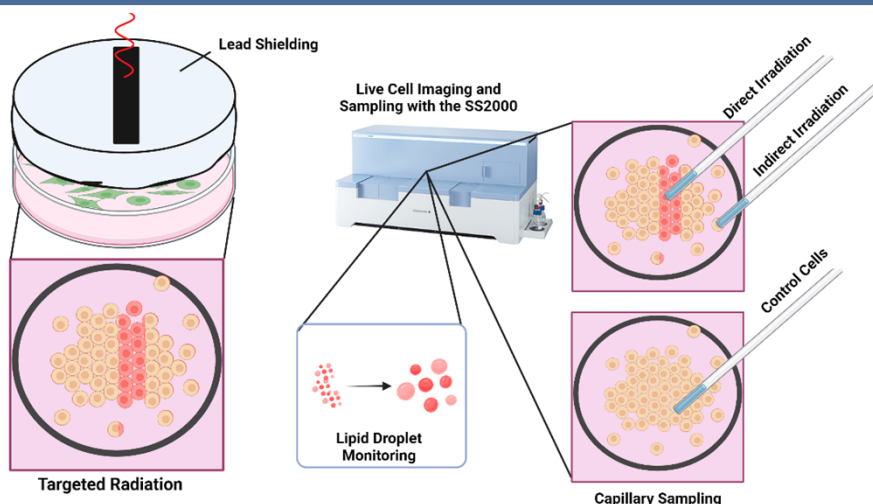


Radiation-Induced Lipid Remodeling at the Single-Cell Level Using LC-MS

Single Cellome™ System SS2000

The importance of multi-omics research at single-cell and sub-cellular levels has grown significantly in recent years, especially for developing cancer treatments. Single Cellome™ System SS2000 supports this research by enabling sampling of single cells or cellular compartments in a controlled environment with simultaneous confocal imaging. For downstream applications, cells can be sampled directly into analytical vessels for storage, shipping, or immediate analysis. We introduce a method where cells exposed to high X-ray doses were incubated for several days while lipid droplet size changes were tracked by confocal imaging. Later, cells were sampled, and lipid composition and abundance were analyzed by LC-MS/MS and LS-MS, respectively.

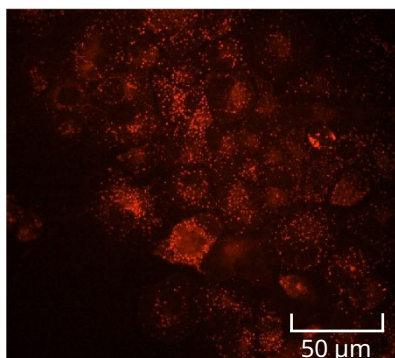
Workflow for cell irradiation, imaging and sampling



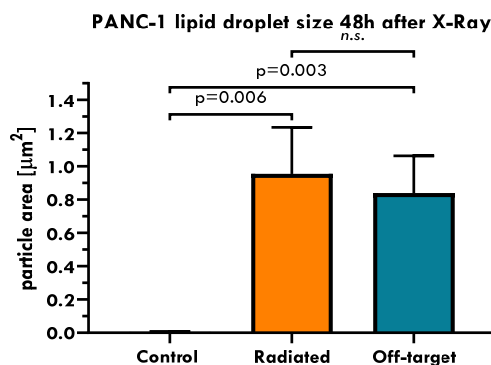
PANC-1 cells were irradiated through a slit in lead shielding and incubated in SS2000 for 48 h with imaging intervals every 2 h. Next, SS2000 was used to sample directly irradiated cells, bystanders, and cells from a control dish. Sampled cells were divided into two categories and either analyzed by analytical flow LC-MS or nanoflow LC-MS in different laboratories, respectively.

Imaging lipid droplets of cells irradiated with high-dose X-rays with SS2000

Aggregation of lipid droplets was observed 48 h after PANC-1 cells were irradiated uniformly. Lipid droplet size was significantly higher in directly and indirectly irradiated cells compared to control cells.

