UNIVERSITY COLLEGE LONDON

Development of a Robotic Chromosome Delivery System

End of Project Report

Prashant Patel

07/07/2014 to 12/09/2014

This report describes a 10 week project in which an automated chromosome delivery system was created and added to a microfluidic setup. Several problems were encountered throughout the project, some requiring major changes to the setup (removing valves) and thus deviating from the original idea. At the end of the project, a functioning proof of concept was created although several improvements have to be made to reach the end goal.

Table of Contents

1. Introduction

This summer project was designed to make the process of extracting chromosomes from a microfluidic chip automated.

The recovery of the screened chromosome into a test tube required the development of a robotic system to feed the exit tube into a 96-well plate of test tubes, after running the void volume into a waste channel.

The long term scientific objective is to perform chemical manipulations of individual chromosomes and save the results for genetic analysis (using the Hi-C crosslinking method). This would be carried out inside a microfluidic device and adding a robotic chromosome delivery system would be greatly beneficial.

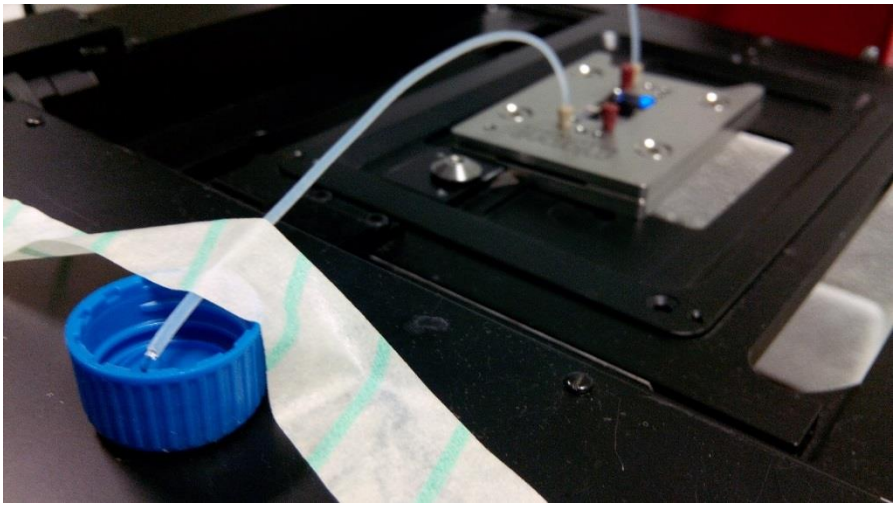

Figure 1. – A microfluidic chip under a microscope.

2. Setup

The early concept of the setup was to have a syringe pump that would lead to the microfluidic chip and from there, lead to a 3-way valve that would either send the chromosome to a waste container or towards a 96-well plate held within the X-Y stage. A computer would control the valve with the user specifying whether a viewed chromosome was valuable or not. If the user asked for the chromosome to be stored, the valve would change to the 96-well output and wait a predetermined amount of time (dwell time). The timing from when the chromosome was seen in the microfluidic chip to the end of the dispensing nozzle had to be accurate, as this would determine if the correct chromosome was captured. After the dwell time had elapsed, the X-Y stage would automatically move to the next well, ready for another chromosome capture.

