

MD ImageXpress Confocal HT.ai (Irchel): Acquiring Z stacks over time

This guide will help you to acquire over time on the MD ImageXpress Z stack.

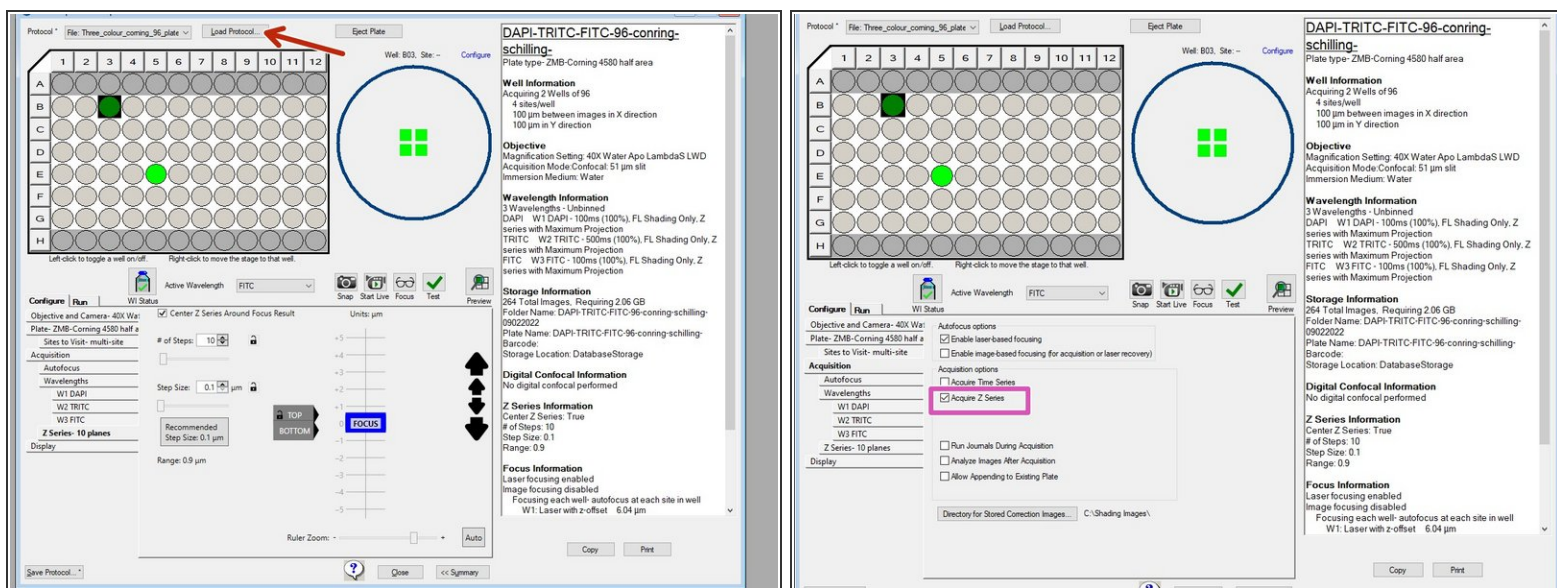
Written By: z mbstaff

The screenshot displays the MD ImageXpress Confocal HT.ai software interface. A red arrow points to the 'Load Protocol...' button. The main window shows a 12x8 grid of wells (A-H, 1-12). Well B3 is highlighted in green, and well E5 is highlighted in blue. A large circular field of view (FOV) is shown in the center, with a 4x4 grid of green squares indicating the acquisition area. The right panel displays acquisition details for 'DAPI-TRITC-FITC-96-conring-schilling-'. The 'Well Information' section shows 'Acquiring 2 Wells of 96' with '4 sites/well' and '100 µm between images in X direction' and '100 µm in Y direction'. The 'Objective' section shows 'Magnification Setting: 40X Water Apo LambdaS LWD', 'Acquisition Mode: Confocal: 51 µm slit', and 'Immersion Medium: Water'. The 'Wavelength Information' section shows '3 Wavelengths - Unbinned' with 'DAPI W1 DAPI - 100ms (100%), FL Shading Only, Z series with Maximum Projection', 'TRITC W2 TRITC - 500ms (100%), FL Shading Only, Z series with Maximum Projection', and 'FITC W3 FITC - 100ms (100%), FL Shading Only, Z series with Maximum Projection'. The 'Storage Information' section shows '264 Total Images, Requiring 2.06 GB', 'Folder Name: DAPI-TRITC-FITC-96-conring-schilling-09022022', 'Plate Name: DAPI-TRITC-FITC-96-conring-schilling-Barcode:', and 'Storage Location: DatabaseStorage'. The 'Digital Confocal Information' section shows 'No digital confocal performed'. The 'Z Series Information' section shows 'Center Z Series: True', '# of Steps: 10', 'Step Size: 0.1', and 'Range: 0.9'. The 'Focus Information' section shows 'Laser focusing enabled', 'Image focusing disabled', and 'Focusing each well- autofocus at each site in well W1: Laser with z-offset 6.04 µm'. The bottom panel shows the 'Configure' tab with 'Active Wavelength' set to 'FITC'. The 'Snap' button is highlighted. The 'Start Live' button is highlighted. The 'Focus' button is highlighted. The 'Test' button is highlighted. The 'Preview' button is highlighted. The 'Z Series' section shows 'Z Series- 10 planes'. The 'Display' section shows 'Display'. The 'Ruler Zoom' section shows 'Ruler Zoom: -' and 'Auto'.

