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Development of Tissue Printed Nitrocellulose Cards/Arrays for Real Time PCR Amplification and Detection

by

Helena Nien-Hwa Chia

Submitted to the Department of Mechanical Engineering in the Partial Fulfillment of the Requirements for the Degree of

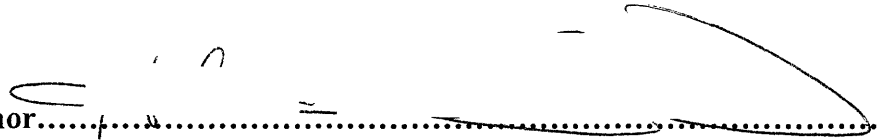
Bachelor of Science

**at the
Massachusetts Institute of Technology**

June 2004

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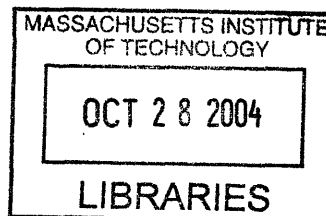
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**Submitted to the Department of Mechanical Engineering on May 7th, 2004
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Abstract

Tissue print technology allows for the transfer of cellular material from tissue onto a nitrocellulose film for immunocytochemical assays. The MIT BioInstrumentation Laboratory is currently developing a novel cancer marker imaging system for detection of cancerous tissue, which will be useful for discerning tumor margins. This research will advance the recent application of tissue print technology in bio-medicine by combining it with imaging and real time polymerase chain reaction (PCR) amplification and detection. A major objective in the design of this instrumentation is to develop the capacity to evaluate much larger areas of tissue. An approach to fulfilling this objective is the creation of a gasket that can seal individual wells of a nitrocellulose array. A gasket was created by laser cutting an assembly of molded silicone rubber and a double-sided tape (silicone-acrylic). Experiments showed when the gasket was adhered to a glass slide and subjected to the PCR, there was no leakage. FAST Slides, nitrocellulose slides provided by Grace Bio-Labs, are cut with a laser to generate the nitrocellulose arrays.

Thesis Supervisor: Ian Hunter

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1.0 Background

Touch preparations or tissue printings are the transfer of cellular material through pressing cut surfaces of tissues or organs onto substrata such as microscope slides or microporous membranes [1, 2, 3]. The process provides a fast, simple, and inexpensive method for collecting tumor cells for use in experimental and diagnostic oncology (Gaston et al., manuscript submitted).

McGrath et al (1991) showed that both the sensitivity and reproducibility of immunocytochemical assays for receptor protein were greatly improved when tissue was thaw-mounted on microporous nitrocellulose membrane rather than on glass slides [3]. Experiments by Varner and Ye (1994) using plant cells suggested that nearest cell localization of proteins, enzymes, and mRNA is possible with nitrocellulose [2]. This success with nitrocellulose films in plant cell printing has provided the animal world with an acceptable tool for immunocytochemistry, *in situ* hybridization patch-clamp ion channel analysis, scanning electron microscopy, and several other cytochemical techniques [2].

Experiments performed by McGrath et al (1991) using pre-wet or dry membrane of different pore size showed that printing to dry nitrocellulose membrane with 0.05 μm pores gave the best cytological resolution. However, he noted that signal intensity was directly related to pore size suggesting that some trade off in signal intensity would be required in order to maximize resolution [3].

Other factors that have undermined the use of nitrocellulose in microscopic applications include inadequate light transmission, its irregular surface, and distortion of the film if not supported. In our studies, light is not transmitted through the film and the small aperture results in a large depth of field so surface roughness will not be a problem (personal communication). Finally, distortion of the film due to swelling was overcome by McGrath et al (1991) and by Conley (1997) when they glued the nitrocellulose films onto microscope slides for cryostat tissue mounting and immunocytochemistry [3,4].

Grace Bio-Labs has since developed a method to cast polymeric nitrocellulose onto a glass microscope for tissue processing and cytochemistry [1]. This product has a stable interface between the film and glass for temperatures, pH, and solvents of all cytochemical assays including the polymerase chain reaction (PCR). The surface of the film has round pores about 0.1 μm in diameter, a thickness less than 20 μm , negligible autofluorescence and capability of being cleared to transparency with mounting media for high resolution imaging. Film slides contain, on average, twice the number of cells found on glass slides [5].

Tissue printing in combination with immunoblotting, DNA/RNA hybridization, or other affinity-based techniques creates a two-dimensional image of biological macromolecules on the surface of the printed tissue. As such, patterns of mRNA and protein expression characteristic of specific cancers in humans can be identified. Since specimens are not damaged by tissue printing, tissue printing provides an opportunity for molecular analysis while preserving the tissue for histopathological study or for research.

Novel tissue print and "print-phoresis" technologies have been developed by Gaston et al (manuscript submitted) and applied to the analysis of aggressive prostate cancers [6]. The focus is on markers of tumor invasion of the prostate capsule, such as prostate specific antigen (PSA) hot spots on the exterior surface of the prostate gland. This new technology provides a practical approach for the rapid detection and classification of

solid tumors in surgical specimens and tissue. This information could be extremely useful in determining surgical margins

The MIT BioInstrumentation Laboratory is currently developing a novel cancer imaging system for detection of cancerous tissue. A major objective in the design of this instrumentation is to develop the capacity to evaluate much larger areas of tissue in real time. While companies such as Grace Bio-Labs and Schleicher and Schuell have developed film slides and gaskets (as discussed above), their use is limited to non-PCR based technologies as the gaskets do not adhere to the slides under the PCR cycling conditions (personal communication). This work discusses the development of gaskets capable of adhering to nitrocellulose arrays under PCR conditions.

2.0 Materials and Methods

Resin gaskets have been produced from molds designed in solid edge and fabricated using a stereolithography printer. Nitrocellulose arrays have been generated by custom cutting film slides using the Firestar series t60 laser engraver from Trotec [7].

