EXPLORATORY ANALYSIS OF SPUTTERED METAL LAYERS FOR APPLICATION TO MASS SPECTROMETRY IMAGING

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Introduction

Mass spectrometry imaging (MSI) is a technique that maps the distribution of molecules in biological tissues [1]. The most widely used MSI technique is Matrix Assisted Laser Desorption/Ionization (MALDI), but organic matrices may impact the quality of the molecular images due to limited lateral resolution and background noise in the low mass range, hindering its use in metabolomics and high spatial resolution applications [2]. Recently, gold and silver sputtered deposited layers have been proposed as a valuable alternative to organic matrices [3,4]. With sputter deposition it is possible to achieve high lateral resolution and low background noise. Here, we present the sputter technology for MSI by exploring its possibilities using various metals and metal-oxides. We tested the use of Au, Ag, FeO, Cu, Pt, Pd and WO_3 with various layer thickness to compare.

Methods

Mouse liver tissues were sliced into 10 μm sections using a cryostat and directly placed on indium tin oxide (ITO)-coated glass slides. Next, various layers of different thickness ranging from 3 to 20 nm of metal nanoparticles were deposited over each tissue section using a sputtering system running in RF mode. This procedure has been repeated for each metal: Au, Ag, FeO, Cu, Pt, Pd and WO_3. MS spectra were acquired with a Bruker UltrafleXtreme MALDI-TOF instrument. The instrument has been adjusted with an accumulation of 1000 laser shots per pixel and laser frequency of 2 kHz. The randomwalk feature was configured with a movement of 200 μm in order to average a large area of the tissue (Fig. 1).
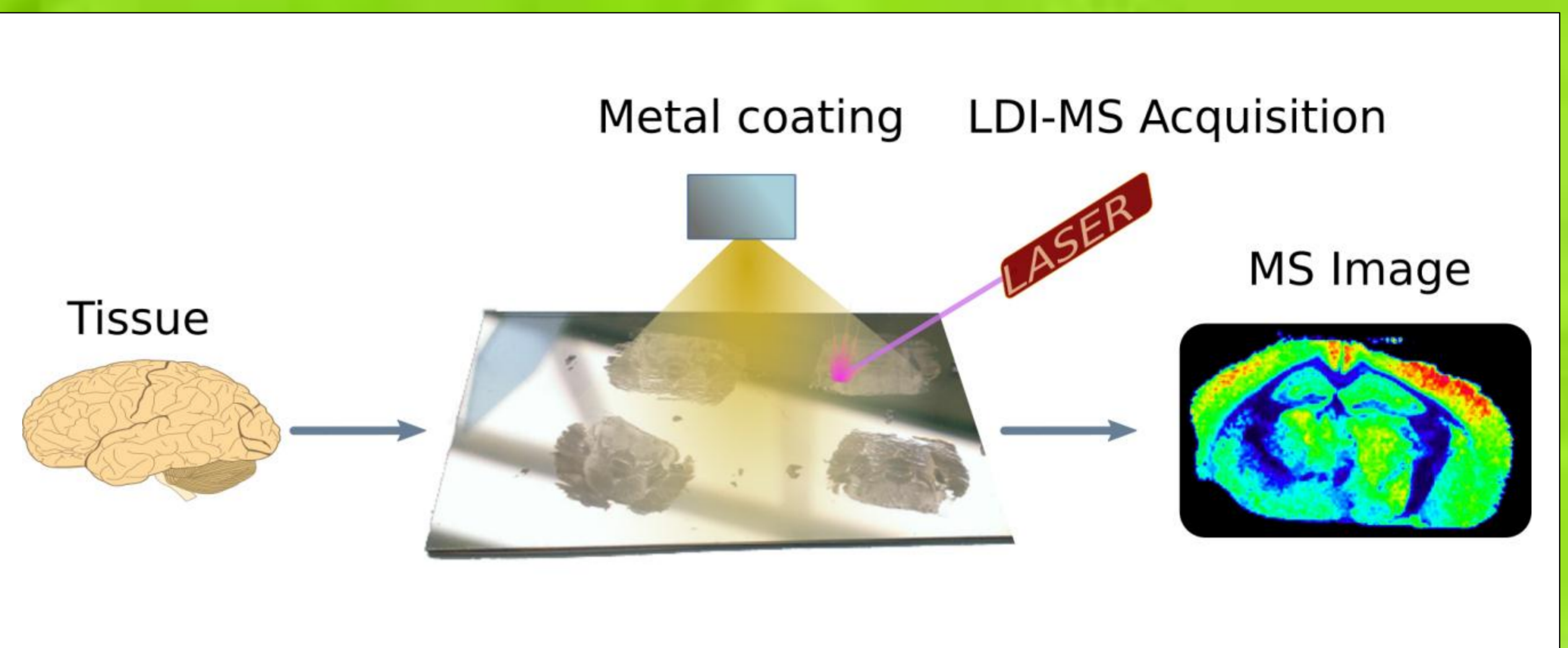

