ZELLMECHANIK DRESDEN

AcCellerator QuickGuide

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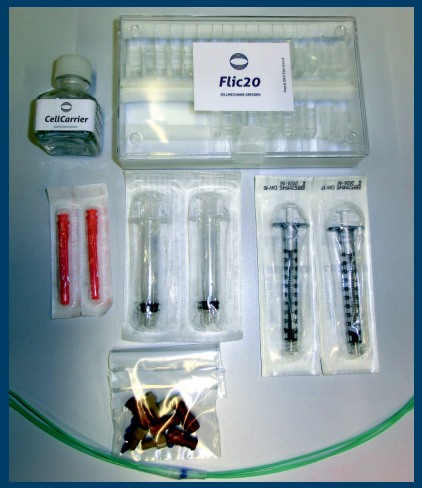
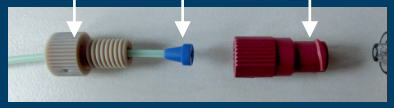
Preparation Checklist



- *CellCarrier* w. appropr. viscosity
- *FlicXX* w. appropr. channel dimension
- 2x blunt fill needle
- 2x 5 ml syringe, Luer Lock
- 2x 1 ml syringe, Luer Lock
- 3x Tubing of appropr. length
- 2x Fitting, 2x Ferrule, 2x Adapter



- StarterKit manual for detailed handling of microfluidic parts and cleaning of tube

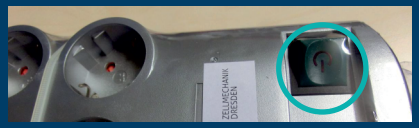
Sample

1. Check your sample requirements
2. Spin and resuspend cells in CellCarrier

Desired sample values:
Cell concentr. min 1M /ml, opt 3M /ml
Cell count min 100k, opt 2M
Sample volume min 50 µl, opt 500 µl

Power on

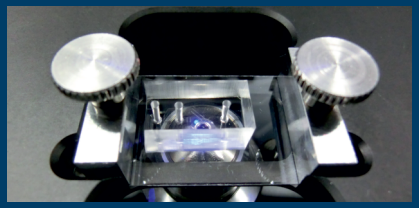
Switch on main power on socket



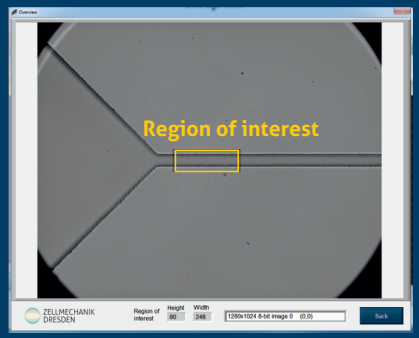
Check power:
microscope, XY stage, computer
syringe pump, camera, LED

Check image

1. Start *ShapeIn*
2. Activate *GetOverview*
3. Place a *FlicXX* on the stage and fix it



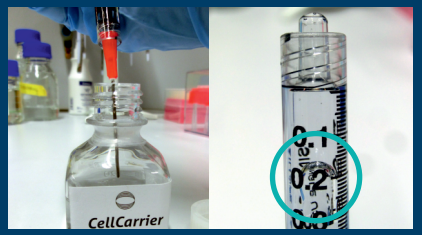
4. Find channel using XY stage
5. Focus channel
6. Align channel along the region of interest (ROI) using the camera rotation
7. position the ROI at the exit



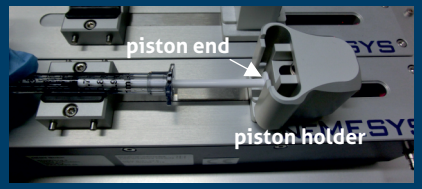
Too dark? Low contrast?
Read the troubleshooting

Syringe Pump

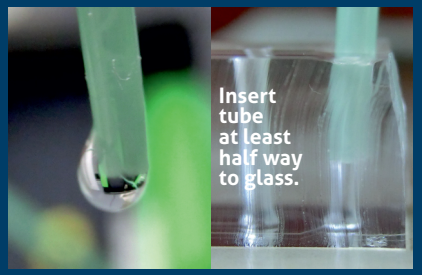
1. Start PumpOperation
2. Initialize pump
3. Syringe piston holder position = Level in the software?
4. Take up CellCarrier/Sample
5. Remove air bubbles



6. Connect tubing via adapter
7. Move piston holder to piston end



8. Fix syringe in syringe pump
9. Fill tube at 3 µl/s until drop forms
10. Slow down to 0.1 µl/s



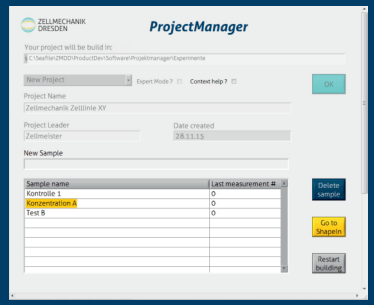
11. Plug into *FlicXX* during pumping
- Order of tube connection to the *FlicXX*: 1st Sheath, 2nd Waste, 3rd Sample

