Video Article

Soft Lithographic Procedure for Producing Plastic Microfluidic Devices with View-ports Transparent to Visible and Infrared Light

Mona Suryana[1], Jegan V. Shanmugarajah[1], Sivakumar M. Maniam[1], Gianluca Grenci[1]

[1]Mechanobiology Institute (MBI), National University of Singapore

Correspondence to: Gianluca Grenci at mbigg@nus.edu.sg

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Abstract

Infrared (IR) spectro-microscopy of living biological samples is hampered by the absorption of water in the mid-IR range and by the lack of suitable microfluidic devices. Here, a protocol for the fabrication of plastic microfluidic devices is demonstrated, where soft lithographic techniques are used to embed transparent Calcium Fluoride (CaF_2) view-ports in connection with observation chamber(s). The method is based on a replica casting approach, where a polydimethylsiloxane (PDMS) mold is produced through standard lithographic procedures and then used as the template to produce a plastic device. The plastic device features ultraviolet/visible/infrared (UV/Vis/IR) -transparent windows made of CaF_2 to allow for direct observation with visible and IR light. The advantages of the proposed method include: a reduced need for accessing a clean room micro-fabrication facility, multiple view-ports, an easy and versatile connection to an external pumping system through the plastic body, flexibility of the design, *e.g.*, open/closed channels configuration, and the possibility to add sophisticated features such as nanoporous membranes.

Video Link

The video component of this article can be found at https://www.jove.com/video/55884/

Introduction

Fourier Transform Infrared Spectro-microscopy (FTIR) has been extensively utilized as a label-free and non-invasive imaging technique to provide detailed chemical information of a sample. This enables the extraction of biochemical information to study the chemistry of biological samples, with a minimum amount of preparation since the absorption spectrum of the specimen carries the intrinsic fingerprints of its chemical composition[1,2]. Recently, FTIR has been increasingly applied to the study of live biological samples, *e.g.*, cells[3]. However, water, which is the medium for living cells in most cases, shows a strong absorbance in the mid-IR region. Even as a thin layer, its presence can completely overwhelm the important structural information of the specimens.

For many years, the common approach was fixing or drying samples to completely exclude the water absorption signal in the spectrum. However, this approach does not allow for real time measurements on living cells, which is essential to study the change of their chemical composition and cellular processes with time. One way to obtain reliable absorption spectra from live biological samples, is to limit the total optical path length in the medium of the IR beam to less than 10 μm[4].

A well-established approach in living cell experiments has been so far, Attenuated Total Reflection (ATR)-FTIR imaging, which enables measurements independent of the sample thickness, allowing cells to be sustained in a thicker layer of aqueous medium. However, the small depth of penetration of the evanescent wave restricts measurements of samples to only the first few microns from the surface of the ATR crystal[5].

Alternatively, the water absorption limitation has been circumvented with the emergence of various microfluidic systems, which are generally classified into two large groups: open channel (where one of the fluid surfaces is exposed to the atmosphere) and closed channel (where two IR-transparent windows are separated by a spacer with a defined thickness).

Loutherback *et al.* developed an open-channel membrane device that enables long term continuous IR measurements of live cells for up to 7 days[6]. The method requires high humidity in the environment to prevent evaporation of the medium from the cell surface. The system works best with cells that naturally grow at air-liquid interfaces, such as epithelial tissues of the skin, lung, and eyes, or microbial biofilms[7].

A closed-channel configuration aims to create a uniform, thin layer between two parallel IR-transparent windows, where cells are maintained in their aqueous media. The thickness of this cavity is such that the water absorption signal is below saturation. Water background can then be subtracted to obtain the correct sample spectra. Most of the closed-channel methods utilize a plastic spacer separating the two windows to form a demountable liquid chamber[3,8,9]. An advantage of this method is that it does not require microfabrication; however, structures that are more complex than a measuring chamber with in- and out-let channels are extremely difficult to realize in the thin spacer. There is also an issue with

the reproducibility of the path length between IR measurements due to its reliance on mechanical clamping. In order to achieve a more precise control of the spacing for a more reliable spectrum acquisition, optical lithography methods have been implemented to pattern photoresist on top of the IR substrate to define the spacer[9,10,11,12]. Even though this makes it possible for more complex structures to be defined in the spacer, the method requires access to a microfabrication facility to produce the pattern on every substrate.

In this paper, we present a simple fabrication technique of an IR-compatible microfluidic device, with the aim to reduce the fabrication cost and the requirement of accessing a microfabrication facility. The method presented here (see **Figure 1**) uses an established process known as soft lithography. Two molds are required in this case. The primary mold is made from a 4-inch silicon wafer using a standard UV lithography process. The secondary mold is its replica made of PDMS, which has a reversed polarity of the pattern in the silicon primary mold and serves as the master mold for subsequent device fabrication.

The device has two separate layers: a first layer with the microfluidic layout (which in the presented case consists of the microfluidic channel, in-let/out-let, and an observation chamber with a CaF_2 viewport), and a second layer with a flat surface (which consists of only a CaF_2 viewport).

