Standard Operating Procedure

TU/e

SOP details

Title	Polymer TopoChip production by hot embossing
Description	This SOP describes how to imprint TopoChips (or any other topographies) on polymer substrates using an OrmoMould via the hot embossing method.
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SOP number	1.6
Version number	1

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Reviewed			
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Version changes

Version	Name	Date	Changes made
1	Mehmet Tas	12-04-2021	First full version
2	Burcu Gumuscu	24-5-2021	Revised
3			
4			
5			

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1 Purpose

To produce the final TopoChip or other topography to be used in cell experiments using the hot embossing method (SOP 1.6).

2 Principle

The hot embossing method is a method of fabricating microscale scale patterns. It is a simple technique process with low cost, high throughput and resolution. Hot embossing is a process that relies on raising the temperature of a type of polymer to up its glass-transition (T_g) temperature and on pressing a heated master mould into the polymer for triggering a local flow of the material to fill the cavities to be replicated. It creates patterns by mechanical deformation of imprint polymer films. The imprint polymers can be different thermoplastic polymers. The glass-transition temperature (T_g) of a material characterizes the range of temperatures over which this glass transition occurs. It is always lower than the melting temperature, (T_m), of the crystalline state of the material, if one exists. It is the gradual and reversible transition in amorphous materials (or in amorphous regions within semicrystalline materials) from a hard and relatively brittle "glassy" state into a viscous or rubbery state as the temperature is increased. This allows controlled “reshaping” of these type of polymers.

This SOP can be used for the following polymer materials: polystyrene (PS), polyurethane (PU) and poly(styrene-block-isobutylene-block-styrene) (SIBS). The master mould used here is a silane treated Ormomould (SOP 1.5).

3 Before You Start

You must complete the OrmoMould Fabrication for Polymer Imprinting step (SOP 1.5) before you start this protocol. The OrmoMould has to be silane treated (SOP 1.3) to allow easy release of the imprinted polymer surfaces after the imprinting to avoid damaging your OrmoMould. Before using this SOP, please get an introduction to the microfabrication lab for the cleanroom (Feynman lab) and the equipment needed for this process from the super users.

4 Required materials

4.1 Workplace

This SOP should be performed in the microfabrication lab (Gemini- Noord, Feynman lab). Follow the safety protocols instructed by the lab managers and perform the experiment accordingly in allocated locations in the lab.

4.2 Equipment and disposables

- Hot Embossing Tool (requires separate training by a super-user)
- Precision Balance
- Polymer material– to be imprinted
- N_2 (Nitrogen) spray gun
- Heat resistant gloves
- PTFE separator sheets

5 Procedure

Hot embossing procedure for polymer topochip making is a simple, manually operated process with a conventional/single-stage platform. The user controls three parameters:

- Pressure
- Temperature
- Time

These three parameters allow the user to precisely imprint different polymeric surfaces with high resolution, yields and reproducible replications.

