Observing the 3rd Dimension

A Simple Way to Upgrade Common Microscopes for Sample Rotation

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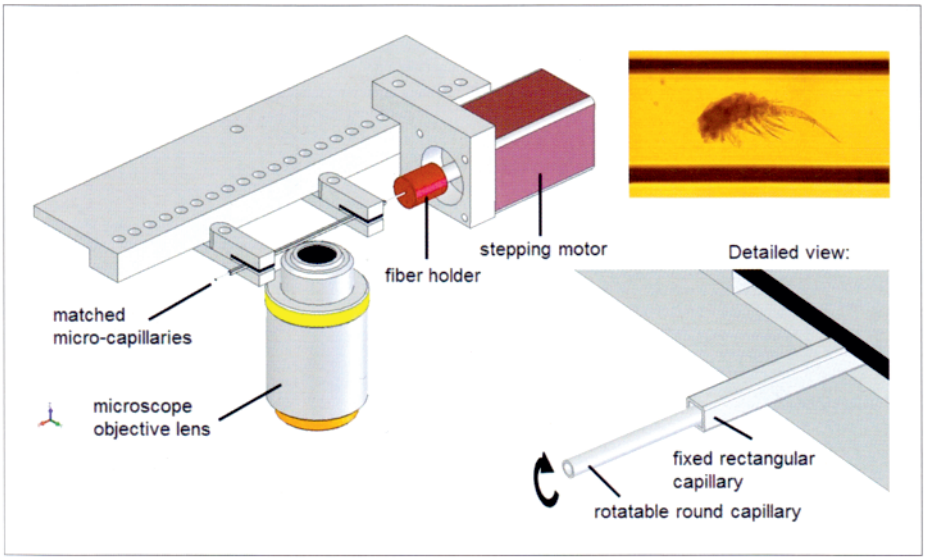

Fig. 1: Device for rotation of three-dimensional samples to be fixed on a positioning stage of a microscope.

In microscopy, samples are usually located on glass slides or in specific dishes and may, therefore, only be observed from one direction. This is especially unfavorable for three-dimensional samples where larger or more complex specimens or structures are to be studied. For that reason we developed a modular device allowing longitudinal axial rotation of the specimen up to 360°, independent of the sample size. It can be easily adapted to a variety of common microscopes for gaining deeper insights into the sample.

Sample rotation in microscopy is getting into the focus. Within the last years some efforts were made to vary the perspective of sample illumination and/or observation (see for example Bradl *et al.* [1], Staier *et al.* [2] and Heintzmann and Cremer [3] for single cells and nuclei and Huisken and Stainier [4] for embryonal organisms).

Our approach was to design a rotation device which (a) can be used together with a wide range of commercially available microscopes [5], (b) extends the possibilities of various 3D microscopy techniques, e.g. light sheet fluorescence microscopy, confocal microscopy and structured illumination microscopy and (c) is suitable for specimens with a size of a few micrometers up to several millimeters. The whole rotation device is mounted on a holder that is inserted into the positioning stage of a microscope. It is helpful for recording images or stacks of images from any desired direction – whether for subsequent multi-view reconstruction or for just having a better chance to pick out the most interesting part of the sample for observation or imaging [6].

Sample Holding

The crucial part for sample rotation is the way of sample holding. The sample has to be freely rotatable providing optimum optical access from every direction. In the presented setup (fig. 1) samples are located in round capillaries coupled to a stepping motor. The round capillary is placed in another rectangular capillary which is fixed. The outer rectangular capillary is made of borosilicate glass (n = 1.47) and its plane surfaces assure optimum illumination and image quality.

Importance of Index Matching

To prevent optical distortion in illumination and detection, the refractive index of the inner round capillary has to match the refractive index of the immersion fluid which fills the space between the outer and the inner capillary as well as the medium surrounding the sample.

For fixed samples: If the samples are fixed in glycerol, round capillaries made of borosilicate glass are a good choice since the refractive indices are almost equal. In this case glycerol should be chosen as immersion fluid, too.

For living samples: Samples located in an aqueous medium like agarose are best hold in round capillaries made of fluorinated ethylene propylene (FEP, n = 1.34) [7]. In this case water is used as an immersion fluid between the two capillaries. Alternatively, the FEP capillary may be used without a surrounding rectangular capillary when observing the sample with a water immersion objective lens. In that case, it is sufficient to couple the FEP capillary and the lens with a drop of water and to use the rectangular capillary only as a retainer at the open end of the FEP capillary.

A wide range of sizes for rectangular and round capillaries are commercially available (e.g. VitroTubes by VitroCom Inc., USA). Thus, the user is free to choose the size of the capillary matching the size of the specimen to be observed. We commonly use outer rectangular capillaries with an inner cross section of 600 µm × 600 µm or 900 µm × 900 µm with a wall thickness of 120 µm or 180 µm [8] in combination with inner round capillaries with an outer diameter of 550 µm or 870 µm with a wall thickness of 75 µm or 85 µm. When observation with illumination wavelengths in the UV range is desired, there is also the possibility to use capillaries made of quartz glass instead of borosilicate glass. Suitable FEP capillaries are also commercially available from different suppliers (e.g. Zeus, Ireland).

Quick Sample Uptake

Sample uptake by the capillary is very easy to perform using the capillary forces, if the sample is located in a liquid. If the sample is located in a gel like agarose it can be taken up by plunging the capillary directly into the agarose. Alternatively, in both cases the capillary can be attached to any kind of syringe or pump to soak in the sample.

Observing the Sample

The stepping motor rotating the inner round capillary is driven by a stand-alone control unit offering different operation modes including a PC interface. Via the control unit rotation speed, angular resolution (from 0.1125° to 1.8°) and direction of rotation can be chosen.