Here an UV-curable optical adhesive, Norland optical adhesive 73 (NOA73, abbreviated as NOA henceforth), is used to form the main plastic body of the device. There are several advantages of using this optical adhesive: low fabrication cost, ease of connectivity to external systems, good optical transparency, low viscosity, and most importantly, biocompatibility[13]. CaF_2 is a suitable choice as the viewport due to its biocompatibility and excellent IR-transparency[14].

With this new approach, access to a microfabrication facility is strictly required only for the fabrication of the primary mold. Subsequent fabrication processes for the plastic microfluidic device can be carried out in any laboratory equipped with an UV-illumination source.

Protocol

1. Preparation of Silicon Primary Mold

NOTE: A photomask is required for the preparation of the primary mold. The photomask can be either purchased from independent providers or fabricated in-house through standard optical mask fabrication procedures. A photomask with bright field polarity is used in this case (**Figure 2a**).

1. **Pattern definition**
    1. Spin coat a 4 inch silicon wafer with SU-8 3010 negative photoresist at 2,300 rpm for 30 s.
    2. Soft-bake the photoresist on a hot plate at 65 °C for 2 min and then at 95 °C for 8 min.
    3. Expose the photoresist to UV light (i-line, 365 nm) through the photomask under the mask aligner for a total energy dose of 100-120 mJ/cm^2; the hard contact mode is preferred to achieve a better resolution.
    4. Remove the wafer and apply a post exposure bake at 65 °C for 1 min and then at 95 °C for 2 min.
    5. Develop the photoresist using a SU-8 developer at room temperature, then rinse with isopropyl alcohol, and gently blow dry with nitrogen; the measured pattern thickness should be 10 µm and below. See **Figure 2b** for the picture of the actual silicon mold.

2. **Silanization of silicon mold**
    1. Treat the silicon mold with oxygen plasma at 60 W for 30 s with 20 sccm of oxygen flow. Set the chamber pressure to 1-10 mbar during the process.
    2. Place the mold in a vacuum jar with 50 µL of silane and leave the jar in the vacuum state (1-10 mbar) for at least 2 h.
    NOTE: The silanization process creates a hydrophobic surface coating which prevents PDMS from sticking to the mold[15]. Note that the primary mold can also be fabricated using an alternative method, which involves dry etching of the silicon. In this case, the photomask will be of opposite polarity (dark field), and the pattern definition in step 1.1 will use a positive photoresist.

2. Preparation of PDMS Secondary Mold

1. **PDMS mixing**
    1. Mix the PDMS elastomer and curing agent, 10:1; the total amount is such that the resulting PDMS thickness is approximately 1 to 1.5 mm.
    2. After thorough mixing, degas the mixture by leaving it in a vacuum jar in the vacuum state (1-10 mbar) for approximately 15 min or until there are no visible bubbles; this is to remove any trapped air within the mixture.

2. **Mold replication**
    1. Pour the PDMS mixture on the silicon mold fabricated in step 1 and degas the mixture to remove any trapped air with the same settings as in step 2.1.2. Heat at 70 °C for 2 h on a hot plate to cure the mixture.
    2. Remove the cured PDMS from the hot plate and let it cool down to room temperature. With a razor blade, cut the PDMS along the edges of the silicon mold.
    3. With a pair of tweezers, pinch one corner of the cut PDMS and carefully peel the PDMS replica off the silicon mold; the resulting microfluidic pattern on this secondary mold is a protrusion, which is the opposite polarity of the primary mold (**Figure 2c**).

3. **Silanization of the PDMS replica (same as in step 1.2)**
    1. Treat the PDMS mold with oxygen plasma at 60 W for 30 s with 20 sccm of oxygen flow. Set the chamber pressure to 1-10 mbar during the process.
    2. Place the mold in a vacuum jar with 50 µL of silane and leave the jar in the vacuum state (1-10 mbar) for at least 2 h.

3. Preparation of PDMS Templates

NOTE: To standardize the shape and size of the final devices and to ease the alignment of the main features in the two halves, two separate PDMS templates were used, which define the geometry of the device, the placement of the transparent window and the in- and out-let connections. The first PDMS template aids the fabrication of the patterned half of the device, while the second helps to ease the fabrication of the flat half of the device.

1. Design the templates using a computer aided design (CAD) software. **Figure 3a** shows the lay-out and dimensions of the template used to fabricate the patterned half of the device. To fabricate the flat half of the device, remove the 1.5 mm diameter holes from the design.
2. Acquire the templates from an external provider or through an internal mechanical workshop if available.
   NOTE: Acrylic was used as the template material due to ease of fabrication and low cost achievable in any standard workshop. There are alternative options, such as 3D printing.
3. Mix the PDMS elastomer and curing agent 10:1; be sure to prepare a sufficient amount of PDMS to entirely submerge the templates.
4. After thorough mixing, degas the mixture by leaving it in a vacuum jar in the vacuum state (1-10 mbar) for approximately 15 min or until there are no visible bubbles (whichever is later); this is to remove any trapped air within the mixture.
5. Pour the PDMS mixture on the acrylic templates until their top-most surface is submerged about 1 mm below the liquid surface. Degas the PDMS again to remove any trapped air with the same settings as in 3.4. Heat this at 60 °C for 2 h on a leveled hot plate to cure the mixture.
6. Remove the cured PDMS from the hot plate and let it cool down to room temperature. With a razor blade, cut the PDMS along the edges of the acrylic templates.
7. With a pair of tweezers, pinch one corner of the cut PDMS and carefully peel the PDMS off the acrylic templates.
   NOTE: **Figure 3b** shows the lay-out and dimensions of the PDMS replica used to fabricate the patterned half of the device.
8. Prepare the second PDMS replica for fabricating the flat half of the device by repeating steps 3.3 to 3.7 but using the acrylic template without the 1.5 mm diameter holes.

