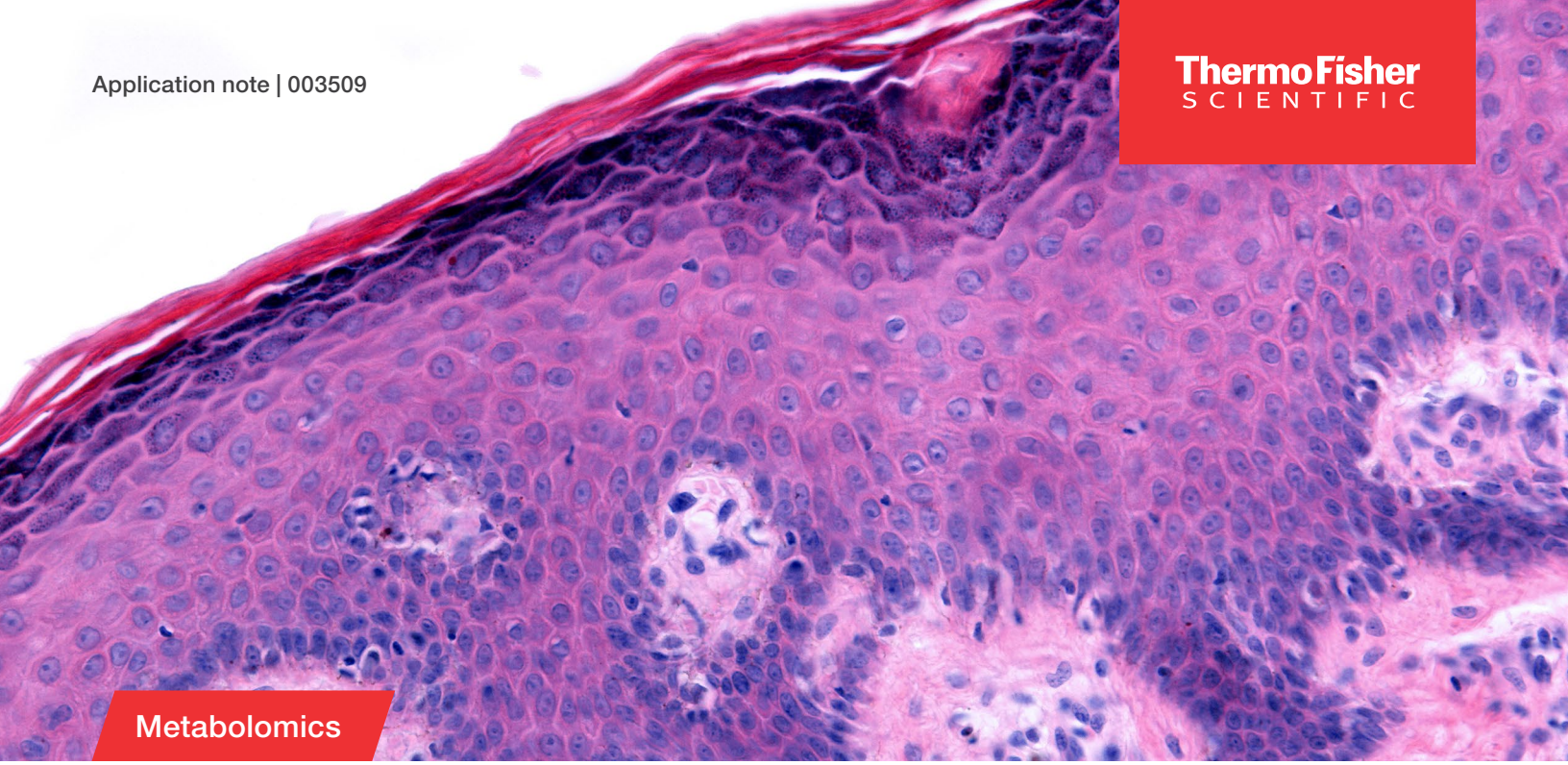
Application note | 003509



Metabolomics

Advancing spatial lipidomics: high spatial resolution mass spectral imaging (MSI) using the MassTech AP-MALDI UHR ion source with the Orbitrap Exploris mass spectrometer

