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Research Note

Method for taking Scanning Electron Microscope photographs of nematodes and meiofauna with the support of a low-cost and easy-made container

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Summary

This paper presents a method for capturing Scanning Electron Microscope (SEM) photographs of small specimens, including nematodes, arthropods, small insects, and other meiofauna. Our method is tailored to handle nematode specimens mounted on permanent slides, an area with relatively limited documentation. Besides, the process of transferring such delicate specimens from one solution to another has historically posed numerous challenges. To address this issue, we introduce a low-cost and easy-made container designed specifically to facilitate the aforementioned procedure, with a particular focus on SEM photography. The newly introduced container offers a practical solution that enhances the efficiency and effectiveness of specimen handling, ultimately enabling high-quality SEM imaging. This method holds significant promise for researchers working in the field of microscopic organism analysis, providing a valuable tool for their investigations with minimum cost.
Keywords: critical point drying; dehydration; hand-made tool; SEM

Introduction

Scanning electron microscope (SEM) pics are considered as one of the most important data in nematology recently, providing valuable insights into their morphology and physiology (Bhat *et al.*, 2022; Coomans, 2000). Achieving high-quality SEM imaging involves several essential steps, with critical drying of specimens being particularly important (Abolafia, 2015; Eisenback, 1985). Up to now, numerous dehydration methods have been described in the literature, each tailored to the specific requirements of different organisms, including nematodes, arthropods, insects, and other meiofauna (Abolafia, 2015; Chandran *et al.*, 2016; Eisenback, 1986, 1985; Nguyen *et al.*, 2021). However, the microscopic nature of organisms like nematodes complicates their transferring and handling procedures, making specimen preparation challenging. To facilitate this step, a number of methods with the support of different containers has been introduced (Eisenback, 1985). However, existing methods for specimen dehydration of the aforementioned organisms often come with limitations, such as needing for living specimens, high costs, or limited accessibility to equipment and materials (Abolafia, 2015; Chandran *et al.*, 2016; Day, 1974; De Grisse, 1979; Eisenback, 1986; Hayunga, 1977; Nguyen *et al.*, 2021; Wergin, 1981). Therefore, there is a need for an efficient and cost-effective solution that can be universally applied by researchers.

In this study, we present an adapted procedure for capturing SEM images, specifically focusing on nematodes in permanent slides. Considering previous methods, we propose modifications that enhance the imaging process. Additionally, we introduce a novel approach to create a hand-made container using inexpensive materials, which facilitates the dehydration process. By addressing the challenges associated with specimen handling and dehydra-

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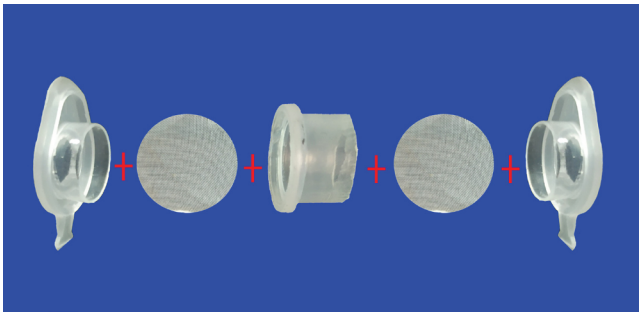

Fig. 1. The hand-made container designed for containing nematodes.

tion, our method aims to provide researchers with an efficient and cost-effective solution for obtaining SEM photographs of nematodes and other meiofauna.

Material and Methods

Procedure for making a hand-made container used in transferring nematodes to different solutions:

1. Remove the conical part of an micro-centrifuge tube using a knife and keep the cylindrical part for the next steps.
2. Make a rounded hole at the lid of an micro-centrifuge using a knife (2 lids are needed for the next steps)
3. Prepare 2 rounded pieces of nylon mesh filter (5µm pore size for working with small nematodes) that slightly larger than the micro-centrifuge opening aperture.
4. Specimens can be kept in the hand-made container by closing the cylindrical part of an micro-centrifuge tube with two lids after covering it with the two pieces of the nylon mesh filters (prepared above) (Fig. 1).