4. Microfluidic Device Fabrication

1. **Fabrication of the patterned half of the device (*i.e.* , with device layout)**
   1. Treat the CaF_2 window with oxygen plasma at 60 W for 30 s with 20 sccm of oxygen flow. This is done to improve the flow of NOA during the following fabrication.
      NOTE: This step is not mandatory.
   2. Place carefully the first PDMS template (one with the 1.5 mm diameter pillars) on a flat surface, *e.g.*, a soda lime plate (**Figure 4a**). Place a CaF_2 window centered on top of the PDMS plug and gently press the window such that it is in good contact with the plug (**Figure 4b**).
   3. Take the PDMS mold made in step 2 and place a thin UV-transparent plate (in this case, a quartz plate, 500 µm thick and 1.5 cm x 1.5 cm in size) on the backside of the mold, aligned with the location of the central chamber (**Figure 4c**). Make sure that the quartz plate is in good contact with the PDMS mold.
      NOTE: The quartz plate prevents the unwanted area of the mold from easily coming into contact with the CaF_2 window.
   4. Gently place this PDMS mold face down towards the CaF_2 window with the fluidic chamber aligned to the center of the CaF_2 window. Make sure that all the elements (template, mold and window) are in good contact and aligned (**Figure 4c-4d**).
   5. Gradually dispense drops of NOA at the in-let of the PDMS template and let it slowly fill the cavity. Once the resin comes into contact with the edge of the window, the capillary flow will fill the thin gap (~10 µm) between the PDMS mold and CaF_2 window (**Figure 4e-4f**).
   6. After the cavity is completely filled, cure the NOA by exposure to UV light (*e.g.*, with a UV-LED exposure system, **Figure 4g**).
      NOTE: The exposure time may vary accordingly with the energy of the UV source. The UV-LED exposure system, which provides a power density of 24 mW/cm^2, requires around 90 s at 100% power and continuous exposure mode.
   7. Carefully remove the thin quartz plate from the back of the PDMS mold and then gently peel the PDMS mold from the top of the NOA layer (**Figure 4h**). Finally, remove the NOA layer from the PDMS template (**Figure 4i**).
      NOTE: The resulting device layout on the cured NOA would have the same polarity of the pattern in the primary silicon mold.

2. **Fabrication of the flat half of the device (*i.e.* , without device layout)**
   1. Treat the CaF_2 window with oxygen plasma at 60 W for 30 s with 20 sccm of oxygen flow.
      NOTE: This step is not mandatory.
   2. Place carefully the second PDMS template (one without the 1.5 mm diameter pillars) on a flat surface, *e.g.*, a soda lime plate. Place a CaF_2 window centered on top of the PDMS plug and gently press the window such that it is in good contact with the plug.
   3. Place a 1 mm thick PDMS sheet with 5 cm x 3.5 cm size on top of the CaF_2 window, with the PDMS sheet aligned with the center of the PDMS template. Make sure that the PDMS sheet is in good contact with the window.
   4. Gradually dispense drops of NOA at the in-let of the PDMS template and let it slowly fill the cavity.
   5. After the cavity is completely filled, cure the NOA by exposure to UV light (*e.g.*, with a UV-LED exposure system).
      NOTE: The exposure time may vary accordingly with the UV energy source. With the UV-LED exposure system, which provides a power density of 24 mW/cm^2, this requires around 50 s at 100% power and continuous exposure mode.
   6. Peel off the PDMS sheet from the top of the NOA layer and carefully remove the cured NOA layer from the PDMS template.

3. **Bonding of the two halves of the device**
   1. Align the two halves of the device such that both CaF_2 windows are aligned. Gently finger-press both halves at the corner of the NOA layers such that the position of the two halves are fixed.
   2. Cut two circular discs out of a 1 mm thick PDMS sheet using an 8 mm diameter puncher (**Figure 5a**).
   3. Cut two rectangles with the same size of the device (4 cm x 2.5 cm) from a 1 mm thick PDMS sheet. On both PDMS rectangles, cut openings corresponding to the channels and the in-let/out-let of the device.
      NOTE: The pre-cut openings in the PDMS rectangles are meant to prevent the channels from collapsing during pressing.