Authors

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Keywords

Orbitrap Exploris mass spectrometer, AP-MALDI MS imaging, mass spectrometry imaging, MSI, high-resolution accurate-mass (HRAM) mass spectrometry, lipidomics, Orbitrap technology, dermatological research

Application benefits

- The combination of AP-MALDI MSI and LC-MS-based lipidomics analyses enables fine (down to 5 µm) spatial characterization and accurate mass determination of lipids of interest in challenging small-sized *in vitro* skin samples, providing unique insights with potential applications in dermatological research and personal-care product development.
- MassTech Inc.'s AP-MALDI UHR (Ultra-High Resolution) ion source makes it possible to switch from an electrospray configuration for LC-MS/MS analysis to a MALDI configuration for MS imaging in less than 2 minutes.
- The high mass resolution and accurate mass (<1 ppm) capabilities of the Thermo Scientific™ Orbitrap™ mass analyzer provide high confidence in lipid annotation.

Goal

To demonstrate the efficacy and utility of the advanced spatial resolution capabilities of the AP-MALDI UHR imaging apparatus coupled with the Thermo Scientific™ Orbitrap Exploris™ series mass spectrometers for the characterization of lipid alterations in *in vitro* reconstructed human epidermis (RHE) models. Additionally, to comprehensively elucidate and localize lipidomic modulation at the cellular level in epidermis subjected to UV radiation and specific sun-filter formulations to gain insights into spatial dermatology, with potential applications in dermatological research and the development of personal-care products.

Introduction

Mass spectrometry imaging (MS imaging, MSI) enables direct analysis of the spatial distribution of molecular species in biological tissues.[1] The approach allows sample analysis without extraction and separation, preserving the morphology of the tissues being studied and enabling researchers to determine the specific location of molecules in specimens. The label-free MSI approach presented in this application note has been applied to various classes of molecules, including metabolites, lipids, peptides, proteins, and glycans.[2]

Because of its ability to ionize a broad range of analyte molecules of different sizes, matrix-assisted laser desorption ionization (MALDI) is a popular MSI ionization technique. While most MALDI MSI experiments are performed under low-pressure (vacuum) conditions, atmospheric pressure (AP)-MALDI MSI offers a game-changing alternative that eliminates source pump-down time, simplifies preparation of hydrated samples, enables analysis of vacuum-incompatible molecules, and allows the use of more volatile matrices without increasing needs for source cleaning (compared to low-pressure MALDI ion sources). Recent breakthroughs in AP-MALDI ion source technology (e.g., novel ion source design) have led to enhanced sensitivity, scan speed, and spatial resolution for imaging experiments.[3,4] Importantly, the sensitivity of AP-MALDI MSI now approaches that of vacuum MALDI MSI, making it suitable for many biological applications.[5]

The AP-MALDI UHR (Ultra High Resolution) ion source (MassTech Inc., Columbia, Maryland, USA) allows the user to switch the mass spectrometer ion source from an electrospray ionization setup (nanospray, high-flow electrospray, or other type of liquid-based technique) to an AP-MALDI setup in just a few minutes. This easy-to-switch feature makes it convenient to perform both LC-MS and MSI workflows using the same mass spectrometer, increasing access to the unique benefits of MSI in precisely locating and imaging individual molecules of interest among other molecules directly from tissue sections. Compared to LC-MS alone, the ability to combine LC-MS and MS/MS analysis with MSI provides significantly more comprehensive insights to answer biologically relevant questions.

Another commercially available AP-MALDI MS imaging solution—TransMIT's AP-SMALDI[5] AF ion source—is available with Orbitrap mass analyzer technology as well.[6,7] Both AP-MALDI ion source manufacturers provide powerful tools for MS Imaging research and applications.[8-10]

Over the last decade, MALDI MSI has emerged as an important tool for skin analysis. Numerous publications have endorsed its usefulness for simultaneously determining the composition of lipids and their respective spatial distributions in skin samples.[11-14] However, only a few describe the feasibility of using MALDI MSI for the analysis of *in vitro* skin models, such as RHE samples.[15-17] AP-MALDI MS imaging of skin models has been described by Feucherolles et al.,[18] and parts of this article are included in this application note.

Experimental

Figure 1 depicts MSI experimental design, starting with the RHE *in vitro model,* to embedding, sectioning, matrix spraying, AP-MALDI analysis, and data analysis and interpretation.

