

autoFISH -

Python controlled fluidics system

- **Before getting started, try to read the entire documentation to get a feeling for how this package works. Some things might otherwise be confusing first.**
- **TESTED FOR WIN 10 only:** most microscope control packages work only under Windows.

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Overview

Here, we provide a brief overview of how autofish works and what components are needed. The subsequent chapters provide a more detailed description.

In short, autofish is a Python package that performs sequential fluidic runs on a microscope, alternating between complex buffer exchanges and automate image acquisition.

- The **used fluidics system** is home-made based on widely available and cheap components. It is strongly based on published approaches (Boettiger et al., 2016; Moffitt and Zhuang, 2016).
- **Image acquisition** is either done with the Python package PycroManager (Pinkard et al., 2021) or by a simple synchronization with a shared text file.

Python controls the entire workflow, which also allows to include additional data-processing steps if required, e.g. some image analysis to verify data integrity or to already launch pre-processing steps to gain time.

Configurations: demo and standard experiments

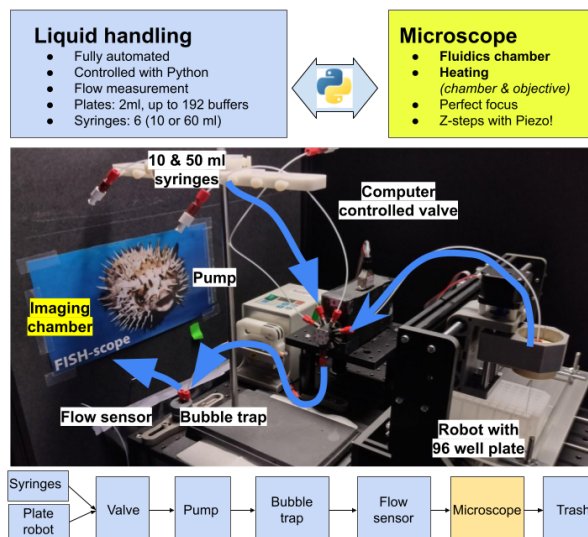
We provide **demo configuration** files that allow testing the different modules without connecting to actual hardware. This allows testing a proper installation of autofish. Demo configurations can be found in the [demo subfolder](#). We also refer to these demo tests in the respective sections.

We also provide the configurations we use on our system (Nikon Ti) to perform standard experiments [here](#).

Fluidics system

We use a custom-build fluidics system to perform the liquid handling.

We provide detailed building plans in the dedicated document found [here](#).



Microscope control

We provide several options to start acquisitions on a microscope.

1. Image acquisition can be performed with the Python package **PycroManager** (Pinkard et al., 2021). This allows launching multi-D acquisition events with Micromanager 2.
2. Synchronization can also be done with a **shared text file**. The value of this text file is set to 1 once the acquisition should be started. The acquisition software has to set the value back to 0 once the imaging is terminated. Then the next fluidic run is started.

New contributions

We develop autofish in a modular and extensible fashion. This design should make it relatively easy to add new hardware components, e.g. a different pump or a different acquisition routine.

If you want to integrate your own development in *autofish*, you can create a pull request on GitHub and after verification, we can integrate these. Alternatively, you can also contact us to discuss how to best integrate your contribution.

Software installation

Code is hosted on GitHub: <https://github.com/fish-quant/autofish>

Below is a recommended workflow for getting started, other ways exist as well.

Install autofish and miniconda

This you only have to do once.

1. Download the latest **version of miniconda** (Python 3.X) from <https://docs.conda.io/en/latest/miniconda.html>
2. Open Anaconda terminal and Create dedicated environment:

```
conda create --name autofish python=3.9
```
3. Activate environment:

```
conda activate autofish
```
4. Install autofish

```
pip install -i https://test.pypi.org/simple/ autofish
```

Starting autofish

1. Activate environment:

```
conda activate autofish
```
2. Start user interface

```
autofish
```

For developers

We use Visual studio code for developments, but of course any Python IDE works. Installation link can be found [here](#).

Change default terminal to Command Prompt

Under Windows, we had some issues when using PowerShell as a Terminal, when changing this to Command Prompt, things worked out fine. To do this, perform the following steps in Visual studio code

1. Open Visual Studio Code.
2. Press CTRL + SHIFT + P to open the Command Palette.
3. Search for "Terminal: Select Default Profile"
4. Select "Command prompt"

Setting up visual studio code

1. Launch Visual studio code
2. From the menu File > Add folder to workspace
3. Select folder containing the Python code.

