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## Introduction

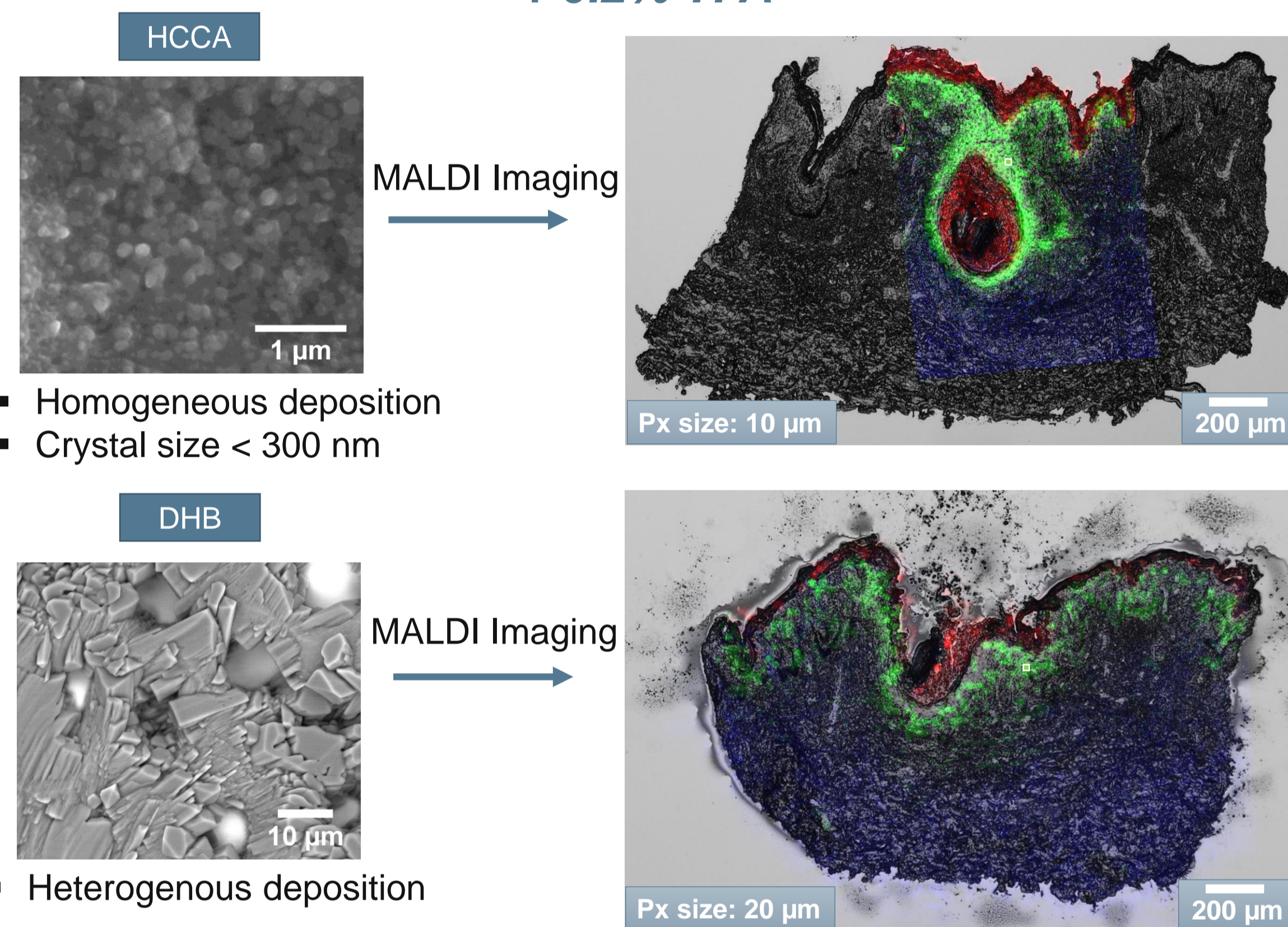
Latest developments in the field of MALDI imaging has led to a significant impact in the pharmaceutical and cosmetics fields. Sample preparation, high spatial resolution and high selectivity are crucial parameters for reliable identification of molecules of interest on biological samples. MALDI UHR coupled with an LTQ/Orbitrap Elite provided the advantage of accurate mass measurements and high selectivity by tandem MS with a spatial resolution down to 10  $\mu\text{m}$  that allowed the detection and localization of untargeted and targeted molecules in the human skin layers.