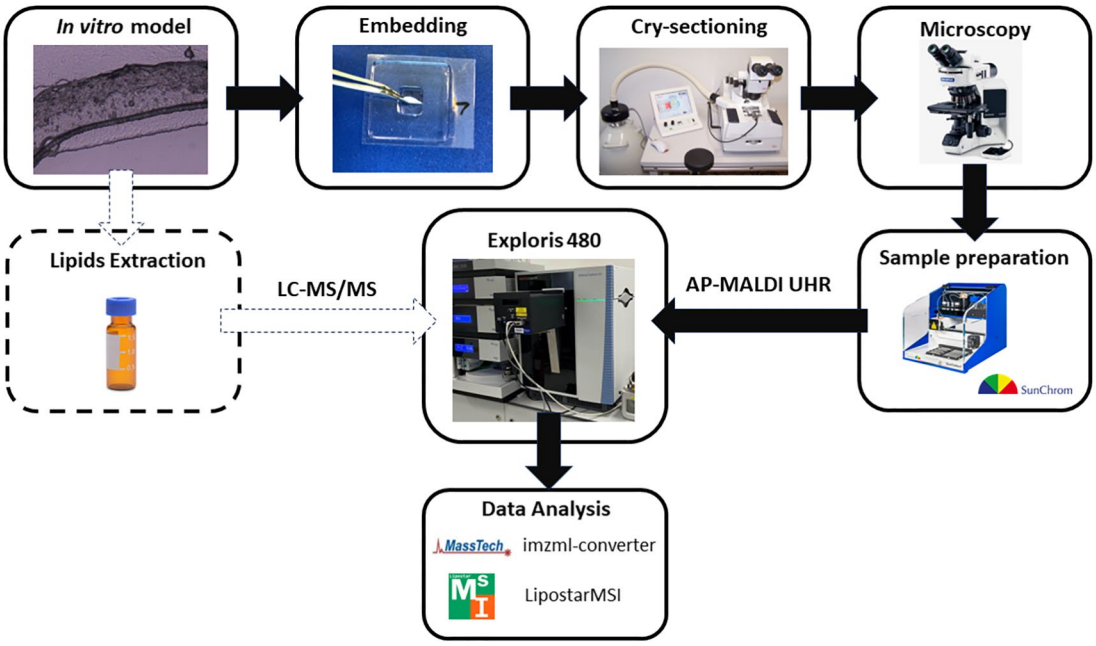

Figure 1. Overview of the experimental MSI design from *in vitro* model to data analysis. Because this application note is focused on the AP-MALDI MSI workflow, the LC-MS/MS workflow is not described here. Refer to Feucherolles et al. for details about the LC-MS/MS workflow.[18]

Glossary

This glossary is intended to help the reader by providing short descriptions for the several abbreviations used in this application note.

RHE	Reconstructed human epidermis
SkinEthic	SkinEthic™ RHE is an *in vitro* reconstructed human epidermis from normal human keratinocytes cultured on an inert polycarbonate filter at the air-liquid interface. It is histologically like the *in vivo* human epidermis. https://www.episkin.com/SkinEthic-RHE
EpiSkin	EpiSkin™ is an *in vitro* reconstructed human epidermis from normal human keratinocytes cultured on a collagen matrix at the air-liquid interface. This model exists at different stages of maturity. https://www.episkin.com/Episkin
PCM	Polycarbonate membrane
CMC	Carboxymethylcellulose (tissue embedding material)
SC	*Stratum corneum*
SG	*Stratum granulosum*
SS	*Stratum spinosum*
SB	*Stratum basale*
TIC	Total ion current
AGC	Automatic gain control
CER	Ceramide
PC	Phosphocholines
SM	Sphingomyelin

Solar simulated radiation sample treatment

RHE models were exposed to solar simulated radiation (SSR) at a dose of 16.5 J/cm^2 using a Suntest Heraus ICPS+ instrument (applying 2x the minimal erythemal dose (MED)). Samples were kept either untouched or treated with a specific sun-filter formulation prior to SSR. After irradiation, the RHE models were incubated for 24 h and then embedded in a mix of 10% gelatin and 2.5% carboxymethylcellulose (CMC) diluted in water for later slicing in the Cryo-Ultramicrotome. Embedded RHE models were frozen in 2-methylbutane and liquid nitrogen and stored at -80 °C until analysis.