4. Double click on the file `coordinator_gui.py` located in the folder `autofish`.
5. Running this script will open the user-interface of autofish by pressing on the Run symbol (arrow) above the code editor window.
6. The first time, you have to specify
 - a. ... the conda environment you defined above.
 - b. ... if you would like to install the Python extension: accept.
7. You can save this workspace to a file, which can then be opened for subsequent usage, and which will also remember the conda environment: *File > Save workspace as*

Typical autofish experiments

Overview

We provide different simple user interfaces to define both the microscopy and the fluidics. These are explained in dedicated sections below. The entry point is a **launch pad** (opening with the command `autofish`), permitting access to these control interfaces, and eventually start complete run.

A typical workflow consists of

1. Open **fluidics interface** to define the fluidics run. This includes specifying the sequence of buffer exchanges, but also preparing the system by priming the tubing. Please note that you can also run selected cycles without an acquisition.
2. Specify acquisition in the **acquisition software**, e.g. micromanager.
3. Setup acquisition in autofish
4. Initiate the controller to permit a fully automated run.
5. Run the full fluidics run.

Some scenarios

Acquisition specification after the first run

Often the **acquisition might be defined after the first fluidics run**, i.e. because no labeling was performed on the bench. Here, you can run the first run manually from the fluidics interface, define your acquisition and acquire the images for this first run, before going into automated mode.

Acquisition of additional channels

If you acquire **additional channels** in the first round, e.g. DAPI, we recommend **acquiring them last**. Like this, the numeric channel identifiers will not get mixed up, e.g. FISH will always be the first channel to be acquired.

A typical autoFISH experiment

Below, we described a typical workflow for an autofish experiment. All steps are explained in more detail in the following sections.

- Only the first round contains a **DAPI** stain. Only for this round the DAPI image is acquired.
- **Imaging settings** are defined after the first round, since here cells can be seen.
- For the remaining rounds, the experiment is automated.

Preparation

1. Get **samples** ready
 - a. Mount imaging chamber
 - b. Prepare all buffers

2. Prepare **autofish** system
 - Define experimental settings in config file.
 - Start fluidics flow sensor and start logging into file.
 - Start autofish and initiate (config files, zero robot)
 - Prime lines and check flow (replace tubing if necessary)
 - Hardware (easy to forget)
 - Temperature control (both objective and chamber)
 - Vacuum pump

Actual experiment

1. Run first hybridization round (including DAPI)
2. Micromanager: define acquisition.
 - a. Include DAPI channel
 - b. Save position list
3. Autofish: setup imaging
 - a. Adjust microscope config file: channels and z-stacks
 - b. Open PycroManager module in autofish
 - i. Load config files (position, microscope)
 - ii. Specify folder to save images
 - iii. Perform acquisition for first round
4. Start automated runs.
 - a. Initiate controller
 - b. Launch all runs

Once done

Turn off hardware.

1. Clean system.
2. Turn off hardware.
3. Detach tubing from peristaltic pump.

Microscopy control

In the pulldown menu of the launchpad, you can select which type of imaging synchronization you would like to use.

PycroManager

Here, the acquisition is performed with micromanager 2 (Edelstein et al., 2014) with the Python package PycroManager (Pinkard et al., 2021). This requires installation of

- Latest nightly build of micromanager 2 from [here](#).

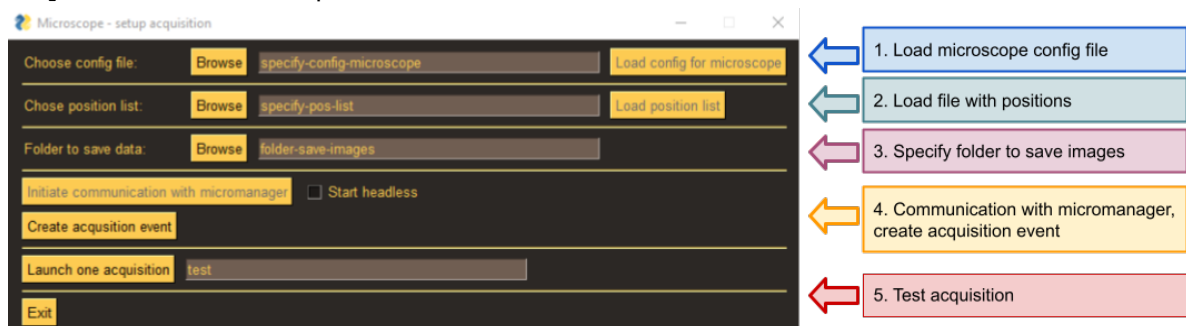
Micromanager, PycroManager, headless

We refer to the excellent documentation of PycroManager, for how to get started and verify that the installation works properly.

