Instruction S4 – 2LabsToGo-Software Manual

2LabsToGo-Software execution

Inside the installation folder execute the following command in a Linux terminal:

python3 run.py (or execute the alias 'go')

To access 2LabsToGo Software open a tab in the explorer and enter to either:

http://127.0.0.1:8000/
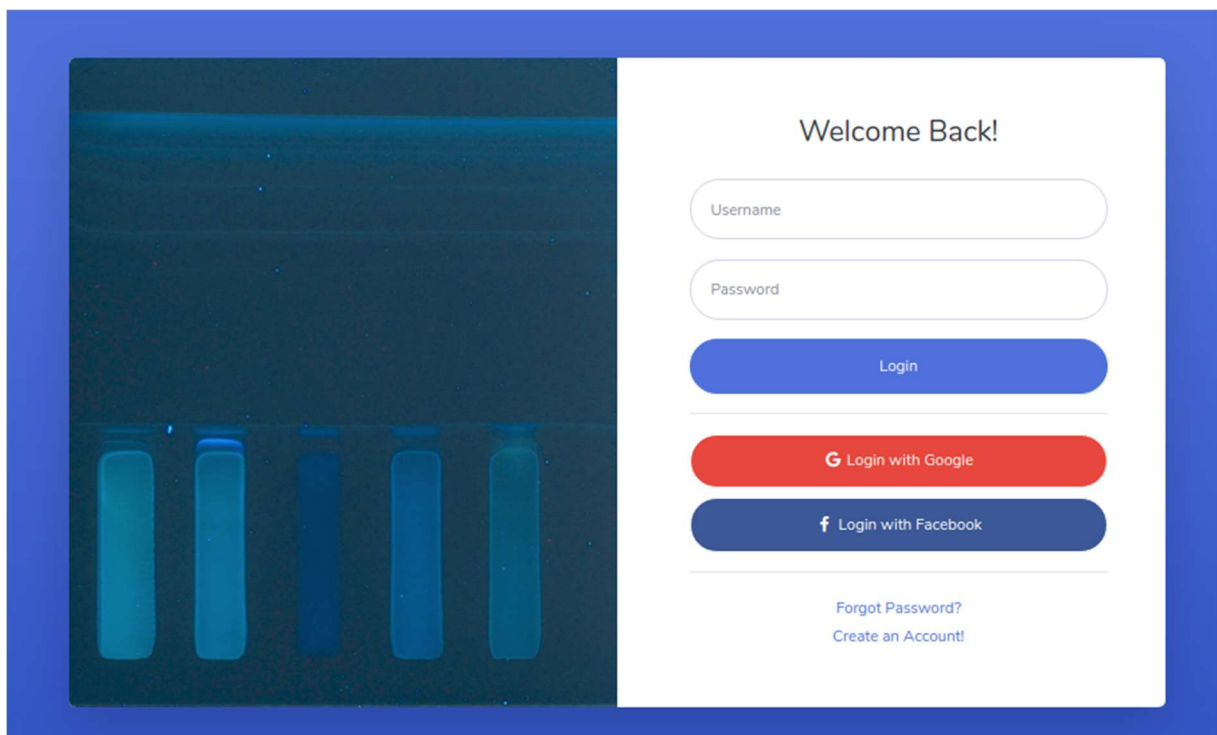
http://localhost:8000/

Register

The **login screen** of 2LabsToGo-Software will be displayed.

To create an account, click 'Create an Account' on the bottom region of the login screen.

The *Register screen* will be shown.

→ Please fill all the fields in order to create a new Account, afterwards press Register Account. If every field was filled with valid data, the *Connection Screen* should be visible by now.
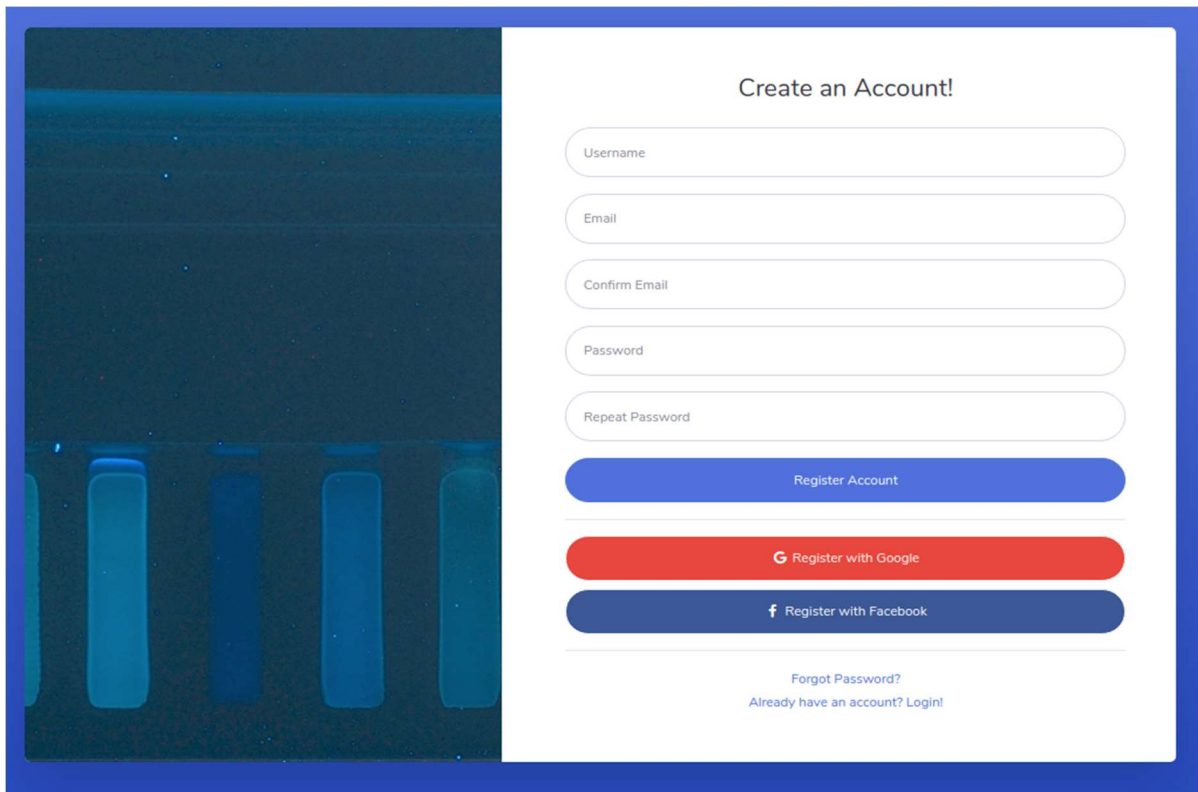
Connection

