ACCESSIBLE AND LOW COST DESIGN AND MANUFACTURING OF “CHAMLIDE” OBSERVATION CHAMBERS FOR MOLECULAR IMAGING

SMS Initiative - Gaus Lab
Lowy Cancer Research Centre

Benjamin Goodnow
Supervisor: Rusty Nicovich
February 2016

ABSTRACT

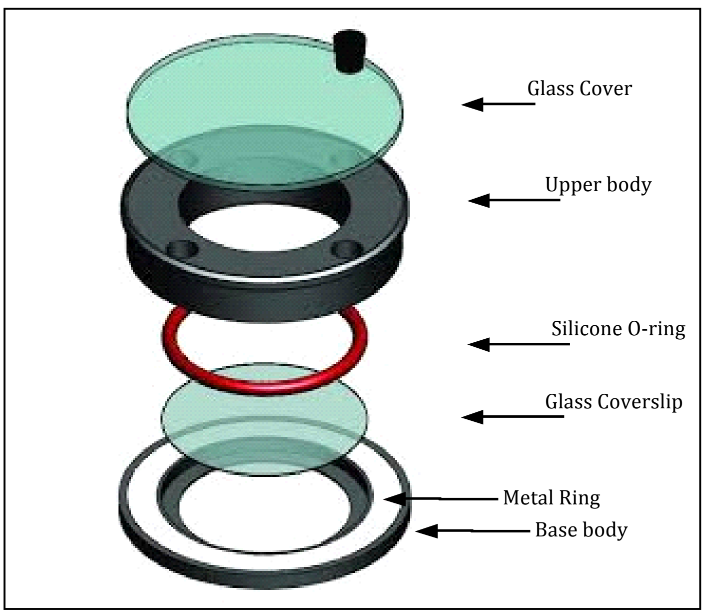
The aim of this project was to replicate the Chamlide Magnetic Chamber by Live Cell Instrument using alternate, cheaper and accessible manufacturing processes and materials. The purpose of the chamber is to allow efficient placement of observation medium over a sample and coverslip to allow for molecular imaging in a microscope. The processes used to create this chamber would include CAD design, 3D printing each half of the chamber, adhering of rare earth magnets into the chamber's designed cuts and finally laser printing silicone gaskets for waterproofing. Prototypes were made and tested, with faults in waterproofing, tolerance, magnetic force and warping to be addressed. A final product resulted, along with custom variations in chamber shapes and sizes depending on need. The final microscopy image results proved successful and comparable to the original Chamlides, whilst the estimated savings for producing ten chambers was over $5000AUD.

TABLE OF CONTENTS

1. INTRODUCTION

Currently the UNSW Single Molecule Science initiative, aimed at developing and applying molecular imaging technologies, use a product called Chamlide (Live Cell Instrument Corp [LCI] 2015) for containing a sample within an observation medium. The system is comprised of two halves (Figure 1): an aluminium base with a steel ring inset and through hole for light to pass, and in the centre a glass coverslip (of varying shapes) is placed. The top half is a larger plastic body with magnets inset, which connects to this base through magnetic force, allowing for a liquid observation medium to be added on top the coverslip. To prevent water leakage, a silicone gasket is used between the two bodies. Finally, a glass cover can be placed on the top of the Chamlide. The overall system improves on other chamber designs which use screws or complex assemblies, reducing leakage and increasing efficiency.

However, the primary drawback of this design is cost. A single unit is priced at approximately $380USD (Quorum Technologies 2014), due primarily to its unique patented design occupying a niche uncontested. With many projects occurring in the SMS labs, and different shape and size coverslips used, many units are required. Thus a lower cost, accessible solution is required to ensure the lab has immediate access to a full range of chambers, increasing efficiency and practicality during experimentation. The product does not have, nor require, high cost materials. The material costs would be less than $20USD. What perhaps may increase expenses is precision. As such the aim of this project is to design a similar, precise chamber for accurate molecular imaging that could be made with readily accessed materials using innovative, low price manufacturing processes.