4. Stack in the following order from the bottom: one PDMS rectangle with pre-cut openings, one PDMS disc (in contact with the bottom window, cut in step 4.3.2), the two finger-pressed halves of the device, the second PDMS disc (sitting on the top window), and lastly the second PDMS rectangle with pre-cut openings (**Figure 5b**).
5. Place this assembly in the vacuum press setup such that it is sandwiched between 2 plates and seal the plastic bag (**Figure 5c**). Turn on the vacuum pump and evacuate the assembly. Let the vacuum pump reach its base pressure or apply the vacuum for at least 10 min.
   NOTE: The base pressure achieved depends on the vacuum pump used and quality of the sealing of the plastic bag.
6. Expose the evacuated assembly to UV with a broad-band Hg gas lamp at 270 W for 15 min. Turn off the vacuum pump and let the assembly slowly vent to atmospheric pressure before removing the final device from the assembly.

Representative Results

Figure 6 presents the transmittance spectra of a brand new CaF_2 window, the patterned half of the device, and the complete device. All three spectra exhibit excellent transparency to mid IR with transmittance larger than 80%. The interference pattern visible in the spectrum of the full device (yellow curve in the figure) is caused by the air gap in the range of 9-10 µm between the two windows. These spectra demonstrate that the fabrication approach presented here does not alter the transparency of CaF_2 in the mid IR range.

Figure 7a shows an example of good replication in NOA of the PDMS secondary mold with the microfluidic lay-out. The structure on top of the CaF_2 window is well formed because of the clean peeling of the NOA layer from the PDMS mold after it is partially UV-cured. No NOA should remain on the mold or on the window surface in contact with the mold protrusions. Any NOA stuck to the mold translates into missing NOA structure on the window, which would cause leaks during flow experiments of the final device. In addition, to achieve good sealing of the two halves, NOA should still be tacky after UV exposure of the half layers. NOA is over-cured if it is non-tacky. The exposure dose should be optimized to achieve such outcome.

Figure 7b instead, shows unsuccessful replication in NOA where the pattern on the CaF_2 window is not properly defined. It is mostly caused by insufficient UV exposure dose, *i.e.*, under-curing of NOA. In such cases, NOA is still somewhat wet, causing some of it to stick to the PDMS mold. However, if NOA still sticks to PDMS mold even though the correct exposure dose has been given, this can be a symptom of silane coating (*i.e.*, anti-stick layer) being degraded over time. As PDMS is a soft mold, it is not as long lasting as the silicon primary mold. It needs to be replaced after a number of uses.

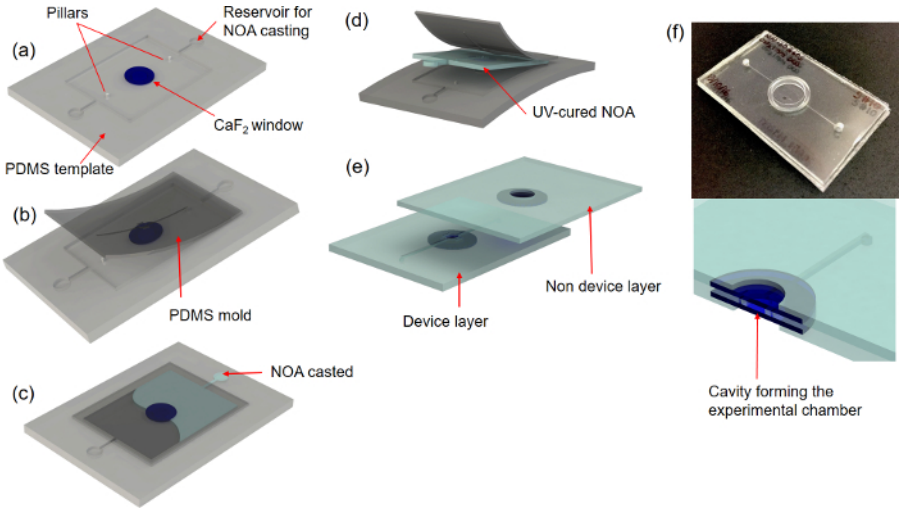

Figure 1: Fabrication process of the plastic microfluidic devices: (**a-e**) Schematic of the fabrication process. (**f**) Picture of an actual device and schematic of its cross-section. Please click here to view a larger version of this figure.