The connection screen is used to connect to the 2LabsToGo. The machine will communicate via the Raspberry GPIO with the app. All interactions between the app and 2LabsToGo will be visible in the Monitor window.
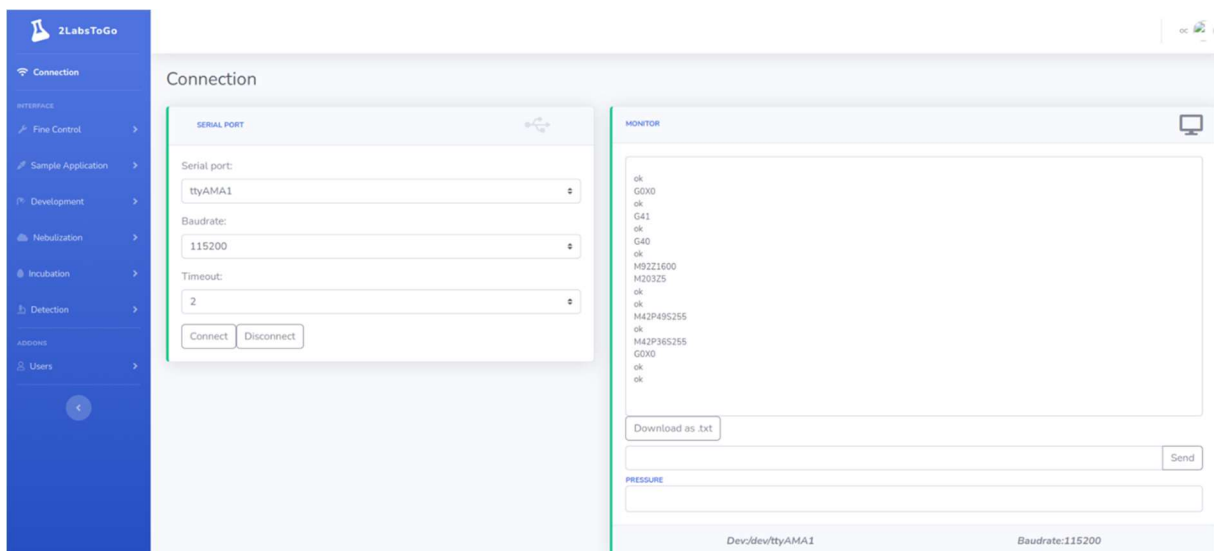
The serial port is automatically selected.

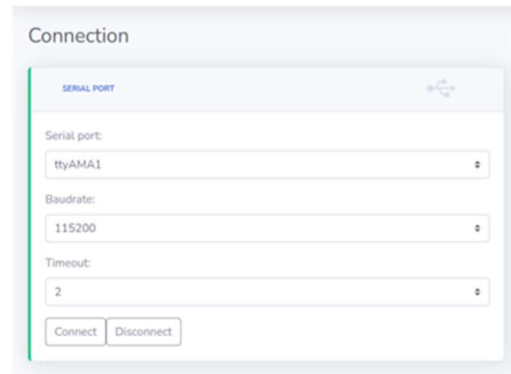The Baud rate is related with the connection speed. Please leave it in the default option of 115200.

→ A slow baud rate will slow down the speed of reaction of the system.

→ If it is set too high, it could produce errors in the messages received and sent.

The Timeout is the maximum time (s) that the app will wait to give a response. Keep the default value.

Press the **Connect** button to send a connection request to 2LabsToGo. The successful established connection is notable because the app will start printing the text sent by the machine on the **Monitor** screen on the right, and the Connection Bar down below the screen will turn to green, and it will display the name of the device to which the app has been connected.

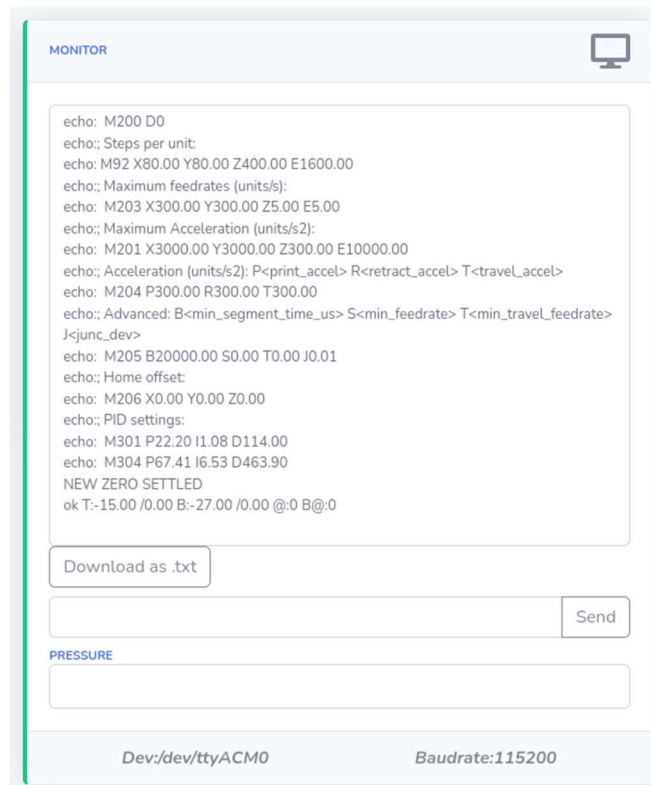This bar is visible on every tab of the software.

After following the previous steps, the connection should have been established and the machine will be ready to work. Test it by sending **G28** to the machine using the dialog box located in the bottom of the Monitor screen. After the machine receives this command, it should start moving.

Operating the software

On the left side, a blue panel with the different processes of 2LabsToGo-Software is displayed (side bar).

Generally, first left-click on a process, for example, Sample Application, when a sub-selection window is opened.

Right-click on a sub-selection and select "Open link in a new tab". A new browser tab will be opened to handle the process.

With this arrow the side bar can be hidden.

Fine Control

Fine Control' provides different tools.

Some of them are exemplarily explained here.

X,Y Motors

Motor control is a simple interface to move the motors a certain amount of mm (Steps) at a certain speed, the motors will move in a specified direction by clicking on the arrows.

The **Home** button between the arrows will move the apparatus into the absolute zero position respective to x and y. This is the position, in which both endstops are triggered.

Plate Heater
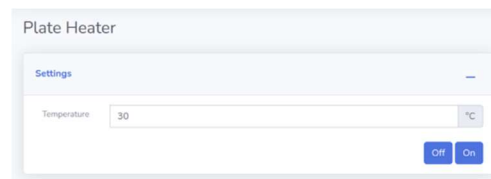
This window allows the user to heat up the multifunctional plate holder up to 130 °C. Set the desired temperature in the field and activate the heating by pressing the **On** button. The heating will now be checked every four seconds, displayed in Monitor screen. By pressing the **Off** button the heating can be deactivated.

