autoFISH: fluidics system

This document describes how to build a computer-controlled fluidics

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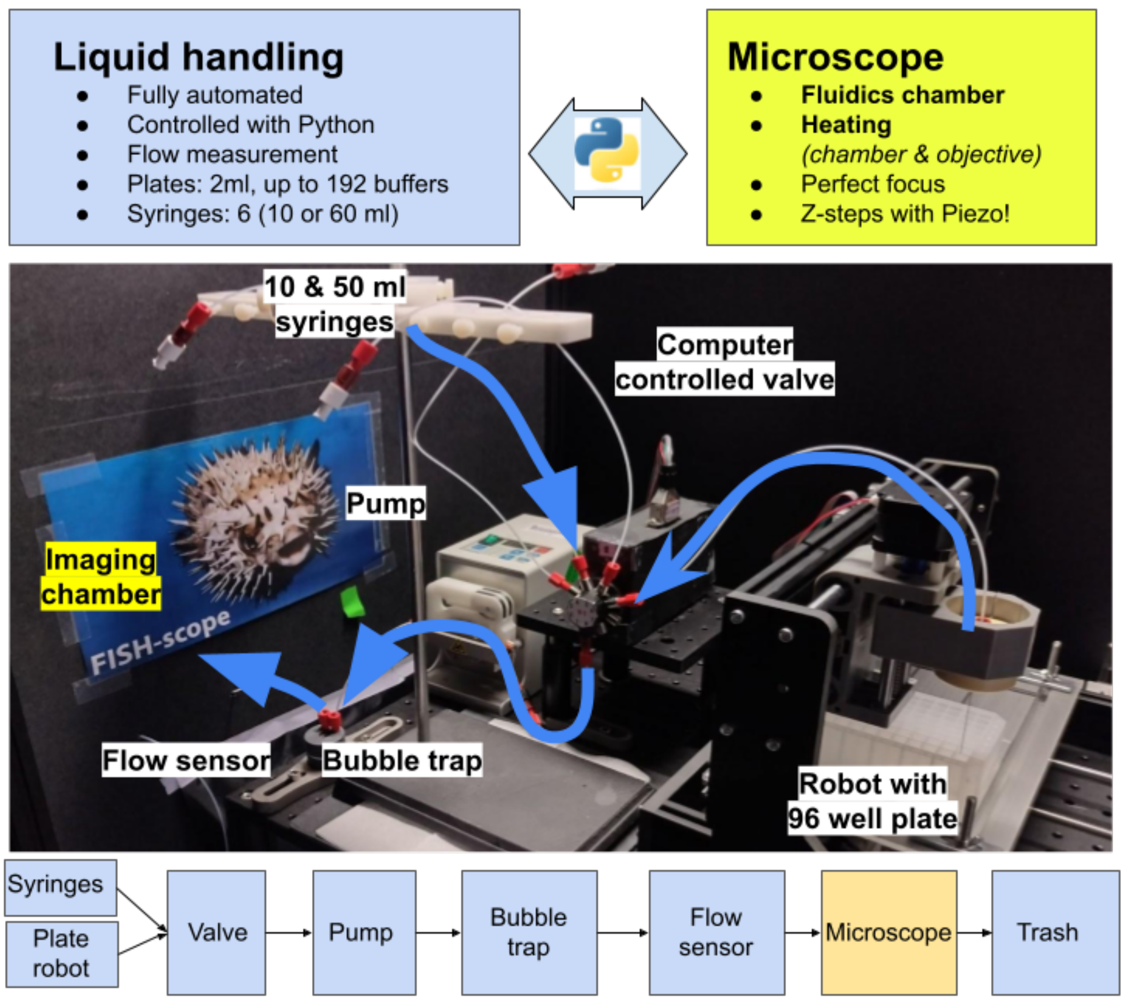
Overview

This custom build fluidics system is based on a published paper for merFISH and ORCA (Moffit JR et al., 2016) but with some modified components as specified below. They main changes are

- **Bubble trap** to remove air bubbles
- **Flow sensor** to measured pumped volume after each step, and stop system in case a problem occurred
- **Pump placement** in the middle of the fluidics circuit. While it pulls liquid out of the different reservoirs, it pushes liquid through both the bubble trap and the imaging chamber. We found that this has several advantages:
    - Reduces air in the system
    - Permits to use the bubble trap efficiently
    - Reduces movement of the coverslip when pump is activated → reduced risk to loss focus
- Combined uses of **computer controlled valve** and **plate robot**: syringes for general purpose buffers, 96 well plate for round-specific buffers.



