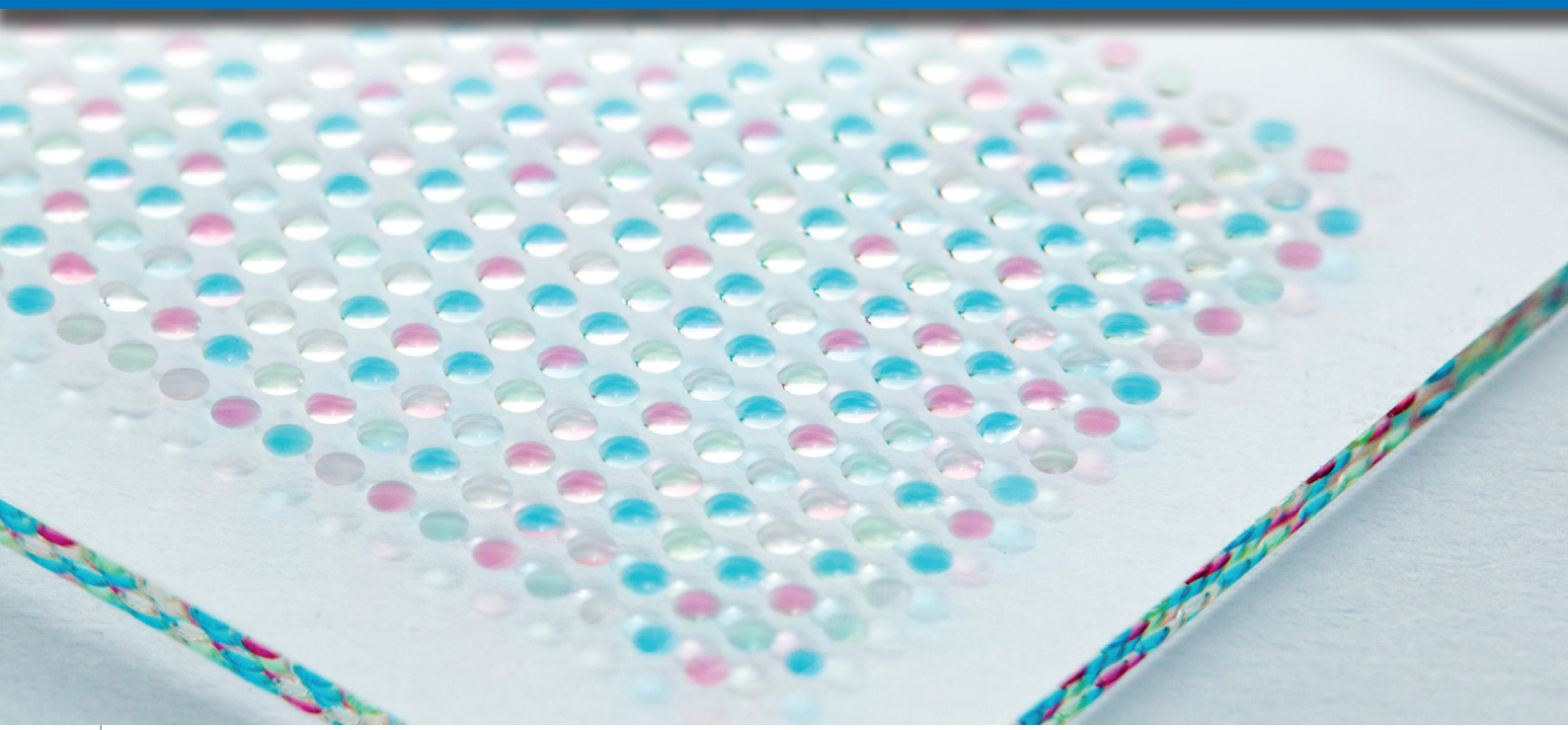

High-throughput MALDI-MS based read-out of high-density droplet microarrays facilitating next generation on-chip drug discovery

On-chip miniaturized and parallelized solution-based synthesis of novel compound libraries followed by on-chip characterization and screening in droplet microarrays has the potential to accelerate the drug discovery process by increasing throughput and reducing material consumption.

