

DROPSEQ FLUIDIC

ODROPSEQFPCK

OBJECTIVE OF THE PROTOCOL

The aim of this protocol is to explain how to reproduce the fluidic part of the Macosco DropSeq protocol using Fluigent pressure controllers instead of syringe pumps.

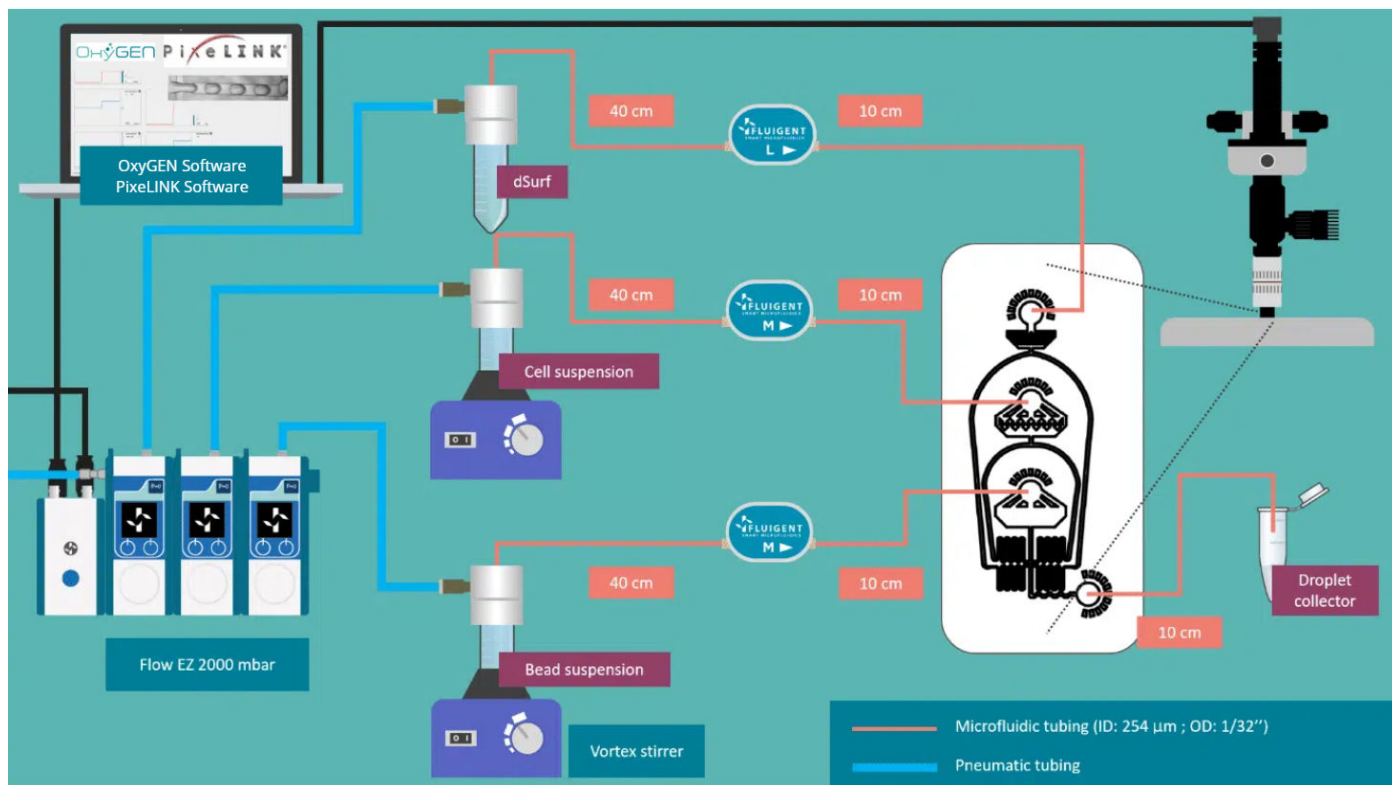
The main advantage of pressure controllers are the very fast response times. Using pressure control, droplets of the right size can be generated in less than two seconds. Using flow sensors, it is also possible to use direct flow control in order to use the pressure controller in the same way you would use a syringe pump.

MATERIALS AND METHODS

To realize this protocol you need the following material:

Product	Reference	Content description
DropSeq package	ODROPSEQFPCK	3 *FlowEZ 2 bar
		1 *Link
		1 *Pcap 15 ml
		2 *Pcap 2ml
		2 *Flow unit M
		1 *Flow unit L
		DropSeq PDMS chip containing 23 designs
		Flow EZ supply kit
		2*Low flow rate kit
		High flow rate kit
Optional product	IMCA001	Digital high speed microscope
	DR-RE-SU-12	12mL dSURF
	FLPG+	Pressure source
External product	Barcoded beads	Barcoded beads: Chemgenes CSO-2011*
	Vortex mixer	Vortex mixer: Sigma, Fisher scientific...

The first step is to recreate the following set up.



PROTOCOL

Preparing the device for a new experiment

When starting a new experiment it is recommended to push out all the air from the chip and the tubings. For that follow the following steps:

1. Before using the PDMS devices, please check the generators carefully. If you have any concerns/questions, please do not hesitate to contact us.
2. Do not fully peel off the film at the beginning: Please cut off the small area for each generator for using. (All of our PDMS chips are covered with Sealing Film, hydrophobic porous sealing film with medical-grade adhesive for tissue culture plates.)
3. Add one drop of dSURF (or Fluorinated oil with other surfactant if not using the dSURF) into the outlet before starting the experiment and wait for a few min. Please increase the flow rates of the F-oil and aqueous phases step by step. Avoid jumping from 0 to ~14 mL/h (or maximum flowrates) directly.
4. Connect the dSURF (or Fluorinated oil with other surfactant if not using the dSURF) tubing to the right entry port on the microfluidic device.
5. Apply a small pressure using the Flow-EZ (about 200 mbar should be enough)
6. Wait until you see some oil coming out of the open in-/outlets of the chip and then set the pressure back to 0 mbar.
7. Start the vortex stirrers and apply a small pressure to the two other reservoirs until you see a small drop of fluid coming out of the end of the tube
8. Stop all pressures and connect all tubings according to the schematic

Launching an experiment

Once the device is connected properly starting a DropSeq run is very fast. Simply apply following pressures to the different reservoirs using the OxyGEN software. The droplets should appear in less than a few seconds.

- P(Oil) = 1300 mbar
- P(Cells) = 540 mbar
- P(Beads) = 580 mbar

These pressures should generate droplets of about 90-130 μm which leads to encapsulation rates from 1 to 3 %).

Tip:

Using the OxyGEN software, pressure configurations can easily be saved. This allows setting all pressures to a given point at the same time.

Flowrate control

Combined with a Flow Unit, the Flow-EZ can be used exactly as a syringe pump. The OxyGEN software allows flowrate commands. A feedback loop on the flowrate then adjusts the pressures to the right value. This technique can be useful to evaluate the necessary pressures for the experiment.

Once in the right state, the pressure configuration can then be saved.

