Report: Establishing Low Cost Microfluidic System for Single Cell Analysis

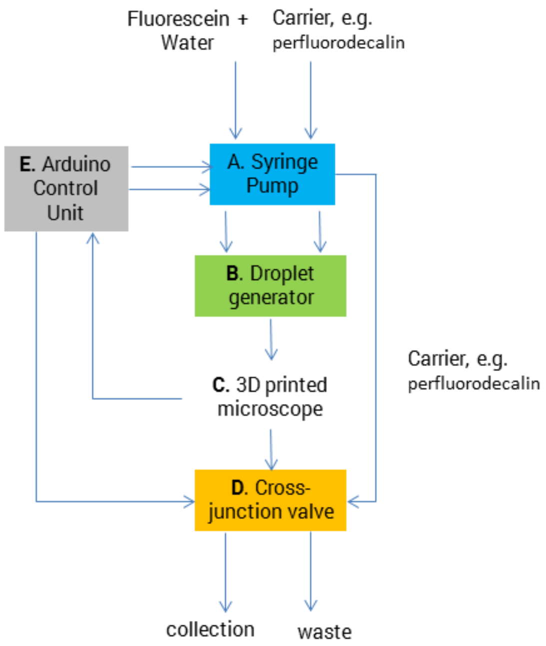

Figure 1: Schematic of the proposed microfluidic device.

Project Outputs:

- 3D printed fluorescent microscope.
- 3D printed syringe pump.
- Arduino controller and software.
- A droplet generator prototype.

Outstanding Outputs:

The following outputs are dependent on generating consistent droplet sizes so are awaiting completion.

- A cross-junction for droplet sorting.
- Detailed information on range of protoplast sizes.
- All designs will be made openly available on microfluidics.org (MIT).
- An open access publication detailing the outcomes.

Additionally, in the original proposal it was stated two working devices would be created. However, as the project is under development only one device has been produced so far.

Unforeseen Circumstances:

Unfortunately the member of the team initially responsible for 3D printing (Neil Pearson) left employment at the Earlham Institute half way through the project. It was therefore not possible to iterate through (1) syringe pump design and (2) 3D printed droplet generator designs during the project. To address this Steven Burgess is awaiting training on Ultimaker 3D printer at Cambridge Makespace.

Part A: Producing a Syringe Pump

The first iteration of a syringe pump was produced and assembled following the open source design from (Patrick et al., 2016)(Figures 1-4). Arduino control of the A4988 Stepper Motor Driver Module (#02258664; http://www.geeetech.com/wiki/index.php/StepStick_A4988_Stepper_Driver_Module) was step up following instructions adapted from: http://howtomechatronics.com/tutorials/arduino/how-to-control-stepper-motor-with-a4988-driver-and-arduino/ and performed as expected. For wiring diagram see Figure 3. Testing of the syringe pump design identified several limitations:

***The use of a flexible connector** to couple the motor and rod resulted in vibrations. This became a problem at small motor step sizes in the motor as the vibrations meant the resulting noise interfered with smooth operation of the pump.
Potential solution: replace connector with rigid alternative.

***The method of syringe attachment to the pump** was a major flaw. Two problems arose – (1) by printing a fixed groove size for the syringe holder, operation is limited to only BD 1ml Plastipak syringes, this was an issue when switching the 1ml Luer-Lok syringes which did not fit. (2) The fitting was too loose, such that when under pressure syringes were wont to jump out of the device. *Potential solution: requires re-design of the syringe casing including adjustable attachment rings to hold syringes in place. Alternatively to switch to a different alternative open source design* (Wijnen et al., 2014)*, or use a solenoid pump.*

***Oscillating flow.** As foreseen in the proposal the stepper motor was insufficiently smooth in operation. The effect of stepping introduced highly variable droplet size in preliminary experiments.