2.1 Gasket

Early attempts to generate silicone gaskets by cutting a 1 mm thick silicone sheet resulted in a lot of burning on the edges of cuts (personal communication). Burning was significant enough that there was some concern about residue interfering with the PCR. Therefore creating a gasket from a mold was determined to be the best method because it allowed for wells to be made without cutting and the resin was a firm but pliable consistency when set.

The gasket was designed to have dimensions of 76 mm in length, 25.4 mm in width, and 1.75 mm in thickness so that it could cover the entire surface area of a microscope slide and create wells each with a volume of 7 μL . Each well had dimensions of 2 mm by 2 mm by 1.75 mm and each gasket had a total of 27 wells. The wells were arranged in a grid with three wells the width of the gasket and 9 wells the length of the gasket. Each well was 4 mm away from the next well on each side.

The gaskets were made by filling molds with resin. The resin was P4 Silicone made by Silicones, Inc., an addition cure two-component silicone rubber that had a service temperature of -51.1°C to 357.2°C. Molds to form the gasket were designed on Solid Edge as two parts, a female and male mold, and formed by the Viper si2 SLA System provided by 3D Systems, a solid imaging system that builds molds made of Accura SI 40 Nd liquid material [8].

After the molds were printed and cleaned, the holes were tapped. The assembly included the two parts of the molds and screws to secure the mold. The edges where the female and mold parts met were taped and a barrier at the top was made with tape to prevent bubbling resin from escaping.

10 mL of P4 base and 1 mL of P4 activator were mixed in a small weighboat until no white streaks were seen. The mixture was poured into the mold and the mold was secured to a large weighboat. The mold was placed in a vacuum chamber and allowed to de-aerate for 30 minutes. After 24 hours of setting, the mold was opened and gasket removed.

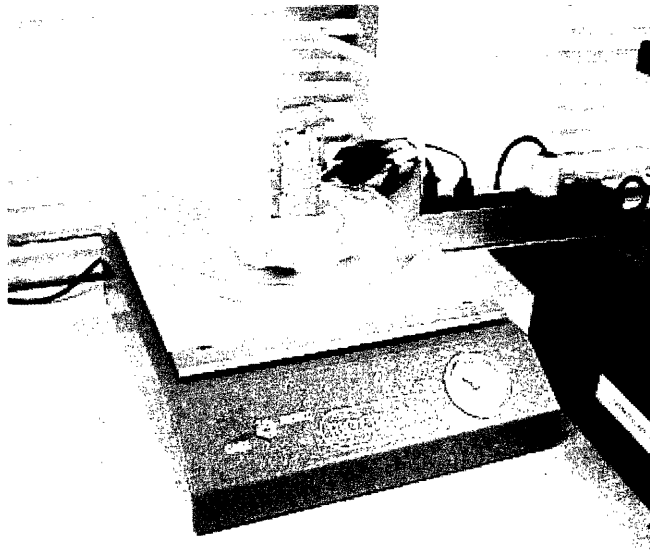


Figure 1. The mold was sealed with tape and placed in a large weigh boat inside the vacuum chamber to de-aerate for 30 minutes.

2.2 Nitrocellulose slide

The nitrocellulose microporous film bonded to premium white glass microscope slides (FAST slides) from Grace Bio-Labs had a layer of casted nitrocellulose that was 40 mm in length and 20 mm in width. Therefore a process was required to remove areas of the nitrocellulose from the glass slide so that a grid of nitrocellulose squares remained for the bottom of the wells made by the gasket. The method used for removing the thin layer was to burn it off using the Trotec. FAST slides with 6 mm by 6 mm square nitrocellulose areas are also available but used only for finding the laser settings for cutting the 40 mm by 20 mm slides.

Since the Trotec engraved the areas of the nitrocellulose that needed to be removed, an image was made as a draft on Solid Edge and then printed to the Trotec. The 6mm by 6mm FAST Slide was placed on the platform and the laser was focused using the red pick. The settings for the frequency, speed, power, and number of passes of the laser were entered. The laser head was positioned to the correct starting point and the job was run. Visual inspection for cracking of the glass and burning of the nitrocellulose was made. Microscope inspections were also made for a few of the settings to understand how changing each condition would affect the cut.

To cut the 40 mm by 20 mm FAST slides, the slide is placed on the laser platform with the nitrocellulose facing upwards. The laser is focused using the red pick and an image of the grid wanted was made as a draft on Solid Edge. The draft was printed to the Trotec and then the settings were adjusted for the job.



Figure 2. A FAST slide has nitrocellulose over a glass microscope slide and can come as one large rectangle as in the photo on the left. The photo on the right is a FAST slide in the Trotec, a laser cutter.

2.3 Adhesive

For the silicone rubber gasket to bind to the glass microscope slide and create a good seal, an adhesive was needed that withstood temperatures of 95°C and could bind to both acrylic and silicone. Preliminary experiments using 3M Hi-strength 90 mounting spray, 3M super77 tape, and 366 Loctite UV curing glue all failed to provide adequate sealing (personal communication).

Orange and Purple Hardman Epoxy were spread onto two pieces of gasket and then pressed onto a FAST slide that was cut. The epoxy was allowed to set for 24 hours. A peel test was performed by trying to lift one corner of the gasket upward.

Double-coated polyester with differential silicon and acrylic adhesive system from Special Tapes (#D969) was cut in the pattern of the wells either by itself or while attached to the gasket to be cut together. The tape was 0.146 mm thick and had a service temperature of -28.9°C to 121.1°C.

To cut the tape with the Trotec, the tape was attached to a large piece of glass with the acrylic liner facing upward. Settings of frequency, power, speed, and number of passes were adjusted to find the optimal conditions to prevent burning of the tape while being able to completely cut through it.

To cut the tape while attached to the gasket, the silicone liner was removed from the tape and the tape was pressed onto the gasket from left to right. Pressure was exerted over the entire surface for a few minutes and then the assembly was placed with the tape side upwards on the laser platform. Settings for power, frequency, speed, and number of passes were adjusted until there was the least amount of visible burning possible and the cut pieces of gasket and tape could be removed easily with tweezers.

