Quick Start Guide

Leica Cell DIVE Imager

(ICTEM room 304B)

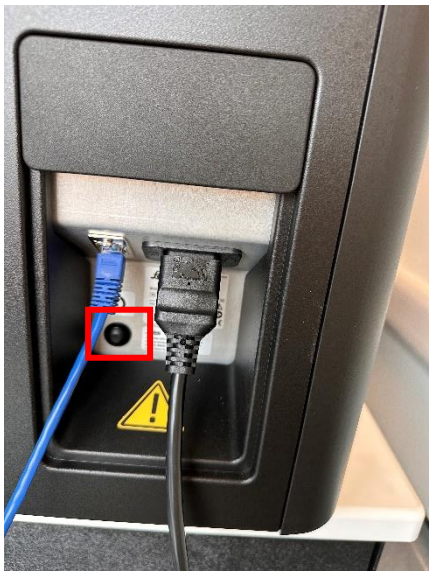
Imperial College London

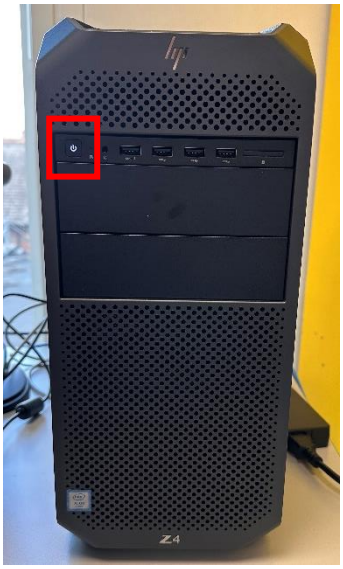
Facility for Imaging by Light Microscopy

Observing Life As It Happens

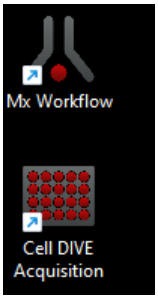
STARTUP

1. On the side of the Cell DIVE Imager opposite the sample door, press the power button to turn the system on. Press the power button to turn it on the PC.





2. On the PC desktop, double-click the Cell DIVE Acquisition software icon to open the acquisition software and double-click the Mx Workflow icon to open the Mx Workflow Software.



BASIC MULTIPLEX IMAGING WORKFLOW

A single round of imaging includes the following steps:

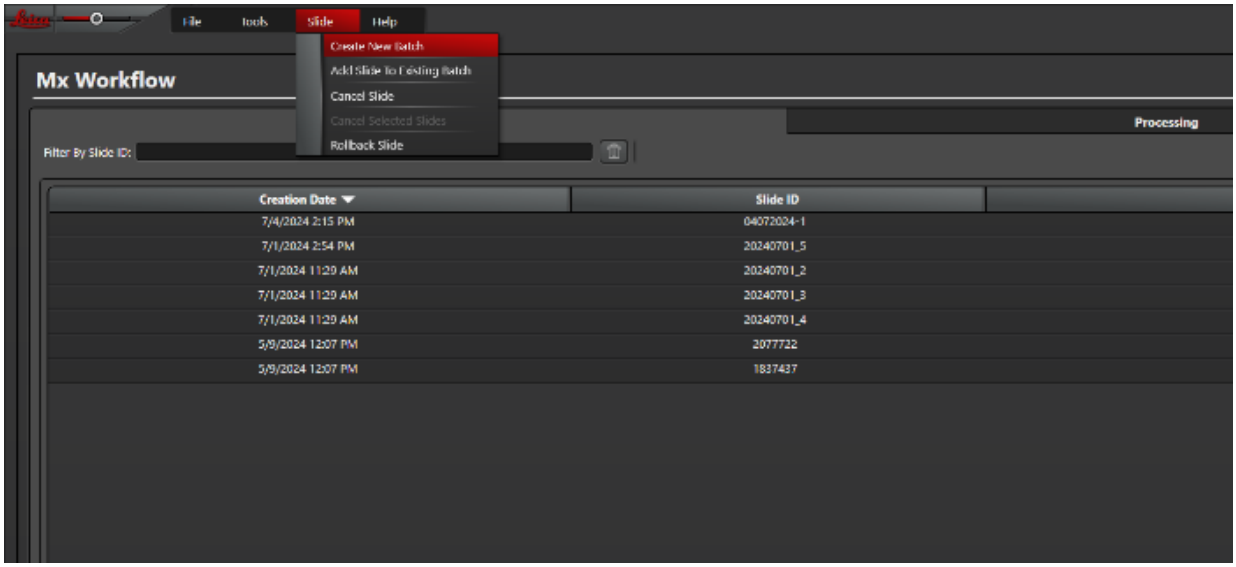
1. Adding slide(s) and creating an imaging batch;
2. Sample preparation (chapter 5 “Slide preparation for multiplex imaging” in Cell DIVE user manual)
3. 2X whole slide imaging and tissue selection
4. 10X whole tissue imaging and QC evaluation
5. 20X region selection
6. 20X autofluorescence imaging and QC evaluation
7. Biomarker labelling (chapter 6 “Multiplex imaging” in Cell DIVE user manual)
8. 20X Biomarker imaging and QC evaluation
9. Dye inactivation

