A Scanning Electron Microscopy Specimen Holder for Viewing Different Angles of a Single Specimen

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KEY WORDS SEM; freedom of rotation; specimen holder

ABSTRACT The specimen holder for scanning electron microscopy described herein allows a single specimen to be examined in any possible view and significantly improves object illumination. The specimen is glued to a fine pin and flexibly mounted on a double-sided adhesive conductive pad on a rotatable pivot. A milled pot placed beneath the specimen acts as an electron trap. This provides a homogeneous black image background by minimizing noisy signals from the specimen's surroundings. Microsc. Res. Tech. 73:1073–1076, 2010. VVc 2010 Wiley-Liss, Inc.

INTRODUCTION

In traditional scanning electron microscopy (SEM), specimens are usually attached directly to stub mounts using conductive adhesives such as tabs, tapes, or sheets (e.g., Beutel and Gorb, 2001; Schmalfuss, 1998; Uhl et al., 2007; Zimmermann et al., 2009). These double-sided adhesives are either polycarbonate, aluminum,copper, or silver-based. The main advantage of this method is that it is not particularly time-consuming.A major disadvantage, however, is the limited viewing angle that results, which prevents analysis even of parts of the sample which are not attached to the stub. Aside from this it is extremely time-consuming and sometimes even impossible to remove the background of an image with image processing software such as Photoshop (Adobe), especially when specimens are densely covered with setae. Moreover, image processing is a controversial field in science as original data are altered. The method described later, however, removes the need for image processing. Wichard et al. (1995) described a method that improves the illumination of arthropod specimens using a special construction called a ‘‘specimen pot.’’ Consisting in the simplest case of a stub wrapped with a cylinder of aluminum tape, a more sophisticated version is composed of a milled aluminum cylinder with a needle holder. To minimize backscatter, the interior of the ‘‘specimen pot’’ is coated with conductive carbon. The upper side, with the exception of a small opening, is sealed with a piece of aluminum foil. Sputtered specimens are fixed to a needle and placed above the opening of the pot, thus assuring that the background of the object is completely or almost completely black. The object can be rotated around the axis of the pin on which it is mounted, but only around this axis. A sample holder which offers a much higher degree of freedom and unlimited views of the specimens has therefore been developed.

DESCRIPTION

The sample holder consists of a base plate and a metal block bolted together at right angles. The metal block is equipped with a rotatable pivot in a borehole (Figs. 1A, 1D, and 1F). One side of this pivot is milled and serves as a holder for the specimen (see below) while the opposite side consists of a screw slot that permits the precise rotation of the specimen (Figs. 1D and 1F). A steel ball pushed by a coil spring holds the pivot in position (Fig. 1D). The tension of the coil spring and thus the rotability of the pivot is controlled by a setscrew (Fig. 1D). The second part of the specimen holder consists of a milled pot internally coated with conductive carbon featuring a rectangular object window (Figs. 1B and 1C). The pot is not firmly attached to the base plate but can be positioned freely below the specimen.

A double-sided conductive adhesive pad (Plano) glued to the bottom of the pot permits flexible mounting on the base plate. Both the sample holder and the pot are made of brass. The sample holder can be easily crafted by a precision engineer on the basis of Figure 1. Before first use both parts should be thoroughly degreased to avoid contamination of the SEM.

USE

The specimen is glued to a fine pin (e.g., ‘‘Minutienstifte’’) with nail polish, which is a very useful adhesive. A piece of double-sided conductive adhesive pad is used to attach the pin bearing the specimen to the milled surface of the pivot (Fig. 1A). The sample is then coated with gold or gold palladium on both sides using a sputter coater. The object window of the pot then has to be positioned below the specimen. By turning the pivot with a screwdriver and moving the needle, a single specimen can be examined from almost any viewing angle (Figs. 2 and 3) while the hollow interior of the pot ensures a completely black background. If the needle is mounted at an angle to the rotation axis of the

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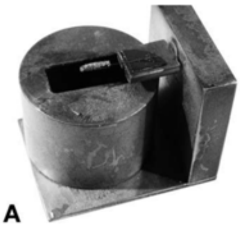

