MD ImageXpress Confocal HT.ai (Irchel) - 1: Multiplexing Introduction

Startup of the system

Written By: Urs Ziegler



INTRODUCTION

Startup of the system

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Step 1 — Hardware



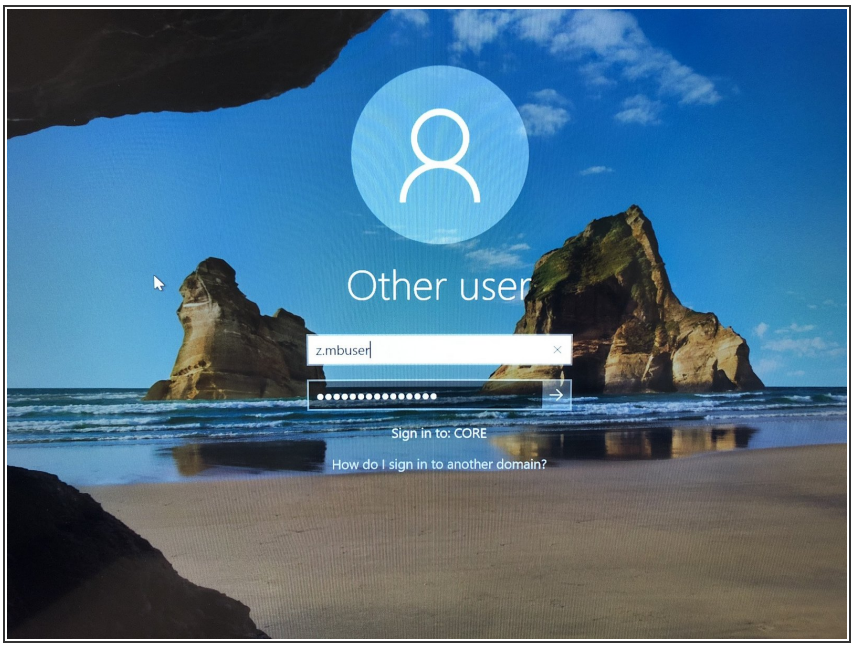


ⓘ All basic hardware components and computer should be on. If not, start the system following this sequence:

- 1. Instrument
  - Optional : if environmental control is needed, turn on sample heater
- 2. Laser (press button powerstatus)
- 3. Turn key to on
- 4. Turn on computer

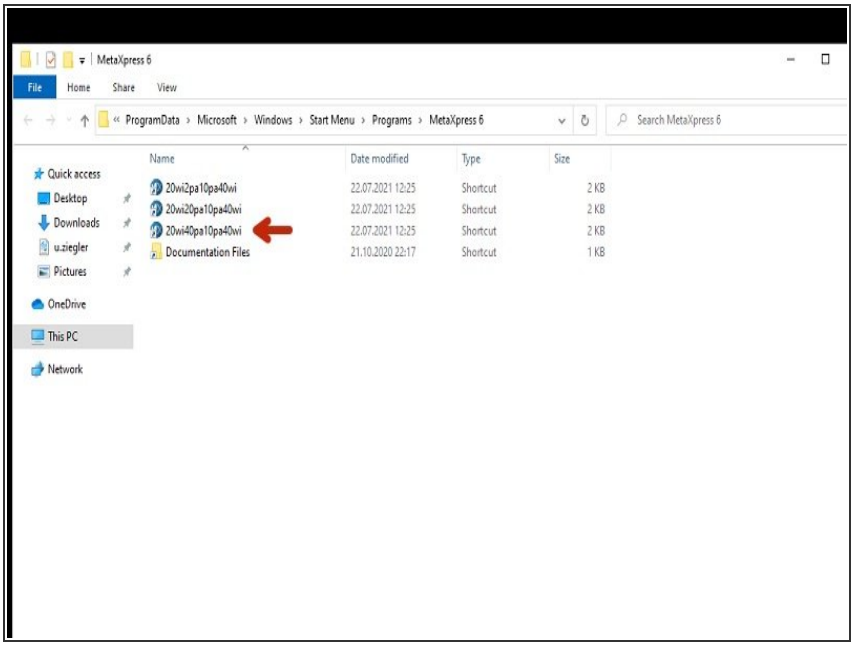
⚠ Turn on computer always last

Step 2 — Log-in



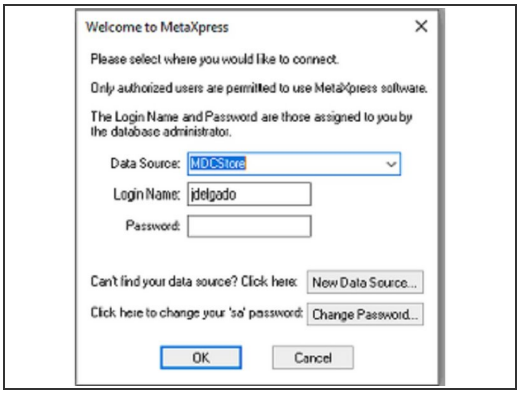
- Log in with your ZMB credentials.

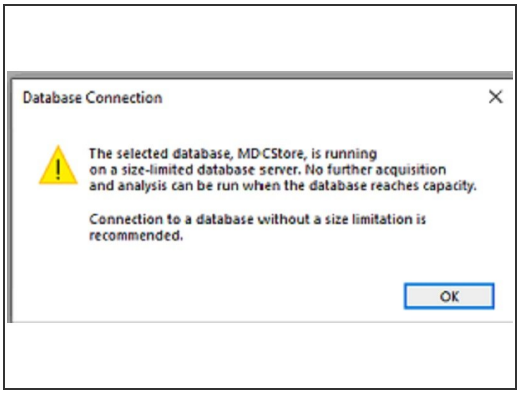
Step 3 — Start software

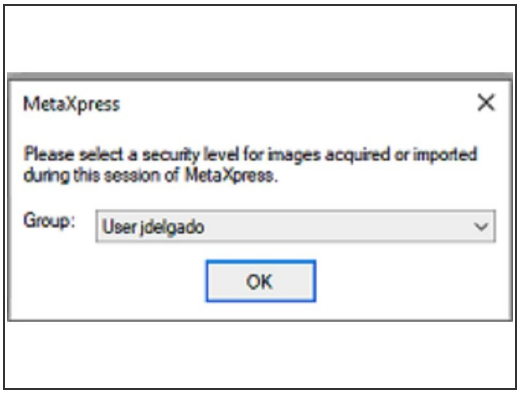


- Start the **profile defined in the feedback form**
  - ⓘ The other profiles can only be used if objectives in the system are / have been changed.
  - ⓘ Available profile are eg **20wi2pa10pa40wi** (2025-08-29)

Step 4 — Login to the SQL database



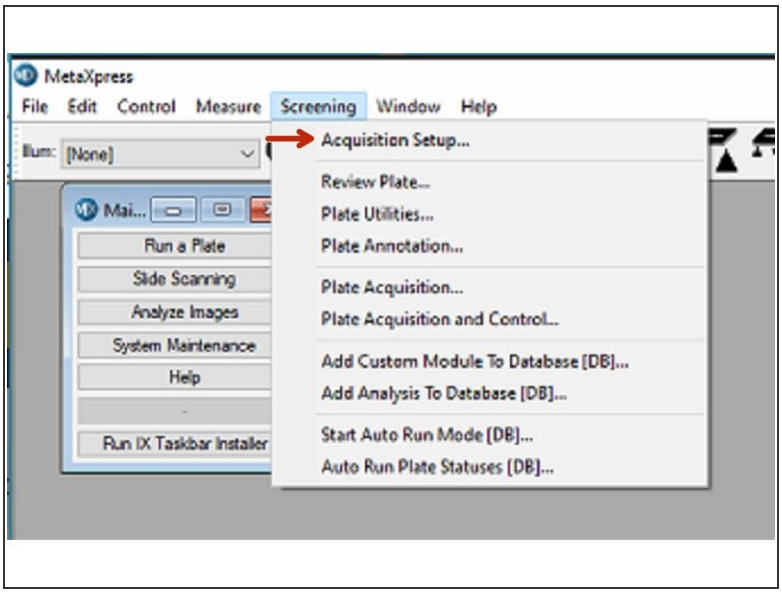


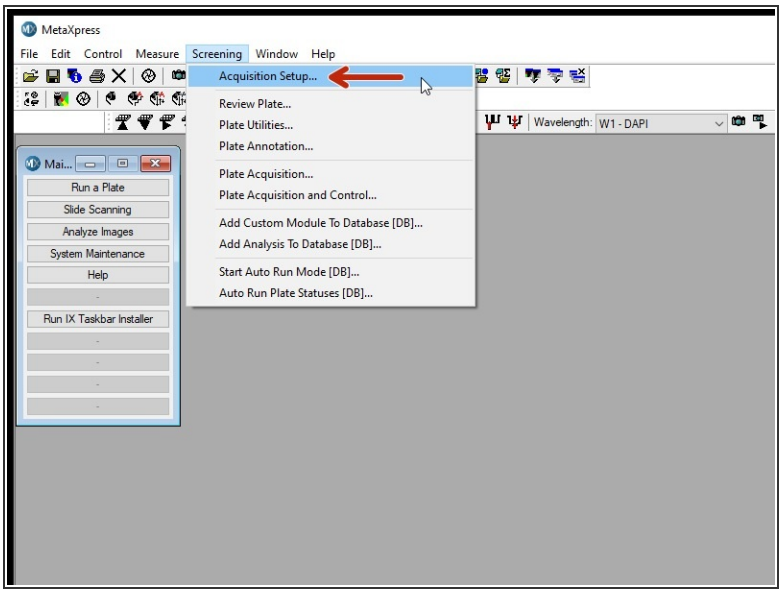


- Login to MDCStore
- username: use the username for the SQL database MDCStore.
  - ⓘ username is different from your windows username. Generally it is the windows username **without** the dot.
- password: use your SQL password
  - ⓘ The SQL password is different from the windows password. If you have forgotten it, the ZMB staff can help you.

ⓘ Accept the two information messages popping up after you connect to the database.

