

Tissue pathology of aging research revealed with lipid analysis using MRMS MALDI Imaging

The synergy between MALDI Imaging techniques and high-resolution MRMS technology allows for high-fidelity detection of analytes and their relative spatial localization.

Introduction

As biological age advances the reduction of the natural cellular regeneration process leads to an accumulation of senescent cells. In skin, this manifests the morphological phenotype of aged skin. Cellular senescence is a state of (typically) irreversible cell cycle arrest resulting from damage or developmental cues, where cellular metabolism is altered, and senescent-specific molecules are released representing the so-called senescence-associated secretory phenotype (SASP). It has been reported that phospholipids (PLs) and their oxidized forms are SASP factors and play a crucial role in skin aging [1]. A candidate for monitoring the skin aging progress is the lipid PAPC (1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine, Figure 1A), a major component of cell membranes and lipoproteins. The oxidation and fragmentation of its arachidonate moiety leads to the production of several oxidation products (oxPAPCs), like LysoSPC (1-stearoyl-2-lyso-sn-glycero-3-phosphorylcholine, Figure 1B) and LysoPC (1-palmitoyl-2-lyso-sn-glycero-3-phosphorylcholine, Figure 1C), which are involved in the modulation of inflammatory processes and contribute to the onset of premature skin aging [2].

Keywords:
MALDI Imaging, scimaX,
Lipidomics, SCiLS Lab

The ability to precisely visualize the relative abundance and distribution of different molecules in biological tissue provides important insights into the structure and function of the system itself. This work focused on studying PAPC and its several oxPAPCs in skin tissue. The eXtreme Resolution (XR) provided by MRMS combined with MALDI Imaging capabilities proved critical for the lateral distribution of molecular species differing only by a few mDa. The resulting images allowed for precise visualization, identification, and observation of the different spatial localizations of PAPC and oxPAPC species for young and aged skin.

Methods

OCT-embedded biopsies from two female patients (aged 31 and 63 years old) were collected following national ethical guidelines, cryosectioned at 10 μm , and mounted on to conductive glass slides. While normal protocols dictate OCT removal, here washing was not done due to the fragility of the sample. The MALDI matrix 1,5 diaminonaphtalene (1,5 DAN) was applied via a home-built sublimation device [3]. MALDI Imaging experiments were performed on a 7T scimaX MRMS instrument (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) in positive ion mode at lateral spatial resolution of 40 μm and 10 μm from m/z 107-2000 with a transient length of roughly 1 second using 2-omega mode detection resulting in a resolving power of 350,000 at m/z 400. Data analysis was performed with SCiLS™ Lab 2023a Pro (Bruker Daltonics), and raw data was normalized to the matrix peak area (m/z 317.17607). Lipid identification was based on accurate mass (below 1 ppm error) and database search using LipidMaps (mass tolerance $m/z \pm 0.005$) [4].