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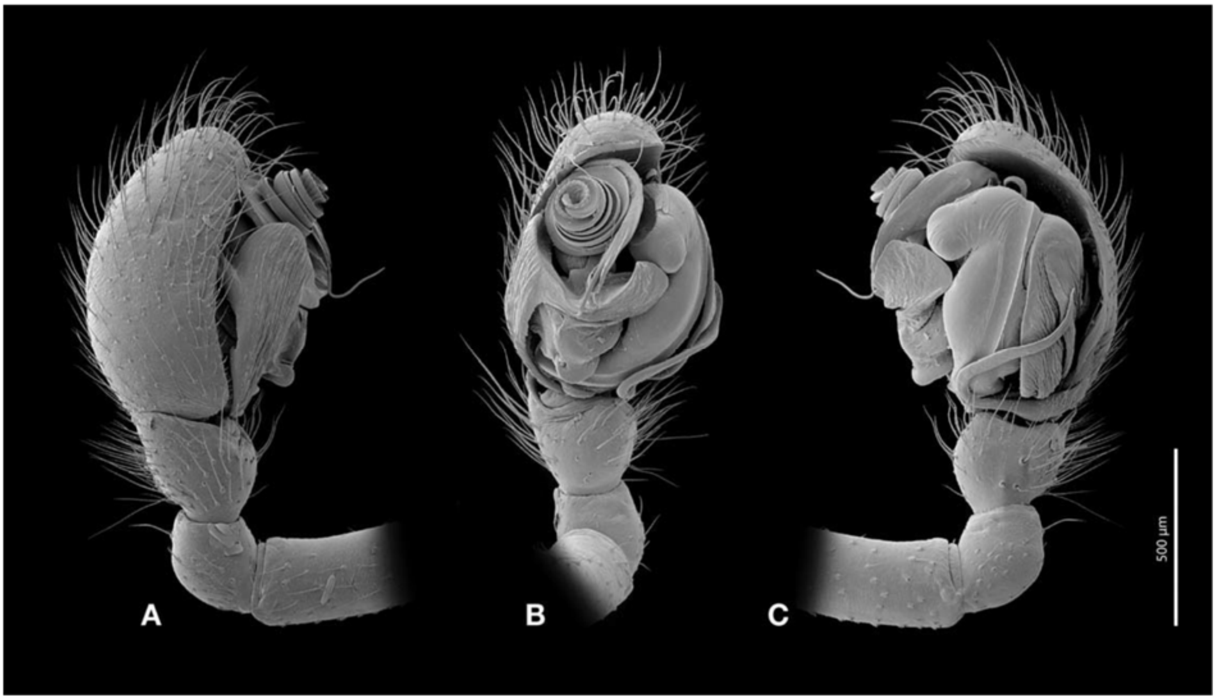

Fig. 2. Male genital (pedipalp) of a long-jawed spider (Tetragnatha sp., Tetragnathidae). (A) Mesal, (B) Caudal, (C) Lateral view of the same left pedipalp. SEM micrographs.

