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# Development of a MALDI imaging mass spectrometry (IMS) approach to bacterial proteomics: first application to *Listeria monocytogenes* biofilms exposed to a desiccation

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## Introduction

Human listeriosis cases are due to the ingestion of contaminated foods with *Listeria monocytogenes* and most cases start with food contamination in industries. The control of *L. monocytogenes* is difficult to achieve due to its survival capabilities to harsh conditions and to its ability to form biofilms. Bacteria are consecutively submitted to daily variations in air relative humidity to cleaning-disinfection procedures. Despite this, part of the population is able to persist, but the molecular mechanisms by which biofilms adapt to desiccation are not well-known.

Matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) is a surface-sampling technology that can determine spatial information and relative abundance of analytes directly from biological samples. Spectra are collected and each peak intensity in the spectra is used to generate an ion intensity map.

This study aims to develop an IMS approach to explore the protein expression and their *in situ* distribution within *L. monocytogenes* biofilms exposed to desiccation.

## Discussion

The profiling showed lower level of background noise and higher intensity of peaks than the classical approach. This could be due to the higher ratio matrix-sample present in the profiling and also the superior signal accumulation obtained in one spot of analysis. For both approaches, an ANOVA test was made and the resultant significant *m/z* intervals were used for the PCA, which separated the spectra from the 3 regions (Figure 4 and 5).

However, it can't be stated that the 3 regions showed clear differences since this result might be related to a technical constraint (Figure 7). The rate and quality of crystallization of the matrix/analyte is affected by humidity. The normal section and the interface are submerged in liquid throughout the workflow, unlike the desiccated section having a lower level of humidity and yielding spectra with higher intensity ions.

The majority of proteins detected by intact cell MALDI methods are small ribosomal proteins, which are ideally suited to make a robust bacteria identification but not ideal to screen for adaptation to stresses. This <15 kDa proteins are abundant and can disturb the ionization of other sparse proteins. Nonetheless, one can assume the possible presence of cold shock proteins within these spectra, like from the CspL family ( $\pm 7,200$  Da).