Abstract

On-chip characterization of newly synthesized compounds is particularly critical, since most existing analytical techniques are incompatible with synthesis platforms operating at low volume and concentration and a high level of parallelization. In this application note, we demonstrate proof of concept for a rapid workflow for highly sensitive chip based compound characterization down to the attomole range in high-density nanodroplet arrays based on AQUARRAY's Droplet Microarray technology as a sample support and read-out by Bruker´s rapifleX® MALDI PharmaPulse® high-speed MALDI-TOF MS system.

Introduction

Rapid and diverse development of novel treatments and diagnostics is crucial especially in exceptional situations such as global pandemics. There is a clear need to accelerate the process of drug discovery, that often takes more than 20 years, and costs on

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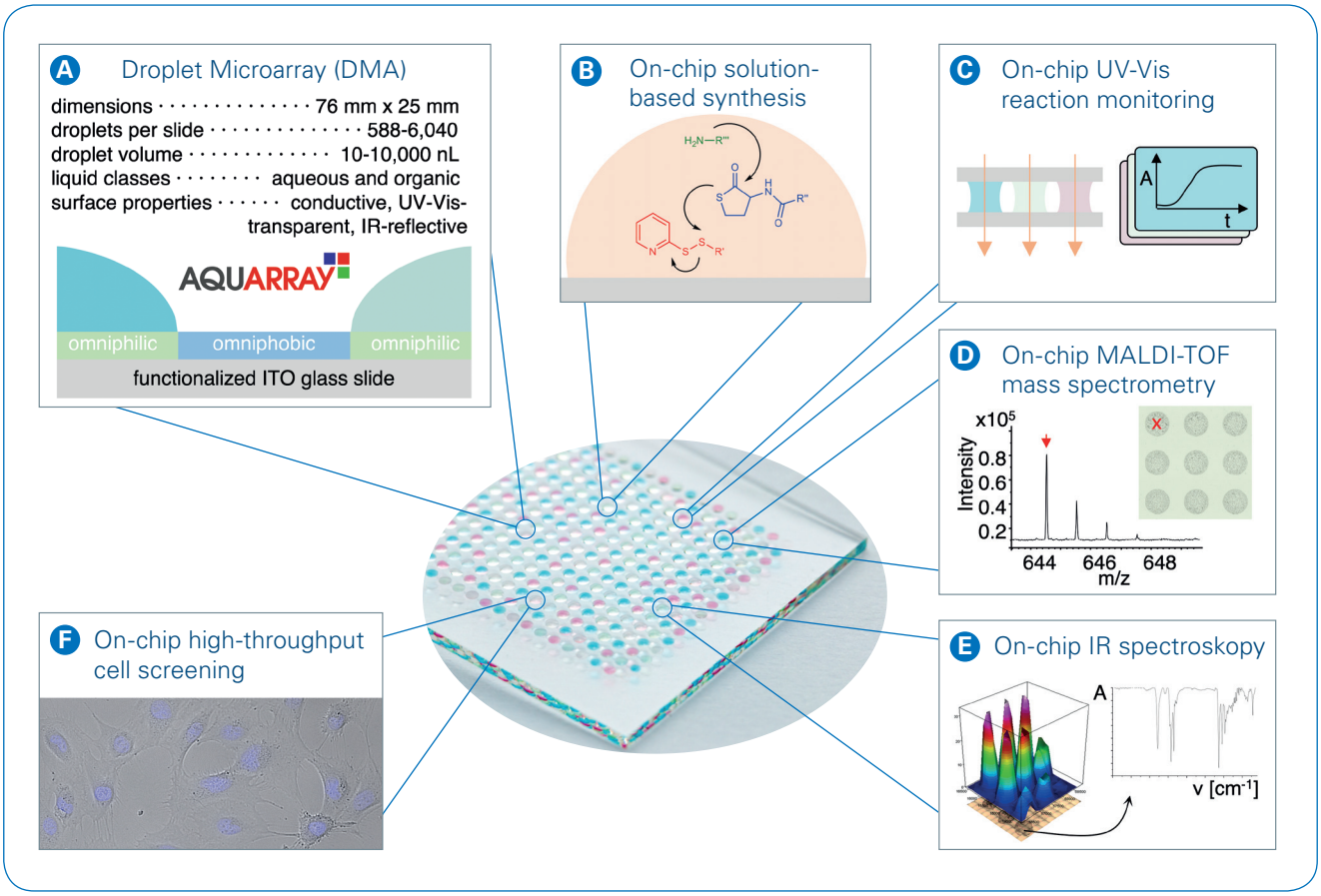