***Controlling Flow Rates:** It became apparent that separate regulation of flowrates for carrier fluids (oil) and buffers are highly desirable to control droplet size.

Potential solution: use multiple syringe pumps, or a solenoid pump.

Part B: Producing a Droplet Generator

A prototype microfluidic chip was produced by cutting 5mm acrylic sheets with a LS 6090 PRO Laser Cutter powered by a 60w CO_2 Water Cooled Laser Tube in Cambridge makespace. Each prototype chip consisted of two components: a bottom piece with channels cut in a cross junction for mixing of oil and water, and a top cover to seal the channel with holes cut for insertion of tubing (Figure 5). The channel width generated by the laser was ~300µm. Testing identified the following limitations:

***Leakage from tube to chip connections:** Inaccuracies in cutting holes for tubing into the top piece of the chip resulted in leaking. Bluetack was unable to provide a proper seal (Figure 7), but this was remedied with the addition of superglue (Figure 8).

***Leakage between upper and lower pieces of the chip:** Three approaches were attempted to hold the upper and lower pieces of acrylic together: neodymium magnets, steel clips (Figure 7) or superglue. All proved ineffective in preventing leakage of liquid out of the microfluidic channels on the lower surface into the interface between the two sheets of plastic (Figure 9). This is presumed to be the result of slight warping on the plastic sheets preventing a tight seal. To address this issue a plastic coating was attached over the lower part of the chip prior to clamping with the top surface to hold the tubes in place. Using this setup it was possible to contain the carrier and buffer liquids within the microfluidic channels and produce water in oil droplets when connected to syringe pump.

Due to the numerous difficulties encountered in the use of laser cut chips, which were also restricted by the lower limit of channel width (~300µm), and roughness of channels cut it was decided to test a capillary system using commercial components.

A 360µm T-junction with 150µm internal diameter was used in combination with 1ml Luer-Lok syringes (Figure 11). This addressed any issue of leaks, and provides a standardized small channel diameter. Full prototyping of the T-junction was prevented by issues relating to the syringe pump – (1) the syringes did not fit to the pump, so where held in place by tape (2) pressure build up on the line from the carrier phase resulted in buckling of the syringe when held in place with tape (3) the requirement for variable flow control over carrier and solute components to facilitate droplet production.

Follow on plans

To demonstrate a working protocol we propose using commercially available components to build a sub £5K device.

Component	Cost
Dolomite (3200319) Pico-Gen (TM) Droplet Chip (60µm width) x2	£636
Dolomite (3000024) Linear Connector 4-way	£255
Travel Costs	£50
Second hand syringe pump from ebay	£1500

Expenditure

Item	Quantity	Cost	Reason
Sylgard® 184	1	82.46	To make PDMS microfluidic chip
PEEK Tubing	1	32.39	To make capillary electrophoresis device, iteration (1)
MicroTee Assay PEEK /16"	1	123	To make capillary electrophoresis device iteration (1)
PEEK Tubing 360µm (Kinesis;1572)	1	36.70 (+VAT)	To make capillary electrophoresis device, iteration (2)
MicroTee Assay PEEK 360µm (Kinesis;P-888)	1	106 (+VAT)	To make capillary electrophoresis device iteration (2)
Luer Lock Connector (VWR; P-662)	4	147.32 (+VAT)	To make capillary electrophoresis device iteration (2)
Luer Lock Syringes (Fisher;10630694)	1 box	27.66 (+VAT)	To make capillary electrophoresis device iteration (2)
3 month Membership of Makespace Cambridge	1	120	Training and access to laser cutter to make prototype microfluidic chips
Raspberry Pi 3	1	35.99	To operate openscope
1x5/6/6.35/8mm Flexible Shaft Coupling Motor Connector	1	1.49	Liquid control iteration 1 (syringe pump)
Screw Rod 150mm	1	4.59	Liquid control iteration 1 (syringe pump)
Screw Rod 300mm	2	10.76	Liquid control iteration 2 (syringe pump)
Nema 17 3D Printer CNC Twophase 4wire Stepper Motor 1.8Deg 17HD3400822B	1	7.82	Liquid control iteration 1 (syringe pump)
Stepper Motor Controller	1	8.99	Liquid control iteration 1 (syringe pump)
Arduino Uno + accessories	1	68.29	Liquid control iteration 1 (syringe pump)
Male-Male breadboard connectors	1	1.13	Liquid control iteration 1 (syringe pump)
HeroNeo® 5pcs Easy Connectors For Led Strip Light 3528 5050 to link Adapter Power Supply	1	1.18	Liquid control iteration 1 (syringe pump)
Raspberry Pi 3 SD CARD	1	7.12	Liquid control iteration 1 (syringe pump)
Fluorinert™ FC-40 (F9755-100ML;Sigma-Aldrich)	1	130.48 (+VAT)	For droplet production
Jeffamine® ED-900 (14527-500ML-F;Sigma-Aldrich)	1	52.67 (+VAT)	For droplet production
Fluorescein (46955-100G-F; Sigma-Aldrich)	1	18.91 (+VAT)	To visualize droplets