2.4 Adhering gasket to slide

The method to assemble the tape, gasket, and slide parts, if they are all made separately, was to first attach the tape to the gasket. The acrylic liner of the tape was then removed and the gasket was then pressed onto the cut nitrocellulose slide from left to right. Pressure was exerted over the entire surface area for a few minutes manually. Special attention was paid to line the holes of the tape and gasket as close as possible and to prevent the gasket from covering any of the nitrocellulose.

If the gasket and tape were cut together on the Trotec, then the acrylic liner was removed and the gasket was pressed onto the cut nitrocellulose slide from left to right. Pressure was exerted over the entire surface area for a few minutes manually.

2.5 Fluorescent experiments

Alexa 488 conjugated to bovine serum albumin (BSA) was diluted in PBS to concentrations of 20 μM , 2 μM , 200 nM, and 20 nM [9]. The gasket, tape, and slide assembly (tape and gasket cut separately) was loaded with 5 μL of the dilutions or PBS in every other well for one row. 2 μL of mineral oil was pipetted on top of the dilutions or PBS. The slide was then spun for 1 minute at 800 rpm and 25°C uncovered in the Eppendorf Centrifuge 5804R. The slide was covered with PCR foil and placed in the PCR instrument (Mastercycler gradient from Eppendorf) for cycling (preincubation at 94°C for 5 minutes, start cycle at 94°C for 30 seconds, 53°C for 1 minute, and 72°C for 40 seconds). The slide was then spun in the centrifuge for one minute at the same settings as before. The foil was peeled back slowly. If there was liquid on the foil, it was pipetted back into the well if possible. The slide was imaged using the “in-house” fluorescence spectrometer at an 8 second exposure time using one LED. The custom-made fluorescence spectrometer excites fluorophores at 491 nm and measures their emission at 515 nm using an LED array, charged couple device (CCD), and a combination of spectral filters. Images were taken immediately after the PCR run, and at 10, 25, and 35 minutes.

The experiment above was run again but with a microscope slide instead of a cut FAST slide and the gasket and tape were cut together. 5 μL of dilutions or PBS and 2 μL of mineral oil are loaded into every other well in one row. Another experiment was performed with just changing the volumes of the solution to 2.5 μL of dilutions and PBS and 2.5 μL of mineral oil.

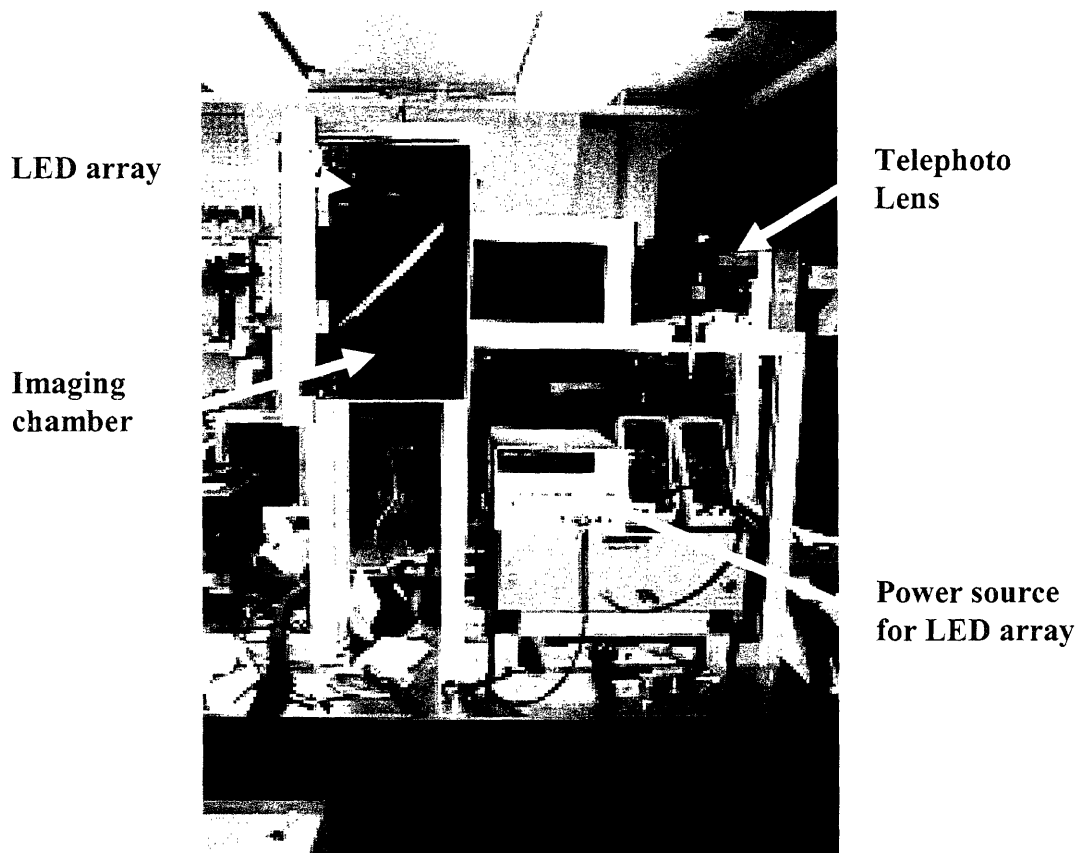


Figure 3. The custom-made fluorescence spectrometer has a power source for the LED array that emits blue light (470 nm peak), an excitation and emission filter, a CCD, and a telephoto lens.

2.6 Fluorescent experiments for evaporation from imaging chamber

After the molded silicone rubber and tape were cut together, the assembly was attached to a glass slide. 2.5 μL of dilutions at 2 μM and 2.5 μL of mineral oil were loaded into one well. A Petri dish is lined with Whatman paper, saturated with distilled water, and placed inside the imaging chamber. The slide is placed on top of the Petri dish bottom. Each image is exposed to light for 8 seconds exposure time with one LED. Between images, the Petri dish top is put on and the lights were turned off. Images were taken at 5, 15, 30 and 45 minutes after addition of fluorescence.

