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PAPER

Integrated three-dimensional filter separates nanoscale from microscale elements in a microfluidic chip†

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We report on the integration of a size-based three-dimensional filter, with micrometre-sized pores, in a commercial microfluidic chip. The filter is fabricated inside an already sealed microfluidic channel using the unique capabilities of two-photon polymerization. This direct-write technique enables integration of the filter by post-processing in a chip that has been fabricated by standard technologies. The filter is located at the intersection of two channels in order to control the amount of flow passing through the filter. Tests with a suspension of 3 μm polystyrene spheres in a Rhodamine 6G solution show that 100% of the spheres are stopped, while the fluorescent molecules are transmitted through the filter. We demonstrate operation up to a period of 25 minutes without any evidence of clogging. Preliminary validation of the device for plasma separation from whole blood is shown. Moreover, the filter can be cleaned and reused by reversing the flow.

1. Introduction

Recent advances in microfabrication technology have enabled the miniaturization of several functionalities of traditional biochemical laboratories and allowed processing samples in micro- to nanolitre amounts, giving rise to the concept of micrototal analysis systems, also known as Lab-on-Chip (LOC).^{1–3} LOC devices are typically composed of a microfluidic system integrating functionalities such as pumping, mixing, trapping and sorting. As a consequence, advancements in LOC technology require improved methods to fabricate integrated microscale active and passive devices. In particular, porous filters are valuable building blocks in LOC systems and have a variety of applications in microbiological studies and biochemical analysis.^{4–7} Unlike alternative approaches such as electrophoretic and dielectrophoretic flow,⁸ optical tweezing,⁹ shear-induced forces,¹⁰ magnetic forces,¹¹ and gravity-driven forces,¹² sorting using a porous filter is simple, inexpensive and does not require extra steps such as labeling. Specifically, filters with microscale pores are useful in biological/biochemical analysis applications, such as isolation of plasma from red and white blood cells,

sorting of cells of different sizes, or purification of samples from impurities or interfering constituents.^{5–7}

During recent years, a variety of different porous filter fabrication techniques have been developed. Emulsion photopolymerization,¹³ salt-leaching techniques,¹⁴ directed assembly and transfer molding¹⁵ methods yield high resolution porous filters with easy integration into microfluidic channels but do not allow regular periodicity or precise control over pore size and shape. Various molding procedures are being developed for fabrication with inexpensive polymer substrates, but these are devices which have limited resolution and geometry.¹⁶ On the other hand, standard photolithographic methods produce well-controlled periodic structures but require complex and often expensive processing techniques.^{6,17} The inherent two-dimensional (2D) nature of these technologies means that the production of three-dimensional (3D) devices requires the alignment and assembly of multiple layers. Having a filter based on a 3D mesh instead of 2D pillars has several advantages. The main ones are: (i) the increased mechanical resistance to shear stresses that enables a higher void ratio and (ii) the possibility to effectively filter disk-shaped elements, *e.g.* red blood cells, without dramatically reducing the pore size. In fact, a 2D micropillar structure would require openings smaller than the minimum dimension of the cell, while 3D meshes require openings smaller than the maximum dimension, thus relaxing the requirements for the spatial resolution of the technology. Photolithographic patterning using a conformable phase mask can generate sub-micron 3D structures, but this method is mostly limited to periodic geometries.¹⁸ In addition, the fabrication techniques described above always require channels that are initially open. Sealing of the channel is performed after the fabrication of the filter. Advanced facilities are therefore needed

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to provide a clean environment where dust does not enter into the channels during the filter fabrication. In addition, the presence of the filter typically poses severe problems in the sealing of the channel, due to uneven surfaces and difficult adhesion of the filter to the cover. As a consequence, in most of the reported experiments the chip is sealed with a soft cover such as a polydimethylsiloxane (PDMS) foil. However, PDMS has several drawbacks in real applications, such as absorption of organic solvents and small molecules, its intrinsic hydrophobicity, and permeability to water vapor.¹⁹ It is therefore of great interest to find ways to integrate porous filters in otherwise standard sealed microfluidic chips which are already optimized for the target application.

