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Standard Guide for Cell Culture Analysis with SIMS[1]

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E673 Terminology Relating to Surface Analysis (Withdrawn 2012)[3]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

7. Procedure

7.1 Cells are grown on silicon wafer pieces (approximately 1 cm^2 area) of any shape. Alternatively, high purity germanium wafer pieces are used for cell growth for studies involving the use of ^{44}Ca stable isotope. These substrates are nontoxic to cells and have been used for growing various cell lines (**1**,**2**,**8**). Sterilize the silicon or germanium pieces prior to cell seeding. After the cells reach about 80 % confluency, replace the nutrient growth medium with new medium containing 11 µm polystyrene beads (approximately 50 000 beads per 100 mm plastic dish, see Ref (**1**) for details on size of the beads). These beads act as spacers during the sandwich-fracture technique. It takes approximately 30 min for the beads to settle down on the substrate. After beads settle down on the substrate the cells can be subjected to desired treatments and cryogenic sampling.

7.1.1 After the desired treatments fast freeze and freeze-fracture the cells by a sandwich technique which involves the following steps: *(1)* remove the silicon piece containing the cells from the nutrient medium, *(2)* remove excess nutrient medium from the cells by touching one edge of the silicon piece with filter paper, *(3)* place a new and clean silicon wafer piece on top, sandwiching the cells between two polished surfaces, *(4)* fast freeze the sandwich in cryogenic fluids (supercooled isopentane, propane, liquid nitrogen, and so forth), *(5)* transfer the sandwich quickly to liquid nitrogen, and *(6)* fracture the sandwich by prying apart the two halves under liquid nitrogen. At this stage the silicon piece used for growing the cells contains a group of cells fractured together at the basal or dorsal cells surfaces, and randomly scattered individual cross fractured cells where the fracture plane has passed through the cytoplasm and/or nucleus (**10**). In a group of cells fractured at the dorsal cell surface the apical plasma membrane fracture removes the extracellular nutrient medium and the EF-leaflet of the plasma membrane on the top silicon piece (**1**, **10**). The fractured cells on the silicon substrate can be analyzed frozen-hydrated or after freeze-drying with SIMS imaging techniques.

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

7.1.3 For correlative optical, SEM and SIMS, fractured freeze-dried cells can be imaged with a reflected light microscope or SEM prior to SIMS analysis (**11**).

7.1.4 For organelle localization in relation to SIMS isotope images, a correlative laser scanning confocal microscopy and SIMS approach has been developed (**2**). This approach relies on labeling the organelles with specific fluorescent markers in live cells and then mapping the organelle localization in 3-D with a laser scanning confocal microscope in a fractured freeze-dried cell prior to SIMS analysis of the same cell (**2**,**4**,**5**).

7.1.5 This sandwich freeze-fracture method has been successfully used for dynamic SIMS studies of quantitative subcellular localization of anticancer agents in human cancer cell lines (**12**, **13**), and 3-D quantitative imaging of subcellular calcium stores in cells undergoing cell division (**14**).

7.1.6 This sandwich freeze-fracture method has found usages in static Time-of-flight SIMS and Laser-SNMS techniques for molecular and atomic localization studies in mammalian cells and single cell organisms (**15-17**).

8. Keywords

8.1 SIMS

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