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► To cite this version:

Nicolas Verplanck, Maxence Wisztorski, Jonathan Stauber, J.C. Camart, Michel Salzet, et al.. Enhanced spatial resolution of MALDI images using silicon masks. 11th International Conference on Miniaturized Systems for Chemistry and Life Sciences, MicroTAS 2007, 2007, Paris, France. pp.41-43. <hal-00284454>

HAL Id: hal-00284454

<https://hal.science/hal-00284454v1>

Submitted on 9 Jun 2022

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ENHANCED SPATIAL RESOLUTION OF MALDI IMAGES USING SILICON MASKS

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ABSTRACT

This paper reports on simple, efficient and economical methods to increase MALDI image resolution. It is not currently possible to focus the diameter of a MALDI laser down to $50 \times 50 \mu\text{m}^2$, i.e. $2500 \mu\text{m}^2$, without decreasing signal intensity. But using Silicon masks openings features, it might even be decreased to about $15 \times 50 \mu\text{m}^2$, i.e. $750 \mu\text{m}^2$. Furthermore, the presence of a mask on the tissue section sample may result in a significant increase in the signal intensity for high m/z ratios. Finally, a huge advantage of the masks is their ease of adaptability on any MALDI mass spectrometer.

Keywords: MALDI imaging, mass spectrometry, silicon mask, tissue analysis

1. INTRODUCTION

MALDI is well adapted to the introduction of raw samples such as tissue cuts. For tissue imaging, the minimum distance between two points must be superior to the laser beam diameter, which corresponds to the irradiation of several cells in the tissue using focalisation systems available on classical commercial instruments. Ideally, the image definition should be at the scale of the cell (10-20 μm for small cells). In the art, various methods have been tried to decrease the area irradiated by the laser beam for MALDI analysis (optical techniques [1], aperture [2], etc). In view of the various drawbacks of known methods to increase MALDI imaging resolution, there is clearly a need for new alternative, simple, efficient and economical methods to increase MALDI image resolution.

2. EXPERIMENTAL

The masks were engineered in silicon substrates (<100> orientation). Several masks of various openings, forms and sizes were fabricated using standard microfabrication techniques. According to the technique used (wet and dry etching), two geometries of masks have been obtained. Dry etching (50 μm) has been performed using Bosch process (ICP) through a photoresist mask: Mask A (Figure 1). For wet etching, we used KOH through a Si_xN_y mask which leads to V-grooves formation (following <111> orientation): Mask B (Figure 2). Both substrates were reduced in thickness to obtain a perforated membrane around 50-100 μm .

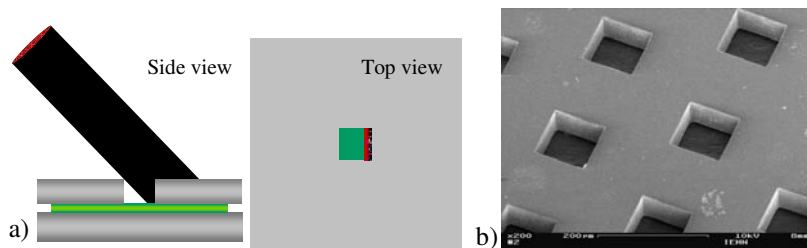


Figure 1 a) Schematic view of the irradiated area through a DRIE mask, b) View of the mask.

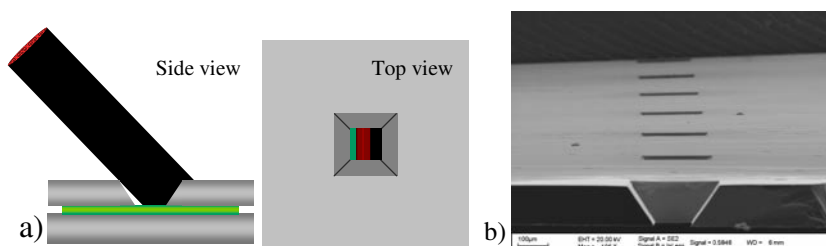


Figure 2 a) Schematic view of the irradiated area through a V-groove mask, b) View of the mask.