2.7 Fluorinert

A gasket-tape assembly was cut with the laser and then placed on a microscope cover slip. Two wells are filled with 2.5 μL of 20 μM dilution of Alexa 488-BSA and 2.5 μL of mineral oil on top. The cover slip was placed inside a plastic box cover that could hold the cover slip and then immersed in about 3 mL of fluorinert, an electronic liquid that has a boiling point of 215°C [10]. An image was taken using the fluorescence spectrometer. The cover slip and gasket were placed in the Mastercycler gradient and subjected to 5 cycles of the PCR. An image was taken after the heating and foil are removed.

2.8 Wicking of nitrocellulose (uncut slides)

A gasket that was cut with the tape was adhered to a glass cover slip. 2.5 μL of 20 μM of Alexa 488-BSA was loaded into three wells, one well apart, and then covered with about 3 mL of fluorinert. It is imaged before heating, then subjected to 5 cycles of the PCR. The cover slip was imaged again while in the fluorinert.

A gasket that was cut with the tape was adhered to a glass cover slip. It is placed in a plastic box cover and dilutions were pipetted into the wells. The cover slip was placed inside of a Petri dish lined with wet Whatman paper. The Petri dish with cover slip was placed in the imaging chamber of the fluorescence spectrometer and images were taken

A nitrocellulose FAST slide was cut to produce a piece that could fit inside the plastic box cover. The Trotec was used to make square grids on the slide and then a gasket with tape cut together was adhered to the piece of FAST slide. Two wells were filled with 20 μM dilution of the Alexa 488-BSA and heated in the PCR for 5 cycles of the HOGANIS program.

3.0 Results

The design of the gasket changed over time as well as the molds for improvement of assembly of the gasket, tape, and microscope or FAST slide. Fluorescence was used to visualize whether the adhesive created a good seal between each well and therefore the results are images.

3.1 Design of gasket

A total of three molds were made that allowed for better de-aeration of the resin and making of the wells. The first mold, similar to an early prototype mold, included pegs to create holes through the gasket. This design also had holes on the female part to allow air bubbles to escape. The second mold had an increased thickness of both the male and female parts and a sloping top to allow resin that had bubbled up to travel back into the cavity of the mold. The third mold had a female and male part that stood vertically and created wells in the gasket by pegs. An additional vertical male mold minus the pegs was fabricated.

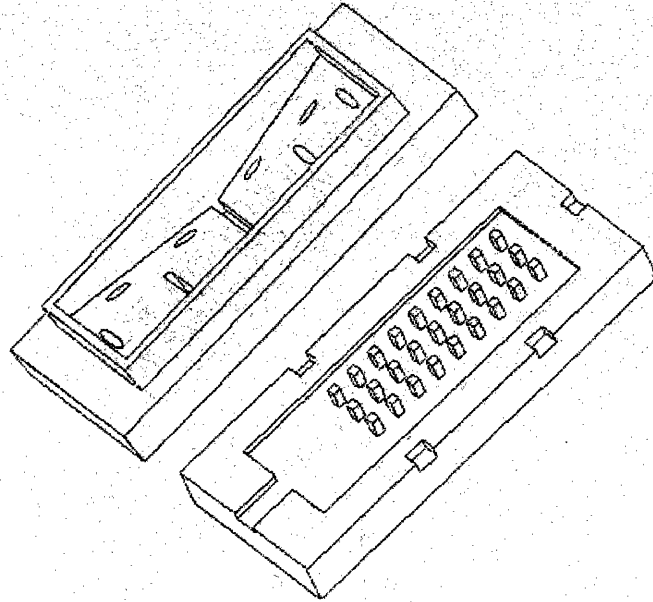


Figure 4. The design of the second mold has a sloping top to allow bubbling resin to re-enter the cavity and air holes for de-aeration.

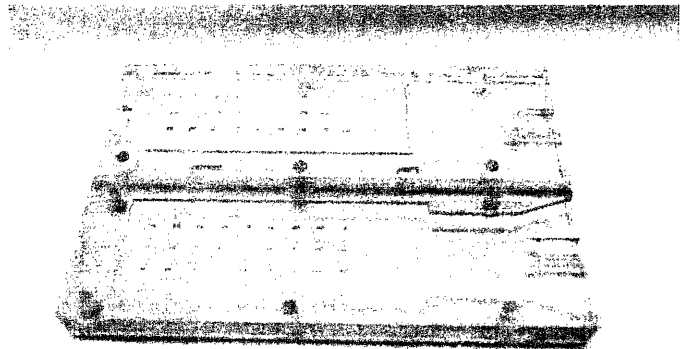
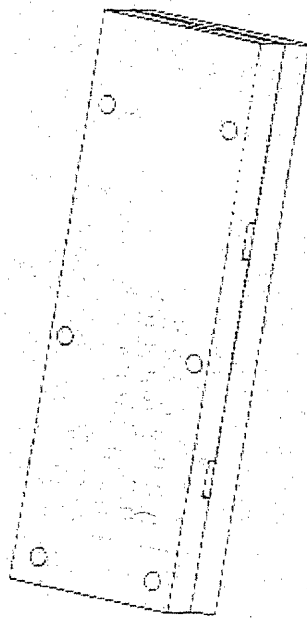


Figure 5 and 6. The third mold stands vertically to allow gravity to pull down the resin while an opening at the top allows for air bubbles to escape. Another male piece was made without the pegs.

The vertical mold with no pegs was used to make the gaskets. The double-sided tape from Specialty Tapes was selected as the best adhesive because both epoxies applied failed a gentle peel test while the tape did not give during a gentle peel test.