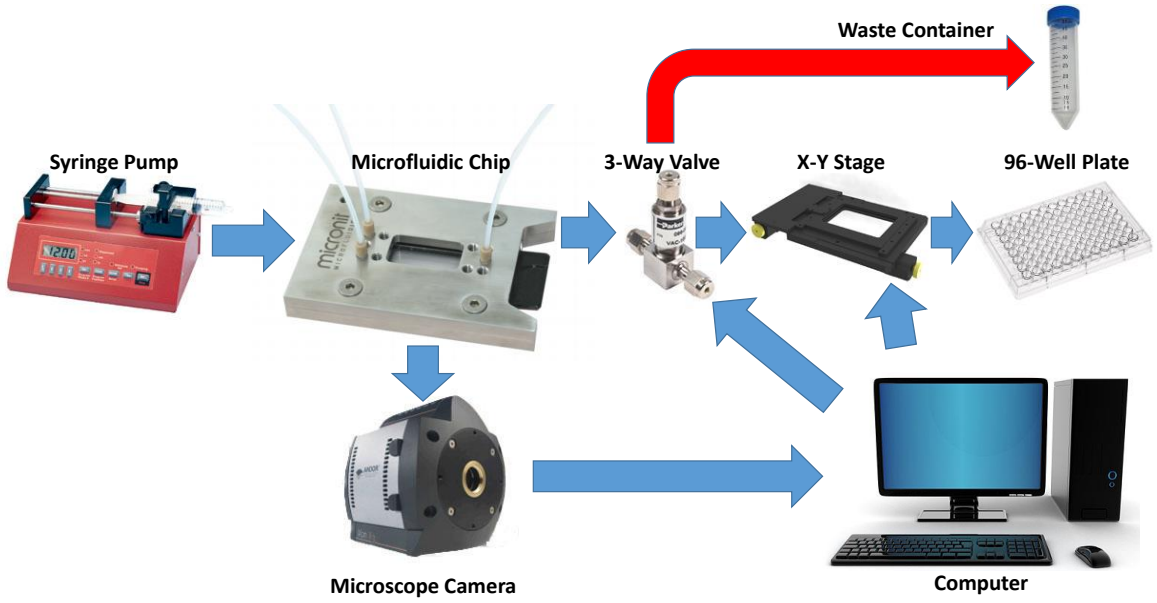

Figure 2. – Early concept of proposed setup.

2.1. – Previous Work

A LABVIEW program created by Benjamin Coles provided a good basis to build on. Its function was to control the X-Y stage and had the capability of conforming to any size well plate with the added benefit of having a configurable dwell time.

2.2. – Initial Setup

The first setup consisted of: an Aladdin syringe pump, a Micronit Fluid Connect 4515 with a microfluidic flow-cell chip (FC_FLC50.3_Pack), a manual 3-way valve, two Parker Series 9 2-way solenoid valves, a Märzhäuser 2-phase X-Y scanning stage, a Nikon Eclipse TE2000-E fluorescence microscope with an Andor iXon3 885 camera and a computer. 1/16” OD (0.5mm ID) was used to connect the pump and microfluidic chip until the 3-way valve where this was

increased to 1/8” OD (1.5mm ID) for the rest of the setup. A 10µL pipette tip was used as a dispensing nozzle.

Since a 3-way solenoid valve wasn’t available at the time, a manual 3-way valve was attached to two, 2-way solenoid valves to create a makeshift, electronically controlled 3-way valve. The valves were powered by a single power supply at 24V, 500mA and were controlled by a TTL SPDT relay that was connected to a National Instruments DAQ USB-6008. The NI data acquisition device was controlled by a modified version of Benjamin Coles' LABVIEW program. The modified program would now control the valves, changing to the 96-well plate output during dwell time and then reversing back to the waste container. A circuit diagram of how the valves were connected is shown below in figure 3.

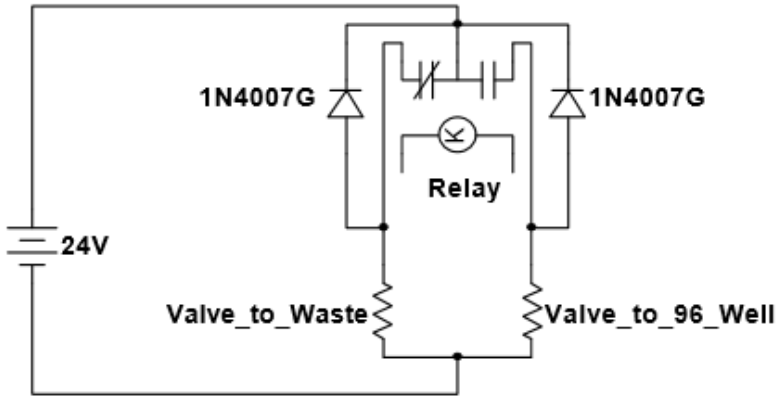

Figure 3. – Diagram of the circuit controlling the valves. Please note that the valves are represented as resistors. The relay has two fly-back diodes to remove voltage spikes when the relay switches. Normally closed, the valve leading to the waste will be open.