Figure 1: Photograph of the chemBIOS platform based on the Droplet Microarray (DMA) technology with an array of droplets of different dyes, and schematic overview over potential chemBIOS applications. Spot diameter: 900 µm; spot distance (border-to-border): 225 µm; droplet volume: 100 nL. (A) Schematic describing the surface functionality of an indium-tin oxide (ITO)-coated glass slide modified by the Droplet Microarray technology. Omniphilic (liquid attracting) spots are separated by omniphobic (liquid repellent) borders. The flat, conductive, UV-Vis-transparent and IR-reflective properties make the chemBIOS platform compatible with (B) on-chip high-throughput solution-based synthesis, (C) on-chip UV-Vis reaction monitoring, (D) highly sensitive on-chip MALDI-TOF mass spectrometry, (E) on-chip IR spectroscopy and (F) on-chip high-throughput cell screening. Adapted from ref[1].

average $2-4 billion for a single new drug [1]. Miniaturization, parallelization and unification are important key parameters to accelerate the drug discovery process by reducing material consumption and therefore overall costs. Recently, Benz et al. introduced chemBIOS, a novel miniaturized platform unifying on-chip high-throughput solution-based combinatorial synthesis in arrays of nanodroplets with straightforward characterization and screening (Figure 1) [1,2]. The chemBIOS platform is based on AQUARRAY's Droplet Microarray (DMA) technology (Figure 1). Arrays of hundreds to thousands of nanodroplets can be accommodated on a conductive DMA microscope slide. Each nanodroplet represents a separate nanovessel that can be utilized for synthesis as well as for chemical, biochemical and biological assays. High contrast in wettability between omniphilic spots (liquid attracting) and surrounding omniphobic parts (liquid repellent), and missing physical walls between the spots enable high-density arrays that allow for more than 20-fold increase in throughput of synthesis and screening when compared to conventional 1536-well microtiter plates commonly used in ultra-high-throughput screening (Figure 1). While high-throughput miniaturized and parallelized synthesis of compound libraries becomes increasingly important in drug discovery, analytical characterization of large compound libraries remains challenging. Most analytical techniques are not designed for parallel analysis, low volume and concentration, and are incompatible with the chemistry infrastructure [1]. In contrast to that, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) is particularly well suited for high-throughput analyses offering high acquisition speed and spatial resolution as well as inherent compatibility to surface immobilized sample formats, e.g. microarrays. MALDI-TOF, therefore, represents a promising read-out technique in next generation on-chip drug discovery.

Here we demonstrate proof of concept for a workflow enabling fast and highly sensitive on-chip compound detection down to the attomole range by combining AQUARRAY's high-throughput DMA technology with ultrafast, and highly sensitive automated read-out utilizing Bruker´s rapifleX MALDI PharmaPulse MALDI-TOF instrument.

