Sample holder for rotation of 3-dimensional specimens in microscopy

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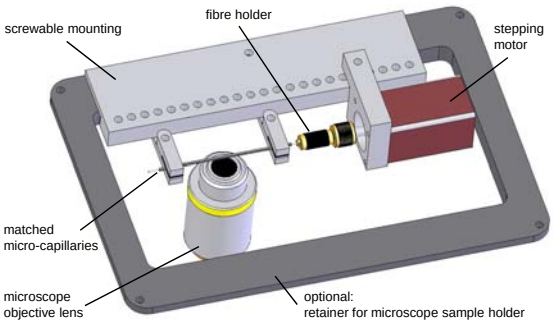
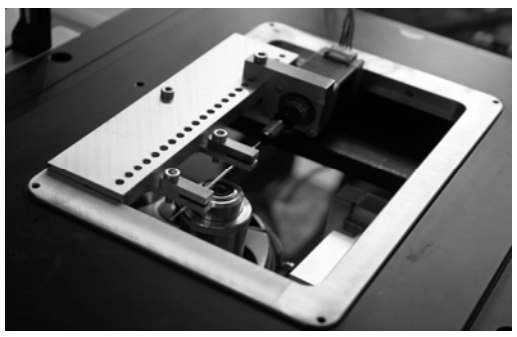
INTRODUCTION

Microscopy has become a well-established and commonly used method in fields of scientific research, medical diagnosis and industrial quality management. In common microscopy, samples are often embedded between glass slides such that observation is only possible from one perspective, namely vertical to the glass slide. However, especially for larger three-dimensional objects observation from different views is desirable. The presented sample holder permits rotating of the object when positioned in the light path of the microscope. Recorded images can be put into a defined three-dimensional context, enabling reliable 3D reconstructions of the specimen. The device can be easily adapted to a great variety of common microscopes and is suitable for various applications in science, education and industry, where the observation of three-dimensional specimens is essential.

SAMPLE HOLDER

For fluorescence microscopy two matched micro-capillaries of borosilicate glass are used. The central part of the device consists of an outer glass capillary of rectangular shape wherein another capillary with a round shape is placed. The specimen is located within this inner capillary and the sample holder is connected to the microscope such that the specimen is positioned in the light path of the microscope. The spacing between inner and outer capillary as well as the inner capillary containing the specimen is filled with an appropriate immersion fluid which is taken up by capillary forces. By rotating the inner capillary, the specimen can be observed from any desired perspective vertical to its longitudinal axis. This allows the perspective of observation to be changed easily with respect to the specimen.

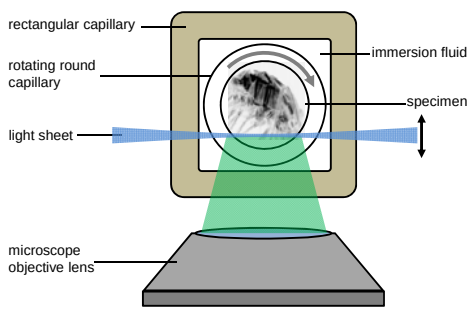




MICROSCOPY METHODS

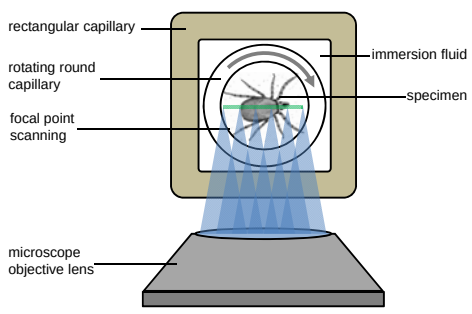
The device for rotation of 3-dimensional specimens supports a wide range of common microscope techniques including epi-illumination and transillumination. Two techniques for detection of specific layers within a specimen are presented in detail.

Single plane illumination microscopy (SPIM)



A light sheet is created perpendicular to the observation path either by a cylindrical lens [1] or by scanning of a laser beam. At SPIM only the plane under investigation is exposed to light, and successive measurements can be performed at low exposure of the whole sample when either the light sheet or the specimen is shifted in axial direction.

