2.1.2 Wafer Production

The masks used for chip production were constructed using a variation on standard photolithography techniques and with materials provided by the Tay Group and Bio Engineering Laboratory at the Bioscience and Systems Engineering department of ETH Zurich. The flow layer was composed of AZ50-XT positive photoresist (MicroChemicals GmbH, Ulm, Germany) with SU-8 negative photoresist (MicroChemicals GmbH, Ulm, Germany) layered on top of a four inch silicon wafer. All photolithography masks were ordered from Fineline Imaging (Fineline Imaging, Colorado Springs, CO, USA) and the final design was a modified version of a design created by Tino Frank. The following section provides the detailed cleanroom protocol for mold production.

AZ50-XT Photolithography

Clean and Dry Wafer

Four inch diameter silicon wafers serve as an atomic-grade flat surface for PDMS chip molds. Silicon wafers have to be absolutely clean before submitting them to the photolithography process:

- Using a squirt bottle rinse each waver in the following order with (1) acetone, (2) isopropanol, (3) water.
- Spin dry wafers for 60s at 2500rpm.
- If necessary wafers can be then blown off using pressurized N_2.
- Place wafers on a hotplate for 5min at 200ºC.

Hexamethyldisilazane Coating

In order to ensure a strong bond between the silicon wafer and photoresist structures the wafer must first be treated with hexamethyldisilazane (HMDS) (Sigma Aldritch, St. Louis, MO, USA). This was accomplished using a standard HMDS Priming procedure described below.

- Program [HMDS-PRIM-1] exposes the wafer to HMDS vapor for 5min.

Spinning AZ50-XT Photoresist

AZ50-XT positive photoresist is capable of forming the rounded edges of the membrane valves when heated needs to be applied evenly across the wafer. A positive resist is a type of photoactive polymer in which the portion of the photoresist that is exposed to ultra violet light becomes soluble to the photoresist developer. The portion of the photoresist that is unexposed remains insoluble to the photoresist developer. Following the removal of polar substrate it can then be heated to 200°C in order to melt the photoresist, producing nicely rounded structures. Spin-coat wafer with AZ50-XT immediately after HMDS exposure. Thus, the spin coater and AZ50-XT need to be prepared during the wafer cleaning step by lining the interior of the spin coater with Aluminium foil and pouring roughly 30ml of AZ50-XT into a sterile plastic cup.

- Place the HDMS treated waver on the chuck of the spin coater with no delay and pour AZ50-XT photoresist over its surface until roughly 2/3 of its area is covered.
- Spin at 2700rpm for 20s to ensure a 23µm thick layer of photoresist.
- Remove the wafer from the spin coater and wipe any photoresist contamination off the back of the wafer with acetone.

Rehydration

The membrane valves of the PDMS chip require uniform membrane thickness in order to properly function. As a result, it is crucial that all photoresist features of the mold are the same height. In order to ensure that the photoresist is evenly distributed across the surface of the wafer the wafer needs to be left on a level surface while the polymer is still liquid. During this time it needs to be protected from ambient light to prevent photochemical conversion from occurring.

- Place the wafer in a petri dish and cover it in Aluminum foil.
- Let the wafer sit on level surface for 10min.

Soft Bake

The wafer was then baked at 115ºC for three min to solidify the photo resist by removing the majority of the solvent. Evaporating the AZ50-XT solvent solidifies the coating and prevents it from contaminating the mask aligner during the alignment step.

- Preheat a level hotplate to 115°C.
- Place wafer on the hotplate and remove it after 3min.

Rehydration

During the softbake step the bulk of the water concentration of photoresist film drops. However, a certain water content in the resist during exposure is required to allow a reasonably high development rate and a high contrast. This missing water has to diffuse from the air into the resist film. Therefore, a delay time between baking and exposure is necessary to rehydrate the complete photo resist film towards the substrate:

- Place wafer in petri dish.
- Place petri dish on level surface.
- Cover petri dish in aluminium foil
- Leave for 2hr or overnight.

Mask Alignment

Exposing AZ50-XT to ultraviolet radiation causes the resin within the polymer to undergo a photochemical conversion from hydrophobic to highly hydrophilic and allows the unexposed photoresist to be washed away using a polar solvent.

- Clean the wafer by blowing it off with N_2 before alignment step.
- Place on alignment tool wafer chuck.
- Engage vacuum clamp.
- Insert mask in mask holder and engage vacuum clamp.
- Insert mask holder into alignment tool.
- Insert wafer.
- Expose wafer for 200s split exposure (20s on 30s off).
- Remove wafer.
- Remove mask.
- Shut down mask aligner.

Develop

During the development step unexposed AZ50-XT polymer is dissolved in a proprietary aqueous solution known as AZ400k (MicroChemicals GmbH, Ulm, Germany). This alkaline solution removes the polar photoresist that has been exposed to ultraviolet radiation.

- Mix 60ml of water with 20ml of AZ400K in a glass dish.
- Submerge the wafer in solution.
- Agitate for 5min.
- Replace solution with fresh 60ml water mixed with 20ml of AZ400k.
- Agitate for one minute. Rinse with water using a squeeze bottle.
- Spin dry wafers for 60s at 2500rpm.