For observation a microscope objective lens with an appropriate working distance should be chosen to reach at least the level of the rotation axis of the sample. Good results can be achieved by objective lenses with low numerical apertures, e.g. 5x/0.15, 10x/0.30 or 20x/0.50 lenses. Alternatively, for higher magnification a 63x/0.9 water dipping objective lens turned out to be suitable.

Exemplary Results

Figures 2 and 3 show some exemplary results. The images depicted are z-projections from eight different rotation angles. The copepod in figure 2 was incubated with rhodamine 6G at a concentration of 10 µM for 24 h. Fluorescence images were recorded by confocal laser scanning microscopy using an excitation wavelength of 488 nm. Fluorescence was detected using a long pass filter with cut-off wavelength at 505 nm. Image stacks from each direction consist of 100 images recorded at distances of $\Delta z = 6$ µm.

Figure 3 shows z-projection images of a zebrafish embryo recorded by confocal laser scanning microscopy at the Institute of Molecular Biology (IMB), Mainz, Germany.

References

All references are available online:
http://bit.ly/IM-Bruns

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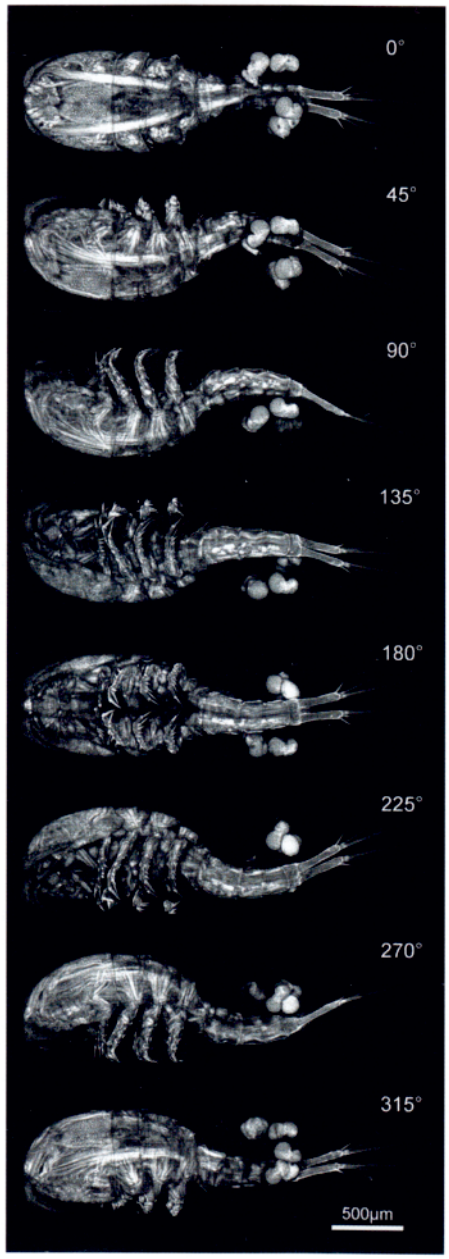

Fig. 2: Fluorescence z-projection images of 8 individual rotation steps of a copepod with half-filled egg sac incubated with rhodamine 6G (10 µM, 24 h) recorded by confocal laser scanning microscopy (excitation wavelength: 488 nm; fluorescence detected at $\lambda \geq 505$ nm; each z-stack: $\Delta z = 6$ µm, 100 images).

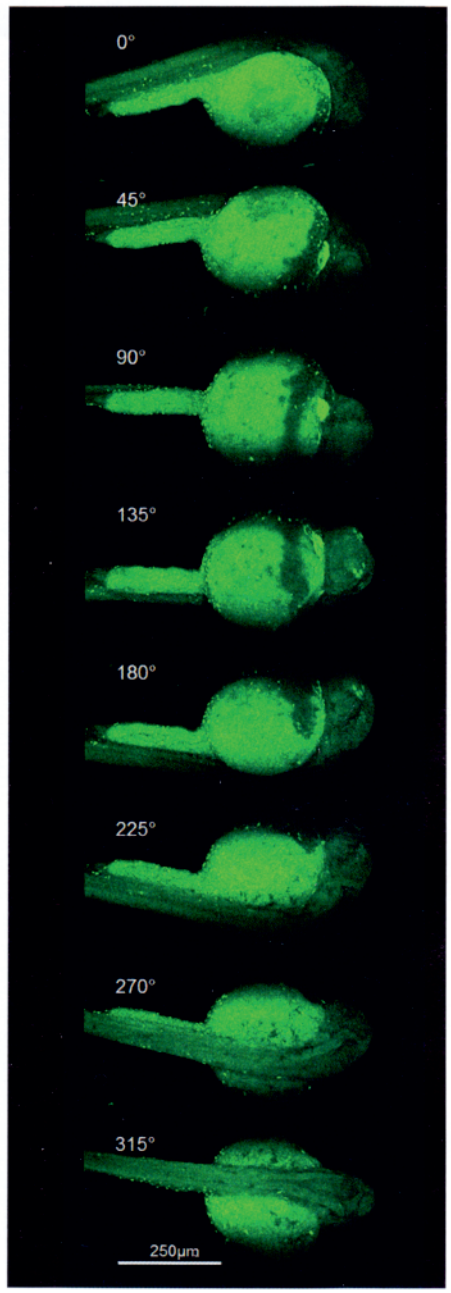

Fig. 3: Fluorescence z-projection images of 8 individual rotation steps of a zebrafish embryo recorded by confocal laser scanning microscopy [Images recorded by Holger Dill, Mária Hanulová, and Sandra Ritz, Institute of Molecular Biology (IMB), Mainz, Germany].