Building the system

Here, we describe how to build the system starting from the buffer storage to the flow-sensor. For a complete list of all required components, please consult the dedicated section ***Component list***.

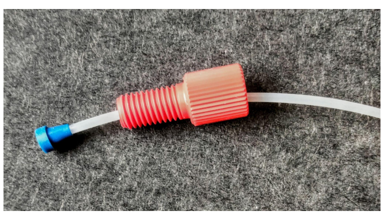
Tubing

We use either **TEFZEL/ETFE** or **PTFE** tubing with a small inner diameter (ID =0.5mm = 0.020") to minimize the amount of required liquid. The other diameter (OD) of 1/16" is standard for many fluidics applications.

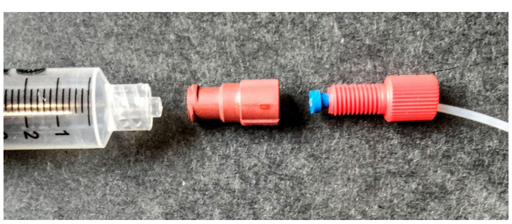
Connections

Several connectors are used to connect the tubing to the different components. Connections are **shown in blue**, we also provide pictures to show how these should be assembled. The two main connection types are:

- Most connections rely on a standard **¼-28 Threaded Connection**, where you use nut (blue) and a threaded connector (red) to connect the tube with another connector



- Other connections are based on **male or female Luer locks** (commonly found on syringes). Show below is how such a Luer connector can be used in combination with a threaded connection to connect the fluidics tubing to a syringe.



Used connectors

P-658	1/4-28 Female to Female Luer	Connects on syringe (male Luer) and connects to XP-202	

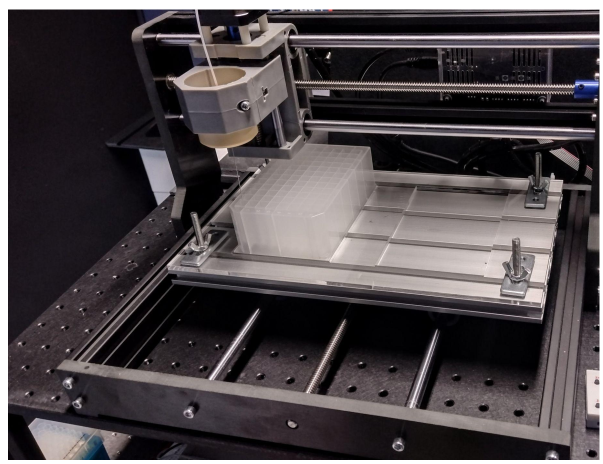
XP-202	Flangeless Fitting Delrin, ● 1/4-28 Flat-Bottom ● For 1/16" OD	Connect tubing to a threaded ¼-28 Port.	
P-655	Luer Adapter Assembly 1/4-28 Female to Male Luer	Connects to output of valve (female Luer) allows attachment of XP-202	
P-646	Barbed to Threaded Male for soft 1/16" ID tubing, ¼ -28 male	To connect soft tubing (imaging chamber)	
P-603	Standard Union Delrin, ¼-28 Port	Screws on IDEX P-646 and allows connection of XP-202	
P-692	Conical adaptor for soft tubing, ¼-28 male.	To connect soft tubing (vacuum pump)	

Buffer storage 1: liquid handling robot

We used a 3D CNC robot, where we replaced the CNC router with a custom printed syringe holder. We also used a plexiglas with 2D cutouts for two 96 well plates.

- **Model**: CNC 3018 Pro
- **Important**: has to be controlled by **GRBL**
- **Hamilton needle**
- **Connection**: **IDEX P-655 and P-202**

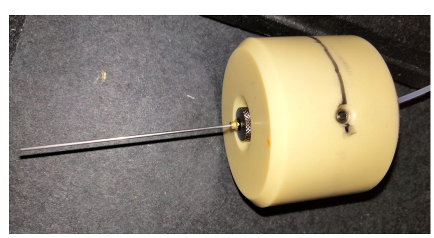
Note: **we glued the connectors together**, since they can wiggle free when the robot moves a lot.



