Integration of MALDI FTICR MSI data with the open-source software Cytomine

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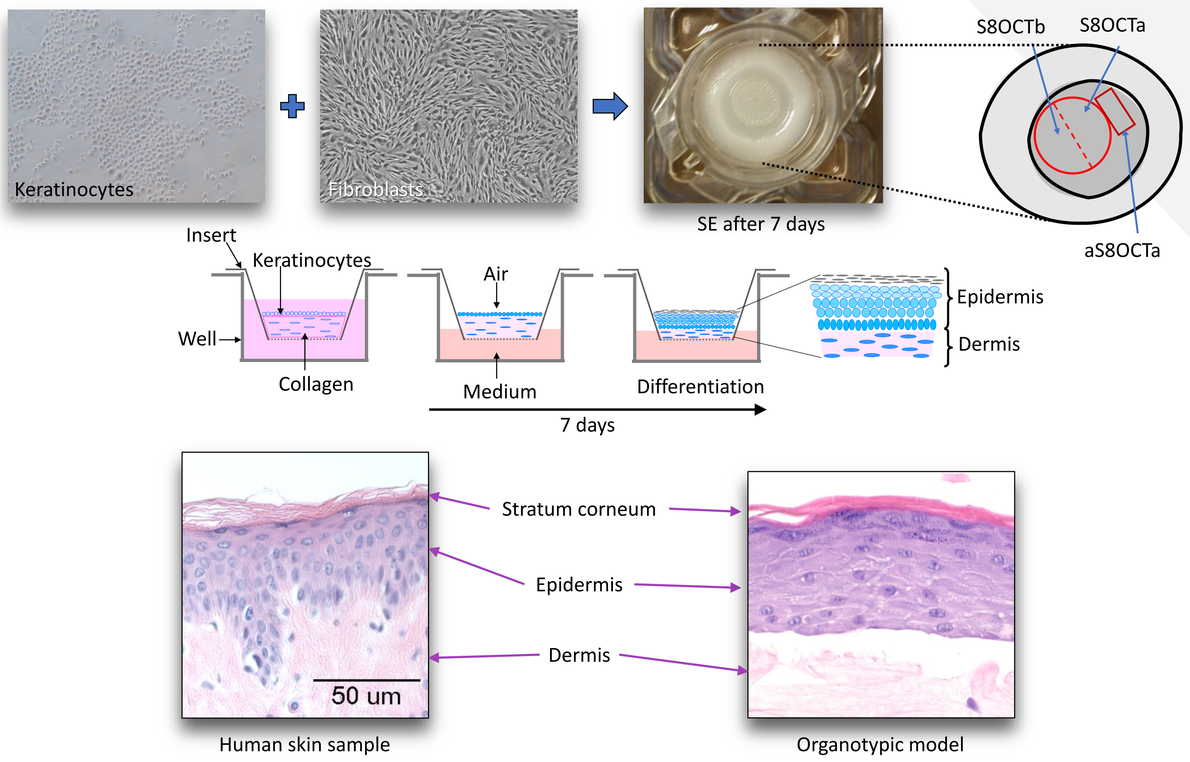
Introduction

Our skin is constantly exposed to solar radiation, high oxygen levels, and environmental pollutants. Our study aims to target specific native (LipS) and oxidized phospholipids (oxLipS) that are known to be senescence-associated secretory phenotype (SASP) related (i.e., LysoPC, oxPAPC).[1] The production of these oxidized species is obtained after exposure to UV light. Here, we employ MALDI FTICR mass spectrometry imaging (MSI) to visualize and identify lipid species of interest in an organotypic model system (Fig. 1) and to integrate this data with the open-source software Cytomine.[2]

