Manual for the LabView Integration UI

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1 User Guide

1.1 Prequisites

- The program is not very fast. In case something does not happen instantly, be patient and try to avoid clicking multiple times. This mostly results in unpredictable behavior.
- If the software is not already installed on the computer, refer to the README.md file.

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. 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.

6. 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)
7. Wait until the status field says “Initialization finished successfully”
8. Now, the program is fully operational.

1.3 Operating a Multiring chip

Make sure you started the program according to section 1.2.

1.3.1 Initalization

1. Start the Elveflow device and the **ESI** software, close all valves
2. Pressurize the Elveflow with 4 bar to 8 bar.
3. Wipe the microfluidic with Acetone, IPA and ddH20 to remove dust etc., which cause imaging artifacts
4. Fill the control tubing with ddH2O with a 1 mL syringe and connect it to the respective inlet
5. Insert the chip carefully into the microscope
6. Ensure that the tubing does not interfere with the other devices and can move freely during stage driving
7. Connect a 40 µM Fluorescein solution 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 1 of the chip
8. 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
9. 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)
10. Start the Labview program
11. Click the **Live** Button, adjust **Filter**, **Intensity** and **Exposure** settings.
12. Pressurize the control valves with 1.5 bar
13. Click the **No Flow** Button to pressurize all quake valves

1.3.2 Loading the chip

14. Check the control inlets for leaks and wait for the channels to fill
15. Pressurize the **ESI** channels for water and fluorescein to 250 mbar
16. Select *Reagent 2* and click the **Flush** Button to inject the fluorescein

1.3.3 Adjusting the valve pressure

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

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

1.3.4 Remove air

20. select *Reagent 1* and click the **Flush** Button again to inject the water

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

    Upon arrival, let it flush as long as air is in the flushing channel between the rings
21. 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.

1.3.5 Performing the pump calibration

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

    if not, hit the **Clear Last** button
24. Move to every ring, starting at the bottom (of live mode) and capture one position of each ring with **Save Pos**
25. Exit *Live Mode* and hit **Capture Blank** of the calibration panel at the bottom; follow the instructions and wait until the acquisition is finished
26. Change to *GFP*, go into **Live** and **Flush** with Fluorescein for 10 s
27. Make sure **All Valves** is still on and load all rings with **Load**
28. Exit **Live** and **Capture Full Intensity**
29. Go into **Live**, make sure **All Valves** is still on and **Load** all rings with Fluorescein
30. Exit **Load** and adjust *Feed* and *Pump* cycles

    Best results were achieved with $>$800 *Pump Cycles* and $\sim$30 *Feed Cycles*

31. 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

32. 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

33. 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* or *10x* or another magnification

34. Save your position list with **Save List** with the suffix *.xml* in a desired location
35. Clear the *Microscope Settings* by clicking the **Clear Last** Button
36. Choose your desired acquisition settings (*Filter*, *Exposure*, *Intensity*) and **Save Settings**

    Repeat it for every color you want to acquire

37. **Save List** with the suffix *.xml* at the same location as the positions
38. Make sure that the Refresh Ratios are set correctly
39. Start the **Program Setup**
40. If you wish to modify a previously created program, click on **Load Old Program**
41. 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

42. 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

43. Hit **Save** and follow the instructions

    The later filename is composed of **YYMMDD_hh_mm_ss_"YOURPREFIX".tif**

    For simple modifications you can also modify the *.xml* file accordingly.