ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/326867695

Improved Method for Handling and Collection of Semi-Thin Sections of Resin-Embedded Biological Specimens for Light Microscopy

Article *in* Microscopy and Microanalysis · August 2018

DOI: 10.1017/S1431927618007444

CITATIONS
0

READS
17

4 authors, including:

Kajal Bose Ghoshroy
University of South Carolina Sumter
13 PUBLICATIONS **118** CITATIONS

SEE PROFILE

Habeeb Alsudani
Cold Spring Harbor Laboratory
7 PUBLICATIONS **44** CITATIONS

SEE PROFILE

Soumitra Ghoshroy
University of South Carolina
55 PUBLICATIONS **1,211** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

BioDetection Work View project

HISTOLOGICAL DIFFERENTIATION IN THE DIGESTIVE SYSTEM AND BRANCHIAL ARCHES OF THE LIVE- BEARING TELEOST , POECILIA SPHENOPS View project

All content following this page was uploaded by Kajal Bose Ghoshroy on 20 August 2018.

The user has requested enhancement of the downloaded file.

1392
doi:10.1017/S1431927618007444
Microsc. Microanal. 24 (Suppl 1), 2018
© Microscopy Society of America 2018

Improved Method for Handling and Collection of Semi-Thin Sections of Resin-Embedded Biological Specimens for Light Microscopy

Kajal Ghoshroy[1], Erika Balogh[2, 3], Habeeb Alsudani[2] and Soumitra Ghoshroy[2]

[1.] Division of Science, Math & Engineering, University of South Carolina Sumter, Sumter, SC.
[2.] Electron Microscopy Center, University of South Carolina, Columbia, SC.
[3.] Current address: South Carolina Dept. of Health and Environmental Control, Columbia, SC.

Light (LM) and electron microscopic (EM) observations of most biological specimens need proper tissue preservation followed by both thick and ultrathin sectioning using an ultramicrotome. During this method, the fresh specimens are generally cut into small pieces, fixed, dehydrated, passed through a transitional solvent, infiltrated in an electron beam-resistant resin epoxy or acrylic, and finally polymerized into tissue blocks. The polymerized specimen blocks are usually trimmed to a trapezoidal shape and sectioned. For many such studies, it might be essential to initially make thick sections of the trimmed resin blocks for LM observations [1]. Subsequently, ultrathin sections may be made for transmission electron microscopy (TEM). This traditional procedure is useful for researchers as well as students learning to section resin-embedded tissue blocks. However, this standard thick sectioning technique is a time-consuming method where the trimmed block is loaded onto a chuck of an ultramicrotome and thick (0.5 to 2 μm) sections are made using a glass or diamond knife. The sections then may be collected, one at a time, by dry retrieval method or by using a drop of water placed on the cutting edge of the knife. This protocol may need different tools namely, a clean eyebrow hair mounted on an orangewood stick, a flattened orangewood stick (spatula shaped), or a fine pair of tweezers. The individual sections are then transferred onto a drop of water placed on a clean, oil free, glass slide. Precautions are taken to avoid wrinkled or folded sections. It is often a daunting task to pick up sections without losing it behind the knife edge, and during the transfer process from knife edge to the water droplet. This is quite frustrating for a new learner and especially a student who has limited time during the semester to master this technique. Even after successful transfers, the sections may be too thick to image or are wrinkled (fig. 1), and as a result the process often needs to be repeated.

We have developed a simpler transfer process for sections that need to be collected from knife edge for LM observations. In this procedure, a well-trimmed specimen block is mounted onto the chuck of a Dupont Sorvall MT2B ultramicrotome and the boat of a diamond knife is filled with water. Once the trimmed block face (that is parallel to the knife edge) comes in contact with the diamond knife, the motor is turned on for sectioning. The section thickness is maintained at 80-120 nm. Once several sections with satisfactory thickness begin to float on water (fig. 2), the motor is turned off and a clean eyebrow-stick is used to park the sections for retrieval. A thoroughly cleaned glass slide is then prepared and a drop of clean water is placed on it. Once a certain number of sections are parked on the surface of water, a Perfect Loop (EM Sciences, PA) is used to collect the sections. The loop with a droplet of water holding the sections is then brought in very close proximity to the water droplet on the glass slide and held right on top of the droplet without touching it. A laboratory compressed air can is then brought over the loop and a controlled whisk or quick burst of air is applied (fig. 3, 4) to transfer the sections and the water droplet onto the larger water drop placed on the glass slide earlier. The glass slide with sections is then placed on a hot plate. As water evaporates, the sections are fixed to the slide (fig. 5). These are later stained with an epoxy tissue stain (EM Sciences, PA) and observed under a LM.

Downloaded from https://www.cambridge.org/core. IP address: 179.61.188.57, on 20 Aug 2018 at 17:49:43, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S1431927618007444

The following images (fig. 6-12) depict the usefulness and application of this proposed method for LM study of various tissue samples. After collection of tissue sections for LM, ultrathin sections can be made immediately for TEM study by simply changing the thickness setting on the ultramicrotome. Thus, this method, not only reduces time for tissue prep, but also simplifies the overall method.

References:

[1] Bozzola J. S. and Russell L. D., Electron Microscopy Principles and Techniques for Biologists 2nd Edition (1999).

