

## ● Progress towards out-of-the-box sub-cellular imaging by pairing microGRID and MALDI-2

Single cell analysis in a spatially resolved context is the gateway to deciphering scientific questions in various application areas. Such an analysis requires the highest available spatial precision combined with highly sensitive detection.

### Abstract

Consistency and robustness in sampling below  $10\ \mu\text{m}$  lateral spatial resolution has eluded commercial MALDI Imaging instruments. Here we present microGRID, the latest technological addition to Bruker's timsTOF

flex platform, which improves the precision of MALDI Imaging to enable high-quality single-cell analysis. Combined with MALDI-2, Bruker provides a package that delivers both spatial resolution and required sensitivity to unlock out-of-the-box sub-cellular imaging.

### Introduction

The understanding of complex processes in biological samples on a molecular level gained increasing interest in recent years. Thus, the requirement to improve imaging techniques to highest achievable spatial

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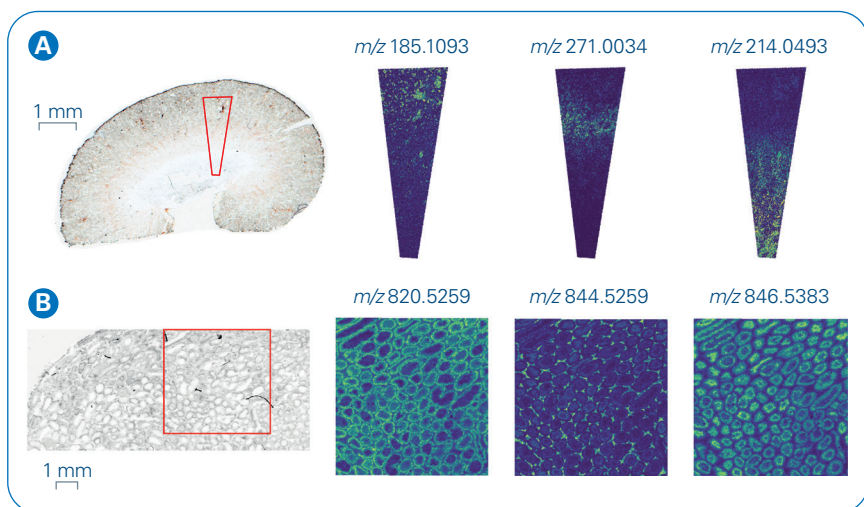


Figure 1: microGRID enabled MALDI ion images using a spatial resolution of 5  $\mu\text{m}$  from (A) rat kidney sample in negative ion mode and (B) a rat testis sample in positive ion mode polarity. Shown are the optical images including the measured area (outlined in red) and 3 respective ion images of metabolites and lipids.

resolution and sensitivity is key to allow for a comprehensive visualization of molecular distributions and to link them to biological functions and origin. However, approaching spatial resolution of only a few micrometers is a challenge and has so far required in-house engineering expertise. Also, with higher spatial resolution comes the demand for more sensitive mass spectrometers. Here, we introduce microGRID, which combines MALDI stage and smartbeam 3D laser beam positioning to facilitate high quality imaging down to 5  $\mu\text{m}$  step size. In combination with MALDI-2, Bruker's unique post-ionization technology, desired robustness and sensitivity at high spatial resolution are available out of the box for the timsTOF fleX platform.

Therefore, data from various biological samples is presented showing the microGRID technology, which utilizes sample stage position feedback and adaptive laser beam positioning to increase the accuracy of high-resolution images down to 5  $\mu\text{m}$  pixel size. In addition, analyte classes that are typically opaque in conventional MALDI were detected by using MALDI-2 post-ionization. This sensitivity boost also enables

the detection of compounds recently only possible with larger pixels when using conventional MALDI.

Combining the above-described technologies with established sample preparation methods including IntelliSlides<sup>®</sup> to enable SCiLS<sup>™</sup> autopilot, robust single-cell resolution can be achieved by beginner and expert users. The workflow is complemented by SCiLS<sup>™</sup> Lab, Bruker's easy-to-use software for analyzing imaging data.

