallel to the x- or y-axis. Different optical and imaging effects (e.g. refraction index matching, object position in the capillary) were analyzed by means of test objects and computer simulation.

The three-dimensional image-forming properties of fluorescent beads in the capillary system were experimentally examined using an inverse confocal laser scanning microscope. The results were compared to measurements obtained from the same microscope with the standard stage for plane slides with cover glasses. The measured point spread function suggested that in spite of the a berration effects, the optical arrangement used allows a gain in the 3D resolution by tilting the object.

We have adapted this device to a Zeiss Standard 25 microscope, equipped with a step motor controlled xy-table with a z-focus unit. The specimen images are registered by a cooled black/white CCD camera CF 8 RCC. All components are controlled by a 80486/50 MHz MS-DOS computer with a frame grabber board under Windows 3.1, with the image acquisition and analyzing software Optimas. With this setup, 3D imaging after optical sectioning of objects in the capillary was performed. To get an optimal perspective of the objects, the capillary can be tilted automatically under any angle desired. For example, this has the advantage to acquire optical sections of an object, rotate this object by 90°, and register an additional set of optical sections. If both sets of sections are merged in an appropriate way, an improved spatial resolution can be obtained (e.g. as described by Shaw et al. 1989) (equal resolution in all three directions x, y, z). Rotating by further 90°C may also be helpful to discriminate shading effects of high absorbing regions in the object.

The device can also be adapted to other conventional microscopes and to confocal laser-scanning microscopes (CLSM). We have been able to combine the tilting device successfully with the following microscopes:

Leitz Orthoplan, Leica TCS 4D, Zeiss Axiophot, Zeiss LSM 10 and 310, Zeiss Standard 25, and with the inverse CLSM at the EMBL described by E.H.K. Stelzer, 1990) In those cases where an automated xy-movement of the microscope table and automated z-focusing were not available, a modification of the basic device for manual tilting was used. Results from cell nuclei with chromosome regions labeled by fluorescence in situ hybridization show that the tilting approach is suitable to improve 3D distance measurements.

For visual analysis, optical sections of two fluorescent beads were recorded by CLSM. One bead of 5.85 μm diameter showed a light microscopically observable internal structure. The other, smaller one had a diameter of 3 μm. At tilting angles of 0°, 10°, 20°,..., 360° the image planes located centrally in the larger bead were registered. After alignment, the entire series was animated on a 80486/50 MHz PC (under the Linux operating system). The online visualization ("movie") of rotating objects indicated the feasibility of this procedure for 3D inspection.

A modification of the tilting device was performed applying glass-fibres instead of capillaries. This has the advantage that the biological objects can be fixed onto the fibre instead of sucking in a suspension into the capillary. The fibres can easily be manufactured with different diameters, and

glass types of different Prefractive indices can be used for appropriate matching to different buffer solutions. During reparation, the biological objects are mounted into a specially designed device which has the same dimensions as a microscope slide. Standard preparation techniques can be applied so that *in situ* hybridization has not to be performed in su-spension. If by any reason the refractive index of the glass-fibre and the buffer medium cannot be matched in an accurate way, the object can be tilted until it is located at its nearest position to the objective. From there, 2D- as well as 3D- images of the object can be acquired under rotation angles of ±40° without major image distortions caused by the refractive index mismatch. It is possible to work either with or without a standard coverslip, which is located between the objective and the glass-fibre. With the coverslip, the immersion oil for different objectives can be used while the object can be mounted into buffer solutions in a similar wayas in the conventional microscopical setup, We thank Dr. T. Cremer and Dr. P. Lichter Heidelberg, for access to the Leica TCS 4D, the Zeiss Axiophot, and the Zeiss LSM, respectively.

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