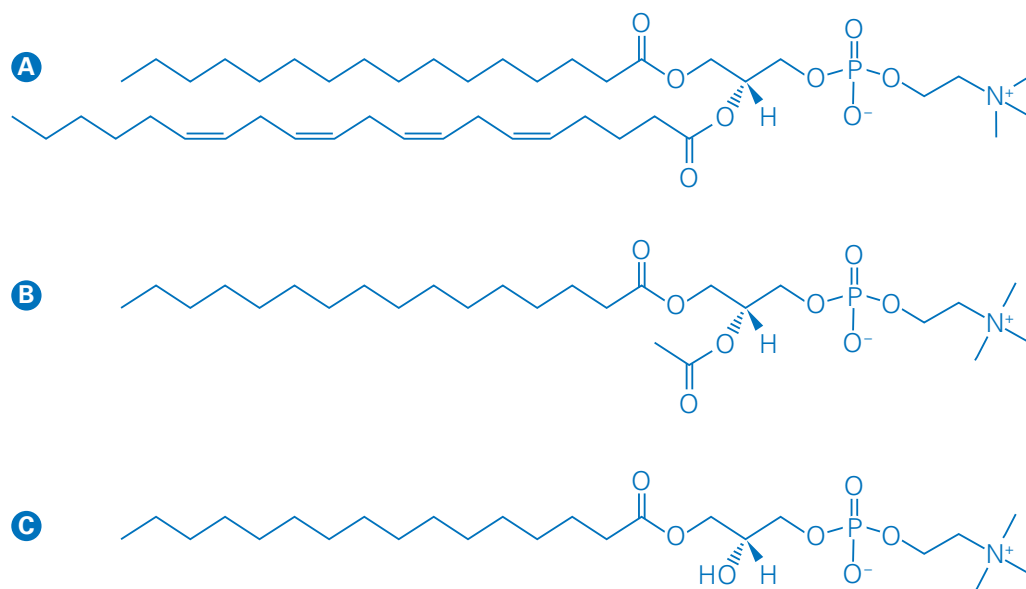


Figure 1
Chemical structure of (A) non oxidized lipid PAPC; (B) interim species LysoSPC; and (C) oxidation end product LysoPC.

Results

The distribution of PLs in different skin compartments is essential for a better understanding of skin aging, making MALDI Imaging an unprecedented tool for an unbiased imaging approach with broad biochemical detection. The synergy with accurate mass measurement at high mass resolution allows for the identification of unknown species.

Distribution of PAPC (m/z 782.56933, mass error: 0.13 ppm) and its subsequent oxidation products, LysoSPC (m/z 524.37100, mass error: 0.13 ppm) and LysoPC (m/z 496.33980, mass error: 0.08 ppm), to name a few, show that the non-oxidized lipid is abundantly present in the sample from the young donor, while being depleted in the old one (Figure 2A). LysoSPC and LysoPC are not only mainly present in the aged sample but show strong opposite compartment distribution (Figure 2B and 2C). High spatial resolution imaging at 10 μm shows that PAPC and both oxPAPC co-localize in the younger skin sample (Figure 3). In contrast, PAPC is no longer observed in the aged skin while LysoSPC and LysoPC co-localize. Additionally, LysoPC is the more abundant species, especially in the upper layer of the dermis. This can be explained by the fact that LysoPC is the final oxidation product of PAPC.