Step 5 — Accessing the Plate Acquisition Setup



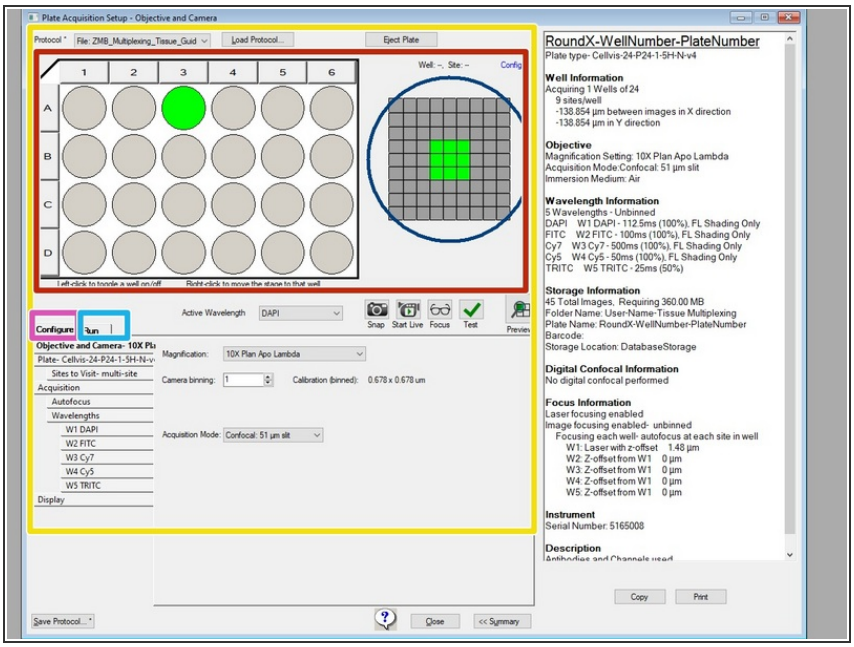


- Enter acquisition setup

ⓘ The Screening menu, which is used for protocol configuration and image acquisition.

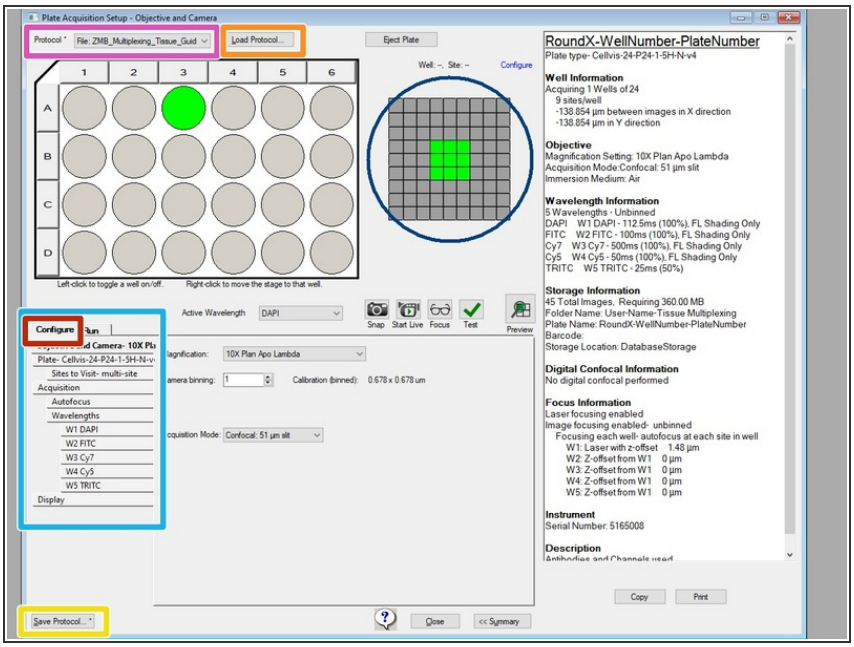
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Step 6 — Plate Acquisition Setup dialog



- Use the Plate Acquisition Setup dialog to create protocols for acquiring images from plates.
- The Plate Acquisition Setup dialog provides three primary functions:
  - Configuration control - for setting up plate acquisition protocols.
  - Run - for doing a plate acquisition according to a selected protocol.
  - The Plate Map that displays the plate layout to control movable components of the system by simply right clicking.

Step 7 — Configure tab / create a new protocol



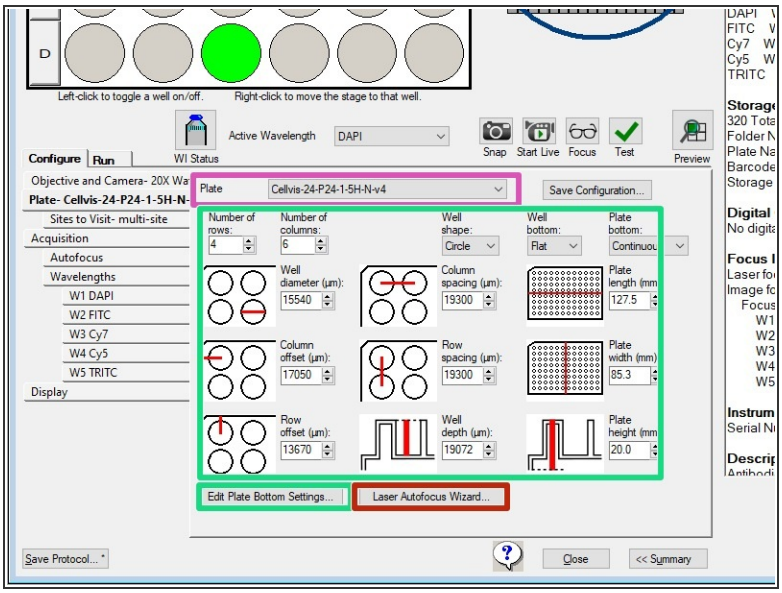
ⓘ Use the Configure tab to configure a plate acquisition protocol. The Configure tab is organized in a “top-to-bottom” tab structure that runs down the left side to guide you through the process ofsetting up the protocol configuration.

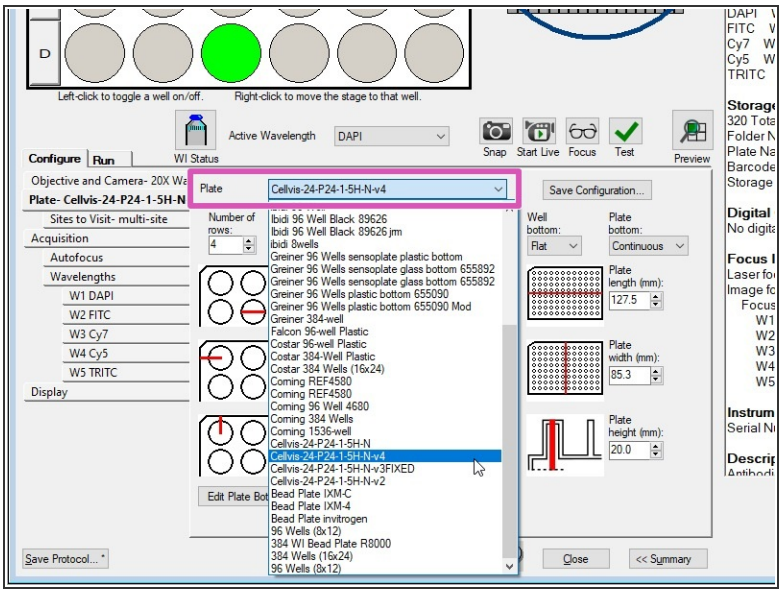
⚠ Make sure to only modify your own protocols!

- Use the Protocol drop-down to select from the eight most recently used plate acquisition protocols.
- Use the Load Protocol button to select a protocol that you saved from a previous session for plate acquisition during the current session.
- If you want to generate a new protocol, save a protocol first before modifying it. Save protocols to file in your private folder and not to database.

  ⚠ Select the **Save to File Rather Than Database** check box.

Step 8 — Plate tab





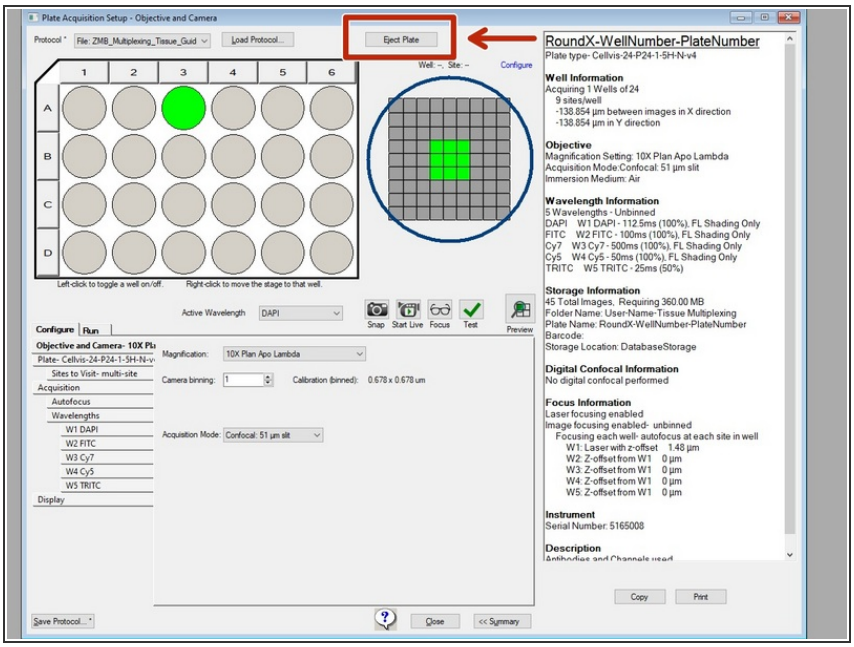
ⓘ Use the Plate tab to configure the plate dimensions to accurately control the X, Y, and Z movements of the system. The plate dimensions you define also ensure that laser-based autofocusing is as accurate as possible.

- The ZMB provides you with common plate types that are already configured, from which you can choose.
- If the plate type is not available in the Plate Name list, you can enter the manufacturer's plate specifications including plate bottom settings (if available).
- The manufacturer generally cannot provide values for the plate bottom settings, including optical thickness, which is not the same as physical thickness, and bottom variation. You must run the **Laser Autofocus Wizard** to measure the values to ensure proper focus.
  - Click **Laser Autofocus Wizard** and follow the instructions to calculate plate bottom measurements.

  ⓘ The wizard guides you step by step through the process of automatically calculating plate bottom dimensions and the focus laser exposure times that are required for each objective with your selected plate type.