## Workflow

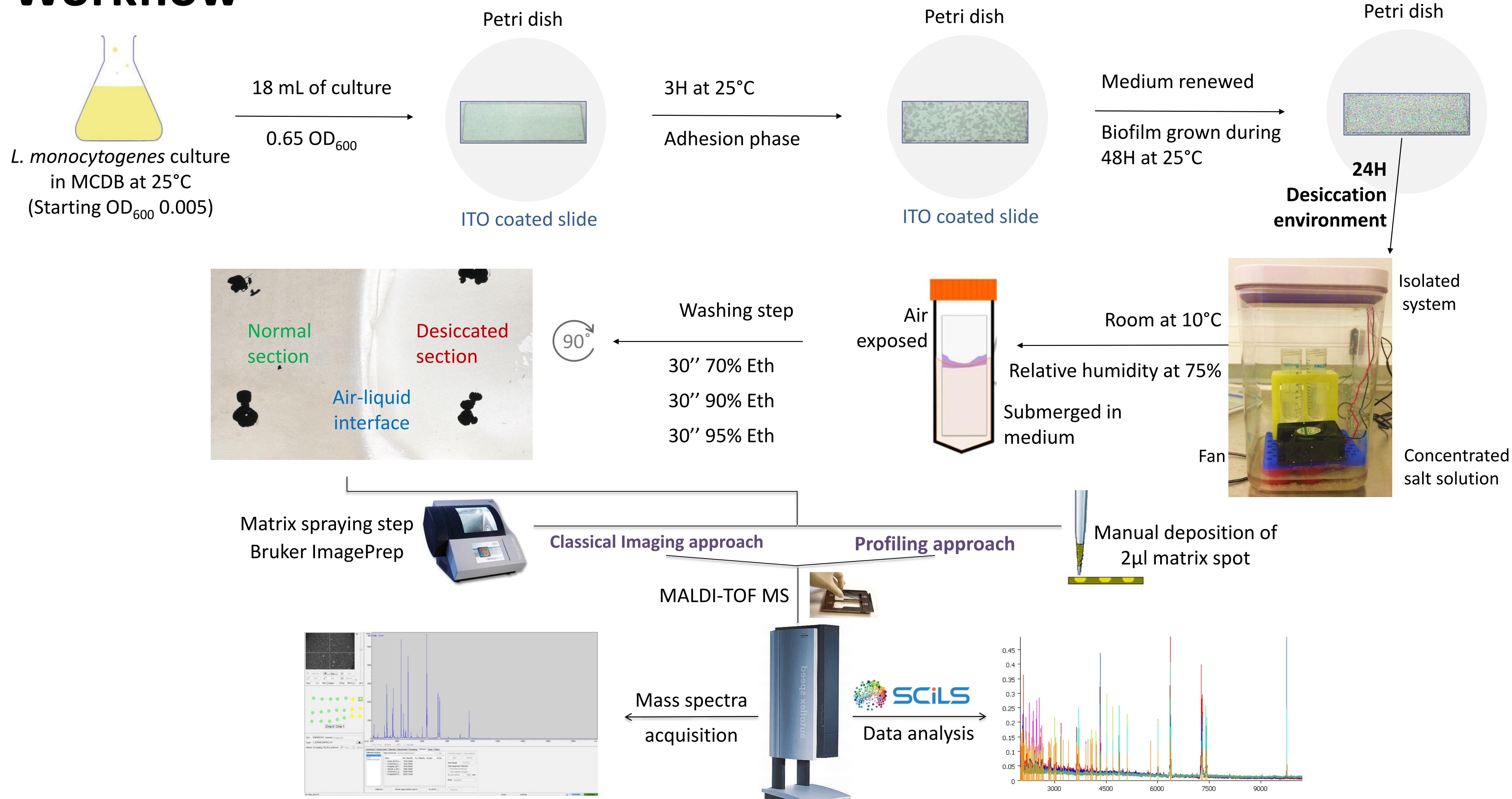


Figure 1: Workflow development for the Imaging Mass Spectrometry analysis of *Listeria monocytogenes* biofilms subjected to desiccation

The workflow development yield *L. monocytogenes* biofilms with 2 sections of analysis and an interface between them, leading to good spectra in both approaches, a classical imaging and profiling (Figure 1). Through the classical approach, a section across the biofilm was screened (Figure 2). The gathered mass spectra combined with the SCiLS software enables visualization of the result across the biofilm (Figure 6). In the Profiling approach the mass spectra are acquired from larger spots, allowing to obtain a better spectral resolution (Figure 3).

## Results

### Classical imaging approach

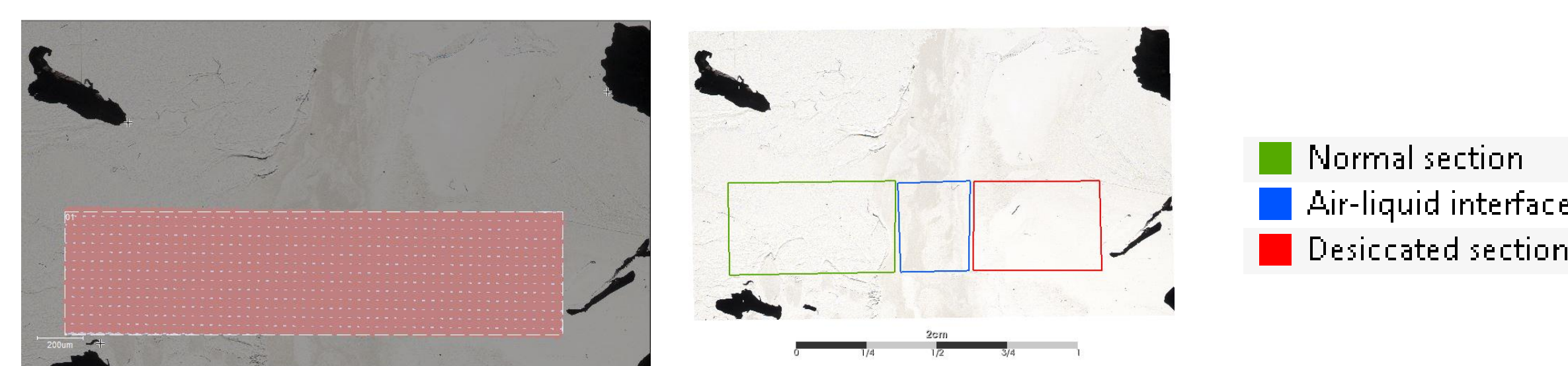


Figure 2: MS analysis across a biofilm section; Resolution: 100 µm; 500 shots per spot