Confocal laser scanning microscopy (CLSM)



For illumination of the specimen a laser beam is focused by the microscope objective lens into the image plane and shifted by a scanning head to generate a 2-dimensional image. By moving the objective lens in vertical direction one can also generate a 3-dimensional dataset (optical sectioning).

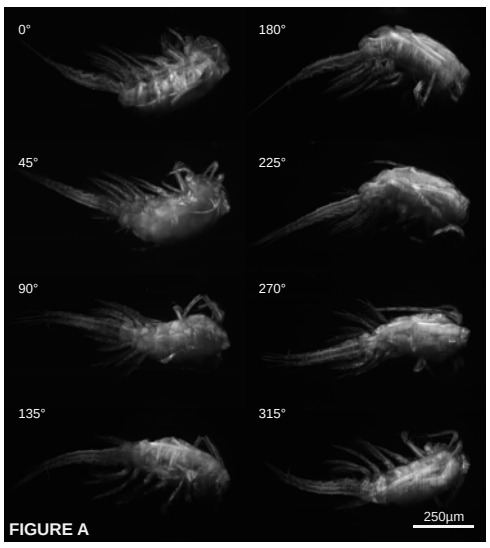


FIGURE A

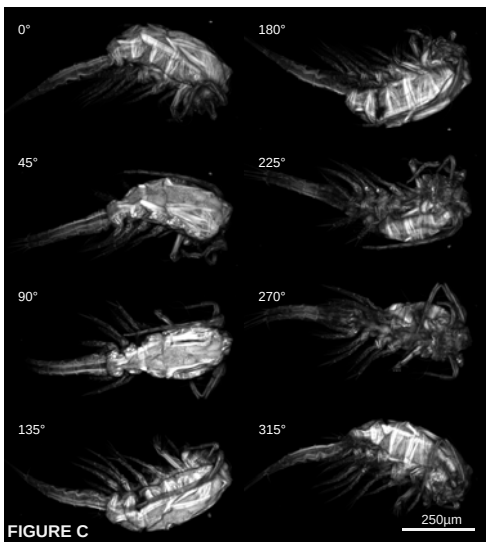


FIGURE C

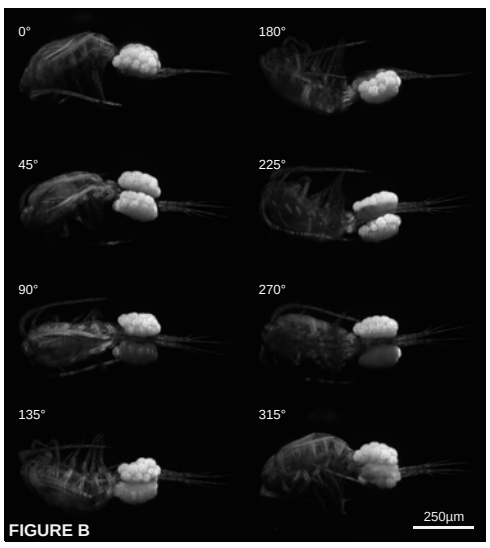


FIGURE B

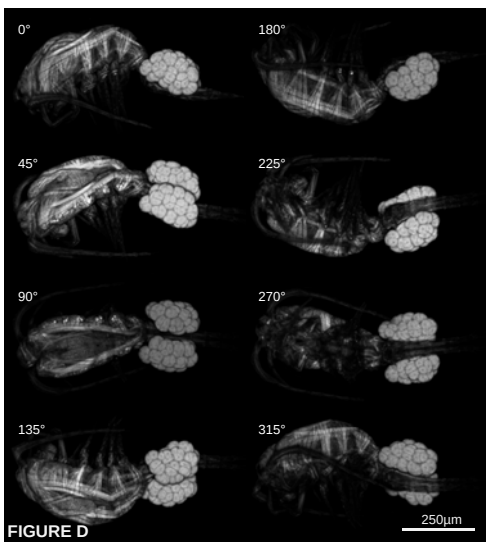


FIGURE D

SPECIMENS

Multicellular tumour spheroids (MCTS), as described e.g. by Bruns et al. [1] are commonly used for live cell experiments. For demonstration of the device, however, two further (fixed) specimens revealed to be appropriate and are described below.