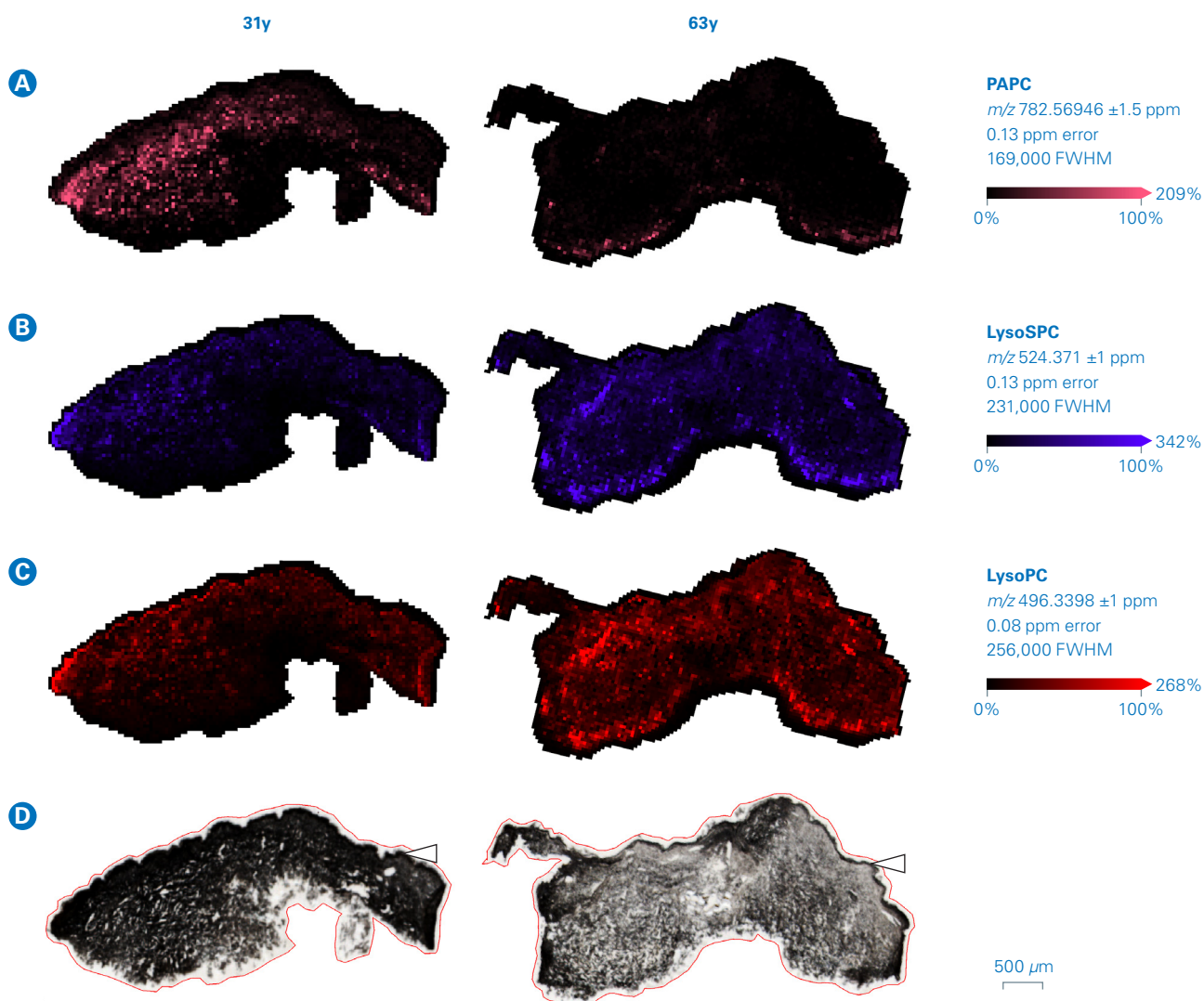


Figure 2

Positive ion MALDI images at 40 μm lateral resolution of selected m/z values in young versus aged skin.

Lipid distribution of (A) PAPC; (B) LysoSPC; and (C) LysoPC; (D) Optical image of the tissue section. White arrow: epidermal layer.

