Imaging Center Biomedical Core Facility Ruth & Bruce Rappaport Faculty of Medicine Technion - Israel Institute of Technology



מרכז דימות מרכז תשתיות ביורפואי

הפקולטה לרפואה ע"ש רות וברוך רפפורט הטכניון - מכון טכנולוגי לישראל

Instruction Celldiscoverer7 2025-03-16.docx 2025-03-16



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 Incubation tab on the right panel check I Heating 37.0 °C and CO2 Small V 5.0 % and make sure pump is set at 5

Dish Calibration

- Clean the bottom of your dish with 70% ethanol and wipe it dry.

-	In Sample tab press		
-	nsert your dish on the stage and make sure it is placed right		
_	In Sample tab		
	- in Sample Carrier press Select. to choose your dish (e.g., Multiwell 24) and check		
	Measure Bottom Thickness		
	- in Carrier Data choose "Material" (polystyrene or glass)		
	- in Prescan Options check Create Carrier Overview and Automatic Sample Carrier Calibration		
	Load Sample		

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

Set Parameters

- tab (on the right panel) choose your objective and optovar, e.g. Celldiscoverer In Acquilition tab check 🗹 Auto Save and go to 💾 Auto Save tab to choose the right folder and name In for your experiment (we recommend also checking Automatic Sub-Folder to create a folder with the current date). Please only use PI names for primary folders. Press Frank Setup and choose fluorophores by double clicking on Contrast N and choosing from the list in Add Dye or Contrast Method; make sure you are on the right camera: Orca-Flash 4.0; click [Ok]. In Acquisition Mode tab make sure you are on Binning 1x1 and recommended frame (1200x1200). Check Stiles Navigation & Tile In tab [below-center panel] choose wells 0 In In **Positions** choose positions: e.g., choose **I** and number of positions and press or tab [below-center panel] choose one well 0 and number of positions, "draw" the ellipse on the well, Choose tab <right-click> Copy Container for Replication select other wells <right-In Selected Container click> Paste Replication to 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 Auto Immersion tab on the right press MEnable Immersion and Every time you change well manually (before starting an experiment), do _ and Definite Focus tab on the right press In Press Likes
 - In <u>A Channels</u> 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 (**Constitution**). 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:
 - 🔺 Channels E Imaging Setup 1. In tab choose the channel you want to change, and in change QBP 425/30. the emission multi filter (TBP 425/30 + 524/50 + 688/145 or **RFP** or [BP 690/50] to or accordingly, and Channels tab select the transmitted light channel (oblique or bright), in 2. In E Imaging Setup tab click on TBP 425/30 + 524/50 + 688/145 or and press Minclude in this Setting Z-Stack tab set number of If acquiring a z-stack, with z-stack ticked (slices and interval and press Center and Center tab choose Combine Software Autofocus and Definite Focus Focus Strategy In and Definite Focus Stabilize as Start for Software Autofocus ; Resolution and Speed: Exact 🖬 🐓 Positions M Repeat Every Position Stabilization ; Synchronized by During Time Series Interval \rightarrow V Enable Periodic Stabilization – period 30s ng Time Series Interval Enable Nericitic Stabiliz Period 30 a Software Autofocus choose: In Automatic B Check Time Series Time Series tab set duration of experiment and interval In 💷 Tiles tab press In Go to first point in the list (Move to Current Point) and focus on it manually Close Press
- Double check your Focus strategy has not reverted to "by Tiles setup".

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Start Experiment

- Press Start Experiment (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 Graphics tab below the image, choose .
- If you **Heavse Experiment** (e.g., to dispense a reagent) and **Elect Tray**, uncheck all selections in **Sample** before returning the dish (**Load Sample**) and pressing **Continue Experiment** -

(press only once, it will take a good few seconds to respond). If you followed the experiment acquisition in the Player tab below, you will have to check

Follow Acquisition in the Dimensions 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"

Fast Acquisitio	Fast Acquisition		
🥏 Interactiv	/e 🥥 Compromise	🥝 Triggered	
and the second se			

- 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)
 - Eject Tray to retrieve your dish
 - If an experiment is starting same day or next day, do not turn heating off when the software asks.
 - Close the software

Acquisition mode

- 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).