G-Code Editor

G-code is the language that 2LabsToGo understands.
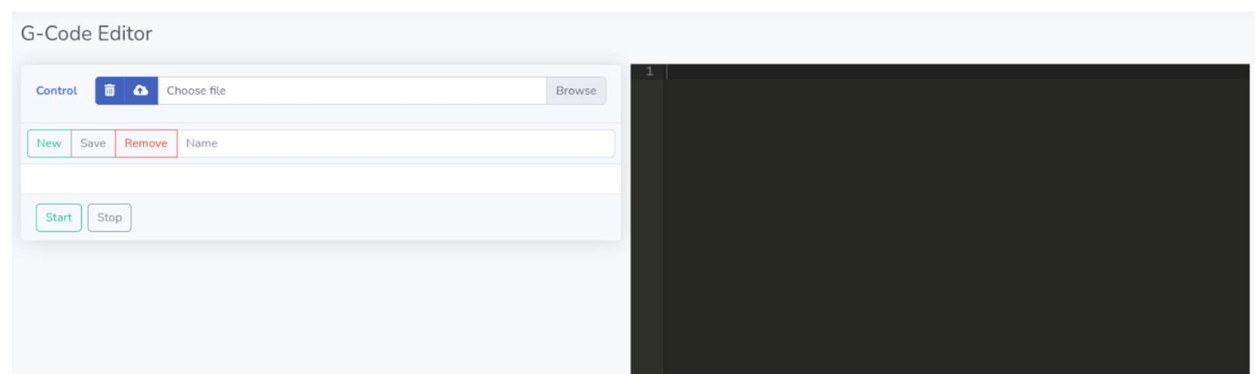
The G-Code editor allows to create new G-code files and to execute them. Files can be created, saved, loaded, removed and uploaded.
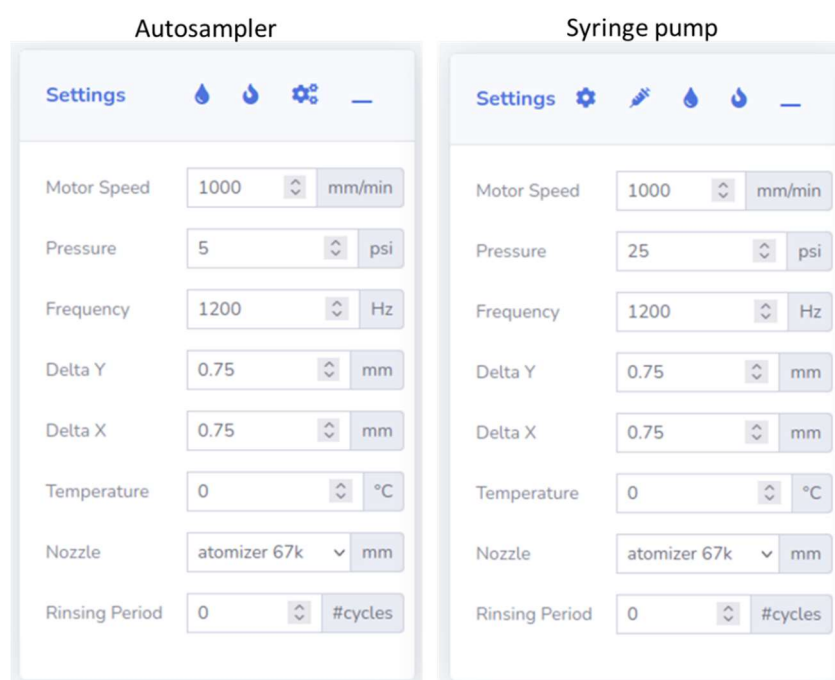
Sample Application

The Sample Application page is used to configure and apply the samples. There are two different ways to apply the samples, displayed in the dropdown list after clicking on **Sample Application** in the sidebar.

The first option is **Autosampler** to proceed with the sample application procedure using the autosampler, *i.e.*, automatically applying up to eight samples. The second option **Syringe Pump** allows to apply sample by sample with the syringe pump (semi-automatic application).

Settings

There are the forms Settings, Parameters, Save and Load and Plate Preview. Note that some forms are different according to the sample application method selected.

The Settings window configures the basic parameters for the sample application, both for the autosampler and the syringe pump. The only differences between the two windows are the functionalities presented by the small icons at the top of the window, explained below.

The following parameters can be selected.

| Motor Speed	Sets the movement speed of the x-motor.
Initial Pressure	Sets the pressure to be reached before opening the dispensing valve. Changing the pressure only has sense for the syringe-pump system. In the case of the autosampler, 5.0 psi should be selected.
Frequency	Sets the time the valve is opened while applying. The frequency is $\frac{1}{Time_{valveOpen}}$.
Delta Y	Sets the minimum movement between drops in the Y direction.
Delta X	Sets the minimum movement between drops in the X direction.
Temperature	If it differs from 0, the heating is turned on. The machine will wait until it reaches the settled plate temperature before starting.
Nozzle	Select the used nozzle. It will affect the volume calculation.
Rinsing Period	Defines the number of cycles (between band passes), whereafter a brief rinsing is inserted to prevent a hanging drop. With '0' no rinsing is performed, with '1' after each pass, with '2' after two passes, and so on.

Parameters

The Parameters define settings related to the plate and band sizes.

It is important to note that the different parameters listed below are limited to each other. For example, it can not be applied on a plate with an X Size parameter of 100 mm if the offset is set to Left 50 mm and Right 50 mm because there will be no space for the bands.

Plate Size	Sets the sizes of the plate (X, Y).
Offsets	Sets a margin on the plate for all sides (Left, Right, Top, Bottom).
Band Calculation	Number of bands creates as many bands as specified in the **Band Calculation** field. The bands' length will be adjusted regarding the space between left and right offsets, and the selected gap between the bands.
	Band Length will create as many bands as possible with the set **Band Length** regarding the space between left and right offsets, and the selected gap between the bands
Height	Sets the height of the bands, taking steps of the already configured **Delta Y**, 0 will apply a band, values >0 will apply an area.
Gap	Sets the distance between the bands. This may be decreased/increased to achieve the desired number of bands.
Pause	Setting a waiting time between the passes to allow the band to dry.