3. RESULTS AND DISCUSSION

Masks were evaluated for MALDI analysis of standard peptide solutions and tissue sections. Mass spectra were either acquired on a Voyager DE-STR (Applied Biosystems) equipped with a nitrogen laser or on an Ultraflex II TOF/TOF (Bruker Daltonics) equipped with a Smartbeam laser.

First tests on standard peptides solutions and tissue sections show that the apposition of masks does not disturb the desorption/ionization process and significantly decrease the dimensions of the irradiated area by the MALDI laser without decreasing the signal intensity of the observed ions. These results demonstrate that it is possible to decrease the analyzed areas down to about $15 \times 75 \mu\text{m}^2$, i.e. $1125 \mu\text{m}^2$. Using other masks openings features, it might even be decreased to about $15 \times 50 \mu\text{m}^2$, i.e. $750 \mu\text{m}^2$. Without masks, it is not currently possible to focus the diameter of a MALDI laser down to $50 \times 50 \mu\text{m}^2$, i.e. $2500 \mu\text{m}^2$, without decreasing signal intensity. Figure 3 describes such results through mask A with different dimensions of openings ($500 \mu\text{m}$, $240 \mu\text{m}$, $100 \mu\text{m}$ and $50 \mu\text{m}$). Figure 4 shows results without mask (Figure 4.a) and through mask B with internal openings respectively of $100 \mu\text{m}$ (Figure 4.b) and $185 \mu\text{m}$ (Figure 4.c).

In addition, it has even been observed that the presence of a mask on the tissue section sample may result in a significant increase in the signal intensity, in particular for high m/z ratios (results obtained both on standard peptide mixtures and on rat brain tissue sections).

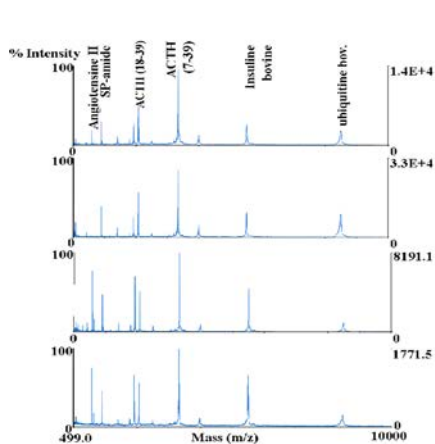


Figure 3 MALDI analysis of peptide standards through mask A with opening of a) 500µm, b) 240µm, c) 100µm and d) 50µm.

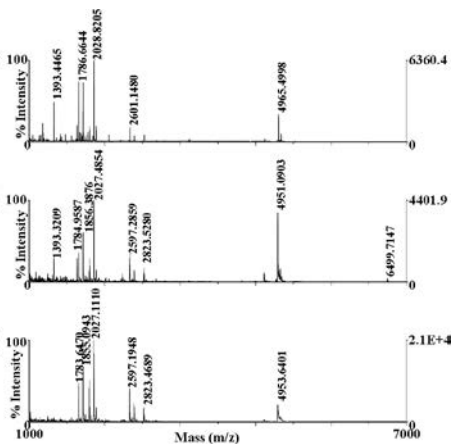


Figure 4 MALDI Direct analysis of rat brain a) without mask, b) and c) through mask B with internal opening of respectively 100µm and 185µm

4. CONCLUSIONS

Although the precise mechanism leading to this surprising increase in signal intensity is not completely elucidated, this result is particularly interesting for tissue section MALDI imaging, since most of the prior art technologies used to improve the resolution tend to result in a signal decrease. Finally, a huge advantage of the masks is certainly their ease of adaptability on any MALDI mass spectrometer, since their use does not involve any material modification or new equipment [3].

The next step will be the realization of a matrix of openings allowing MALDI imaging by point-to-point mass spectrometry.

ACKNOWLEDGEMENTS

We thank for its technical support Mr. Christophe Boyaval (SEM imaging). The European Union, the Centre National de la Recherche Scientifique (CNRS) and the Nord-Pas de Calais region are gratefully acknowledged for financial support.

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