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microGRID Technology For Robust High-resolution Imaging Down To The (Sub)cellular Level

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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 an 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.

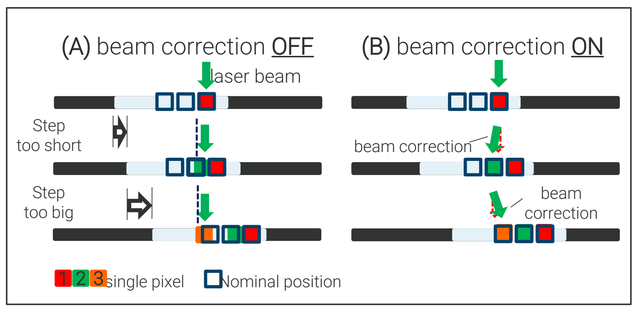

Fig. 1 Combination of sample stage movement and laser beam positioning. With (A) beam correction off, limitations in stage precision lead to artifacts in pixel positioning. (B) *microGRID* exploits the laser beam steering to correct to nominal pixel position, enabling artifact-free imaging.

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. MALDI-MSI data were visualized using SCiLS Lab. Tentative annotations were obtained by MetaboScape based on accurate mass.

Results

- The *microGRID* laser beam adjustment leads to the reduction of oversampling artifacts like checkerboard patterns and striping and thus increases the validity of high-resolution imaging data.
- The increase of achievable and robust lateral resolution in MALDI-MSI now enables one to clearly visualize small structures within the testicle, which are not distinguishable at bigger pixel sizes (Fig. 2). This emphasized the need for robust tools to perform MALDI-MSI with ≤ 5 µm spatial resolution.
- The combination of laser positioning robustness and fast scanning rates of 10 pixel/sec allows for imaging of large sample areas like mouse brain or rat kidney while maintaining significant spatial information (Fig.3).
- *microGRID* imaging can resolve target structures, such as the Purkinje cell layer in the cerebellum (a) and the glomerular system of the kidney (b). This makes a new histological depth accessible for MALDI-MSI.
- MALDI-2boosts sensitivity to compensate for decreased ablation material at smaller pixel sizes and online calibration guarantees stable MS confidence for elongated run times.
- The significant 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 limit and allows for the visualization of cellular fine structures and organelles like the nuclei (Fig. 4).

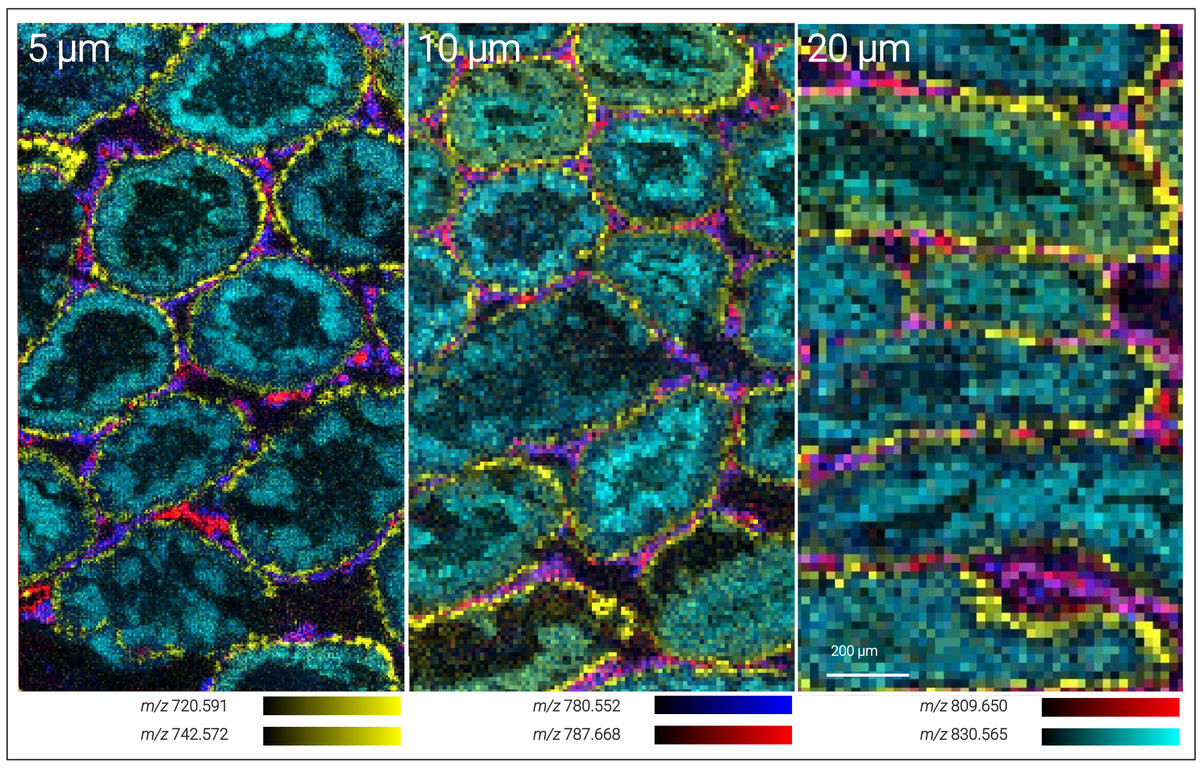

Fig. 2 MALDI-2-MSI data of a rat testis, imaged with 5, 10 and 20 µm pitch of the MALDI sample stage movement with laser beam correction by *microGRID* technology.