The resulting bands' design is shown in the Plate Preview.
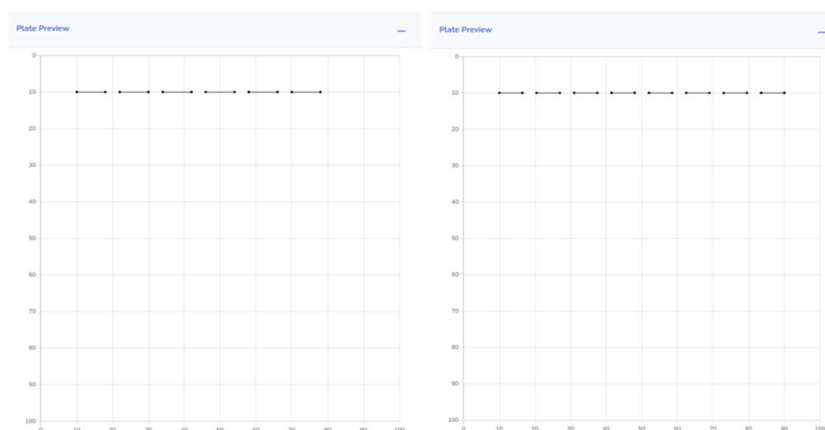
Save and Load
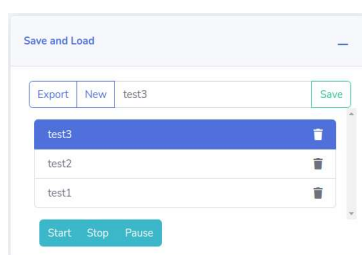
The sample application settings can be saved and loaded to and from the database.

In the text entry field in the Save and Load window, the application settings can get a project name.

After all settings have been performed, click on **New**, type a name, and click on **Save**. If the settings are changed thereafter, they cannot be saved under this name by clicking **Save**; create a new project with the changed settings.

Clicking the **Export** button will export all settings as csv file.

Check the browser' setting for downloads and activate "Ask where to save …".

Otherwise the file will automatically saved as output.csv in the download folder.

To load a project, click on one of the already saved projects and all settings will automatically be reloaded.

To import a sample application project, choose a file and click on *Import CSV*.

Clicking on **Start** will start the application process. It also can be stopped with **Stop**, if something went wrong. It is important to note that the application process will not be finished immediately, but only after the already sent Gcode commands are processed.

Syringe-pump

By selecting this application method, some G-codes will automatically be sent to set the movement parameters for the syringe pump motor and to switch the 3-way valve, indicated by the LED's *SP* and *3WV* in front of the electronic box. Some features are also available once clicked on Syringe Pump, displayed in the upper right corner of the Settings form, like in the image below. There also is a mouse-over information.

With the **Gear** icon, the syringe size must be selected: 2 mL, 5 mL or 10 mL. According to this selection, the movement calculations are carried out. In the pop-up window it is possible to see how the Length value changes, but the user cannot modify this value here. By clicking *Ok*, the values are saved and the syringe piston movement can be calculated correctly.

The **Syringe** icon stands for Syringe Load, and it refers to how much volume (in mL) was loaded into the syringe. Once selected and clicking *Move*, the syringe pump pusher will move to a position that the syringe can be inserted. If the syringe does not fit exactly, increase or decrease the set volume slightly (use the arrows) and click **Move** again. The volumes can also be saved and will be presented in a table on the left side of this window, to rapidly be selected.

The **Drop** icon stands for Rinsing, thus flushing the tubings and the nozzle. In the pop-up, there are two options, one is automatic rinsing, and the other is manual rinsing. For Automatic Rinsing, it is possible to select a volume in µL to rinse and a speed in mm/s, then just click the *Start* button. In the case of manual rinsing, there is only the **Open Valve** button, which means that the valve will open, and the rinsing process is performed by manually pressing the syringe piston. The valve will automatically be closed after 30 s!

The **Fire** icon refers to Warm Up. Here, the dispensing valve frequency, number of drops and the pressure that must be reached before starting the warm up, are selected. Then it is possible to **Start**, **Stop** and **Pause** this process.

After filling in the above settings, the Sample List section will display a table with as many rows as bands have been selected, to be applied with the <u>same sample in the syringe</u>. In the table, the properties related with each band can be set.

Sample List

Band	Sample	Volume (uL)	Calculations
1	Name	1 / Water	Volume: 0.997, DropVol: 0.002, MinVol: 0.026
2	Name	1 / Water	Volume: 0.997, DropVol: 0.002, MinVol: 0.026
3	Name	1 / Water	Volume: 0.997, DropVol: 0.002, MinVol: 0.026
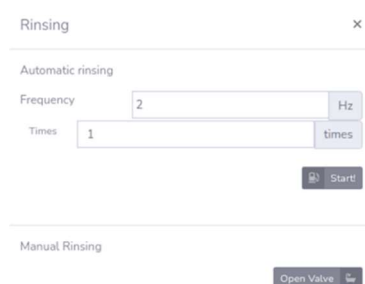
Band	Displays the band number to which the row refers.
Sample	A text box to write a comment or a name to identify the sample. It can be copied and pasted.
Volume	The desired volume in µL to be applied. The type of solvent it is also used to calculate the drop volume; therefore, it must be set (acetone, water, methanol, …). If it is not listed, consult the appendix at the end of this manual.
Calculations	The *minVol* (minimum volume to be applied) and the *DropVol* (volume of a single drop, depending on the nozzle type, pressure, and solvent type) are shown. The calculated *Volume* is a multiple of the *DropVol*, why it generally cannot match the desired volume exactly. The *minVol* is the minimum volume to be applied as a band regarding the band setting.

Autosampler

In the case of the autosampler, some forms are similar to those in the Syringe pump view. The different forms including functionalities are explained here.
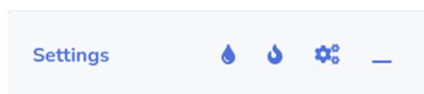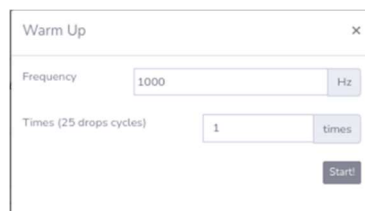

The **Drop** icon refers to Rinsing (as for Syringe Pump), but the way it rinses is different for the autosampler micropump. In this window, as before, automatic rinsing or manual rinsing can be selected. In the case of automatic rinsing, a number of **Times** can be selected, *i.e.*, the number of pump strokes; each stroke of the micropump is 50 µL. Regarding the suggested Frequency of 2, Times also depends on the nozzle type to rinse the entire tubing-pump-valve system (about 400 µL needed). Clicking the **Start** button starts the rinsing process.


For manual rinse, there is only the **Open Valve** button to open the dispensing valve. This option allows to rinse the tubings, the 3-way valve, the dispensing valve, and the nozzle with <u>any solvent not suitable for the micropump</u> (Instruction S5 – Solvent-compatibility of 2LabsToGo). Therefore, disconnect the tubing from the OUT port of the micropump, connect it to a syringe (with the Luer-Lock adapter) filled with solvent, and manually press the syringe piston, while the valve is open. Note that the valve will automatically be closed after 30 s.