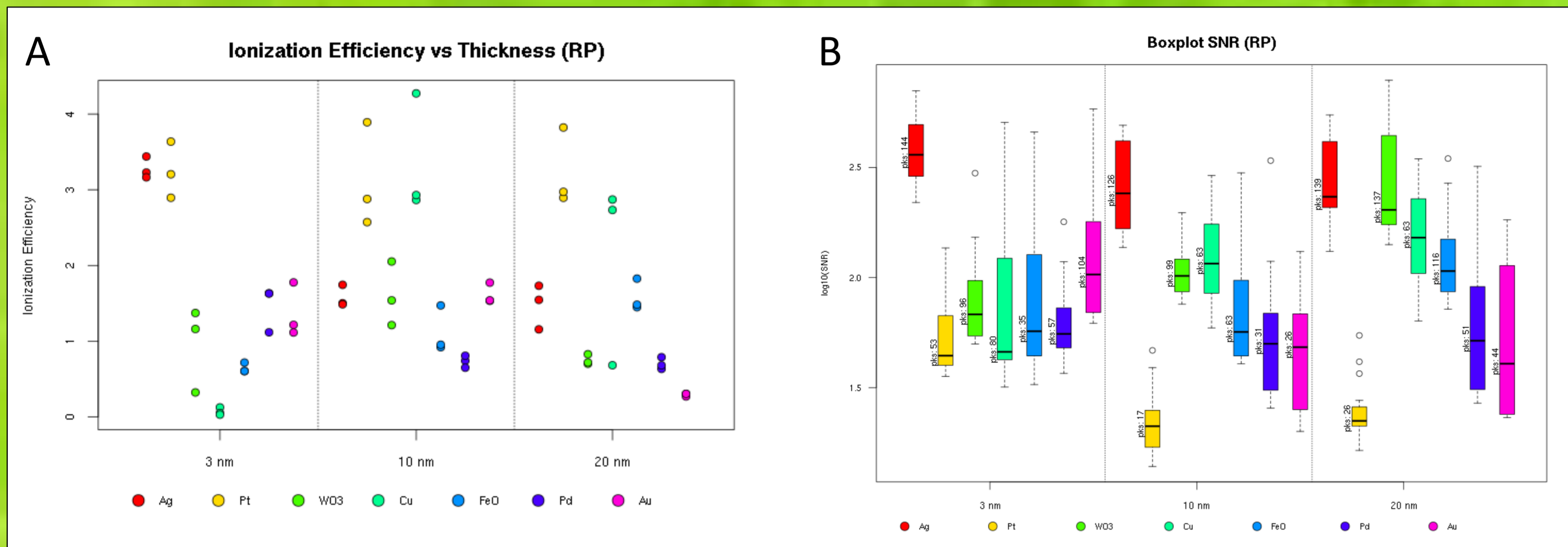**Fig. 1.** Experimental workflow of sputtered metal for MSI.

Fig. 2. Sputtered layer thickness optimization. **A:** IE factor of various layers. **B:** Total number detected peaks.

Sputtered layer thickness optimization

Metal sputtered layers are compared using ionization efficiency (IE) (Fig. 2A), the total number of detected peaks and its SNR (Fig. 2B). IE factor is calculated as a ratio of total ion count (TIC) and the used relative laser power to acquire a spectrum. Following thickness are selected as the best for each metal:

Ag	3 nm	**FeO**	20 nm
Pt	3 nm	**Pd**	3 nm
WO₃	20 nm	**Au**	3 nm
Cu	10 nm		

Metal layer evaluation

The MS spectra obtained for each metal (Fig. 3A-G) presents slightly different features. This suggests that, as it happens with organic matrices, each metal promotes the ionization of different molecules. Therefore, a selection of a metal over another may result in an improvement of the MS sensitivity for a given MSI experiment. The detected peaks of each metal layer is plotted in Fig. 3H to show which spices are able to detect each metal layer. The isotopic pattern of each metal have to be taken in consideration because in some cases (e.j. WO_3) most peaks are product of multiple stable isotopes.
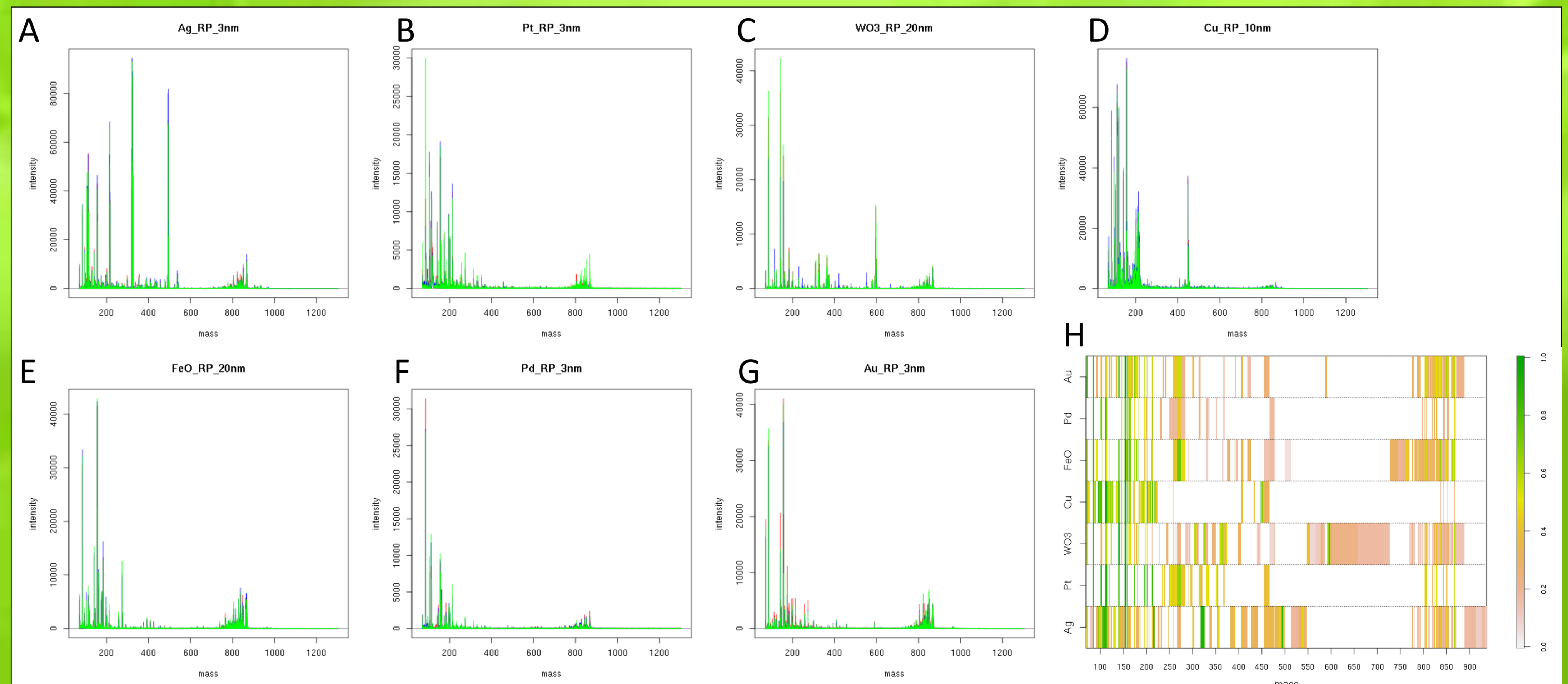

Fig. 3. Spectra acquired with the optimal sputtered layer of each metal. **A:** Ag 3nm. **B:** Pt 3nm. **C:** WO_3 20 nm. **D:** Cu 10 nm. **E:** FeO 20 nm. **F:** Pd 3 nm. **G:** Au 3 nm. **H:** Spectral complementarity of all metals layers.

Conclusion

Various sputtered materials have been tested and compared as an alternative means of laser desorption ionization for MSI. Most of the peaks corresponding to the sputtered metal layer clusters were also detected. This means that, metals with less stable isotopes may result more appropriate for MSI, since metal clusters will contain fewer peaks. These detected peaks of metal clusters are useful as references for internal *m/z* calibration. These metals together with previously reported Ag and Au open up a new field for exploration of sputter coated materials for MSI analysis.

Acknowledgments

References

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