Methods

Three different lipidoids were selected as model compounds here. Lipidoid **1** (*m/z* 644.522), **2** (*m/z* 686.569) and **3** (*m/z* 672.553), dissolved in 2-propanol (Merck Millipore), were dispensed using an I-DOT Mini AQ liquid dispenser (Aquarray GmbH) onto conductive DMA slides (Aquarray GmbH) with round shaped spots with a diameter of 2.83 mm (500 nL per spot) and 900 μm (50 nL per spot) in a concentration to reach an absolute amount of compound per spot of 1000, 100, 10, 2, 0.3, 0.1 and 0.05 fmol. The solvent was evaporated at ambient conditions. 500 nL α-cyano-4-hydroxycinnamic acid (CHCA; Alfa Aesar) matrix solution (5 mg mL^{-1} in 70% 2-propanol supplemented with 0.1% trifluoroacetic acid (Sigma-Aldrich) and 1 mM ammonium phosphate monobasic (Merck Millipore)) were dispensed to 2.83 mm spots (resulting in an area concentration of 397 ng mm^{-2} CHCA). 50 nL CHCA matrix solution (2.5 mg mL^{-1} with the same solvent and additive conditions) were dispensed to 900 μm spots (391 ng mm^{-2} CHCA). The solvent was dried at ambient conditions. Measurements were performed in triplicate of each sample dilution level using a Bruker rapifleX MALDI PharmaPulse MALDI-TOF/TOF mass spectrometer (reflector positive operation mode; laser repetition rate 10,000 Hz; 10,000 laser shots were accumulated per spectrum from 50 different raster positions within a sample spot) (Figure 2). A centroid peak finder was used to determine peak areas/intensities and S/N ratios. S/N ratios of each sample dilution level were determined and the limit of detection (LOD) was estimated by comparing the sample spectrum with a blank matrix spectrum as a reference [1].

Results and discussion

Lab-on-a-chip systems are developed to miniaturize and parallelize chemical and biological methods and thereby reduce the consumption of valuable reagents and solutions. While