The **Fire** icon refers to Warm Up. In this pop-up window it is possible to eject a number of drops, where the value **Times** means 25 drops cycles, thus, Times 2 means 50 drops, and **Frequency** sets the desired frequency of the dispensing valve to drop.

The **Gear** icon refers to Needle Movement. It is possible to move the needle up or down, to be able to place it correctly in the initial position if it is not. It should be noted that the movement is relative (moves the selected steps from the current position). Once the needle is at home height, this position can be set to zero by clicking the **Home** button.
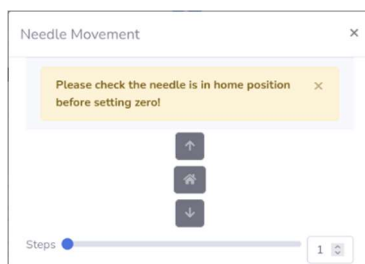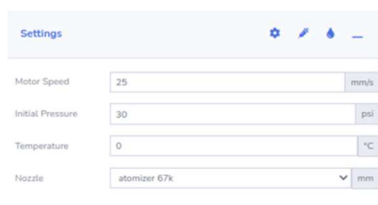
The autosampler Sample List form differs slightly from the Syringe Pump Sample List. Besides the sample name, sample volume and sample solvent, the vial number (up to 8 samples) can be selected for each band. To apply a solvent blank from the rinsing vial, select vial number 9.

Development

First place the **Cover Development** on top of the plate holder to built the development chamber.

Settings

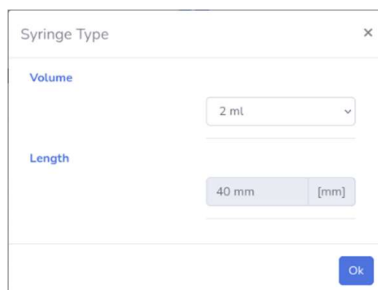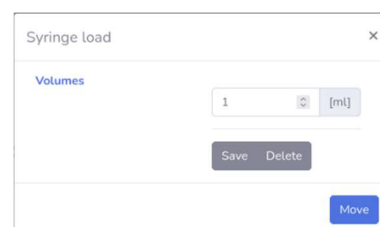| Motor Speed	Sets the speed of the syringe motor.
Initial Pressure	The minimum pressure to reach before the valve is opened.
Temperature	Sets the temperature of the plate during the development (option). It may delay the start of the process.
Nozzle	Only for documentation of the nozzle used.

Syringe type

With the **Gear** icon it is possible to select the Syringe Type: 2 mL, 5 mL or 10 mL. According to this selection, the movement calculations are carried out. In the pop-up window it is possible to see how the Length value changes, but the user cannot modify this value. By clicking **Ok**, the values are saved, and the syringe load can be calculated correctly. This is the first setting that the user should modify.
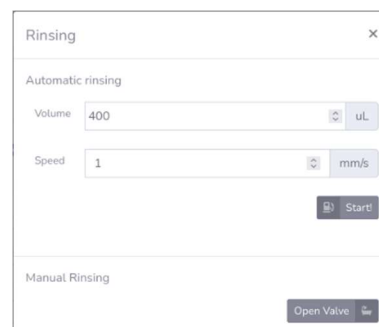

Syringe load

After click on the **Syringe** icon located in the top-right corner, the **Syringe load** screen will open. It refers to how much volume (in mL) of mobile phase was loaded into the syringe. Once selected and clicking **Move**, the axis of the syringe pump will move and the syringe can be inserted. If the syringe does not fit exactly, increase or decrease the set volume slightly via the arrows and click **Move** again.
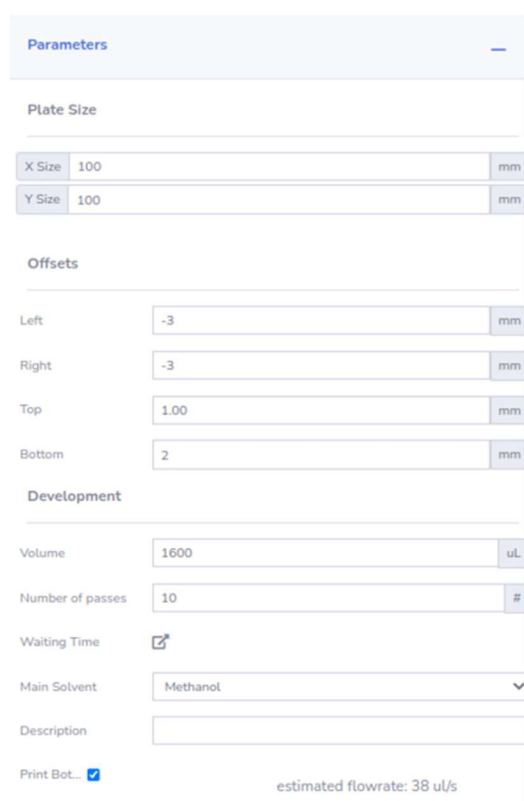
Rinsing

After click on the **Drop** icon located in the top-right corner, the **Rinsing screen** will open. Rinsing is a quick way especially to exchange the solvent and to remove air bubbles from the microfluidic system. Rinsing can be done automatically with a set volume by pressing **Start** or manually by clicking **Open Valve** and pressing the syringe.

Development Parameters

Detailed information on plate sizes and offsets can be found under the analogous parameters used for Sample Application.

Volume	Total amount of mobile phase volume ejected in the development process.
Number of Passes	The number of passes in which the total volume will be applied.
Waiting Time	Sets the sleep time between each pass not to overload the plate, depending on the migration speed. Available in a new window (see below).
Main Solvent	Only for documentation of the main solvent of the mobile phase.
Print Bothways	If this option is checked, the solvent will be ejected on the way back also.
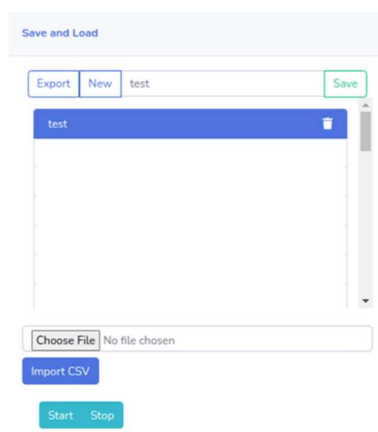
Save and Load

As for the the Sample Application, in the text entry field in the Save and Load window, the development settings can get a project name.

After performing all settings, click on **New**, type a name, and click on **Save**. Thereafter, parameter changes cannot be saved under this name (clicking Save). Create a new project with the changed settings.