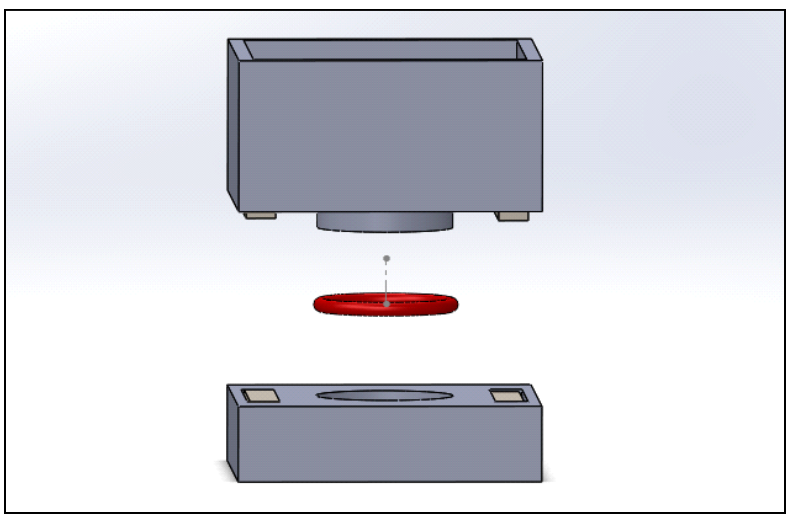
(Figure 1. Exploded view of LCI's Chamlide CMB commercially available. Quorum Technologies 2014)

2. CONCEPTUAL DESIGN AND FIRST PROTOTYPE

The conceptual design of the chamber followed the fundamentals of the Chamlide, with the key constraints and solutions transferable, in particular the use of coverslips, waterproofing for the observation medium, and magnetic connection of the two halves. However, two key changes were made: no glass cover, deemed a non-crucial aspect increasing material costs; and no inset steel ring, instead utilising magnets in the base just as the top, again reducing the bill of materials and manufacturing processes required.

The design of the chamber's plastic body underwent multiple iterations in order to achieve a refined design, with problems in precision, tolerance and material choice to be overcome. The flexibility of CAD software and 3D printed prototypes allowed for an efficient iterative process. All CAD design was completed in Solidworks 2014 (Dassault Systemes 2014) and the manufacturing of the body used a mid-range UP Plus 2 Desktop 3D printer (UP3D 2015). This printer utilised 1.75mm ABS filament, a high-temperature-resistant and durable plastic, suitable for the range substances the chamber may require, and suitable for use in an autoclave. A kilogram of ABS can be purchased for $50AUD (with one chamber requiring as little as 15g). It is to be noted that PLA (a common 3D Printing material) is less temperature-resistant and known to deform at higher temperatures, hence the preference of ABS (B Tymrak et al. 2014)

The first prototype printed (Figure 2) was designed primarily around an 18mm round coverslip and medium sized rectangular block (2.2x0.5x0.5cm) ceramic magnets, chosen due to low price and high accessibility ($5AUD from hardware outlets). Due to the shape and size of the magnets a cube shape was required, 40x40x30mm.



(Figure 2. CAD design of prototype Chamlide [note the top of the upper body had a cut made to allow for the possibility of a glass cover])

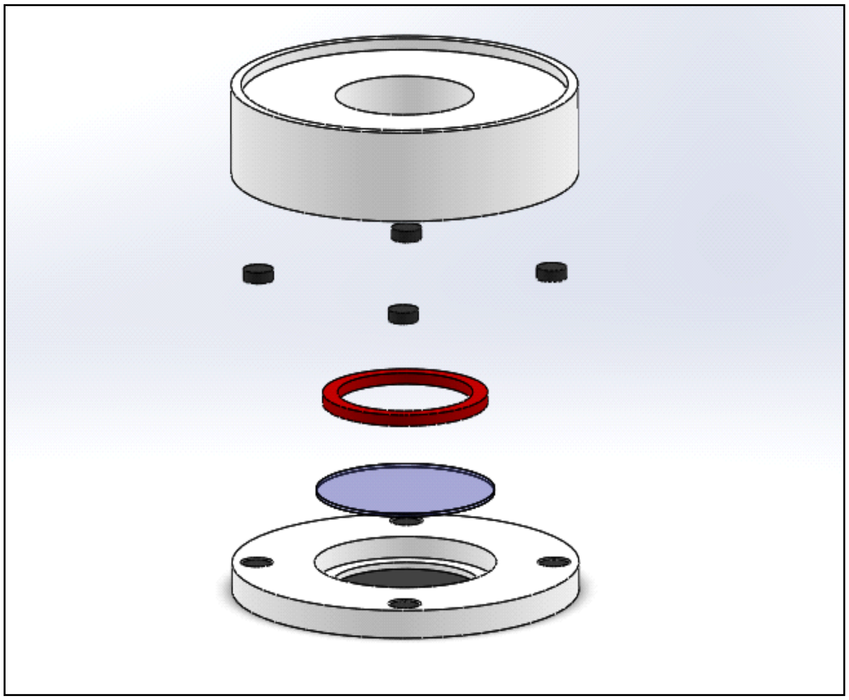
For testing, an 18mm glass coverslip and commercial, common silicone o-ring (via hardware outlet) were placed using forceps in the base half successfully, with enough clearance to be carefully lowered, but tight enough such that neither component shifted. The magnets could also be inset easily, with flush edges to allow uniform attraction, and kept in place with no glue required.