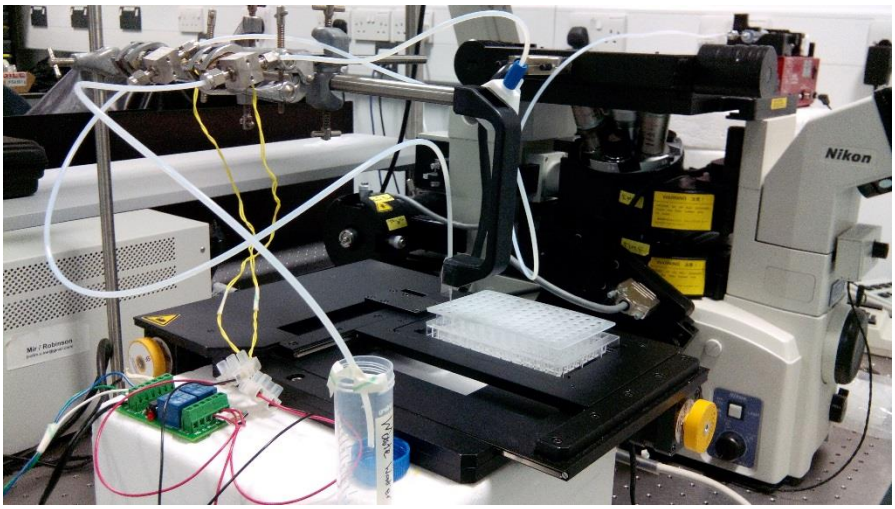

Figure 4. – Picture of the initial setup.

2.2.1. – Setup Testing

During a first trial run of this setup, using the 1000µm channel on the flow-cell and Milli-Q water pumped through the system, the valves were reaching close to their operating temp of 105°C. This caused the valves to act elastically when opening and closing, causing the droplet rate through the dispensing nozzle to be erratic. A more significant problem was that the chromosomes passing through the valves were at risk of being denatured. An aluminium heat sink was added to try cool the valves, but to no avail.

2.3. – Second Setup

The temperature issue with the valves was solved by having a secondary power supply at 5V that the valve would switch to after receiving a brief pulse of 24V (show in figure 5). The lower voltage would reduce power consumption and therefore decrease heat produced while still allowing the valve to remain open. A second TTL SPDT relay was added to the system for this functionality and the LABVIEW program was again modified. The circuit diagram of the modified setup is shown below.

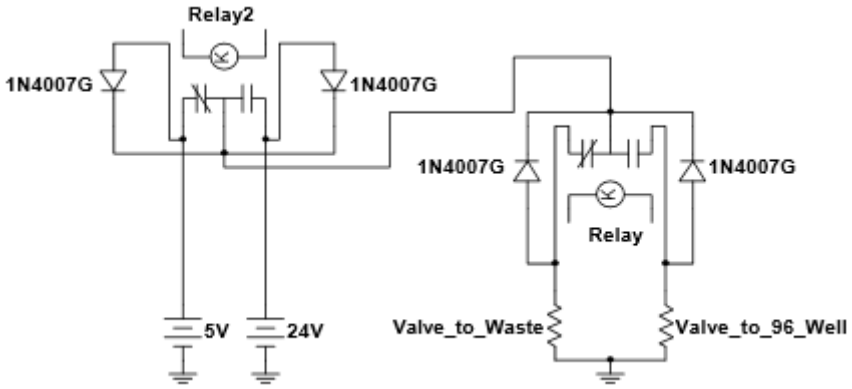

Figure 5. – Diagram of modified circuit controlling the valves. The second relay (Relay2) now can provide two voltages to a valve. Again, please note that the valves are represented as resistors and fly-back diodes have been added to remove voltage spikes.