Watch the Overview window
Wait 100 s for the cells to arrive in ROI
Set measurement flow rate
Wait 60 s for equilibration

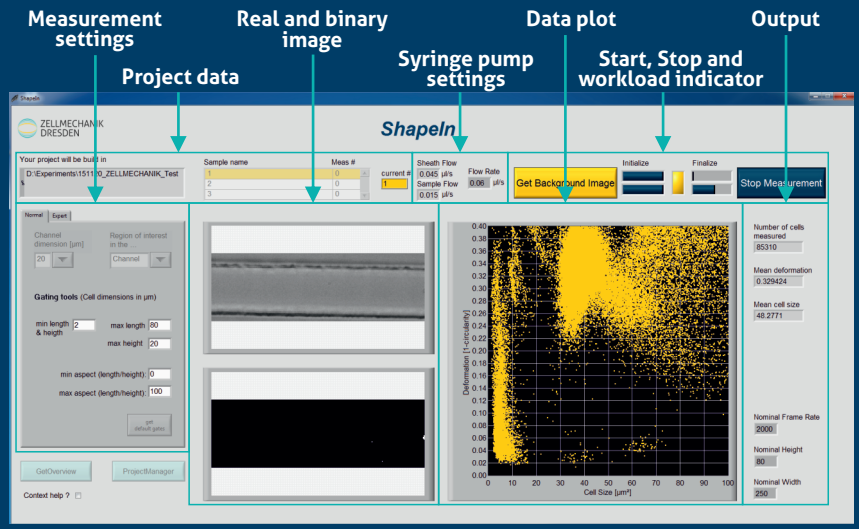
READ THE SAFETY INSTRUCTIONS

ShapeIn and ProjectManager

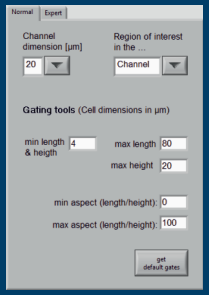
1. Go to *ShapeIn*
2. Activate *ProjectManager*
3. Create new or continue project
4. Follow on-screen instructions (use context help for quick tips)
5. Press Go to ShapeIn to transfer data

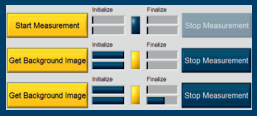


Data structure:
1. Project
1.1 Sample
1.1.1 Measurement

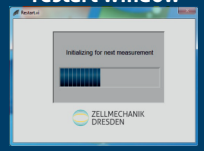


1. Choose the used channel dimension (e.g. *Flic20* = 20 µm channel)
2. Choose if you measure in channel or in reservoir
Default gatings are set for your choice. Changes can be made.
3. Check alignment of channel in ROI
4. Check the flow rate settings
5. Press Start Measurement
Starts when Initialize slider is filled





restart window



When binary image gets static white pixels press Get Background Image
High numbers of measured cells fill the Finalize slider
6. Press Stop Measurement (Finished when Finalize slider is empty)
7. Wait for restart of *ShapeIn*

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AcCellerator Troubleshooting

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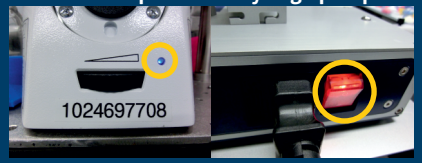
Missing Power?

Here you can see if a device has power.

microscope | syringe pump

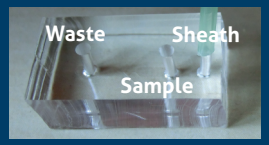


camera | LED

Software only runs with plugged dongle.

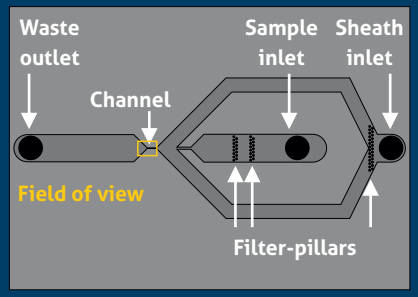
LED off? Connected to camera? Connected to power?

Finding the channel



Overview of *FlicXX* and inlets

This is the micro-structure of the *FlicXX*. Yellow square = Area in GetOverview

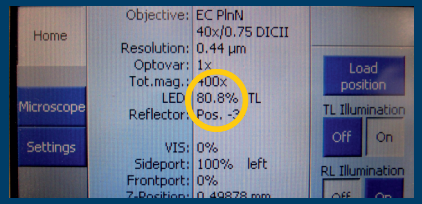


Try to find any egdge, focus and move stage in X and Y to the channel.

Dark Image?