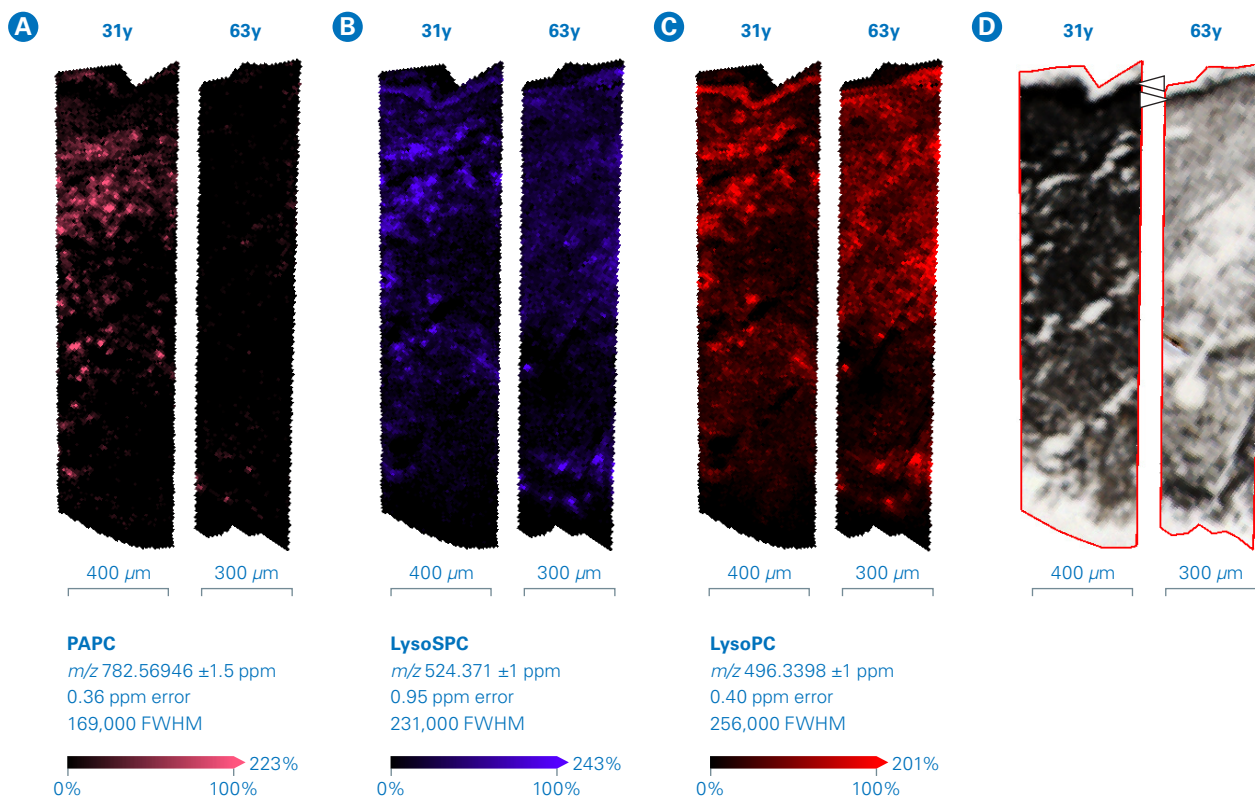


Figure 3

Positive ion MALDI images at 10 μm lateral resolution of selected m/z values in young versus aged skin.

Lipid distribution of **(A)** PAPC; **(B)** LysoSPC; and **(C)** LysoPC. **(D)** Optical image of the tissue section. White arrow: epidermis layer. Young and aged skin samples are shown at a lateral resolution of 40 μm for sample survey (Figure 2) and at 10 μm to highlight cellular details (Figure 3).