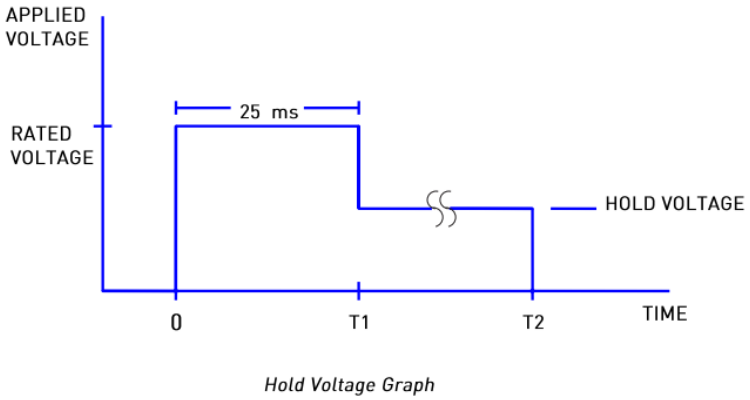

Figure 6. – Graph showing the minimum time required to stay at the rated voltage (24V) before dropping down to the hold voltage (5V) [1]. In the LABVIEW program this was set to 100ms.

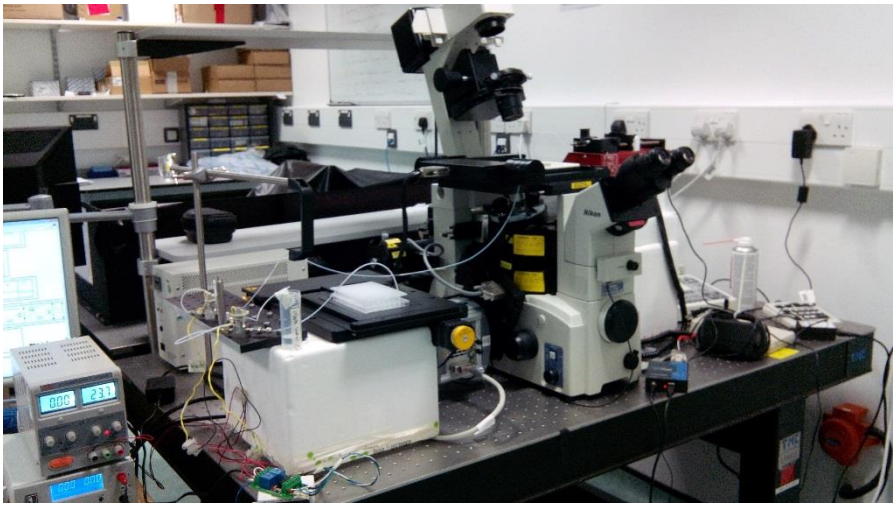

Figure 7. – Picture of the second setup (secondary power supply and heat sink added).

2.3.1 – Setup Testing

For this trial run, the 1000µm channel was used again and Milli-Q water was pumped through the system. This time, the valves stayed just slightly above room temperature. Flow rate was set to 10µL/min (same as the previous trial). The time for a droplet to form at the dispensing nozzle and to drop into the well was recorded. These results were compared to the previous trial as shown in figure 8.