INTRODUCTION

In this guide of the Center for Microscopy and Image Analysis (University of Zurich, Switzerland) we describe how to acquire Z stacks over time on the MD ImageXpress.

Step 1 — Set up your Plate acquisition protocol

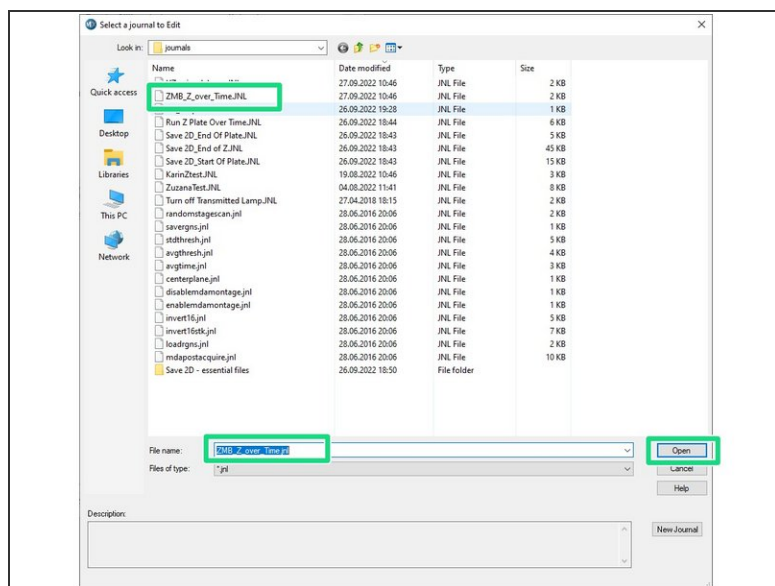
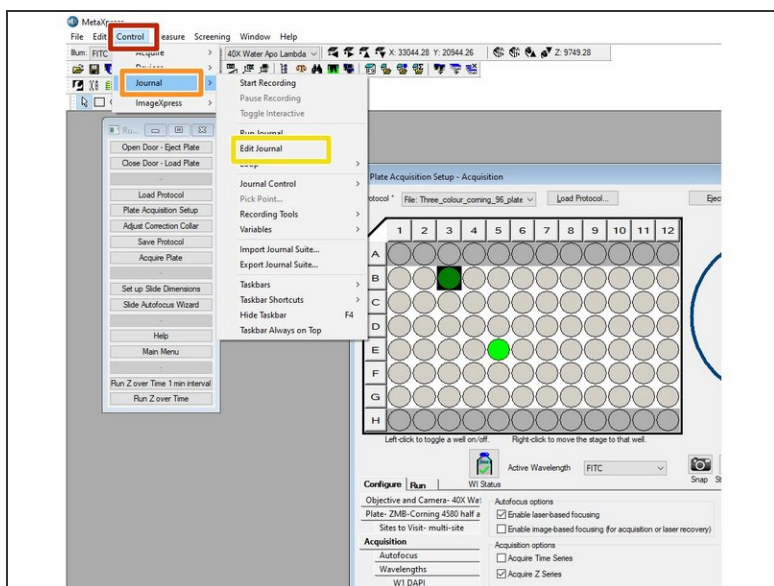


● Create your plate acquisition protocol or load it from a previously saved file.

● Make sure you include Z-stacks in your acquisition protocol.

