Enhanced scanning electron microscopy images using muscovite mica, an example with Rhizaria

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Abstract
Muscovite mica sheets were used as a support to capture scanning electron microscopy pictures of marine biological samples. The physical properties of the cleaved muscovite mica provide a clean background, which greatly reduces the post-processing of images, thereby enhancing them and resulting in impressive images. We chose siliceous Rhizaria for this investigation due to their morphological diversity and elaborate skeletons.

Research highlights
• Muscovite mica sheet was used as a SEM support for biological sample
• Physical properties of muscovite mica sheet can provide native dark, gray, and white background of SEM images
• Muscovite mica sheet should be considered for image production in the context of art and science intersection

KEYWORDS
muscovite mica, Rhizaria, scanning electron microscopy

1 | INTRODUCTION
Good images are essential for the investigation and interpretation of the morphological features of biological species. While drawings can be a good option for artistically gifted specialists, like Haeckel (1904), their production is very time-consuming and requires exceptional skills. Fortunately, for less artistically talented scientists, other means of illustrating the intricate details of small and morphologically complex creatures have been developed.

Scanning electron microscopy (SEM) is one of the techniques that has contributed to the elucidation of many scientific questions through observations. The application of SEM in the biological sciences has improved the understanding of developmental, physiological, and structural mechanisms of living organisms (Karr, 1971). This technique can also be essential for the taxonomic identification of microorganisms (Pachar and Cameron, 1992). The interaction of an electron beam with an object allows SEM to provide observations at very high magnification and remarkable resolution, resulting in a spectacular level of detail. Thus, SEM images are often used to illustrate scientific publications.

Microscopic observations allow the observer to explore a world invisible to the naked eye, revealing the hidden wonders of nature. SEM imaging also assists in bringing out the hidden artistic side of scientists thus enhancing the interest of their work to the general public. Due to their graphic quality, SEM images are a major contribution to scientific articles and exhibitions, providing an attractive and attention-drawing means of presenting scientific topics (Tyurina, 2020).

SEM images are usually reprocessed on postproduction, especially when they are used for exhibits aimed at highlighting the artistic aspects of the organisms dealt with. A major part of this work is blurring or removing the background of the object imaged, that is, the support on which it was placed. Samples for SEM are routinely mounted on supports with filters for microorganisms or with silver adhesive or conductive adhesive tapes for larger objects, that often degrade que quality of the overall image and include unwanted distracting details in it. These usually involve an unsightly background (e.g., pores in filters create a perforated surface and adhesive tapes
are irregular and can capture dust and fibers), resulting in irregularities which divert the observer’s eye away from the centerpiece. Postproduction is a long and tedious process, and the result, although more attractive, may decrease the accuracy of the specimen figured.

In order to circumvent this problem, we sought a technique capable of highlighting the specimen observed with SEM, and minimizing the postproduction work required. Several criteria were kept in mind for the selection of the support material: an easy mounting set up, a smooth and dust free surface at the micro/nanometric scale, and the ability to keep the targeted object immobile within the observation chamber while being compatible with electron microscopy. After various preliminary trials, we settled on the cleaved muscovite mica substrate initially commercialized for sample preparation in atomic force microscopy and already used in SEM for nanomaterials characterization (Ociejea et al., 2013).

Siliceous rhizarians (Polycystina and Phaeodaria), collected in the Atlantic Ocean and in the Ross Sea with the use of plankton nets, were chosen to test the performance of the method. Siliceous rhizarians are single-cell marine protists which form unique and intricately detailed biogenic silica skeletons (Takahashi et al., 1983). SEM can be used as a complementary technique for thorough analysis of these organisms (Boltovskoy et al., 1983). The incredible beauty and diversity of their skeletons makes them the ideal objects for this test. However, the use of cleaved muscovite mica sheets as a support for SEM observations should be tested for other types of objects, hopefully facilitating the production of flawless images.

**Figure 1** Scanning electron microscopy images of rhizarians. (a)–(c), Spumellaria of the family Actinomidae collected in the Pacific sector of the Southern Ocean. (d)–(f), Phaeodaria of the family Medusettidae (Euphysetta lucani) collected in the Atlantic Ocean. (a), Top view of specimen, (b) Sample observed at ~75° tilt. (c) and (d), sample observed at ~85° tilt. Scale bars, 50 μm. Acquisition conditions are presented in Table 1.
Our protocol provides a basic and cost effective adjustment that produces excellent results for scientific SEM observations coupled with artistic edge of biological sample. Cleaved muscovite mica sheets offer a clean surface making it possible to take accurate pictures with clean dark, gray, or white background, respectively, depending on observation angle.

2 | METHODS

A disk of muscovite mica sheet (V4 grade) is glued with double-sided tape on the SEM support stub.

1. The muscovite mica sheet is peeled off with adhesive tape to remove a layer and obtain a clean, smooth surface.
2. A cleaned skeleton is handpicked (Mendez Sandin, 2018) using a dissecting microscope, and placed on the muscovite mica sheet.
3. The sample is then dried at 37 °C until all moisture is eliminated.
4. The SEM stub is coated for 30 s with excess heavy metal (Au/Pd) using a SC7620 Mini Sputter Coater.
5. The coated specimen on the stub is observed and photographed in SEM (in our case, a Hitachi S3200 N SEM).

3 | RESULTS AND DISCUSSION

Our results are presented in Figure 1. When placed perpendicular to the electron beam (tilt = 0°), the mica sheet presents a very clean, soft, and deep dark black background (Figure 1a,d). One surprising and artistically appealing result appears when the support is tilted to observe the sample from the side. When the tilt angle is around 75°, the cleaved muscovite mica sheet produces a gray background (Figure 1b,e). At angles around 85° to 88°, the background turns white (Figure 1c,f). The uniform background can be explained by the extreme planar properties of the cleaved muscovite mica and by the inclination of the support to the detector. The tilt enables an even background not only on a black background (De Wever 1980) but also with brighter colors (gray, white).

Because of the bright background, we can appreciate the shadow of the skeleton (Figure 1b,c,e,f). Indeed, a phenomenon similar to the shadow created when an opaque body intercepts the sun’s rays is observed. In this case, the incident electrons are obstructed by the sample, resulting in an aesthetic shadow effect, which cannot be observed on a dark background, when the support is perpendicular to the incident electron beam.

Also, it is apparent that cleaved muscovite mica sheets provide an efficient adhesive surface for our specimens whose dimensions are between 150 and 300 μm, even at vertical inclinations (tilt angle = 90°). This is probably due to an accumulation of capillary adhesion forces during drying, and Van der Waals and electrostatic forces with negatively charged mica.

The main advantages of this technique are: (i) low cost and, (ii) clear background. In conclusion, the use of cleaved muscovite mica sheets seems to be a perfect technique to facilitate the link between scientific and artistic observations of specimens when utilizing SEM for biological sample. Preliminary tests have shown that this support, can also be used for smaller samples than presented here.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

Natalia Llopis Monferrer collected and isolated specimens. All authors designed the experimental setup. Philippe Elies performed image analysis. All authors commented on and revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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