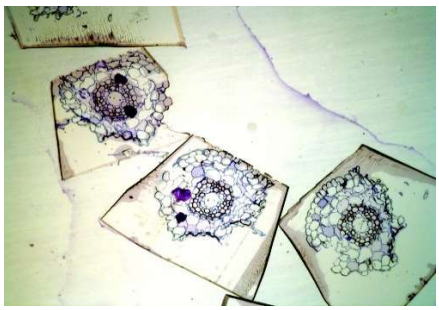

Fig 1. Thick mustard root cross sections made using conventional method (0.5-2 µm thick)

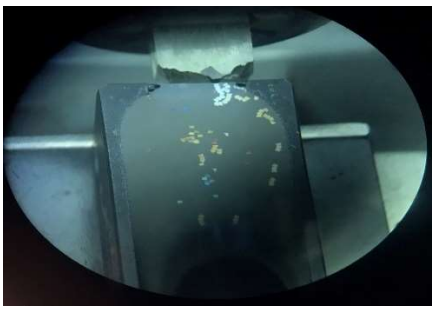

Fig 2. Sections floating on diamond knife boat

Fig 3. Sections being transferred from Perfect Loop using compressed air can

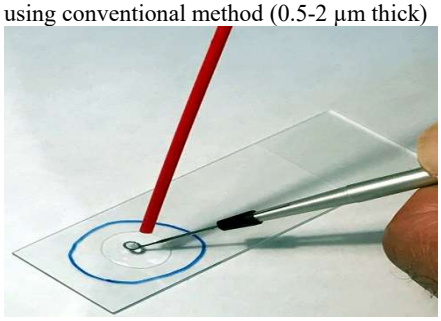

Fig 4. Close-up view of transfer procedure

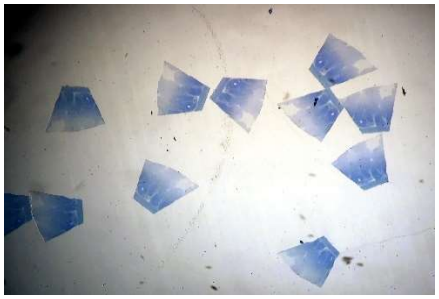

Fig 5. Low mag view of stained sections attached to glass slide x100

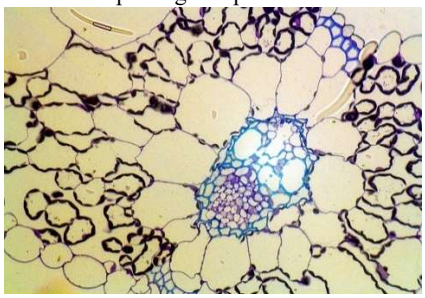

Fig 6. Giant Reed *Arundo donax* leaf section made using new method x400

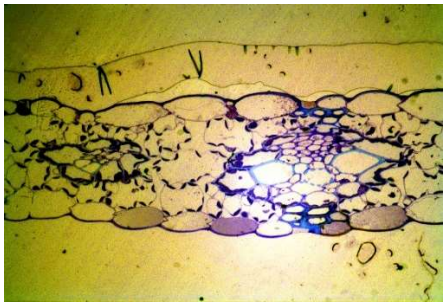

Fig 7. Corn leaf section made using new method x400

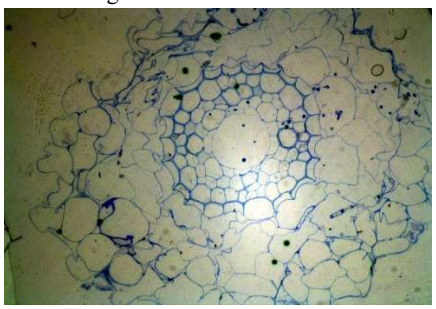

Fig 8. Jalapeno pepper root section made using new method x400

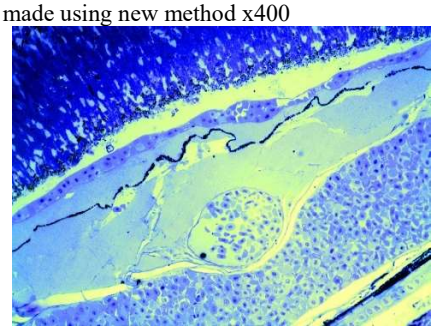

Fig 9. Fish eye section made using new method x400

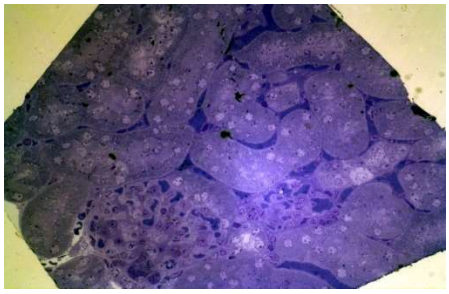

Fig 10. Mouse kidney section made using new method x400

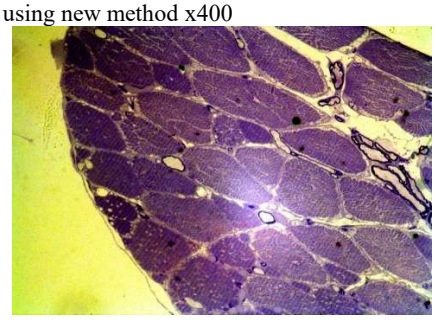

Fig 11. Fish muscle section made using new method x400

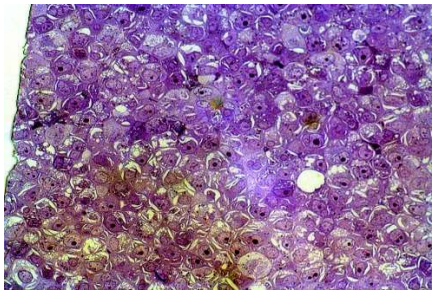

Fig 12. Cultured animal cell section made using new method x400

Downloaded from https://www.cambridge.org/core. IP address: 179.61.188.57, on 20 Aug 2018 at 17:49:43, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/S1431927618007444

View publication stats