## Experimental

To illustrate the potential of this methodology and to highlight the possible spectrum of future applications, we present data from different exemplar sample sets. First, the lateral distribution of different phospholipids in mouse brain, testis and kidney sections using an on-tissue laser focus diameter of 5  $\mu\text{m}$  was studied. Second, we demonstrate improvements in ion yield when using MALDI-2 and last, we show results from imaged sub-cellular distributions of lipids and metabolites using different cell lines.

Tissue (mouse brain, rat testis and rat kidney) were cut into thin sections of 10  $\mu\text{m}$  thickness and prepared on IntelliSlides<sup>®</sup> (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) using the thaw-mounting technique. Slides were stored in a vacuum box until further usage. Samples were prepared with DHB matrix (brain and testis) and NEDC matrix (kidney) using the standard protocols on a M3+ sprayer by HTX technologies.

Cultivation and preparation of THP-1 and Caki-2 cells was done according to Bien *et al.* [1,2] and kindly provided by the Dreisewerd lab (University of Muenster, Muenster, Germany). In short, cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Brunswick, Germany; DSMZ numbers ACC 16 and ACC 54) and directly cultivated in 8-well chamber slides (Millicell EZ Slide, Merck, Darmstadt, Germany) until they reached sub-confluence. For THP-1 monocytes, differentiation to macrophages was achieved by stimulation with 200 ng/mL phorbol 12-myristate 13-acetate (PMA) for 72 h, followed by cultivation in pure media for additional 72 h. After cultivation, cells were washed with PBS (Lonza, Basel, Switzerland) and chemically fixed with formalin (4% formaldehyde in PBS, low methanol, Carl Roth, Karlsruhe, Germany) according to the protocol in [1]. For fluorescence microscopy, additional staining of the cell nuclei and the cell membrane with Hoechst 33342 (Invitrogen, 10  $\mu\text{g}/\text{mL}$ , 0.5 mL) and Wheat Germ Agglutinin (WGA; Oregon Green 488 Conjugate, Invitrogen, 5-50  $\mu\text{g}/\text{mL}$ , 0.5 mL) for 10 min was carried out, followed by additional washing steps with PBS and 150 mM ammonium acetate (Fisher Chemical, Schwerte, Germany). The chambers were then removed, the samples dried overnight, and the DHAP matrix sublimated using a custom-made sublimation chamber.

Data were recorded on a timsTOF fleX MALDI-2 system equipped with microGRID technology enabling highly accurate 5  $\mu\text{m}$  spatial resolution with enhanced sensitivity using MALDI-2 post-ionization.

## Results

The depth and quantity of information obtained from a biological system is mainly determined by the analytical sensitivity of the mass spectrometer and the achievable lateral resolution of molecular distributions.

Achieving the maximum lateral resolution not only requires a suited minimal laser focus diameter, but equally importantly a precise sample positioning, i.e. by a precise MALDI stage. Precise mechanical positioning in the  $\mu\text{m}$  range, at 1/50 of a hair's diameter, gets increasingly challenging. Measuring the actual position, however, is robustly possible. The microGRID technology exploits these properties and determines the exact position of the MALDI stage in a 100 nm range, using linear encoders, to actively compensate the laser focus position to the correct spot on the sample. The entire process is fully automatic and requires no further user input. This results in highly accurate images at spatial resolution down to 5  $\mu\text{m}$ , demonstrated on two sample systems in Figure 1. Here, ion distributions measured at a spatial resolution of 5  $\mu\text{m}$  on a rat kidney in negative ion mode demonstrates resolving very fine structures in medulla and pelvis regions (See Figure 1A) without over-sampling artifacts such as striping or signal fading. In Figure 1B a small section from a rat testis sample was imaged at 5  $\mu\text{m}$  using microGRID. With the highly robust spatial resolution, small structures within the testicle can be clearly visualized, e.g. Leydig cells can be distinguished from the seminiferous tubules in the interstitium.