AP-MALDI MSI sample preparation

Non-destructive AP-MALDI MSI sample preparation was based on the protocol described by Feucherolles et al.[18] Briefly, slices with thicknesses of 6 µm were sectioned from embedded samples using a Cryo-Ultramicrotome Leica EM FC6 (Leica Microsystems GmbH, Germany) set at -20 °C. Slices were thaw-mounted onto a stainless-steel plate coated with 3-aminopropryltriethoxsilane (APTES) to promote glass-slide adhesion. Air-dried tissue sections were washed with distilled water chilled to 4 °C. RHE tissue sections were coated with 24 layers of either:

- HCCA matrix (α-cyano-4-hydroxycinnamic acid, 3 g/L in acetonitrile/H_2O (1:1, v/v) solution + 0.2% trifluoroacetic acid)

or

- DAN matrix (1,5-diaminonaphthalene), (3 g/L in acetonitrile/H_2O (1:1, v/v) solution + 0.2% trifluoroacetic acid)

using a SunCollect MALDI-Sprayer (SunChrom GmbH, Germany) set at a flowrate of 15 µL/min, velocity of 600 mm/min, and Z axis position of 25 mm.

AP-MALDI MSI analysis

MSI analyses were performed using an AP-MALDI UHR ion source coupled to a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer operated in both positive and negative ion modes. The Orbitrap Exploris mass spectrometer was equipped with the Thermo Scientific™ EASY-IC™ source to provide an in-spectrum lock mass for scan-to-scan mass scale correction. For the MSI experiments, the ion source was operated in the Constant Speed Raster motion mode with a step size of 5 µm. The laser was operated at a frequency of 400 Hz and 3% laser energy. Spectra were acquired using an ion injection time of 490 ms, mass range of 205 to 2,000 Da, and a mass resolution setting of 240,000 at *m/z* 200. The automatic gain control (AGC) (typically used to control ion injection) was disabled to ensure equal injection time for all pixels and across the entire tissue area.

Data processing and analysis

Using MassTech's imzML converter, raw mass spectrometer data files and position information from the Constant Speed Raster ion source mode were merged by conversion into imzML format.[19] The imzML files were then imported into LipostarMSI software (v.2.1.0b1) (Mass Analytica, Spain) for image processing, visualization, and lipid annotation based on the LipidMaps database. All images were normalized to the total ion current (TIC).

Results and discussion

The *stratum corneum* (SC) is the skin's primary barrier that functions to prevent water loss and protects against environmental hazards, such as bacteria, chemicals, and sun exposure.[20] Recent advances in skin biology research have increased the understanding of the many skin functions and mechanisms involved in inflammation.[21] The importance of lipids in skin disease pathogenesis has been brought to the forefront of as a result of these studies.

Within a 100 µm dimension across the skin model, RHE models can be divided into the following regions:

- polycarbonate membrane (PCM), i.e., support for the growing cells
- *stratum corneum* (SC)
- *stratum granulosum* (SG)
- *stratum spinosum* (SS)
- *stratum basale* (SB)

Leveraging the segmentation and *m/z* co-localization capabilities of the LipostarMSI software, the molecular structure of RHE was defined (Figure 2). Segmentation was based on clustering algorithms. Clustering segmentation showed four main layers, which were consistent with the histological structures of RHE (i.e., the SB, SS, SG, and the interface between the SS and the SG). In this study, a sphingomyelin (SM), two phosphocholines (PC), and a ceramide (Cer) were identified in the cross section of the layers of SB, SS, and SG, respectively (Figure 2c).

The imaging experiments allowed visualization of the spatial localization of lipid modulation. Overall, seven main categories of lipids were identified in the different model sections, including lipids from the glycerolipids, sterols, fatty acyls, glycerophospholipids, and sphingolipids classes. The high mass resolution and accuracy of the Orbitrap mass analyzer made it possible to confidently annotate molecules based on accurate mass measurements. For instance, 25-hydroxy-cholesterol 3-sulfate ($C_{27}H_{46}O_5S$; *m/z* 481.2993 $[M-H]^-$), known to play an important role in lipid metabolism, inflammatory response, and cell survival, was annotated with a 0.6 ppm mass accuracy.