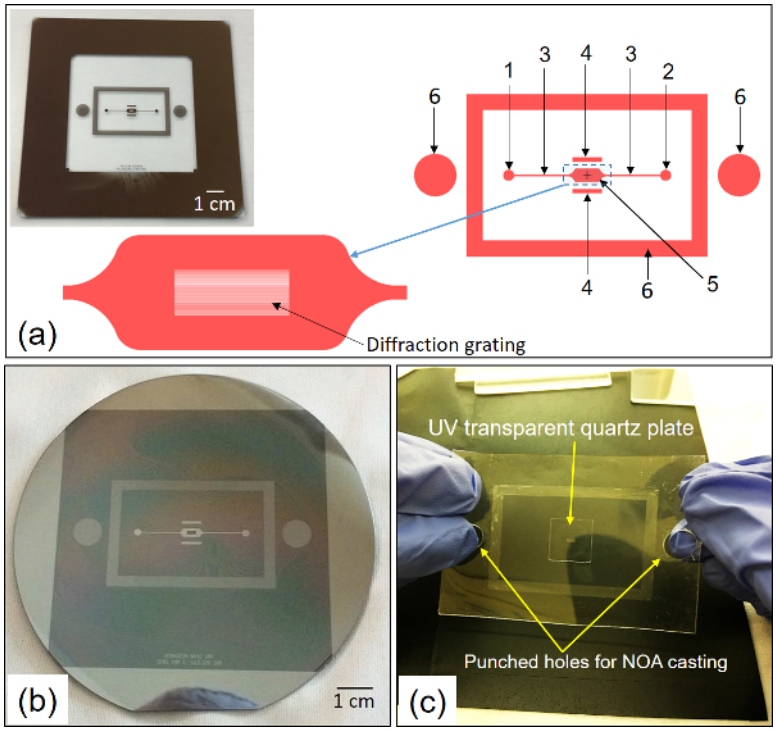

Figure 2: Overview of the photomask, silicon primary mold, and PDMS secondary mold replicated from the silicon mold: (**a**) Photomask with bright field polarity (top left); Lay-out of the pattern in the photomask (right) consisting of: in-let and out-let with 2 mm diameters (1 and 2) and distance of 3 cm between them, channels with 300 µm width (3), two reference chambers with 5.5 mm x 0.75 mm size (4), central chamber with 5 mm x 2.5 mm size (5), diffraction gratings as a visual guide with lines 10 µm wide and 20 µm gap (6); Zoom-in lay-out of the central chamber showing the diffraction grating (bottom left). (**b**) Picture of the silicon primary mold with the pattern defined in the SU-8 photoresist. (**c**) Picture of the PDMS secondary mold with reverse polarity with respect to the primary mold. Please click here to view a larger version of this figure.

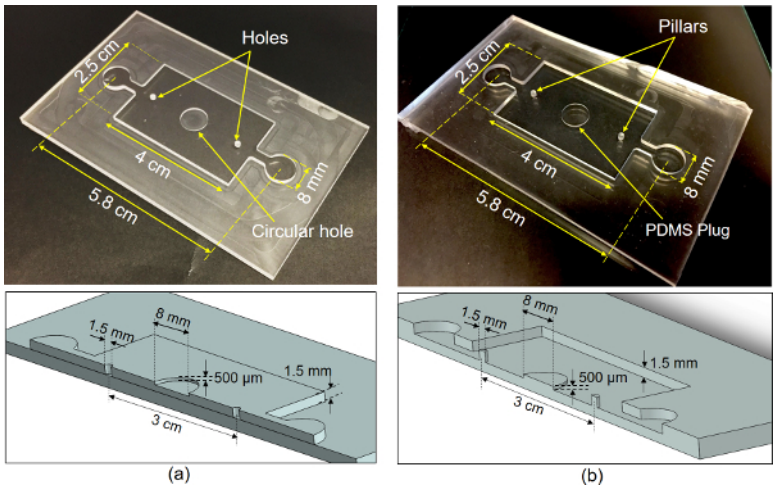

Figure 3: The templating tools prepared to ease the device fabrication: (**a**) Acrylic template: actual template (top) and its schematic cross-sectional view (bottom). (**b**) PDMS replica of the acrylic template: actual replica (top) and its schematic cross-sectional view (bottom). Please click here to view a larger version of this figure.