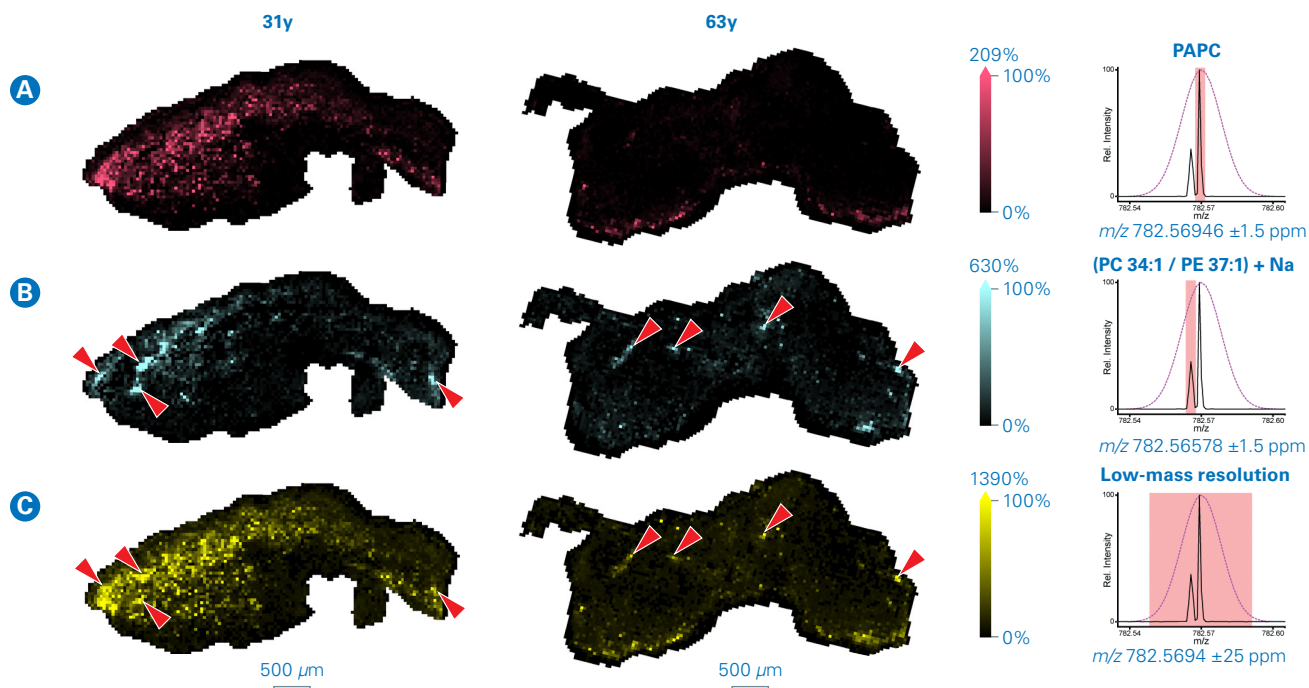


Figure 4

Positive ion MALDI images at 40 μm lateral resolution and relative profile spectra in young versus aged skin.

Lipid distribution for **(A)** PAPC and **(B)** the tentatively assigned signal at m/z 782.56578 are shown together with **(C)** the ion image resulting from an average m/z signal as measured on a lower resolution instrument. Black line spectral profile: overview SCiLS MS spectra. Magenta dotted line simulated profile: simulated mass spectra at FWHM 40K. Red arrows: areas containing high abundance signal present in both **(B)** and **(C)**.

The high mass resolving power of the MRMS instrumentation reveals an m/z value in proximity to PAPC, differing by only 3.4 mDa. This ion signal (Figure 4B and 5B) shows a remarkably different spatial distribution compared to PAPC. Figures 4C and 5C show simulated mass spectra for PAPC at a resolving power of FWHM 40,000, as can be achieved on modern time-of-flight instrumentation. In contrast, the eXtreme Resolution of MRMS enables clear differentiation between the different ion distributions, ensuring accurate ion images.

Tentative identification of this unknown m/z value is currently based on LipidMAPS database search. The closest result (Δ 0.0012 Da, 1.5 ppm error) belongs to the sodiated form of either phosphatidylcholine (PC 34:1) or phosphatidylethanolamine (PE 37:1). Unambiguous identification of this component would require MS/MS head group analysis.