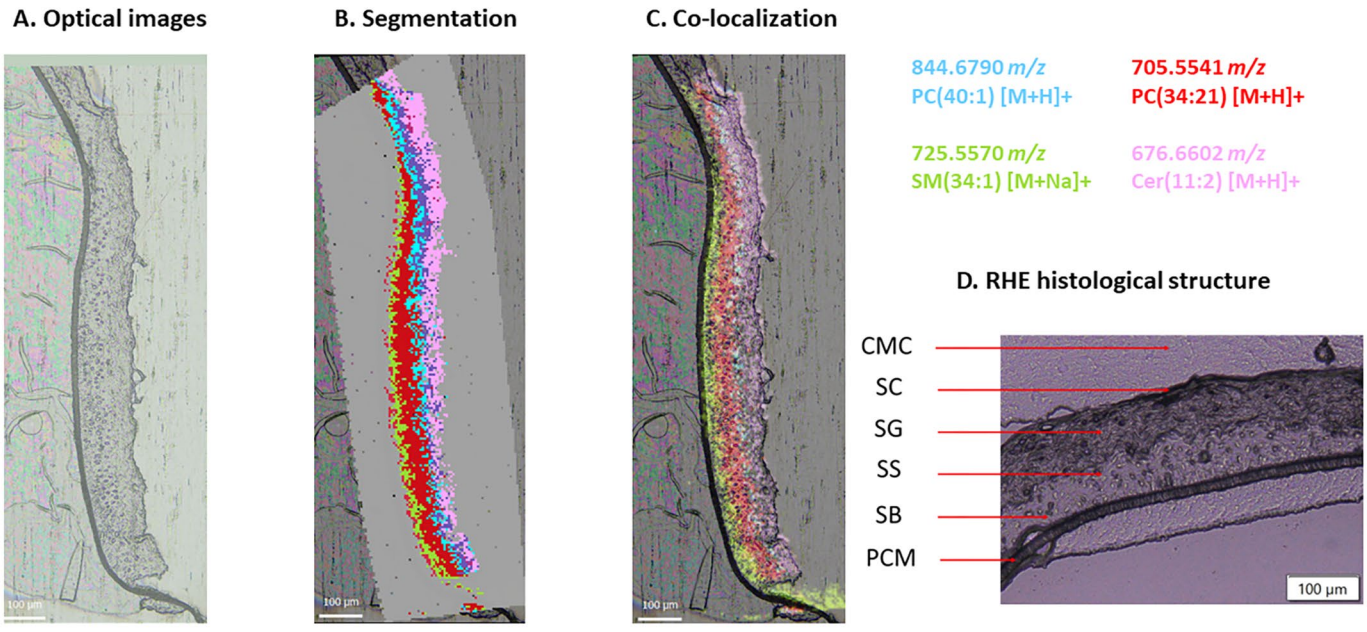

Figure 2. *In vitro* skin model molecular architecture. Visualization of a RHE section by (A) brightfield microscopy, (B) after clustering algorithm segmentation, and (C) after co-localization of *m/z* values based on segmentation cluster. The *m/z* values are overlaid on the microscopy snapshot. (D) EpiSkin reconstructed human epidermis (SkinEthic) histological structure. **CMC:** Carboxymethylcellulose (tissue embedding material added to slice the tissue prior to MSI analysis), **SC:** *Stratum corneum,* **SG:** *Stratum granulosum,* **SS:** *Stratum spinosum,* **SB:** *Stratum basale,* **PCM:** Polycarbonate membrane. Scale: 100 µm. See Feucherolles et al. for additional details.[18]

The role of this compound (at *m/z* 481.2993) was clearly observed when UV-irradiated and non-irradiated RHE samples (p-value: 0.00028) were compared (Figure 3). Localization of the 25-hydroxy-cholesterol 3-sulfate was weakly detected at the interface between the SS and SG when the tissue was not subjected to UV stress (Figure 3, left). However, when UV-irradiated prior to MSI analysis, the molecule's signal was significantly increased and principally localized in the SB and SS regions (Figure 3, center). Interestingly, in the RHE protected with a sun filter formulation, the intensity and the localization of the 25-hydroxy-cholesterol 3-sulfate was identical to that of the RHE not subjected to UV irradiation (Figure 3, right).

Therefore, the authors hypothesized that the sun filter protected the RHE from UV-induced inflammation, which is in agreement with the intended purpose of sun-filter personal care products.