  ⚠ Do not use the Laser Autofocus Wizard for slides because this feature is not compatible with slides.

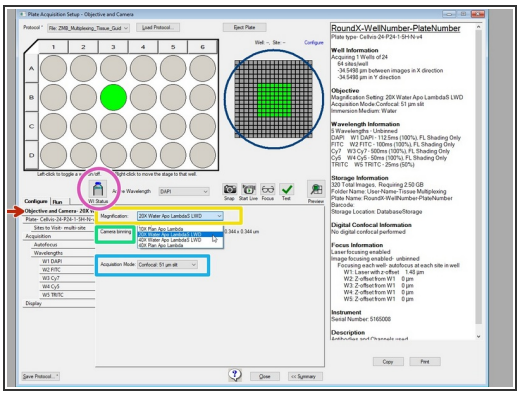
Step 9 — Load a plate

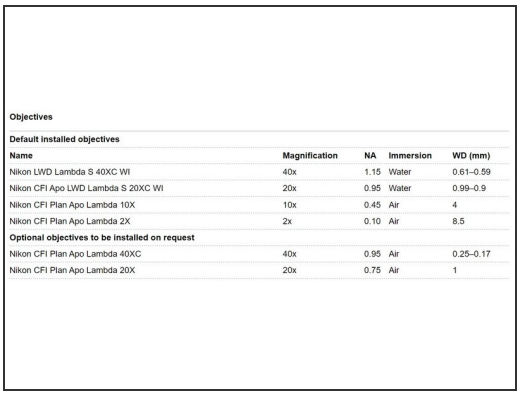


- The Eject Plate/Load Plate button switches between the following two options:
  - **Eject Plate** - Opens the top door and plate clamp to load or remove a plate.
  - **Load Plate** - Closes the top door and the plate clamp so that the instrument can acquire the plate.

ⓘ Note: The plate clamp closes within 2 minutes

Step 10 — Objective and Camera tab

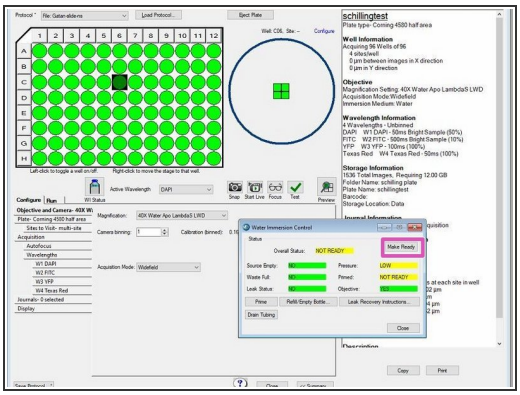




Objectives

Default installed objectives

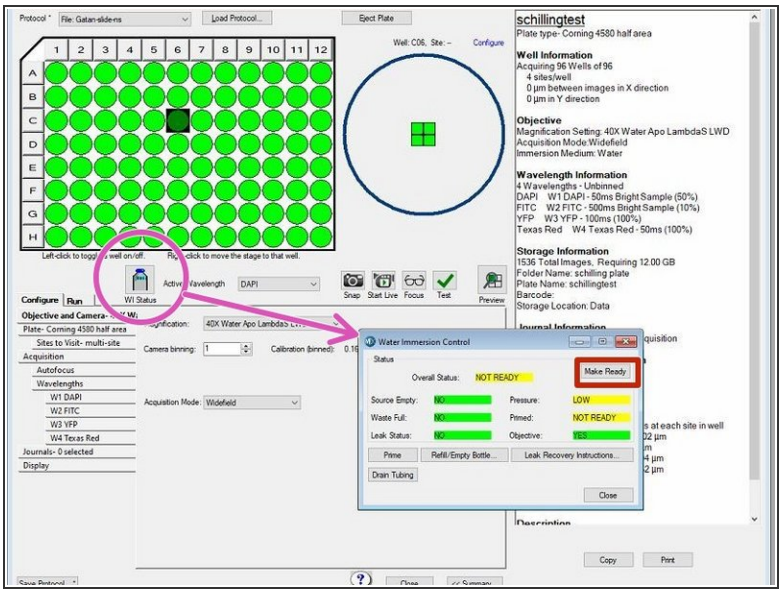
Name	Magnification	NA	Immersion	WD (mm)
Nikon LWD Lambda S 40XC WI	40x	1.15	Water	0.61–0.59
Nikon CFI Apo LWD Lambda S 20XC WI	20x	0.95	Water	0.99–0.9
Nikon CFI Plan Apo Lambda 10X	10x	0.45	Air	4
Nikon CFI Plan Apo Lambda 2X	2x	0.10	Air	8.5
Optional objectives to be installed on request				
Nikon CFI Plan Apo Lambda 40XC	40x	0.95	Air	0.25–0.17
Nikon CFI Plan Apo Lambda 20X	20x	0.75	Air	1

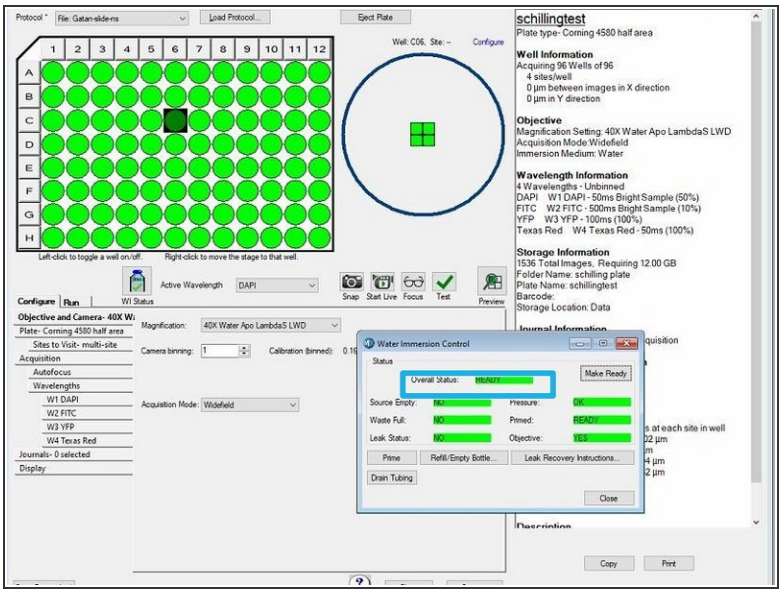


- Click the Magnification drop-down and select the setting of the objective to use for the protocol.
  - For 20x and 40x water immersion: control the water immersion system and add water immersion by clicking on **Make Ready**.
- Change camera binning if a different resolution is required or to increase the signal to noise signal-to-noise ratio.
  - ⓘ Higher binning might improve the speed of the acquisition, but reduces image spacial resolution.
- Dual disk unit allowing selection between 3 modes:
  - **Widefield** imaging mode.
  - **50 micron pinhole** disk for your basic **confocal** requirements (better optical sectioning but less sensitive). Spacing between pinholes 500 um
  - **51 micron slit** for **high throughput** confocal requirements (faster acquisition but lower optical sectioning). Spacing between pinholes 250 um

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Step 11 — Water immersion control



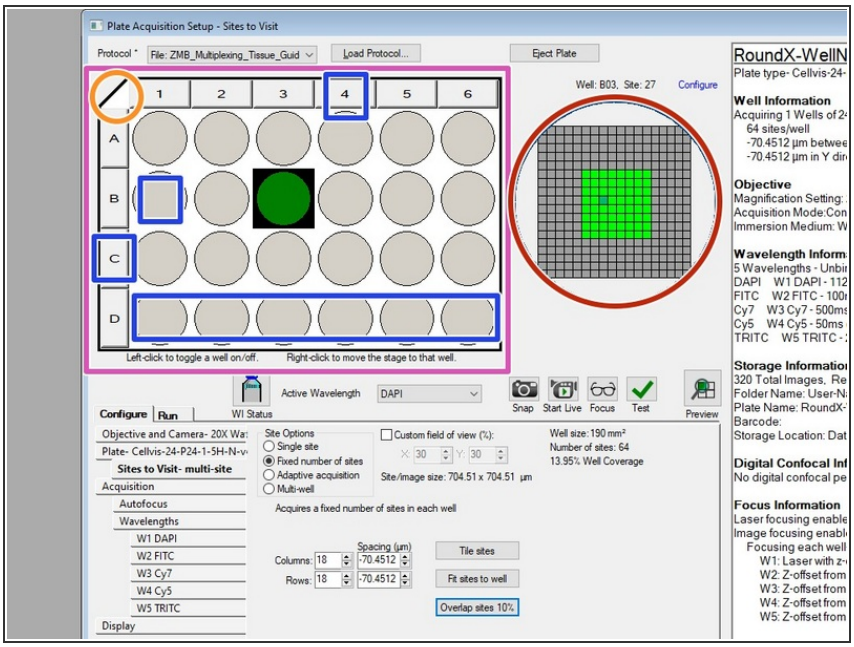


- When you select a water immersion objective the WI Status icon displays. Click on the **icon** to access the water immersion control.
  - Create a water bolus by clicking on "**Make Ready**".
  - As soon as water bolus is created and objective is ready to use the overall status will turn green "ready".

ⓘ When you switch from a water immersion objective to an air objective, the system drains the water and the software opens the instrument top door so that you can dry the bottom of the plate before using the air objective.

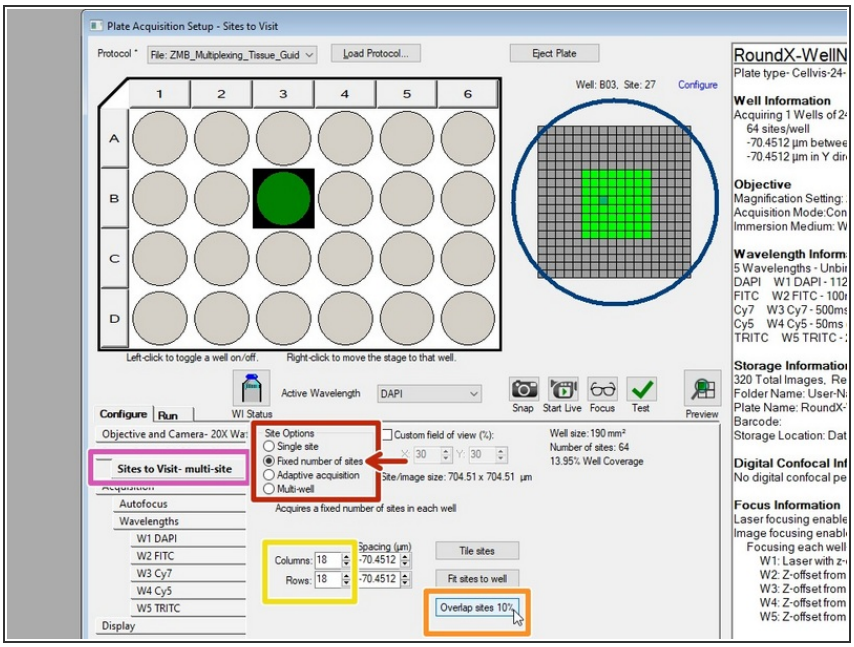
⚠ If you do not dry the plate bottom, you run the risk of getting water on the air objective and potentially into the instrument.