PycroManager allows **headless connections** to micromanager. This means, no open instance of micromanager is required and only essential elements of the micromanager core are started from within Python. In our hands, this allowed for more stable acquisitions

Typical workflow

1. We usually open a micromanager instance to **set up the acquisition**. This involves
 - a. Finding adequate **exposure times** for the different channels.
 - b. Specifying the **different positions** that should be acquired. These positions are then exported as a position list for import into autofish.**ToDo: show screen shot.**
2. At this stage, micromanager can be closed if a headless acquisition is performed.
3. Update the microscope configuration file (see below) to specify the z-range and the channels that should be acquired.
4. In the Python autofish GUI, select `pycromanager` from the dropdown menu and press `Specify acquisition`. This will open the window shown below:



- a. Load configuration file
- b. Load position list
- c. Specify folder to save images
- d. Connect to micromanager
- e. Create the acquisition event

- f. [Optional] launch a test acquisition, this can also be used to acquire the images of the first fluidics run.
5. The interface can now be closed (unless you want to perform different acquisitions for the first round as for the remaining ones)..

Microscope configuration

All acquisition parameters, except the position lists, are specified in the file `microscope_config.yaml`

Some of the parameters are specific to your microscope, such as the name of the XY and Z drive, the provided names are for an inverted Nikon Ti. You might need to adapt this for your microscope.

A typical file is shown below:

```
Microscope:
  type: 'NIKON_TI'
  xy_drive_name: 'TIXYDrive'
  z_drive_name: 'TIZDrive'
  channel_group: 'channels'
  channels: ['Cy3_75', 'DAPI_5']
  channel_exposures_ms: [100,50]
  channel_blank: DAPI_0
  z_start: 0
  z_end: 6
  z_step: 0.4
  mm_app_path: 'C:\Program Files\Micro-Manager-2.0-nightly'
  mm_config_file: 'MMConfig_demo.cfg'
```

The different fields are describe next:

Command	Action
type	Specify your microscope. This will essentially determine how the position list is read (see function <code>load_position_list</code> in <code>imager.py</code>) Currently implemented are <ul style="list-style-type: none"> • Demo: for test purposes only with demo MM config file. Small position list is created. Allows testing the PycroManager installation. • NIKON_TI: for an inverted Nikon Ti.
xy_drive_name z_drive_name	Names of the XY and Z drives. Will depend on your microscope. These are used to interpret the loaded positions lists.
channel_group	Name of channel group containing the channels that will be acquired.
channels	List of all channels that will be acquired.
channel_exposures_ms	List of exposure times (in ms) of the acquired channels specified in channel.

<code>channel_blank</code>	[optional] Channel name used for a blank acquisition. When specified one additional image will be taken at the first position without a z-stack using this channel. This can be necessary when the light source is not automatically turned off after acquisition.
<code>z_start</code> <code>z_end</code> <code>z_step</code>	Positions of z-stack relative to specified Z-position in position list.
Only needed for headless start of micromanager:	
<code>mm_app_path</code>	Path to micromanager
<code>mm_config_file</code>	Name of micromanager config file

Position list

The different positions that should be acquired are stored in a .pos file. In autofish, these positions are then read into python as XYZ coordinates (`imager.py > pycromanager > load_position_list`). This routine might need to be adapted to your microscope.

Demo

You can load the demo file `microscope_config_demo.yaml`.

You have two different options.

1. Open micromanager, and connect normally
2. Update the micromanager path and name of the config file and connect headless.

