

## Universal Deposition Device for Off-line Coupling of LC to MALDI MS and MS/MS

Tomas Rejtar, Hsuan-shen Chen, Eugene Moskovets, Lingyun Li, Viktor Andreev and Barry L. Karger

Barnett Institute and Department of Chemistry, Northeastern University, Boston, MA, USA

The analysis of complex proteomic samples requires high resolution separation processes in order to reduce the sample complexity prior to the MALDI MS analysis. Depending on the characteristics and amount of the sample, various separation techniques are available. Typically, separation of protein digests is achieved by reverse phase LC. In order to preserve separation resolution of LC, it is necessary to deposit sample onto the MALDI plate in a way that it can be interrogated in the MS mode at a rate of at least 5 MS spectra per chromatographic peak. For high resolution separations, it can be accomplished by a continuous deposition, where MS spectra can be acquired at a rate of more than one mass spectrum per second of chromatography, thus suitable for separation with peak half-heights approximately 3-5 sec. On the other hand, for applications where high chromatographic resolution is not required (e.g. limited sample complexity) sample deposition can be performed at a lower rate.

In order to utilize different modes of deposition, we have modified the previously demonstrated vacuum deposition interface to enable continuous deposition (streaking) at various flow rates as well as deposition of discrete droplets (spotting). Compared to the vacuum deposition, the MALDI plate was spin coated with nitrocellulose prior to sample deposition, which allowed deposition at a pressure of 200 Torr (vs. the previous 1 Torr) without widening of the trace. Microcrystals formed using continuous deposition were similar to those found in the standard dried-droplet method resulting in MS and MS/MS characteristics comparable to the sample prepared by dried-droplet procedure. In the discrete droplet mode, it was found that the volume of the deposited liquid had to be approximately 100 nL in order to achieve reliable and reproducible deposition. This droplet volume corresponds to spotting every 3 sec at a flow rate of 1.5  $\mu\text{L}/\text{min}$ .

The off-line deposition device was used in both the continuous and spotting modes for nano and capillary LC separations. Different operating modes and experimental conditions of the deposition interface are listed in Table 1. The deposition interface was used in the continuous mode for analysis of a strong cation exchange fraction of tryptic digest of yeast soluble proteins using nano LC [1]. Coupling of the cap LC to MALDI MS in the continuous mode was demonstrated on the analysis of a digest of a 10 protein mixture, Fig. 1. A combination of spotting and continuous deposition was used to deposit a narrow streak of external mass calibrants in close proximity of nano LC separation deposited in the form of spots (1 spot = 3 sec of chromatography) [2]. This procedure allowed achieving mass accuracy of  $\pm 10$  ppm for approximately 90% of peptides identified by Mascot searching using MS/MS, Fig. 2.

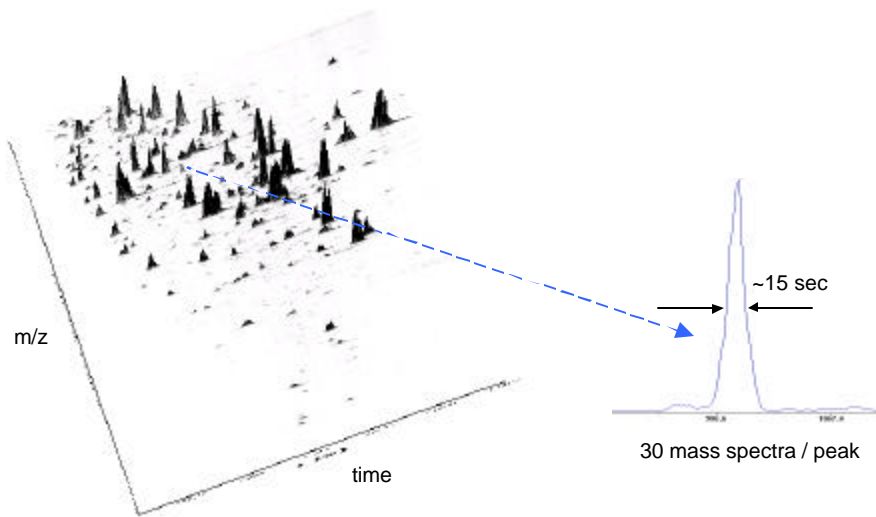
**Table 1:** Deposition conditions using different operating modes of the deposition interface.