## WORKFLOW FOR SAMPLE PREPARATION AND ANALYSIS



## IMPACT OF MATRIX CHOICE AND PREPARATION ON SPATIAL RESOLUTION AND SENSITIVITY

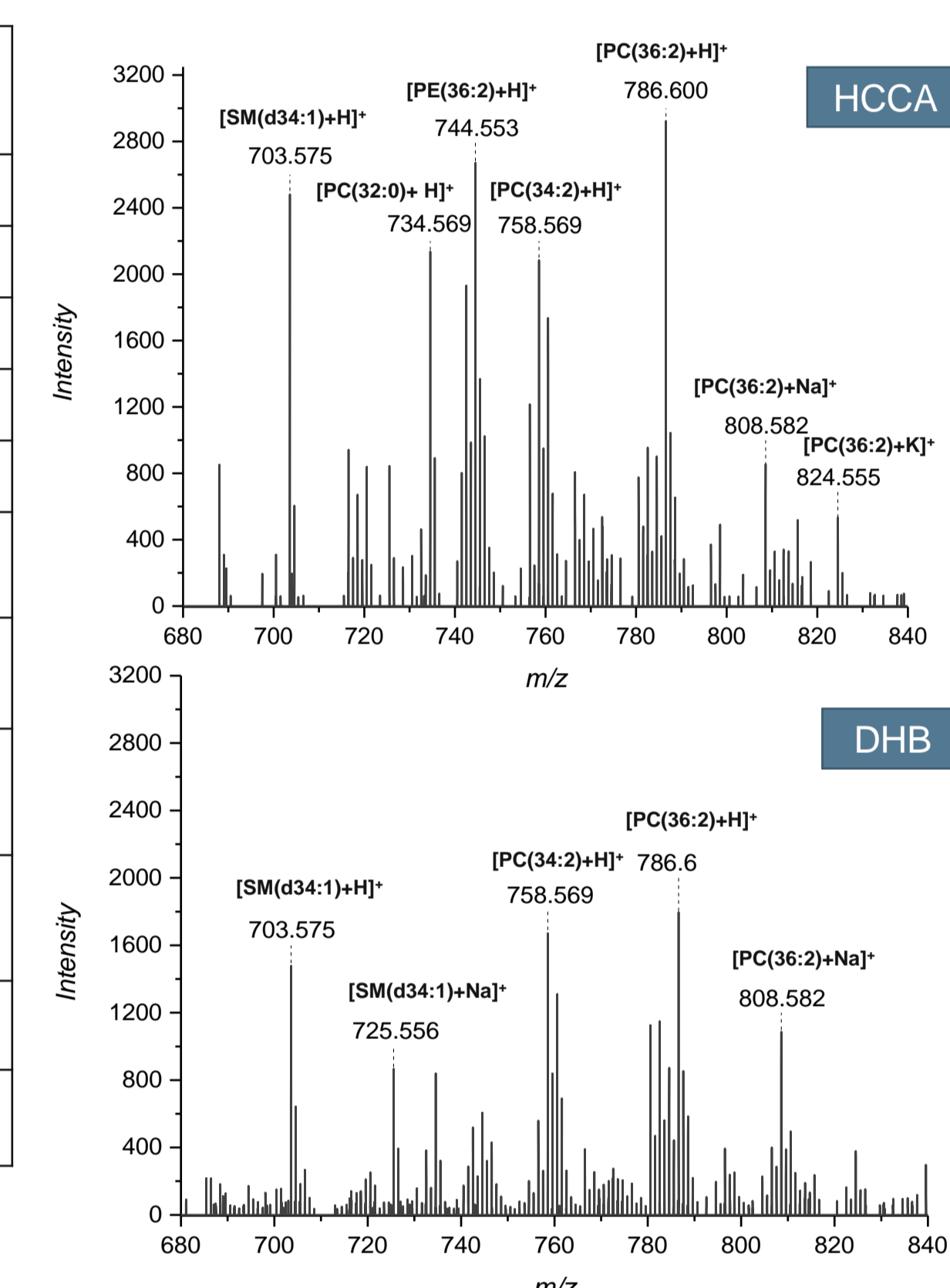
Two matrix preparations in 1:1 v/v Methanol/Chloroform + 0.2% TFA