Step 12 — Plate Map well selection



- Use the Plate Map to select the wells to acquire:
  - To exclude wells from the image acquisition, click on a well, click a row or column header, or drag the cursor over a section of wells. Excluded wells display in gray.
  - To include or exclude all wells, click the upper left corner triangle.
  - ⓘ To move the stage to a specific well, right-click a well.
  - The Site Map represents the number of sites in each well and the position of the sites to acquire for each well. Sites you select to acquire display green and sites you choose to not acquire display gray.

Step 13 — Sites to Visit Tab



- You configure the number of sites that are to be acquired for each well in a plate acquisition protocol on the Sites to Visit tab
  - ⓘ Depends on the size of the well, the objective magnification, the distribution of sample material in the well, the type of plate, and the fluid content of the well.
- For Multiplexing Click a **Fixed Number of Sites**, configure the site layout, and then deselect the unwanted sites.

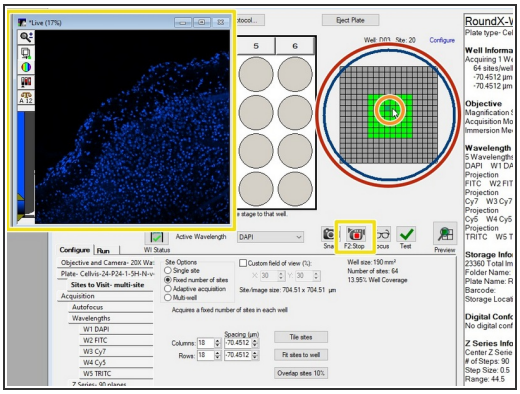
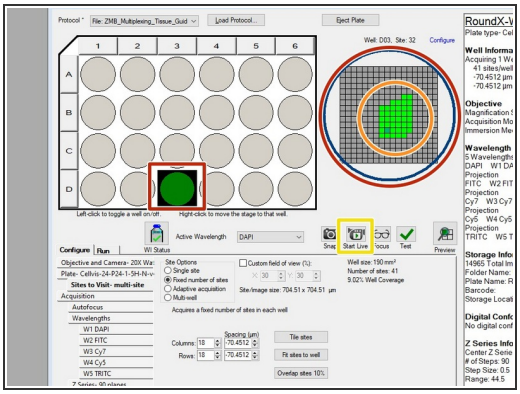
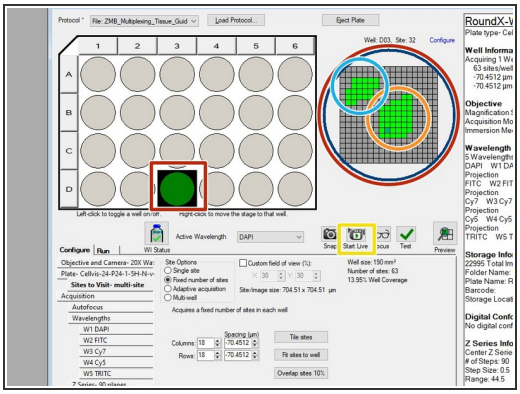
ⓘ Click to acquire a **Single site**.

ⓘ Click to acquire an **Adaptive Acquisition** is a computational algorithm that counts the number of cells on the fly during sample acquisition. This increases the chances of collecting valid data in every well. The number of sites the software acquires per well is based on the number of cells per well.

ⓘ Click **multi-well acquisition** to acquire high density plates faster. This option uses the size of the camera field of view to acquire several wells simultaneously while taking a single image, thereby reducing plate acquisition time. The software automatically calculates the number of wells in which a site can be simultaneously acquired.

- Adjust Column & Row Numbers
- Critical to select **Overlap sites 10%**

Step 14 — Selecting ROIs

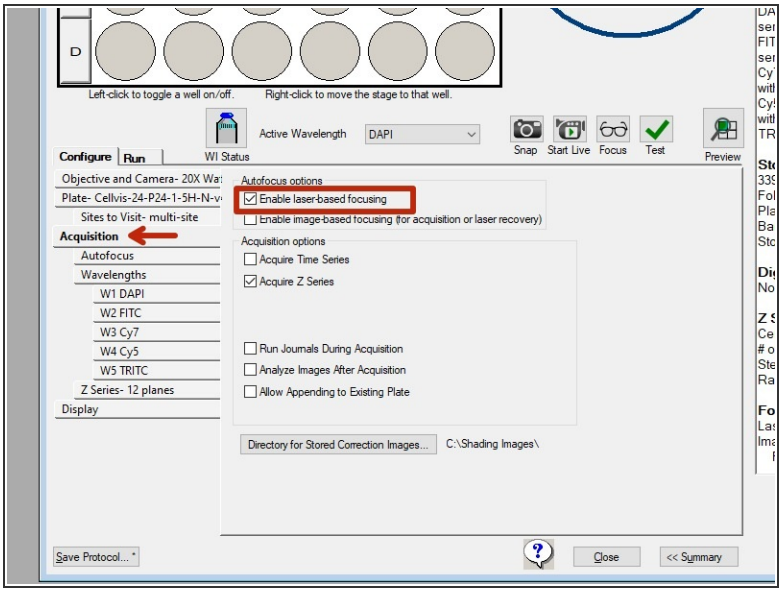
ⓘ Select ROIs including tissue boundaries.

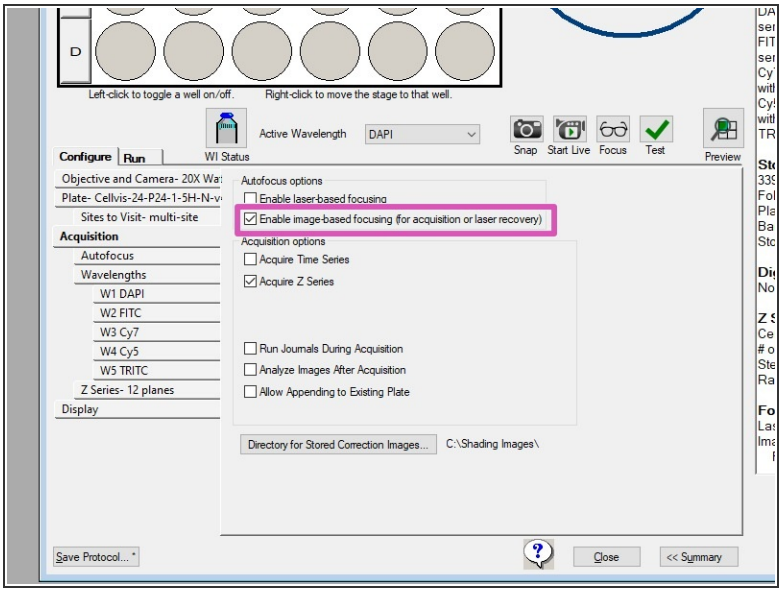
- Start Live to see tissue boundaries

⚠ Keep in mind the shutter closes after 30 seconds, so you have to stop and start live to keep seeing your tissue edges

- Stop live when you finish or to reset the shutter if it closes while you are checking tissue boundaries.
- Precise tissue borders are important as you will reacquire same regions in each multiplexing cycle and would like to avoid black tile acquistions.
- Avoid noncontinuous ROIs

Step 15 — Acquisition Tab (Autofocus options)





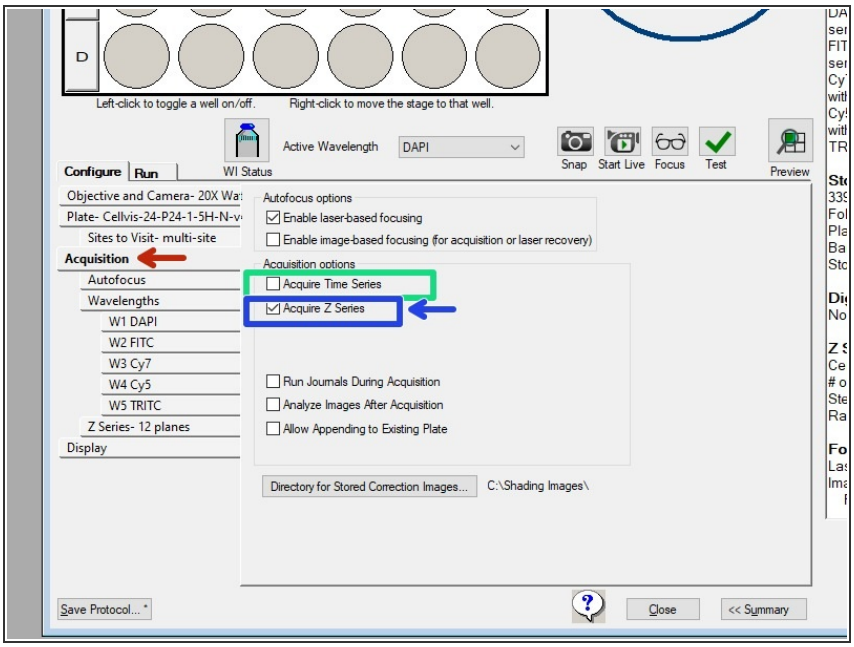
ⓘ Use the Acquisition tab to specify the autofocus options and additional acquisition options for the protocol.

- The Acquisition tab provides two autofocus options:
  - **Laser Based Focusing** is generally set to find the bottom of the well, and then moves the objective a distance up from the well bottom. This method is the fastest and does not cause photo damage to your wells. This method might not be sufficient if the distance above the bottom of the well varies in your sample.
  - **Image Based Focusing** uses a contrast-based algorithm to identify the best focus image. This option works best for experiments that use low-power objectives or when the sample distance above the bottom of the plate varies. Performance can be slower than Laser Based Focusing and focusing can fail if out-of-focus debris is in a sample.

