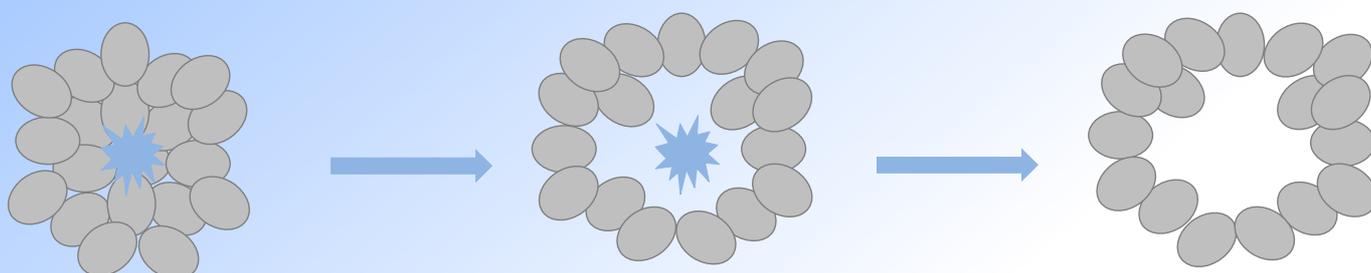


MALDI Imaging

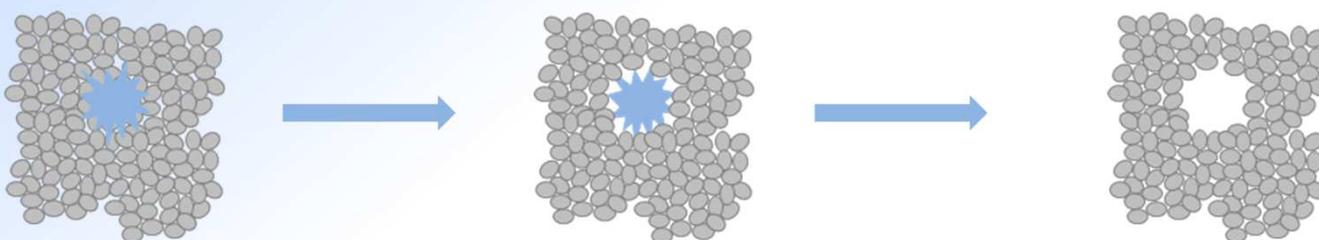
Influence of crystal size of matrix layer on resolution in MALDI Imaging

SunChrom is proud to announce the assignation of a world patent (WO 2017/025205 A1) on its SunCollect spray nozzle and spray procedure. The sprayer in combination with a unique spray procedure ensures to produce the smallest crystal size ever seen –
just around 100 nm only!

Obviously there is a relationship between the crystal size of the matrix coverage and possible resolution in the MALDI Imaging process. The pictured schema shows the dependence of the resolution as an example for 5 μm laser beam diameter; Fig. 1



- Crystal size: 30 μm
- Laser diameter: 5 μm
- Hole diameter: up to 100 μm



- Crystal size: 5 μm
- Laser diameter: 5 μm
- Hole diameter: up to 20 μm

Calculation of the area: $F = \pi * r^2$
The burned areal is 25x larger !!!

Fig. 1 Dependence of the hole diameter resulting after a laser shot of 5 μm diameter on different matrix crystal sizes.

Laser beam diameter and hot zone

The edge or resolution shown theoretically can be worse if the laser beam shows abnormalities known by older MALDI MS instruments, where besides a “hot inner zone” also an additional second hot zone exists, where the majority of matrix layer is completely removed; Fig. 2