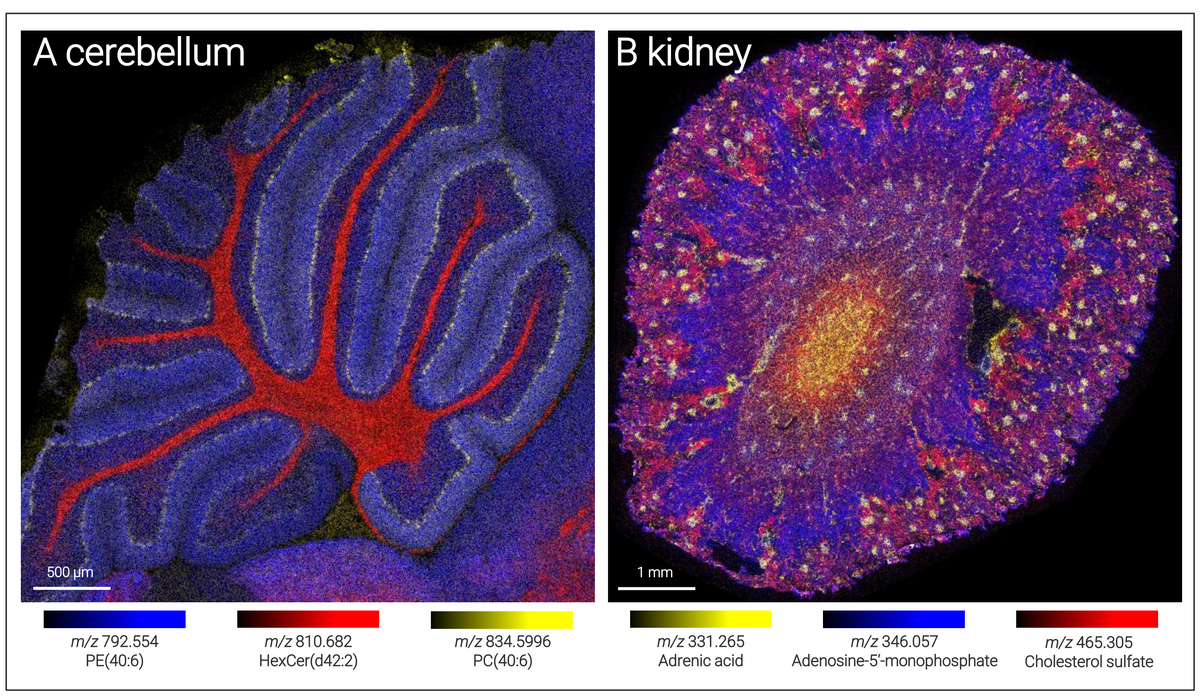

Fig. 3 5 µm *microGRID* imaging data of (A) lipids in mouse cerebellum measured with MALDI-2 (pos), and (B) metabolites in rat kidney measured with MALDI (neg). The data demonstrates the robustness of the *microGRID* technology even with enlarged sample areas.

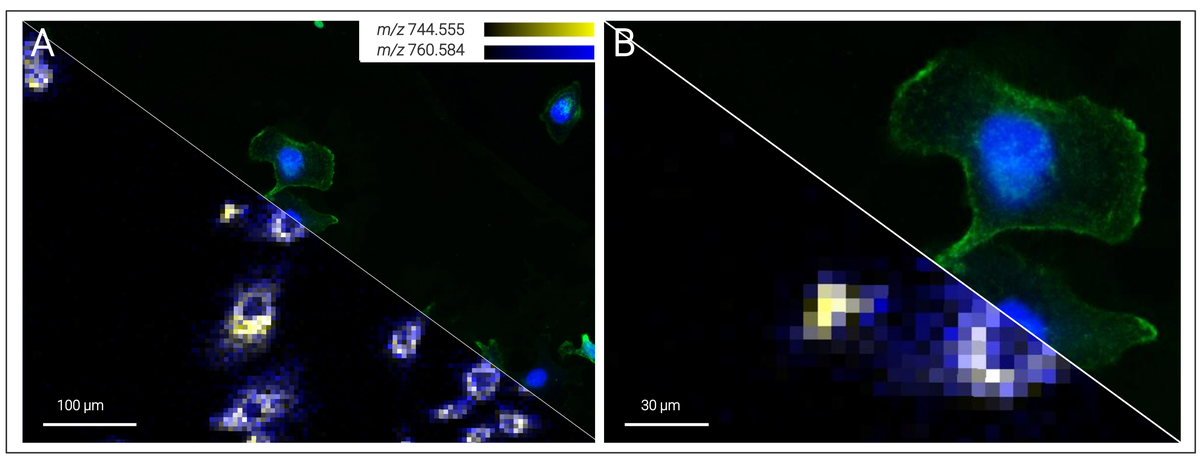

Fig. 5 MALDI-2-MSI data imaged at 5 µm resolution using *microGRID* of (A) Caki-2 cells, overlayed with fluorescence microscopy data showing subcellular resolution (zoom-in in B). Cells were kindly prepared by Jan Schwenzfeier, University of Münster.

Conclusion

- *microGRID* allows for true high-spatial resolution MALDI-MSI down to 5 µm
- Stage movement is compensated by a high-resolution positional readout and correction by the laser beam accordingly via smartbeam™ 3D technology
- Sensitivity enhancement with MALDI-2 allows for visualizing of numerous molecules in various tissue types and even on the single (sub)cellular level

Technology