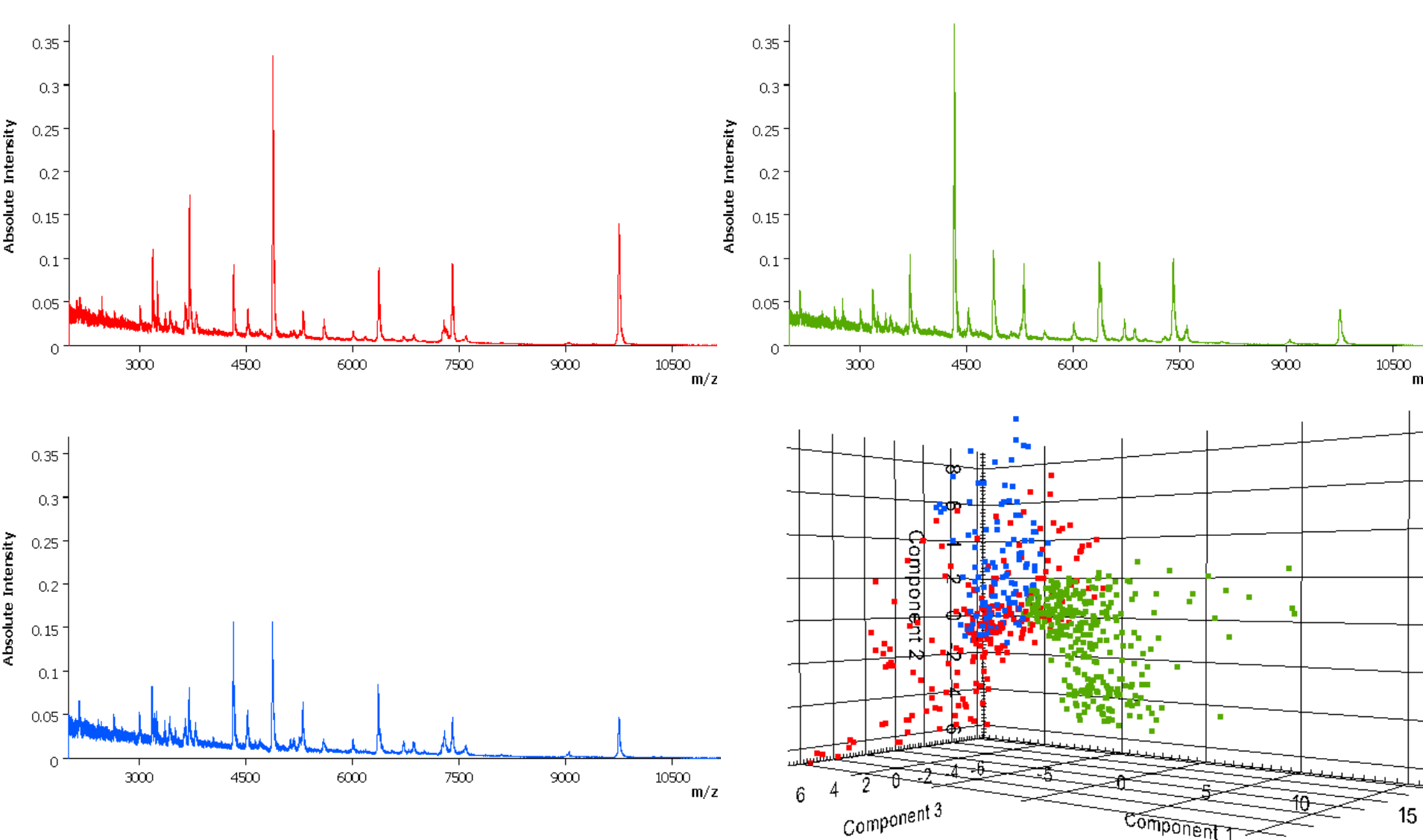


Figure 4: Average spectrum for each of the 3 sections and principal component analysis