Femtosecond laser fabrication of micro/nanostructures by two-photon polymerization (2PP) is a powerful method for realization of high resolution, 3D devices with applications to photonics, micromechanical systems and LOC technology. In contrast with existing fabrication technologies for microfluidic filters, 2PP enables precisely controlled direct-write of 3D porous structures with sub-micron resolution, simple processing and easy integration. 2PP is performed in a photosensitized resist where simultaneous absorption of two degenerate photons from a near-infrared femtosecond laser results in the crosslinking of monomers and oligomers.^{20–22} The two-photon absorption mechanism enables selective polymerization in the small focal volume, allowing sub-diffraction limited resolution in both the lateral and axial directions as well as fabrication at different depths with little or no collateral effects. 2PP has been used to fabricate woodpile structures,²³ periodic scaffolds for tissue engineering and cell migration studies,^{24–26} and photonic crystal structures.^{27,28}

Despite existing work,^{29–31} 2PP integration of structures into LOC devices has still much potential to be explored. Here, we present the fabrication by 2PP of a 3D porous filter, separating nanoscale from microscale elements, directly integrated in an already sealed commercial LOC. The filter is tested with a suspension of 3 μm polystyrene spheres in a Rhodamine 6G solution and is capable of complete removal of the beads from the fluorescent solution in continuous operation for a time period of up to 25 minutes; no clogging was observed during operation time. The filter can be cleaned by reversing the flow and reused several times. Proof-of-principle results on filtering whole blood are also presented.

2. Experimental

The fabrication setup for 2PP is shown in Fig. 1a. The laser irradiation source is a home-built mode-locked Ti:sapphire laser with 87 MHz repetition rate, 40 fs pulse width, 790 nm central wavelength and 400 mW average power. The writing beam is directed through a power attenuator, a beam expander and a high speed shutter (LS6T2-NL-100, Uniblitz), and is then focused onto the sample using a 1.4 numerical aperture objective (Plan-APOCHROMAT, 100 $\times$ oil immersion, Carl Zeiss AG). The sample is mounted on a three-axis piezoelectric motion stage (P-517.3CL, Physik Instrumente) with nanometre resolution and 100 μm $\times$ 100 μm $\times$ 20 μm travel range. The irradiation for 2PP is performed in continuous mode at a constant velocity. The beam is overlapped with a 633 nm helium–neon laser and a CCD

camera is used to observe the back-reflections from the sample in order to achieve fine alignment of the writing beam to the desired area and depth.

The negative 2PP resist is a hybrid organic–inorganic sol–gel (SZ2080) developed in recent years for this technology.^{32,33} The photoinitiator 4,4'-bis(diethylamino)benzophenone at a 1% concentration provides an absorption band in the UV and triggers the 2PP process. SZ2080 has high optical quality, good post-processing chemical and electrochemical inertness, and good mechanical stability. Its low shrinkage properties make it suitable for applications sensitive to structural deformations, since additional design steps are not needed to compensate for distortions.

An important advantage of the fabrication technology presented here is the direct integration of the porous filter into a commercial microfluidic system where channels are already sealed. This enables addition of filter elements into already optimized microfluidic systems without having to modify their design, thus greatly improving the flexibility of the layout and the simplicity in the fabrication of the final device. The microfluidic chip used in the present experiment is a commercial cross-channel LOC (X3550CH.3, Micronit Microfluidics) made of borosilicate glass. A schematic of the chip and a cross-sectional view showing the shape of the channels are shown in Fig. 1b and c. The chip has four wells, with wells 1 and 4 located at the ends of the long channel and wells 2 and 3 located at the ends of the short one. The lengths of the two channels are 40 and 10 mm, respectively. The channels are 20 μm deep and 50 μm at their widest points. An important feature of this chip is that its channels are sealed with a thin 145 μm glass top layer, which has the thickness of a coverslip glass. This enables fabrication using the short working distance 1.4 NA objective as well as high resolution imaging inside the channel.

Initial fabrications are performed on flat substrates to define and characterize the filter geometry. The resist is drop-cast onto a cleaned coverslip glass of 145 μm thickness and soft-baked at 100 $^{\circ}\text{C}$ for 1 hour to remove the solvent. The sample is then mounted on the piezoelectric motion stage and aligned to the femtosecond laser beam. In particular the depth positioning is performed by observing the back-reflected light from the air–glass interface. Fabrication is then performed by translating the sample with respect to the laser beam at a constant speed. The exposure power is 5.2 mW after the objective and the speed is 40 $\mu\text{m s}^{-1}$. Following exposure, the structures are immediately visible and are checked under a standard bright-field microscope, equipped with a suitable yellow filter to prevent unwanted polymerization. Structures are then developed by immersion in a 50 : 50 mixture of 4-methyl-2-pentanone : isopropanol.