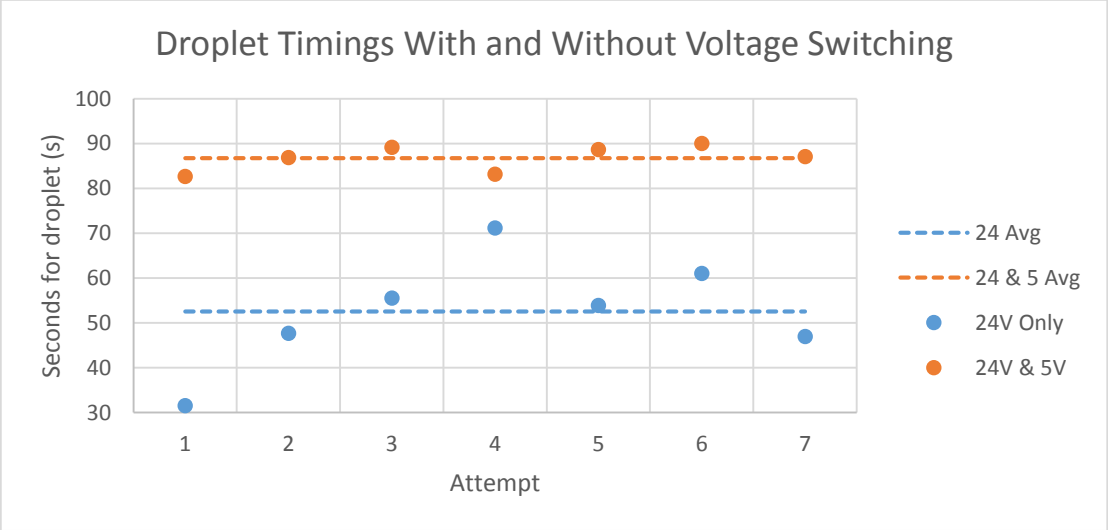

Figure 8. – Time for droplets to form and drop (at 10µL/min) with and without voltage switching (24V & 5V and 24V only respectively).

As seen in figure 8, although the mean time for a droplet is larger without voltage switching, the timings are much more consistent with it.

After the temperature problem had been solved, analysis began on the time taken for the chromosome to traverse the setup after it had been viewed. For testing purposes 1µm red

latex beads were used (Life Technologies F-8819). Using equation 1, a water to bead solution was created of 2,000,000:1 which contained approximately 18 beads/µL.

$$Number\ of microshperes/mL = \frac{6C \times 10^{12}}{\rho \times \pi \times \phi^3}\ (eq.1)\ [2]$$

Equation 1. – Where C is the concentration of suspended beads in g/mL (0.02 g/mL for a 2% suspension), ϕ is the diameter of microspheres in µm and ρ is the density of polymer in g/mL (1.05 for polystyrene) [2].

Using a flow-cell chip with a channel of 1000µm width and 20µm depth (1.9µL channel volume), and a 20x air objective, a rate of dispensing had to be found that would allow the beads to be visible. 100µL/min was too fast and so was 10µL/min. 1µL/min seemed to provide the best rate at which the beads were visible for a reasonable amount of time to the user.

With a viewing rate now found, the time to traverse the setup was calculated theoretically using the volume held inside the tubing, within the valves and also half of the channel volume (half as the objective should be placed in the middle of the chip). Using the combination of 1/16" and 1/8" tubing, the volume was calculated to be approximately 1280µL. At a pumping rate of 1µL/min, the user would have to wait almost 21 hours before the wanted chromosome reached the dispensing nozzle.

Clearly this was unacceptable and so reducers were bought to bring the tubing size down to 1/16" (0.5mm ID) throughout the setup. The volume was calculated again and came to be about 536µL. Although better than the previous setup, the user would still have to wait around 9 hours.

The valves presented a problem in that they had a volume capacity of 342.7µL. At a pumping rate of 1µL/min, it would take the chromosome 6 hours to pass through the valve alone. Since the waste was in the order of a few hundred µL, the decision was made to drop the valves from the setup. Waste would now be stored in specific wells on the 96-well plate.

The piping was shortened from 95cm to 39cm and the volume was calculated to be 82.5µL. This was chosen to be the basis of the final setup.

Piping Size	**1/8" & 1/16"**	**1/16"**	**1/16" (Shortened & No Valve)**
Internal Volume (µL)	1280	536	82.5

Table 1. – Internal volumes held by the different piping sizes and setups. (1/8" ID 1.5mm, 1/16" ID 0.5mm, Valve held 342.7µL)

2.4. – Final Setup

Although there was a significant reduction in waiting time, more improvements were needed. By controlling the pump, the time taken to traverse the setup could be reduced by speeding up the pump after viewing the chromosome. This was done through the use of the RS232 interface of the pump. A custom DB9 RS232 to 6P4C RJ14 cable was created for this purpose. A LABVIEW program was also created to control the pump.