MALDI-2 has already proven to enable or increase the detectability of various analyte classes by orders of magnitude [3]. With decreasing laser spot diameter on the sample, and thereby a decreasing amount of ablated material, the use of MALDI-2 becomes crucial to fully exploit the information stored at small pixel sizes. Figure 2 shows data comparing MALDI and MALDI-2 measurements using consecutive

rat brain sections at 5  $\mu\text{m}$  pixel size in positive ion mode. Also here, the fine structures, distinguishing white matter, grey matter and granular layer in the cerebellum, are clearly visible. Ion images of selected lipid masses show nicely the 3 different scenarios that can occur when using 5  $\mu\text{m}$  pixel sizes in traditional MALDI mode. Figure 2A displays the ion distribution of  $m/z$  734.5703 showing nearly no difference in MALDI and MALDI-2,

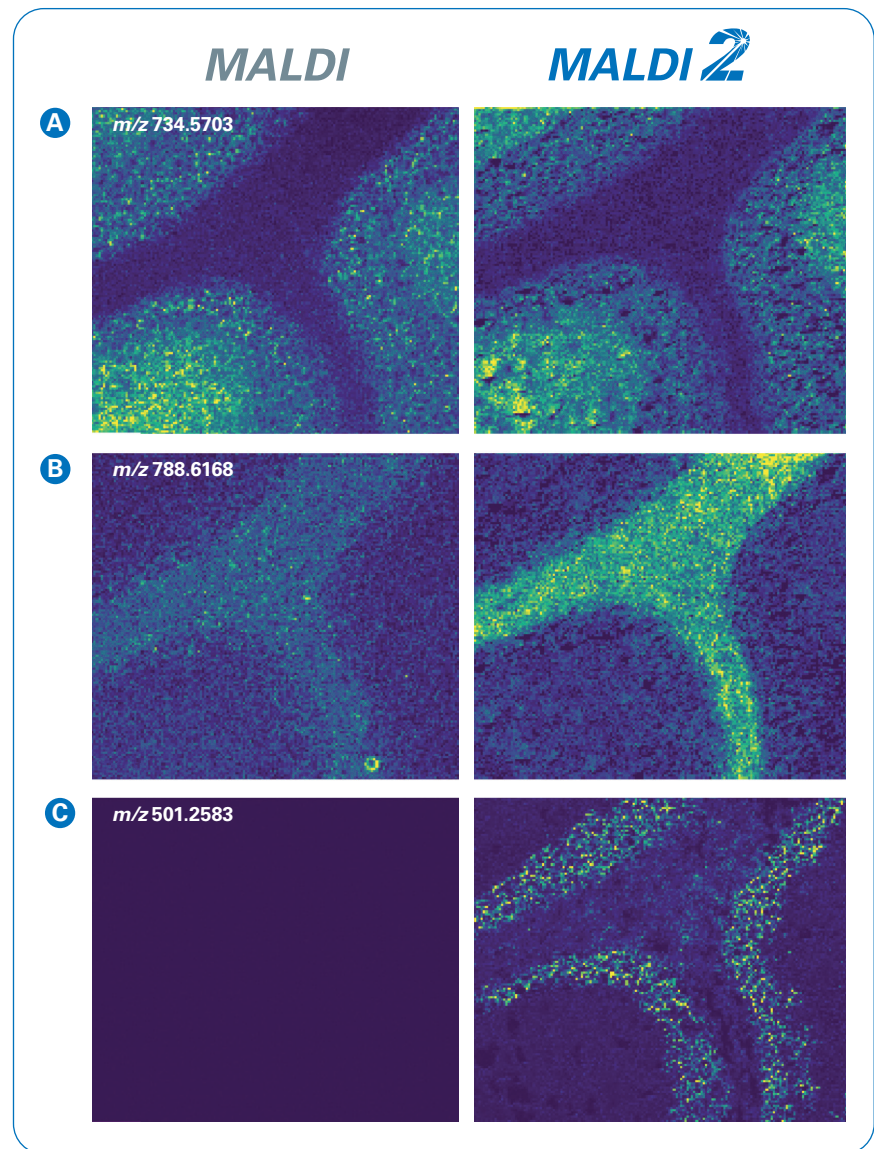


Figure 2: Comparison of microGRID imaging in MALDI (left) and MALDI-2 (right) mode. Ion images from a small region of the cerebellum of rat brain are shown. The data were recorded with 5  $\mu\text{m}$  spatial resolution in positive ion mode from consecutive sections. (A) Ion distribution of  $m/z$  734.5703 showing nearly the same intensity in MALDI and MALDI-2 (B) ion image of  $m/z$  788.6168 where intensity is a factor of  $\sim 2$  higher in MALDI-2 and (C) ion image of  $m/z$  501.2583 where sensitivity is not sufficient in MALDI mode at 5  $\mu\text{m}$  pixel size. Only with the increased sensitivity of MALDI-2 it is possible to detect the fine structures in this case.