For structures inside the microfluidic chip, the fabrication procedure is more complex. A specific protocol has been developed to introduce the resist into sealed microfluidic channels, which is schematized in Fig. 2. To complete this operation one must find the best compromise between two opposite requirements: on the one hand, the resist should be liquid enough to flow inside the channels; on the other hand, as much solvent as possible should be evaporated before the resist is inserted in the chip in order to facilitate the soft-baking step, which must be completed before the irradiation process. The optimized procedure involves a partial soft-bake of the resist for 3 min at 80 $^{\circ}\text{C}$

Fig. 1 (a) Fabrication setup. (b) Layout of the microfluidic chip used in the experiments. (c) Cross-sectional view of the chip showing a channel, the thin sealing layer and the laser irradiation direction. (d) Layout of the filter in the channel junction and filtering geometry (not to scale).

before insertion into the chip. To minimize thermal shock effects, the chip is also heated to the same temperature. The partially baked resist is then placed inside three of the four access wells (1, 2 and 3 in Fig. 1d) and is flowed into the channels by creating a pressure depression at the fourth well (4). Once the resist reaches the desired position inside the channels, the soft bake is completed by baking the filled chip at 105 °C for 90 minutes. A higher temperature is used to compensate for the fact that the resist is now thermally insulated by the chip. A longer bake time is used to ensure that the whole solvent can evaporate from the resist and escape through the wells.

Alignment to the desired position on the chip is performed by observing the low-power back-reflected spot at the glass–air and glass–resist interfaces. Repeatability error of the alignment procedure is on the order of 3 μm in the three spatial directions. Filters are written by irradiating intersecting lines in continuous mode along the three axes, *x*, *y* and *z*. Removal of unexposed resist is performed by immersing the entire chip in the development mixture and allowing the propagation of developer into the channels *via* the wells. Development time depends on the amount of resist that has been inserted in the chip. A typical time in our case was about 24 hours for about 20 nL of resist in the channels, but this could be significantly shortened if a smaller amount of resist could be handled. The chip is then dried in open air at 50 °C for 10 minutes. The fabricated structures are imaged by standard bright-field microscopy, and

for higher resolution, by scanning electron microscopy (SEM) (EVO 50 EP, Carl Zeiss AG).

The filter is tested by flowing 3 μm polystyrene spheres suspended in a 100 mM phosphate buffer (pH 7.2). In order to clearly visualize the flow, the spheres have also been suspended in a fluorescent version of the buffer which has 40 μM of Rhodamine 6G. The chip is mounted in a cartridge providing optical accessibility to the chip and connection of the channels to 4 reservoirs where the solutions are placed. Imaging is performed using an inverted microscope (DMI3000 M, Leica Microsystems) in bright-field and fluorescence imaging modes. Hydrostatic pressure differences in the 4 reservoirs are used to precisely control the flow of the test solution in the channels and through the filter. First, all channels are filled with buffer, then a controlled amount of the microsphere suspension is placed in well 1, while the flow is controlled by filling the other access holes to different heights with buffer solution. Time-lapsed images and videos of the solution and test spheres flow are captured by a CCD camera. Flow rates are determined by measuring the advancing speed of the forefront of the fluorescence solution in the channels (details are provided in the ESI†).

3. Results and discussion

A physical filter in a microfluidic channel is intrinsically prone to clogging. In fact, all the material that is not able to pass through

Fig. 2 Schematic of the procedure to fabricate 3D structures in sealed microchannels by 2PP.

the filter will be blocked in front of it and will eventually close all exposed pores. In order to improve this situation we decided to fabricate the filter at the intersection of two channels and to give it a triangular shape as represented in Fig. 1d.

This geometry has two advantages: on the one hand it allows the blocked material to flow away to the sides of the filter, thus increasing the filter lifetime before it is clogged; on the other hand, it allows us to arbitrarily define the portion of the flow actually going through the filter and that going into the side channels, irrespective of the speed of input flow. This second feature can be used for filtering small portions of a rapidly flowing solution over long periods of time with limited clogging of the filter.

