This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

Designation: E1880 – 12 (Reapproved 2020)

Standard Practice for Tissue Cryosection Analysis with SIMS[1]

This standard is issued under the fixed designation E1880; 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E673 Terminology Relating to Surface Analysis (Withdrawn 2012)[3]

3. Terminology

3.1 *Definitions:*

3.1.1 See Terminology E673 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 (**1**).[4]

5.2 The procedure described herein has been successfully used for SIMS imaging of calcium and magnesium transport and localization of anticancer drugs in animal models (**2**, **3**, **4**, **5**).

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

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

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

[4] The boldface numbers in parentheses refer to a list of references at the end of this standard.

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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 mm^2 to 25 mm^2 surface area, which can be irregularly shaped. A simple mechanical Pellet Press[5] can be used effectively for pressing for pressing sheet indium onto the surface of polished silicon wafer. Next, vapor deposit an approximately 1 µm thick layer of high purity (99.999 %) indium onto the pressed indium sheet. The high purity of indium is emphasized only due to the fact that it should not impart any significant contamination to the sample. The vapor deposition can be achieved by vacuum-based processes such as evaporation from a heated filament or sputtering from an indium target. The indium substrates are now ready for use.

7.1.1 Chill an individual indium substrate by immersing it into liquid nitrogen prior to its use for pressing cryosections. Quickly transfer the indium substrate to the cryomicrotome and keep at the desired temperature of cryosectioning. Place a frozen tissue cryosection on the indium substrate and gently press by using a new chilled silicon piece. Make sure that the polished surface of the top silicon piece is used to press the cryosection onto indium substrate in order to avoid introducing the irregular topography of the rough silicon surface. Remove the top silicon piece by sliding it off using chilled tweezers. The pressed frozen cryosection on the indium substrate is now ready for frozen-hydrated analysis with a cold stage in the SIMS instrument. Alternatively, the pressed cryosection on the indium substrate can be freeze-dried by transferring the indium substrate to a freeze-drier.

7.1.2 Upon completion of freeze-drying, the freeze-drier should be opened by introducing dry gasses (N_2, Ar, etc.) in order to avoid rehydration of tissue sections. The indium substrates containing freeze-dried tissue sections should be quickly transferred to a desiccator for storage. The freeze-dried cryosections are now ready for SIMS analysis.

7.1.3 Depending on the need of a particular SIMS analysis, the freeze-dried cryosections may be analyzed directly or gold coated to enhance electrical conductivity.

7.1.4 A quick visual inspection of the cryosection surface should be made prior to its insertion into the sample chamber of the SIMS instrument. A reflected light microscope can be used to observe any folds, ripples or loosely attached regions in the section. At this stage, it is always desirable to "repress" the freeze-dried section gently into the indium with a polished silicon piece. It is also desirable to remove the loosely attached pieces of tissue section from the substrate by using tweezers.

7.1.5 Correlative morphological information to complement the SIMS analysis can be made by using adjacent cryosections for optical microscopy and SIMS analysis (**2**).

8. Keywords

8.1 SIMS

[5] The sole source of supply of the Pellet Press apparatus known to the committee at this time is Parr Instrument Co., Moline, IL. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

REFERENCES

(**1**) 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.

(**2**) 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.

(**3**) 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.

(**4**) 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.

(**5**) Smith, D. R., Chandra, S., Barth, R.F., Yang, W., Joel, D.D., and Coderre, J.A., "Quantitative Imagining and Microlocalization of Boron-10 in Brain Tumors and Infiltrating Tumor Cells by SIMS Ion Microscopy: Relevance to Neutron Capture Therapy," Cancer Research, Vol 61, 2001, p. 8179.

(**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.

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