Sample preparation

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Sample preparation is a fundamental step in most biomedical assays. Except for a few direct measurement methods, almost all biological samples need some kind of operation or preparation to enable the precise and accurate detection of the target analytes. As in traditional laboratory or bench methods, sample preparation is also an essential function in many Point-of-Care (PoC) devices. Performing on-chip sample preparation has the advantage that the entire process can be realized with minimal user intervention, reducing errors, costs and time thus enabling full sample-to-answer processes.

Lab automation

The aim of sample preparation is to process the sample in such a way that the target analyte(s) can be correctly detected and measured by a specific analytical technique. This sounds simple, but each particular sample can have different sample preparation techniques depending on what we are looking for and the sample itself. Sometimes the analyte is too diluted and we need to concentrate it, or vice versa: too concentrated and we need to dilute it. Or perhaps the analyte is shielded behind many 'interferents' present in the sample that block its detection and we need to perform extraction steps. Sample preparation does not always involve the conditioning of the sample for a particular analytical detection. Sometimes, and particularly when working with cells, we only need to separate a cell type from a pool (or cell populations with certain characteristics) that will be used in other downstream processes such as cell culture, cell therapy, etc.

Benchtop sample preparation methods usually involve several steps that need to be performed by expert users relying on highly specialized equipment in controlled laboratory environments. After these steps, the user needs to manually present these 'conditioned samples' to analytical equipment. All of these steps add time, costs and risks associated to the manual handling of the samples. Microfluidic devices can integrate and automate sample preparation steps directly into analysis platforms, thereby reducing time, costs and risks. Further, these miniaturized systems can reduce dead volumes and sample waste due to the reduced geometries on which they typically operate. However, there is not a generic microfluidic solution that can be

applied to all samples. An exhaustive analysis of the sample type, analytes and analytical technique needs to be done to find the right solution for each particular application.

In this white paper, we take a look at some of the most common sample preparation operations that can be performed on-chip. We also provide some guidelines to help one determine the best approach for specific assays.

Looking at the whole assay

At first, we need to have a very clear view of the following factors.

- Starting sample type: is your sample whole blood? Plasma? Urine? Saliva? A cell suspension? What are the typical volumes? Is your sample stable in time? Is your sample a biohazard? Do you need to maintain sterility? What is (are) your target analyte(s)?
- Associated benchtop sample preparation steps: does your sample need separation? Extraction of genomic material? Dilution? Mixing? Lysis? Labelling? Does the sample contain any interferents? Does it involve coupling to a magnetic substrate?
- Analytical techniques or associated downstream processes: is the detection based on an electrochemical reaction or an optical principle? Does it require molecular amplification? Do you need to retrieve the prepared sample for downstream processing or is it an end-point assay?

The choice of on-chip sample preparation techniques depends heavily on the abovementioned parameters. Due to the extensive variety of samples, analytes and analytical techniques, there is no one-fits-all solution. This is why a separate study has to be done for each case to come up with the right solution.

On-chip sample prep

There is an extensive variety of sample preparation techniques that can be integrated on-chip. We will discuss some of the most relevant and provide examples of applications.

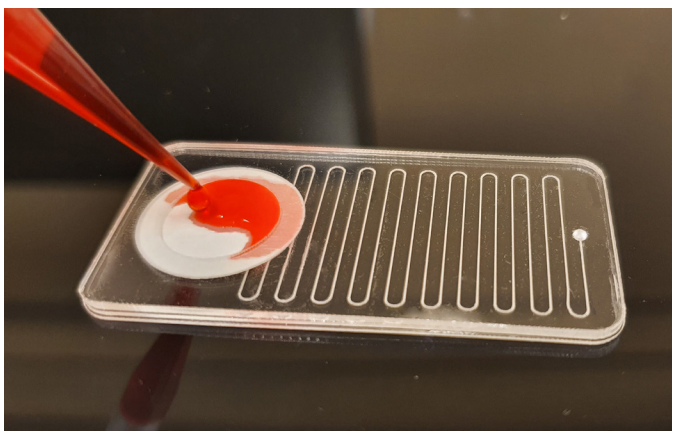
1: Filtration

Depending on the sample type, the analyte and the analytical technique, it might be required to separate certain components from the sample to prevent inhibition of detection or clogging of the microfluidic channels for example. A typical example of filtration is the use of membrane filters to separate plasma from whole blood. These filters can quickly capture red blood cells, white blood cells and platelets leaving pure, cell-free and hemolysis-free plasma that can be used for downstream detection.

