Enhanced Scanning Electron Microscopy Images Using Muscovite Mica, an Example with Rhizaria

Natalia Llopis Monferrer (✉ natalia.llopismon@univ-brest.fr)

Université Bretagne Occidentale

Valentin Foulon

Université Bretagne Occidentale

Philippe Elies

Université Bretagne Occidentale

Research Article

Keywords: Muscovite Mica sheets, electron microscopy pictures, cleaved Muscovite Mica

Posted Date: April 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-386630/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Enhanced scanning electron microscopy images using Muscovite Mica, an example with Rhizaria

Natalia Llopis Monferrer^{1,2,*,#}, 0000-0003-4894-031X, natalia.llopismon@univ-brest.fr

Valentin Foulon^{1,#}, 0000-0002-9597-7628, valentin.foulon@univ-brest.fr

Philippe Elies³, philippe.elies@univ-brest.fr

¹ Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280 Plouzane, France

² Sorbonne University, CNRS, UMR7144, Ecology of Marine Plankton Team, Station

Biologique de Roscoff, Roscoff, France

³ Plateforme d'Imagerie et de Mesures en Microscopie, Université de Bretagne Occidentale,

29200, Brest, France

*Corresponding author

#First co-authors

Abstract

Muscovite Mica sheets were used as a support to capture scanning electron microscopy pictures. 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.

Main text

Good images are essential for the investigation and interpretation of the morphological features of biological species. While drawings can be a good option for the artistically gifted specialists, like Ernst Haeckel¹, 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. This technique can also be essential for the taxonomic identification of microorganisms². 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.

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, i.e., 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 the 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 fibres), resulting in irregularities which divert the observer's eye away from the centrepiece. 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 minimize 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, which met all our criteria, and many more.

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 skeletons³. Scanning Electron Microscopy can be used as a complementary

technique for thorough analysis of these organisms⁴. 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.

Our protocol provides a basic and cost effective adjustment that produces excellent results for scientific SEM observations coupled with artistic edge. Cleaved Muscovite Mica sheets offer a clean surface making it possible to take accurate pictures with clean dark, grey or white background.

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 handpicked⁵ 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 seconds 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).

Our results are presented in Fig. 1. When placed perpendicular to the electron beam (tilt = 0°), the mica sheet presents a very clean, soft, and deep dark black background (Fig. 1a and 1d). 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 grey background (Fig. 1b and 1e). At angles around 85° to 88° , the background turns white (Fig. 1c and 1f). The uniform background (black, grey, white) can be explained by the extreme planar properties of the cleaved Muscovite Mica and by the inclination of the support to the detector.

Because of the bright background, we can appreciate the shadow of the skeleton (Fig. 1b, 1c, 1e and 1f). Indeed, for some points on the image, the incident electrons are obstructed by the sample, resulting in anaesthetic shading 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 $150\text{ }\mu\text{m}$ and $300\text{ }\mu\text{m}$, 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.

99

100 In conclusion, the use of cleaved Muscovite Mica sheets seems to be a perfect technique to
 101 facilitate the link between scientific and artistic observations of specimens when utilising

scanning electron microscopy. Preliminary tests have shown that this support, can also be used for smaller samples than presented here.

Acknowledgements

The authors would like to thank the captain and crew of RV Tangaroa and the RRS James Clark Ross for their efforts in facilitating the sampling during TAN1901 and AMT28 oceanographic expeditions. This study is a contribution to the international IMBER project and was supported by the UK Natural Environment Research Council National Capability funding. We thank N. van Horsten and H. Whitby for their valuable help. We are particularly grateful to D. Boltovskoy for his always helpful comments. This work was supported by the "Laboratoire d'Excellence" LabexMER (ANR-10-LABX-19) and co-funded by a grant from the French government under the program "Investissements d'Avenir".

Author contributions

NLLM collected and isolated specimens.
All authors designed the experimental setup.
PE performed image analysis.
All authors commented on and revised the manuscript.

Competing Interests Statements

The authors declare that they have no conflict of interest.

References

1. Haeckel, E. *Kunstformen der Natur*. (Olaf Breidbach, 1904).
2. Pachar, J. & Cameron, J. Scanning Electron Microscopy: Application in the Identification of Diatoms in Cases of Drowning. *Journal of Forensic Sciences* **37**, 860–866 (1992).
3. Takahashi, K., Hurd, D. C. & Honjo, S. Phaeodarian Skeletons: Their Role in Silica Transport to the Deep Sea. *Science* **222**, 616–618 (1983).

4. Boltovskoy, D., Kotzian, S. B. & Pedrozo, F. L. Some New Techniques for the Preparation and Illustration of Polycystina (Radiolaria). *Micropaleontology* **29**, 382 (1983).
5. Mendez Sandin, M. M. Scanning Electron Microscopy imaging for Opaline Silica Single Cell Skeletons (Polycystines Radiolaria). *protocols.io* (2018)
doi:dx.doi.org/10.17504/protocols.io.ug9etz6.

Figure legends

Figure 1 | **Scanning electron microscopy images of rhizarians. a-c**, Spumellaria of the family Actinommmidae 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 approximately 75° tilt. **c-d**, sample observed at approximately 85° tilt. Scale bars, 50 µm.

Figures

Figure 1

Scanning electron microscopy images of rhizarians. a-c, Spumellaria of the family Actinommidae 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 approximately 75° tilt. c-d, sample observed at approximately 85° tilt. Scale bars, 50 μm