After the set resin was bonded to the silicone side of the double-sided tape, the gasket/tape was cut using the following laser settings: power of 22 W, speed of 17.75 mm/s, frequency of 1500 Hz, and 1 pass. The acrylic side of the tape is then bonded to the glass slide. This assembly is used for experimentation.

3.2 Fluorescence experiments

3.2.1 Glass slides with gaskets

Before running any PCR experiments, experiments were set up to determine whether gaskets with double sided tape would adhere to glass slides without heating. Equal volumes ($2.5 \mu\text{L}$) of Alexa 488-BSA or PBS and mineral oil were added to a row of wells formed by simply adhering a gasket to a glass slide. Fluorescence was imaged using the CCD camera over a range of time points. Signal was only detected in wells containing $20 \mu\text{M}$ and $2 \mu\text{M}$ Alexa 488-BSA. The volume of liquid in the $2 \mu\text{M}$ well decreased over time and was noticeably decreasing from 5 minutes to 15 minutes after addition of the sample.

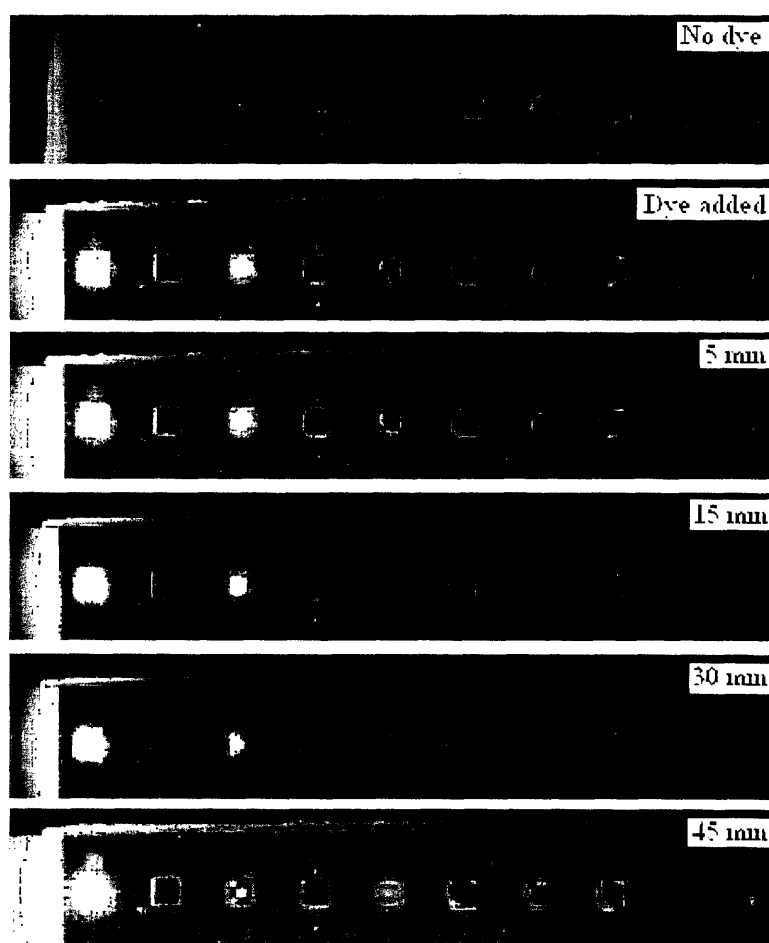


Figure 7. The most left well has the $20 \mu\text{M}$ of Alexa 488-BSA and every other well to the right contains the next ten fold dilution. The last well on the right has PBS.

Given that we observed no leakage in the absence of heating using our custom-designed gaskets, we then decided to assess the behavior of the gaskets under PCR

conditions. Dilutions of Alexa 488-BSA in PBS were loaded into individual wells created by adhering gaskets to glass slides. The wells were topped off with 2 μL of mineral oil to prevent evaporation, covered and subjected to 5 minutes at 94°C followed by 2 cycles of the PCR. Fluorescence was accessed over time using the fluorescence spectrometer. Figure 8 shows that signal was only observed in wells containing 5 μL of 20 μM and 2 μM Alexa 488-BSA consistent with earlier results. More importantly, no leakage between wells is observed indicating that the gasket has withstood the conditions of PCR. A bubble was observed on top of the two visible concentrations when the slide was removed from the imaging chamber which explains the halo effect observed in this figure.

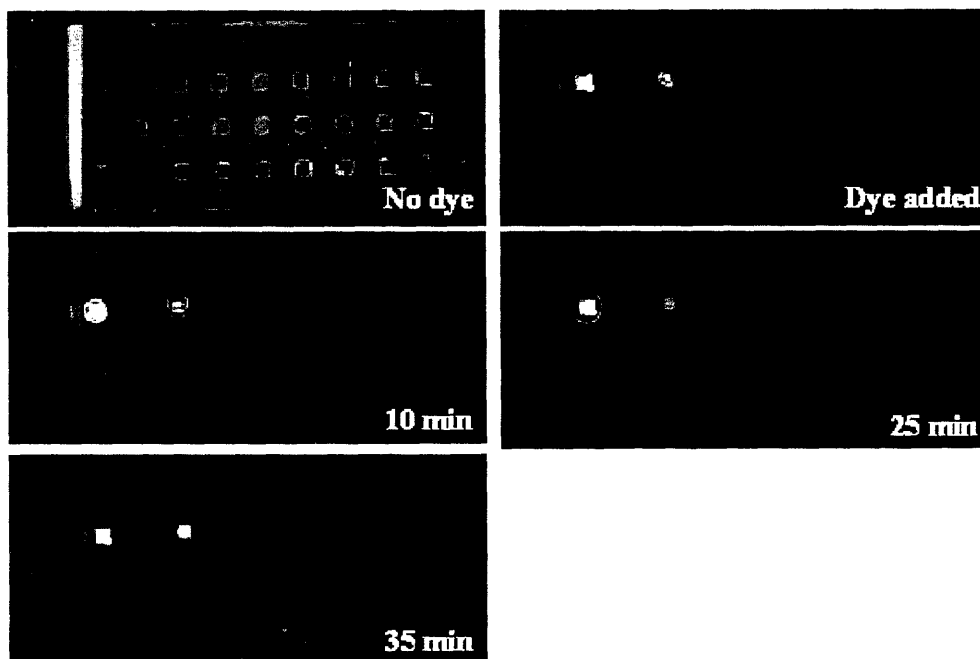


Figure 8. The addition of the concentrations of Alexa 488-BSA and PBS to the gaskets adhered to a glass slide showed the 20 μM and 2 μM concentrations were visible. The total volume of liquid added into each well is 7 μL .