Copepods belong to the group of small crustaceans resident in sea water as well as in fresh water. They put the main disposal of marine zooplankton and play therefore an important role within the marine food chain. Copepods are typically 0.2-2mm long and have a teardrop-shaped body with an armoured exoskeleton and large antennae. They possess a single median compound eye, and the thorax has up to five segments, each with limbs. The narrow abdomen contains five segments without any appendages. Copepods do not have any heart or circulatory system.

Ticks are small arachnids in the order of Ixodida. Along with mites, they constitute the subclass Acarina. Ticks are ectoparasites (external parasites), living by hematophagy on the blood of mammals, birds, and sometimes reptiles and amphibians. They are vectors of a number of diseases. Ixodida ticks undergo three primary stages of development: larval, nymphal, and adult. Within the larval state they have a size of 500µm x 400µm and possess six legs (compared to eight legs for the further states).

RESULTS

Results obtained by single plane illumination microscopy (SPIM)

FIGURE A & FIGURE B: Fluorscence z-projection images of 8 single rotation steps of copepod without egg sac (A) and with egg sac (B) incubated with acridine orange (10µM, 30min) recorded by single plane illumination microscopy. Light incidence from top to bottom (excitation wavelength: 470nm; fluorescence detected at $\lambda \geq 515$nm; each z-stack: $\Delta z = 5$µm, 80 images).

Results obtained by confocal laser scanning microscopy (CLSM)

FIGURE C & FIGURE D: Fluorescence z-projection images of 8 single rotation steps of a copepod without egg sac (C) and with egg sac (D) incubated with acridine orange (10µM, 30min) recorded by confocal laser scanning microscopy (excitation wavelength: 488nm; fluorescence detected at $\lambda \geq 505$nm; each z-stack: $\Delta z = 4$µm (C) and 6µm (D), 100 images).

FIGURE E: Autofluorescence z-projection images of 16 single rotation steps of Ixodidae ticks of the larval state recorded by confocal laser scanning microscopy (excitation wavelength: 488nm; fluorescence detected at $\lambda \geq 505$nm; each z-stack: $\Delta z = 8.5$µm, 50 images).

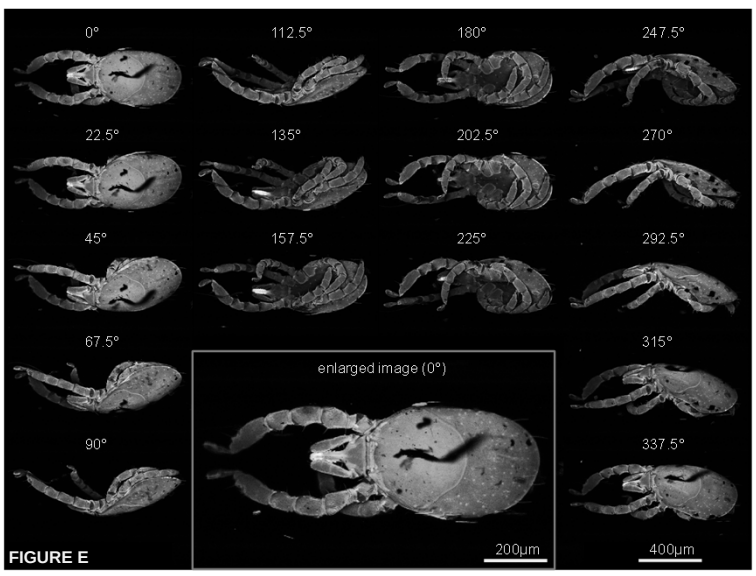


FIGURE E

REFERENCES

[1] T. Bruns, S. Schickinger, R. Wittig and H. Schneckenburger, “Preparation strategy and illumination of 3D cell cultures in light sheet-based fluorescence microscopy,” *J. Biomed. Opt.* **17**, 101518 (2012).