Manual for the LabView Integration UI

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1 User Guide

1.1 Prequisites

If the software is not already installed on the computer, refer to the README.md file.

What NOT to do

- The program is not very fast. In case something does not happen instantly, be patient and avoid clicking multiple times. This mostly results in unpredictable behavior.
- Never, I repeat, **never** use the **Close**-Button (On Windows $\rightarrow$ the X) on the top right corner of any window. This will crash the program in every case. The same applies to the intrinsic *LabVIEW-Abort Program* button near the top left corner. Always use the special **Abort**. **Finish** or **Stop** buttons inside the UI.
- Normally, the COM ports can stay unmodified. However, (mainly after Windows Updates) these ports tend to change. Their correctness can be verified in the Device-Manager (Geräte-Manager) by plugging them out and back in.
- If a device does not turn green upon program start, do not worry. This happens (unfortunately) quite often. Just click the big **STOP** Button and restart. If the device is still not recognized try switching the respecive and/or your computer off and back on again.
- Do not open a .tif file, while an acquisition is running.

1.2 Starting the software

1. Ensure that the following devices are booted and connected to the PC
   - Olympus Microscope
   - TANGO Stage Controller
   - SOLA Light Engine
   - Valve Controller
2. Start the LabvView project UI_Project.lvproj
3. Expand the VIs subfolder and start the latest version of the UI
4. Click the Run or Run-Continously button on the top left corner
5. The red lamps on the top-left corner turn green upon successful connection to the respective device.

   If a lamp stays red:
   - Hit the STOP-Button and start over
   - If the lamp is still red, switch the respective device off and on again.
   - Ensure the correct COM-Port is selected. (Via Device-Manager or NI-Device Monitor)
6. Wait until the status field says “Initialization finished successfully”
7. Now, the program is fully operational.

1.3 Operating a Multiring chip

Make sure you started the program according to section 1.2.
A general remark: You can capture and modify the positions as well as the microscope lists with the buttons in their proximity. To select a specific configuration, double click on the desired row. A single click will only select the row for a modification by **Replace** or **Clear Selected**.

1.3.1 Initalization

1. Start the Elveflow device and the **ESI** software
2. Disable all 4 valves in the software
3. Pressurize the Elveflow with 4 bar to 8 bar by turning the right valve (blue) between the pressure regulators above the microscope
4. Wipe the microfluidic top and bottom with Acetone, IPA and ddH20 to remove dust etc. (otherwise it will cause imaging artifacts)
5. Fill the control tubing at least (!) 20 cm with ddH2O with a 1 mL syringe and connect it to the respective inlet
6. Insert the chip carefully into the microscope
7. Ensure that the tubing does not interfere with the other devices, corners or edges and can move freely during stage driving. Move a sufficient length into the microscope to avoid pulling at the inlets.
8. Connect a 40 µM Fluorescein solution to the Elveflow
   - Use the small diameter PTFE tubing for the whole length.

- Apply 10 mbar to 20 mbar at the respective **ESI** channel to move the fluid to the end of the tubing until a drop starts to form
- Insert the connector to Inlet 1 of the chip

9. Connect ddH_2O or nfH_2O to the Elveflow
    - Apply 10 mbar to 20 mbar at the respective **ESI** channel to move the fluid to the end of the tubing until a drop starts to form
    - Insert the connector to Inlet 2 of the chip
10. Build a connector from small-diameter PTFE-tubing to big-diameter PTFE-tubing; connect it to a waste container and the outlet of the chip (To minimize the hydraulic resistance and capacitive air effects)
11. Start the Labview program
12. Click the **Live** Button, then adjust **Filter**, **Intensity** and **Exposure** settings.
13. Open the controle pressure with the left, blue valve on top of the microscope and pressurize the control valves with approx. 1.5 bar by turning the center and left pressure regulator.
14. Click the **No Flow** Button to pressurize all quake valves

1.3.2 Loading the chip

15. Check the control inlets for leaks and wait for the channels to fill, control it in the Live mode. Now would be a great time for experienced users to save the different calibration positions.
16. Pressurize the **ESI** channels for water and fluorescein to 250 mbar
17. Select *Reagent 1* and click the **Flush** Button to inject the fluorescein

1.3.3 Adjusting the valve pressure

18. Upon arrival, deactivate **Flush** and switch to the *GFP* filter; adjust *exposure* (exit live mode) and *intensity* as well as the histogram sliders
19. Use the *10x Objective* and move to a valve in the reagent-multiplexer.