	STREAKING		SPOTTING	
	0.2	2.5	0.2	2.0
LC flow rate [ $\mu\text{L}/\text{min}$ ]	0.2	2.5	0.2	2.0
Total flow rate [ $\mu\text{L}/\text{min}$ ] (LC + MALDI matrix)	1.5	5.0	1.5	4.0
MS sampling rate [sec/MS spectrum]	1	2	3	5
LC time [min] per plate	120	20	45	30
Total no. of possible MS per plate	7,000	1,000	900	400
No. of plates to deposit 60 min of LC	1/2	2	2	2
Time to acquire MS signal from 60 min LC [hours]	4.5	2.0	1.5	1.0
Comment	nanoLC	capLC	nanoLC	capLC

MALDI plate: Area available for deposition 45 x 45 mm. MS instrument: AB 4700 Proteomics Analyzer

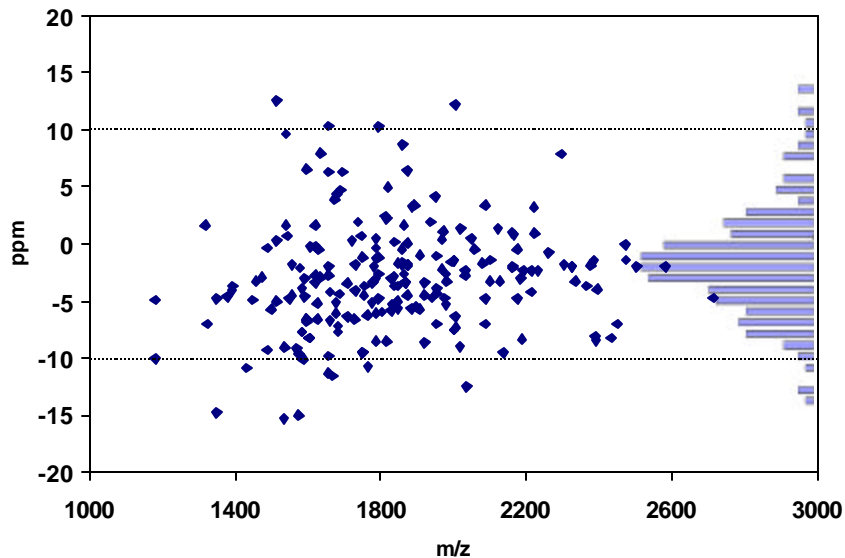
### References:

1. For more applications of the deposition interface, see posters: WPW 449, ThPE 064
2. E. Moskovets, H.-S. Chen, A. Pashkova, T. Rejtar, V. Andreev and B.L. Karger *Closely placed external standard: a universal method of achieving 5 ppm mass accuracy over entire MALDI plate in MALDI-TOF MS* submitted.



**Figure 1.** Capillary LC-MALDI MS using continuous deposition.

*Sample:* 5  $\mu$ L 2  $\mu$ M tryptic digest of 10 protein mixture. *LC separation:* 300  $\mu$ m i.d x 5 cm column packed with 3  $\mu$ m ODS particles using 30 min gradient. *Flow rate:* 2.5  $\mu$ L/min + 2.5  $\mu$ L/min matrix solution. MS spectrum acquired every second of chromatography.



**Figure 2.** Distribution of mass errors for peptides identified by MS/MS in nano LC-MALDI MS separation using spotting and closely placed streak of mass calibrants.

*Sample:* 5  $\mu$ L SCX fraction (15th out of 20) of yeast digest. *LC separation:* 75  $\mu$ m i.d x 15 cm column packed with 3  $\mu$ m ODS particles using 50 min gradient. *Flow rate:* 0.2  $\mu$ L/min + 1.2  $\mu$ L/min matrix solution. MS spectrum acquired every 3 sec of chromatography. Distance between sample spots and streak of calibrants < 400  $\mu$ m.