### Profiling approach

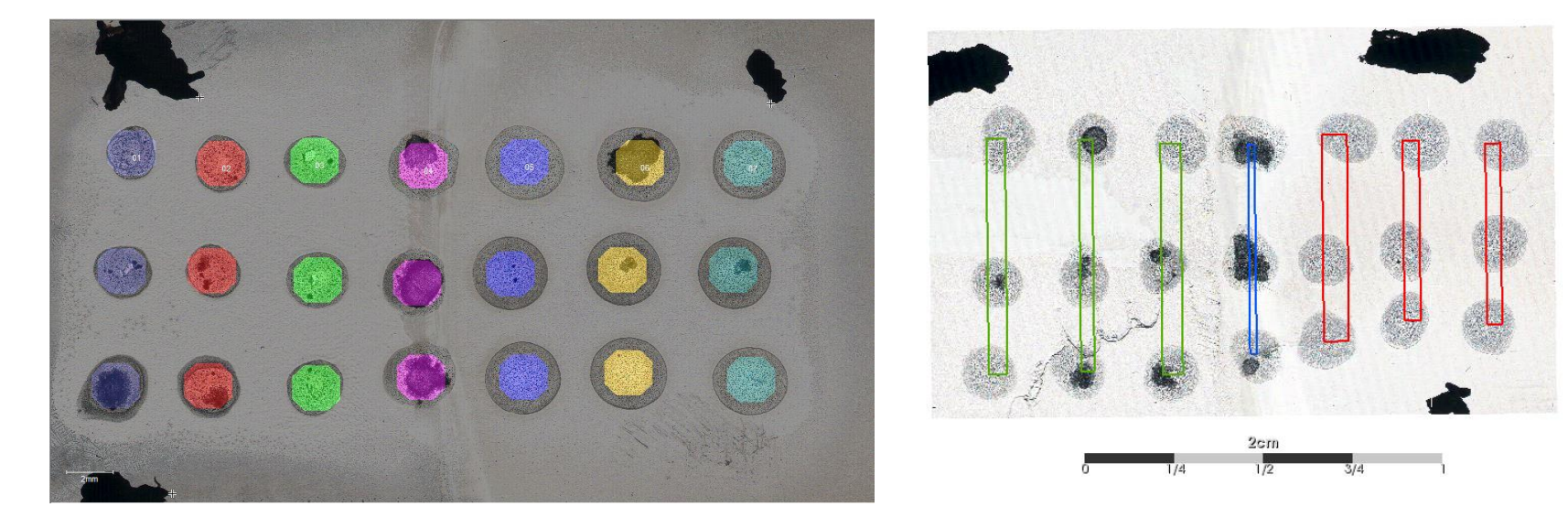


Figure 3: MS analysis in the drops of matrix; Resolution: 2000 µm; 4000 shots per spot