ⓘ You can configure one or both autofocus options for a plate acquisition protocol. **It is recommended primarily using Laser Based Focusing.**

ⓘ Some samples can benefit from using both focus options including live organisms, suspension cells, tissue samples, and assays where the best focus position varies with the phenotype. When you select both focus options, the software uses LBF to move the objective to a position above the bottom of the well, and uses the IBF to fine tune the focus.

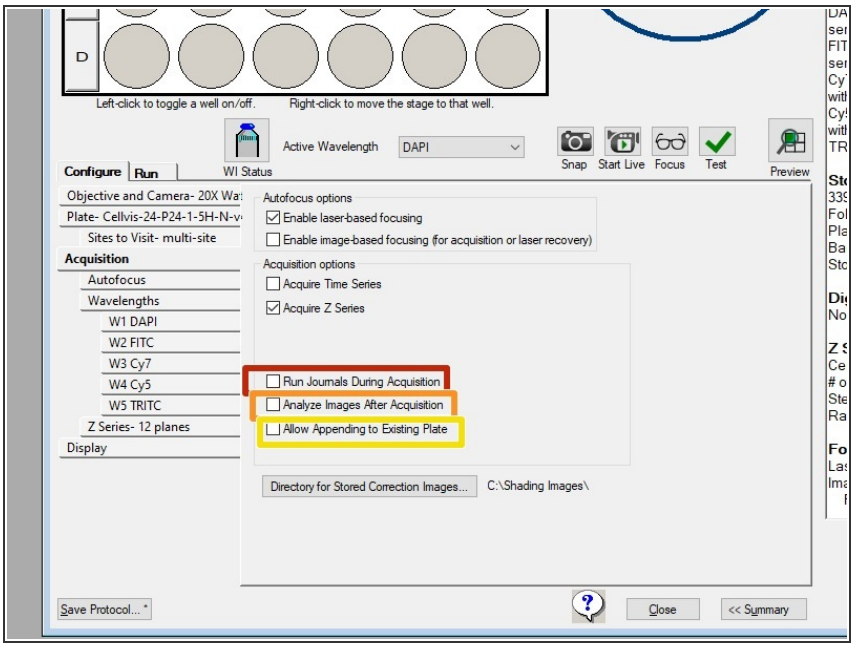
Step 16 — Acquisition Tab (Acquisition options)



The Acquisition tab provides two series acquisition options (you can select one or both):

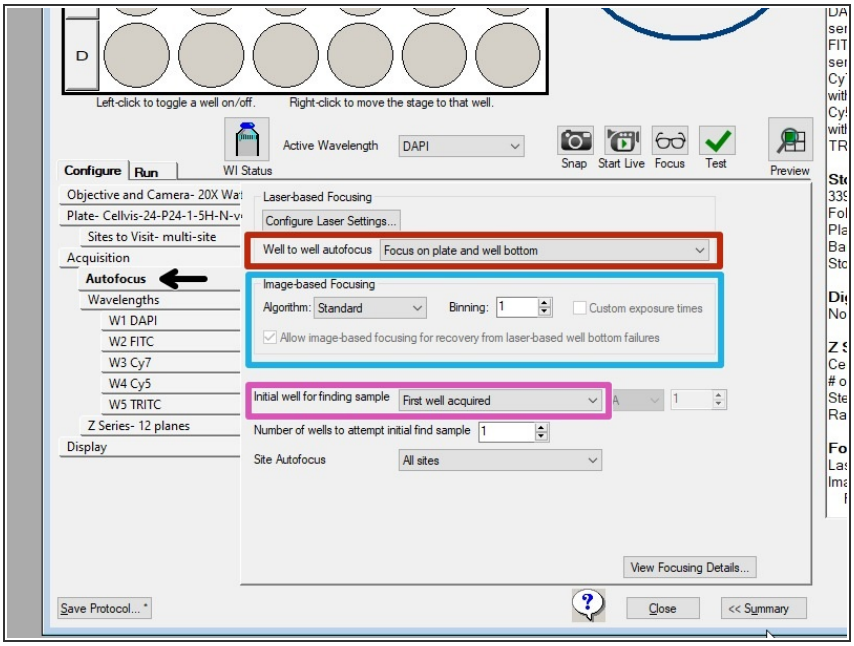
- **Acquire Time Series** acquires images at multiple time points. When you select this option, specify the set of images to acquire at each time point.
  - **Continuous** time series acquisition
  - With **discontinuous** time lapse acquisition, you can return the sample plate to the incubator in between time points, and you can read other plates in the interim.
- **Acquire Z Series** acquires individual opticalsections (planes) in sequence through a sample that can produce a 3-D image of the sample. When you select this option, specify the number of steps and the step size to move through the sample.

Step 17 — Acquisition Tab (Acquisition options)



- Journals: macro based complex tasks that should be run while acquiring (normally not selected).
- Analyze images after acquisition (normally not selected).
- Allow appending to existing plate: data will be added to an already acquired plate. Can be used e.g. for multiplexing experiments.

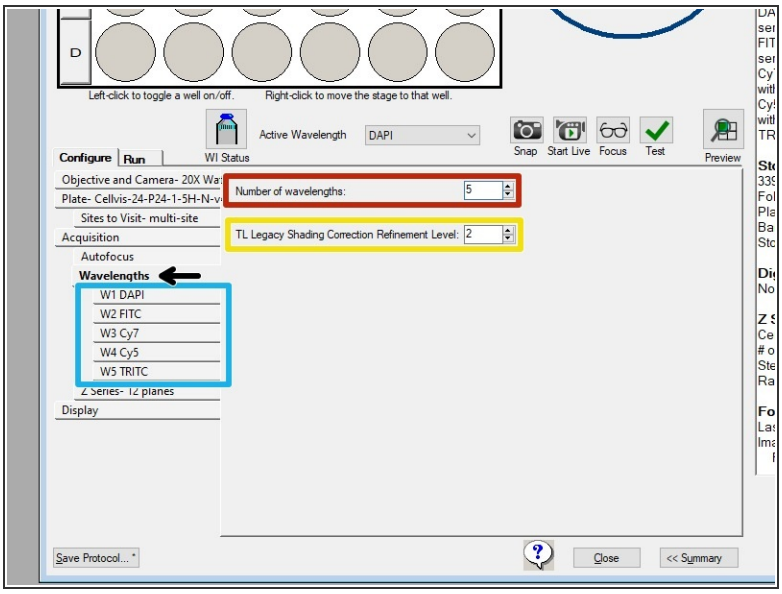
Step 18 — Autofocus tab



- Adjust focusing routine with laser settings and where the focus will be acquired.
- Configure image based focusing (if selected in the previous step)
- Specify the finding of sample and where to focus

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Step 19 — Wavelengths tab



Filter Set[1]	Wavelengths[2,3,4]	Common Fluorophores	Shading Correction Plate	Calibration Slide
DAPI	Excitation: 405/20 nm Emission: 452/45 nm Dichroic: FF409-Di03	DAPI, Hoechst, Alexa Fluor 350, BFP	Pink	Green (GP-8)
CFP	Excitation: 445/20 nm Emission: 483/32 nm Dichroic: ZT 445/520	CFP	Pink	Yellow (GP-11)
FITC	Excitation: 467.5/21 nm Emission: 520/28 nm Dichroic: ZT 405/470/555/640/730 or FF506-Di03	FITC, GFP, FAM, Alexa Fluor 488, BODIPY, Calcein, Fluo-4, MitoTracker Green	Pink	Yellow (GP-11)
YFP	Excitation: 520/10 nm Emission: 562/40 nm Dichroic: ZT 445/520	YFP	Green	Red (GP-7)
TRITC	Excitation: 555 nm Emission: 598/25 nm Dichroic: ZT 405/470/555/640/730	Cy3, TRITC, Alexa Fluor 532 & 546 & 555, Rhodamine, TAMRA, LysoTracker Yellow, MitoTracker Orange	Red	Red (GP-7)
Texas Red	Excitation: 555 nm Emission: 624/40 nm Dichroic: FF593-Di03-25	Texas Red, mCherry, Alexa Fluor 568 & 594, Calcium Crimson, Cy3.5, HcRed, MitoTracker Red, Propidium Iodide	Red	Red (GP-7)
Cy5	Excitation: 638/17 nm Emission: 692/40 nm Dichroic: ZT 405/470/555/640/730	Cy5, Alexa Fluor 647 & 660, DRAQ5, APC (Allophycocyanin), BODIPY 650/665, DiD, SYTO Red, TOTO-3, and TO-PRO-3	Pink	Red (GP-7)
Cy7	Excitation: 725/30 nm Emission: 794/32 nm Dichroic: ZT 405/470/555/640/730	Cy7	not supported	not supported

- Use the Wavelengths tab to specify the total number of acquisition wavelengths for an experiment.

ⓘ You must specify at least one acquisition wavelength and you can specify up to eight.

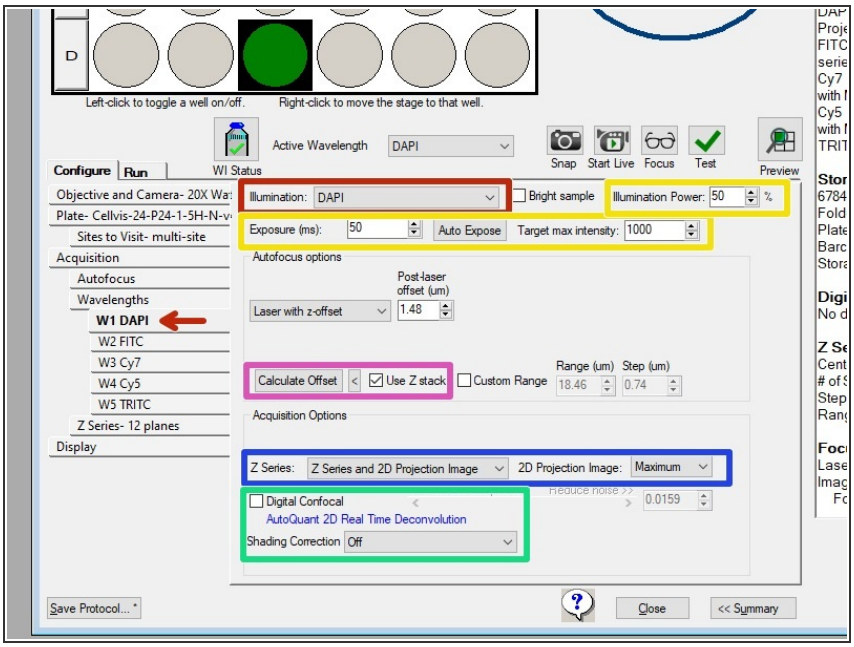
- When you specify more than one wavelength, subtabs display below the Wavelength tab where you configure exposure time, autofocus, and time lapse settings for each acquisition wavelength.