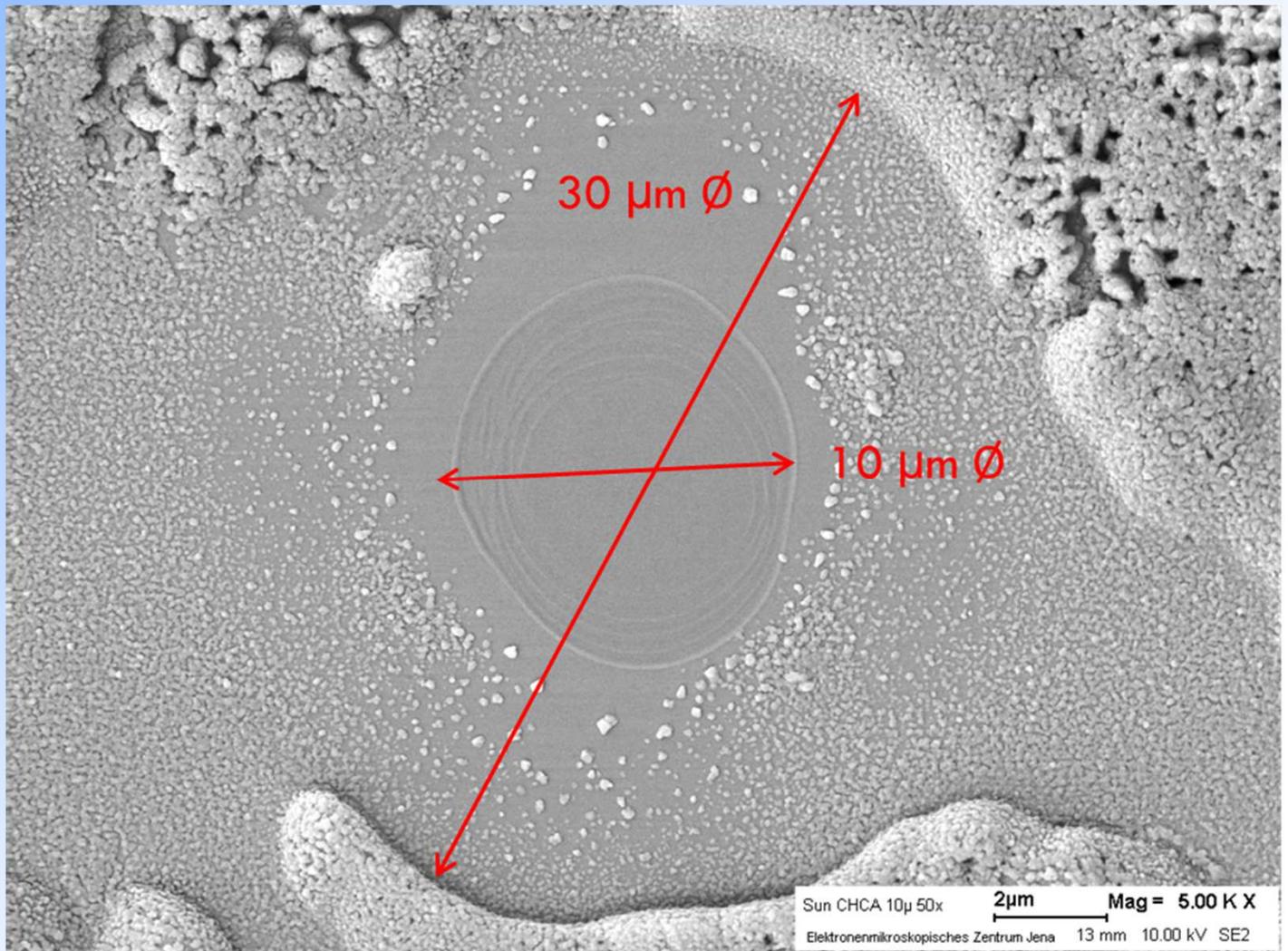


Fig. 2 Dependence of the hole diameter resulting after a laser shot of 10 μm diameter (“Hot zone”) Bruker AutofleXtreme.

CONDITIONS:

- Standard ITO slide
- Matrix: CHCA (5 mg/mL) 10 layers with increasing matrix donation
- Sprayed with SunCollect
- Crystal size approx. 100 nm !!
- 50 shots of 10 μm laser

Patented spray procedure

Besides the absolute crystal size it is also important how the crystals are oriented on the tissue surface. Ideally and especially for DHB they should be vertically oriented instead of normally horizontal oriented, when the matrix layer is applied with a high matrix/solvent dosage.

The huge advantage of this patented unique spray procedure is that it prevents an undesired washout of any compounds from the tissue. Whereas this phenomenon is frequently observed when the matrix solution is applied at high flow rates from the first layer onwards.