Reflow

AZ50-XT is required during the wafer production step as it forms the "arched" surfaces of the flow layer valves when it is heated. In order to achieve this "arched" structure the developed AZ50-XT is melted and allowed to cool slowly, producing a uniform parabolic profile. The wafer was covered with a glass petri dish and baked at 200ºC for 13hrs, proceeding from room temperature to 200ºC at 15ºC per hr. This causes the AZ50-XT to melt and form parabolic surfaces.

- Place the wafer on room temperature hotplate and cover with petri dish.
- Set target temperature to 200°C and temperature ramping to 15°C.
- Remove the wafer after 13hr and allow it to cool to room temperature.

SU-8 Photolithography

Clean and Dry Wafer

Four inch diameter silicon wafers serve as an atomic-grade flat surface for PDMS chip molds. Silicon wafers have to be absolutely clean before submitting them to the photolithography process:

- Using squirt bottles rinse each waver in the following order with (1) acetone, (2) isopropanol, (3) water.
- Spin dry wafers for 60s at 2500rpm.
- If necessary wafers can be then blown off using pressurized N_2.
- Place wafers on a hotplate for 5min at 200ºC.

Hexamethyldisilazane Coating

In order to ensure a strong bond between the silicon wafer and SU-8 structures the wafer must first be treated with hexamethyldisilazane (HMDS). This was accomplished using a standard HMDS Priming procedure described below.

- Program [HMDS-PRIM-1] exposes the wafer to HMDS vapor for 5min.

Spinning Negative Resist

SU-8 (MicroChemicals GmbH, Ulm, Germany) is a high contrast, epoxy based photoresist designed for micromachining and other microelectronic applications, where a thick, chemically and thermally stable coating is desired. When it is exposed to ultraviolet light the thermally cross-

linked portions of the film are rendered insoluble to liquid developers. As developed SU-8 is thermally stable to very high temperatures it is an ideal tool for producing high aspect ratio structures on silicon master molds.

- Place the HDMS treated waver on the chuck of the spin coater with no delay and pour SU-8 photoresist over its surface until roughly 2/3 of its area is covered.
- Spin at 3100rpm for 20s to ensure a 23μm thick layer of photoresist.
- Remove the wafer from the spin coater and wipe any photoresist contamination off the back of the wafer with acetone.

Soft Bake

The SU-8 layer must be heated a first time after it has been applied to the surface of the wafer. In order to partially solidify the photoresist before exposure the wafer needs to be baked on a hotplate for two min at 65ºC followed by ten min at 95ºC. Evaporating the SU-8 solvent solidifies the coating and prevents it from contaminating the mask aligner during the alignment step.

- Preheat a level hotplate to 65°C and another to 95°C.
- Place wafer on 65°C hotplate for 3min.
- Transfer it to 95°C hotplate for 10min.
- Remove the wafer and allow it to cool to room temperature.

Mask Aligner

Exposing SU-8 to ultraviolet radiation causes the resin within the polymer to undergo a photochemical conversion from hydrophobic to highly hydrophilic and allows the exposed photoresist to be washed away using a nonpolar solvent. To obtain vertical sidewalls in the SU-8 resist, use an I-line filter to eliminate UV radiation below 350nm.

- Clean wafer by blowing it off with N_2 before alignment step.
- Place on alignment tool wafer chuck.
- Engage vacuum clamp.
- Insert mask in mask holder and engage vacuum clamp.
- Insert mask holder into alignment tool.
- Insert wafer.
- Expose wafer using an I-line filter for 13s.
- Remove wafer.
- Remove mask.
- Shut down mask aligner.

Post Exposure Bake

The SU-8 layer must be heated again after the exposure phase in order to accelerate SU-8 polymerization. The cross linking process may engender significant residual stress in the SU-8 layer and this residual stress is a major source of potential cracks Therefore it is preferable to avoid a rapid cooling of the SU-8 after the PEB.

- Place wafers on a hotplate.
- Heat to 95°C by ramping 110°C/hr for 45min.

- Maintain wafer at 95°C for 5min.
- Turn off hotplate and allow to cool to room temperature for at least 40min.

Develop

During the development step unexposed SU-8 polymer is dissolved in a proprietary organic solution known as mrDEV 600 (MicroChemicals GmbH, Ulm, Germany). This acetone solution removes the nonpolar photoresist that has been exposed to ultraviolet radiation.

- Pour 40ml of mrDEV 600 in a glass dish.
- Submerge wafer in solution.
- Agitate for 3min.
- Using squirt bottles rinse each wafer in the following order with (1) acetone, (2) isopropanol, (3) water.
- Spin dry wafers for 60s at 2500rpm.
- If necessary wafers can be then blown off using pressurized N_2.
- Place wafers on a hotplate for 5min at 200ºC.

Hard Bake

After development, the SU-8 master may be heated a third time to further cross link the SU-8 mold and make sure the SU-8 is not damaged during the soft lithographic steps with PDMS. The temperatures involved during the SU-8 hard baking process are usually higher than for the soft baking and PEB processes. Longer baking times, however, may be required depending on the thickness of the SU-8 layer.

- Preheat hotplate to 65°C.
- Place wafer on hotplate.
- Cover it with glass petri dish.
- Allow to heat for 2min.
- Ramp to 160°C at 120°C/hr for 2hr.
- Turn off hotplate.
- Retrieve Wafer.