⚠ Red indicates that you have yet to configure the acquisition wavelengths.

- Enter the refinement level.

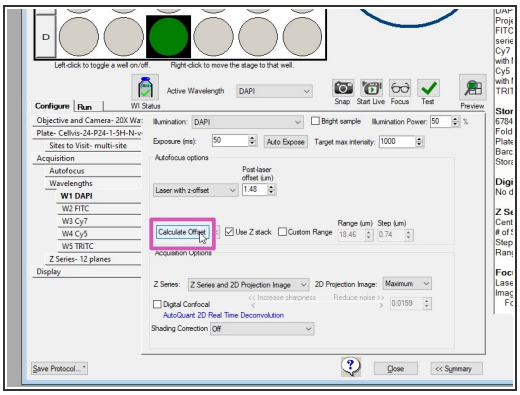
  ⓘ Increasing this value slows the acquisition routine. The single value that you enter applies to each acquisition wavelength for which you select Auto Correction for TL Legacy as the shading correction method.

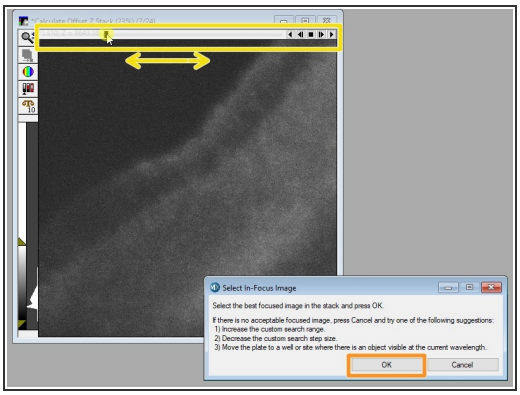
Step 20 — W# tabs

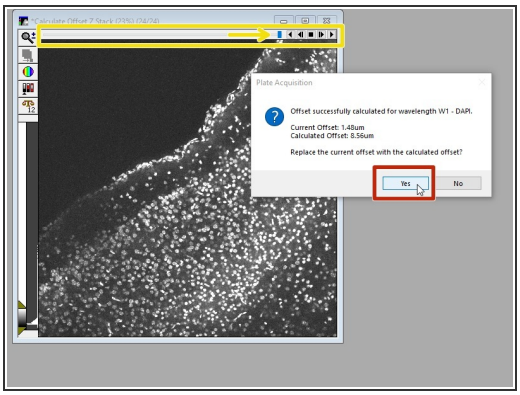


- Use the W# tabs below the Wavelength tab to configure acquisition wavelengths.
- Click the Illumination drop-down and select the illumination setting; for example, DAPI.
- Specify the exposure and illumination power.
- Calculate Offset to calculate the offset for all subsequent wavelengths.
- Specify the z-series (3D, projection) and how the projection should be calculated
- Digital confocal is a deconvolution computation. Normally this is not selected

Step 21 — Calculate Offset





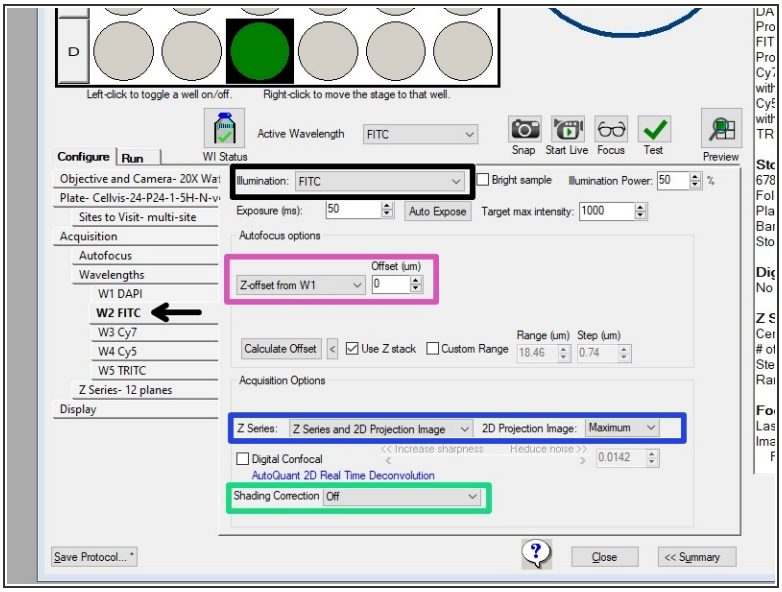


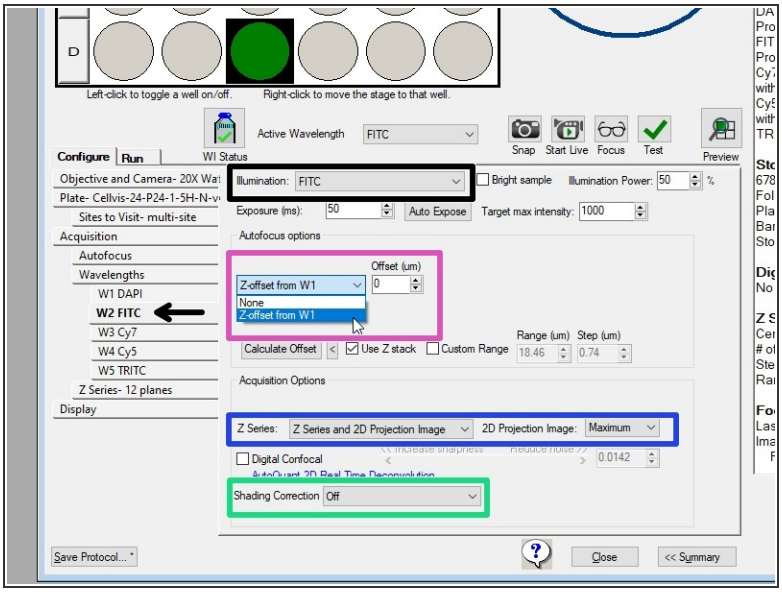
- **Calculate the Offset of W1** to calculate the offset for all subsequent wavelengths; for example, DAPI

ⓘ Pop up window of WI appears and z-series of focus points is acquired

- Use Slider to adjust and select best focus
- At best focus select **OK** or cancel if you want to select a different tile region or to readjust exposure/illumination power.
- Click **Yes** to replace the current offset with the calculated offset

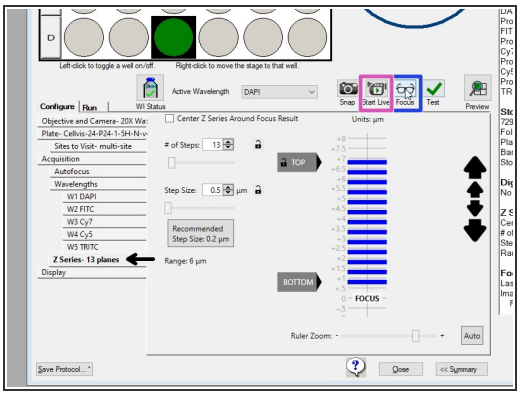
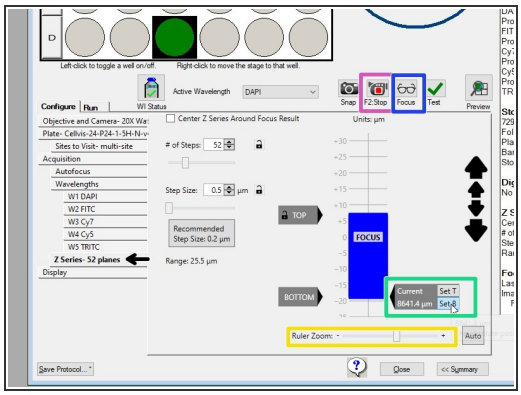
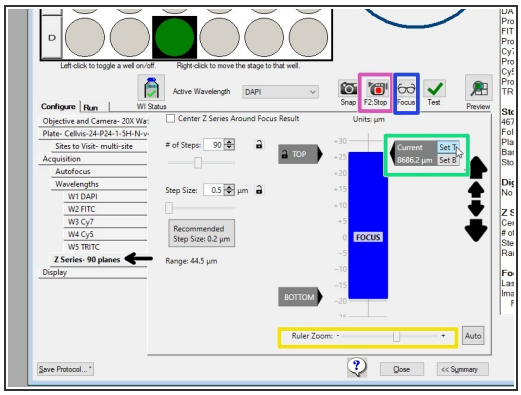
Step 22 — Use W1 offset for other wavelengths





- For W2- ; for example, FITC select **Z-offset from W1**.
- For W2 and each subsequent Wavelength specify Z series and 3D Projection Image
- Keep Shading Correction consistent for all Wavelengths
- ⓘ Repeat for all remaining wavelengths W3...W8.

Step 23 — Z-Series tab

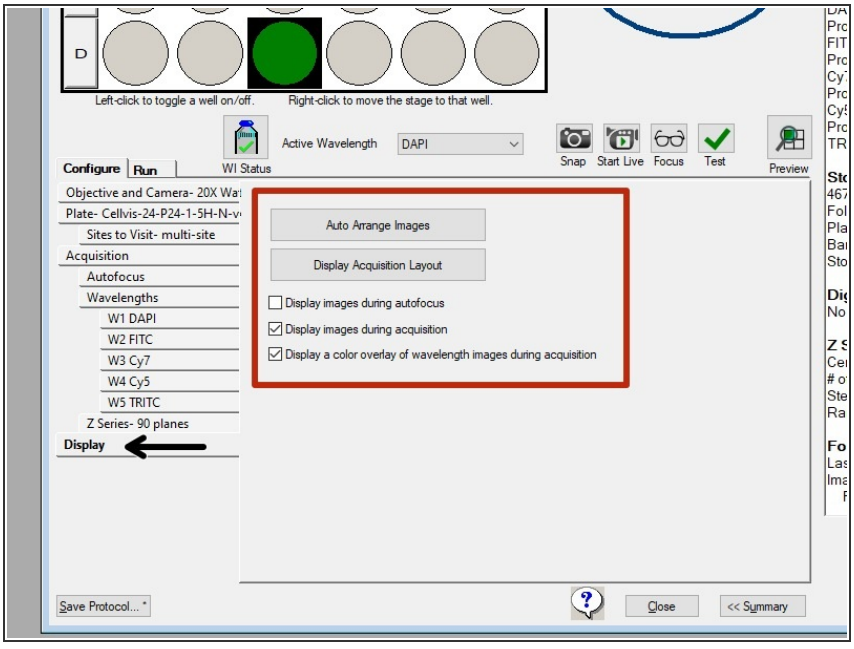
ⓘ Use the Z Series tab to configure Z series options (only if activated previously).