Filtration is typically one of the first steps in an assay and can replace centrifugation for low-volume samples. At Micronit we have experience integrating filter modules on-chip for whole blood and urine samples.

2: Cell sorting

For many assays, cell sorting is a fundamental sample preparation step. This is typically done to remove interferents (cell types that are not of interest) or to fractionate cell populations (based on their mechanical properties or size for example). There is a wide variety of sorting techniques available that can be implemented in microfluidic devices.



Example of a blood plasma separation filter integrated into a microfluidic device.

Think of the following techniques:

- Acoustophoresis, a cell sorting method based on acoustic waves that depends on cell properties such as size, density and compressibility (relative to the surrounding medium)
- Dielectrophoresis, in which cell sorting is conducted using the electrical properties of the cells
- Pinched flow fractionation, a size-sorting method based on the spreading flow profile inside a microchannel
- Deterministic lateral displacement (DLD), in which an array of micro-pillars is used to separate cells based on their size and shape

3: Dilution

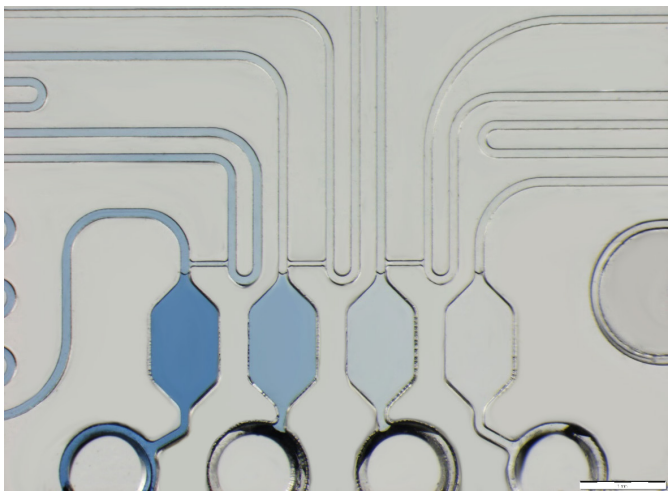
Dilution is sometimes a key step in sample preparation. This is usually done to dilute interferents but also to dilute the analytes to an optimal concentration for the analytical technique. Dilution can also be used to promote better separation efficiencies when using complex samples such as whole blood.

Dilution steps can be integrated on chip. These can be performed using purely capillary forces, hence abolishing the need for external actuation.

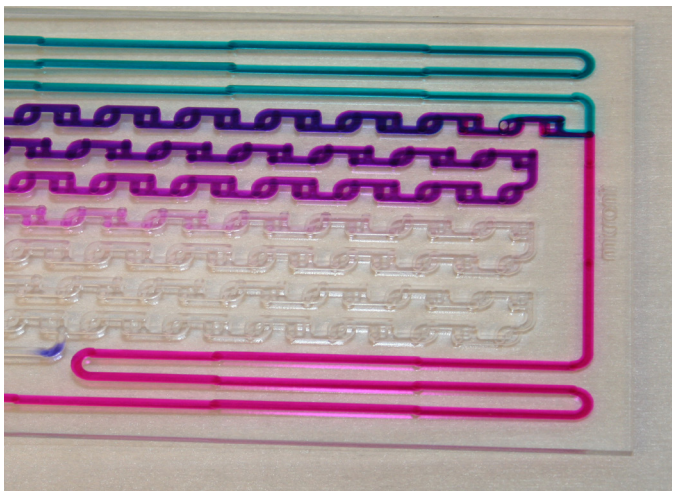
4: Mixing

Mixing is often an intermediate step between other sample preparation steps. For instance, after dilution or resuspension of reagents, it is usually needed to mix the sample to homogenize the concentration across the whole volume. A variety of active and passive micromixers can be implemented, which can be tailored for each application's needs.

