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Development of a robotic system interfaced to a microfluidic device to isolate a single chromosome

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Abstract

Polyamine chromosomes in solution are used for various applications where the quality of the isolated chromosomes used as starting material is a key factor. Here we describe our current efforts for obtaining a single chromosome using microfluidics and collecting using a robotic device. Beads of similar size to the hydrodynamic radius of chromosomes were used to characterize and describe the instrumentation development as well as to aid in future improvements as needed. The procedures described here have the potential to provide a rapid and efficient way to isolate highly pure human chromosomes as starting material for many applications thus offering exciting new possibilities.

Keywords: Chromosome, microfluidic, micromanipulation, microscopy, chromatin

Introduction

Mitotic chromosomes are used for a number of different applications such as generation of chromosome paints (Yang *et al*., 2009), chromosome structure studies (Yusuf *et al*., 2014)), extracting proteins (Uchiyama *et al*., 2005) and genome analysis (Fan *et al*., 2011, Marie *et al*., 2013). Such applications require an efficient collection of purified chromosomes in high yields using several methods (Sone *et al*., 2002, Wray and Stubblefield, 1982, http://dx.doi.org/10.1038/nprot.2008.166, Tulp *et al*., 1980, Yusuf *et al*., 2014). The collection of a single chromosome would also be beneficial for many applications such as new Hi-C studies (Nagano *et al*., 2013). Currently the best methods for collecting single chromosomes includes using laser microdissection and flow cytometry (Dolezel *et al*., 2012). Even though these methods are useful they can be time-consuming, involve specialised equipment, can be costly, require trained technicians and the recovered chromosomal material can be damaged. Recently, Quake and co-workers (Fan *et al*., 2011) used a microfluidic system for partitioning chromosomes into different chambers within a device before genotyping and sequencing individual chromosomes. This is a relatively complex device performed in-chip separation and amplification but did not make the intact chromosomes available for use outside the device. This highlights the need for a much quicker, simpler and cheaper method to obtain purified chromosomes. This report describes our progress and attempts for developing a robotic microfluidic delivery system that we demonstrate using beads and should be useful for collecting individual chromosomes.

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Material and methods

Microfluidic device

The microfluidic setup was purchased from Micronit (Netherlands). The microfluidic flow-cell chip (Connect 4515) consisted of channels of 1000 μm width (Micronit Thin Bottom Flow-Cell (FC_FLC50.3_Pack)). This had 1 inlet and 1 outlet. The height of the channel is 20 μm such that particles as large as 15 μm can be handled with little difficulty.

Bead samples used for microfluidic device

For system characterisation purposes 1 μm red latex beads were used (Life Technologies F-8819, 535-575nm emission wavelength). Using equation 1, a water to bead solution was created of 2,000,000:1 which contained approximately 18 beads/μL.

$$\frac{\text{Number of microspheres}}{\text{mL}} = \frac{6C \times 1012}{\rho \times \pi \times \varphi 3} \quad \text{(eq.1)}$$

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); (Invitrogen Detection Technologies (2005)).

A mixture of 4μm yellow-green (Life Technologies F-8859) and red (F-8858) beads were also used at different concentrations.

Instrumentation used for microfluidic robotic system

The setup consisted of an Aladdin syringe pump (Model No. AL-1000, USA), a manual 3-way valve, two Parker Series 9 2-way solenoid valves, a Märzhäuser 2-phase X-Y scanning stage, an inverted Nikon Eclipse TE2000-E fluorescence microscope with an Andor iXon3 885 camera and a computer, see Figure 1. 1/16" OD piping (0.5mm ID) was used to connect the pump to the inlet of the microfluidic chip and from the outlet to the 3-way valve where this was increased to 1/8" OD (1.5mm ID) for the rest of the setup. A 10 μL pipette tip was used as a dispensing nozzle. The valves were powered by a single power supply at 24V, 500mA and were controlled by a TTL Single-Pole-Double-Throw (SPDT) relay that was connected to a National Instruments DAQ USB-6008. The NI data acquisition device was controlled by a LabVIEW program.

Setup of the microfluidic robotic collection system

The syringe pump was used to pump samples at the rate of 1, 10 or 100 μL/min to the microfluidic inlet via tubing. Once the sample was observed in the microfluidic channel using fluorescence from the beads at the microscope stage and a 20x objective, the sample flowed to the outlet via tubing. A 3-way valve was used to send the sample either to a waste container or towards a 96-well plate held within the X-Y stage. The setup can be seen in Figure 1. A LabVIEW program was used to control the X-Y stage and had the capability of utilising any size well plate. 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 LabVIEW program. The program controlled the valves, changing to the 96-well plate output when the user specified and then reversing back to the waste container. To prevent valve heating, an aluminum heat sink was used to aid in heat dissipation. This solution proved to be inadequate and so a secondary power supply at 5V was added. The valves switch to the 5V supply after receiving a brief pulse of 24V resulting in a ~82% reduction in power consumption (3 watts at 24V, 0.52 watts at 5V). A second TTL SPDT relay was added to the system for this functionality and the LabVIEW program was modified.