However, when the upper and base parts were connected, it was found the size of the magnets provided a strong attractive force, far stronger than the steel-to-magnet combination of the LCI system. Consequently, the coverslip was shattered. The diagnosis of this fault led to multiple possibilities. Firstly, magnets were only placed on two of the four sides, leading to higher pressure on only these sides, resulting in a bending moment on the coverslip. Secondly, and likely the largest contributor, the magnet size was simply too large for a chamber of this size, creating an overwhelmingly stressful load on the coverslip. Thirdly, the durometer (hardness) of the o-ring was high, at 70, meaning more force was required to compress it and hence more force transferred to the coverslip.

Another possible issue was also detected when analysing the chamber's implementation. The square-shaped and 4 cm^2 footprint proved to be unable to fit within some of the microscope systems used in the SMS Labs. Furthermore, the distance (5mm) between the bottom of the base section and the coverslip reduced flexibility in the minimum distance the objective lens could be to the sample. In addition to this, the base and inner-cut where the coverslip rested were not completely flat due to warping during the 3D printing process. The coverslip must be parallel with the lens in order to not produce imaging errors; hence reducing this warp is crucial to the design's precision and reliability.

3. SECOND PROTOTYPE - ADDRESSING KEY FAULTS

Consequently all issues were to be addressed in the second iteration of the design (Figure 3). The most immediate change was the magnet choice. The large ceramic magnets were changed for much smaller, 3x1mm disk-shaped rare earth magnets. These were inset into the 3D printed body by making four cylindrical cuts (in CAD) about the circumference, allowing a clearance of 0.2mm to be tolerant of the lower accuracy in finer 3D printed details. The small size meant a lower coefficient of friction, requiring the magnets to be glued into the cuts, simply using a small amount of store-bought superglue. Care was taken in ensuring all four magnets in the each half of the body had the same polarity outwards, and in ensuring the magnets in the opposite half were the opposite polarity for magnetic attraction. Some sanding was required if glue built up over the magnet, meaning the two chambers would not connect uniformly. A fine balance had to be made for the magnet inset depth, as too deep reduced the strength of the attraction, yet too shallow and the magnet/glue may protrude. Both problems lead to a situation in which the o-ring did not seal and, when tested with water, there was leakage from the chamber. A prototype using six magnets was also tested, no significant benefits were observed and as such it was more cost effective to use four.

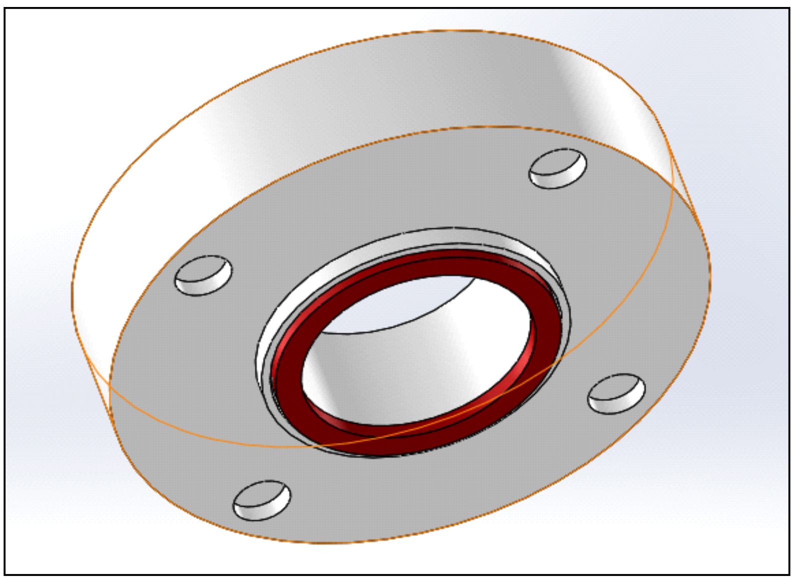


(Figure 3. Exploded view of the second iteration. From top to bottom: the upper ABS half [*note cylindrical extrusion cannot be seen], 4 rare earth magnets evenly spaced, silicone gasket, glass coverslip, ABS base half with inset magnets)

With the new magnets, a body designed for an 18mm coverslip could now be reduced to a 35mm disk footprint, fitting for microscope platforms and reducing plastic cost in printing. The depth of the chamber (both halves) was also reduced. Furthermore, this smaller, lighter design led to reduced warping. Warping during the 3D printing process is caused when the outer and lower (older) layers of a solid begin to cool before the interior and newer layers, which are more thermally insulated. Consequently, in a smaller design, there is less time for the solid to cool un-uniformly. Nevertheless, to ensure minimal warping, a heated platform (85C) was used, along with an enclosure to heat the air around the piece, and the print speed set to fast (up to 30mm^3/s) for the base half. A "raft" support structure was also used, in which a layer of ABS is printed first with a lower nozzle height to press the plastic firmly into the base plate.