Screening of endogenous molecules in the skin layers

Skin Layers	m/z	Tentative assignment with Metlin database
Stratum Corneum Ceramide are mainly detected	386.399	[Cer fragment + H] <sup>+</sup>
	526.519	[Cer(d33:0) + H] <sup>+</sup>
	554.551	[Cer(35:0) + H] <sup>+</sup>
	740.712	[Cer(t46:0(2OH)) + H] <sup>+</sup>
	754.620	[GlcCer(d38:2) + H] <sup>+</sup>
Epidermis Different lipids classes Phosphatidylcholine (PC), Sphingomyelin (SM), Phosphoethanolamine (PE)	703.575	[SM(d34:1)+H] <sup>+</sup>
	734.569	[PC(32:0) + H] <sup>+</sup>
	744.553	[PE(36:2)+H] <sup>+</sup>
	758.569	[PC(34:2)+H] <sup>+</sup>
Dermis Mainly proteins and peptides are located	582.273	[C <sub>20</sub> H <sub>37</sub> N <sub>11</sub> O <sub>8</sub> + H] <sup>+</sup>
	600.283	[C <sub>33</sub> H <sub>37</sub> N <sub>5</sub> O <sub>6</sub> + H] <sup>+</sup>

Mass Spectra in a comparable 400  $\mu\text{m}^2$  surface area in the epidermis

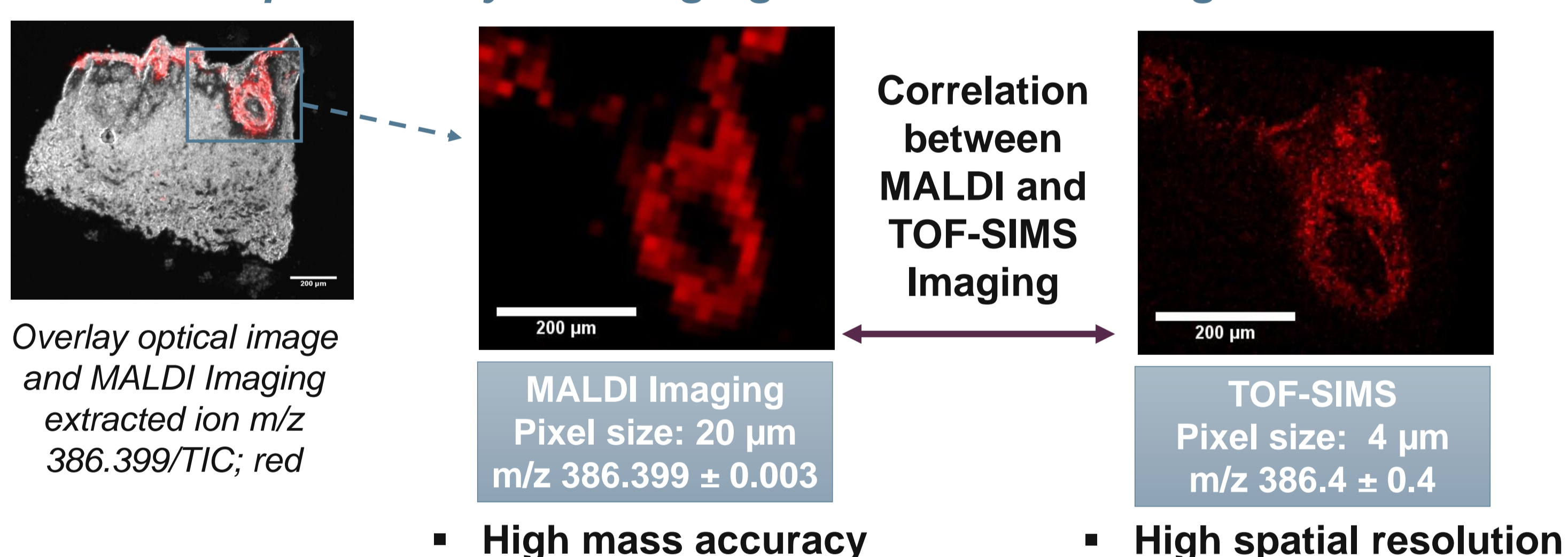


**MATRIX PREPARATION:**

- Significant impact on crystals size and homogeneity
- Direct effect on spatial resolution
- Better results with HCCA matrix
- Detection of endogenous molecules in the different layers of the skin

