Designation: E 1880 – 97

Standard Practice for Tissue Cryosection Analysis with SIMS[1]

This standard is issued under the fixed designation E 1880; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice provides the Secondary Ion Mass Spectrometry (SIMS) analyst with a method for analyzing tissue cryosections in the imaging mode of the instrument. This practice is suitable for frozen-freeze-dried and frozen-hydrated cryosection analysis.

1.2 This practice does not describe methods for optimal freezing of the specimen for immobilizing diffusible chemical species in their native intracellular sites.

1.3 This practice does not describe methods for obtaining cryosections from a frozen specimen.

1.4 This practice is not suitable for any plastic embedded tissues.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 673 Terminology Related to Surface Analysis[2]

3. Terminology

3.1 *Definitions:*

3.1.1 See Terminology E 673 for definitions of terms used in SIMS.

4. Summary of Practice

4.1 This practice describes a method for the analysis of tissue cryosections with SIMS. Tissue cryosections for SIMS analysis need to be mounted flat on an electrically conducting substrate. Cryosections should remain flat and adhere well to the substrate for SIMS analysis. This is achieved by pressing frozen cryosections into an indium substrate. Indium, being a malleable metal (Moh hardness = 1.2, Young's modulus = 10.6 GPa), provides a "cushion" for pressing and holding the frozen cryosections flat for SIMS analysis. Indium substrates are prepared by pressing sheet indium onto a polished silicon wafer. An approximately 1 µm thick layer of indium (99.999 % purity) is then vapor deposited on this surface. This top layer provides "fluffy" indium that helps in holding cryosections flat for SIMS analysis.

5. Significance and Use

5.1 Pressing cryosections flat onto a conducting substrate has been one of the most challenging problems in SIMS analysis of cryogenically prepared tissue specimens. Frozen cryosections often curl or peel off, or both, from the substrate during freeze-drying. The curling of cryosections results in an uneven sample surface for SIMS analysis. Furthermore, if freeze-dried cryosections are not attached tightly to the substrate, the impact of the primary ion beam may result in further curling and even dislodging of the cryosection from the substrate. These problems render SIMS analysis difficult, frustrating and time consuming. The use of indium as a substrate for pressing cryosections flat has provided a practical approach for analyzing cryogenically prepared tissue specimens.[3]

5.2 The procedure described herein has been successfully used for SIMS imaging of calcium transport in intestinal tissue.[4,5]

5.3 The procedure described here is amenable to soft tissues of both animal and plant origin.

6. Apparatus

6.1 The procedure described here can be used for tissue cryosection analysis with virtually any SIMS instrument.

6.2 A cold stage in the SIMS instrument is needed to analyze frozen-hydrated specimens.[6]

7. Procedure

7.1 Prepare the indium substrate by pressing sheet indium onto polished silicon wafer pieces of approximately 15 to 25 mm^2 surface area, which can be irregularly shaped. Next,

[1] This practice is under the jurisdiction of ASTM Committee E-42 on Surface Analysis and is the direct responsibility of Subcommittee E42.06 on SIMS.

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[2] *Annual Book of ASTM Standards*, Vol 03.06.

[3] Sod, E. W., Crooker, A. R., and Morrison, G. H., "Biological Cryosection Preparation and Practical Ion Yield Evaluation for Ion Microscopic Analysis," *Journal of Microscopy (Oxford)*, Vol 160, 1990, p. 55.

[4] Chandra, S., Fullmer, C. S., Smith, C. A., Wasserman, R. H., and Morrison, G. H. "Ion Microscopic Imaging of Calcium Transport in the Intestinal Tissue of Vitamin D-deficient and Vitamin D-replete Chickens: $A^{44}Ca$ Stable Isotope Study," *Proceedings of the National Academy of Sciences (USA)*, Vol 87, 1990, p. 5715.

[5] Chandra, S., and Morrison, G. H., "Sample Preparation of Animal Tissues and Cell Cultures for Secondary Ion Mass Spectrometry (SIMS) Microscopy," *Biology of the Cell*, Vol 74, 1992, p. 31.

[6] Chandra, S., Bernius, M. T., and Morrison, G. H., "Intracellular Localization of Diffusible Elements in Frozen-hydrated Biological Specimens with Ion Microscopy," *Analytical Chemistry*, Vol 58, 1986, p. 493.