Needle holder

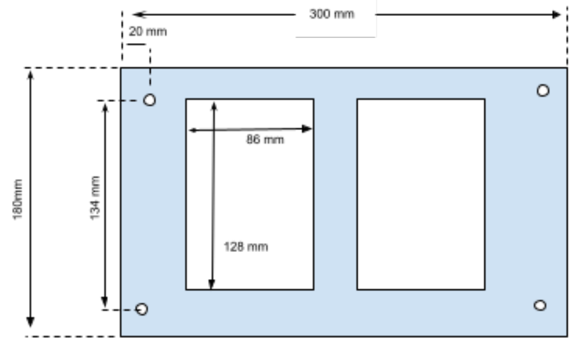
We 3d printed a needle holder that fits in the spindle holder. This is essentially a cylinder with a central hole to accommodate the needle.

We further added a headless screw that can be used to secure the needle firmly.



96 well plate holder

To securely place the 96 well plates on the robot, we use a holder made of sturdy cardboard or plexiglass.

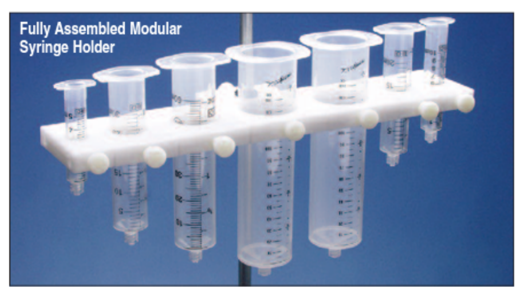


Construction and testing of robot

1. Build according to instructions.Once build, you should already be able to move the robot with the provided joystick.
2. Install provided driver (for our robot it was named `CH340SER.exe`)
3. Connect USB to computer.
4. Determine your Machine's COM port (see below).
5. Open provided control software (for our robot `grblControl`)
6. Attempt to move the robot from within the software.
7. If this works, you should be able to use the robot in our Python control software.

Buffer storage 2: syringe holder

For general purpose buffers, e.g. for washing or imaging, we use syringes (from 10ml to 140 ml) with a female Luer lock. We use a modular syringe Holder from Warner instruments permitting to assemble a syringe holder accepting different volumes, as well as the corresponding Support Stand (**W3 64-0162**).



Connection: **P-658 and XP-202**

When the system is not used, we wash all fluidic lines with water and remove the syringes. We close the connectors (P-658) with a lid (the ones shown on the right come from a purification kit and are used to close purification columns).

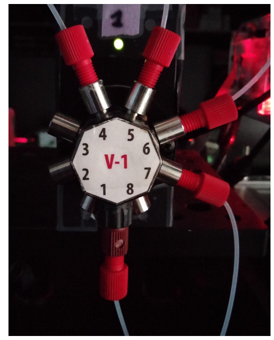
Automated valve

We use computer-controlled valves with several inputs (8 for the one listed below) and one output. Several companies produce such valves, we use the ones of **Hamilton**. Here you will need the valve positioner as well as the valve itself. The valve positioner is delivered with a power supply and control cable.

Shown on the right is the valve itself with 4 connected inputs (ports 4 to 7) and one output.

Inputs are connected with threaded connections, output is connected with a female Luer.

- **Entry**: **Idex XP-202**
- **Exit**: **Idex P-655 + XP-202**

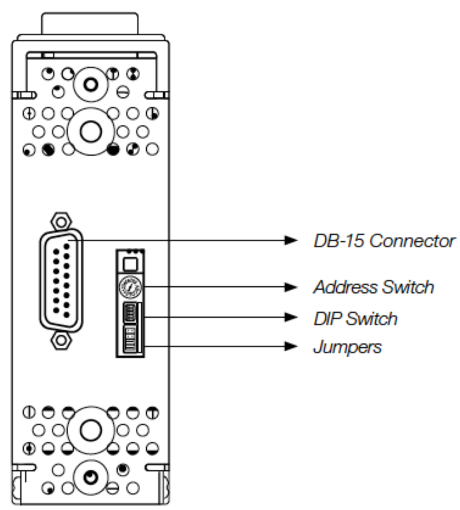


Input/output valve

Certain valves permit flow in both directions. Then can thus either be used to select from several incoming fluidical lines, e.g, to select from different buffers, or to select for different output lines, e.g. to direct buffer to a specific sample. We use the latter when working with Ibidi multi-channel slides. Our control software autoFISH supports both input and outlet valves.

Configuring Hamilton MVP/4 valve

Address switch
The Address Switch is set such that the first MVP/4 is set to “0,” second to “1,” and so forth.



DIP and termination switches

You have to change the

- **DIP switches** (4-6) to indicate that the used valve has 8 ports (Table 2-2, page 14).
- Termination switch (7-8) have to be set to indicate that we only have one valve.