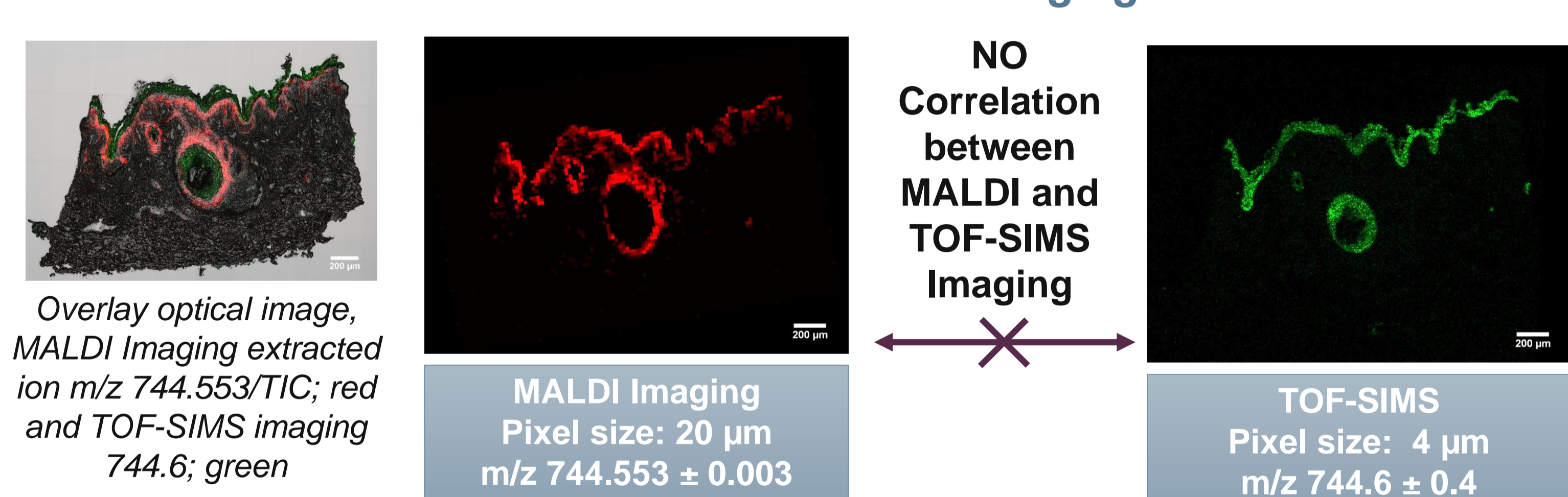
## IMAGING CORRELATION BETWEEN MALDI-HRMS AND TOF-SIMS

Complementary MS Imaging modalities on a fragment ion