Clicking the **Export** button will export all settings as csv file to the user's download folder.

To load a development project, click on one of the already saved projects and all settings will automatically be reloaded.

A csv file can also be imported.

- Choose a file and click on **Import CSV**.

Nebulization

Mini-Incubation

Concerning the devices for the bioassay lab, software operation of the Nebulizer and Mini-Incubator is self-explanatory (Instruction S1). Cultivation with the Mini-Shaker is not integrated in software.

Detection

In the side bar menu, Detection offers **Capture Image** and **Process Image**. Right-click on Capture Image opens the Detection tab to capture plate images and to save them.

After setting User Controls, Camera Controls and LED Controls, the images to be captured can get a project name in the text entry field in Plate Preview window. Click on **New**, type a name, and click on **Save**. Thereafter, parameter changes cannot be saved anymore under this name (clicking Save). Create a new project with the changed setting.

Clicking the **Export** button will export all settings as csv file to the user's download folder.

Capture Plate Image
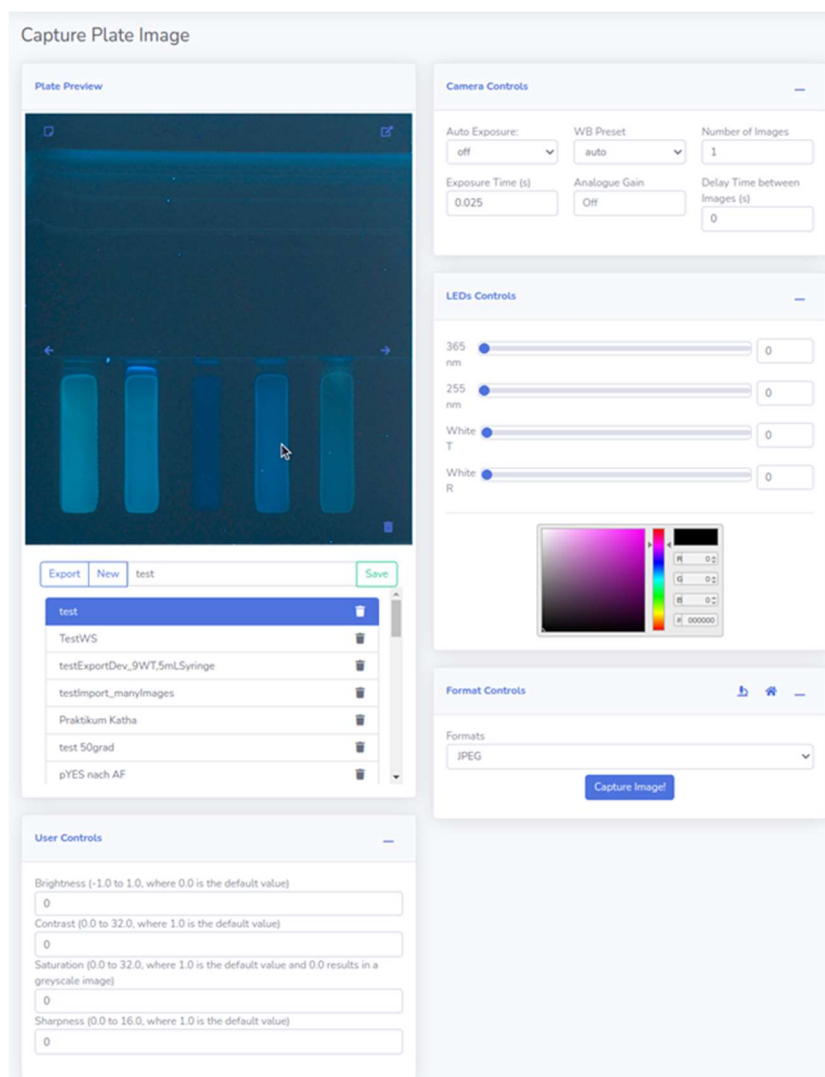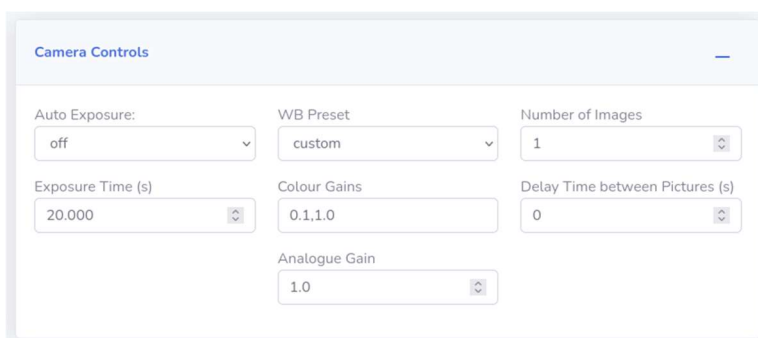
The User Controls include a list of settings that will be applied to the image, once it is already taken, so it is post-processing of the image.

Brightness	Adjust the image brightness [-1.0 to 1.0], where 0.0 is the default 'normal" value, -1.0 is very dark and 1.0 is very bright.
Contrast	Sets the contrast [0.0 to 32.0] of the image, where 0.0 is the default 'no contrast" value, 1.0 is the default 'normal' contrast, and larger values increase the contrast proportionately.
Saturation	Amount of colour saturation [0.0 to 32.0] of the image, where 1.0 is the default 'normal saturation" value, 0.0 produces greyscale images saturation, and higher values produce more saturated colours.
Sharpness	Sets the image sharpness [0.0 to 16.0], where 1.0 is the default 'normal" level of sharpening, 0.0 implies no additional sharpening is performed, and larger values apply proportionately stronger sharpening.

Camera Controls

Camera Controls includes a list of configurations that will affect the camera settings.

| Auto Exposure	on – off
Exposure Time	Time in seconds, only when Auto exposure is off, range [0.0001 to 200.0000].
WB Preset	Set the Auto White Balance (AWB) mode to any of the following: off, auto, tungsten, fluorescent, indoor, daylight, cloudy, or custom.
Colour Gains	Only when WB Preset is Custom, it is possible to set the colour gains [0.0 to 32.0], a pair of numbers where the first is the red gain (the gain applied to red pixels by the AWB algorithm) and the second is the blue gain.
Analogue Gain	Proportional to ISO is the analogue gain applied by the sensor, [1.0 to 30.0], where 1.0 is the default value.

In the case a set of consistent plate images is required by a multishot, select the number of images and the delay time. While multi-shotting, the images are consistent each other, there are no settings changed between images, even the camera itself does not autogain between images.

| Number of Images	Multishot possibility, from [0 to 200], where 1 is the default value.
Delay Time between Images	Time in seconds between images in case of multishot [0 to 200], where 0 is the default value.