2.2 Assembly of a Multilayer Microfluidic Chip

2.2.1 Soft Lithography

Wafer Cleaning

Structures embossed in PDMS (Techsil, UK) can occur on a nanometer scale. Therefore any particles or debris present on the master molds surface will be transferred into the final PDMS layer. As a result, wafers need to be thoroughly cleaned before any PDMS is applied to them.

- Using adhesive scotch tape, gently remove any dust or debris from the wafer by applying it evenly to the chips surface and peeling it away.
- Blow off any remaining contamination using a pressurized air gun.

Nonstick Functionalize

Silanizing the PDMS master is important as many typical silicon surfaces result in PDMS adhering to the master, making peeling and preserving the PDMS more difficult. A drop of trimethylchlorosilane (TMCS) (Sigma Aldritch, St. Louis, MO, USA) is placed in a vial and placed in a wafer carrier with the PDMS master. TMCS then evaporates and forms a monolayer on the surface of the silicon master that prevents the PDMS contacting and bonding to the master.

- Place the wafers inside a closed wafer carrier box with a small beaker containing a few drops of TMCS.
- Incubate at room temperature for 15-60min.
- Dispose of TMCS by opening wafer carrier box and letting the TMCS evaporate under a fume hood.

Clean and Dry Slides

The glass slides used in wafer production need to be thoroughly cleaned in order to ensure a complete chemical bond to the PDMS chip. While the design can remain functional with some inconsistent bonding most irregularities such as trapped bubbles or laminar separation will result in non-functional chips. In order to prevent these defects glass slides (Paul Marlenfeld GmbH, Germany) are rinsed with DI water from Genpure before being rubbed with Micro-90 (Sigma Aldritch, St. Louis, MO, USA) cleaner sonicated to ensure complete cleanliness, and vacuum dried.

- Prerinse with Deionized water from Genpure, water purification machine.
- Wash with Micro-90 cleaner. Place small amount of Micro-90 cleaner on gloves, and rub onto glass.
- Rinse with Deionized water.
- Place in slide holder.
- Sonicate for 5min at 35°C.
- Rinse after sonication.
- Blow off water.
- Dry in oven at 80°C for at least 45min.

Flow Mould Polydimethylsiloxane

The flow layer of the chip provides structure and support. As a result it needs to be both thick and sturdy. The standard PDMS mix is 10:1 polymer to curing agent by mass. This produces an elastic yet durable polymer when cured in an oven.

- Make an aluminum foil container by using the black cup moulder.
- Place aluminum foil container in a large glass petri dish.
- Mix 60g of PDMS with six g curing agent in a plastic cup for each flow layer wafer.
- Place aluminum cover on cup so PDMS does not spill out into mixer.
- Weigh total mass of PDMS, cup, cover, and plastic adaptor and set counterweight in mixer accordingly.
- Mix PDMS in Thinky mixer (Thinky USA Inc, Laguna Hills, CA USA) for three min at 2000rpm mix & 3min at 2200rpm defoam. [Program 1]
- Pour the mixed PDMS and curing agent over wafer.
- Place in vacuum chamber.
- Degas for 90min or until no more bubbles are present.
- Place in oven on level surface, and cure for 45min at 80°C. Take out after 45min.

Control Mould Polydimethylsiloxane

The control layer forms the membrane between the low pressure flow channels and high pressure control channels and as a result needs to be highly uniform across its surface.

- Make an aluminum foil container by using black cup moulder.
- Mix 10g of PDMS [Momentive Performance Materials, Germany] with 1g of curing agent in a cup.
- Place aluminium cover on cup so PDMS does not spill out into mixer.
- Weigh total mass of PDMS, cup, cover, and plastic adaptor and set counterweight in mixer accordingly.
- Mix PDMS in Thinky mixer for 3min at 2000rpm mix & 3min at 2200rpm defoam. [Program 1]
- Place the mixed PDMS in a vacuum chamber for 30min for degassing.
- Place wafer on spin coater vacuum chuck and turn on vacuum.
- Rotate wafer to ensure it is well centred. If it's is not centred turn off vacuum and move to centre of vacuum chuck.
- Pour PDMS onto wafer covering at least 3/4 of substrate the photoresist features.
- Pour with laminar flow to not introduce bubbles as close as possible above the wafer.
- Spin at 2300rpm for 60s for 25µm tall PDMS layer.
- Turn off vacuum and place wafer in aluminium cup.
- Let sit on a level surface for at least 15min.
- Place on a level surface in the oven and cure for at least 45min at 80°C.

Cut-out and Punch Flow Layer

Once the flow layer has been fully polymerized in the incubator it needs to be cut into the desired shape and punctured to allow the insertion of pressurized metal tubes.

- Take the flow layer from the incubator and allow it to cool until it can be handled safely.
- Carefully peel the aluminum cup from the PDMS disk and discard it.

- Slowly cut away any of the PDMS covering the back side of the silicon wafer using a razor or a scalpel and discard it.
- Gently peel the flow layer PDMS disk off of the silicon wafer.
- Mark the edges of the chip using an office pen.
- Carefully cut each edge using the guillotine (Figure 1).
- Align and punch each of the holes in the flow using the custom perforation tool (Figure 1).
- Fully raise the lever of the hole punch after punching to ensure that the PDMS plug is fully ejected from the chip.
- Thoroughly clean the flow layer with adhesive tape to remove any remaining PDMS particles.