In the above experiments, loss of sample was observed over the fluorescence time course. Given that the LED array was on during the imaging period, evaporation could have been due to the heat generated by the LED. To address this, we loaded an equal volume of 20 μM sample and mineral oil into a single well and then placed the slide into a Petri dish containing saturated 3MM Whatman filter paper. Images were taken over a similar time course except that in this case, the LED was turned off and the assembly covered between measurements. No loss of signal intensity was observed in this experiment indicating that the heat generated from the LED was in fact causing a loss in sample volume during the imaging period (see Figure 9).

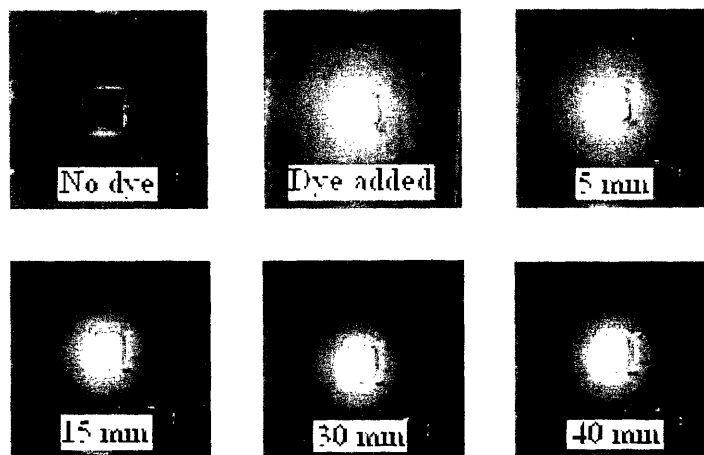


Figure 9. The intensity of the 20 μM of Alexa 488-BSA does not change over time as the slide is sitting in a damp Petri dish inside the imaging chamber.

During the course of experiments designed to test the adherence of the gasket under PCR conditions, it was noted that condensation was occurring on the PCR foil and a visible difference in volume could be seen. This change in volume could be due to the condensation or evaporation of sample during the PCR (see Figure 10).

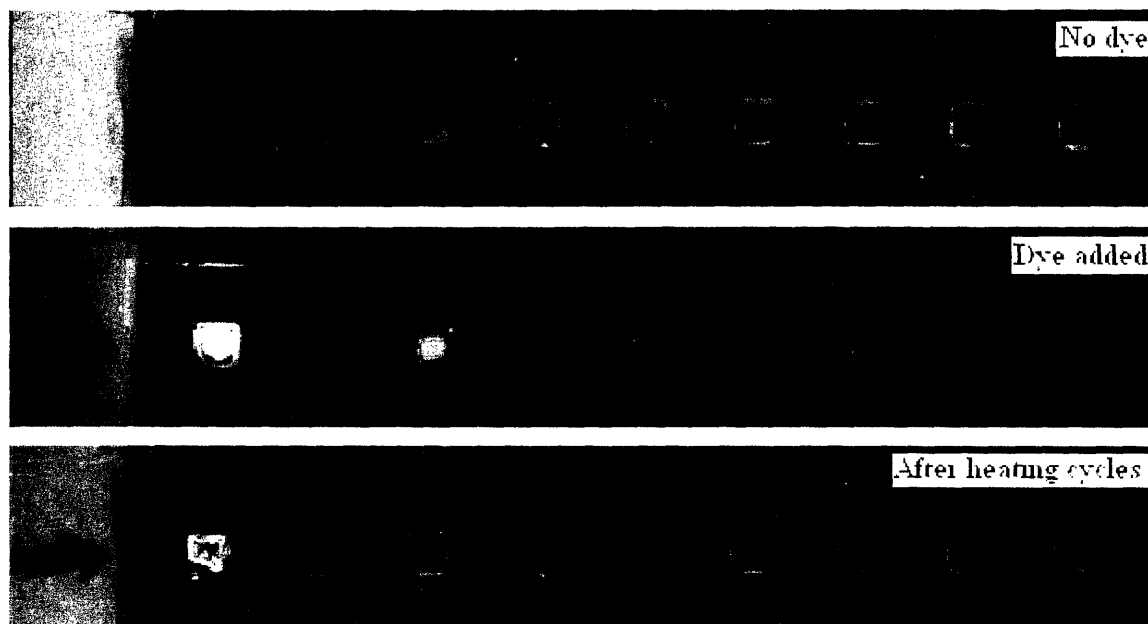


Figure 10. Dilutions of Alexa 488-BSA with mineral oil were added to individual wells and subjected to PCR conditions after which signal intensity was determined by fluorescence spectroscopy.

In an attempt to prevent the loss of sample, we removed the problem of condensation by getting rid of the PCR foil and addressed the problem of evaporation by immersing the slide in fluorinert. Individual wells were loaded with equal volumes of 20 μM Alexa 488-BSA or PBS and mineral oil. The slides were then placed in a plastic container that would fit in the *in situ* holder and completely covered with fluorinert.

Samples were subjected to 5 cycles of the PCR followed by imaging. Figure 11 shows that signal intensity before and after heating are comparable. No loss of sample was observed under these conditions.