LEDs Control
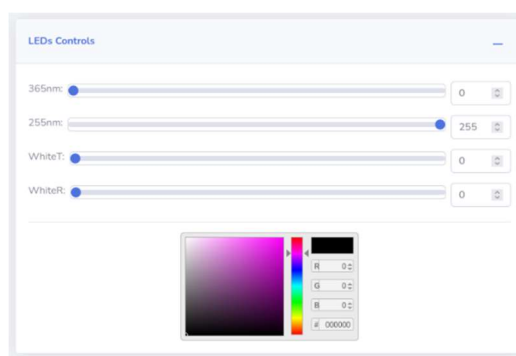
Allow to illuminate the plate with the UV-light (365 nm and 265 nm) and visible light.

The UV LED power can be settled from 0 (minimum) to 255 (maximum), the same applies in the case of WhiteT (White Transmission) and WhiteR (White Reflection).

The RGB selector allows different combinations of red, green, and blue light intensities.
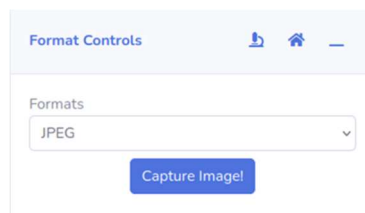
Allows to select a format between JPEG, PNG and BMP, where JPEG is the format by default. The image resolution generally is 2028 x 1520 px and cannot be changed.

Clicking on the **Microscope** icon will start to move the plate under the camera cabinet.

Clicking on **Capture Image** will take a photo with the current configurations.

Clicking the **Home** icon will move the plate holder to the front.

Plate Preview
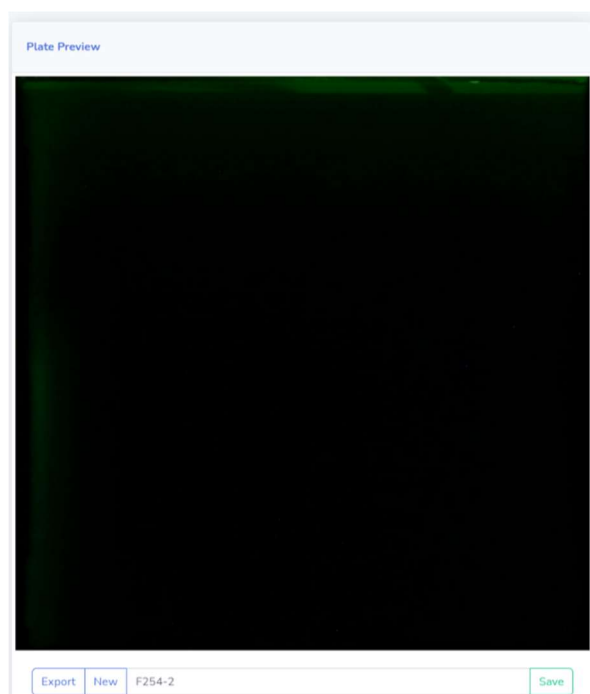
Every photo is saved in the database with a timestamp and displayed in the Plate Preview.

The image can be enlarged in a new browser window (click in the upper right corner).

To delete the image click in lower right corner.

For multishots, each image is saved with the same timestamp but a consequtive number is added.

Clicking the arrows to the left or to the right in the previewed image switches between the images of a project while displaying all settings of the particular image taken.

The camera and image settings can get a project name to be saved.

After performing all settings, click on **New**, type a name, and click on **Save**. Thereafter, parameter changes cannot be saved under this name (clicking Save). Create a new project with the changed settings.

Clicking the **Export** button will export all settings as csv file.
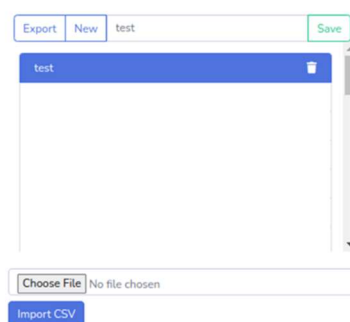
A csv file with all image settings can also be imported.

- Choose a file and click on **Import CSV**.

Process Image
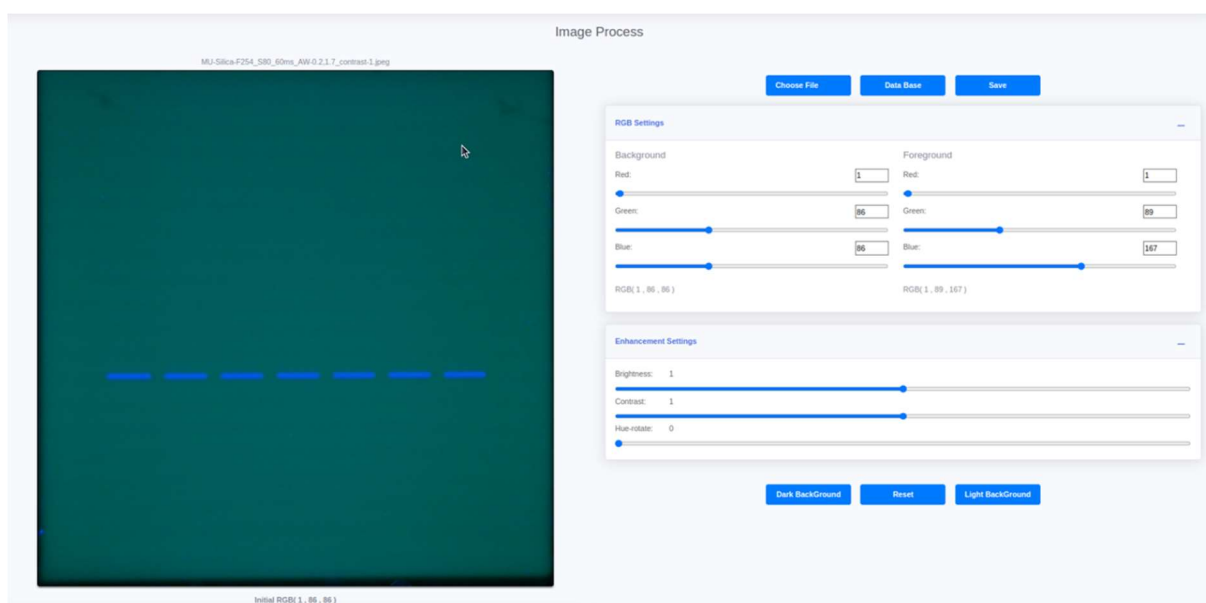
Opening the Process Image tab, the last captured plate image (timestamp) will be loaded. With the arrows (right and left of the image) it can be toggled between the images in the database.

