



HAL
open science

Ultra-sensitive detection method with droplet based microfluidic device coupled to MALDI-TOF

Kiarach Mesbah, Sarah Bregant, Florent Malloggi

► **To cite this version:**

Kiarach Mesbah, Sarah Bregant, Florent Malloggi. Ultra-sensitive detection method with droplet based microfluidic device coupled to MALDI-TOF. *MicroTAS*, Oct 2016, Dublin, Ireland. cea-02346351

HAL Id: cea-02346351

<https://cea.hal.science/cea-02346351>

Submitted on 5 Nov 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Ultra-sensitive detection method with droplet based microfluidic device coupled to MALDI-TOF

Kiarach Mesbah¹, Sarah Bregant², Florent Malloggi¹

¹LIONS/NIMBE, CEA, CNRS, Université Paris-Saclay, CEA Saclay, Gif sur Yvette, France

²SIMOPRO CEA Saclay, Gif sur Yvette, France

We present an automated microfluidic droplet generator/depositor for MALDI-TOF analysis allowing attomol detection of peptides from sub-nanomolar solutions: a sensitivity which is a paramount importance for proteomics and diagnosis field.

Droplet based microfluidic devices offer many advantages in integration of multiple biological or chemical processes, essential tools for biomarker/biological fluids analysis [1]. However the detection mainly relies on optical methods such as fluorescence which implies a labelling of target molecules but also suffers from a lack of sensitivity. At the opposite mass spectrometry is a highly sensitive technic based on a label free detection of analytes but interfacing droplet-based microfluidic and mass spectrometry is challenging and up to now there are few studies reported [2-3].

We focus on the integration of a droplet based microfluidic system with a MALDI-TOF. We develop methods which prevent the use of surfactants: a crippling factor since surfactants led to high background noise, and then are not compatible with high sensitivity detection method. To circumvent this effect, we designed a chip which prevents droplets coalescence by spacing each droplet with a controlled volume of oil (Figure 1). This simple geometry allows to finely tune the amount of spotted droplets and controlled the coalescence. The microfluidic chip outlet is then connected to a capillary, and the droplets are transferred to a MALDI plate mounted on a motorized xy-stage. To estimate the potential of the platform, we developed a full optimized method (sample composition, matrix composition, droplet generation, deposition and analysis) of relevant proteomics biomarker: a native peptide Angiotensin II involved in several diseases [4-5]. First, we compared our system with a standard MALDI deposition procedure *i.e.* manual pipetting. The spectra of Figure 2 show 500nl of a solution of 10fmol/ μ l Angiotensin II *-left* pipette deposited and *-right* platform deposited. In both cases, the 1046.2 m/z peak of Angiotensin II is visible but in the former case the intensity is increased by one order of magnitude. A closer look to the deposits shows a concentration effect with the platform deposition (see insets figure 2).

In a second step, we made a sensitivity test by depositing different concentrations of analytes. The Figure 3 shows the average peak intensity as a function of n the quantity deposited in femtomole. We record good intensity signal down to 100 attomoles (see inset figure 3). Below this threshold the spectra are very noisy (Figure 4-*left*).

In order to increase the sensitivity of the method, we developed a multi-spotting deposit. By repeated deposition of droplets, we increase drastically the amount of peptides on the spot, and can then reach very low level of detection. As a proof the Figure 4 shows the spectra of sub-nM solution before (*left*) and after the multideposit (*right*).

We developed a method which allows to generate single droplet in a microchip coupled with a MALDI-TOF analysis. We control the volume of sample deposit on each spot, and by multi-spotting we reached a very high sensitivity compatible with physiological concentration of proteomic biomarkers.