Figures

Figure 1: Syringe Pump Motor Assembly

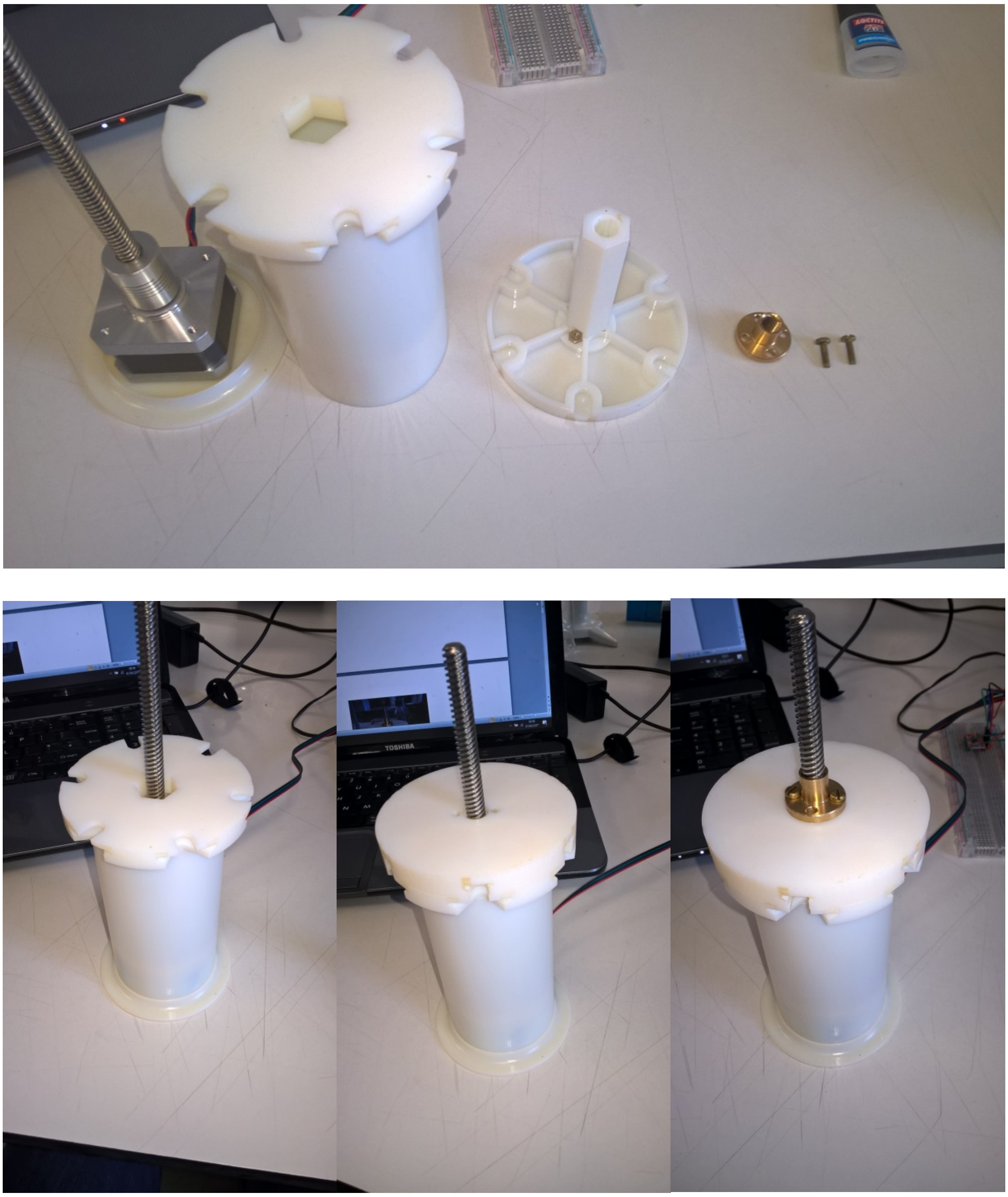

Figure 2: 3D Printed Syringe Pump Assembly

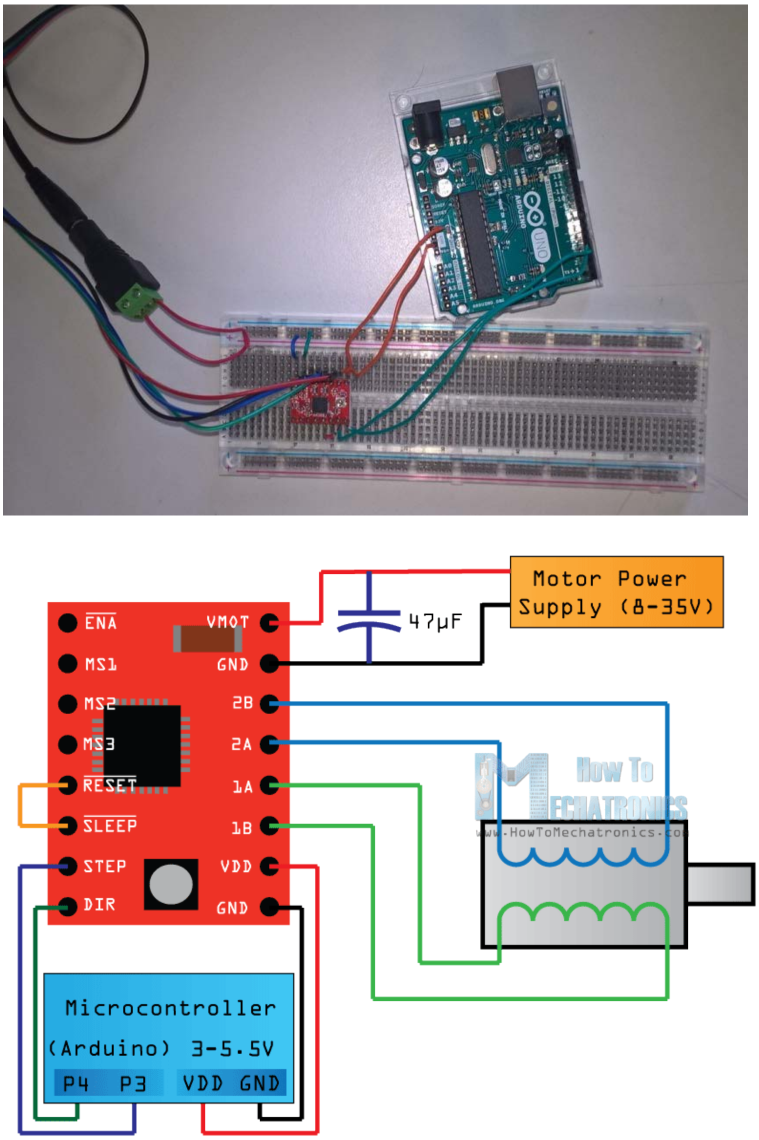

Figure 3: A4988 Stepper Motor Driver Module wiring diagram. Source: Dejan Nedelkovski (http://howtomechatronics.com/tutorials/arduino/how-to-control-stepper-motor-with-a4988-driver-and-arduino/)