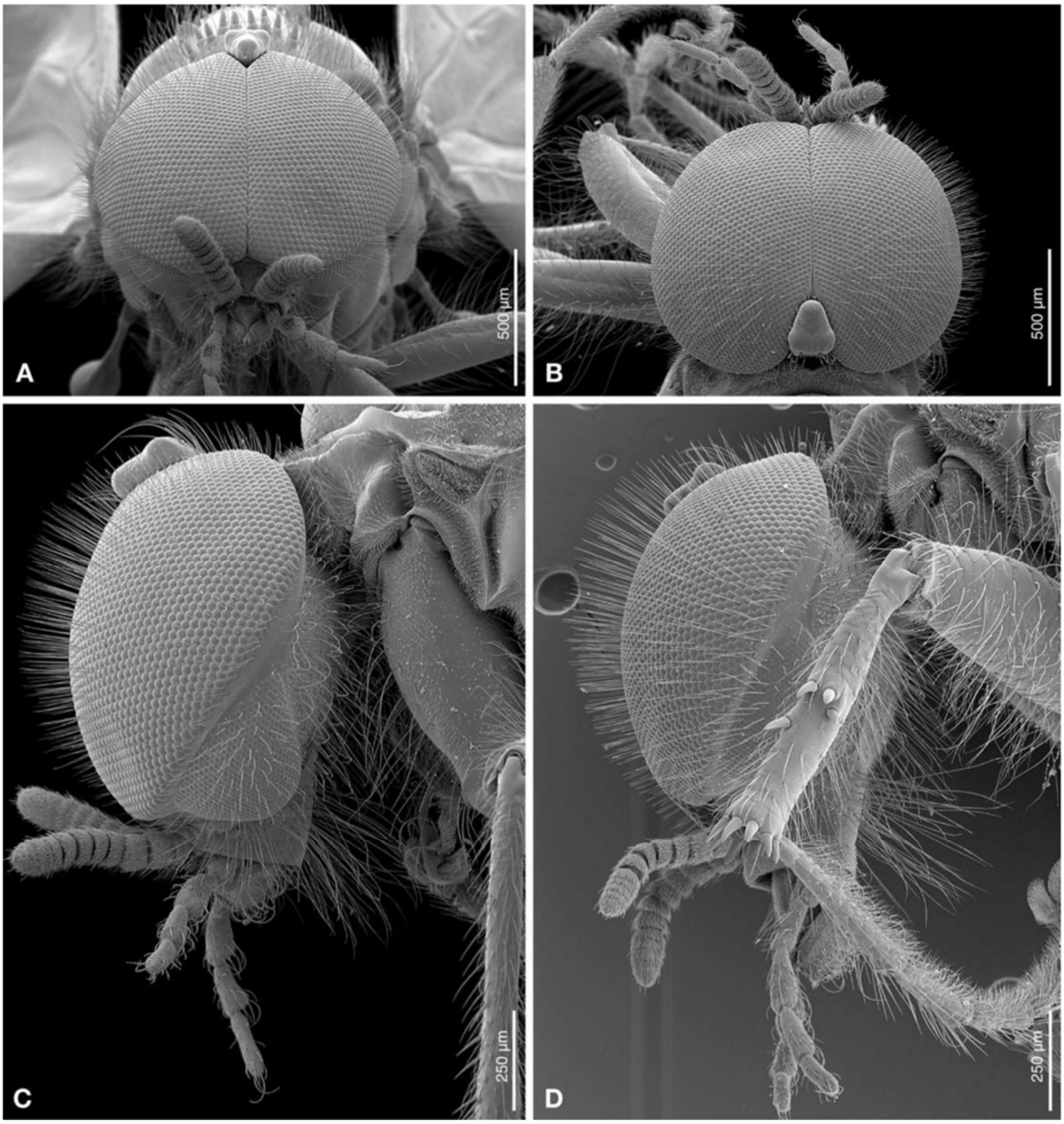

Fig. 3. Male head of a March fly (Dilophus sp., Bibionidae). (A) Frontal, (B) Dorsal, (C) Lateral view of the same specimen; (D). Lateral view of another specimen, traditional mounting on a stub. SEM micrographs.

holder the possible viewing angle is greatly increased in comparison to the method proposed by Wichard et al. (1995). When placing the specimen on the sample holder into the SEM it is very important to take into account the maximum heights in order to avoid damage to the lens.
Stored on their pin and stuck into a small piece of plasticine on an SEM stub, specimens can easily be archived permanently in a stub sample container (Plano).

DISCUSSION

In terms of object illumination, this method is a significant improvement on traditional mounting on a stub (see Fig. 3). The interior of the pot acts as an electron trap minimizing the signal surrounding the specimen, thus providing a homogeneous black background. As both sides of the specimen are coated, spurious charges occur very rarely. Rotating the specimen holder on the stage of the SEM alters the illumination of the specimen. Changes in the view of the specimen, however, require a removal of the specimen holder from the microscope. Because the SEM chamber has to be vented and pumped, this procedure is more timeconsuming than simply tilting the object stage as is possible in the standard set-up where specimens are mounted on a stub. A second disadvantage compared to standard stubs is that the specimen can be more easily damaged or fall off the pin during preparation. In contrast to the specimen holder presented by Wichard et al. (1995), the holder presented here significantly increases the possible viewing angle of specimens thanks to the flexible mounting of the specimen on a rotating pivot and the free movement of the pot on the base plate. The specimen holder as described in Figure 1 can be used for objects of _1–12 mm in length. Smaller objects are difficult to glue to a fine pin while larger ones protrude from the object window of the pot. The ultimate limitation factor, apart from specimen size, is the distance between the specimen stage of the SEM used and the lens. Both overview images and detailed micrographs with enlargements of over 10,0003 can be taken using the construction described. We obtained excellent results in an FEI XL30 ESEM and a Zeiss DSM 960A.

Examples of SEM images taken using the device described can be found in Pohl and Beutel (2004, 2005, 2008). ACKNOWLEDGMENTS

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