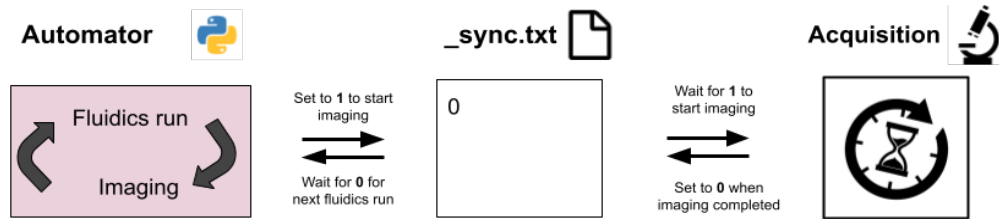
Known issues

If you see error messages complaining about ZMQ, a likely explanation is that you don't have the latest nightly build of micromanager and/or the latest version of PycroManager. Try to download the latest micromanager version, and update PycroManager:

- o 1. Open conda terminal
- o 2. Activate environment: `conda activate autofish3`
- o 3. Update pycromanager: ``pip install -U pycromanager``

Text file synchronization

Here, a shared text file is used to establish communication between autofish and the image acquisition software. This text file contains 1 to indicate that the acquisition should be launched, and it has to be set back to 0 by the acquisition software once the imaging is terminated.



Typical workflow

1. In the main interface select `text file sync` from the dropdown menu and press 'Microscope'
2. In the interface select the text file that you want to see to communicate with the acquisition software.
3. Create the acquisition object by pressing on the corresponding button.
4. You can then close the interface.

Demo

If you want to test alternating sequences of fluidics run and imaging, you could load the demo config for the fluidics. When you launch the full run, you can manually set the sync file back to 0 to indicate that the imaging is completed.

Adding your own acquisition routine

The code of autofish is modular, and you can add your own acquisition routine. However, this will require some coding. As a guidance, you can already look at how the existing two imaging routines are implemented.

- You will need to add a new Class in `imager.py` (using Microscope) as a parent class. As a minimum, this class needs to provide a function called `acquire_images`. This function will launch the desired acquisition routine.
- In `coordinator.py`, you will need to add your new acquisition class to the function `run_all_rounds` to correctly handle the potential input parameters.
- In `coordinator_gui.py`, you will need to add you class to the dropdown-menu of the launch GUI (`make_window_control`), and then write the GUI for your calls that collects the relevant user inputs.

Setting up the fluidics system

We describe next how you can set up the fluidic system and define a sequence of steps that will be performed automatically. The fluidics system consists of at least a **pump** and the **pipette robot**, optionally it can further contain a computer-controlled valve and flow sensor

Configuration files

For a user, 2 files are important which define how Python can interact with the different components and how the actual experiment is defined.

- **system_config.json**: specifies how to communicate with the different hardware components (pump, valve, CNC robot). This has to be adapted to a given fluidics system only once, and then remains unchanged. For more details see the dedicated section in the [Appendix](#).
- **experiment_config.yaml**: specifies where the buffers are and the sequence of a run. Has to be adapted for each experiment. For more details see dedicated section about [experiment specifications](#).

Basic usage of fluidic system

The fluidics system can be controlled and initiated via the user interface shown below. We next detail a typical workflow, more details on the config files are provided in subsequent sections.

The screenshot shows the Automator GUI with the following sections and callouts:

- 1. Specify fluidics configuration file & initiate robot**: Points to the 'Choose config file' and 'Initiate robot & open serial ports' buttons in the 'Fluidics configuration [serial ports]' section.
- 2. Place CNC needles over well A1 and zero robot**: Points to the 'Zero stage' button in the 'Zero pipette robot over well A1' section.
- 3. Specify & load robot configuration file**: Points to the 'Load robot file' button in the 'Robot properties' section.
- 4. Choose a buffer & start pump to primer buffer line**: Points to the 'Start pump' button in the 'Prime / wash fluidics lines' section.
- 5. Choose cycle and run it**: Points to the 'RUN sequence' button in the 'Run sequences' section.

The GUI also displays the 'Pipette robot status' at the bottom: <id>|VPos:0.000,0.000,0.000|Et:15.126|FS:0.0>

1. **Make sure hardware specifications** stored in **system_config.json** are correct.
2. Update **robot configuration file** *robot_config.yaml* for your experiment..
3. Turn on the fluidics system (pump, robot, (if present) valve, (if present) start flow measurement.
4. Start autofish, this will show the GUI.