Figure 4: Final assembly of the Syringe Pump with 1ml BD Plastipak syringes attached.

Components:

1x5/6/6.35/8mm Flexible Shaft Coupling Motor Connector
8mm Lead Screw Rod Z Axis Linear Rail Bar Shaft 150mm +Nut T8
Nema 17 3D Printer CNC Twophase 4 wire Stepper Motor 1.8Deg 17HD3400822B
A4988 StepStick Stepper Motor Driver
Male-Male breadboard connectors
HeroNeo® 5pcs Easy Connectors For Led Strip Light 3528 5050 to link Adapter Power Supply
Arduino Uno
Laptop
USB Connector

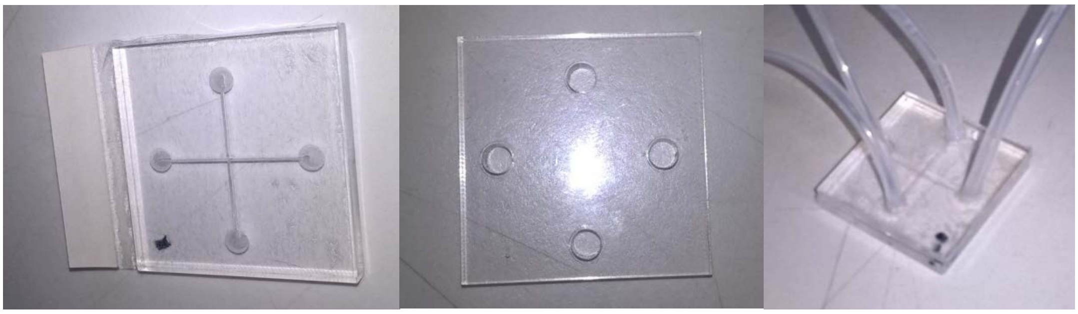

Figure 5: Prototype laser cut microfluidic chip, showing bottom and top pieces of 5mm acrylic cut separately and assembled with tubing (left to right).