Conclusion

AP-MALDI MSI was used to investigate the lipidomic changes associated with the application of UV radiation to *in vitro* reconstructed human epidermis (RHE) samples (skin model systems). The RHE models were studied for their lipidomic changes following UV-radiation with and without a sun-filter formulation. Lipidomic modulation at the cellular level was comprehensively elucidated and localized, providing unique dermatology insights such as the spatial characterization of the lipids involved in inflammatory pathways. The approach offers significant potential for dermatological research and personal care product development using challenging-to-analyze, small-sized samples like *in vitro* skin. The AP-MALDI UHR ion source-Orbitrap Exploris mass spectrometer setup provides unparalleled mass and spatial resolution, high mass accuracy, and high sensitivity, providing scientists with a versatile and complete workflow for dermatology lipidomics.

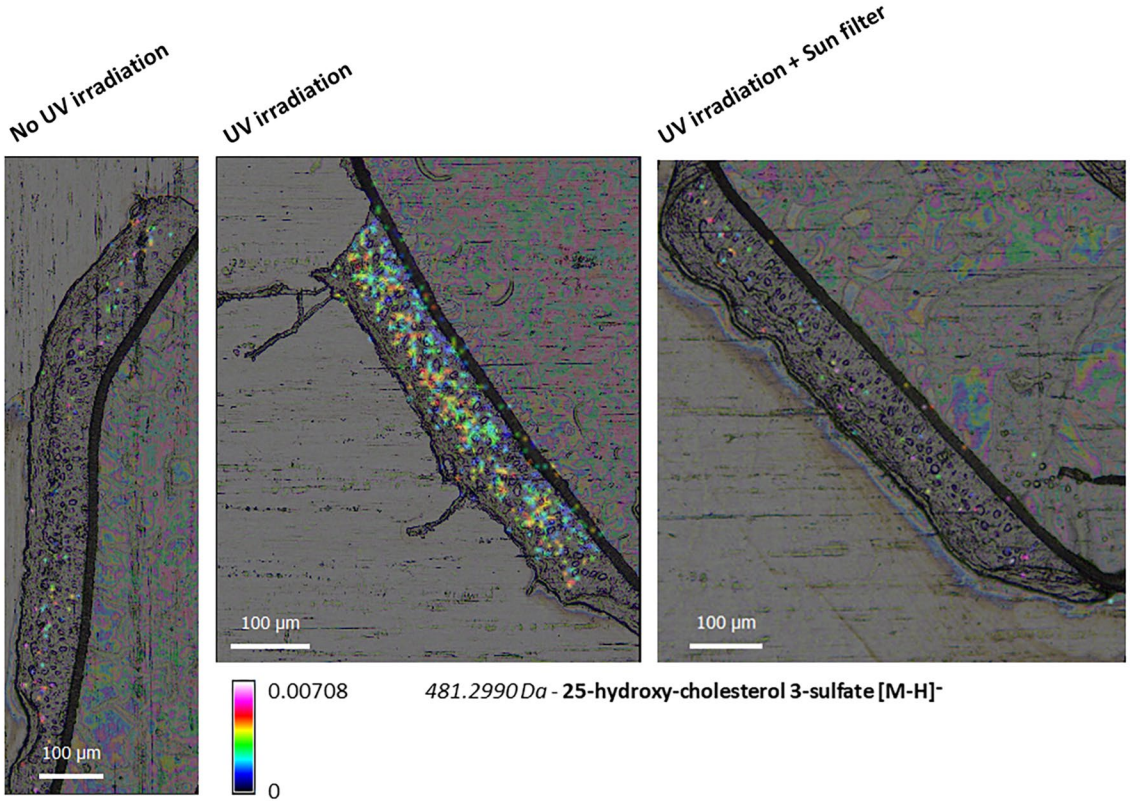

Figure 3. Localization of 25-hydroxy-cholesterol 3-sulfate [M-H]$^-$ with *m/z* 481.2990 across the different RHE section preparations. Left: not exposed to UV irradiation. Center: exposed to UV irradiation. Right: sun filter protection with a sun-filter formulation and exposed to UV irradiation.

Acknowledgements

This application note is based on the scientific paper by Feucherolles et al,[18] which contains additional details on sample preparation, data processing, LC/MS analyses, further discussion, and useful references.

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