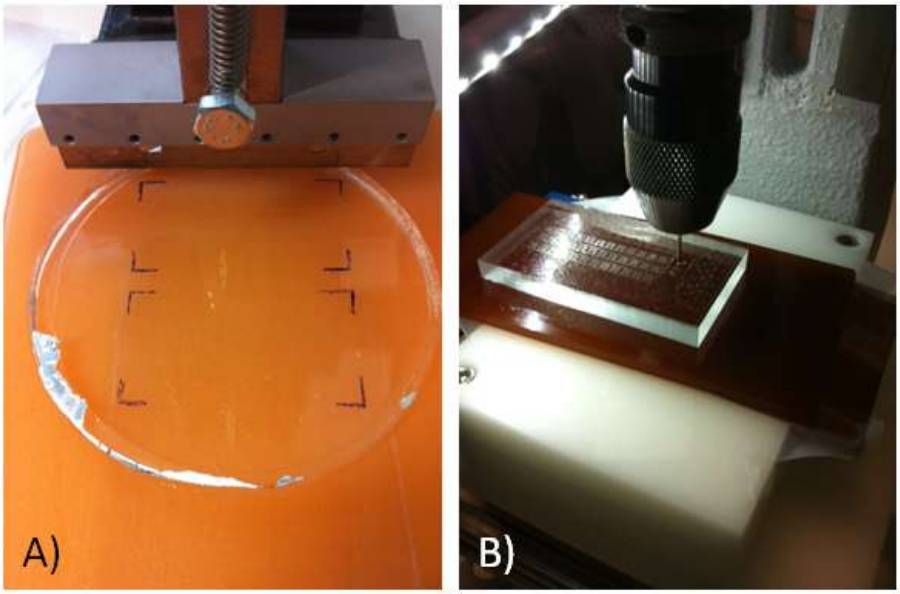

Figure 1 Cut-out and Hole Punching the SCCC Flow Layer. A) Mark the edges of the chip outline with a pen to improve cut-out reliability. B) Align and punch pin insert ports visually.

Plasma Treat Flow and Control Layers

The plasma bonding step enables to finish your microfluidic chip fabrication. To permanently bond the PDMS components to each other, researchers use a plasma cleaner to change the surface properties of PDMS. The mechanism of bonding involves the oxidation of the surface layer, which increases the concentration of hydroxyl groups, leading to the formation of strong intermolecular bonds upon contact. Bonding the flow layer to the control layer requires both components to be exceptionally clean at all times and requires precision and speed to avoid incomplete chemical bonding between the two layers.

- Wash the nitrile gloves worn while handling PDMS using isopropanol and water to remove any residual detergent powder or other potential contaminants.
- Clean the metal plate used to hold the flow layer PDMS, the alignment tool, and the area around the alignment tool using isopropanol to remove PDMS and dust particles.
- Place the flow layer on the metal plate with the microscopic features facing up.
- Clean flow layer blocks with tape.
- Clean control wafer with air gun.
- Insert metal plate into plasma generator.

- Insert PDMS coated control layer wafer into the plasma generator.
- Vacuum seal the chamber of the plasma generator and fill it with oxygen gas for 2min.
- Run the plasma generator at 20W for 15s.

Bond and Align Control and Flow Layers

The hydroxyl groups exposed on the surface of the PDMS by the oxygen plasma are inherently unstable and therefore the two layers should be brought into contact as quickly as possible. Contact between the two layers results in immediate covalent bonding between the two surfaces.

- Immediately after plasma treatment place the control layer wafer on the stage of the alignment tool.
- Place the thick flow layer blocks on the suspension mounting block tool.
- Orient the 2 layers such that the multiplexer and waste components of both layers are parallel to each other.
- Position the flow layer blocks directly above the control layer using the XY adjustment knobs.
- Lower the flow layer blocks until they are roughly 300μm apart.
- Adjust the alignment using the peristaltic pump component as a starting reference point.
- Adjust the yaw using the multiplexer inputs as a reference.
- Check all valves on the chip.
- Adjust XY and yaw.
- Lower the flow layer into direct contact with the control layer while periodically making adjustments to the alignment.
- Inspect each chip for bubbles trapped between the 2 layers.
- Press all bubbles out of the chip by placing the wafer on a flat surface and pushing down.
- Incubate wafers at 80ºC for a minimum of 2hr.

Cut out and Punch Chip

After the two layers are sufficiently chemically bonded together the control layer inlets are punched and the whole chip is covalently bonded to a glass slide.

- Use a scalpel to cut the thin control layer PDMS around the edge of the chip.
- Gently peel each chip off of the control layer wafer.
- Align and punch the control layer ports for each chip.
- Thoroughly clean each chip using adhesive tape to remove all PDMS particles.
- Place the glass slides inside the plasma generator.
- Vacuum seal and pressurize the plasma generator with oxygen for 2min.
- Expose glass slides to plasma at 20W for 5min.
- Store glass slides in a petri dish until the PDMS chips are ready.
- Expose PDMS chips to plasma at 20W for 15s.
- Lower PDMS chips onto the glass slides, features down, until one edge makes contact.
- Let the chip fall onto the glass slide.
- Place all chips on a flat surface and apply pressure evenly to each until no bubbles remain.
- Incubate completed chips at 80ºC overnight.