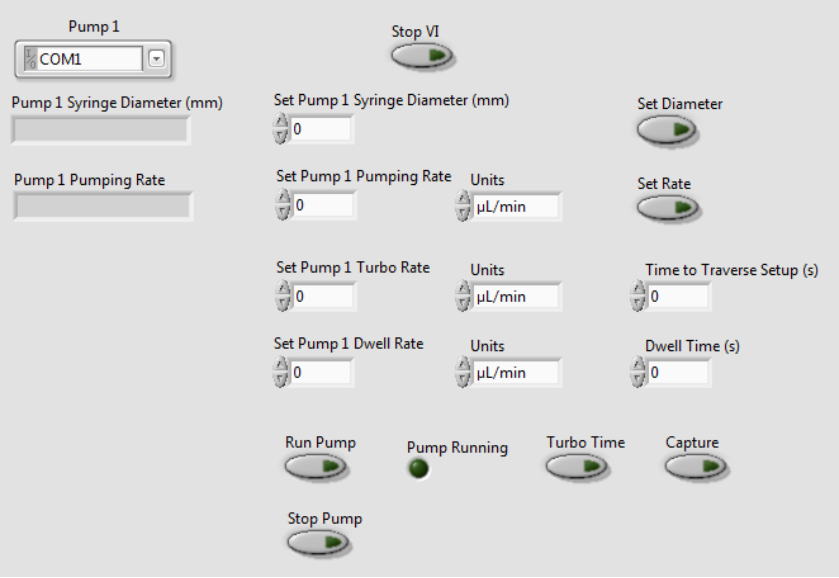

Figure 9. – User interface of the LABVIEW pumping program.

When the turbo button was pressed, the pump would accelerate to the turbo rate for a specified amount of time, with both parameters being determined by the user. With the turbo rate set to 100µL/min, the wait time was calculated to be 49.5 seconds, much faster than previously. The user would now have to manually select his/her own save and waste wells through the X-Y stage LABVIEW program, although this can be automated with further programming.

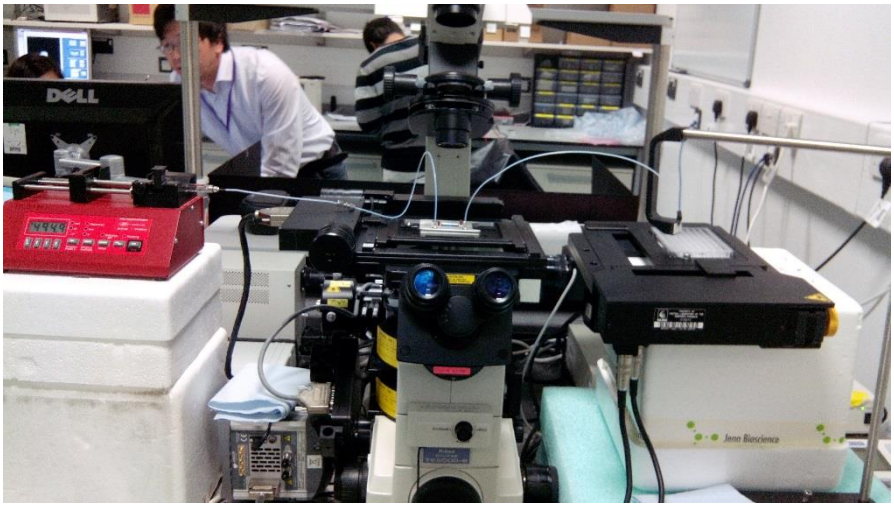

Figure 10. – Picture of the final setup (valves removed & shorter piping).

3. Results and Discussion

Beads were used again for testing purposes. To make sure the correct beads were being captured, a mixture of 4µm yellow-green (Life Technologies F-8859) and red (F-8858) beads were used at different concentrations.

Bead	4µm Yellow-Green	4µm Red
Ratio (Water : Bead Solution)	1,000,000:1	100,000:1
Beads/µL	0.568	5.68
Beads/s (at 1µL/min)	105	10.5

Table 2. – The properties of the two different solutions of beads that were mixed together.

The viewing rate was set to 1µL/min and turbo rate to 100µL/min with the time to stay at the turbo rate set to 49.5 seconds as calculated previously.