5. **Initiate system** (Step 1 in GUI, red).
 - a. Press `Browse` to select fluidics specification file.
 - b. Press `Initiate robot & ...` to initiate communication with the different components.
6. **Zero CNC robot over well A1** (Step 2 in GUI, blue)
 - a. Move robot with jog buttons for indicated distance in mm. Center over well A1 (a few mm above)

IMPORTANT: don't move too much, robot doesn't know the limits of the different motors.
 - b. Once needle is centered over A1, press `Zero stage`
7. Load robot specification file (Step 3 in GUI, green). This will verify which buffers are used for the sequential runs, estimate the total run time, and calculate the position of each well. Some information is provided in the terminal, more details in the log file
8. **Prime buffer lines** (without the chamber attached) (Step 4, purple)
 - a. Select the buffer you want to prime from the pulldown menu. Press `Go to buffer`.
 - b. Press `Start pump`, which will turn on the pump for indicated duration in seconds. Pump can only be **started once**. To start again, the buffer has to be selected again.
9. **Connect chamber and prime**
 - a. Fill with buffer
 - b. Chase bubbles.
 - c. Mount on support
10. [Optional] If you want to run a selected round, select it from the drop-down menu, and press `RUN sequence` (Step 5, orange)
 - a. Progress will be shown in the terminal. A more detailed log is created in a file.
 - b. To stop the run, press `STOP sequence`

Once round is terminated, it will be removed from the dropdown list

Experiment specification: experiment_config.yaml

This file contains information about the buffers, the sequence of buffer exchange steps and the layout of the plate robot.

- Stored as a yaml file
- Strict formatting: spacings and indents have to be respected.
- File contains three sections defining the entire experiment. Each section starts with the respective keyword followed by a ". Elements of each section have an indent of 4 spaces.
 - **buffers:** name and position of all buffers
 - **sequence:** sequence of steps the robot has to perform for each run
 - **well_plate:** properties of the plate robot

buffers

Here, the names and position of all buffers are specified. **Two types of buffers** can be specified:

- **General purpose buffers:** they are the same for each round. Typically stored in syringes.
- **Hybridization round specific buffers:** they change during each round.

- These are typically buffer with readout oligos, or specific wash buffers. Several such buffers can be defined for a round.
- The buffers are declared in the 'sequence' section and terminate with an **ii**, e.g., `w_ii`. In the buffer list, they are specified with a unique name for each round, e.g., `w_r1` for round `r1`.
- **At least one** hybridization specific buffer must be specified. Autofish analyses the settings file and then automatically determines the round identifies.
- **Runs are listed and executed in the order the buffer list is provided.**

Names

- Each name must be **unique**.
- Round-specific buffers are indicated with an **ii** at the end of their name.
 - They have to be unique as well, e.g. using `w_ii` and `w_stringent_ii` will lead to conflicts since the string `w_` can be matched to both buffers.
- Please note that after the name, you have to add ":" and then the position.

Examples

<code>buffer: wash</code>	general purpose buffer "wash"
<code>buffer: w_r1</code>	round specific buffer <code>w_</code> for <code>r1</code> (is defined as <code>w_ii</code> in the protocol sequence)
<code>buffer: h_r1</code>	round specific buffer <code>h_</code> for <code>r1</code> (is defined as <code>h_ii</code> in the protocol sequence)
<code>buffer: w_r1</code>	round specific buffer <code>w_</code> for <code>r2</code> (is defined as <code>w_ii</code> in the protocol sequence)

Positions

Positions are provided with a tuple with 3 elements:

[valve-id, plate-id, plate-pos]

- **valve-id:** which port of computer-controlled valve, e.g., with 8 entries.
 - 0: no valve used (because no valve in fluidics system)
 - >0: port entry number
- **plate-id:** several plates could be put on the robot
 - 0: no plate -> position as to be specified with XYZ coordinates (see below)
 - 1: plate 1 -> position must be specified with the well number (A1, ...)
- **plate-pos:**
 - For absolute positions you must specify the position relative to 0 position when homing the robot. To go to position (X=135mm, Y=36mm, Z=-39mm), you must specify `X135_Y36_Z-39`. Important are the underscores, and the minus (-) for the Z coordinate since the robot will move down.
 - For the well position, you can use A1, A2, ... The program calculated the absolute position based on the [well_plate specification](#) that you provided.

Note: For buffers on the syringe stand, plate-id and plate-pos must be set to null.