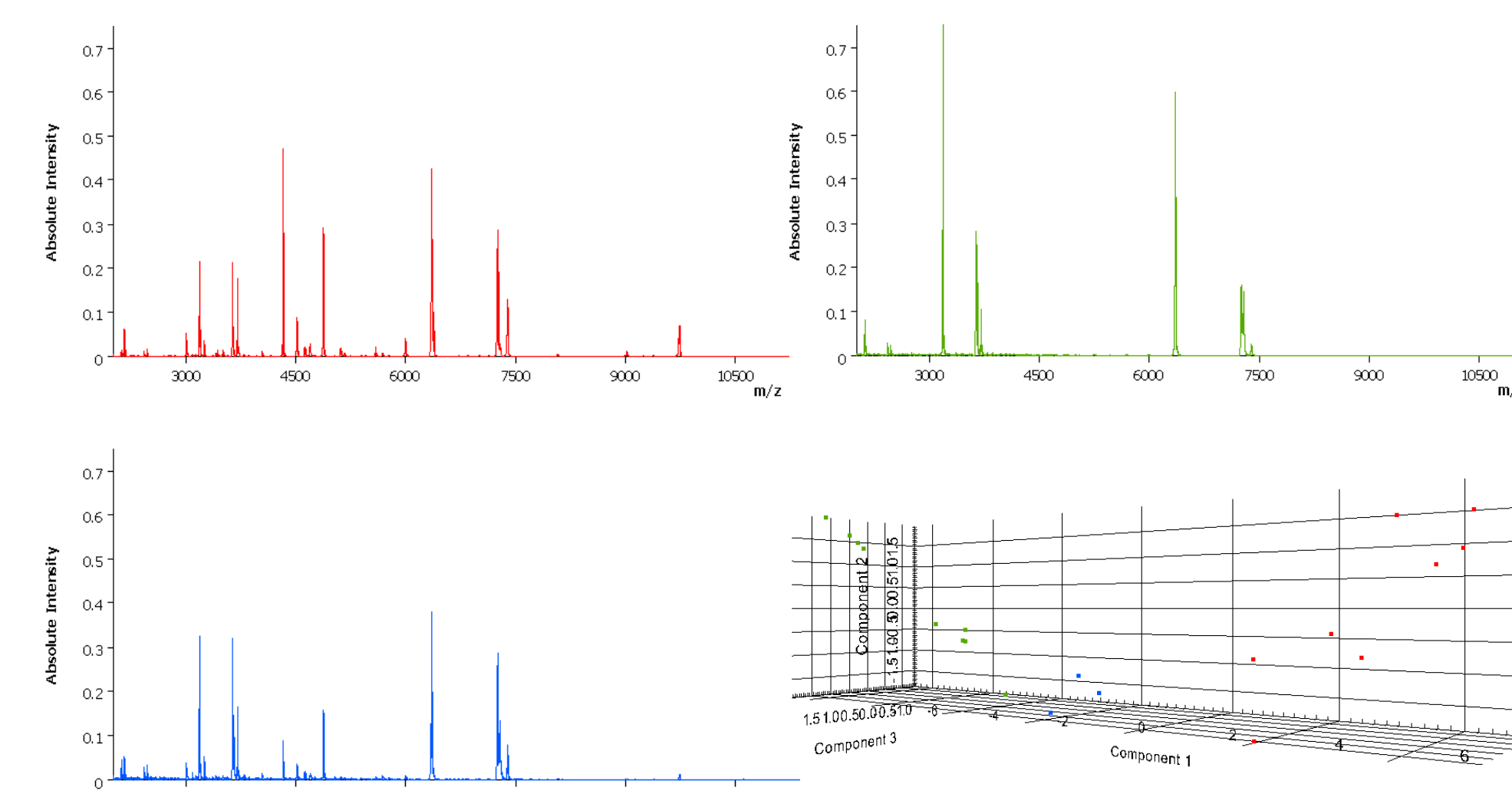


Figure 5: Average spectrum for each of the 3 sections and principal component analysis

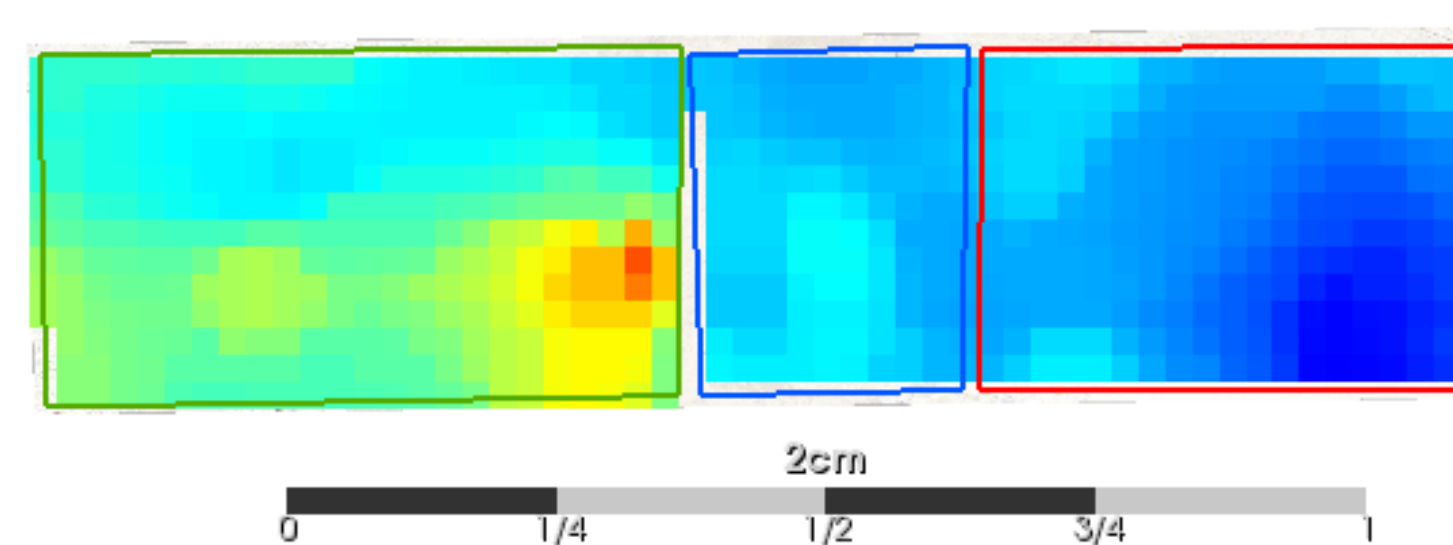


Figure 6: Visualization of PCA's component 1 on top of the section of analysis.