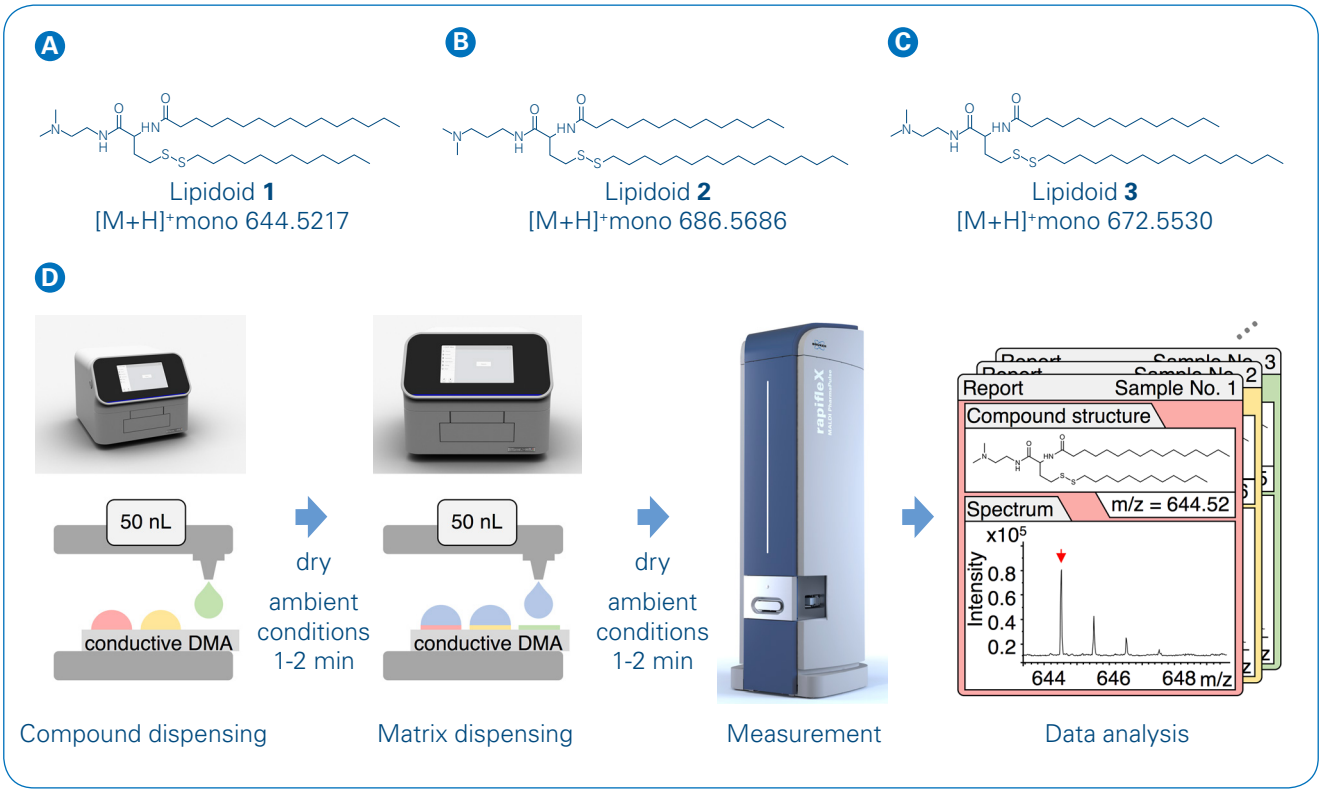

Figure 2: (A)–(C): Chemical structures of the analysed compounds; (D): Schematic presenting the workflow of on-chip drug discovery read-out process by MALDI-TOF MS. An array of different drug compounds can be applied to the omniphilic-omniphobic patterned, conductive chemBIOS slide or synthesized on-chip by non-contact liquid dispensing. After evaporation of the solvent at ambient conditions the precipitated compounds are prepared with MALDI matrix by dispensing the matrix solution to the dried spots. The rapifleX MALDI PharmaPulse mass spectrometer enables fast and sensitive characterization of large compound libraries from the slide directly.

synthesis and screening of hundreds to thousands of compounds can already be performed simultaneously on-chip requiring only nano- to microliters of reagents [1,2], chemical characterization remains a major challenge in those systems. Ideally, on-chip analytical methods should be fast, compatible with low volumes and concentrations, and high-density arrays to fit the criteria of the on-chip high-throughput synthesis platform. The chemBIOS platform featuring an omniphilic-omniphobic surface pattern meets all these critical requirements and is compatible with MALDI-MS read-out performed on a rapifleX MALDI PharmaPulse instrument. This enables highly time-efficient mass spectrometric characterization of high-density arrays comprising thousands of compounds at high spatial resolution. Arrays of compounds can be synthesized on-chip or applied by liquid dispensing using a fast, high-precision, low-volume liquid dispenser, for example I-DOT Mini AQ Edition (Aquarray GmbH). We tested three lipid-like small molecule compounds lipidoid **1** (*m/z* 644.522), **2** (*m/z* 686.569) and **3** (*m/z* 672.553) with a final amount of substance per spot in the range of 0.05-1000 fmol using two different array spot sizes with a diameter of 2.83 mm (80 spots per slide) and 900 µm (1152 spots per slide), respectively, in order to validate the entire workflow and to investigate