Procedure for taking scanning electron microscope (SEM) photograph:

1. Put a permanent slide in the fridge for 15 minutes and open it using a dissecting knife. Add approximately 50 µl of glycerin

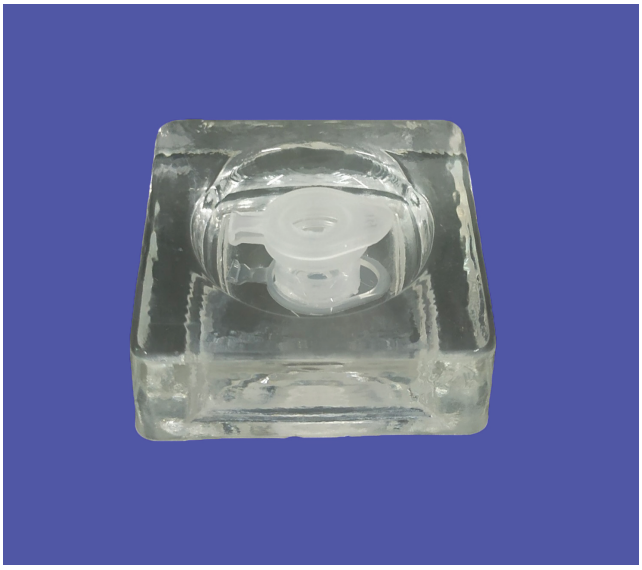

Fig. 2. Nematodes kept in the container placed within an embryo disc with glycerin.

before transferring nematode specimens using a picking needle to a drop of glycerin (approximately 50 µl) placed in a hand-made container and close the lid (Fig. 2).

2. Place the container with nematodes on an embryo disc with 200 µl of glycerin and add 150 µl of 95 % ethanol every 10 minutes (repeat this process ten times) (Fig. 2).
3. Transfer the hand-made container through a graded ethanol concentration series of 95, 98, 100 % (1 h each), 100 % (overnight), and 100 % absolute ethanol (20 min, next morning) (Fig. 3).
4. Critical point dry specimens in a hand-made container with liquid CO_2
5. Mount specimens on stubs with carbon discs and coated with gold before observation with a scanning electron microscope. Results can be seen from Fig. 4.

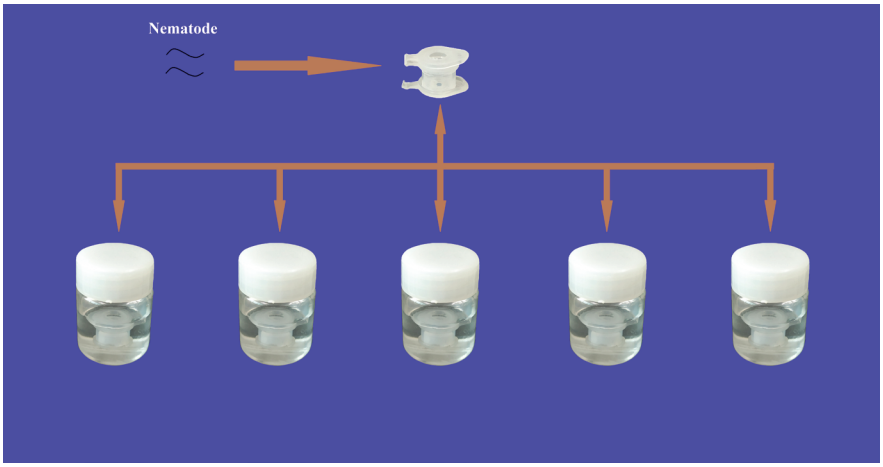

Fig. 3. Nematodes can be easily transferred to the hand-made container, facilitating subsequent transfers to different solutions.

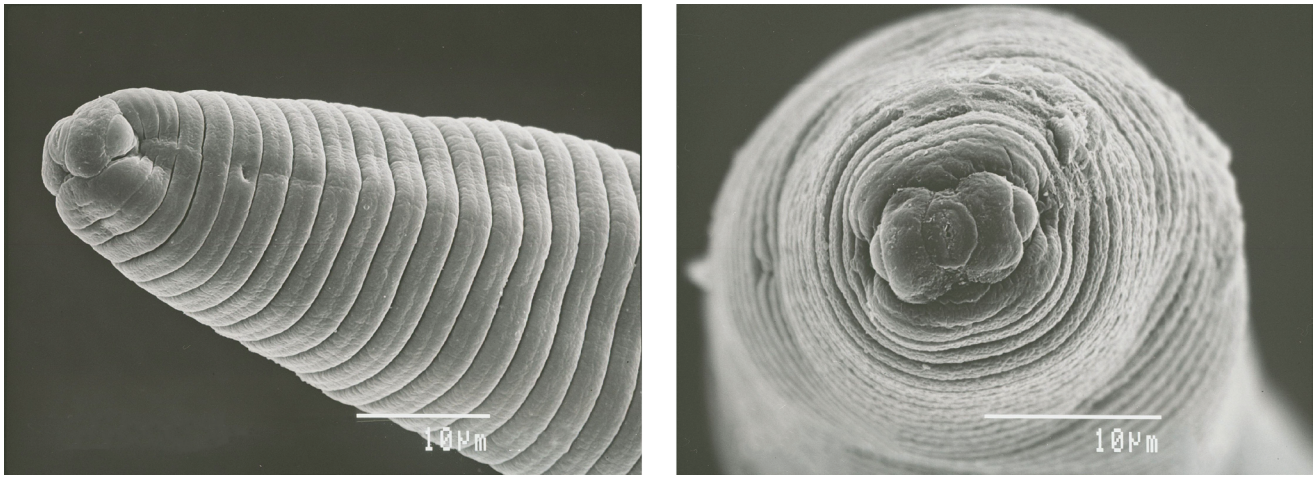

Fig. 4. Scanning Electron Microscope (SEM) images of nematodes captured using our proposed procedure.

