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A sample stage design for MALDI Imaging with adaptive laser optics improves positioning accuracy for high resolution imaging

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Introduction

MALDI imaging is a powerful technique to map biomolecules in tissue. To create a spatially resolved ion image, most MALDI instruments move the sample using x-y stage relative to a stationary laser beam, to create a mass spectrum for every pixel. However, approaching spatial resolution of only a few micrometers, over wide travel range, poses a challenge for the mechanical accuracy of most stages, affecting the quality of the MALDI-images.

Here, we introduce *microGRID*, a new instrument design which combines both stage and laser beam positioning to eliminate imaging artifacts down to about 5 µm. In combination with MALDI-2, this new technique enables highly sensitive imaging at high spatial resolution without compromising on pixel fidelity.

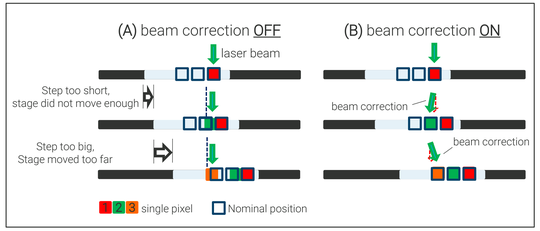

Fig. 1 Combination of sample stage movement and laser beam positioning. With (A) beam correction off, limitations in stage precision lead to artifacts in pixel positioning. (B) *microGRID* exploits the laser beam steering to correct to nominal pixel position, enabling artifact-free imaging.

Methods

Optical encoders with sub-micron resolution were integrated into a regular stepper driven MALDI sample stage and monitor the actual position. Any deviation from the ideal raster is precisely detected by the encoders and sent to the adaptive smartbeam™ 3D laser optics for automatically on-the-fly correction and irradiating precisely the targeted pixel within µm accuracy.

Images were collected using smartbeam™ 3D systems with ~5 µm laser spot size at raster spacing of 5-20 µm. We used three different kinds of samples with CHCA and DHAP matrix-coating by sublimation. Ablation craters were analyzed by high-resolving optical microscopy. MALDI-MSI data were visualized using SCiLS Lab.

Results

- Fig. 2 shows the effect of the *microGRID* technology on ablation craters and MALDI images. For the imaging raster, 10 µm nicely illustrates the increase in raster precisions, already visible by eye. Backlash, start-stop and wave-like distortions are eliminated with active correction, delivering a regular burn pattern. The same effect applies to 5 µm pitch but is less visible by eye due to almost complete sample consumption.
- For the MALDI images, at a pitch of 5 µm, this results in a reduction of oversampling artifacts like checkerboard patterns or striping. With laser beam correction, more accurate ion images are generated, and the observed ion distribution is expected to better reflect the biological distribution. However, achieving 5 µm resolution with MALDI-2 is more sensitive for optimal acquisition parameters, due to the increase of primary laser energy.

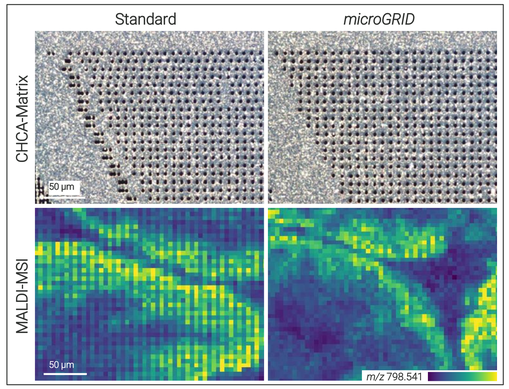

Fig. 2 Demonstration of the new *microGRID* technology on position accuracy of ablation craters (10 µm pitch, top) and artifacts in MALDI-2-MSI (5 µm pitch, bottom).

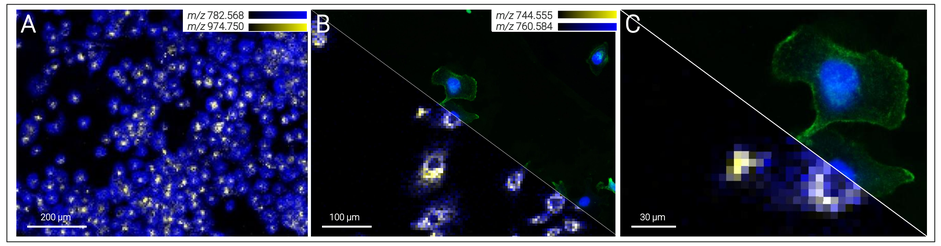

Fig. 5 MALDI-2-MSI data imaged at 5 µm resolution using *microGRID* of (A) THP-1 cells, differentiated to macrophages, and (B) Caki-2 cells, overlayed with fluorescence microscopy data showing subcellular resolution (zoom-in in C). Cells were kindly prepared by Jan Schwenzfeier, University of Münster.

- The increase of achievable and robust lateral resolution in MALDI-MSI now enables to clearly visualize small structures within the testicle, which are not distinguishable at bigger pixel sizes (Fig. 3). This emphasized the need for robust tools to perform MALDI-MSI with ≤ 5 µm spatial resolution.
- The drastically increase in positional accuracy leads to improved spatial resolution and thereby opens the field for the analysis of the smallest unit of life – single eucaryotic cells. With dedicated sample preparation strategies like matrix-sublimation, *microGRID* is able to push imaging resolution to its outer limit and allows for the visualization of cellular fine structures and organelles like the nuclei (Fig. 4).

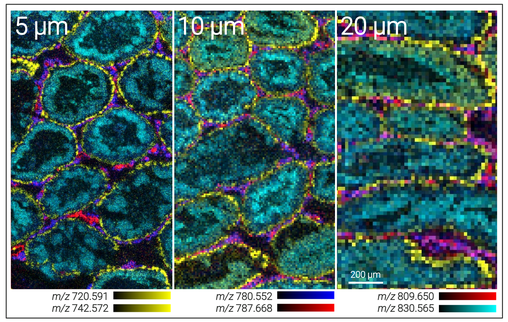

Fig. 3 MALDI-2-MSI data of a rat testis, imaged with 5, 10 and 20 µm pitch of the MALDI sample stage movement with laser beam correction by *microGRID* technology.

- Co-registration with optical microscopy demonstrates the high quality and accuracy of MALDI images, without artifacts generated by sample preparation or MALDI acquisition.

Summary

For most sample stages in imaging mass spectrometers, approaching a true spatial resolution of a few micrometers poses a challenge for the mechanical accuracy. By using position feedback and adaptive laser beam positioning, the fidelity of high-resolution images down to 5 µm pixels can be increased. The combination with MALDI-2 results in a drastically increased sensitivity, which allows for generating of high quality MALDI-MSI data despite of the decrease in analyzed sample material. The proof-of-principle data from different relevant sample systems show, in combination with adequate sample preparation strategies, artifact-free images of fine structures in rat testis tissue and even single eucaryotic cells.

Conclusion

- *microGRID* allows for true high-lateral resolution MALDI-MSI down to 5 µm pixel size
- Inaccurate stage movement is compensated by using high-resolution linear encoder for positional readout of the MALDI stage and correct the laser beam accordingly via smartbeam™ 3D technology
- The combination with sensitivity enhancement by MALDI-2 allows for visualizing of numerous molecules in various tissue types and even on single (sub)cellular level

Technology