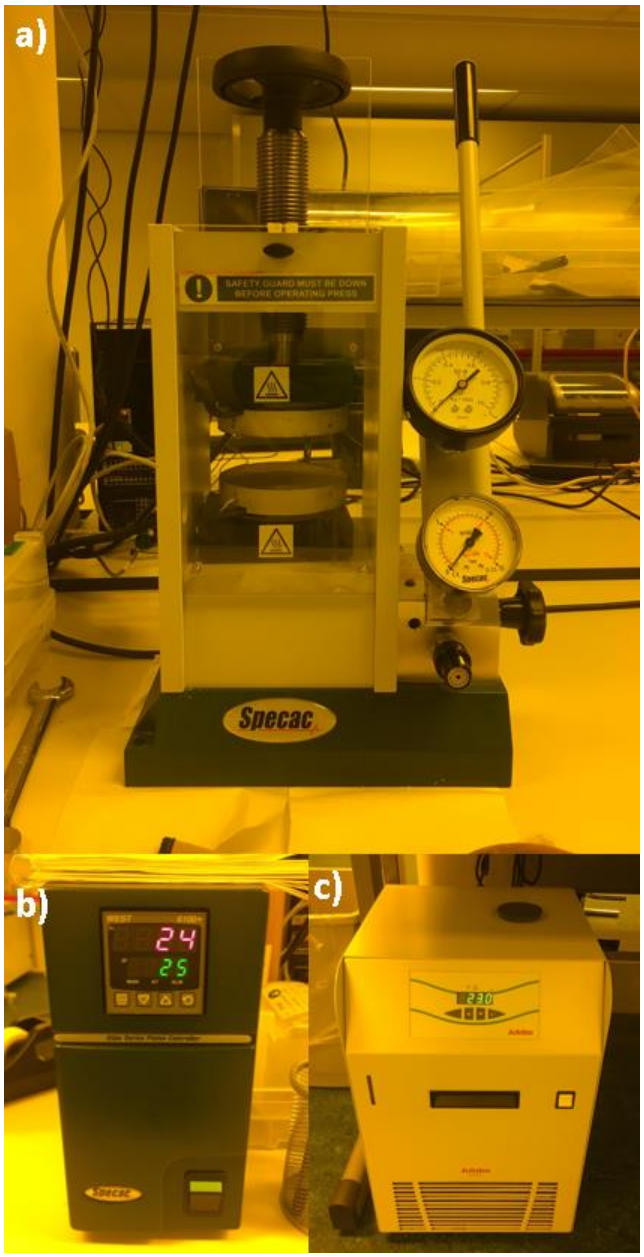

Figure 1. (a) Hot embossing tool, (b) Temperature Controller, (c) Chiller for cooling

Briefly, a master OrmoMould is placed on the lower plate and the work material (generally polymer) is placed on top of the master mould, which is then covered by an upper plate. The polymer is heated above its glass transition temperature (T_g) of polymer using a heater. Next, the pressure is applied to the polymer through the lower plate and the plates are held under pressure for some time. After this, the temperature is lowered below the T_g whilst the applied load is removed. Finally, the mould is separated from the substrate. Through these series of steps, the topographical structures on the OrmoMould are transferred onto the polymer substrate.

The step-by-step guide to using the hot embossing tool is as follows:

5.1 Working procedure

5.1.1 Hot Embossing of PS Sheets:

Step 1: Check whether the chiller, which is connected to the hydraulic press is switched on. It prevents the heating plates from overheating. Set the chiller temperature to 4-5 ^{o}C. The fluid inside the chiller is not just water, so do not top up if you think the fluid level is low. Talk to the technicians.

Step 2: Prepare your sample to be imprinted, by stacking custom-made brass plates which were designed for 4-inch glass wafers (100 mm in diameter) for the embossing tool.

Step 3: If you are using polystyrene (PS) as your polymer substrate, see Figure 2 as a guide to stack up your OrmoMould and PS with PTFE sheets, which are utilized to prevent your PS surfaces and the OrmoMould from sticking to the brass plates. Follow the steps described in Figure 2, polystyrene (PS) sheets should be cut into 100 mm x 100 mm squares.