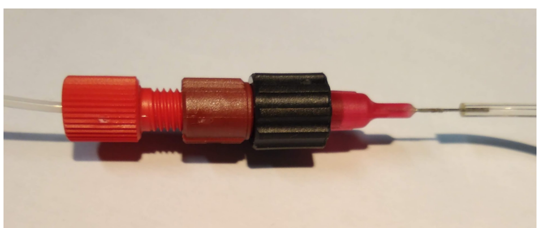
SWITCH	4	5	6	7	8
	ON	ON	OFF	ON	OFF

Pump

We currently support two different options.

Peristaltic pump

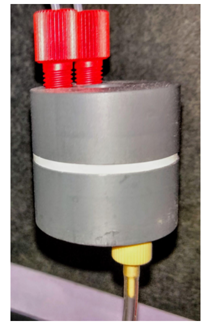
- Ismatec REGLO DIGITAL MS2/12-160, 230 or similar
- Needs to be controllable via the serial port.
- **Entry & exit**: **Blunt Gauge 25 needles (OD 0.54mm) with Luer Lock. + P-655 + XP-202**



Bubble trap with vacuum pump

We use a **bubble trap** to remove air bubbles from the buffers (especially EC is degassing). The bubble trap can be connected to a **vacuum pump** which will help to increase bubble removal. We use a small piezo pump, the pump itself is very cheap, the controller is comparatively expensive, but it is very convenient. The starter set listed in the components section contains the vacuum pump, controller and soft wall tubing.

Shown on the left is the bubble trap with the entry/exit of the fluidics on the top, and the vacuum line connected at the bottom.



- **Target flow rate:** 4 ml / min (sine signal, 250V, 50 HZ)
  More settings see below.

- **Connection of fluidics**: **XP-202**
- **Connection of vacuum line:** **P-692 + soft tubing**

Target flow rate	Amplitude	Frequency		Target flow rate	Amplitude	Frequency
7 ml/min	250 V	100-110 Hz		2 ml/min	220-240 V	20 Hz
6 ml/min	250 V	80-9 0Hz		1 ml/min	125-135 V	20 Hz
5 ml/min	250 V	55-65 Hz		0,5 ml/min	90-100 V	15 Hz
4 ml/min	250 V	40-50 Hz		0,25 ml/min	85-95 V	8 Hz
3 ml/min	250 V	30-35 Hz		0,1 ml/min	80-90 V	3 Hz

As a general guideline the amplitude should be kept as high as possible while varying the frequency.

Flow sensor

We use a flow sensor from Sensirion. The proposed evaluation kit comes with a USB cable that permits measuring flow with a provided software package. Measured flow can be saved in a csv file, which can be read by the Python control software.

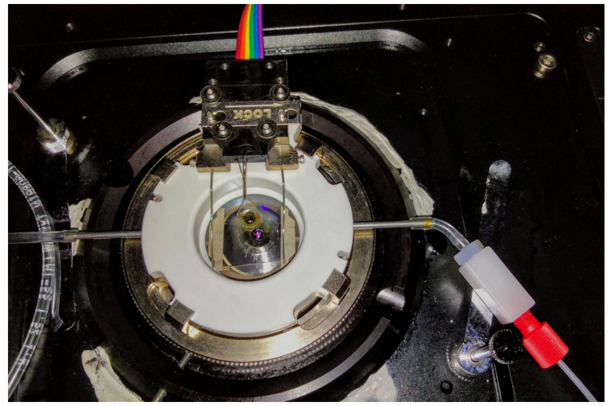
- **Entry and exit**: **XP-202**

Imaging chambers

BIOPTECHS FCS2

We use the Bioptechs FCS2 flow chamber for imaging on the microscope. We recommend purchasing the starter set, which also contains the temperature control and several silicon gaskets.

Show on the left is the mounted chamber, with the fluidics entry on the right, exit on the left. Temperature control is connected on the top./



- **<u>Entry</u>: soft tubing + IDEX P-646 + IDEX P-603 + XP-202**

- **<u>Exit</u>:** we use only the soft tubing without any connectors to avoid clocking up, when parts of the samples dislodge.

Ibidi multi-channel slides

The advantage of these multi-channel slides is that multiple conditions can be tested in parallel, e.g. WT vs drug treatment. They can be connected with a female adaptor sold by Ibidi connected to softwall tubing.