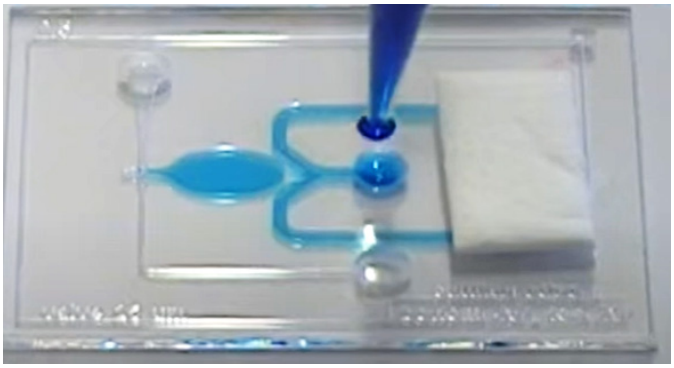
Examples of active mixers include the use of pneumatic microvalves and micropumps, acoustic microstreaming and the use of magnetic-responsive microstructures. Examples of passive mixers include 3D folding microstructures, micro obstacles and special microstructures that induce chaotic advection.



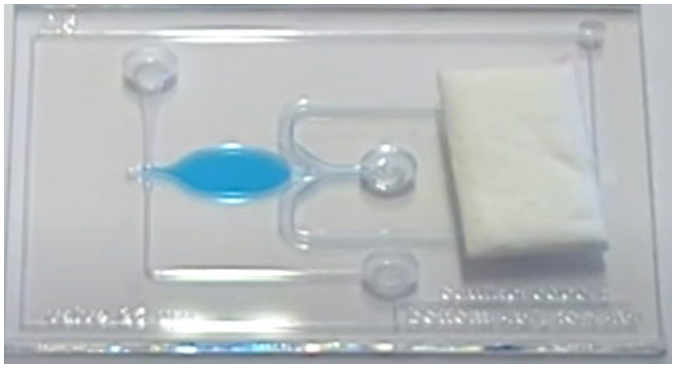
An automated serial dilution chip based on capillary action (project ScaleTime).



3D passive microreactor based on the folding flow technique. The turquoise and pink liquids are rapidly homogenized obtaining a uniform blue liquid.



Volume metering, step 1: the metering chamber is filled by capillary action and the liquid stops at the capillary stop valve. The excess liquid flows through the overflow branches on the sides.



Volume metering, step 2: the wicking pad absorbs the excess liquid of the fluidic network, leaving the chamber isolated with a defined 'metered' volume that can be used for other steps.

5: Volume metering

Volume metering is fundamental in quantitative tests where the exact volume of a sample needs to be determined and controlled. This is very important when doing dilutions or resuspension of dried reagents for example. To achieve this, specially designed microchambers can be filled on-demand, using capillary flow, burst valves, and splitter channels. In this way, precisely defined volumes of liquid can be contained at a defined location of the cartridge which will not be released until a specific trigger occurs.

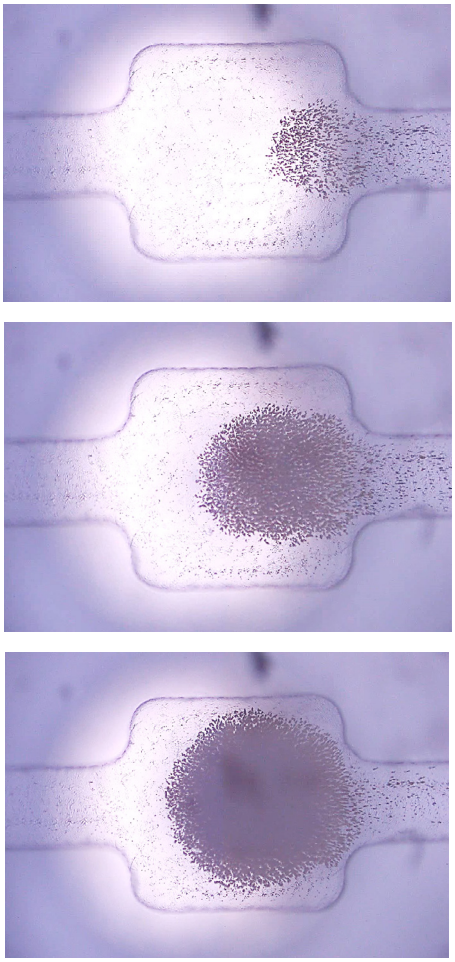
This type of sample preparation enables one to work with reliable volumes, which is an important step in the workflow automation that microfluidic platforms can offer. We can demonstrate many different types of metering solutions that can be adapted to your needs.

