ABSTRACT
We report here a novel and easily scalable microfluidic platform for the parallel analysis of hundreds of individual cells, with controlled single cell trapping, followed by their lysis and subsequent retrieval of the cellular content for on-chip analysis. The device consists of a main channel and an array of shallow side channels connected to the main channel via trapping structures. Cells are individually captured in dam structures by application of a negative pressure from an outlet reservoir, lysed on site and the cellular content controllably extracted and transported in the individual side channels for on-chip analysis.

KEYWORDS: single cell analysis, platform, microfluidics, large scale trapping

INTRODUCTION
Cell populations are heterogeneous: processes are not synchronized in a cell population and individual cells are at different stages of the cell cycle, for instance [1]. Consequently, conventional analysis methods provide averaged information about the cell population as an ensemble [1] and this does not give useful information on the state of individual cells. A single cell analysis approach [2] looks more attractive in that respect; however, the analysis of a single cell in a population appears to be a biased approach as one cannot extrapolate information about the state of a population. Therefore, a more relevant approach consists of analyzing cells of a population in an individual manner, so as to collect information not only the single cell level but also at the population level. This approach reveals a population heterogeneity, which is thought to be indicative of disease development [3]. In that context, we have developed a novel integrated platform for the analysis of a large number of single cells; cells are individually trapped in dedicated structures, lysed on site and the lysates transported to separate channels for their analysis.

EXPERIMENTAL
System fabrication. We use an hybrid PDMS-glass platform. Channels are molded in PDMS using conventional casting technique and a silicon mold with two layers of structuration to define the main (~30 µm height) and the shallow channels (< 5 µm height). Reservoirs are punched in PDMS which is subsequently activated using plasma treatment and bonded to a glass substrate.
Cell sample preparation. Cells are stained and fixed prior to their introduction in the microfluidic device for their analysis. Cell fixation is achieved using a 2-min treatment with ice-cold methanol (-20°C) followed by thorough washing of cells using PBS. A dual cellular staining is subsequently performed to separately label DNA and RNA materials in the cell using Hoechst and SYTO RNA Select, respectively.

Protocol for cell trapping and on-chip cell analysis. Cell trapping is monitored using a home-made pressure manifold fixture as described in the next section. Once every trapped is filled with a single cell, a lysis solution is introduced in the main channel and the individual cell lysates progressively pulled in the analysis channels.

RESULTS AND DISCUSSION
In a first step we optimized cellular trapping towards the reproducible isolation of a large number of cells at the single cell level. This included the optimization of the geometry and dimensions of the trapping sites and the development of a reliable trapping protocol. Trapping sites consist of a cup behind which a short and shallow neck is found connected to a 100-µm wide shallow channel as illustrated in figure 3. Cells are introduced in the inlet reservoir and a low cell flow-rate is established in the main channel by application of a mild negative pressure on the second inlet reservoir. Subsequently, a mild negative pressure is applied in the different suction ports until all cells are present in all trapping sites. The excess of cells in the trapping sites is removed by increasing the pressure in the main channel while maintaining a mild suction in the side channels. With these optimizations, we demonstrate a 95% trapping efficiency (>10 chips presenting 32 traps each). Figure 4 shows trapping results for 4 fixed cells in a 32-trapping sites device.
Once cells are trapped (fig. 4), they are individually lysed. The lysis protocol is flexible and can be electrical or chemical, or a combination can be applied. We use here a detergent solution (SDS or LiDS) which is flushed in the main channel. Simultaneously, the cell’s RNA content is pulled in the analysis channels by application of a negative pressure. For visualization purposes, their RNA had been previously stained using a specific probe, and the stained material is observed at the entry of the analysis channel after cell lysis (fig. 5). Using mild lysis conditions (low amount of LiDS), we are able to lyze the extracellular membrane of the cell while leaving the nuclear membrane intact; this notably enable selective analysis of RNA without risks of contamination of other nucleic acids contained in the nucleus.

![Figure 5: In situ lysis of cells using a chemical approach with the introduction of a detergent solution in the main channel. Lysis is done here using a LiDS solution at 1%. Left: successive pictures illustrating the “slow” process of cell swelling upon action of the detergent followed by cell lysis. Right: photographs of a cell stained with a RNA specific probe before (top) and after (bottom) lysis; after lysis the RNA material is found at the entrance of the analysis channel (enhanced contrast).](image)

**PERSPECTIVES**

We are currently investigating a controlled protocol for the extraction of cellular content; this is achieved through the application of an electroosmotic flow in the analysis channels while cells are lysed. We are notably calibrating the electro-osmotic flow and applying it for early experiments on hybridization of model mRNA samples on integrated microarrays placed in the analysis channels.

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**REFERENCES**