3.1 Fabrication of the filter

After initial optimization of the writing parameters, a series of 2D and 3D structures are fabricated on flat glass substrates using the procedure outlined in the previous section. The requirements of the filter fabrication are: creation of small pores with high accuracy and reproducibility (our target is to separate microscale from

nanoscale elements and thus $\sim 1\text{ }\mu\text{m}$ pores are required) and robustness of the structures against high pressures and local forces exerted by the filtered microscale elements. 2PP is probably the best technological choice to fulfil such requirements since its 3D capabilities and extreme resolution allow defining pores with a square shape of $\sim 1\text{ }\mu\text{m}$ dimension on each side. In addition, to further improve the structure robustness the filter is fabricated by stacking several filtering grids interconnected as a cubic structure. An accurate characterization of the porous structure fabricated on a flat glass sample is performed by SEM. Fig. 3 shows the results of this analysis. The triangular structure is extremely regular in the yz plane (Fig. 3a) as well as in the xy and xz planes (Fig. 3b). The pore dimensions are $1.3\text{ }\mu\text{m} \times 1.3\text{ }\mu\text{m}$ in the yz plane and $1.5\text{ }\mu\text{m} \times 1.5\text{ }\mu\text{m}$ in xy and xz planes (Fig. 3c). The minimum width of the polymerized lines in the yz plane is equal to 500 nm , while along the x -axis the polymerized lines are slightly larger due to the ellipsoidal shape of the voxel.³⁴ Voxel size can be reduced by optimizing exposure parameters and currently a lateral resolution for SZ2080 of less than 200 nm has been reported.³³ However, we prefer to have thicker lines to improve the structure robustness. In addition, irradiation with intensity well above the threshold condition provides a negligible shrinkage during development.³³ In fact, we observe that distortion of our structure in any linear direction is less than 5%. Our fabrication technique allows easy on-demand tailoring of the pore sizes. To show this, we produced structures with almost square pore sizes ranging from $\sim 3\text{ }\mu\text{m}$ (Fig. 3d) down to $\sim 0.5\text{ }\mu\text{m}$ (Fig. 3e). It can be noted that in the structure with $3\text{ }\mu\text{m}$ pore size (Fig. 3d) the polymerized lines are thicker than in the other cases. This is achieved on purpose by increasing the power in the laser irradiation to 6.5 mW , in order to maintain a high robustness of the structure notwithstanding the larger void ratio.

Once the structure is optimized on a flat glass sample, it is fabricated inside a commercial microfluidic chip at the junction between two channels according to the experimental procedure described in Section 2. It is important to stress that the channels in the chip are already sealed and the fabrication is performed using exposure through the top layer of the chip, exploiting the non-linear absorption of the 2PP process. The main issue in replicating the above structure in the microfluidic chip is the alignment to the channels. Actually, exploiting the back-reflection of a low intensity laser beam from the refractive index discontinuities on the chip, it is possible to properly position the filter with accuracy better than $3\text{ }\mu\text{m}$ in all directions. On the other hand, perfect tailoring of the filter to the channel cross-section is easily achieved by irradiating a 3D structure with dimensions exceeding those of the channel. In this way, 2PP is initiated only when the beam moves inside the resin and is terminated when the beam enters the glass, without the need of a shutter. Continuous scanning performed at each glass–resin interface also ensures perfect adhesion to every channel wall. Fig. 4 shows a microscope image of the filter fabricated at the channel junction. One can appreciate the good alignment and quality of the filter in the channel, which is comparable to the results obtained on a flat glass sample. We chose to replicate inside the microchannel the same filter geometry as that represented in Fig. 3a–c (*i.e.* the one with $\sim 1.5\text{ }\mu\text{m}$ pore size), in order to have, on one hand, a good filtering resolution and, on the other hand, the possibility to clearly image the embedded structure with an optical microscope.

Fig. 3 (a) En-face SEM image of the porous filter, identical to the one integrated into the microfluidic channel, fabricated on a flat glass substrate. (b) The tilted SEM image. (c)–(e) Close-up showing top views (dashed box) and side views (solid box) of different structures with (c) ~ 1.5 μm , (d) ~ 3 μm and (e) ~ 0.5 μm pore sizes.

Fig. 4 Bright-field microscope image of the filter inside the microfluidic chip at the junction of the two channels. Inset shows the filter in the junction at a higher magnification.

3.2 Flow experiments

The fabricated filter is tested in several flow conditions to assess its performance. A mixture of 3 μm beads in buffer or fluorescent solutions is used to demonstrate the capability of the filter to stop

the microscale elements while letting the solution flow through. The test sample is injected at well 1 (see Fig. 1d) and a controlled portion of it is filtered to well 4. The rest of the flow is directed to wells 2 and 3.