Method

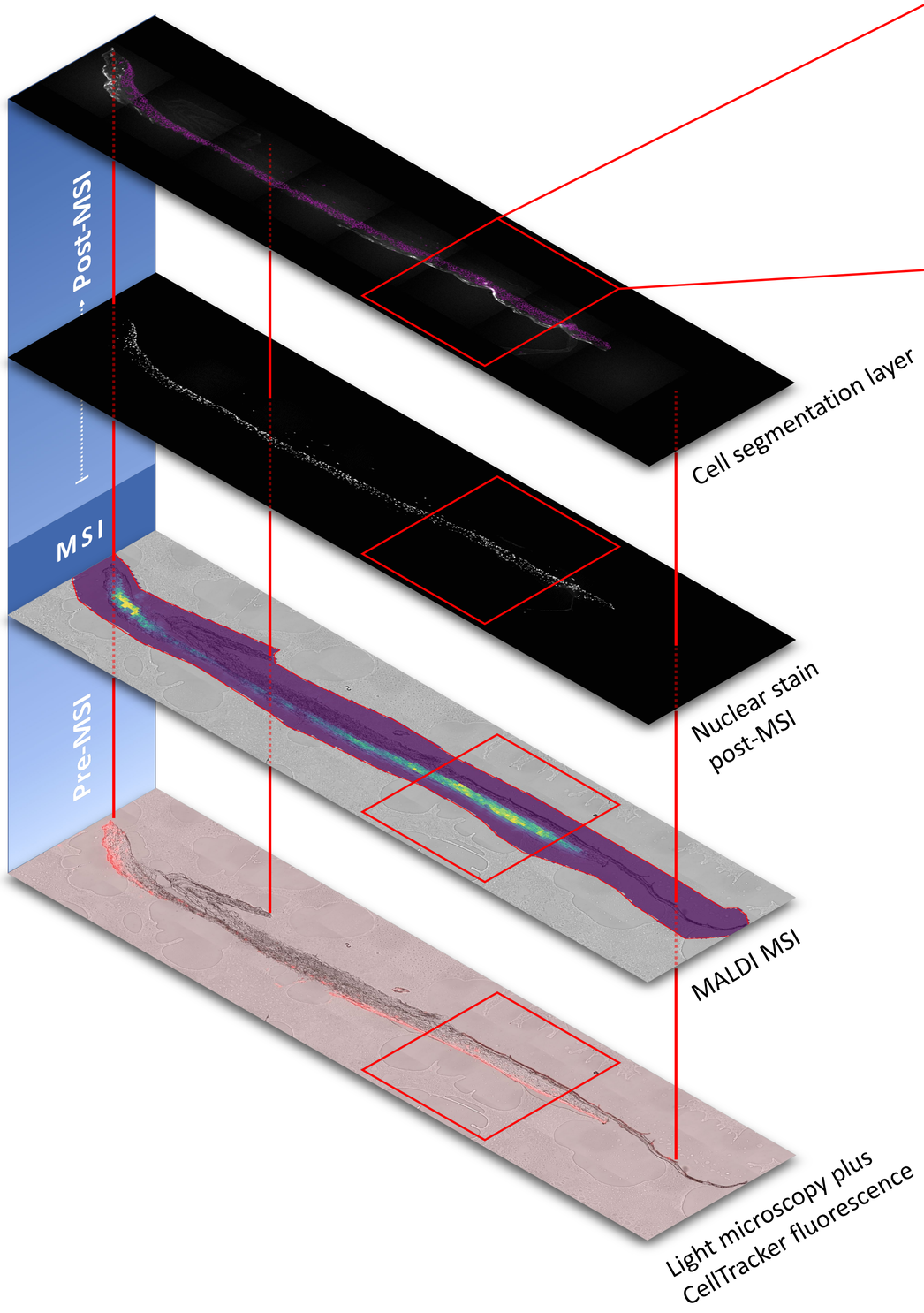
- Sample characteristics:
  - Sample code: S8OCTa
  - Epidermis contains 6% pre-irradiated keratinocytes (20 mJ/cm² UVB)
  - Snap-frozen and embedded in OCT, 6µm cryosections
- MALDI FTICR MSI settings:
  - 7T MRMS (scimaX, Bruker), home-built sublimation device
  - MALDI MSI settings: 1,5 DAN @0.26 mg/cm², 4M, positive polarity, 5 laser shots @50 Hz, lat. res. at 20 µm, matrix peak area normalization [3]