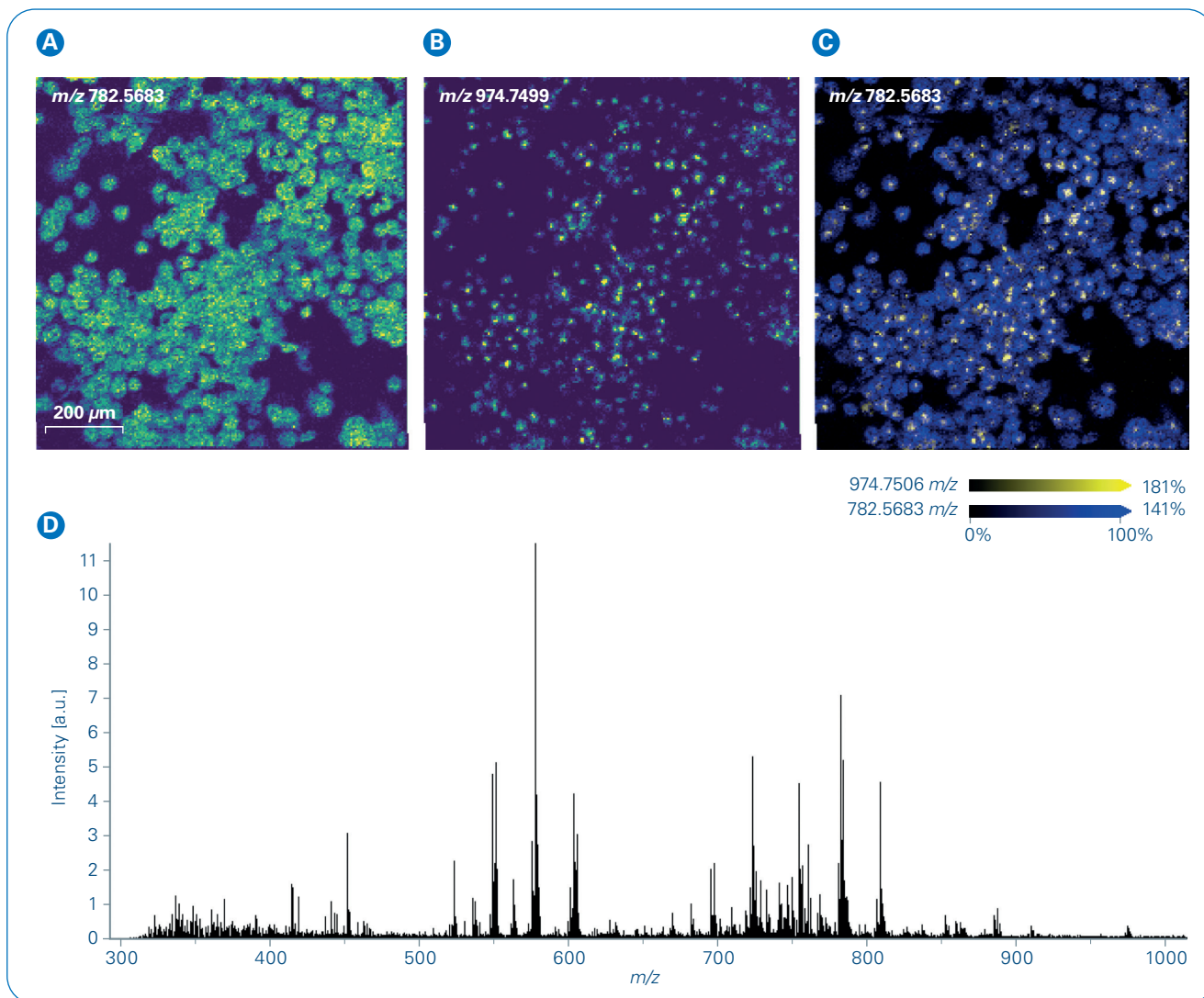


Figure 3: MALDI-2 ion images of THP-1 cell culture, differentiated to macrophages, using a  $5\ \mu\text{m}$  spatial resolution enabled by microGRID Imaging. Localization of 2 different structures in the cell (A, B) and the respective overlay (C). The  $m/z$  spectrum of a single cell shows nice signals over the whole mass range without much noise (D).

whereas the example from Figure 2B shows a gain in sensitivity by a factor of  $\sim 2$  for using MALDI-2. In the third example (Figure 1C), sensitivity is not sufficient anymore and no signal is detectable using traditional MALDI, but with the increased sensitivity using post-ionization, the ion distribution at  $m/z$  501.2583 becomes visible.

With eukaryotic cells being the smallest unit of life, high resolution imaging is inevitable to resolve these structures in the micrometer-range and to identify biomolecule distributions between and inside individual

cells. The vast potential of microGRID imaging in combination with MALDI-2 in this emerging field is demonstrated in Figure 3 and Figure 4.

At  $5\ \mu\text{m}$  spatial resolution, single THP-1 cells with cell size of  $30\text{--}50\ \mu\text{m}$  can clearly be differentiated and identified by the visualization of different  $m/z$  values (Figure 3). Here, PC 34:1, a common glycerophospholipid detected at  $m/z$  782.5683  $[\text{M}+\text{Na}]^+$ , shows a homogeneous distribution in both, the complete cell culture, and in the plasma membrane of individual cells (Figure 3A). However, a glycosphingolipid detected

at  $m/z$  974.7506  $[\text{M}+\text{H}]^+$ , most likely Hex2Cer 18:1;O2/24:0, shows a more discrete distribution (Figure 3B). Being localized in the center of the individual cells, it allows for identification of intracellular structures and association of this lipid to cell organelles (Figure 3C). Although the decrease in pixel size to  $5\ \mu\text{m}$  results in a drastically decrease of ablated and ionized sample material, instrument sensitivity and MALDI-2 boost allow for generating single-cell  $m/z$  spectra with a representable amount of lipids and metabolites in sufficient intensity without being “noisy” (Figure 3D).