3.2.1 Beads in bright field. Frame shots in Fig. 5a show a functionality test of the porous filter with a suspension of 3 μm polystyrene spheres in 100 mM phosphate buffer (see full video in the ESI†—Video S1). As mentioned previously, flow is controlled by different hydrostatic pressures applied at each well by filling the reservoirs at different heights. The channels are first filled with 100 mM phosphate buffer, and the test solution is flowed from well 1 through the input channel with an estimated rate of 50 pL s^{-1} . It should be noted that because of the presence of the side channels, not all of the sample encounters the filter—some simply flows into the side channels because there is hydrostatic pressure driving it towards wells 2 and 3, as well as towards well 4. These side flows should help in washing away the filtered elements from the filter.

The concentration of the test beads is 0.172% by weight, or about 120 beads nL^{-1} . As can be seen in Fig. 5a, 100% of the beads that encounter the filter are blocked and the stiffness of the polymer material ensures that they do not pass through it. The polystyrene spheres that are blocked by the filter tend to adhere to it or to the channel walls. This gathers a large amount

Fig. 5 (a) Test of the porous filter with 3 μm polystyrene spheres suspended in a 100 mM phosphate buffer. (b) Time-lapsed fluorescence microscope images of the slow filtering test with 3 μm spheres suspended in 40 μM Rhodamine 6G. (c) Time-lapsed fluorescence microscope images of the fast filtering test. Frame shots in panels (b) and (c) are chosen to represent three significant situations: when the Rhodamine solution is approaching the filter, when it first passes through the filter and when it has filled the channel after the filter; these three conditions occur at different times depending on the flow rates. Full videos are available in the ESI†.

of spheres in front of the filter and limits the washing effect that was expected by the side flows. The sticking of the particles could be addressed by coating the filter and channel walls or may be less relevant if the elements to be filtered have less affinity to the filter and substrate materials.

3.2.2 Beads in fluorescent solution. The experiment described in the previous section clearly shows that the filter is capable of blocking all microscale elements in the solution. However, no indication can be deduced on whether the flow through the channel is present and how it changes with time. To study this aspect we add Rhodamine 6G molecules to the bead solution and observe the flow under a fluorescence microscope.

Two regimes of sample filtering are explored: one with most of the flow being redirected away from the filter in the side channels (named as slow filtering) and a second one with most of the flow being directed through the filter (named as fast filtering). The two regimes are achieved for the same input flow by changing the relative pressures at wells 2, 3 and 4. These two situations respectively represent the case when a small amount of sample needs to be continuously filtered for a long time (*e.g.* when monitoring a reaction process without depleting the solution) and the case when most of the sample needs to be filtered as fast

as possible (*e.g.* when extracting plasma from whole blood before performing an analysis).

3.2.2.1 Slow filtering. Fig. 5b shows time-lapsed fluorescence microscope images of the filtering test (see full video in the ESI†—Video S2). Images show the times when the fluorescent solution is approaching the filter, when it first passes through the filter and when it has filled the channel after the filter. Once again, 100% of the polystyrene spheres are stopped by the filter while the Rhodamine molecules pass through it. When the fluorescent solution reaches the junction there is a delay of about 20 s before the fluorescent molecules start to appear beyond the filter. This is attributed to the fact that the dye molecules attach to the filter walls and, since in the porous structure the surface to volume ratio is quite high, we observe some time delay before the surface is coated and the dye molecules can finally flow freely through the filter. After this transient, flows are measured to be about 51 pL s^{-1} in the channel approaching the filter, 18 pL s^{-1} toward well 2, 18 pL s^{-1} toward well 3, and 15 pL s^{-1} after the filter toward well 4. The splitting ratios toward wells 2, 3 and 4 are 35%, 35% and 30%, respectively. Therefore, in this filtering condition, only about one-third of the total sample encounters the filter. Similarly to the case discussed in Section 3.2.1 and

Fig. 6 Blood cells are filtered from diluted blood.

shown in Fig. 5a, the polystyrene spheres accumulate before the filter. Because of their slightly higher density with respect to the buffer, the spheres tend to flow at the bottom of the channel, and thus they settle at the bottom of the filter or are stopped by other beads which have been previously blocked by the filter so they do not clog the entire height of the channel even after a long operation time. In fact, the filter is operated for up to 25 minutes and no clogging is observed. As a matter of fact, the measured flow rate toward well 4 is reduced to about half the initial value after 5 minutes and then remains constant for the remainder of the observation time.