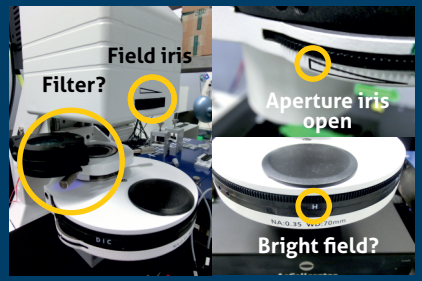
Is the image in the GetOverview window too dark or do you see low contrast? Check the following steps:

1. LED gets power 12 V (100%)? Increase with microscope power control



Example for Zeiss Z1

2. Aperture open? Condenser set to bright field (H)? No filters in?



3. Objectiv fixed in position? Distance to *FlicXX* approximately 0.5 mm?

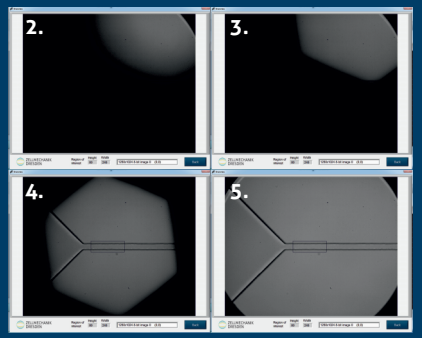
4. No liquid on the *FlicXX*? No obstacles in the light path?

5. 100% transmission to the microscope side port?

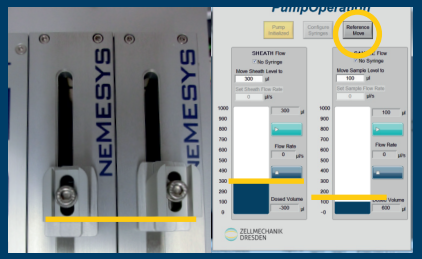
Köhler Illumination

Image still to dark? Köhler! See also microscope manual

1. Open field iris completely. Image gets brighter?
2. Close field iris until image gets darker
3. Move condenser to focus iris edges
4. Center iris
5. Open iris just until edges disappear



Syringe piston holder ≒ Level in the software?



Syringe piston holders at position 0
Level in software at 300 and 100
Do a Reference Move to recalibrate.

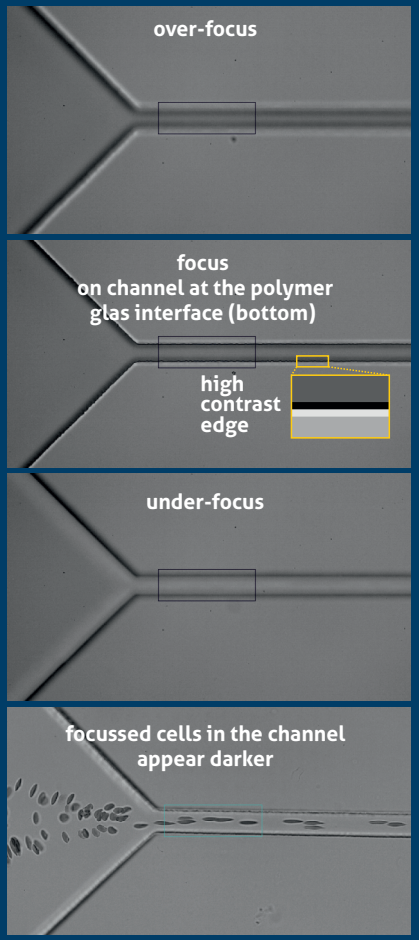
Cells not centered in channel?

Stop measurement if running.
Check for obstacles at the channel entrance.
Check if sheath-flow/sample-flow ratio is 3:1.

READ THE SAFETY INSTRUCTIONS

Finding Focus

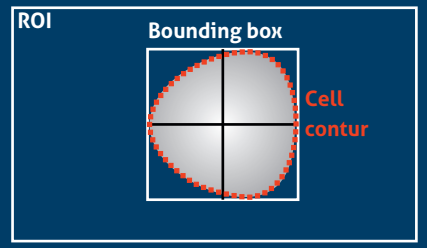
The right focus is characterized by a high contrast. The channel wall will appear in deep black. The adjacent polymer will show a white stripe.



Cells pass channel in the center, not at bottom. So the focus has to be slightly increased. Cell always need to appear darker than the surrounding area.

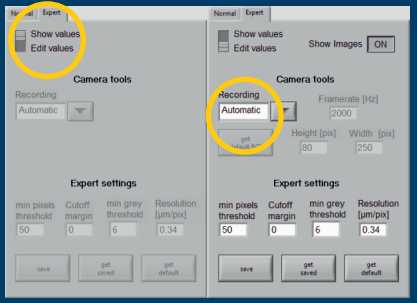
Gating cells

You can edit the settings for cells sizes to be ignored during measurement. Cell size is determined by a box. Edit min and max size or the ratio between X and Y extension of the cell.



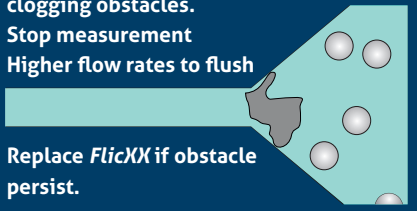
Camera settings

In expert mode you can edit the frame rate and the ROI size when changing from automatic to manual recording.



No cells in channel

Check channel entrance for clogging obstacles.
Stop measurement
Higher flow rates to flush



Replace *FlicXX* if obstacle persist.

Further instructions on zellmechanik.com and in the manual.
Call for support +49 351 41884430