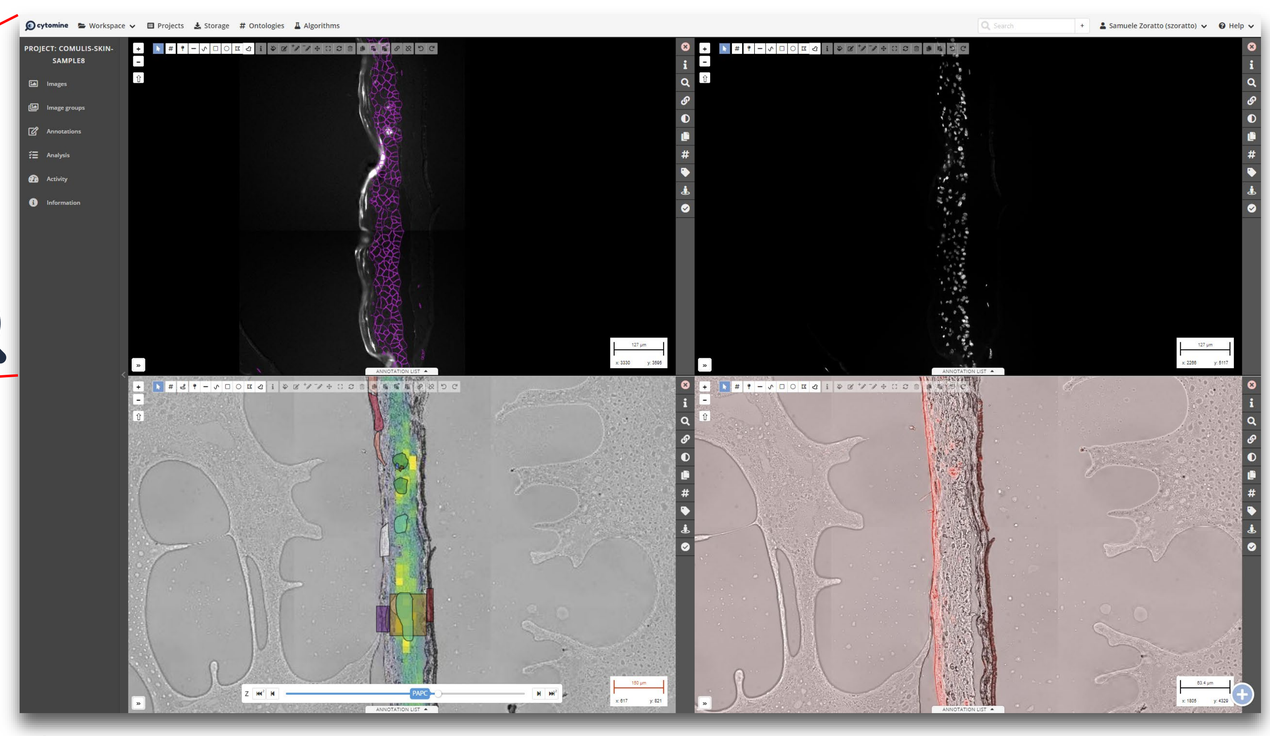
Fig 1: Organotypic model system containing human cells



Graphics M. Mildner and C. Kremslehner

Results





On the top:

- Main view of the Cytomine web-based platform.
- Several images from different sources can be imported into a single project and displayed together.
- Images' coordinates can be linked together, assisting ROI visualization, identification, and annotation.
- Annotations of ROI are collaborative and shared by different team members.

On the left:

- Concept representing the multimodal imaging approach.
- At the bottom: high-resolution bright-field microscopy images are overlayed with fluorescently-labeled keratinocytes. CellTracker™ Red fluorescent dye is used to localize the 6% of UVB pre-irradiated keratinocytes used to generate the skin equivalent model.
- Second level: MALDI FTICR MSI at 20 µm lateral resolution. The image represents the m/z value corresponding to PAPC; a known lipid prone to radical oxidation.
- Third level: post-MSI analysis, the sample is stained to localize the nuclei, after immunofluorescence images are acquired.
- Top-level: based on the nuclear stain images, cell segmentation is generated.

Table 1. Name and mass accuracy of the oxLipS identified.

Lipid Name	Theoretical mass (m/z)	Avg meas. mass (N=6, m/z)	ppm error
1,5 DAN (matrix peak)	317.1761	317.1763	0.62
LysoPPC	496.3398	496.3407	1.87
LysoSPC	524.3711	524.3719	1.64
PC 18:1	550.3503	550.3513	1.79
PONPC	650.4392	650.4408	2.47
PC 16:0	664.4184	664.4200	2.42
PAzPC	666.4341	666.4351	1.57
DPPC	734.5694	734.5705	1.45
PLPC	758.5694	758.5712	2.32
PAPC	782.5694	782.5694	-0.03
SLPC	786.6007	786.6018	1.42
PAPC-OH	798.5644	798.5640	-0.43
PDHPC	806.5694	806.5696	0.26
SAPC	810.6007	810.6003	-0.55
isoPGJ2	812.5436	812.5439	0.35

Conclusion

- ✓ OxLipS identified from MALDI FTICR MSI on skin equivalent models
- ✓ OxLipS correctly localized according to skin structure
- ✓ Multimodal results integrated in Cytomine
- ✓ Teamwork ROI annotation for future AI-based application

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