    Increase the pressure until you see two meniski, which indicate that the valve closes completely.
20. Repeat the same procedure for the ring pump

1.3.4 Remove air

21. select *Reagent 2* and click the **Flush** Button again to inject the water

    For faster flushing, the **ESI** pressure can be increased **temporarily** up to 500 mbar

    Upon arrival, let it flush as long as air is in the flushing channel between the rings
22. Deactivate the **Flush**, click **All Valves** and load all rings with **Load**

    Wait until all rings are partly filled

    Activate manually the valve *A5* to load another side of the ring

    Deactivate *A5* and open *B5* to close the outlet.

    This will pressurize all channel and remove remaining air bubbles.

Test the RemoveAir button. It should do the same.

1.3.5 Performing the pump calibration

23. Click the **No Flow** Button
24. Ensure the *Position List* is empty

    if not, hit the **Clear Last** button
25. Move to every ring, starting at the bottom (of live mode) and capture one position of each ring with **Save Pos**. (The bottom most ring corresponds to RingNr. 1) For this step, the 4x objective is sufficient. However, if you want to use the calibration pictures as reference for your measurement, you should use the same settings as in your later program.
26. Exit *Live Mode*, wait some time and hit **Capture Blank** of the calibration panel at the bottom; follow the instructions and wait until the acquisition is finished
27. Change to *GFP*, select a desired *intensity*, go into **Live** and **Flush** with Fluorescein for 10 s
28. Make sure **All Valves** is still on and load all rings with **Load**
29. Exit **Live** and **Capture Full Intensity**
30. Go into **Live**, make sure **All Valves** is still on and **Load** all rings with Fluorescein
31. Exit **Load** and adjust *Feed* and *Pump* cycles

    Best results were achieved with $>$800 *Pump Cycles* and $\sim$30 *Feed Cycles*
32. Hit **Capture Dilutions**

    After the first dilution cycle, the (a bit under-)estimated remaining time is displayed

    At finish, an input dialog with 8 refresh ratios is displayed
33. Start MATLAB and run the *CalibrationScript.m* under *lib* $\rightarrow$ *Calib*

    Write the according **Refresh Ratios per Pump Cycle** back into Labview

    This is not ideal, I know...

1.3.6 Creating a program

34. **Optionally:** Clear the *Position List* and capture your desired positions.

    NOTE: As the focus is always saved with the position, it makes a difference if the positions are captured in *4x*, *10x* or another magnification
35. Save your position list by **Save List** with the suffix *.xml* in a desired location
36. Clear the *Microscope Settings* by clicking the **Clear** Button
37. Choose your desired acquisition settings (*Filter*, *Exposure*, *Intensity*) and **Save Settings**

    Repeat it for every color you want to acquire
38. **Save List** with the suffix *.xml* (preferably) at the same location as the positions
39. Make sure that the Refresh Ratios are set correctly. This is crucial for any further step!

    If you want to change the Refresh Ratios, you can either double click into the respective ratio and change it or hit the **Input Refresh Ratios** to input all ratios again.

40. Start the **Program Setup**
41. If you wish to modify a previously created program, click on **Load Old Program**
42. Select the previouly created *Microscope* and *Position Settings* by clicking the **Folder Symbol**

    If the lists have been loaded correctly, the LED beneath the Folder turns green
43. Now you can modify the program by clicking the respective buttons:

    Feed: Exchanges a defined fraction of a single ring

    Pump: Mixes all rings by actuating the ring pump. $\left(\text{Formula: Time} = \frac{\text{Pump Cycles}}{4\ \text{Hz}}\right)$

    Change Reagent: Flushes the outer channels with the selected reagent for a defined time

    Loop Start: Begins a unique Loop with a defined number of iterations

    Loop End: Closes a unique Loop with its specified ID

    Acquire: Acquires an image at all positions each with every microscope setting.
44. Hit **Save**

    For simple changes you can also modify an existing *.xml* file accordingly.
45. When program reaches the **Total Acquisition Time**, it terminates automatically
46. Until the **Total Acquisition Time** is reached, the outermost loop is repeated every **Interval**. We mostly use 15 min
47. The later filename is composed of **YYMMDD_hh_mm_ss_"YOURPREFIX".tif**
48. All images which are captured during the program will be stored in the **Image Output Folder**. You can either select an existing directory by moving into the desired folder and selecting **Verzeichnis wählen** or move to another folder, type in the folder name and selecting **Save.** Make sure that there is enough free space on the drive.
49. The previously created program will be saved in a *.xml* file. Navigate to the desired location, type in the file name with its ending and hit **Save**.

1.3.7 Starting a program

50. Check if the refresh ratios are still there and valid
51. Hit the **Run Program** button
52. Select the previously created *program.xml* file and hit **Load**
53. Check if all values and steps are correct
54. Start the continuous loop via the **Start** button

After starting the Program, a queue with all program steps is initialized and the timing is computed. Please be patient. This may take some time.