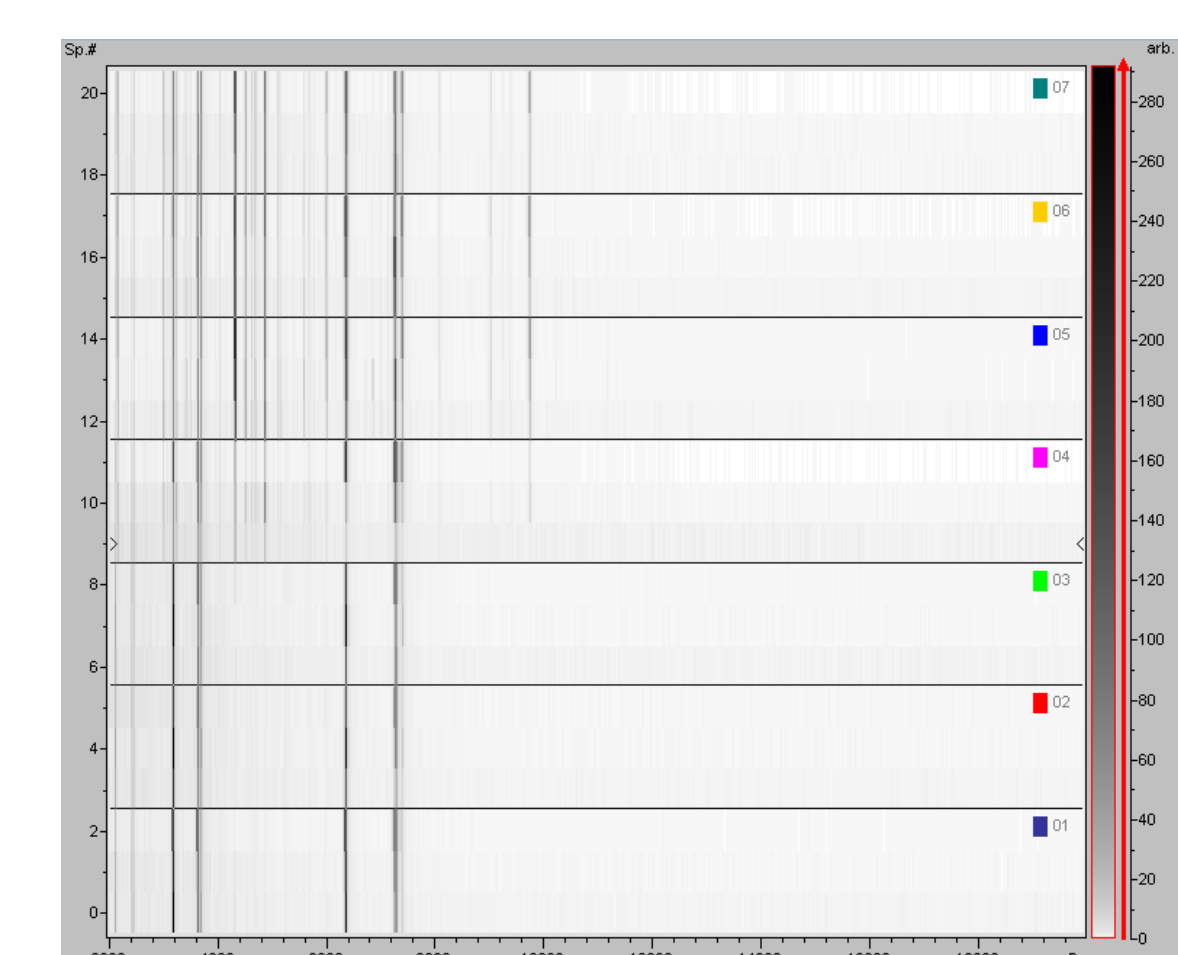


Figure 7: Gel stack view from the average spectra of all the analysed spots in Figure 3.

## Conclusions

The profiling workflow revealed better spectral resolution, making it easier to detect differences between the conditions. The next step will involve testing for protein and peptide identification detected here, either by a traditional shotgun approach or a Liquid Extraction Surface Analysis (LESA-MS). This future identification will dissect the spatial proteome of an intact bacterial biofilm giving a new insight into protein expression relating to biofilm adaptation.