For the final setup and to speed up the collection process, reducers were used to shorten the tubing size down to 1/16" (0.5mm ID) throughout the setup. Valves were also removed and the waste was stored in specific wells on the 96-well plate. Faster collection was also achieved by speeding up the pump after viewing the sample under the microscope. This was done through the use of the RS232 interface of the pump. A LabVIEW program was also created to control the pump. Upon initiating the pump function, it accelerated to the turbo rate for a specified amount of time, with both parameters (acceleration and turbo rate) being determined by the user. With the turbo rate set to 100 μL/min, the save and waste wells were manually selected by the user through the LabVIEW program. For the final collection of beads (yellow and red) the viewing rate was set to 1 μL/min and turbo rate to 100 μL/min with dwell time at the turbo rate set to 49.5 seconds as calculated.

Results and Discussion

In the present work we describe a microfluidic flow approach that could be used to isolate single chromosomes. For the microfluidic robotic system development, the concept of the setup was to configure a syringe pump system that would allow the development of a microfluidic chip and incorporating a 3-way valve to either transfer the chromosome to a waste container or towards a 96-well plate held within the X-Y stage. The valve with the user specifying whether a viewed chromosome was valuable or not would be under a computer control. If the user requests for a certain chromosome to be stored, the valve operation automatically changes to the 96-well output, waiting a predetermined amount of time (dwell time). The timing from the initial sample observed in the microfluidic chip to the end of the dispensing nozzle needs 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 sample capture. The initial proposed setup for the robotic microfluidic system can be seen in Figure 1.

Following pumping Milli-Q water through the microfluidic device (as a cleaning routine) and the entire system, the valves were reached close to their operating temperature of 105°C. This caused the valves to act in a non-systematic manner when opening and closing, causing the droplet rate through the dispensing nozzle to be erratic. This was problematic for biological samples such as chromosomes as they could be denatured when passing through the valves. A circuit diagram of how the valves were connected is shown below in Figure 2.

Figure 3 shows a circuit diagram after adding the supply at 5V that reduced power consumption and therefore decreased the heat produced while still allowing the valves to remain open.

The fly-back diodes were added to prevent voltage spikes that occur when power is removed from an inductive load such as an electro-magnetic valve. With the addition of the 5V supply, the valves remained slightly above room temperature (~29°C). The time for a droplet to form at the dispensing nozzle and to drop into the well was recorded (Figure 4).

As seen in Figure 4, although the mean time for a droplet is larger without voltage switching, the timings are much more consistent with it. To find the rate of dispensing that would allow the beads to be visible, several pumping speeds were investigated. Both 100 μL/min and 10 μL/min appeared too fast. Operating the pump at 1 μL/min provided the best rate at which the beads were visible. With the viewing rate optimised, 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 microscope 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 1280 μL. At a pumping rate of 1 μL/min, the user would have to wait almost 21 hours before the chromosome of interest to reach the dispensing nozzle. To

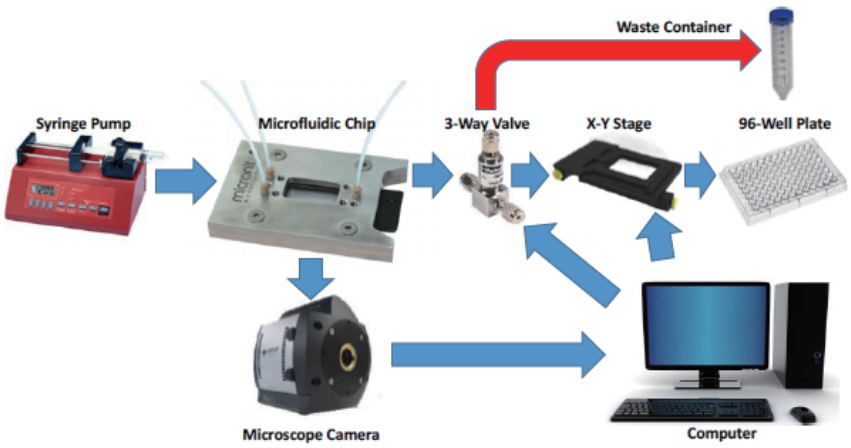

Figure 1. Schematic of initial concept of proposed setup.