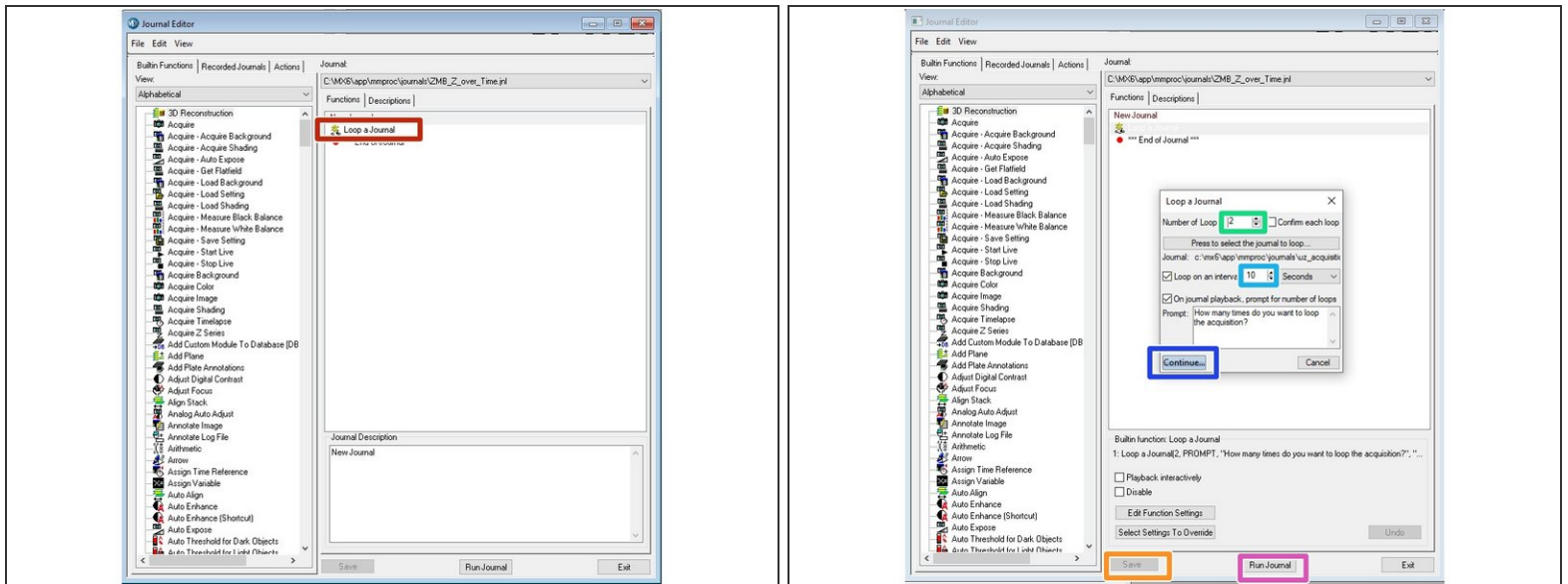
⚠ With the present version of our Z over time pipeline single site acquisitions are not possible.

Step 2 — Open Z over Time Journal



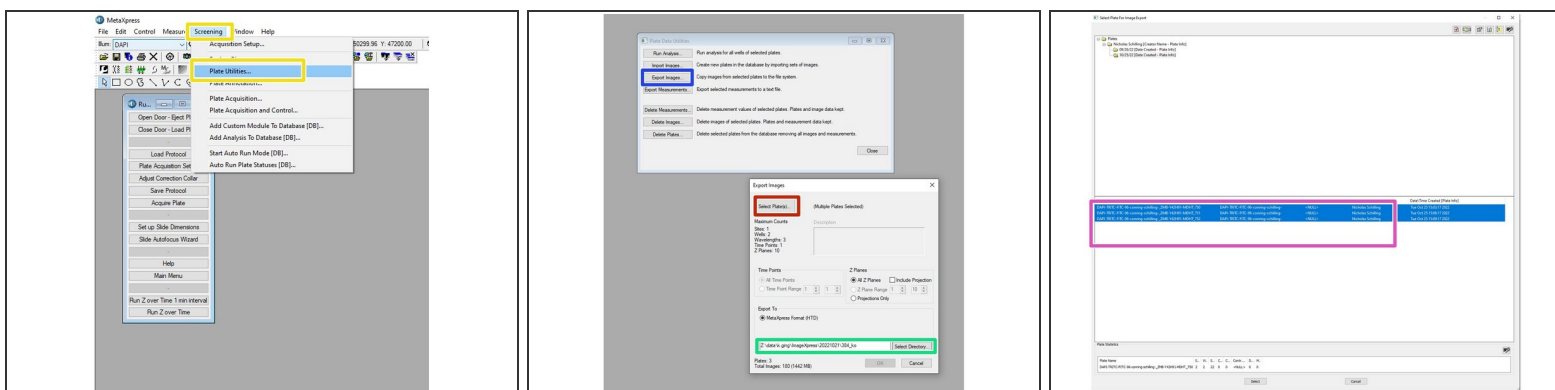
- Open **"control"**.
- Go to **"Journal"**.
- Click **"edit Journal"**.
- Open **"ZMB_Z_over_Time.jnl"**