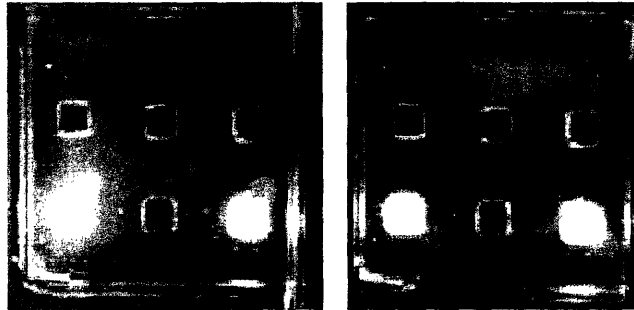


Figure 11. The addition of fluorinert to the dilutions and mineral oil prevented the change in intensity of the fluorescence, even after heating of 5 cycles. The left picture is before heating and the right picture is after heating.

3.2.2 Nitrocellulose slides with gaskets

The next step was to replace the glass slides with the nitrocellulose film slides (FAST slides). First, experiments were conducted to determine whether the nitrocellulose had to be cut or if the gasket could be laid on top of a nitrocellulose film. Therefore 2.5 μL of 20 μM of Alexa 488-BSA was added to three wells and the slide was flooded with fluorinert. Figure 12 shows that sample fluorescence is not contained within the walls of the gasket.

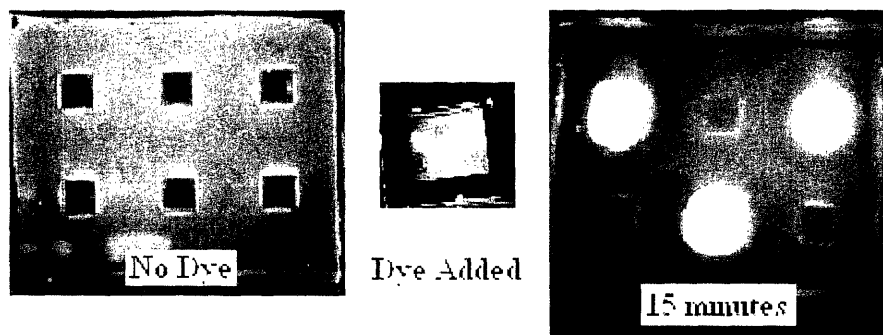


Figure 12. 20 μM Alexa 488-BSA and fluorinert are added to three wells on a piece of nitrocellulose slide, showing fluorescence not contained within the walls of the gasket.

To determine whether the fluorinert or the fluorescence is wicking on the nitrocellulose film (the latter being more likely), 2.5 μL of 20 μM of Alexa 488-BSA was added to two wells and imaged 15 minutes after the addition of sample. Figure 13 shows that the sample is diffusing from the wells. This diffusion is most likely due to wicking by the nitrocellulose.

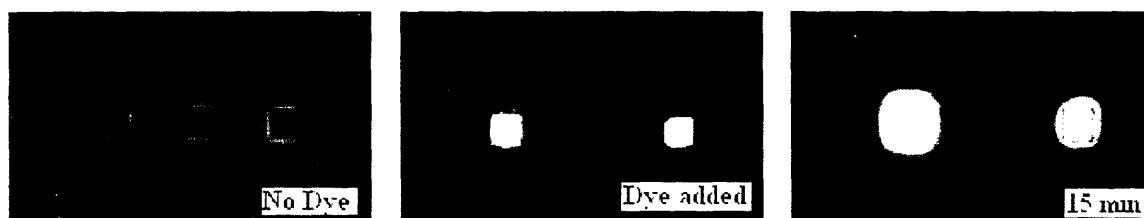


Figure 13. 20 μM of Alexa 488-BSA is added to two wells and then imaged 15 minutes later.

3.2.3 Removing nitrocellulose from the film slides

Samples added to nitrocellulose could wick across the film. In order to present this, we attempted to remove the nitrocellulose connecting the individual wells using the Trotec laser. The parameters of frequency, speed, power, and number of passes of the laser made a very large difference in the result of the cut nitrocellulose film. To find the optimal settings, a slide with 6 mm by 6 mm squares of nitrocellulose film was used so that 1 mm by 1mm squares could be cut to find the best settings. The best results were when the laser had the settings of: power of 0.5 W, speed of 31.95 mm/s, and frequency of 300 Hz, over 2 passes (see Figure 14, right panel). Under the microscope the edges of the cut are extremely clean and there is no cracking of the glass. However, when these settings were translated to an entire slide, visible cracking in the glass was observed.



Figure 14: The result of trying to use the same settings as the trials made from the 6mm by 6mm squares was cracking of the glass (left panel). The settings of the trial produced edges comparable to the edges of the nitrocellulose that was cast (right panel).

Although there was cracking in the glass, a gasket was attached and dilutions of Alexa 488-BSA and PBS were added to the top row of the slide. The slide was heated for 5 minutes at 94°C and subjected to 5 cycles of the PCR followed by imaging. The image taken in the fluorescence spectrometer showed that the solutions had leaked on the FAST slide (see Figure 15) that was cut using the laser (refer to Figure 14, left panel). The high concentration of Alexa 488-BSA (20 μM) in the left well of the first row spread to wells below it and to the right of it.

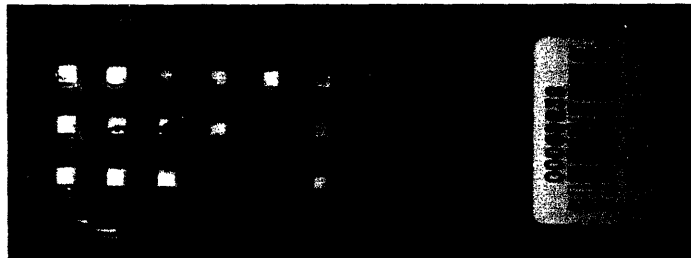


Figure 15. Only the top row was filled with dilutions of Alexa 488-BSA and PBS in every other well, starting with the well on the left. The fluorescence spread over a large section of the slide.