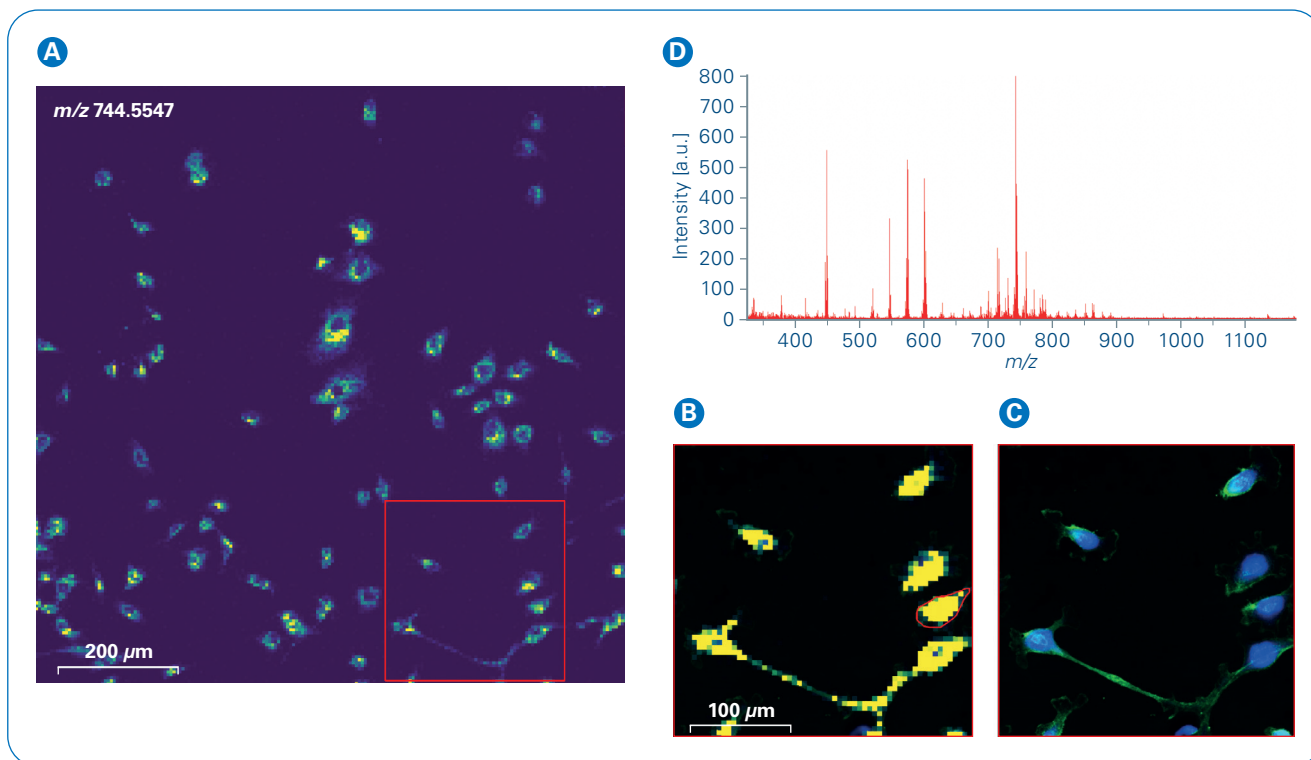


Figure 4: MALDI-2 ion images of Caki-2 cell culture in positive ion mode using a 5  $\mu\text{m}$  spatial resolution enabled by microGRID Imaging. (A) Ion distribution of a PE 36:2 at  $m/z$  744.5547 showing the localization of Caki-2 cells, with a zoom in the red outlined area (B). Comparison of the  $m/z$  image of the zoomed area (B) and the respective fluorescence image (Hoechst 33342 staining of cell nuclei and WGA staining of cell membrane) (C) shows good correlation of the very fine structures. (D)  $m/z$  spectrum of a single cell, highlighted in (B).

At imaging resolution capabilities in the low-micrometer range, sample preparation strategies are becoming increasingly important, as artifacts like lipid-delocalization and matrix-inhomogeneities occur in the micrometer range. With optimized sample preparation strategies as described by Bien *et al.* [1], microGRID is now able to push

imaging resolution to its outer limit and allows for the visualization of cellular fine structures. As an example, Caki-2 cells are chosen, as they develop a variety of cell sizes in the range of 20–80  $\mu\text{m}$ , with elongated fine structures of  $\leq 5 \mu\text{m}$  (Figure 4). Here, not only the cell nuclei become visible at appropriate  $m/z$  values (Figure 4A), but also fine

structures can be observed with those signals only being expressed in single pixels (Comparison of Figure 4B and 4C). Again, single-cell mass spectra (Figure 4D), consisting of approx. 40 pixel are rich in information and show a tremendous variety of  $m/z$  signals in the lipid range.

## Conclusion

The introduction of microGRID technology to the timsTOF fleX platform allows for high-lateral resolution MS imaging down to 5  $\mu\text{m}$ . By determining the exact position of the MALDI-stage in a 100 nm range using linear encoders, the MALDI laser beam will automatically move to the optimal position. This fully automatic technology unlocks high spatial resolution of biomolecules regardless of the user experience. In combination with sensitivity enhancement by use of MALDI-2, microGRID allows now for the visualization of fine structure analyte distributions in various tissue types and even on the single sub-cellular level.



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### References

- [1] Bien T, et al. (2021). *Anal. Chem.* **93**, 4513–4520.
- [2] Bien T, Koerfer K, et al. (2022). *PNAS*, in review.
- [3] Soltwisch J, et al. (2022). *Anal. Chem.* **92**, 13, 8697–8703.

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