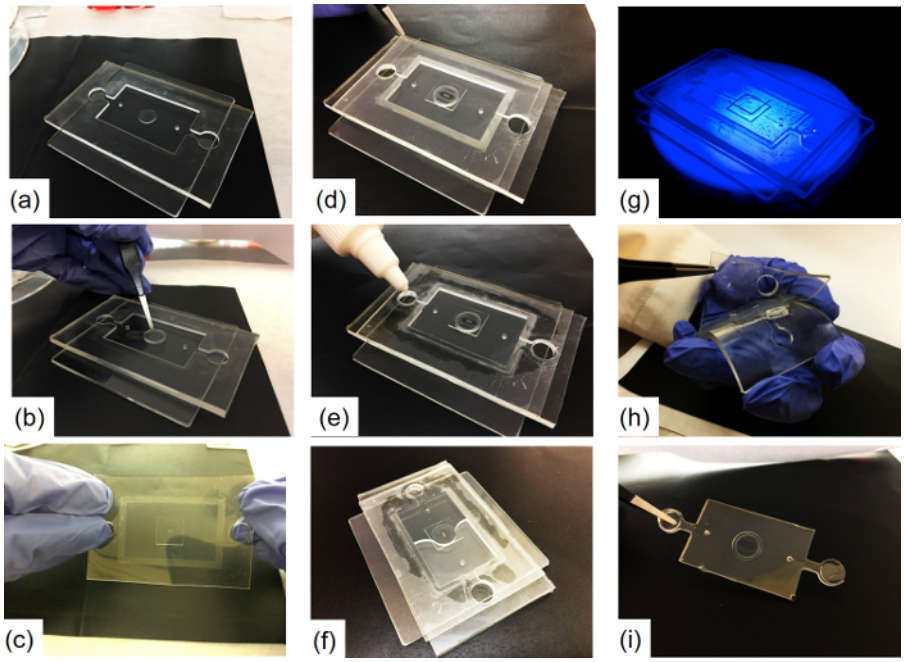

Figure 4: Process flow for the fabrication of the patterned half of the device: (**a**) Place the PDMS template on a flat surface. Here, we used soda lime glass. (**b**) CaF_2 is placed centered on top of the PDMS plug. (**c-d**) PDMS mold is placed face down towards the CaF_2 with the fluidic chamber aligned to the center of the window. Ensure that all the elements are in good contact and aligned. (**e-f**) Casting of NOA via the in-let and allowing it to slowly fill the cavity. (**g**) NOA is cured by exposing it under UV light. Exposure dose may vary depending on the energy of the UV source used. (**h**) Carefully peel the PDMS mold and template to release the cured NOA. (**i**) Completed device layer with microfluidic structures in NOA. Please click here to view a larger version of this figure.

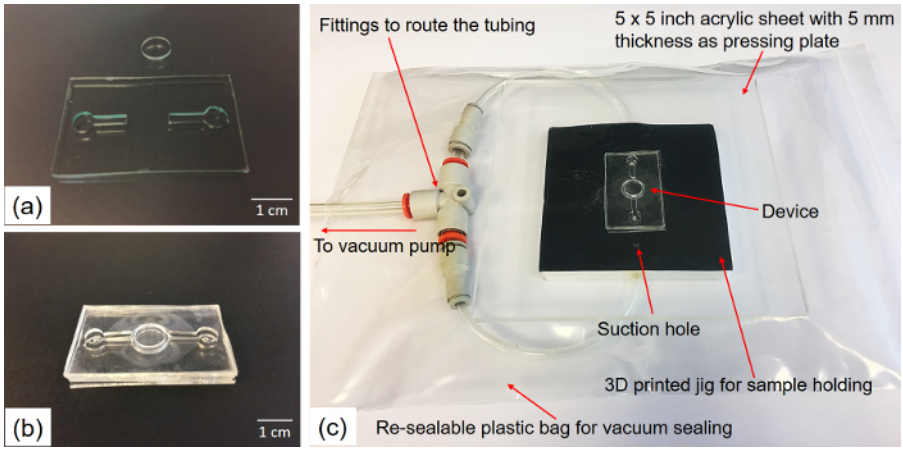

Figure 5: Vacuum press setup for pressing the two halves of the device to form a complete device: (**a**) PDMS circular disc (8 mm diameter) and PDMS rectangle (4 x 2.5 cm) with pre-cut openings. Both are cut from a 1 mm thick PDMS sheet. (**b**) Overview of the layer-stack before pressing. (**c**) Overview of the vacuum press. Please click here to view a larger version of this figure.

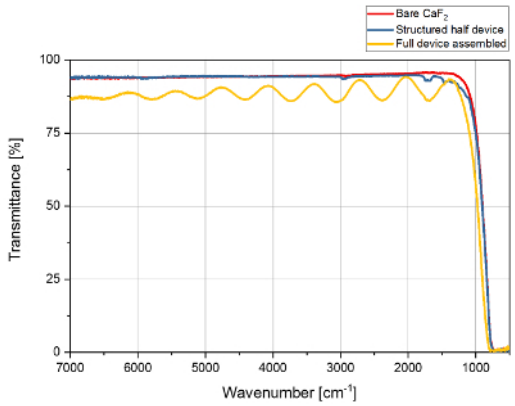

Figure 6: Mid-IR transmittance spectra of a bare CaF_2 window (red), a patterned half of the device (blue), and a complete device (yellow). All three spectra exhibit excellent transparency to mid IR with transmittance larger than 80% Please click here to view a larger version of this figure.

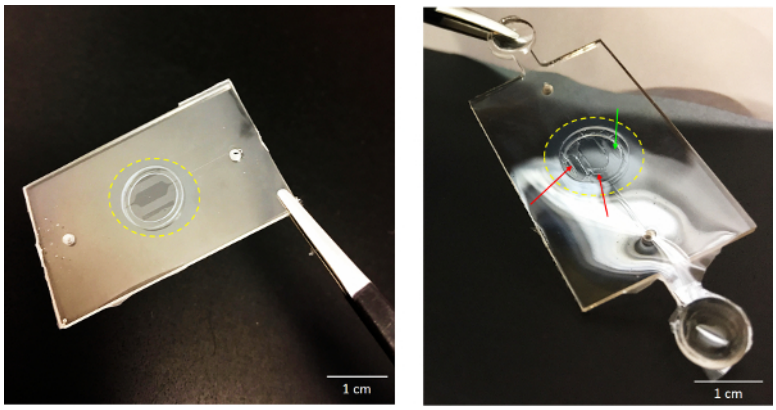

Figure 7: Patterned half of the device: (**a**) Example of a well formed microfluidic structure in NOA. Darker area on the window shows a well defined structure, showing clearly the central chamber, two reference chambers, and the channels. (**b**) Example of a poorly formed microfluidic structure in NOA due to undercuring. There is reflow of NOA as indicated by red arrows. One of the reference chambers is also missing (green arrow). Please click here to view a larger version of this figure.

