

# Development of a novel MALDI-MS based liquid biopsy detection platform

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## Background

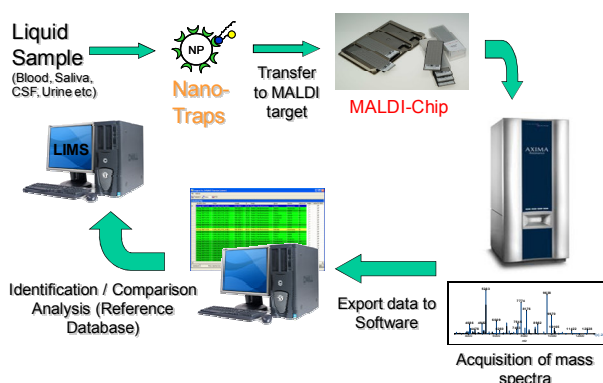
- An increasing number of researchers, start-ups and leading companies have turned their attention to the identification and detection of **biomarkers from biological fluids** (e.g. blood, sputum, saliva, urine, etc.). This highly dynamic field of research is called **liquid biopsy**.
- Cell-derived components** (e.g. CTCs, EVs, Exosomes, etc.) containing the molecular signatures of their diseased parent cells and tissues represent very promising sources of novel biomarkers and attractive targets for **non-invasive diagnostics**.
- MALDI-MS** represents a robust and easy-to-use tool for the analysis of different types of biological molecules (e.g. proteins, glycans, lipids, etc.) which shows high potential to be used as **routine screening tool for clinical applications**.

## Aims

- Translation of liquid biopsy biomarkers to a **MALDI analysis platform**, in particular focused on circulating particles and cell-derived protein and lipid biomarkers (e.g. lipoproteins, circulating cells, microparticles, etc.).
- To develop a **software platform** for the **qualitative and quantitative statistical analysis** of MALDI mass profile data from liquid samples (e.g. blood plasma) based on the MS-platforms and software tools provided by our company partner.

## Methods

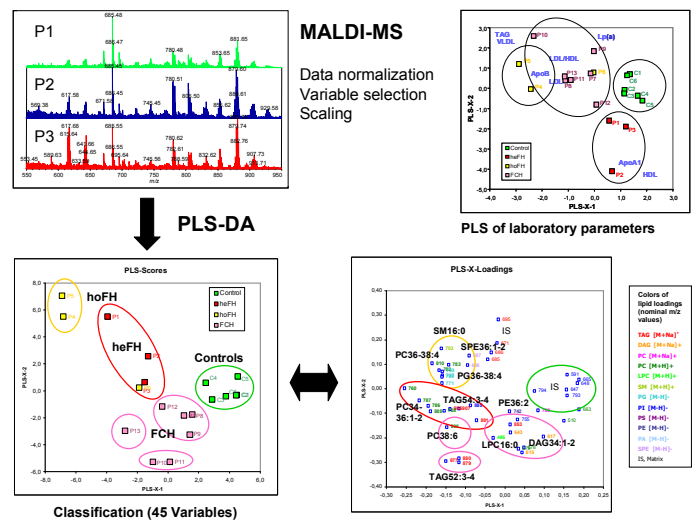
- Development of an **MALDI-MS based workflow for monitoring of biomarkers from liquid biopsy**. Method setup will be focused on potential biomarker candidates identified based on a detailed proteomics and lipidomics characterisation of different samples (e.g. lipoproteins, EVs) in combination with nano-structured biomarker enrichment devices in chip-format and software-based sample analysis (Figure 1).



**Figure 1.** Overview of the workflow of the MALDI-MS based liquid biopsy detection platform. Potential biomarker candidates from liquid biopsy samples will be specifically trapped by nano-structured devices (e.g. nanoparticles) and transferred to MALDI target plates designed to capture different arrays of molecules (i.e. MALDI-Chip). Samples will be analysed using MALDI-MS to obtain specific mass spectra which will be statistically evaluated based on specific algorithms for identification of sample specific mass profiles compared to reference database entries.

## Expectations and Results

- Optimisation of **protocols for biomarker isolation, sample preparation and analysis** (e.g. matrix substances) from liquid biopsy samples (e.g. blood plasma).
- Development of **selective biomarker trapping tools** (e.g. nano-traps) from liquid biopsy for transfer to MALDI targets and direct analysis using different MALDI-MS platforms.
- Establishment of **software tools to guide the liquid biopsy analysis workflow** and the statistical analysis of MALDI data sets for identification and detection of the biomarker molecules. An example for the feasibility of using **MALDI-MS based screening of lipid biomarkers from plasma** for the differentiation of patients with different types of lipid metabolism disorders (e.g. familial hypercholesterolemia) using a multivariate statistical data analysis (e.g. PLS-DA) approach is shown in Figure 2.



**Figure 2.** Example for MALDI-MS based screening of clinical samples using a multivariate data analysis approach. After data normalization, variable selection and scaling of individual MALDI mass spectral data from patients (P1, P2, P3, etc.), PLS-DA delivers a 2D-map of the samples enforced by a predefined grouping of e.g. disease types. Relevant lipid species (corresponding to *m/z* values) can now be detected by evaluating the PLS loadings of the variables. Differentiation of 19 individual hyperlipidemic patients from the different study groups (e.g. hoFH, heFH, FCH, controls) was possible solely based on MALDI-MS analysis. Results adapted from [1, 2].

## Conclusion

- Liquid biopsy samples (e.g. human plasma) represent a valuable source of molecules (e.g. membrane proteins and lipids) to serve as potential biomarkers for clinical diagnosis
- MALDI-MS combined with statistical software tools (e.g. MVDA) represent a promising technique for routine screening of liquid biopsy derived biomarkers for clinical diagnosis
- The developed techniques and SOPs will provide the basis for the establishment and commercialisation of a novel MALDI-MS based liquid biopsy detection platform for clinical applications.

## Acknowledgments

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[1] 59<sup>th</sup> ASMS Conference on Mass Spectrometry and Allied Topics, 2011, Denver, CO, USA. [2] 6<sup>th</sup> Mass Spectrometry Applications to the Clinical Lab (MSACL) Annual Conference & Exhibits 2014, San Diego, CA, USA.