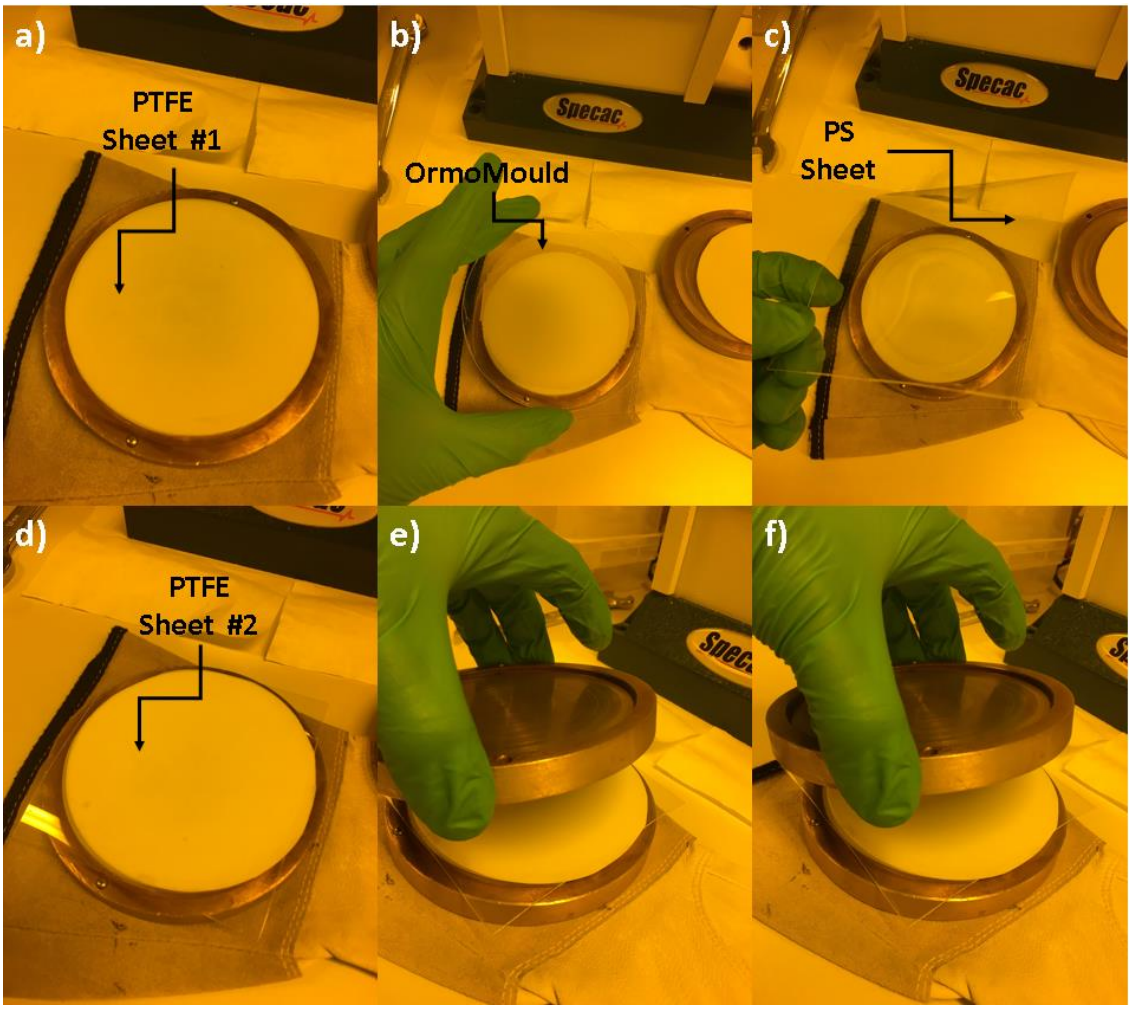

Figure 2. The steps of hot embossing of polystyrene (PS) sheets for hot embossing.

Step 4: Place the brass plates between the heating plates and tighten the top screw of the hydraulic gauge manually. Be careful not to tighten it too hard as that could break your OrmoMould since the plates are not heated at this stage yet. Make sure that the OrmoMould is leveled and there is nothing below the OrmoMould creating a height difference.

Step 5: Now switch on the temperature controller and change the set temperature to the required T_g of the polymer (140 °C for polystyrene, see Table 1) and wait until it reaches and stabilises at the required temperature. The display on the control unit, as shown in Figure 1b shows both the set temperature (green) and the actual temperature (in red).

Step 6: Now close the valve on the right-hand side so that you can operate the hydraulic press to apply the required pressure. When the valve is closed, hydraulic oil is trapped and it can convey the applied load. The load is applied by the large metal bar with the handle. Use the handle to slowly increase the pressure whilst keeping an eye on the pressure gauge. For PS, the required pressure is around 5 tonnes.

Step 7: Keep the system at constant pressure and temperature for 10 minutes to perform the imprinting. The pressure could go down initially due to polymer reflow and also if the hydraulic fluid valve is not tightened enough since some fluid will escape resulting in pressure drop. Maintain the pressure accordingly.

Step 8: After 10 min, lower the temperature to 90 °C, which is below the T_g of PS polymer. Keep the pressure at 5 tonnes.

Step 9: Once the temperature reaches 90 °C (~15 min), open the hydraulic fluid valve slowly to release the pressure. The pressure gauge will go back to zero. Do not loosen the valve too much, that could result in hydraulic fluid leaking, or you could introduce bubbles into the liquid.

Step 10: Unscrew the top plate so you have enough room to take your sample out. Put on the heat resistive gloves and remove the brass plates from the press. You can now release your polymer with the imprints from the Ormomould.

Note:

[1]PS imprints are oxygen plasma-treated before cells are cultured on them. This is explained in SOP 1.2.

[2] If you are going to perform more hot embossing after the first run, be aware that both brass plates and the heaters will still be around 90 °C, therefore always use the heat resistent gloves to load/unload your polymer surfaces.

5.1.2 Hot Embossing of SIBS 2000 (or PU):

Step 1 and **2** described in section 5.1.1 are identical for any type of polymer hot embossing.

Since both SIBS and PU come as small granules, the following procedure will work for hot embossing of both types of polymers. However, since SIBS is more widely used in the BiS group, therefore this section is described with SIBS as an example.