Octet Puncher

Aligning and punching chip inputs is very time consuming. To facilitate this, an octet puncher (Figure 2), was developed by M. Etzrodt and T. Frank in collaboration with the D-BSSE mechanical workshop. This instrument dramatically increases the throughput of chips that can be produced and standardizes the distances between holes in the chip facilitating subsequent chip setup on the microscope and microfluidic control rigs.

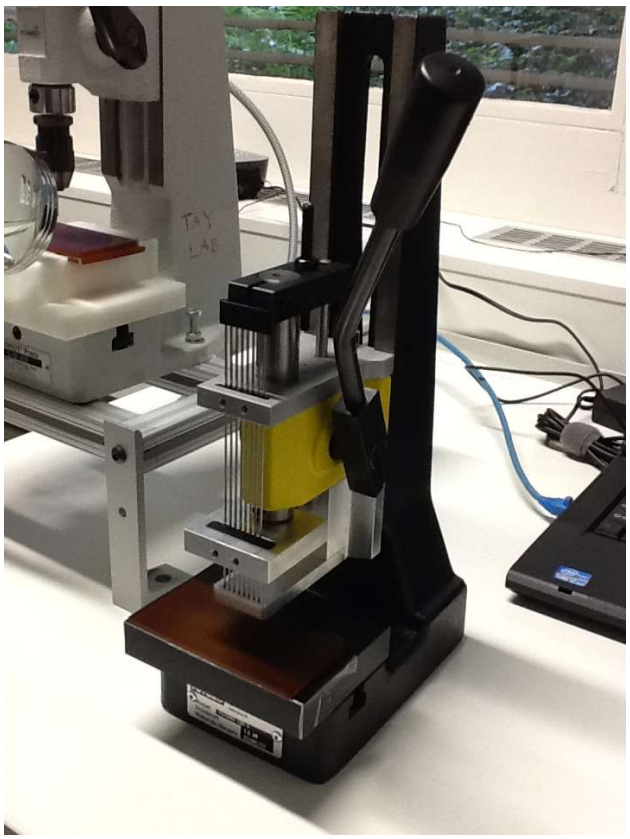

Figure 3 Octet Hole Puncher. The octet puncher is comprised of 8 parallel hole punches similar to the singlet hole puncher.

2.3 Microfluidic Chip Control

The microfluidic control manifolds used for all chip experiments are based on a custom design of the Systems Biology and Bioengineering group of the ETHZ/D-BSSE. We further optimized the design for performance by ensuring that the supply chain was sourced from as few companies as possible and mainly European suppliers rather than from overseas, thus increasing reliability of components, reducing costs and increasing the speed of delivery. Standardization using exclusively FESTO pneumatic components makes the rig extremely modular and changes can be performed quickly using FESTO pneumatic quick plug-in connectors without the need for sophisticated tools. A rig controlling 48 individual valves on a microfluidic chip amounts to a cost of roughly 5.000 CHF.

The basic design for each control manifold is based on a Tay Group design although each manifold was modified according to the available parts and intended location (Figure 4). The pneumatic solenoid valves used to control the PDMS membrane valves of the chips control layer along with all other components can be found in an itemized list in **Error! Reference source not found.**. Six sets of solenoid blocks are attached to the manifold, each containing eight independently controlled solenoid valves, and all connected to a central pressure regulator. These solenoids are controlled using a USB control box built in house specifically for 12V switching.