SunChrom's newly patented spray procedure starts with a very low flow rate for matrix/solvent delivery and increases the flow rate within the first three layers. By this method the surface of the glass becomes porous, similar like tissue surface. It prevents the delocalization of analytes out of the tissue to the glass surface (no wash-out).

The possible effects are shown and compared in Fig. 3

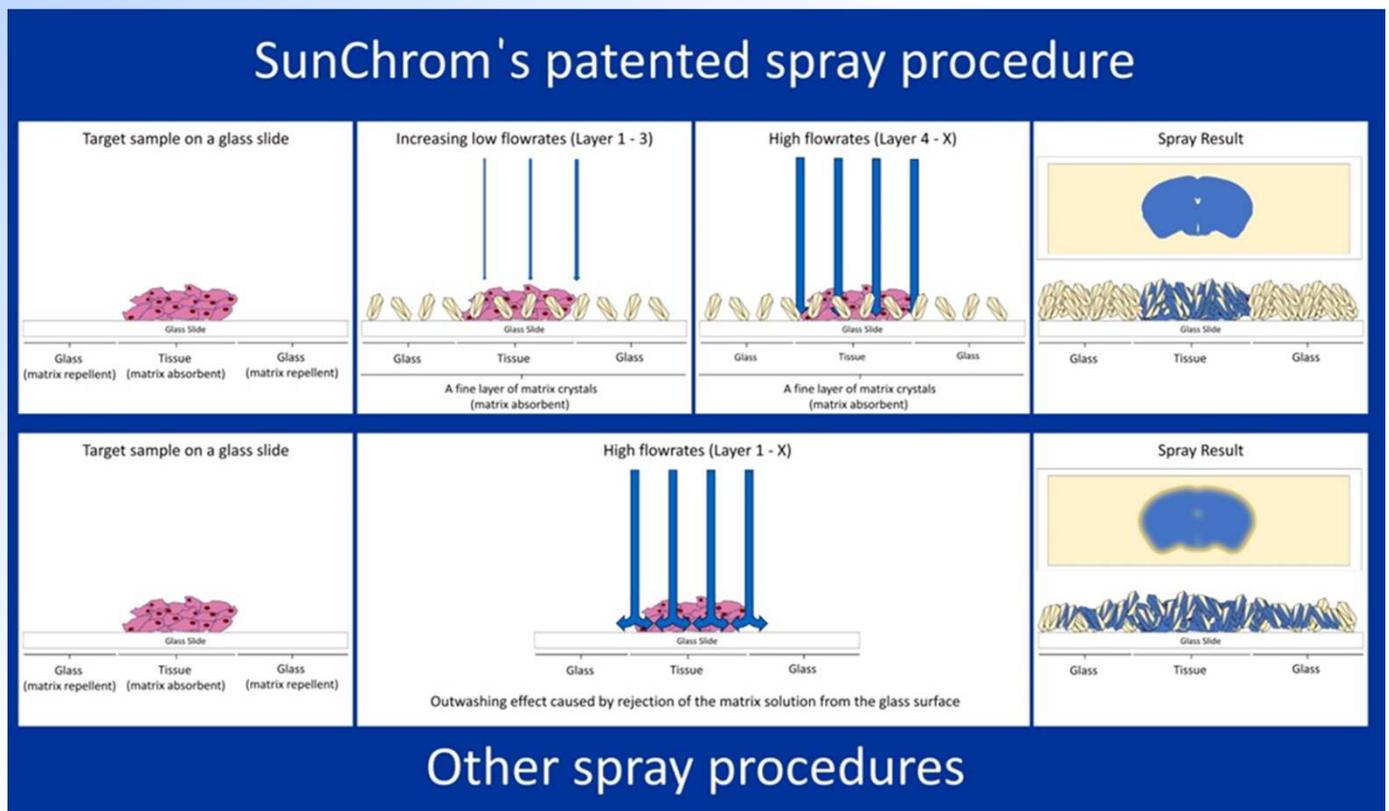


Fig. 3 The influence of matrix application on the resulting resolution and washing-out effects.