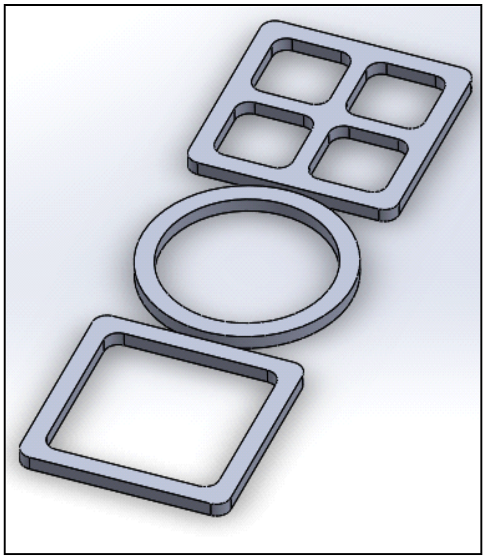
The inner-cut (supporting the coverslip) of the base section was also reduced from 5mm to a minimal thickness of 0.6mm. Multiple, incremental prototype prints were carried out to test this, with anything thinner found to be too fragile to be removed from the support raft in-tact. A print layer thickness of 0.15mm was required in order to achieve this.

The final problem to be resolved was the waterproofing gasket. The upper chamber half has a cylindrical/ring extrusion (Figure 4) with an inner cut (along with the through-hole) for a gasket to lie within. This extrusion, in combination with the base cut for the coverslip, required multiple prototypes in order to determine both the ideal width and depth. A clearance of 0.5mm (generally 0.2mm is considered tight/unmoveable whilst 0.4mm used for kinetic connections) was used in the base cut for the coverslip, to ensure ease of placement while allowing only very slight movement. The extrusion then had to have an 0.4mm clearance of the base cut to ensure friction did not reduce the force from the magnetic system. Finally, the gasket would sit within this, and could not have an inner radius below 14mm as this would reduce the viewable sample area. It also needed to protrude past the extrusion to ensure that it was in contact with the coverslip (whilst in compression), instead of the plastic (for the coverslip would shatter). Thus, there was a very small margin for error in this part, and as such standard commercial o-rings of low durometer did not have the variety to allow for a precise size. This was augmented when considering the need for square and multiple-chamber gaskets to enable other chamber designs. Multiple alternatives were subsequently considered, including professional moulding or purchasing replacement O-rings from LCI. Both options proved to be highly cost-ineffective, moulding only effective for high volume (over 500 gaskets), and LCI maintaining a high price for a single replacement gasket.



(Figure 4. Red silicone gasket lies within the shallow extruded cylinder of the 3D printed body's top half. Note how the gasket slightly protrudes)

Laser cutting using silicone rubber sheets was the chosen alternative. It is a relatively low-cost manufacturing process and material for a cut this size. The gaskets were designed in Solidworks and exported to a useable format for the Laser Cutter (Dxf), with particular care taken to ensure the kerf of the laser (in this case 0.15mm) was accounted for, as precision was key. Silicone gaskets in circular, square and multi-chamber shapes (Figure 5) were successfully cut, using 1mm silicone sheets with 50 durometer. Despite an unusual flame caused by vulcanisation of the rubber, they were of the required precision and hardness.



(Figure 5. Three forms of laser cut silicone gaskets. From top to bottom: multi-chamber, circular and sqaure. Each are 1mm thick and 18mm wide/diameter)

Furthermore, during 3D printing very small nodules or bumps can occur when the 3D printer retracts and changes directions during a print. In a fine detail part, this effect can lead to larger error than anticipated. Slight sanding was used in a few cases (on corners particularly), however after running nozzle height calibrations and incrementally altering settings in the printer (i.e. increasing retraction, setting to a slower print speed) later designs printed very well. As insurance, the gaskets were made slightly smaller to fit regardless of nodules, and in the case where there were no imperfections; light stretching can be used to ensure the correct fit. In doing this there is a greater tolerance to both the 3D printing and O-ring processes.

