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StandardGuide for Cell Culture Analysis with SIMS[1]

This standard is issued under the fixed designation E1881; 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 guide provides the Secondary Ion Mass Spectrometry (SIMS) analyst with a cryogenic method for analyzing individual tissue culture cells growing in vitro. This guide is suitable for frozen-hydrated and frozen-freeze-dried sample types. Included are procedures for correlating optical, laser scanning confocal and secondary electron microscopies to complement SIMS analysis.

1.2 This guide is not suitable for cell cultures that do not attach to the substrate.

1.3 This guide is not suitable for any plastic embedded cell culture specimens.

1.4 *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:*[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E673 Terminology Relating to Surface Analysis (Withdrawn 2012)[3]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 Guide

4.1 This guide describes a cryogenic freeze-fracture method of sample preparation for cell culture specimens for SIMS analysis. In brief, cell cultures are grown on a conducting substrate, such as silicon. When cells reach about 80 % confluency, they are fast frozen and fractured by using a sandwich method (**1**).[4] This allows freeze-fixation of cellular contents and removal of the EF-leaflet of the apical plasma membrane. Since this kind of fracture occurs in groups of cells growing together, fractured cells are easily recognized for optical, SEM and SIMS imaging.

4.2 By correlative laser scanning confocal microscopy and SIMS, the same frozen freeze-dried cell can be analyzed for organelle localization in relation to elemental content (**2**).

5. Significance and Use

5.1 The presence of cell growth medium complicates a direct analysis of cells with SIMS. Attempts to wash out the nutrient medium results in the exposure of cells to unphysiological reagents that may also alter their chemical composition. This obstacle is overcome by using a sandwich freeze-fracture method (**1**). This cryogenic method has provided a unique way of sampling individual cells in their native state for SIMS analysis.

5.2 The procedure described here has been successfully used for imaging Na^+ and K^+ ion transport (**3**), calcium alterations in stimulated cells (**4,5**), and localization of therapeutic drugs and isotopically labeled molecules in single cells (**6**). The frozen freeze-dried cells prepared according to this method have been checked for SIMS matrix effects (**7**). Ion image quantification has also been achieved in this sample type (**8**).

5.3 The procedure described here is amenable to a wide variety of cell cultures and provides a way for studying the response of individual cells for chemical alterations in the state of health and disease and localization of isotopically-labeled molecules and theraputic drugs in cell culture models.

6. Apparatus

6.1 This guide can be used for the analysis of cell cultures with virtually any SIMS instrument.

6.2 A cold stage in the SIMS instrument is needed to analyze frozen-hydrated specimens (**9**).

[1] This guide 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 guide.