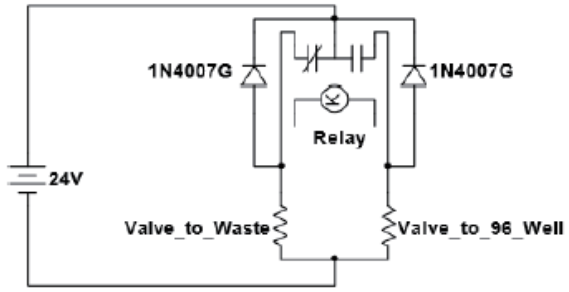

Figure 2. Diagram of the circuit for the valves control. Valves are represented as resistors. The relay has two fly-back diodes to remove voltage spikes when the relay switches are active. Normally closed, the valve leading to the waste will be open.

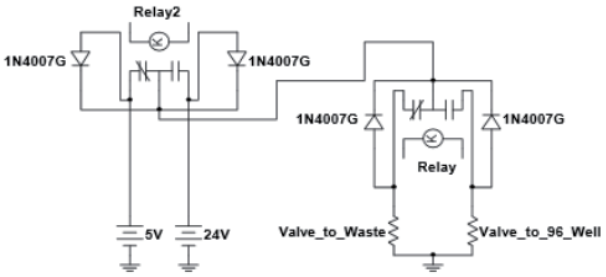

Figure 3. Diagram of modified circuit controlling the valves. The second relay (Relay2) provide two voltages to a valve. Again, valves are represented as resistors and fly-back diodes have been added to remove voltage spikes.

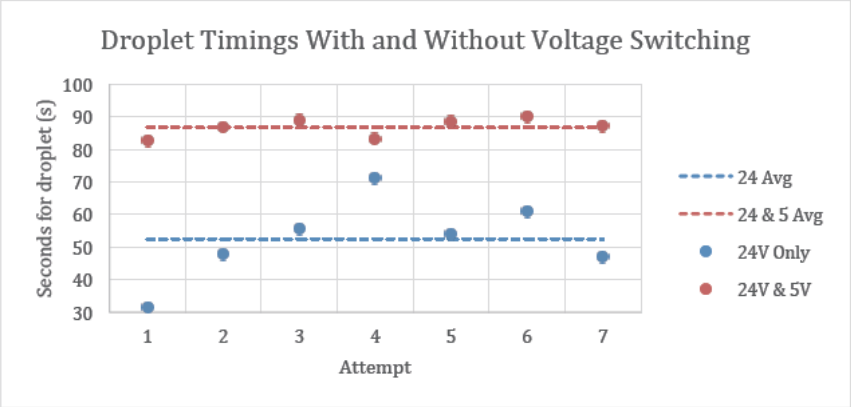

Figure 4. Time for droplets to form and drop (at 10µL/min) with and without voltage switching (24V & 5V and 24V only respectively, frosted). The dashed lines represent the mean time.

solve this problem reducers were used to bring the tubing size down to 1/16" (0.5mm ID) throughout the setup. The volume was calculated again and was found to be about 536 µL which would still take approximately 9 hours. The valves presented a problem in that they had a volume capacity of 342.7 µL. At a pumping rate of 1 µL/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 valves were removed from the setup and waste was stored in specific wells on the 96-well plate. The piping was shortened from 95 cm to 39 cm and the volume was calculated to be 82.5 µL. This was chosen to be the basis of the final setup. By controlling the pump, the time taken to traverse the setup could be reduced by speeding up the pump after viewing the sample with the microscope. The turbo rate accelerated the speed to 100 µL/min that would provide the sample after 49.5 seconds after viewing.

To test the system a mixture of 4µm yellow-green and red beads corresponding to the average size of chromosomes were mixed and collected into the 96 well plate. Due to the frosted nature of the bottom side of the well, imaging the beads directly through the plate proved challenging (Figure 5a, b, c). 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 6a,b,c, there seems to be more beads than previously thought.