Step 3 — Modify Z over Time Journal



- Double click on **"Loop a journal"**.
- Specify **how many times** you would like to **loop** your plate acquisition protocol.
- Specify the **loop interval** you need.
- ⚠ Make sure your loop interval is sufficient. Keep in mind it depends on the duration of your plate acquisition and that you won't receive an error if the interval is too short.
- Click **"Continue"**.
- Click **"Save"**.
- Click on **"Run Journal"** if you are ready to start acquisition.
- ❗ Please note that the journal can be modified by any user in terms of loop time and loop interval, hence check and modify the journal before each experiment.

Step 4 — Export all your looped plate acquisitions



- Once your acquisition is done, export the data by clicking on screening.

- Choose **"Screening" > "Plate Utilities...."**

- Click **"Export Images"**.

- Specify your saving location.

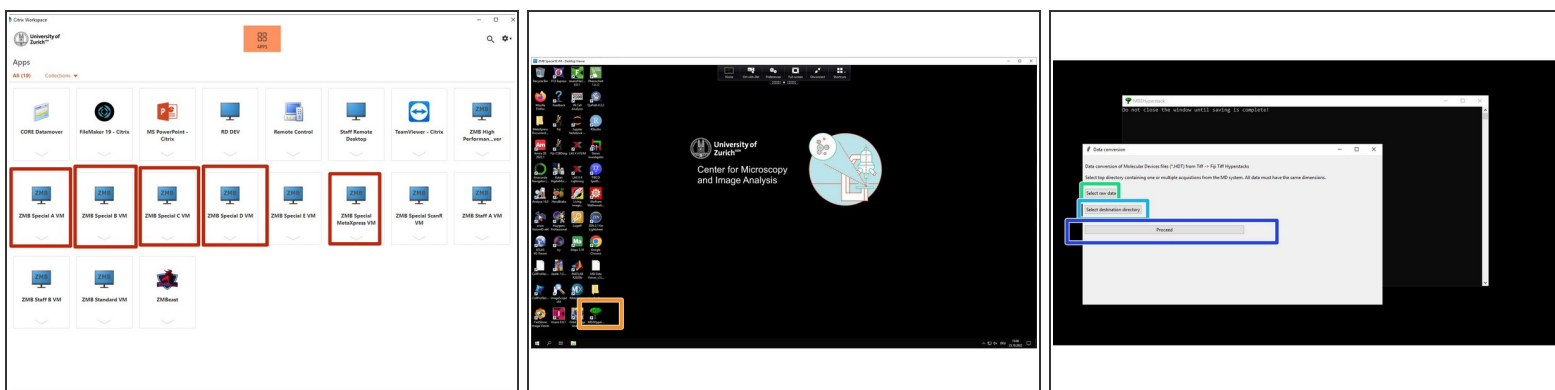
- Click **"select plate"**.

- Choose all looped plate acquisitions.

⚠ Make sure you choose all looped plate acquisitions of your time lapse experiment.

- i** With this setup your individual time points will correspond to individual plate files that will need to be exported.

Step 5 — Convert MD data to tiff Hyperstacks



- Open any virtual machine.
- ① Make sure you have a booking for the virtual machine.
- ① The MD2Hyperstack converter is available on any virtual machine, except for Special VM E and ScanR VM.
- Open *MD2Hyperstack* from desktop.
- Select the location of the data you have exported.
- ① Select the top category folder containing at least one MD dataset.
- Specify the saving directory.
- Click "**Proceed**".

Step 6 — Check your data

- In the end you have a tif file containing a hyperstack for each site of your wells.
- ① Note that the MD2Hyperstack converter also works with Z-stacks (without time), or 2D data and finally converts the MD data to a tif hyperstack that can be opened in Fiji.