Step 3a: Use the precision balance to weigh 2g of SIBS (or PU) granules using a disposable weighing boat which is stored next to the balance. Figure 3 shows the simple steps to prepare a SIBS Topochip for hot embossing. Similar to the PS procedure, an OrmoMould is placed on top of a PTFE sheet and then SIBS granules are poured and evenly spread on top of the Ormomould, another sheet of PTFE is placed on top of the SIBS polymer to prevent SIBS topochip from sticking to the top brass plate (see below Figure 3).

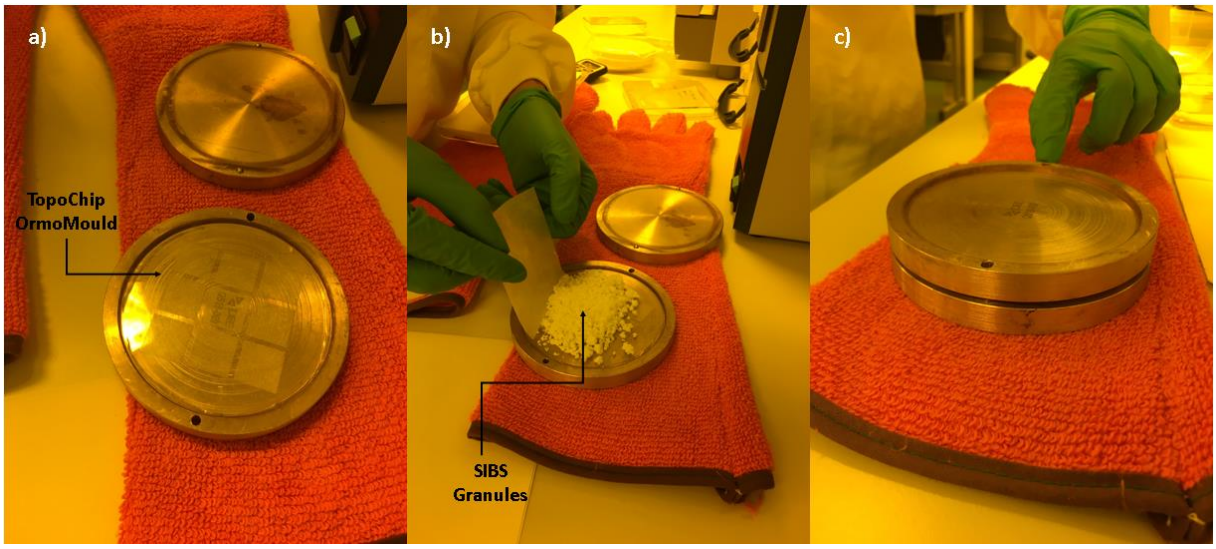

Figure 3. (a) A Topochip OrmoMould, (b) SIBS 2000 being placed on top of the OrmoMould, (c) A SIBS sample ready for hot embossing.

Further steps are very similar to PS imprinting, the only differences are operating parameters, see Table 1 for different polymers. Nevertheless, the steps are as follows:

Step 4a: Place the brass plates between the heating plates and tighten the top screw of the hydraulic gauge manually. Be careful not to tighten it too hard as that could break your ormomould since the plates are not heated at this stage yet.

Step 5a: Now switch on the temperature controller and change the set temperature to the required T_g of the polymer (**160°C** for SIBS 2000 or PU, see Table 1) and wait until it reaches and stabilises at the required temperature. The display on the control unit, as shown in Figure 1b shows both the set temperature (green) and the actual temperature (in red).

Step 6a: Now close the valve on the right-hand side so that you can operate the hydraulic press to apply the required pressure. When the valve is closed, hydraulic oil is trapped and it can convey the applied load. The load is applied by the large metal bar with the handle. Use the handle to slowly increase the pressure whilst keeping an eye on the pressure gauge. For SIBS 2000, the required pressure is around 3 tonnes.

Step 7a: Keep the system at constant pressure and temperature for 10 minutes to perform the imprinting. The pressure could go down initially due to polymer reflow and also if the hydraulic fluid valve is not tightened enough since some fluid will escape resulting in pressure drop. Maintain the pressure accordingly.

Step 8a: After 10 min, lower the temperature to 90 ^{o}C, which is below the T_g of PS polymer. Keep the pressure at 3 tonnes.

