Celldiscoverer7 Simple Time Lapse Experiment Instructions

Switch On and Start Software

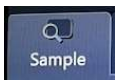
- On the front of the machine:
  - turn on the black button ()
  - press the blue button next to the LCD panel ()
- Turn on the computer and log in:
  User name: **multilabs**
  Password: **123456**
- <u>After the system has finished initializing</u> – see panel on the machine:
  Log into **BookitLab** and activate your reservation to start **Zen Blue 3.5**
- In 

tab on the right panel check 

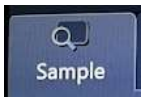
and 

and make sure pump is set at 5 

Dish Calibration

- Clean the bottom of your dish with 70% ethanol and wipe it <u>dry</u>.
- In 

tab press 

- Insert your dish on the stage and make sure it is placed right
- In 

tab
  - in 

press 

to choose your dish (e.g., Multiwell 24) and check 

  - in 

choose “Material” (polystyrene or glass)
  - in 

check 

and 

  - Press 

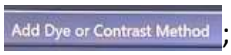
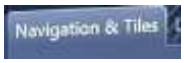
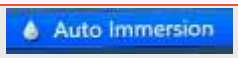
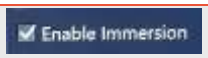
and wait <u>patiently</u> for calibration to finish

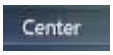
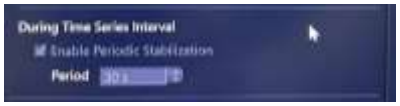
Reuse of old experiment

- If you have an old experiment, you can open the file and press 

.
  When it asks whether to synchronize the new calibration to the new vessel - agree.

Set Parameters

- In  tab (on the right panel) choose your objective and optovar, e.g., 
- In  tab check  and go to  tab to choose the right folder and name for your experiment (we recommend also checking  to create a folder with the current date). Please only use PI names for primary folders.
- Press  and choose fluorophores by double clicking on  and choosing from the list in  ; make sure you are on the right camera:  ; click [ok].
- In  tab make sure you are on  and recommended frame (1200x1200).
- Check 
- In 
  - In  tab [below-center panel] choose wells
    - In  choose positions: e.g., choose  and number of positions and press  or
  - In  tab [below-center panel] choose one well
    - Choose  ,  and number of positions, “draw” the ellipse on the well,
    - In  tab <right-click>  select other wells <right-click> 
- Move individual positions to fall inside the color coded rectangle according to your objective of choice  ()
- Move the stage to a position inside a well.
- If you are using the x50 objective, in  tab on the right press  and 
- Every time you change well manually (before starting an experiment), do  and 
- In  tab on the right press  .
- Press 
- In  tab set the exposure for each channel. **Consult with previous experiments and BCF staff.** In general, live experiments should be carried out with minimal LED intensity (5-15%) and enough exposure to produce at least 1000 gray levels (to check, go to  [below-center panel] and do min-max for each channel).

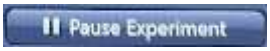
- Set the reference channel first with the arrows below the list  and right click to set it as the reference channel for autofocus (). This may be Oblique, or may be Brightfield (unchecked to remove acquisition) or a second fluorescence channel at even lower exposure (unchecked to remove acquisition).
- If you suspect there will be channel cross talk (e.g., Red signal contaminates Green channel), consider changing the filter for the contaminated channel to the single band emission (Green only or Red only) channel:
    1. In  tab choose the channel you want to change, and in  change the emission multi filter ( or ) to  or  or [BP 690/50] accordingly, and
    2. In  tab select the transmitted light channel (oblique or bright), in  tab click on  or  , and press  .
- If acquiring a z-stack, with z-stack ticked (), in focus, in the  tab set number of slices and interval and press  and  .
- In  tab choose  and  ; Resolution and Speed: 
- Stabilization  ; Synchronized by 
- During Time Series Interval → √ Enable Periodic Stabilization – period 30s
- 
- In  choose:

- Check 
- In  tab set duration of experiment and interval
- In  tab press 
- Go to first point in the list () and focus on it manually
- Press 
- Double check your Focus strategy has not reverted to “by Tiles setup”.

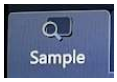
Start Experiment

- Press 

(the warning it issues means that you need to be in focus at the first position at this time – of course you have done this already)
- If you want to follow the progression of the scanning with indication of the well on the images in real time, in 

tab below the image, choose  .
- If you 

(e.g., to dispense a reagent) and 

, uncheck all selections in 

before returning the dish (

) and pressing 

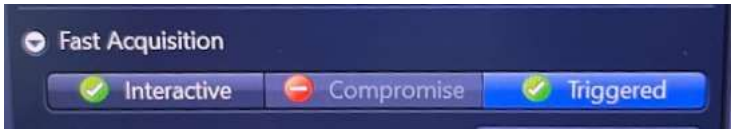
- (press only once, it will take a good few seconds to respond).
- If you followed the experiment acquisition in the 

tab below, you will have to check 

in the 

tab below to see acquisition in real time again.

Fast Experiment

- Use only one channel, consider lowering exposure time as much as possible making sure increasing laser does not bleach or kill your experiment, focus strategy “by tiles setup”
- Acquisition mode → 

End of Session

- At the end of the experiment copy your files to the server (no USB disks on any system computer please) in the folder under your PI’s name. You can access the server from your lab computer (ask Masha mdmasha@technion.ac.il)
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to retrieve your dish
- If an experiment is starting same day or next day, do not turn heating off when the software asks.
- <u>Close the software</u>
- Exit your reservation in BookItLab
- Press the blue button  on the system (it will retrieve the tray)
- **<u>After it shuts down completely</u>** also the black button  below (if you left the heating on do not press the black button below – if unsure, ask BCF staff whether to shut down completely).