- Click **Focus** to see top/bottom scroll bar, if it disappears click Focus again.
- Click **Start Live** to see last visited wavelength; ex. W1 Dapi

⚠ Shutter closes after 10 seconds to reopen click **Start Live** again.

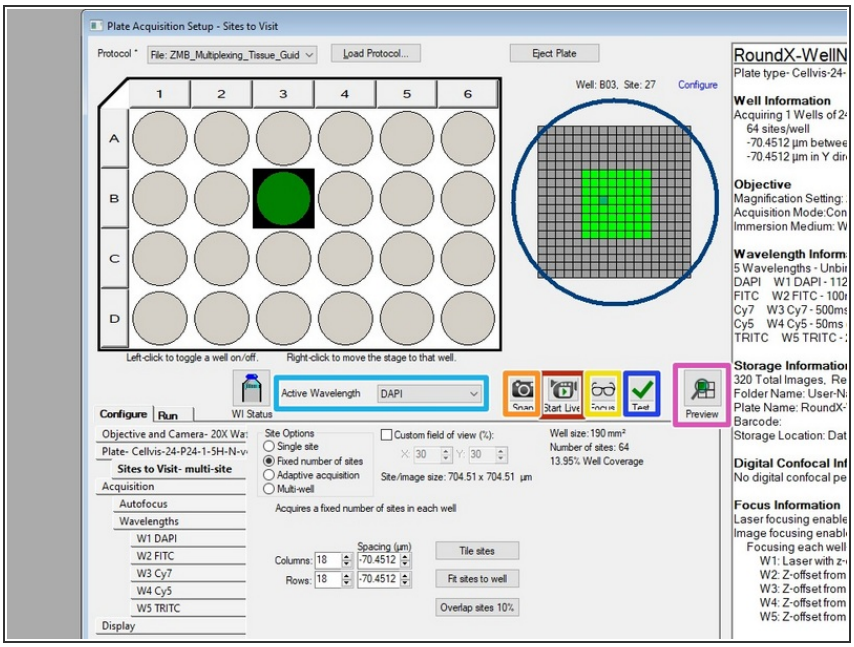
- Select **Top and Bottom** z positions
- Use **Zoom** scroll bar to adjust scale of blue Focus z-series

Step 24 — Display images



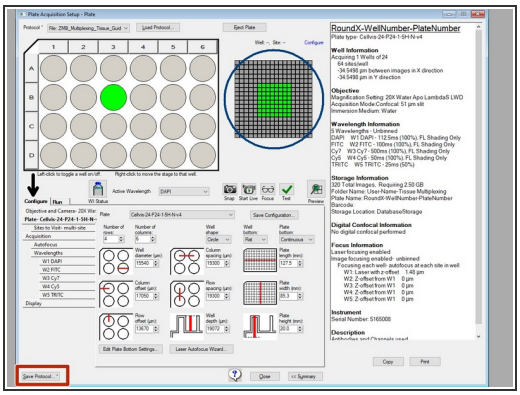
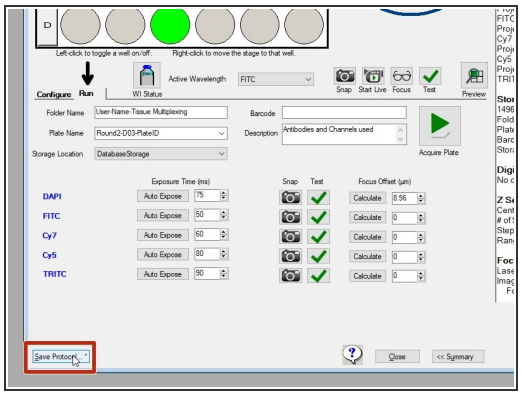
- Specify how and if images should be displayed during data acquisition

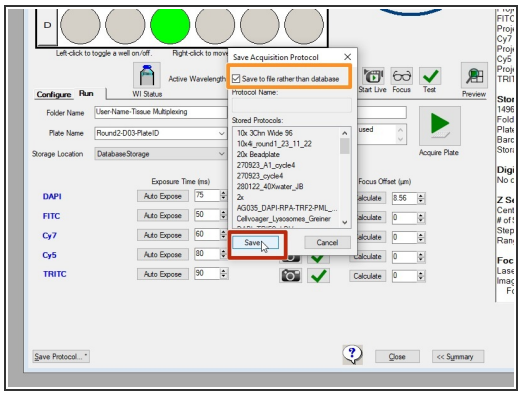
Step 25 — Shortcuts tools



- Each shortcut icon performs the action at the well where the stage is currently located and in the wavelength that currently displays in the Active Wavelength field.
- Use the Active Wavelength drop-down to select an acquisition wavelength.
- Use the Snap icon to acquire an image without performing (or re-performing) an autofocus.
- Use the Start Live icon to start and stop Live mode
- Use the Focus icon to perform the Autofocus routine and then acquire an image
- Use the Test icon to perform the Autofocus routine and then acquire a Z series and present the corresponding 2D projection when you set up a z acquisition.
- Use the Preview icon to perform the Autofocus routine and then acquire a Z series at each of the wavelengths you set up in the protocol and present the corresponding 2D projection when you set up a Z acquisition.

Step 26 — Saving Plate Acquisition Protocols



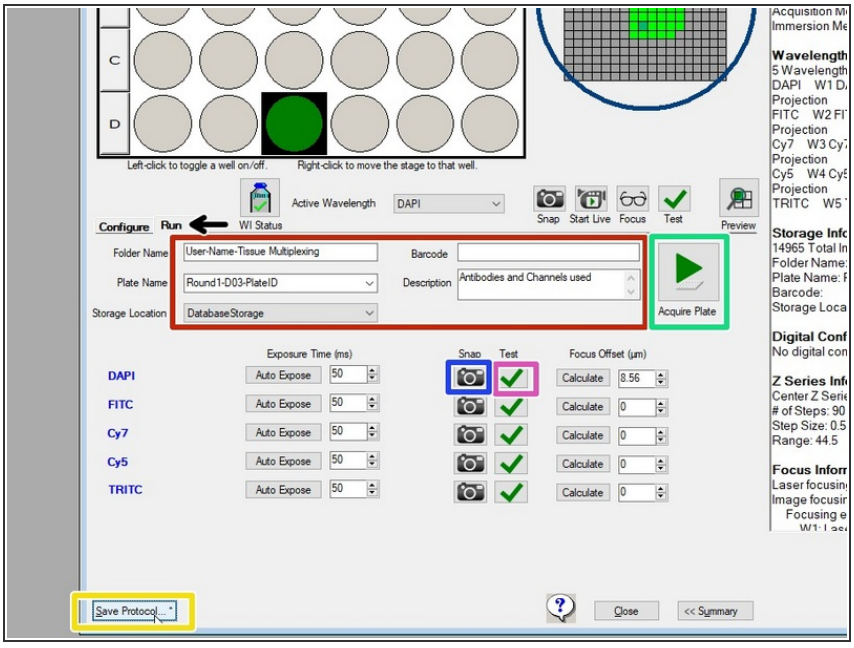
⚠ You should save a plate acquisition protocol to a file (and not to database).

- Click Save Protocol to display the Save Acquisition Protocol dialog. Located on bottom left of both the Configure tab and the Run tab.

  ⓘ * Asterisks appears when protocol changes are made and not yet saved.

  - Select the Save to File Rather Than Database check box.
  - Navigate to the location to save the file in your private data folder (transfer server will be deleted within 7 days).

Step 27 — Running Plate Acquisition Protocol



ⓘ Use the **Run tab** in the Plate Acquisition Setup dialog to run the plate acquisition protocol and acquire the plate data.

- Specify folder and plate names for organizing the data within the database.

  ⓘ Names become part of the image file paths. If the overall path length is too long, it causes errors.

This document was generated on 2025-09-03 09:12:34 AM (MST).

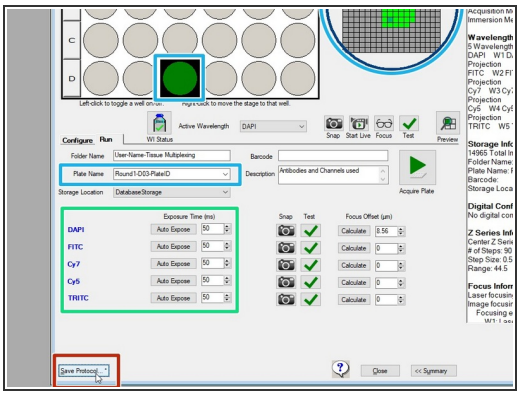
⚠ Please include your Core user name in the "Folder Name" eg. d.duck_test1

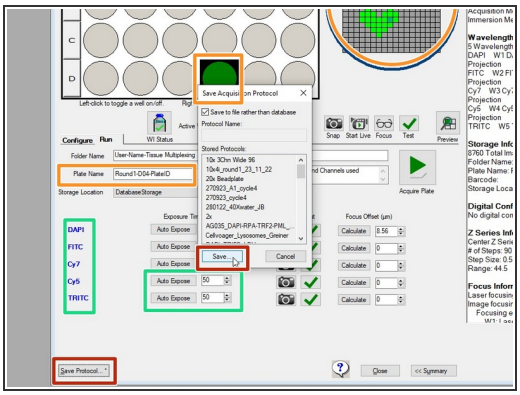
- Click to snap an image.
- **Save Protocol** before you Acquire Plate, to remove the * Asterisk and to be sure your selected Configure settings are run.
- Click **Acquire Plate** to acquire images from a plate based on the settings for the loaded protocol. If you want to run multiple wells with different ROI areas selected instead of starting acquisition here run from journal feature described in Step 2X.