Crystal orientation and laser beam diameter

According to the patented matrix application protocol first a very tiny layer of matrix seeds is created on the tissue/target surface. On these seeds the crystals will grow vertically with every following layer with increasing matrix/solvent concentration. As a result of this very specific and patented procedure even DHB crystals, which usually tend to create very long horizontal crystals, here in our case create predominantly vertical crystals. According to this fact and the better laser beam of the latest MS instruments, the holes after a laser shot correspond exactly to the laser beam diameter.

In this case a desired resolution of 10 μm will be ensured; Fig. 4

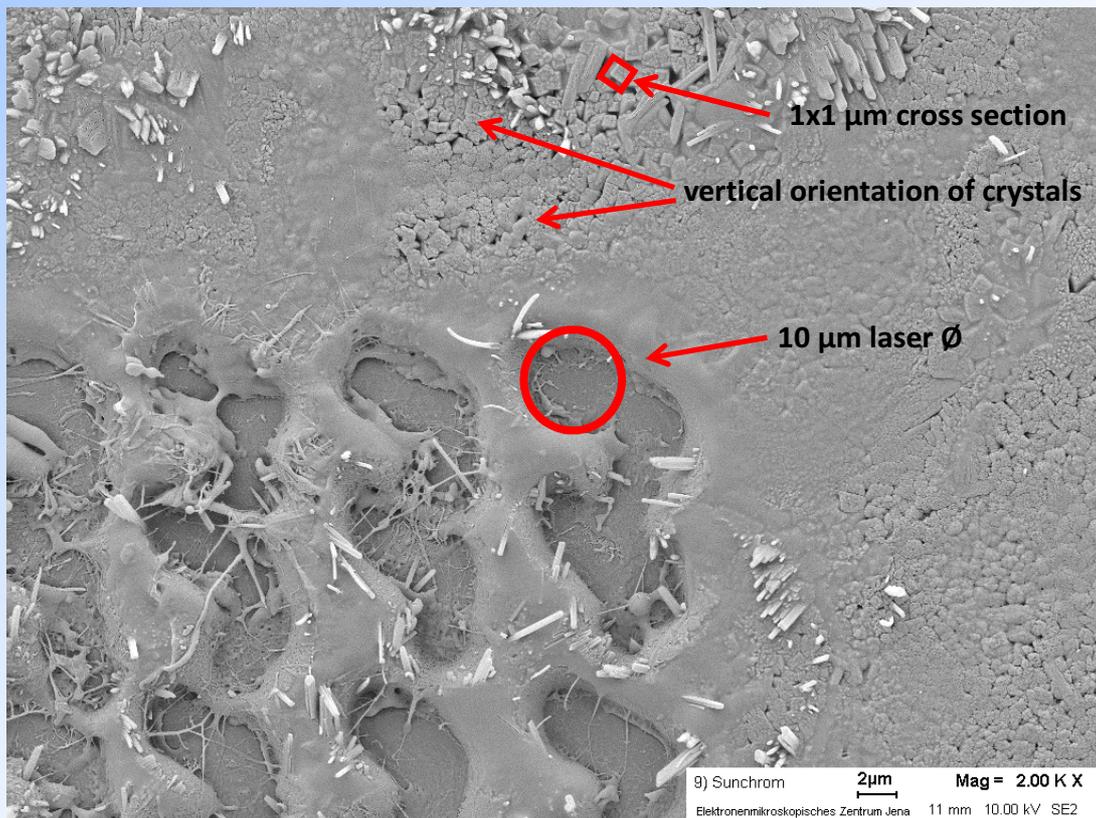


Fig. 4 The hole diameter corresponds exactly to the laser beam diameter; DHB forms predominantly vertical crystals and ensures the maximum achievable resolution.

Besides this special sample preparation technique SunCollect sprays matrix or expensive enzyme solutions extremely economical. Less than 200 μl enzyme solution is needed for a standard microscope or ITO glass slide for absolutely homogeneous 10 layers. This procedure is crucial for a successful sample preparation for MALDI MS.

The crystal size of approx. 1 μm corresponds to sublimation technique; whereas the most important advantage of the SunChrom's patented spray technique is that it is not only applicable to lipid analysis, but for biomolecules like proteins/peptides or other small molecules like drugs and their metabolites as well.