Example

We defined two buffers, the general buffer `wash_valve4` which can be found on the valve 4, and the buffer `w_r1`, which will be used in an iterative run which is on the first 96 well plate, on valve 6 in well A1:

```

buffers:
  wash_valve4: [4,null,null]
  w_r1: [6,1,A1]

```

sequence

Here, the sequence of steps then will be execute is specified. Each step starts with a dash and the steps are executed in the order listed from top to bottom,

Command	Action	Example
wait	Waits for the user to press ENTER. Can be used if some changes have to be done to the system.	- wait:
pause	Take a break for the specified duration in seconds.	- pause: 1200
pump	Activate the pump for the indicated duration in seconds.	- pump: 180
buffer	Select the specified buffer. Buffer names are listed with their position in the dedicated section “buffer” explained below. Buffer names containing ‘ii’ are buffers that are hybridization round specific and can be selected (more below).	- buffer: wash_valve4
round	Specify conditional steps that are performed only for the indicated identifiers for a round (e.g. to perform a DAPI stain). Note that command has an additional indent. Multiple rounds can be defined and have to be listed separated by a “,” and nothing else.	- - round: r1,r4 - buffer: dapi_ii
zero_plate	Moves plate to position 0. This can be useful if during an experiment a plate should be changed (can be combined with wait to stop the system)	- zero_plate:

Example

The to select the buffer wash and the activate the pump for 90s, you can write:

```

sequence:
  - buffer: wash
  - pump: 180

```

Conditional steps

You can define conditional steps, which will be only executed for a certain hybridization run. For example, you want to include a DAPI wash for the first round. For this you have to use an indented sequence as shown below, where the first action is “round” followed by the identifier of the round, where these steps are

to be performed. Please also note how the dashes are used. You can use all actions detailed above, including round-specific buffers, which will then only be used for these conditional runs.

In the **example** below, an additional buffer (`dapi_ii`) is used for rounds r1 and r4.

```
- - round: r1,r4
- buffer: dapi_ii
- pump: 180
- pause: 180
```

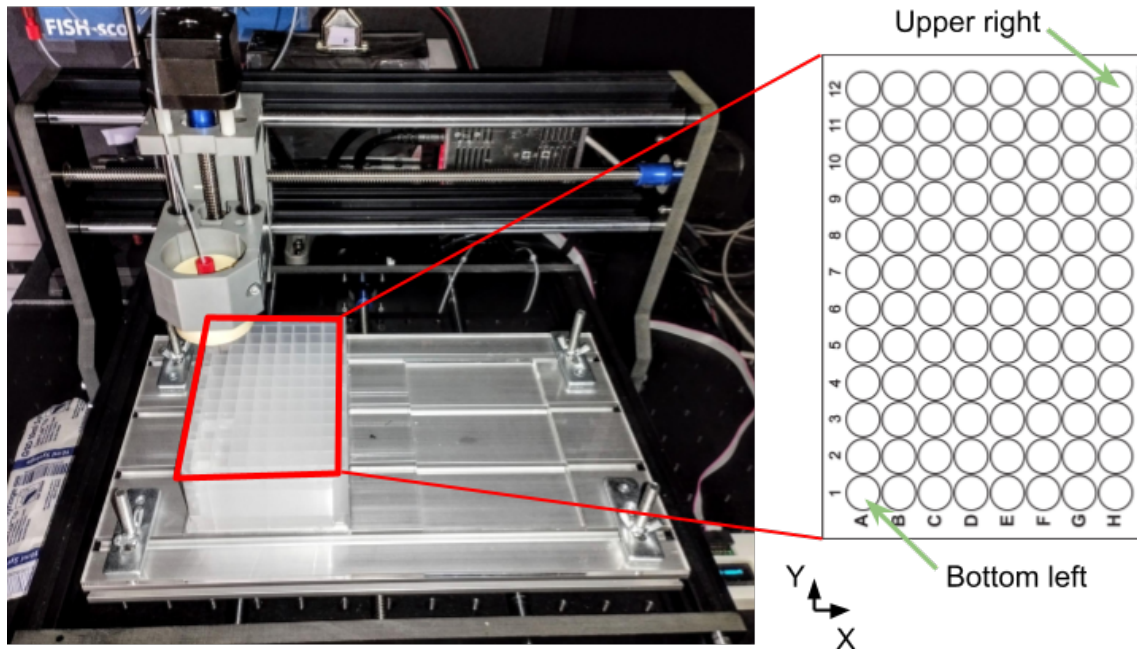
well_plate

Here the specifics of the used well plate are specified. This usually has to be done once for a given configuration.