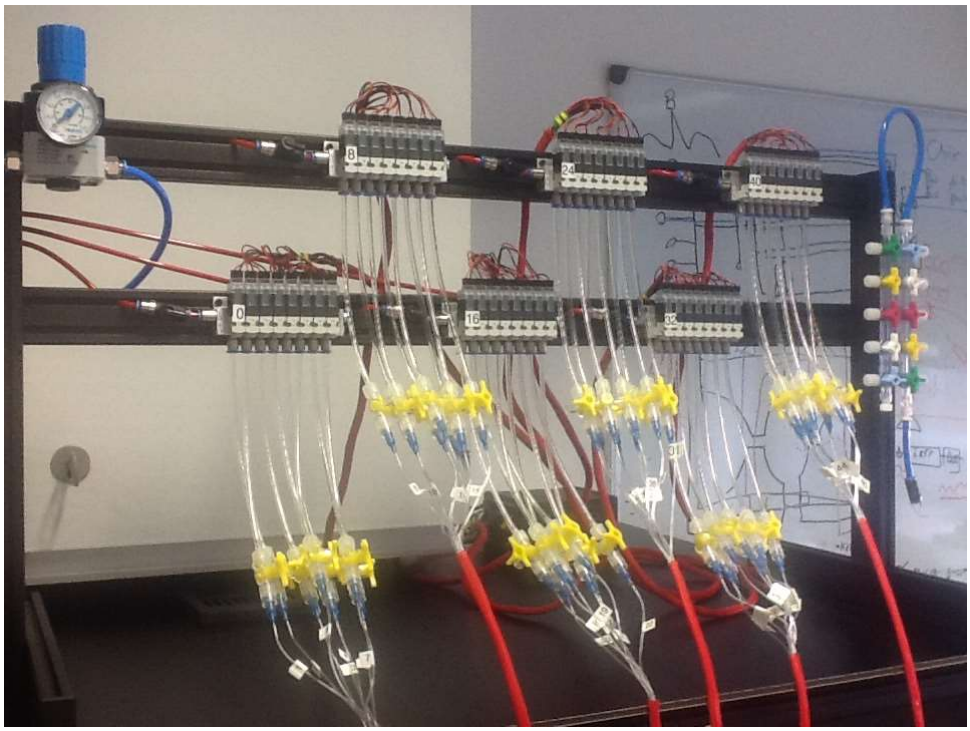

Figure 4 A Microfluidic Control Manifold. The control manifold features six sets of eight solenoid pressure valves for the control layer elements and ten ball valves for the flow layer elements.

2.3.1 Control software design

Using standard Matlab routines provided by the Tay Group, the graphic user interface was programmed according to the needs of the CSD group:

- Intuitive chip manipulation interface.
- Coding free experimental automation.
- Experimental recording and duplication.
- Simplified valve groups.

The major philosophy of the SCCC graphic user interface (GUI) is modularity. As such it is divided into two modules, manual control and process automation, which are further divided into their relevant modules.

Manual Chip Control

The manual control module of the SCCC GUI is divided into six individual modules:

- Direct Valve Module – Allows advanced users to control individual valves in an unassisted manner.
- Input Module – Controls flow from individual liquid inputs into the chip via pre-established multiplexer valve combinations.
- Channel Flushing Module – Facilitates chip cleaning during chip operation.
- Chamber Manipulation Module – Allows users to select specific chambers and chamber components for manipulation.
- Output Module – Selects between chip exit points: emergency vent, waste, and harvest ports.
- Pump Module – Controls the peristaltic pump as well as which valves are used in different pumping mechanism procedures.

Dividing the GUI into these groups reduces the number of objects new users are required to interact with and therefore improves their capacity to use the SCCC with minimal training.

Experiment Automation

Similar to the manual chip control group, the experiment automation panel is divided into tabs:

- Treatments Tab – Users define a one-time chamber treatment event.
- Experiments Tab – Users construct experimental protocols using previously defined treatments.
- Chamber Selection Tab – Users apply specific experimental protocols to certain chambers.

These experimental settings can be saved as a retrievable document and loaded into the GUI for future use (Figure 5).