Step 9a: Once the temperature reaches 90 °C (~19 min), open the hydraulic fluid valve slowly to release the pressure. The pressure gauge will go back to zero. Do not loosen the valve too much, that could result in hydraulic fluid leaking, or you could introduce bubbles into the liquid.

Step 10a: Unscrew the top plate so you have enough room to take your sample out. Put on the heat resistive gloves and remove the brass plates from the press. You can now release your polymer with the imprints from the Ormomould.

Table 1. Parameters used at BiS for common polymer surfaces for imprinting. Duration refers to the time the sample is kept at the required temperature and pressure.

Material	Amount	Temperature (°C)	Pressure (tonne)	Duration (min.)
PS sheets	100 x 100 mm	140	5	10
SIBS granules	2 g	160	3	10
PU granules	2 g	160	5	10

5.2 Safety

Work in the Feynman lab in Gemini-Noord according to the safety regulations. Follow the instructions given in the lab introduction by the lab managers

6 Waste

When working in the Feynman lab, handle waste according to guidelines which are labelled at the waste disposal based on its categories as given below in Table 2.

Technische Universiteit **Eindhoven** University of Technology

Categorisation of hazardous waste substances

HELP PROTECT SAFETY, HEALTH AND THE ENVIRONMENT

Category I (diluted) inorganic acids with heavy metals pH ≤ 7	*Category II* (diluted) inorganic caustic solutions pH ≥ 7	*Category III* low halogen content organic substances	*Category IV* high halogen content organic substances	N/A *Category V* special waste substances	N/A N/A *Category VI* waste substances containing special risks
laboratory chemicals (original packaging for chemicals still containing residues), contact Waste management & logistics, phone no 4343					Radioactive substances
Diluted inorganic acids (nitric acid, hydrochloric acid, etc.)	Diluted inorganic hydroxides	Liquid organic substances (alcohol, acetone, toluene, etc.)	Liquid halogenated organic substances (i.e. substances containing fluorine, chlorine, bromine or iodine)	Preparations and specimens	Biological waste
Heavy metal cations in solution (e.g. zinc, copper, nickel, lead)	Heavy metal ions in solution (e.g. zinc, copper, nickel, lead)	Solid and pasty low-halogen organic substances (including plastics)	Solid and pasty high-halogen organic substances (including plastics)	Chemically contaminated packaging/equipment	gas cylinders and pressurised containers
Cations and anions of heavy metals	Anions of metalloids	Mineral lubrication and system oils (such as sump oil)	Pesticides (containing halogen-based compounds)	Small, hazardous waste	
Solutions containing fluoride	Solutions containing cyanide	Oil emulsions (such as drill, grinder, roller and cutter oils containing water)	Waste oils contaminated with substances containing halogens	All other chemical waste that cannot be placed in categories I to IV or VI . *See the TU/e Science Park Waste Substances Manual*	For the disposal of the waste substances listed above, contact the SBD, BVF or Waste management & logistics
Waste liquid from plating and pickling baths (acid), fixing salt	Photograph developer and activator	Pesticides (halogen-free)			

For the disposal of the waste substances listed above, contact the **Waste management & logistics**

General tips
Read the 'Waste Disposal' instructions and the TU/e Science Park Waste Substances Manual (AMSO website)
Make sure waste is categorised correctly
Correctly label waste
Watch out for accidental reactions (if you have any doubts, see the list of hazardous combinations in the TU/e Science Park Waste Substances Manual)
Be aware of the fire risks associated with Category III and IV substances
Work neatly and tidily, use personal protective equipment
Use the correct packaging for collecting waste
Always close (empty) packaging
If possible, only open packaging in a fume cupboard
Fill packaging to a maximum of 90% (up to the indicator strip)
For the temporary storage of packaging, follow the 'Storage of hazardous substances in accordance with PGS 15' procedure (Appendix 1 of the TU/e Science Park Waste Substances Manual)
Call the TU/e Waste Centre before collection
If you have any doubts about specific types of waste or questions concerning (the above) procedures, etc., you should always contact your faculty's local health, safety and environment co-ordinator

Important telephone numbers:	
Waste Management & logistics (AMSO)	(040 247) 4343
	(040 247) 3500 (if there is no answer call 3355)
Radiation Protection Service (SBD)	(040 247) 3355 (if there is no answer call 3500)
Biological agents safety officer (BVF)	(040 247) 2239
Emergency telephone number	**(040 247) 2222**

PDO/AMVS/2014

Table 2: Categories of hazardous liquid waste