Command	Action	Default
<code>trop_right</code>	Position of upper right well (center)	x: 63 y: 99
<code>bottom_left</code>	Position of bottom left well (center)	x: 0 y: 0
<code>columns</code>	Number of columns	8
<code>rows</code>	Number of rows	12
<code>well_spacing</code>	Distance between well	9
<code>z_base</code>	How much robot will go down in z [mm]	-37
<code>feed</code>	Speed at which robot moves [not implemented yet]	500

Information will be used to automatically calculate the positions of each well (this can also include a tilt, i.e. a plate that is not aligned with the X, Y axis of the robot). The assumed orientation of the plate is indicated in the figure below. This means

- Columns are letters (A-G for a 96 well plate)
- Rows are numbered (1-12 for a 96 well plate)



Flow sensor

As an optional step, flow rates can be measured, and compared to the expected flow when the pump is active. By defining tolerance limits, the fluidics system can be automatically stopped, when the measured flow is outside of this tolerance.

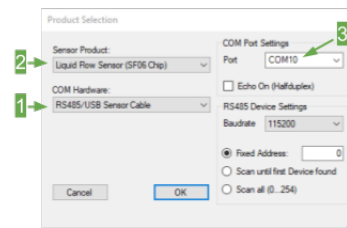
Sensirion SLF3x Flow Sensor

We use a flow sensor from Sensirion (SO-SLF3S-0600F-EVAL-KIT). The evaluation kit connects it directly to a USB port. Rather than reading the flow-rates directly into Python, we use the provided Viewer software available on the Sensirion website. This software allows you to log the flow rates into a CSV file. In autofish, we read this csv file and extract the expected volume for the specified pump duration.

In the system config file, you then have to specify the location of this log file. Also, before activating the pump, make sure that you record flow into the file. The image below shows how to start the viewer software and start logging to a file (in this example the flow sensor is on COM10):

1. Start software "USB RS485 Sensor Viewer" 

2. In interface, select (order is important!):
 - a. COM Hardware: **RS485/USB Sensor Cable**
 - b. Sensor Product: **Liquid Flow Sensor (SF06 Chip)**
 - c. COM port: **COM10**



3. In new interface, start logging by
 - a. Measurement control
 - i. Sampling interval: **50ms**
 - ii. To start measurement, press button **Run**
 - b. Data logging
 - i. Select file: **C:\Temp\DataLog.csv** (default)
 - ii. To start logging, press button **Start logging**



Appendix

System configuration: system_config.json

In this json file, the communication protocol with the different hardware components is specified. Currently four different components are permitted, for each one a parent Python class is defined in autofish, specifying the minimal requirements for required methods. Then a child class can be defined for a specific hardware.

- **pump**: defines a class for a pump. It has to support at least the methods
 - **start**: start the pump
 - **stop**: stop the pump
- **valve**: computer-controlled valve
- **plate**: plate robot permitting to access a large number of buffers, either in 96 well plates or in larger reservoirs.
- **flow_sensor**: sensor to measure flow rates.

The only required field is '**type**', permitting to identify which exact component is connected. All other fields are component-specific. For instance, for components connected via the serial port, information about the COM port and serial port protocol (baud rate) is provided.

Below, the configuration file for our fluidics system is shown:

```
{
  "pump": {
    "test_command": "l#\r",
```

```

        "response": "REGLO DIGITAL",
        "type": "REGLO DIGITAL",
        "baudrate": 9600,
        "COM": "COM10",
        "Revolution": "CCW",
        "Flowrate": 0.5
    },
    "valve": {
        "test_command": "/1h30001R\r",
        "response": "/0",
        "type": "HAMILTON MVP",
        "baudrate": 9600,
        "COM": "COM11"
    },
    "plate": {
        "wake_up": "\r\n\r\n",
        "test_command": "$I\n",
        "response": "VER:",
        "type": "CNCRouter3018PRO",
        "baudrate": 115200,
        "COM": "COM8"
    },
    "flow_sensor": {
        "type": "Sensirion_csv",
        "log_file": "C:\\Temp\\DataLog.csv",
        "kernel_size": 20,
        "flow_min": 20
    }
}

```

Serial port communication

- <https://pythonhosted.org/pyserial/>
- <https://github.com/pyserial/pyserial>
- <https://www.com-port-monitoring.com/> (be careful to download the free version)

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