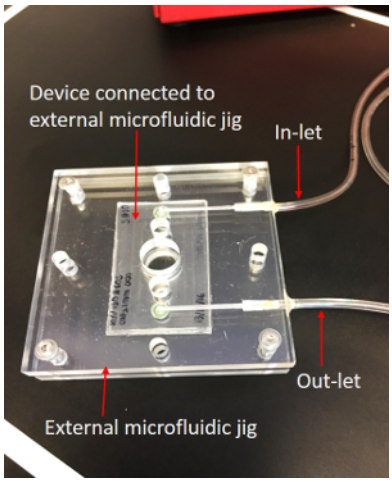

Figure 8: Microfluidic jig connecting the fabricated device to external fluidic circuitry. Please click here to view a larger version of this figure.

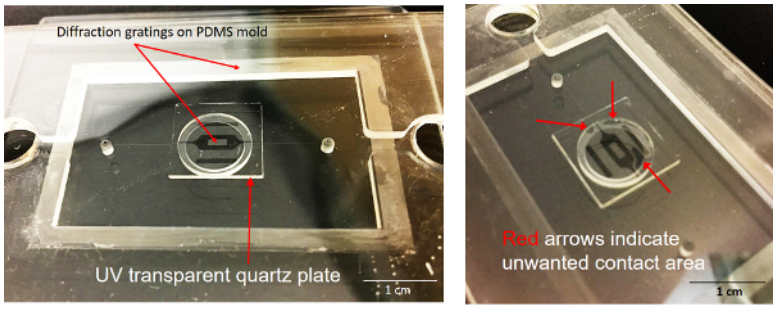

Figure 9: Placement of PDMS mold during fabrication of the patterned half of the device: (**a**) Example of good placement of the PDMS mold on the CaF_2 window. The window is in contact with only the protrusion on the PDMS mold, showing clearly the central chamber, two reference chambers, and the channels (indicated by the darker area). The diffraction gratings around the device and at the center of the central chamber are meant for a visual guide during placement of the PDMS mold. (**b**) Example of a bad placement of the PDMS mold on the CaF_2 window. The darker area shows that there is also an unwanted area of the mold, which is in contact with the window, as indicated by the red arrows. Please click here to view a larger version of this figure.

Discussion

In order to assess and optimize the fabrication protocol, we used a simple layout for the microfluidic pattern with a large rectangular chamber (5 mm x 2.5 mm size) at the center, two small rectangular chambers (5.5 mm x 0.75 mm size) separated from the main circuit on the upper and lower sides, and 300 µm wide in-let/out-let channels. The central chamber is used for the seeding and observation of the cells, while the two separated smaller chambers are used to measure the air background during FTIR experiments as a reference chamber, as discussed in a previous publication[13]. The in-let and out-let connect the central chamber to the external fluidic system.

Here, templating tools (see protocol step 3) are introduced to ease the fabrication process. Previously, it was difficult to consistently place the CaF_2 windows exactly at the center of the final device, which caused issues when aligning the two halves of the device. The use of the template provides a visual guide for placing the window and ensures that the location is always similar. As the geometrical requirements for these templates are not very stringent, standard and cheap production technologies can be used. In this case, we made them out of acrylic plastic through machining in a workshop, but an equally viable and cheap alternative is 3D printing.

The design of the acrylic templates is such that they feature the following (**Figure 3a**): (a) one template is designed with two small holes on each side, while the second is without holes; the holes are 1.5 mm diameter in size and 1.5 mm in depth, (b) both templates have a circular hole with 8 mm diameter and 500 µm depth in the center, and (c) both templates have a rectangular protrusion to define the size, shape, and thickness of the half device; the rectangle is 4 cm x 2.5 cm in length/width and 1.5 mm in thickness.

The resulting PDMS templates replicated from the acrylic templates will have the opposite polarity (**Figure 3b**): (a) one template will have two pillars on each side with 1.5 mm diameter and 1.5 mm height to form the in-let and out-let of the patterned half of the device after NOA casting; the second template will be without pillars, (b) both templates have a 500 µm tall pillar in the center to ease the placement of the CaF_2 windows; this feature will be referred to as "PDMS plug", and (c) both templates are with a cavity, which defines the final shape and size of each half of the device: a rectangle shape with 4 cm x 2.5 cm size and 1.5 mm thickness in this case.

