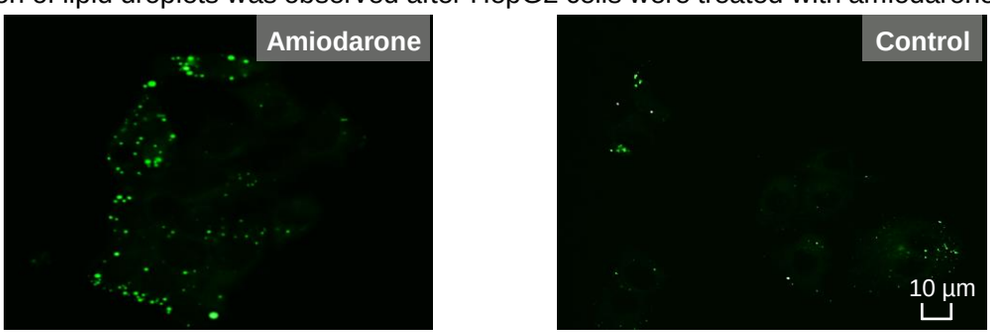
Application Notes

Single Cellome™ System SS2000 Lipid Droplet Sampling and Live Single-cell Mass Spectrometry

In recent years, research on single cells has become increasingly popular. With more sensitive analytical techniques, it has become possible to analyze specific intracellular components such as organelles, even at the single-cell level.
The SS2000 is an innovative system that can sample sub-cellular intracellular components using a glass capillary having a tip diameter of only a few micrometers while imaging with a confocal microscope. In this application note, we introduce a case where after drug treatment, intracellular components were sampled by the SS2000 and analyzed by single-cell mass spectrometry.

Aggregation of lipid droplets by amiodarone treatment

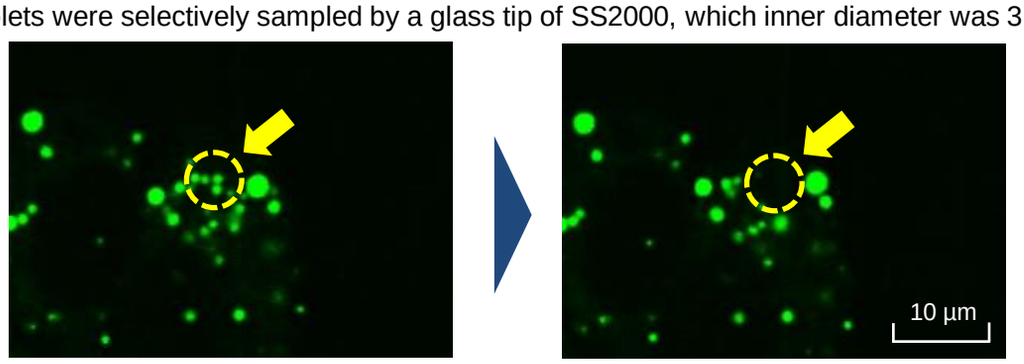
Aggregation of lipid droplets was observed after HepG2 cells were treated with amiodarone.



(Green: lipid droplet)

Sampling of lipid droplet

Lipid droplets were selectively sampled by a glass tip of SS2000, which inner diameter was 3 µm.

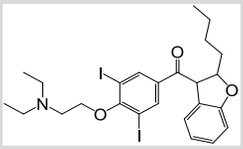


(Green: lipid droplet)

Data provided by Professor Hajime Mizuno, Analytical Chemistry lab of Pharmacology department, Meijo University

Amiodarone

This antiarrhythmic drug has serious side effects including liver damage, and induces intracellular phospholipidosis, resulting in the aggregation of lipid droplets

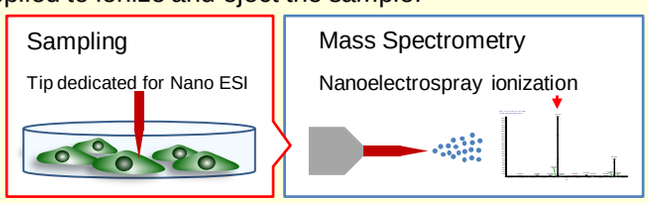


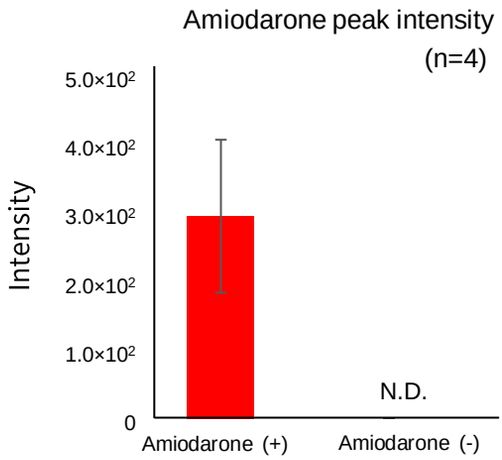
Detection of Amiodarone by Live Single-cell Mass Spectrometry

When single-cell mass spectrometry was performed on the collected sub-cellular lipid droplets, Amiodarone was detected only in samples from drug-treated cells, confirming that Amiodarone accumulated in the lipid droplets.

Single-cell Mass Spectrometry[3)]

Intracellular components were extracted by adding a mix of organic solvent and water into the glass capillary tube. This was then attached to the mass spectrometer and voltage applied to ionize and eject the sample.





Data provided by Professor Hajime Mizuno, Meijo University

Experimental Condition

Cell :	HepG2（35 mm Dish）
Drug :	Added 10 μM Amiodarone or 5.4 μL Ethanol (control) and cultured cells overnight
Reagent :	LipiDye (Ex:488 nm, Em:525 nm)
Sampling :	Sampled the lipid droplets by the tip with an inner diameter of 3 μm dedicated for nano ESI, then this tip with samples was directly applied to the mass spectrometer.
Mass Spectrometry :	Xevo TQ-S, Waters Ion Mode; nanoESI positive、Capillary Voltage; 1.0 kV MS Mode; MRM (646.05 > 58.16)、Collision Energy; 40 V

Subcellular Sampling and Live Single-cell Mass Spectrometry

By selectively sampling intracellular components in a specific region at the single-cell level and analyzing them by mass spectrometry, it is possible to determine the subcellular localization and metabolic levels of administered drugs and their metabolites.
The SS2000 enables the selective sampling of intracellular “micro-regions” by using confocal microscopy and precise tip positioning technology. The combination of SS2000 and single-cell mass spectrometry is expected to contribute greatly to a more detailed understanding of biological phenomena and new drug development.

Expert Comment from Professor Hajime Mizuno

“The SS2000 has enabled us not only to observe cells with induced phospholipidosis by confocal microscopy but also to accurately detect drugs and their metabolites localized in lipid droplets by selective sampling and performing mass spectrometry at a single-cell level.
Currently, our major challenge is to elucidate more detailed cellular mechanisms by analyzing metabolites localized in various intracellular organelles such as mitochondria.”

Reference

1) N. Anderson, et al. Drug-induced phospholipidosis. *FEBS Letters*, 2006 Oct.; 580 (23): 5533-5540.
2) K. Yahata, H. Mizuno, et al. Analysis of the intracellular localization of amiodarone using live single-cell mass spectrometry. *J. Pharm. Biomed. Anal.*, 2021 Oct 25;205:114318.
3) H. Mizuno, et al. Live single-cell video-mass spectrometry for cellular and subcellular molecular detection and cell classification. *J. Mass. Spectrom.*, 2008 Dec.; 43 (12): 1692-1700.

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Printed in Japan, 310 [Ed:01]

AN 80S01B03-01E