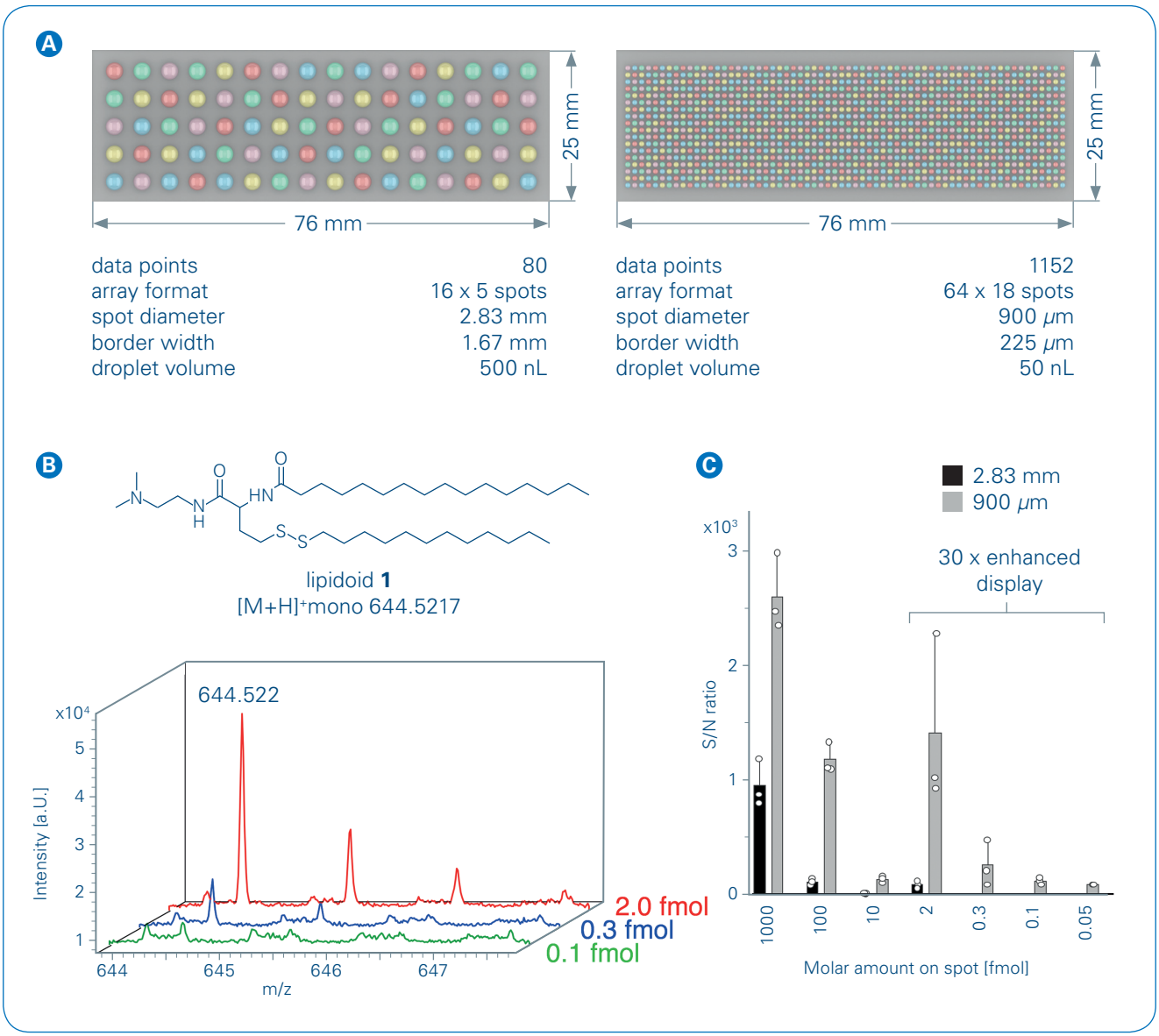

Figure 3: Results of on-chip MALDI-TOF MS analysis using the chemBIOS platform. (A) Schematic showing the array format with a spot pattern of 80 spots (spot diameter: 2.83 mm) and 1152 spots (spot diameter: 900 µm) and corresponding core data of the platform. (B) Spectra of 2.0, 0.3 and 0.1 fmol lipidoid 1 in spots with a diameter of 900 µm. (C) Bar chart comparing the signal-to-noise (S/N) ratio obtained from MS analysis of lipidoid 1 in spots with a diameter of 2.83 mm and 900 µm, respectively.

the method sensitivity depending on spot dimensions (Figure 3). On average, we observed a 100-fold increase in sensitivity for compound detection when comparing array spots with a reduced diameter of 900 μm (LOD ~0.1 fmol) to those with a diameter of 2.83 mm (LOD ~10 fmol) (Figure 3). The enhancements in sensitivity achieved by reducing the spot size obviously come along with the desire to work at higher array density to increase throughput.

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Conclusion

- ChemBIOS unifies miniaturized and parallelized organic synthesis with highly sensitive characterization and high-throughput screening and, thus, covers all aspects of early-stage drug discovery in a single on-chip platform.
- AQUARRAY's Droplet Microarray (DMA) technology enables application of minor amounts of chemical compounds on small-sized, well defined sampling areas. Using a spot diameter of 900 μm, DMAs allow to accommodate up to 1152 spots on a conductive pre-patterned ITO slide (6144 spots when taking into account a standard microtiter plate formatted sample support) enabling high sample throughput.
- Bruker´s rapifleX MALDI PharmaPulse MALDI-TOF enables ultrafast and highly sensitive detection of compounds down to the attomole range from the chemBIOS DMA platform directly without the need for additional sample transfer steps.
- The chemBIOS platform enables multimodal read-out by further spectroscopic methods, such as UV-Vis and IR, and, particularly important for cell screening, fluorescence microscopy.

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