6: Cell lysis

Sometimes the analytes of interest are inside cells (e.g. RNA, DNA) and protected by the cell membrane. This membrane needs to be disrupted in order to release the analytes and allow their detection. There is a wide variety of methods to induce cell lysis including electroporation, acoustic forces, chemical lysis and heat lysis. These steps can also be integrated on-chip as part of an automated lab workflow.

7: Analyte extraction

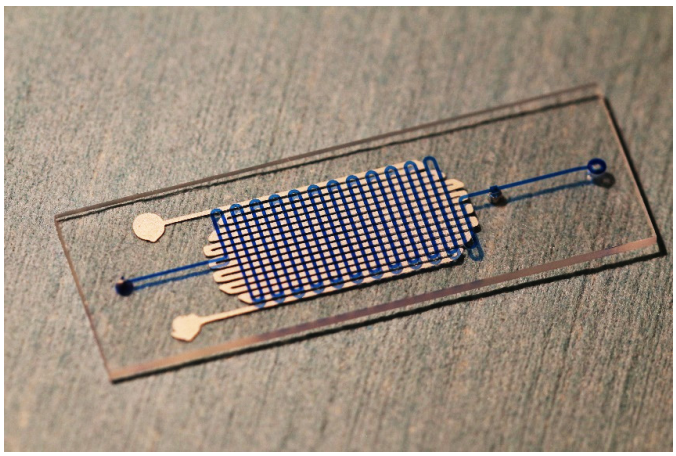
In many assays, the analytes of interest are usually extremely diluted or shielded between other interferents that prevent their accurate detection and quantification. In order to concentrate and remove interferents, an extraction step is usually needed. This step can involve the use of solid supports such as silica beads, silica membranes or paramagnetic beads that create the adequate binding selectivity for the target analytes. These beads-based assays can be integrated in microfluidic chips. The extraction steps are routinely used to selectively capture and purify DNA/RNA fragments, antibodies, proteins, etc.



Example of a beads capture chamber. The images show the accumulation of captured paramagnetic beads inside a microfluidic chamber (project SAMOSS).

8: Sample heating

From DNA hybridization to cell lysis, heating is a fundamental step in many biological assays. This process can involve the integration of on-chip heaters, but at Micronit we also have the expertise to develop off-chip solutions that are tailored to the needs of specific devices.



Example of a polymer microfluidic chip with an integrated heater, hybridization channel and magnetic beads capture chamber (project SAMOSS).

Integration

In this white paper we have discussed a number of sample preparation techniques. The future of labs-on-chips lies in the highest possible form of miniaturization and integration. As a result, these sample preparation techniques should not solely be seen as individual assay steps, for in many cases it will be possible to integrate several of these consecutive steps within one microfluidic platform.

Please contact us to find out what is the best solution for your assay. Our team of experts is always happy to discuss your application and take on the challenge!

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Alvaro J. Conde is an R&D Scientist at Micronit with more than ten years of experience in the development of microfluidic solutions for projects at the interface of biology and engineering. Alvaro completed his Engineer's degree in Biomedical Engineering and a PhD in Microfluidics at the National University of Tucumán (Argentina). Before joining Micronit in 2020, he contributed to numerous interdisciplinary projects powered by lab-on-a-chip technologies, with previous roles at the National Atomic Energy Commission (CNEA, Argentina), National Industrial Technology Institute (INTI – Argentina), Technical University of Denmark, The University of Edinburgh and Heriot-Watt University.

About Micronit

Micronit bv, founded in 1999, with development and manufacturing facilities in the Netherlands and Germany, provides innovative lab-on-a-chip and MEMS solutions using micro- and nanotechnologies. Solutions that help customers improve their products and research, contributing to the quality of life.

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