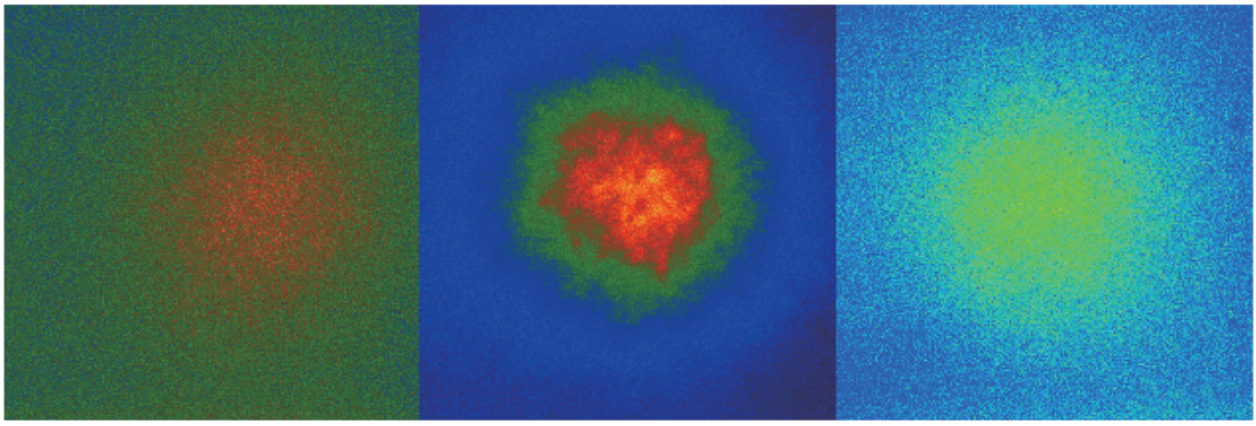

Figure 5a. Green bead captured in well A1. (False Colour)

Figure 5b. Red bead captured in well A2. (False Colour)

Figure 5c. Green bead captured in well A3. (False Colour)

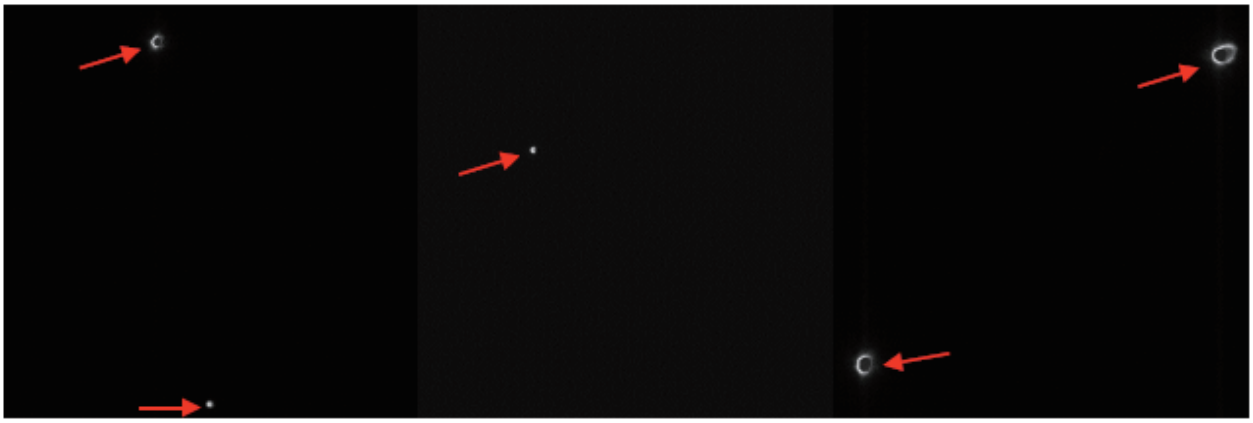

Figure 6a. A1 contents on a microscope slide. (Both Beads)

Figure 6b. A2 contents on a microscope slide. (Red Bead)

Figure 6c. A3 contents on a microscope slide. (Green Beads)

This could have been caused by the limited field of view of the camera versus that of the microscope. The current objective (20x) with the camera resolution (1004 x 1002, 8 µm x 8 µm) provides approximately 400 µm of field of view, along the width of the channel, leaving 600 µm of unviewed space. This also may be due to the concentrations of the bead solutions being too high in conjunction with the droplet size (≈15 µL). Theoretically at 15 µL, there should be 9 green and 85 red beads in each droplet but in reality this wasn't the case. Another 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. Figures 5a,b,c show beads that were captured and imaged through the 96-well plate. Figures 6a,b,c show beads that were pipetted out from the wells and put onto a microscope slide.

Conclusions

Obtaining a single bead from the microfluidic setup proved to require more time than anticipated using microfluidic flow alone. 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 for post imaging. 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. However optimization for 4µm beads may not be optimum for chromosomes. A more expensive solution would be to fabricate custom 400µm flow-cell chips. Using a 10x objective would also alleviate the problem although the user would find it harder to view the bead. 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 be advantageous. 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 program. For example, integration of the pump and stage programs under the same LabVIEW program and having automatic stage movement would increase productivity and usability. The co-authors believe that this setup may eventually be used to capture a single chromosome inside a sample tube following several upgrades made to the current setup.

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