- Good imaging correlation between TOF-SIMS and MALDI on some fragment ions

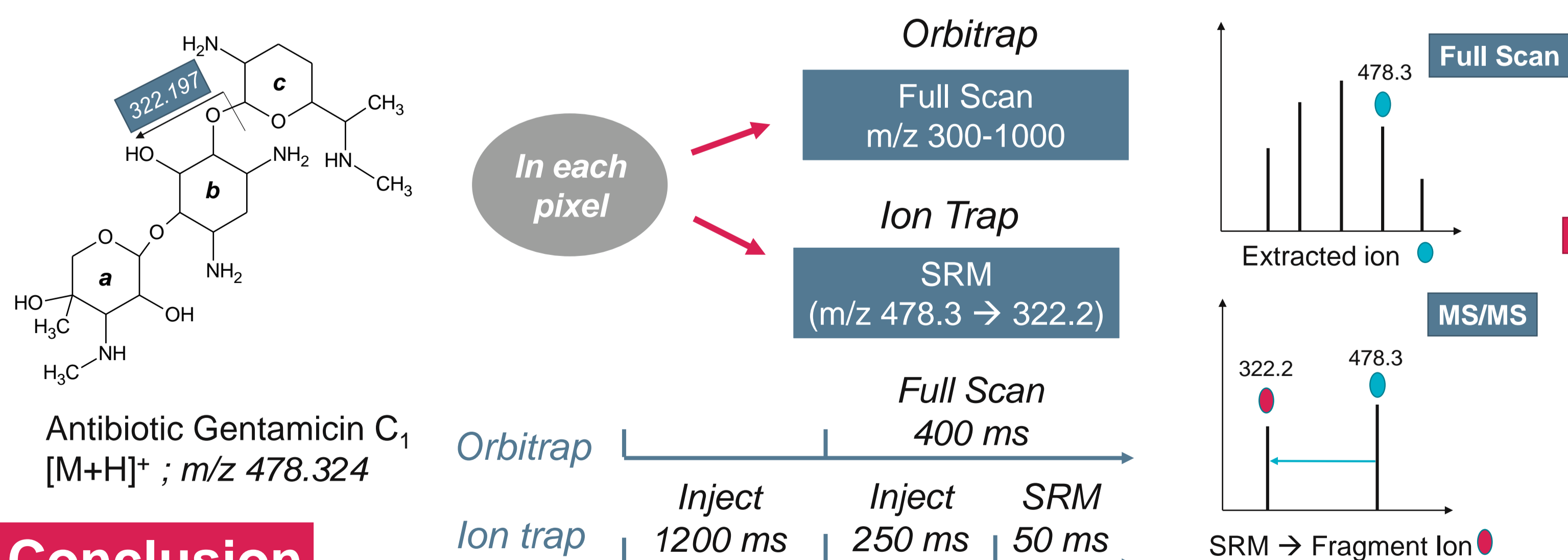
Mismatch between MALDI and TOF-SIMS imaging on intact molecules