ADD PICTURES

A **few important considerations**

- We found it difficult to obtain consistent flow rates in each channel when using a manifold to distribute liquid. We therefore use an **automated outlet valve** to flow liquid subsequently through each channel. This leads to an only marginal increase of experimental duration, since flow duration is longest only for the first channel where the entire system is filled, for subsequent channels a shorter duration is required since only the liquid between the valve and the channel must be replaced.
- To **avoid air bubbles** when connecting the Luer locks to the channel slide, we recommend filling the slide completely and gently pressing the Luer lock inside to displace the extra liquid.
- For temperature control, we use the chamber slides in small top stage incubator, e.g. from Okolab.
- Imaging multiple channels means **moving the objective over larger distances**. This can be problematic with respect to loss of focus and immersion medium.
  - Ideally the system is equipped with an autofocus.
  - High NA water objectives with an automated water dispenser can help to maintain immersion medium, otherwise generously covering the underneath of the channel slide with oil can also help.

Component list

Note: we listed the minimum number of IDEX connectors you will need, ordering a few extra for security might be good.

What	References	Quantity
Tubing (fluidics)	Tube TEFZEL/ETFE 1/16" (OD) x 0.50mm (ID)	5m
Clear PVC tubing	McMasterCarr 5233K51. ID1/16“ ID To connect the imaging chamber	1m
Tubing to threaded port	IDEX XP-202	20
Syringe adapter	IDEX P-658	1 per syringe
Male Luer	IDEX P-655	2
Barbed to threaded Male	IDEX P-646	1
Standard Union for threaded ports	IDEX P-603	1
Conical to threaded male	IDEX P-692	1
	LIDS for closing when done	
Syringe holder	Warner Instruments : stand, base unit, additional units	1
Robot	CNC 3018 Pro, important to have GRBL support	1
Needles	Hamilton™ 90523 (Kel F Hub Needles) Ga25 (ID 0.26mm), needle style 3 (blunt), 51mm	1 (sold by 6)
Imaging chamber	BIOPTECHS FCS2 Starter set	1
Valve positioner	Hamilton MVP/4 Valve Positioner	1
Valve	Hamilton CERAMIC, HVCX, 8-5	1
Flow sensor	Sensirion Flow Sensor evaluation kit SLF3S-0600	1
Bubble trap	ELVEFLOW Autoclavable Bubble Trap for Microfluidics	1
Vacuum pump + control	Bartels mp-Lab! Evaluation Set	1
Serial-to-USB adaptor	USB to Straight-Through RS232 Serial Adapter	2

Serial port control

Which COM port?

In order to control the different components, you will need to know their **COM port** when connected to a computer. This port usually doesn't change . To see on which port your components are

1. Right click on the Windows Start Icon and select "`Device Manager`."
2. Open the "`Ports (COM & LPT)`" Section.
3. Locate the appropriate Device (if you don't know which one, disconnect and reconnect it) and note which COM port it is connected to.

Hardware test

To test a hardware component and if you can communicate with it, you can use dedicated programs that allows you to send commands to serial ports. This allows to test different commands. In the next sections we list some typical commands that can be send to these components.

- **Termite**: simple program to send and receive data to a USB port (https://www.compuphase.com/software_termite.htm)
- **Portmon**: a tool to monitor activity on serial ports https://docs.microsoft.com/en-us/sysinternals/downloads/portmon
- Some more information for how to **monitor serial port activity** https://technet.microsoft.com/en-us/sysinternals/bb896644

Once this works, you can then use our Python control software **`automator`**. If you buy other hardware than the one listed above, you will likely need to write a new Python class for this hardware. For more details, see the dedicated documentation of **`automator.`**

MVP Valves

To communicate with the MVP valve,

- **Baud rate :** 9600 bps
- **Data bits :**8
- **Parity :** none
- **Transmitted text :** append CR

Commands below are for Valve 1

Command	Serial command	Return
Initialization commands	/1h30001R /1h20000R /1h10000R	
Set valve type 8 way 45 degrees	/1h21003R	
Move to valves position 1 (fastest way)	/1h23001R	

RETURN /0@[03]

REGLO Pump

- **Baud rate :** 9600 bps
- **Data bits :**8
- **Parity :** none
- **Transmitted text : append CR**

Command	**Serial command**	**Return**
Software version	1#	*String containing REGLO*
Set control panel inactive	1B	*
Set flow rate to 1 ml/min fmmmmee ; m Mantisse, e Exponent	1f1000-3	Returns 1000E-3
Set revolution counter-clockwise	1K	*
Set revolution clockwise	1J	*
Dispension time in 1/10 of a second → for longer times this has to be in minutes (VM)	1V2400	
Starts pump	1H	
Stop pump	1I	