3.1. – Results

Figures 11 to 13 show beads that were captured and imaged through the 96-well plate. Figures 14 to 16 show beads that were pipetted out from the wells and put onto a microscope slide.

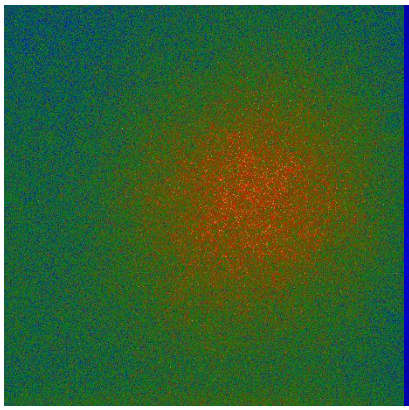

Figure 11. – Green bead captured in well A1. (False Colour)

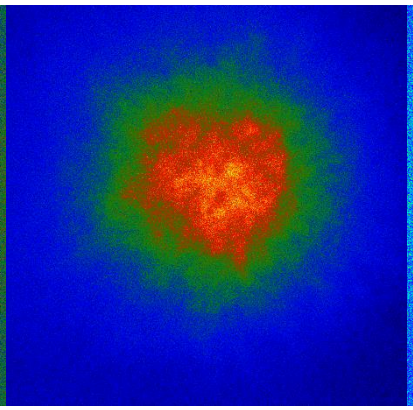

Figure 12. – Red bead captured in well A2. (False Colour)

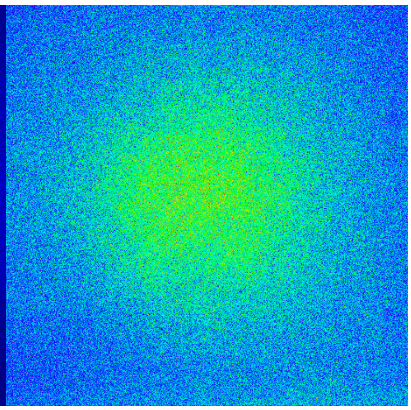

Figure 13. – Green bead captured in well A3. (False Colour)

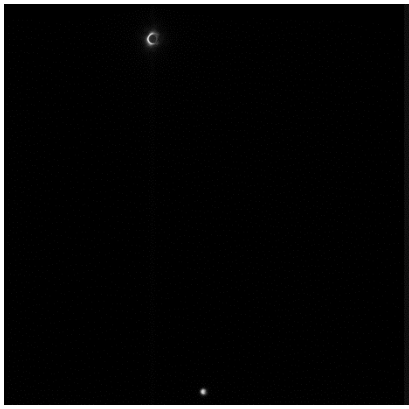

Figure 14. – A1 contents on a microscope slide. (Both Beads)

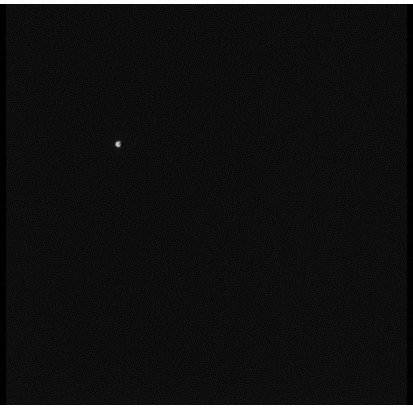

Figure 15. – A2 contents on a microscope slide. (Red Bead)

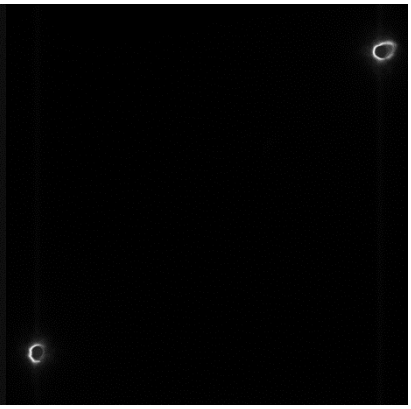

Figure 16. – A3 contents on a microscope slide. (Gr. Beads)

3.2. – Discussion

Due to frosted nature of the bottom side of the well, imaging the beads directly through the plate proved to be difficult. As seen in figures 11 – 13, the images are out of focus and false colour had to be used to improve the contrast of the images. It was difficult to tell whether the correct bead had been captured and so the contents of the well were transferred to a microscope slide.