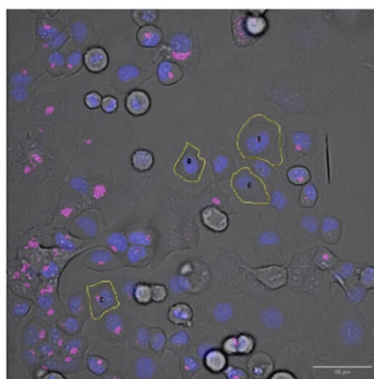


Lipid droplets stained with Nile Red



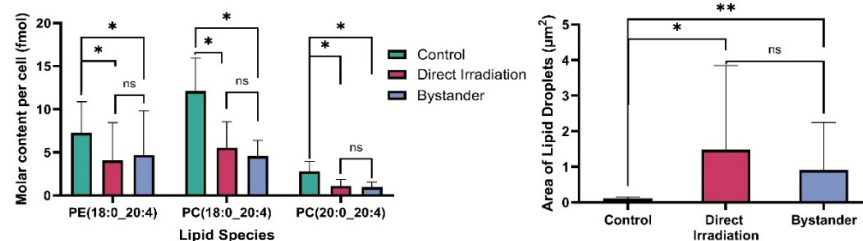
Data from Professor Melanie Bailey, King's College London, London

Single-cell sampling and LC-MS/MS analysis of lipid compositions



Pink: BODIPY – lipid droplets
Blue: Hoechst 34580 – nuclei

Complete cells were sampled using an SS2000 glass tip with a 10 μm inner diameter. Single-cell mass spectrometry revealed that lipid composition in irradiated, and bystander cells differed significantly from controls, exemplified by arachidonic acid-containing PC lipids (left). Imaging confirmed this, showing larger lipid droplet areas in irradiated and bystander cells compared to controls (right).



Experimental Conditions

For more details, please see Reference 1

Cell:	PANC-1
Irradiation:	195 kV, 10 mA for 346 s, delivering a uniform dose of 6 Gy
Dyes:	Nile Red (Ex: 480 nm, Em: 617 $\pm$ 73 nm), BODIPY (Ex: 488 nm, Em: 525 $\pm$ 50 nm), Hoechst 34580 (Ex: 405 nm, Em: 447 $\pm$ 60 nm)
Sampling:	MT-04 glass tips (I.D. 10 μm) were dedicated to LC-MS and LC-MS/MS analyses, respectively.
Collection:	Collected cells were eluted in Qsert LC-MS vials and freeze-dried prior to analysis.
Mass Spectrometry:	Analytical flow LC-MS: UltiMate™ 3000 UHPLC and Q Exactive™ Plus Nanoflow LC-MS/MS: Vanquish™ Neo and Orbitrap Exploris™ 240

Conclusion

The combination of selective single-cell sampling with single-cell lipidomics by LC-MS and LC-MS/MS allowed the demonstrated that bystander cells exhibited lipid composition changes similar to those of directly irradiated cells and significantly different from controls, especially in arachidonic acid-containing PC lipids. Notably, the lipidomic alterations in bystander cells closely mirrored those in directly irradiated cells, suggesting that radiation-induced effects can propagate to neighboring cells. The findings were consistent across two laboratories despite differences in sample handling and analysis and were further supported by live-cell imaging of lipid droplets. This work highlights the possibilities of SS2000 supporting single-cell-lipidomics to study cellular responses to stress, thus increasing its potential for advancing cancer research and radiation biology.

Expert Comment from Professor Melanie Bailey

"SS2000 gives a unique opportunity to relate the morphology of a cell, or the expression of particular fluorescent markers to its lipid profile. At the SEISMIC facility we are using SS2000 with researchers from all over the UK to apply this capability to understand and control viruses and bacterial infections, immune conditions and cell ageing. Another exciting prospect of the instrument is the possibility to extract sub-cellular samples, and we are also working to develop its sub-cellular sampling in conjunction with mass spectrometry."

Reference

- 1) Saunders et al., Single-Cell Lipidomics by LC-MS Interlaboratory Study Reveals the Impact of X-ray Irradiation on a Pancreatic Cancer Cell Line and Its Bystanders. *Anal. Chem.*, 2025, 97, 13532–13541. doi:10.1021/acs.analchem.5c02010.

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