3.2.2.2 Fast filtering. Fig. 5c shows time-lapsed fluorescence microscope images of the filtering test with a different set of hydrostatic pressure conditions (see full video in the ESI†, Video S3). The images again show the relevant situations when the solution has traveled to the filter, passed it and filled the channel after the filter. These are the same situations reported in Fig. 5b, however, they occur at different times due to the different flow rates. Once the fluorescent solution passes through the filter (in this configuration we observed a delay of about 9 s—see discussion in the previous section) we monitored the flow rates in all the channels. The initial values that we measured are: 47 pL s^{-1} in the channel approaching the filter, 4 pL s^{-1} toward well 2, 6 pL s^{-1} toward well 3, and 37 pL s^{-1} after the filter toward well 4. In this case the splitting ratios toward wells 2, 3 and 4 are 8%, 13% and 79%, respectively. Most of the flow volume is therefore directed through the filter. Also in this case the filter is capable of blocking 100% of the beads while letting the fluorescent molecules flow through. After 5 minutes of operation the

Fig. 7 (a) Image of the filter after 25 minutes in the slow filtering regime. (b) The filter after cleaning by injection of buffer solution from well 4 with a syringe.

flow after the filter is decreased to almost half the initial value and then remains constant (see Video S4 in the ESI†). As discussed in the previous section, only the lower portion of the filter is blocked by the beads since they tend to flow at the bottom of the channels due to gravity. Therefore, notwithstanding the faster accumulation of beads in front of the filter, the flow reduction in this case is of the same proportion as that observed in the slow filtering regime. No clogging was observed even after 25 minutes.

3.2.3 Filtering of red blood cells from whole plasma. A preliminary validation experiment towards real applications of our integrated 3D filter is also performed. Diluted whole blood is filtered in order to extract plasma.⁶ A human blood droplet is diluted by a factor of 1000 in physiological buffer. The blood is flowed in the microchannel at a rate of 90 pL s^{-1} , controlled by hydrostatic pressure difference (see Section 2). The filter embedded in the microchannel has a pore size of $\sim 1.5 \text{ μm}$. Imaging of the cells flowing in the chip is achieved with a phase contrast microscope. As shown in Fig. 6, all the cells (mainly red blood cells) are blocked by the filter, thus separating them from the plasma that passes through the filter. The filter proves effective also with deformable and disk-shaped particles (as red blood cells) without the need for openings much smaller than the cell thickness.⁵

3.2.4 Chip cleaning and reuse. After a long operation time, the flow rate is decreased and the filtering area of the microfluidic chip needs to be cleaned before a new experiment. Cleaning is performed by injecting 100 mM phosphate buffer solution from well 4 with a syringe. The flow is reversed across the filter with a high pressure of about 200 kPa. After a few seconds, all beads have been cleared out of the filtering area and the nearby channels, and the filter proves robust to the high pressure flow it experiences. The chip can now be reused (as was done at least 10 times for the experiments here presented). Fig. 7a and b show the filter before and after cleaning.

4. Conclusions

In conclusion, we have demonstrated femtosecond laser 2PP fabrication as an effective tool for the integration of porous filters into a LOC system. The filter has been fabricated inside an already sealed channel of a commercial microfluidic chip, thus proving to be a post-processing add-on that can be implemented in many available devices. The very high spatial resolution of 2PP has allowed us to fabricate a filter with a pore size of about 1 μm . The filter has been tested with a suspension of 3 μm polystyrene spheres in a fluorescent solution. We have shown separation of nanoscale elements (dye molecules) from microscale elements (polystyrene beads) and have demonstrated that 100% of the beads are stopped by the filter. The filter operation has been monitored for a period up to 25 minutes, at a flow rate up to 37 pL s^{-1} after the filter and a sample concentration of $120 \text{ beads nL}^{-1}$, without significant clogging of the channel or jamming of the spheres into the pores. Preliminary validation of the filter for plasma extraction from whole blood has been performed, demonstrating that the filter is effective also for softer particles such as red blood cells. The filter is completely clean

after reversing the flow with an injection of buffer solution at high pressure and can be reused several times.

Future work will explore the use of photoresists with lower viscosity and which do not require any baking step, for better compatibility with the microfluidic channels. A class of materials with these properties are acrylic resins, which are typically used in the pure liquid form and do not require a complex prebaking step.²² This will allow straightforward insertion in the microchannels and a significant simplification of the fabrication process. In particular, one class of acrylate resins, poly(ethylene glycol) diacrylate (PEGDA), can be developed using only water,³⁶ offering excellent compatibility with lower-cost polymer substrates.

The 3D and direct-write capabilities of 2PP technology enable simple integration of porous filters into microfluidic chips with customized pore shape and size, with potential for several applications. Moreover, these results pave the way to the straightforward embedding of several other components, e.g. microvalves,³⁵ in already-made microfluidic chips, thus further boosting device integration.

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