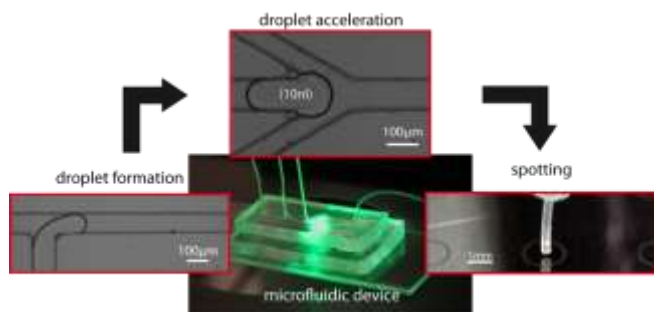


Figure 1. Principle of the droplet formation and MALDI deposition

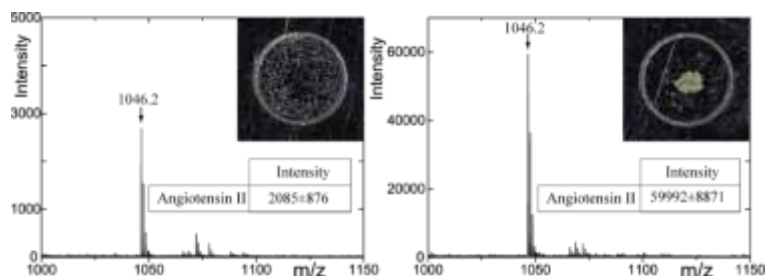


Figure 2. MALDI spectra. *Left.* Standard pipette deposit. *Right.* Microfluidic platform deposit. Inserts: Dried mixture of peptide/matrix before laser desorption.

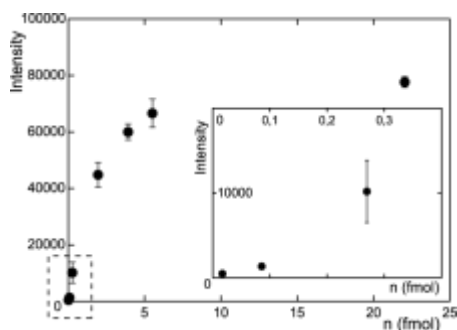


Figure 3. Average MS signal intensity as a function of the amount n (n from 22 femtomoles to 17 attomoles) of Angiotensin II deposited on the MALDI plate by the microfluidic platform deposit.

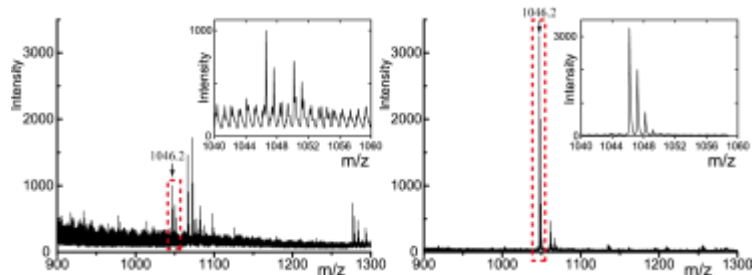


Figure 4. MALDI spectra of a 100 attomoles solution of Angiotensin II. *Left.* Microfluidic platform deposit. *Right.* Microfluidic platform multi-spotting deposit. Inserts: zoom on Angiotensin II peaks.

REFERENCES:

1. "Digital microfluidics." Choi, Kihwan, Alphonsus HC Ng, Ryan Fobel, and Aaron R. Wheeler., *Annual review of analytical chemistry*, 5: 413-440 (2012).
2. "Recent advances in microfluidics combined with mass spectrometry: technologies and applications." Gao, D., Liu, H., Jiang, Y., & Lin J. M., *Lab on a Chip*, 13(17), 3309-3322 (2013).
3. "Interfacing droplet microfluidics with matrix-assisted laser desorption/ionization mass spectrometry: label-free content analysis of single droplets." Küster, S. K., Fagerer, S. R., Verboket, P. E., Eyer, K., Jefimovs, K., Zenobi, R., & Dittrich, P. S., *Analytical chemistry*, 85(3), 1285-1289 (2013).
4. "Identification of neutrophil-derived proteases and angiotensin II as biomarkers of cancer cachexia." Penafuerte, C. A., Gagnon, B., Sirois, J., Murphy, J., MacDonald, N., & Tremblay, M. L. *British journal of cancer*, 114, 680-687 (2016).
5. "Angiotensin II plasma levels are linked to disease severity and predict fatal outcomes in H7N9-infected patients." Huang F, Guo J, Zou Z, Liu J, Cao B, Zhang S, Li H, Wang W, Sheng M, Liu S, Pan J., *Nature communications*, 5:3595 (2014).