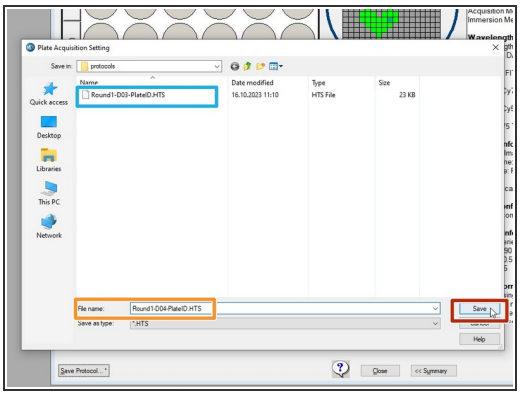
ⓘ Images are saved to the MD database. Please export and mark your files for deletion **no longer than 1 month after acquisition**. Once marked, we need to manually remove them and thus space is not available immediately. Once exported, tiff files will be available in your data folder for additional 180 days.

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Step 28 — Saving Unique ROIs in Multiple Wells





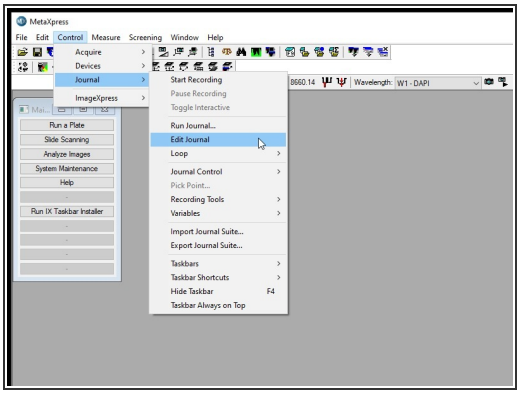


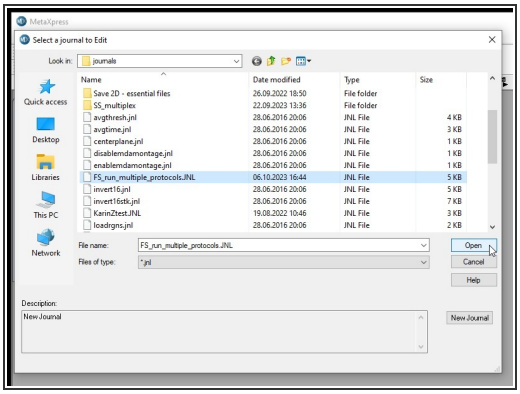
ⓘ With the MD ImageXpress software, a user can only choose one tile setup for all wells. To catch all regions of interest (ROIs) in multiple wells, one would need to either capture a large tile set or accurately place tissue in the center for all wells. Here we describe how one can save unique ROIs for individual wells prior to Journaling in Step 29.

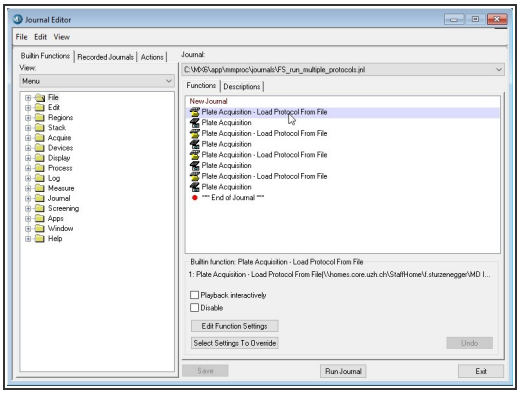
⚠ For Multiplexing, you should setup an initial well with accurate settings for...

- Example Well D3 ROI
- Save Protocol, with File Name...
- Keep All Settings consistent between wells, ex. Wavelengths, Illumination Power, Exposures, etc.
- Reuse D3 well settings, then after selecting new ROI in another well, example Well D4 ROI
- Save Protocol, with New File Name corresponding to Well RO1 #2

Step 29 — Journal to acquire multiple wells

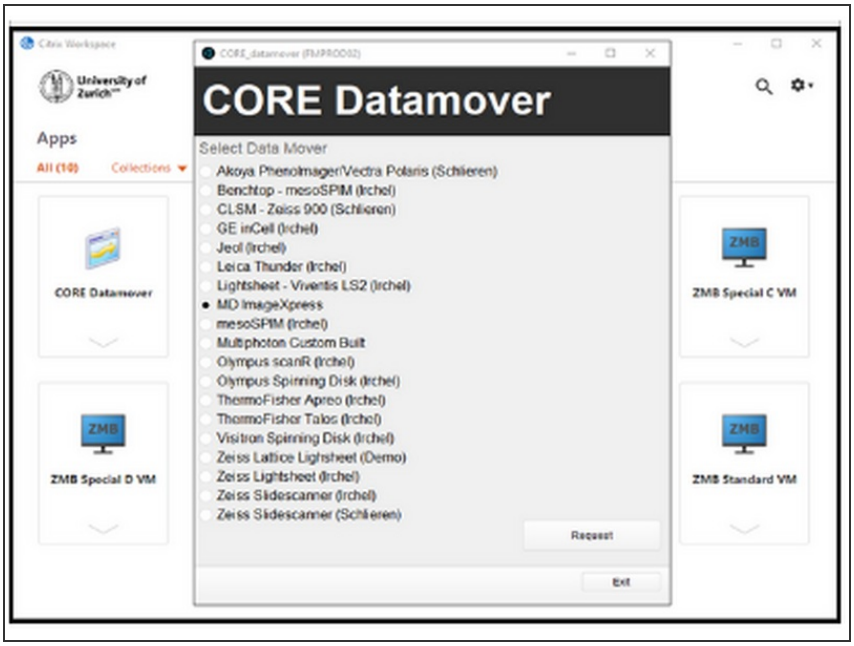






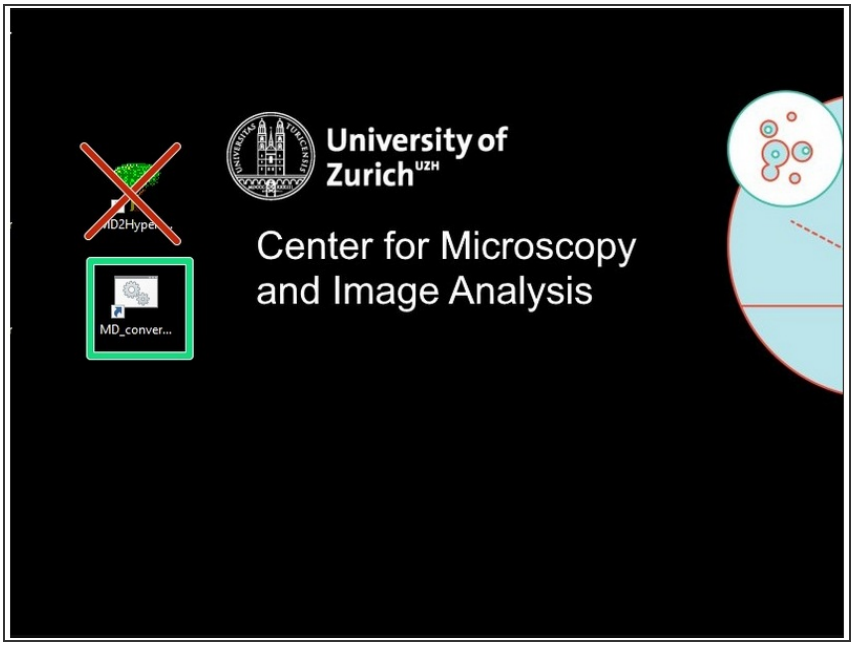
- Specify the journals that should be run.
  - ⓘ Only available if you previously selected Run Journals during acquisition in the Acquisition tab.
  - ⚠ Note: The Journal function is the only way to **combine time-series** and **Z-series** with each other in one acquisition for this system

Step 30 — Export all your plate acquisitions with Datamover and Convert



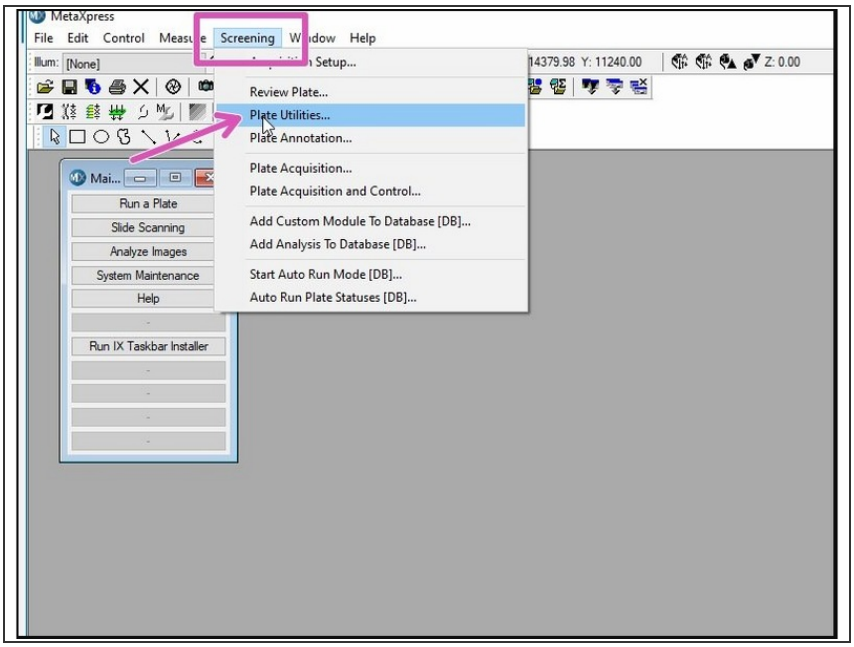
- Instructions for using the datamover to export your plate acquisitions: MD ImageXpress Confocal HT.ai (Irchel) - Datamover & file conversion

Step 31 — Convert MD data to Tiff Hyperstacks



- Instructions for file conversion: MD ImageXpress Confocal HT.ai (Irchel) - Datamover & file conversion

Step 32 — How to mark your data for deletion



- Data deletion instructions here: [MD ImageXpress HT.ai: How to mark your data for deletion]