New chemical FAST slides (distinct from the above) were obtained from Schleicher and Schuell. A section of FAST slide was cut, using the same laser settings used above. Cracking was not observed under the microscope although some nitrocellulose remnants were observed on the slide. A gasket was applied to the slide section and 2.5 μL of 20 μM Alexa 488-BSA was added to two of the wells. PBS was added to the remaining wells. The slide was immersed in fluorinert and subjected to 5 minutes at 94°C followed by 5 cycles of the PCR. Fluorescence imaging shows that sample has diffused away from the wells (most evident in the upper row where sample has clearly diffused to a neighboring well).

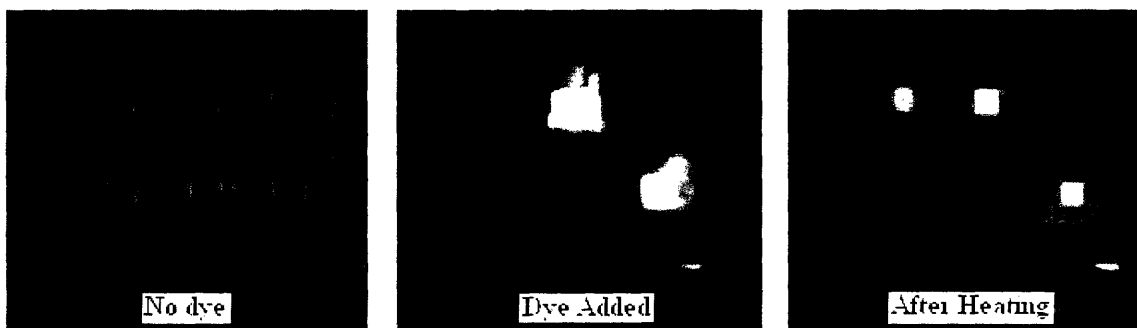


Figure 16. A piece of FAST slide is cut so that a gasket is attached. 20 μM of Alexa 488-BSA are loaded in two wells and the fluorescence has traveled into other wells and between wells.

4.0 Discussion

To create the gaskets, different molds were made so that de-aeration could occur and bubbling resin could not escape. The final design of the vertical mold was far superior to the molds that laid flat on the table because gravity filled the cavity completely with resin after the de-aeration. Although the female and male parts were screwed together, there was still some resin that escaped from the sides of the mold. Addition of tape to the sides of the mold, where the two parts meet, and to the top will prevent resin from escaping. The material the molds were made of was very brittle. Drilling holes was especially difficult due to the material cracking.

After the gasket was made, the ability of the tape to adhere to the glass slides was tested by running experiments in the presence or absence of the PCR conditions. The glass slide provided a smooth, even surface that would mimic the manner in which the gasket would attach to the nitrocellulose arrays cut on the FAST slides. These

experiments demonstrated that the double-sided tape (acrylic-silicone) provided an adequate seal between the wells in the gasket under PCR conditions. No leakage was observed (Figures 7-9).

However, evaporation of sample was noted in initial experiments (Figure 7). Given that this evaporation was observed in the absence on the PCR, it was deemed to be a result of the continual exposure to heat generated by the LED. To circumvent this problem, the LED was turned off between exposures in the time course. Additional measures that included placing the slide in a Petri dish containing saturated Whatman filter paper were also adopted. The Petri dish was covered between exposures.

Sample loss was also noted in experiments where the slides had been subjected to PCR conditions (Figure 10). In this case, condensation of sample onto the PCR foil used to seal the slide during the PCR or evaporation of sample during the PCR due to insufficient sealing may have caused the loss of sample volume. We removed the PCR foil and therefore the problem of condensation and tried to address the issue of evaporation by immersing the slide in fluorinert, a fully fluorinated liquid with excellent heat transfer properties and high boiling point. Figure 11 shows that, at least to the naked eye, the use of fluorinert combats the problem of evaporation. Additional experiments to quantify the amount of fluorescence observed in the wells before and after the PCR should be carried out.

In order to immerse the slides in fluorinert, they had to be placed in some form of container to prevent loss of fluid. We chose to use thin-walled plastic boxes for our experiments. Further studies should be done to determine what effect the thickness of the plastic has on heat transfer.

Having demonstrated that the gasket-tape-glass slide assembly worked well with heating cycles similar to PCR, we attempted to transfer this methodology to nitrocellulose arrays. We first tried to adhere the gasket directly to the FAST slides (no cutting) and found that nitrocellulose wicks the sample. We then tried to cut arrays in nitrocellulose slides by finding optimal laser settings for removing small squares of nitrocellulose. After these settings were found, they were applied to full-sized nitrocellulose slides. However, the result was not the same for the two since cracking was observed on the full-sized slides (see Figure 14). Clearly there is some, as yet undetermined reason, as to why scaling is problematic. If cracking is due to localized heating then cutting the slides/nitrocellulose while wet may be helpful. Alternatively excess power may be absorbed by cutting through a thin sheet of material overlaid on the slide. Grace Bio-Labs also offers a custom design service that could be used to fabricate the nitrocellulose arrays.

5.0 Conclusion

The double-sided tape from Specialty Tapes is a good adhesive to bond the silicone rubber gasket and acrylic slide. It works even when heated up to 94°C for multiple cycles. Future work would involve selecting optimal settings for the laser to cut full nitrocellulose slides without cracking or leaving remnants of nitrocellulose.

6.0 Future work

Finding the optimal settings of the laser cutter or another effective method to remove areas of unwanted nitrocellulose film without burning or compromising the

integrity of the nitrocellulose will be valuable to moving forward. Scaling the nitrocellulose slides and gasket fabrication and assembly will require some troubleshooting. PCR trials should be carried out to determine what, if any, of the materials used in the fabrication process will interfere with the PCR. Some of these experiments could be done by running the PCR in tubes or plates containing small pieces of the fabrication materials. Further investigation into simplifying the process for making this assembly and for using the assembly in tissue printing will benefit those using this technology.

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