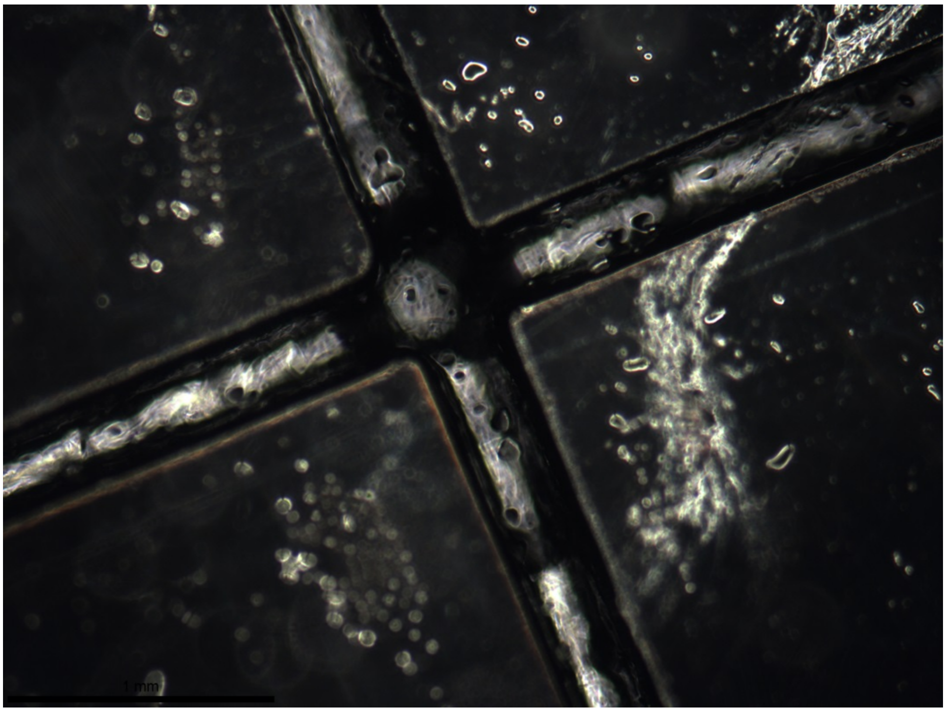

Figure 6: Prototype laser cut microfluidic chip (detail). 4x objective image showing channel width (~300µm) and cross junction.

Figure 7: Prototype laser cut microfluidic chip held together by steel clamps. Showing attempt to seal wires with Bluetack (right) and the resulting leak (left).

Figure 8: Superglue seal of tubing to laser cut microfluidic chip

Figure 9: Prototype Microfluidic chip, leakage of liquid between top and bottom components when sealed with superglue.

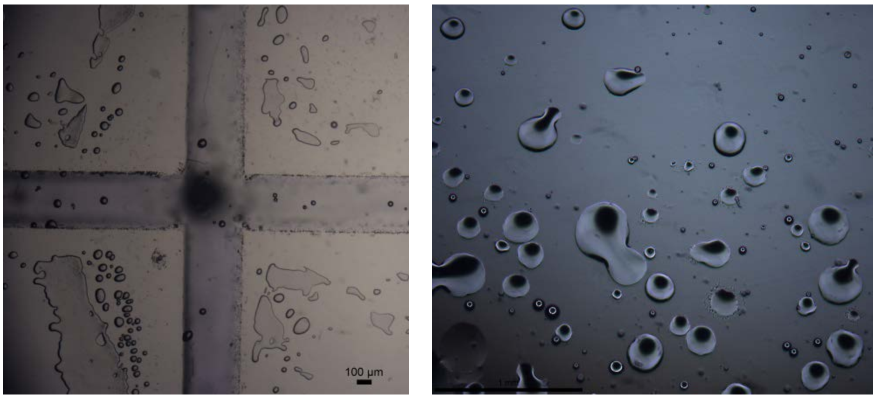

Figure 10: Prototype microfluidic chip. (Right) Liquid contained within channels when plastic cover placed between top and bottom acrylic components. (Left) Water in oil droplets produced from chip.

Figure 11: Capillary droplet generator

Components:

PEEK Tubing 360µm
(Kinesis;1572)

MicroTee Assay PEEK 360µm
(Kinesis;P-888)

Luer Lock Connector
(VWR; P-662)

Luer Lock Syringes
(Fisher;10630694)

Arduino Stepper Motor Script

```
/*     Simple Stepper Motor Control Example Code
 *
 *  by Dejan Nedelkovski, www.HowToMechatronics.com
 *
 */
// defines pins numbers
const int stepPin = 3;
const int dirPin = 4;

void setup() {
  // Sets the two pins as Outputs
  pinMode(stepPin,OUTPUT);
  pinMode(dirPin,OUTPUT);
}
void loop() {
  digitalWrite(dirPin,LOW); // Enables the motor to move in a particular direction
  // Makes 200 pulses for making one full cycle rotation
  for(int x = 0; x <10; x++) {
    digitalWrite(stepPin,HIGH);
    delay(100);
    digitalWrite(stepPin,LOW);
    delay(100);
  }
  delay(1000); // One second delay

}
```