From figures 14 & 16, there seems to be more beads than previously thought. This could have been caused by the limited viewing scope of the camera. The current objective (20x) with the camera resolution (1004 x 1002, 8µm x 8µm) provides approximately 400um of viewing space, leaving 600um of un-viewed space. A 500um channel was on the chip but was blocked with no replacements onsite. This also may be due to the concentrations of the bead solutions being too high in conjunction with the droplet size (≈15µL). Theoretically at 15µL, there should be 9 green and 85 red beads in each droplet but in reality this wasn't the case.

Another slight problem was that when switching back to the viewing rate from the turbo rate, there was a slight delay in the beads returning to their original speed. When accelerating to the turbo rate however, the beads have an almost instantaneous increase in speed.

4. Conclusion

During this project, obtaining a single bead from the microfluidic setup proved to be quite difficult. Certain enhancements could be made to the setup to make it more accurate.

Switching to a either a non-inverted fluorescence microscope or using a 96-well plate without frosted plastic would greatly improve the quality of images taken and would remove the need for transferring the contents to a microscope slide.

Additionally, to improve the accuracy of capturing beads, switching to a channel of 500µm width would reduce the likelihood of error as there would less space for beads to pass unnoticed. A more expensive solution would be to ask Micronit to fabricate custom 400µm flow-cell chips. Obtaining and using a 10x objective would also alleviate the problem although the user would find it harder to view the beads.

To reduce the amount of beads that accumulate in the well, lower concentrations of bead solutions could be used. Furthermore, instead of using a 10µL pipette tip, purchasing a dispensing nozzle which creates a droplet size of less than ≈15µL would help. Droplet size could also be reduced by using the X-Y stage to "cut" the droplet using the side of the well before it is fully formed.

There is also room for improvement in the LABVIEW programs. Integration of the pump and stage programs and having automatic stage movement would increase productivity and usability.

The author believes that this setup can eventually be used to capture a single chromosome inside a well but not before several upgrades are made.

5. Acknowledgements

I'd like to thank Professor Robinson and Yusuf for making this project possible. Special thanks to Benji who helped me with LABVIEW and letting me borrow a lot of equipment and also to Deep who made the lab a brighter place and taught me how to use a pipette correctly.

6. References

[1] – **Parker.com**, *Series 9 Solenoid Valve Datasheet*, Parker, September 2014, retrieved 10th October 2014 from www.parker.com

[2] – **LifeTechnoliges.com**, *FluoSpheres ® Fluorescent Microspheres*, October 2005, retrieved 11th October 2014 from www.lifetechnologies.com[i]

[i] Equipment Used:
Nikon Eclipse TE2000-E Fluorescence Microscope with FITC filter
Andor iXon3 885 Microscope Camera
Märzhäuser 2-Phase X-Y Scanning Stage
Aladdin Syringe Pump (941-371-1003)
Exmire CMA/Micro-Dialysis Type 1 2.5ml Glass Syringes
Micronit Fluidic Connect 4515
Micronit Thin Bottom Flow-Cell (FC_FLC50.3_Pack)
Greiner bio-one Crystalquick 96 Square Well Plate
Parker Series 9 2-way Solenoid Valves (SN: 009-0270-900)
Mastech DC Power Supply (HY3003D)
DC Power Supply (EA-PS 2042-10 B)
TTL Twin Relay Board (can be replaced by PPUSB-RLY02)
NI DAQ USB-6008 (can be replaced by PPUSB-RLY02)

Piping:
1/8” OD, 1.5mm ID
1/16” OD, 0.8mm ID (Syringe Fitting)
1/16” OD, 0.5mm ID

Life Technologies Fluorescent Beads:
1.0µm Red (F8819)
4.0µm Yellow-Green (F8859)
4.0µm Red (F8858)
10µm Orange (F8833)