Ethical Approval and/or Informed Consent

The result of this work has not been published previously and is not under consideration elsewhere.

Results and Discussion

Using our hand-made container, 100 % nematodes were recovered after dehydration. Typically, transferring tiny samples from one solution to another has been a laborious process, especially when managing a large number of samples. By placing all the nematodes in a container once, this simplified technique makes it possible to handle specimens more efficiently, especially when working with a huge number of specimens, and it enables researchers to process more samples in a shorter period. In the context of SEM imaging, the container design's time-saving feature is very advantageous. It might be laborious to prepare the specimens for SEM imaging, which includes dehydration using a graded ethanol concentration series. The container's capacity to transfer numerous specimens at once drastically cuts down on the time needed for this procedure, enabling researchers to speed up their workflow and boost production. Furthermore, the hand-made container developed in this study has the advantage of attachment without the need for glue, making it extremely tolerant to the many chemicals used in specimen preparation. This flexibility improves the container's performance, reusability, and adaptability, giving researchers a dependable instrument for handling specimens in a range of chemical conditions.

On the other hand, our newly developed container is quite small, and therefore, can be placed inside a critical drying machine to ensure a controlled drying condition and minimizes the risk of losing nematodes or other small organisms. This streamlined process allows for a smooth transition from the early phases of specimen preparation to critical drying, reducing the likelihood of specimen loss, contamination, or damage. Overall, this container can improve specimen preservation, minimizes handling, and optimizes the SEM imaging workflow.

Methods for constructing containers suitable for handling small specimens are frequently under-documented and unnecessarily intricate. For example, the method of Abolafia (2015) and Westheide and Purschke (1988) requires numerous materials and can be challenging to work with. Researchers may find it difficult to come up with useful and effective methods due to the absence of readily available information. This work aims to fill this gap by providing an easy and affordable approach for making a container that makes it easier to handle tiny specimens. The method outlined in this study is workable and affordable since the materials required are easily accessible. In general, our methodology, in contrast to existing methodologies that require expensive or specialized equipment, is practicable for researchers in resource-limited contexts.

On the other hand, the container proposed in this study could be somehow similar to other previously described containers, such as the one described by Eisenback (1985). However, it's important to underscore that these containers serve the common purpose of facilitating fluid exchange, minimizing specimen loss, mitigating tissue shock, preventing contamination, and sometimes offering a suitable substrate for SEM observation. Given this shared objective, structural resemblances are expected. It's noteworthy that our container can be made from widely available micro-centrifuge tubes, commonly found in biological laboratories, in contrast to the less prevalent BEEM capsules. Moreover, our container showcases a simplified design with a notably reduced number of lid holes, featuring just one hole in contrast to the 2 – 6 holes seen in Eisenback's design. This modification is rooted in the objective of minimizing unnecessary openings that could potentially interfere with fluid exchange. By streamlining the structure, our container aims to optimize functionality while maintaining its efficacy in facilitating fluid exchange. The simplicity of our container's design renders it a

valuable asset for SEM processes, offering cost-effectiveness and streamlined implementation.

Besides, the size of micro-centrifuge tube and mesh aperture can be adjusted to be compatible with different sizes of organisms. Although the focus of our investigation was on SEM imaging of nematodes, other small life forms may be able to employ the suggested method and container, broadening its usefulness beyond the scope of this study. Our method might be useful for researchers investigating arthropods, insects, and other meiofauna, especially when there are financial limitations and equipment shortages. To improve particular facets of specimen preparation or imaging quality, the methodology might also be changed or coupled with other approaches.

Although our modified method and the inexpensive container appear to be useful, certain drawbacks should be noted. While our modifications improved the imaging process, other improvements could be needed for particular species or a variety of research goals. Some organisms with unusual sizes or shapes would require adaptation. The container design should be modified to accommodate the demands of each study by looking at different possibilities. When modifying the technique, researchers should carefully evaluate the specific characteristics and requirements of their target specimens.

Acknowledgments

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Conflict of Interest

Authors state no conflict of interest.

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