4. RESULTS AND DISCUSSION OF FINAL PRODUCT

Having corrected the critical faults of the original prototyped design, and incrementally refined the chamber to a precise size and fit, the second design proved to be the final product. The 3D printed chamber halves locked magnetically together uniformly, such that the laser cut gasket was successfully compressed against the coverslip, with no leakages occurring. Furthermore, the design footprint was refined and could be successfully tested in the lab, producing clear images comparable to LCI's Chamlide, proving there was minimal warping, with the coverslip both parallel and at an acceptable distance to the objective lens. Having successfully proved the viability for an accessible, low cost design, multiple alternate designs could be produced from this base product. This included a square and multi-chamber design (Figure 8) along with a larger, 21mm coverslip design. The options and variety are vast with each component in the manufacturing process providing great flexibility.

Table 1 shows a cost analysis of the design, including materials and manufacturing.

MATERIALS	**COST (AUD)**
ABS UP Premium* Filament - 7.5g *Less “nodules” than cheaper filaments and less warping	$0.6 ($35/500g)
8 Rare Earth Disk Magnets	$1.32 ($16.5 for 100)
Super Glue	$2
Silicone Gasket	$0.1 ($10 Silicone sheet, makes 100)
MANUFACTURING	**COST (AUD) *Estimation**
3D Printing	Desktop printer is of small size and commercially available to all. Running costs is only ABS plastic. $1240 (variable) for printer, however sites such as 3D Hubs allow for community-based printing services charging for amount of ABS and a small service charge ($10-$15 per Chamber)
Laser Cutting	Used Universal 24 by 18 inch laser Cutter. Would be too costly to buy, however Laser Cutting services are also available. Most companies charge $3 per unique part and $1 per replica, however this is lowered for size, simplicity and material thickness. Gaskets would hence cost less than $1 each to print. Most companies charge $25 minimum however.

(Table 1. Cost Estimations for both material and manufacturing)

It is clear from this analysis the prices are minimal. For a higher volume of Chambers the price will inevitably reduce per unit. For fabricating only a single Chamber the maximum cost (assuming printing services are used) would be $103.5. AUD. For the SMS Lab, ten were made, utilising the free UNSW services for manufacturing. This was at an approximate cost total (given a minimum 500g of abs can be bought) of $63.5 for all ten, or $6.35AUD each. All prototypes could also be made enclosed within this budget.
As of late February, $380USD equates to $514AUD, the price of a single Chamlide CMB Chamber from LCI (via Quorum Technologies). Thus the Lab saved $5076.5, whilst further opening opportunity for custom designs and sizes as required.

In terms of areas of improvement, the design is not as high a quality in terms of finishing and precision as the Chamlide. The gaskets may require slight adjustment through stretching, and some friction may occur when connecting the halves, requiring the halves to be pressed together lightly to ensure waterproofing. Aesthetically they are marginally rougher. Care must be taken in gluing the magnets in particular to ensure a refined visual and functional appeal. Cleaning may also be more difficult, with the more porous ABS layers and imperfections in printing leading to grooves and pits which could hold substances. The magnet cuts are also not completely sealed off, a recommendation for future improvement, achievable by using an ABS and acetone "slurry" to back-fill after magnet placement.

5. CONCLUSION

The flexibility of CAD design software along with the accessibility and low cost of manufacturing through 3D printing and Laser Cutting are key advantages in the chamber's design. It will allow lab workers the option to order an alternate design required for their project and have it available with little time and cost, far surpassing the less flexible and high cost Chamlide. Above all, the custom chamber achieves this without reducing imaging quality.

6. SOURCES FOR MATERIALS AND MANUFACTURING

CAD: http://www.solidworks.com/

Silicone rubber sheets: http://www.ebay.com.au/itm/301621017741
Magnets: http://aussiemagnets.com.au/product/--3mm-x----1mm-Disc-%28Rare-Earth%29--%28Pack-of-100%29.html

Premium UP ABS Filament: http://store.3dprintingsystems.com/filaments/up-abs
UP Plus 2 3D Printer: http://store.3dprintingsystems.com/up-plus-2-3d-printer
3D Hubs for Print Service: https://www.3dhubs.com/

Universal Laser Cutter: http://www.ulsinc.com/en-au/products/pls475/
Laser Cutting Service: https://onlinelasercutting.com.au/

Original Chamlide: http://www.chamlide.com/
Chamlide via Quorum: http://www.quorumtechnologies.com/index.php/2014-06-19-13-10-00/2014-06-19-13-15-39/environment-chambers/chamlide-cmb-for-18mm-round-coverslips-1-detail