Alternatively, any plate image can be uploaded from a local storage ('Choose File').

This image processing tool allows to modify the RGB values of both the background and the foreground (zones) to obtain an optimal contrast. Additionally some Enhencement Settings can be changed for optimization.

Clicking **Dark Background** will set the mean of background pixels to black. Here, it is best to exclude image parts not of interest (Skip Pixels (%)) below the application (Bottom) and above the solvent front (Top). The value of Skip Pixels (%) is identical with mm from the bottom and the top.

Initial Settings —

Skip Pixels (%)

Top: [0]   Bottom: [0]

Threshold

Red: [20]   Green: [15]   Blue: [18]

[Reset & Apply]

The result can be like this:

before
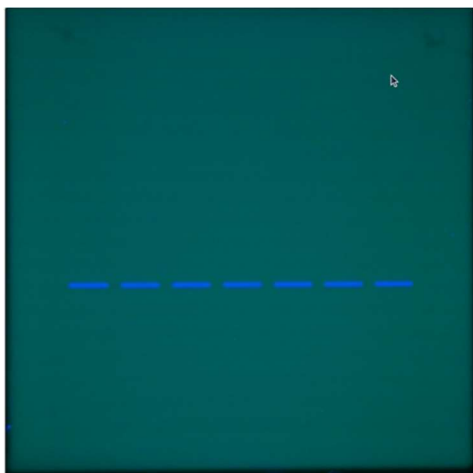
after Process Image
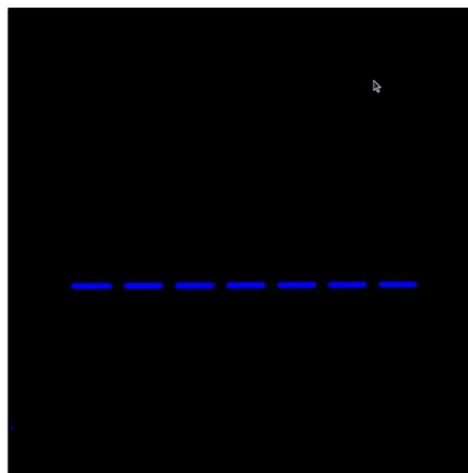
Clicking **Reset** returns to the original image.

Clicking on **Save** will save the corrected image under a selected name, if the browser is set correctly.

Clicking on **Database** returns to the images saved in 2LabsToGo database.

Appendix

Adding solvents

To add a solvent not provided by the 2LabsToGo-Software (here Beer), four files have to be edited.

Density table

2LabsToGo-Software/app/finecontrol/calculations/flow.py#L16

insert a new line in the section:

```
 density_table = {
        "Beer": 1.0,    #(1.0=density for the example Beer)
```

Development options

2LabsToGo-Software/app/templates/modules/development/devproperties.html#L50

add a new line:

```
 <option value="Beer">Beer</option>
```

SampleApp options

2LabsToGo/app/templates/modules/sampleapp/table/table.js#L1

```
const OPTIONS = [""Water", "Methanol", "Acetone", "2-Butanol", …, "Beer",….]
```

2LabsToGo/app/templates/modules/sampleapp/table/tablesp.js#L1

```
const OPTIONS = [""Water", "Methanol", "Acetone", "2-Butanol", …, "Beer"]
```

Adding nozzles

To add a nozzle not provided by the 2LabsToGo-Software (here 0.25), four files have to be edited.

Development

2LabsToGo-Sofware/app/templates/modules/development/settingsform.html#L38

add a line:

```
<option value="0.25">0.25</option>
```

SampleApp

2LabsToGo-Software/app/templates/modules/sampleapp/dinamicpropertiesform.html#L60

add a line:

```
<option value="0.25">0.25</option>
```

2LabsToGo-Software/app/templates/modules/sampleappsp/dinamicpropertiesform.html#L60

add a line:

```
<option value="0.25">0.25</option>
```

2LabsToGo-Software/app/finecontrol/calculations/flow.py#L43-L44

add two lines:

```
if nozzle_diameter == '0.25':
        self.nozzle_lohms = 7500
```

<u>Data/system backup</u>

At least, regularly save the database „2LabsToGo-Software/app/db.sqlite3" and the media folder.
In case a new installation is required, both can be imported.

The SD card of the Raspberry Pi is not as stable as an SSD drive. A power failure, for example, can make the SD card unreadable.
Therefore, it is good to have a full backup of the SD card with all installations, from time to time.

There is an „SD Card Copier" tool on the Raspberry Pi under „Accessories".
Connect an USB SD card reader with a fresh SD card (64 GB) to the Raspberry Pi and run the tool.

However, the backup medium is still an unstable SD card (magnetic field, heat).

It is therefore the best to prepare a compressed image file to be saved on a PC or server.
The Linux dd command (disk dump) and pishrink are the used tool.

Insert an NTFS or exFAT formatted USB stick (128 GB free space) in the Raspberry Pi.
Open a new Linux terminal and type
      df -l (I like List)
The last line of the output gives the information, under which name the USB stick was mounted, for example:
      /media/"*username*"/AAA4B3EBA4B3B7E1
["*username*" is the username selected during the Raspberry Pi OS setup.]

Then type the following dd command:
      sudo dd bs=4M if=/dev/mmcblk0
of=/media/"*username*"/AAA4B3EBA4B3B7E1/2LabsToGo.img status=progress and press ENTER.
The process takes a while. After finishing, there is a file "2LabsToGo.img" on the USB stick.

Compressing the file, is the job of pishrink.
In the terminal type:
      sudo pishrink.sh -z /media/"*username*"/AAA4B3EBA4B3B7E1/2LabsToGo.img
The former img file will be overwritten by a compressed 2LabsToGo.img.gz file, which also takes a while.

With the Raspberry Pi Imager, this file can simply be flashed on an SD card. Thus, the complete installation on the date of the image file is restored.

There also is a <u>bash script</u> available in the folder "2LabsToGo-Software/Operational_qualification", running both the dd and pishrink command.

In a Linux terminal change to this folder by typing
      cd ~/2LabsToGo-Software/Operational_qualification
Then type
      ./dd_pishrink.sh (and press ENTER)

The script first asks for the name of the USB stick to be typed (or copied and pasted), then the formerly described commands are running automatically ending up in the compressed img file.

The backup procedure can also be automated (for example each Sunday on 6 o'clock), if the Raspberry Pi statically is connected to an external server/NAS box via LAN/WLAN (setting up a cronjob).

https://raspberrytips.com/schedule-task-raspberry-pi/

https://www.bing.com/videos/riverview/relatedvideo?q=raspberry+pi+cron+job+setup&mid=96EB7A66C14CF5B5A30E96EB7A66C14CF5B5A30E&FORM=VIRE