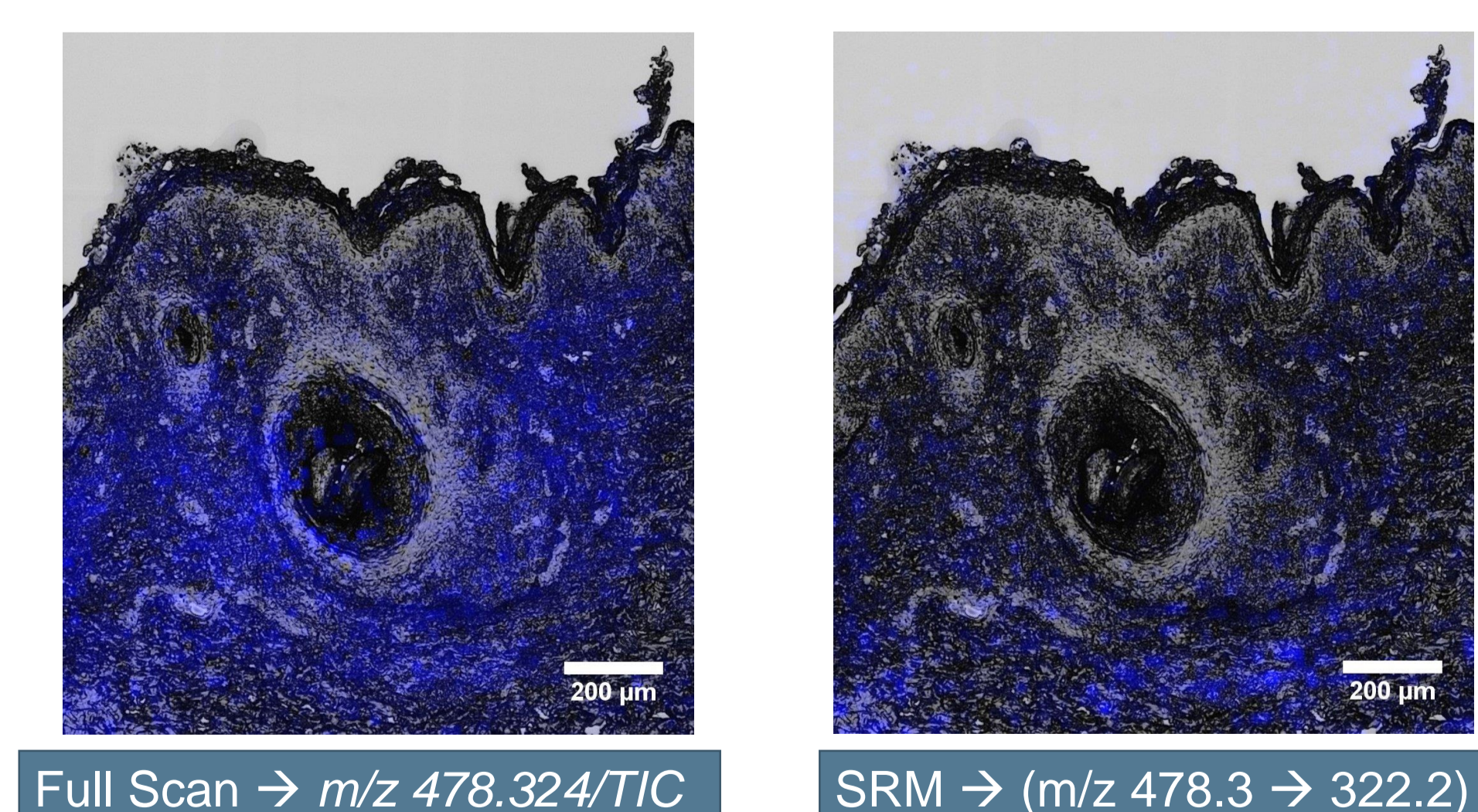
- Mismatch possible on intact molecules due to mass interferences, a higher fragmentation and a different ionization in TOF-SIMS

## TARGETED LOCALIZATION OF AN EXOGENOUS MOLECULE IN THE SKIN BY SIMULTANEOUS FULL SCAN/SRM

Method Development on simultaneous Full Scan/SRM by MALDI-Imaging



Specific localization of the antibiotic by SRM



**SIMULTANEOUS FULL SCAN/SRM**

- High selectivity
- Targeted localization of an antibiotic in the skin
- Interest in pharmaceuticals and cosmetics field

## Conclusion

- Sample preparation with HCCA Matrix enabled to obtain MALDI Imaging on skin sections with a spatial resolution down to 10  $\mu\text{m}$  while keeping a good sensitivity for the detection of endogenous molecules in the different skin layers.
- TOF-SIMS/MALDI MS Imaging correlations may be suitable for multimodal image fusion in the case of fragments ions to achieve higher spatial resolution.
- A method providing simultaneous Full Scan/SRM was developed to specifically localize targeted molecules such as active substances in the skin.