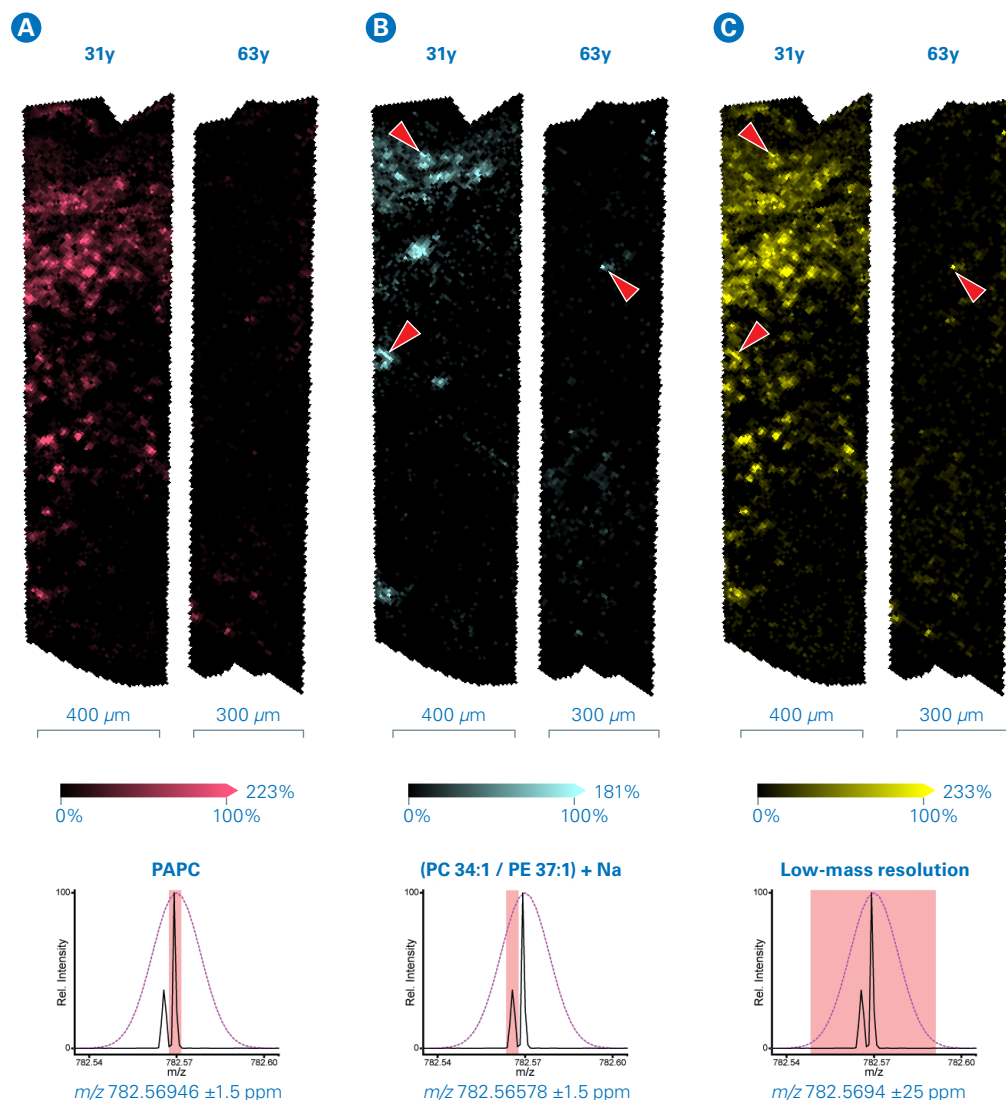


Figure 5

Positive ion MALDI images at 10 μm lateral resolution and relative profile spectra in young versus aged skin.

Lipid distribution for **(A)** PAPC and **(B)** the tentatively assigned signal at m/z 782.56578 are shown together with **(C)**, the ion image resulting from an average m/z signal as measured on a lower resolution instrument. Black line spectral profile: overview SCILS MS spectra. Magenta dotted line simulated profile: simulated mass spectra at FWHM 40K. Red arrows: areas containing high abundance signal present in both **(B)** and **(C)**.

Conclusion

The presented data show the advantages provided by MRMS technology for MALDI Imaging. Lipids are very diverse molecules, and accurate, high-resolution mass measurement is of utmost importance to ensure correct distribution maps. It is shown that eXtreme Resolution provided by scimaX MRMS enables separation of features in the mDa range and is necessary to distinguish different lipid classes and their spatial localization, hence assuring accurate and meaningful data interpretation.

References

- [1] Gruber F, Marchetti-Deschmann M, Kremslehner C., and Schosserer M (2021). *The Skin Epilipidome in Stress, Aging, and Inflammation*. *Front. Endocrinol.*, **11**:607076, DOI: 10.3389/fendo.2020.607076.
- [2] Narzt M, Pils V, Kremslehner C, Nagelreiter I, Schosserer M, Bessonova E, Bayer A, Reifschneider R, Terlecki-Zaniewicz L, Waidhofer-Söllner P, Mildner M, Tschachler E, Cavinato M, Wedel S, Jansen-Dürr P, Nanic L, Rubelj I, El-Ghalbzouri A, Zoratto S, Marchetti-Deschmann M, Grillari J, Gruber F, Lämmermann I (2021). *Epilipidomics of Senescent Dermal Fibroblasts Identify Lysophosphatidylcholines as Pleiotropic Senescence-Associated Secretory Phenotype (SASP) Factors*. *Journal of Investigative Dermatology*, **141**, 4, 993-1006.e15, DOI: 10.1016/j.jid.2020.11.020.
- [3] Holzlechner M, Bonta M, Lohninger H, Limbeck A, Marchetti-Deschmann M (2018). *Multisensor Imaging—From Sample Preparation to Integrated Multimodal Interpretation of LA-ICPMS and MALDI MS Imaging Data*. *Anal. Chem.*, **90**, 15, 8831–8837, DOI: 10.1021/acs.analchem.8b00816.
- [4] Sud M, Fahy E, Cotter D, Brown A, Dennis E, Glass C, Murphy R, Raetz C, Russell D, Subramaniam S (2006). *LMSD: LIPID MAPS® structure database*. *Nucleic Acids Research*, DOI: 10.1093/nar/gkl838.

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