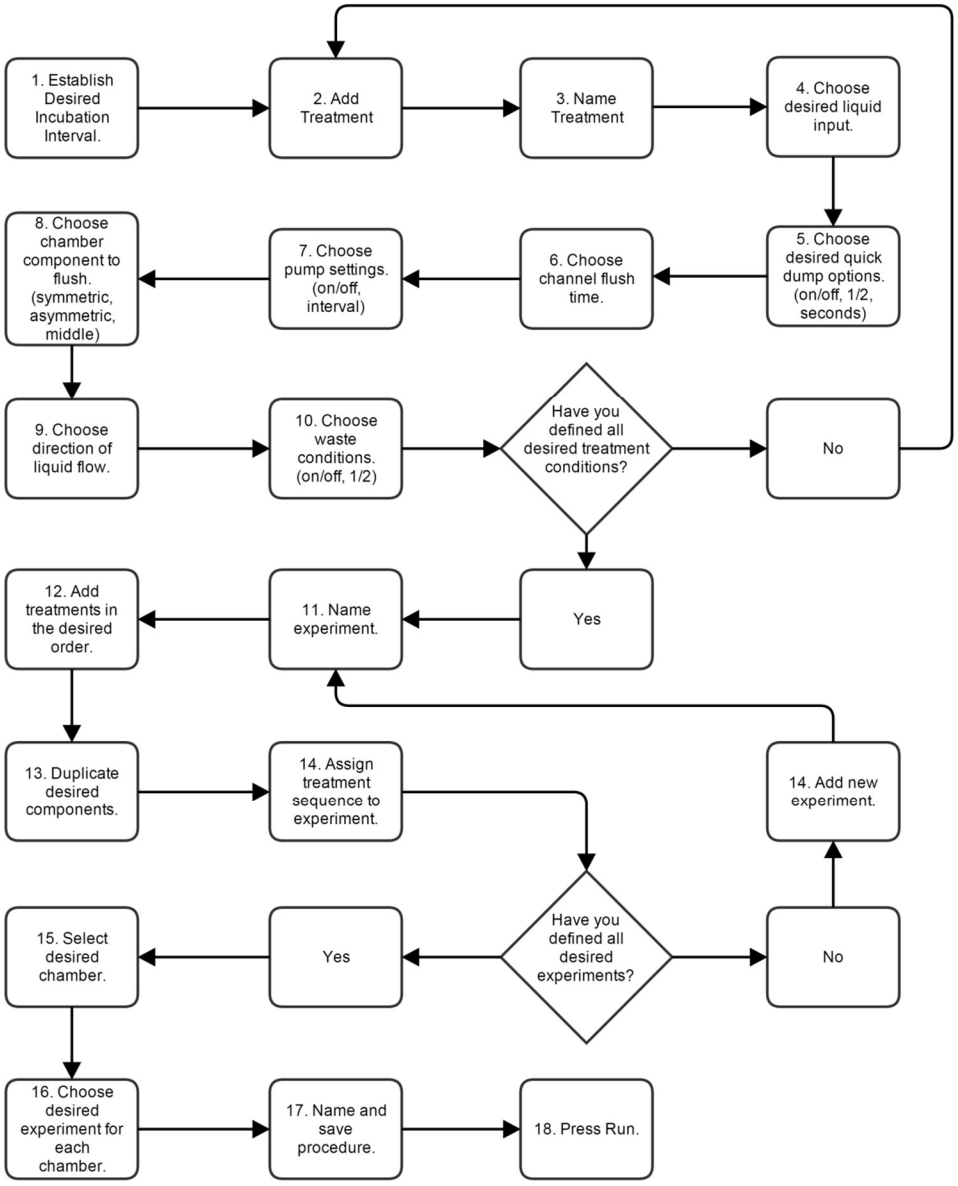

Figure 5 SCCC GUI Experimental Automation Flow-Chart.

2.4 Chip Setup

2.4.1 Microscope Setup

The control of environmental conditions during cell culture requires special attention as stem cells have proven to be highly susceptible to variations in culture conditions which can have drastic results on cell metabolism. Currently the need for accurate and robust control of temperature, humidity, and atmospheric compositions is only partially met by available commercial products. Fluctuations in temperature, humidity, and dissolved gases can alter media composition unpredictably through evaporation and degradation of temperature sensitive media components thereby introducing an unnecessary element of variability between experiments. Combatting this variability during this project required the introduction of novel humidity and temperature control mechanisms.

PDMS is susceptible to gas exchange which is one of the reasons that it is ideal for cell culture. However, gas exchange has the consequence of allowing evaporation to occur very rapidly if humidity in and around the chip is not tightly maintained. Media evaporation can significantly alter the osmotic balance of the media which in turn causes cells to experience potentially toxic osmotic pressure. In order to control the humidity surrounding the chip Sodium Polyacrylate (Sigma Aldritch, St. Louis, MO, USA) was employed as a humidifying gel. Sodium Polyacrylate particles can absorb up to 500 times their mass in water and are ideal for maintaining a large water reservoir in a microscope enclosure without leaking water into sensitive microscope components.

Stem cells are also susceptible to temperature changes. Therefore, in addition to the insulation provided by the microscope enclosure, chips were operated within a much smaller enclosure with an embedded Sensirion USB temperature and humidity logger (Sensirion AG, Switzerland). This configuration added extra insulation and the ability to identify problems in temperature or humidity maintenance over the course of a cell culture experiment.

2.4.2 Control Layer Setup

In order to provide the pressure required to open and close PDMS membrane valves as well as push liquids into the flow layer of the chip, the control manifold needs to be connected to the chip via a network of polyethylene tubes. Each is solenoid is connected to the chip via a four piece water reservoir and injection system composed of 10cm of 2.5mm polyethylene tubing connected to a three way ball valve for easy refilling, 150cm of 0.5mm polyethylene tubing and finally a metal tube bent at a right angle. This water reservoir system allows the solenoids to transmit pressure to the PDMS membrane valves without injecting air directly into the chip. The solenoids and tubing connected to the control layer of the chip were operated between 25psi and 30psi depending on the membrane thickness. The liquids which were injected into the flow layer of the chip were pressurized to 7.5psi using a set of three directional ball valves connected to two meters of 0.5mm polyethylene tubing.

- Before any other components could be attached the user needed to fill each control tube with water and insert each pin of the control tube into the holes punched according to the numbering scheme shown on the map Figure 6.
- After all control tubes were inserted into the chip the entire system is pressurized to 5psi and left alone for 30min while water fills the channels of the flow layer by forcing air through the porous structure of the PDMS.
- After air has been removed from the control lines the pressure on the control layer is increased to 20psi.
- Slowly increase control layer pressure while visually inspecting each membrane valve.
- Continue increasing the control layer pressure until all valves are closed.
- The chip is now ready to be treated with a protein coating or otherwise treated for the necessary experiments.