To further ease the fabrication process, diffraction gratings were incorporated in the design of the photomask for the primary silicon mold. In the PDMS replica of this silicon mold, the shallow protrusions (which are less than 10 µm in height) are hard to see due to the optical transparency of PDMS. However, shallow protrusions if arranged in a grating of 10 µm wide lines with 20 µm gap generate an easily visible interference pattern[16]. This interference pattern was exploited as a visual guide to define the position of the microfluidic lay-out in the PDMS mold. The design of the mold incorporates a frame made of the grating to define the overall geometry of the device. Another grating was further added in the middle of the central chamber of the microfluidic layout in order to make alignment with the CaF_2 window easier. It is worth noting that the grating in the central chamber will not be reproduced in the final device as it consists of a series of short cavities which are not connected to the edge of the central chamber. Therefore, the flowing NOA would not be able to access these cavities.

For the cell injection and medium exchange, every device needs its in-let and out-let manually punched before sealing. This resulted in non-reproducible positioning of the holes. This did not limit the operation of each device since the connection was achieved by gluing a small metallic pin at one of the openings and attaching a plastic reservoir at the other end as a waste collector. Therefore, varying positions of the holes is not of concern except at the cost of a more complex fabrication scheme. To simplify and to standardize the testing and final usage of every device, a custom-made fluidic jig with fixed in-let/out-let locations is introduced (see **Figure 8**), removing the need to attach the pin and reservoir. Hence, in-let/out-let holes with inconsistent positions would present an issue here. By incorporating two 1.5 mm diameter holes in the fabricated acrylic template (protocol step 3), which will become pillars in the PDMS mold, the need to manually punch holes at the ends of the microfluidic channels is removed. Moreover, their position is fixed and the same for every device.

The absence of leaks in the device can be checked with the same procedure as discussed elsewhere[13] *i.e.*, by feeding the device with a solution of fluorescein in de-ionized water.

Critical steps in the protocol
During fabrication of the patterned half of the device, placement of the PDMS mold on top of the CaF_2 window should be carefully carried out. The only structures on the mold which are allowed to contact the window are the 10 µm high protrusions. Whenever there is an unwanted

contact area, the mold placement step should be redone. To illustrate, **Figure 9a** shows careful placement of the mold on the window, while **Figure 9b** shows an example of poor placement, where an unwanted area of the mold is in contact with the window. **Figure 9b** would result in a missing NOA structure on the window. The small quartz plate mentioned in the protocol (step 4.1.3, **Figure 2c**) helps the mold placement as it prevents the unwanted area of the mold from easily coming into contact with the CaF_2 window. Both the quartz plate and PDMS mold are also thin so that they are sufficiently light-weight and transparent to UV[17,18].

Finding the correct UV exposure dose for the half layers is also crucial for the fabrication process. When NOA is exposed with an insufficient dose, the uncured NOA will reflow during the peeling off, causing a loss of definition of the structure and possibly overflowing onto the CaF_2 surface. On the other hand, too high of an exposure dose results in over-curing of the NOA, turning NOA into a non-tacky state. Subsequent bonding of the two halves would suffer because non-tacky NOA on the patterned half layer would not bond to the CaF_2 window on the flat half layer. Ideally, the correct dose should be the shortest exposure dose which allows NOA to replicate the structure reliably while still maintaining its tacky state. Besides determining the correct exposure dose, bonding of the two halves should be done as soon as possible, within 30 min, as the stickiness of NOA gradually decreases with time until it is no longer possible for bonding.

Other points to note are when using the vacuum press system for bonding. The compliance layers (*i.e.*, thin PDMS sheets sandwiching the device) should have relatively uniform thickness for uniform pressure distribution on the device. For this purpose, a custom-made acrylic jig with a defined spacer thickness was used to cast such PDMS sheets. The compliance sheets should also be clean to avoid introducing local pressure, especially on the brittle CaF_2 window.

Advantages of the method compared to existing methods
Our fabrication approach demonstrated the production of a plastic device compatible with FTIR measurements. Because the micro-fabrication technique provides good control over the dimension of the feature, the achievable control over the height of the microfluidic channels is much more precise than what can be obtained with other fabrication approaches (*e.g.*, plastic spacers).

A crucial advantage of this protocol is that it results in a plastic device with UV-Vis-IR transparent view-ports; all the previously demonstrated microfluidic devices for FTIR were produced on top of a large IR transparent substrate, requiring lithography steps for each and every device[10,11,12]. In the present approach, the cost and complexity of the fabrication is reduced since only the production of the Si mold requires lithography.

Finally, using a UV curable resin (NOA73 in this demonstration) as the main plastic body eases the connectivity of the device to an external liquid delivery system, either by gluing or attaching the connections to the plastic body or by using a fluidic jig for more rapid device production or usage.

Future applications of the method
Of the possible improvements and development that can be explored, two are the most immediate and important. First, the broadband optical transparency of the view-port suggests the coupling of FTIR with high-resolution fluorescence microscopy on the same platform. This can be easily pursued by reducing the thickness of one of the CaF_2 windows in order to comply with the requirements of a working distance with high numerical aperture and high magnification objectives. Second, this fabrication scheme can allow for more complex fluidic layouts. Multiple observation chambers and functional elements connecting them such as mixers and sorters, can be implemented as long as their geometry defines the open cavities below the windows.

Disclosures

The authors have nothing to disclose.

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