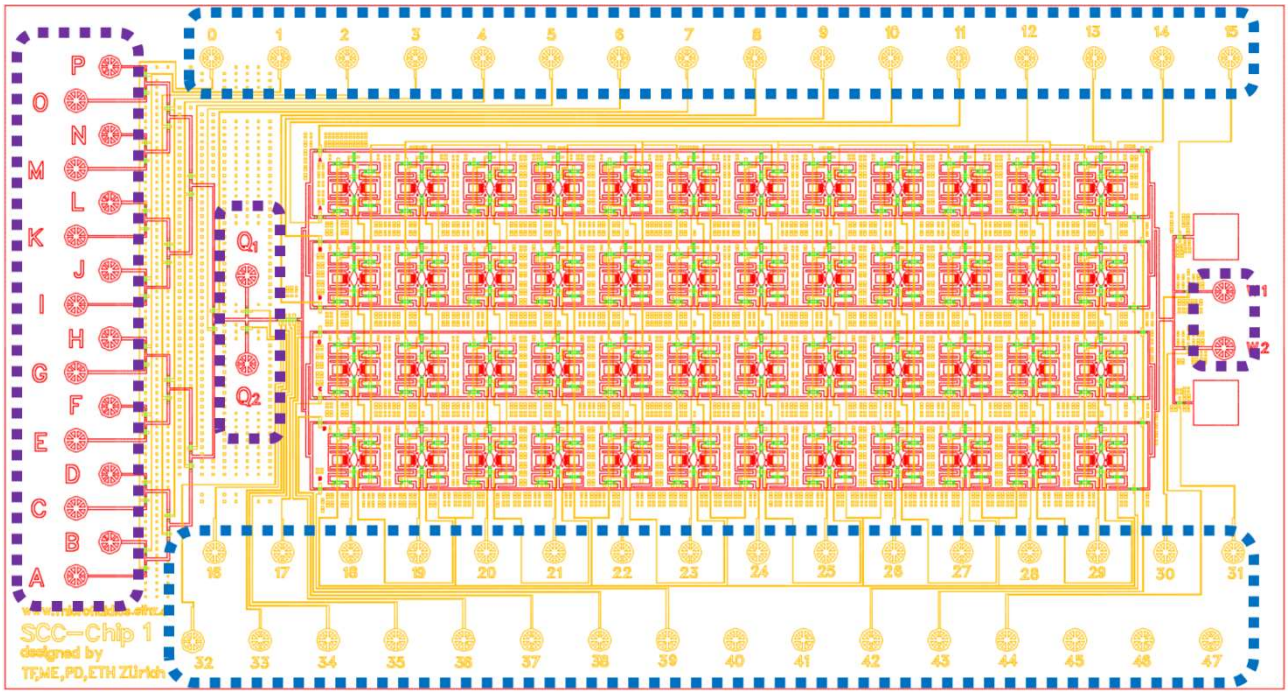

Figure 6 SCCC Schematic and Pin Insertion Map. Insert control layer pins into their corresponding ports punched during the flow layer cut-out and punch step.

2.4.3 Flow Layer Setup

Setting up the flow layer chip components requires pressurized microcentrifuge tubes of the desired liquids to be connected to the multiplexer inputs. Furthermore, an empty reservoir which is open to the atmosphere must be connected to the waste outlet, allowing liquid to enter it from the chip without resistance. These components can be seen highlighted in purple in Figure 6.

- 20cm of 0.5mm Teflon tubing is connected to each microcentrifuge tube by piercing it's rubber stopper lid with a metal pin.

- The interior side of this pin is then connected to a 3cm long segment of Teflon tubing as depicted in.
- Metal pins are then used to connect the microcentrifuge tube to the multiplexer via the 20cm segment of Teflon tubing.
- The 2m polyethylene tubing from the flow layer ball valves is then connected to each microcentrifuge in order to pressurize each one.
- In order to connect the waste collection microcentrifuge tube to the chip, pierce its rubber stopper lid with two metal pins.
- Connect one of these to the chip via a 20cm segment of polyethylene tubing and another metal pin.

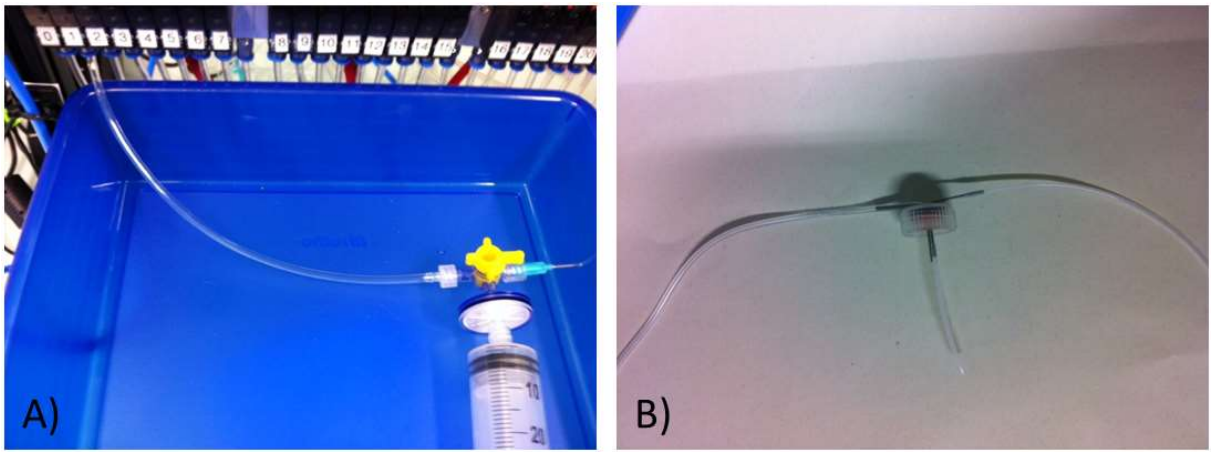

Figure 7: Setting up Manifold Control Layer and Flow Layer Components. A) The water reservoir should be willed to within two cm of the solenoid valve. B) Pierce the rubber stopper lid with two pins before connecting a short segment of tubing to the inside and connecting the microcentrifuge tube to the chip.