Repeat Steps 6-9 multiple times for unique probes.

There is a sample protocol (**Cell DIVE default**) in the Cell DIVE Acquisition software but you can create a new protocol.

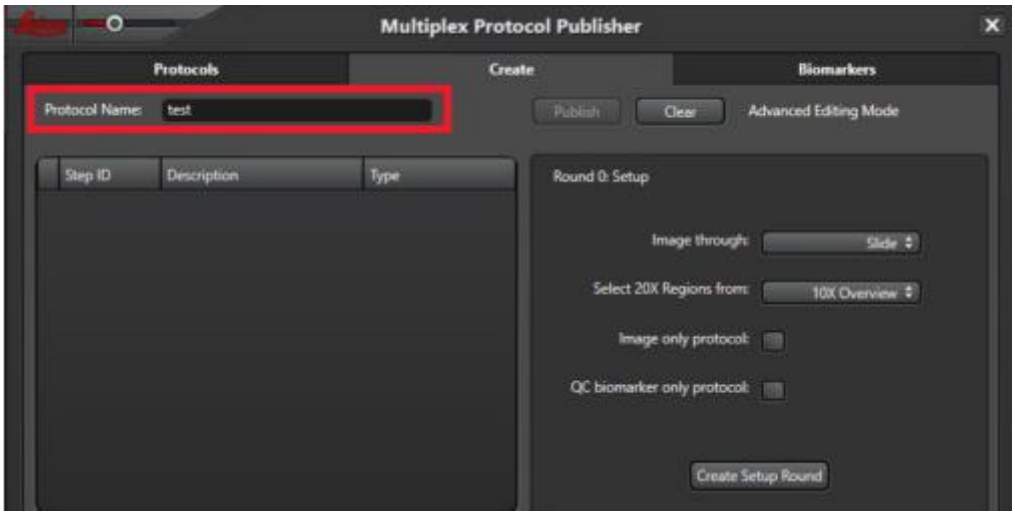
Adding slide(s) and creating an imaging batch

1. On the Mx Workflow menu bar, click **Slide** and then select **Create New Batch** from the drop-down menu.
2. Select “Cell DIVE default” from the **Protocol** drop-down menu or create a custom protocol.



3. To create custom protocols for multiplexed imaging experiments, go to **Tools | Protocol Publisher** to access the Protocol Publisher**.** Use the Biomarkers tab to add or remove biomarker names prior to setting up a new protocol.
    a. In the Multiplex Protocol Publisher window, click the Biomarkers tab.

    b. In the Biomarker Name field, enter a biomarker name then click Add. For the biomarker name, consider including the dye conjugate or secondary antibody in addition to the name of the biomarker.
    c. To create a protocol, click **Create tab.**
    d. In the Protocol Name field, enter a descriptive protocol name.



    e. In the Round 0: Setup section of the Create tab, select the following:
        i. **Image through.** Indicates slide orientation for the duration of the study.
            1. **Slide.** For through-slide imaging, the slide is loaded into the system with the tissue side facing up. Through-slide imaging can be performed in either the ClickWell or dual slide cassette, as long as the slide is loaded in the correct orientation (tissue side facing up).
            2. **Coverslip.** Through-coverslip imaging is not supported on the Cell DIVE imager. Coverslipped samples may still be imaged, but they should be imaged through the glass slide. Do not select this option.
        ii. **Select 20X Regions from.** Indicates where imaging areas for multiplexed imaging rounds will be selected.

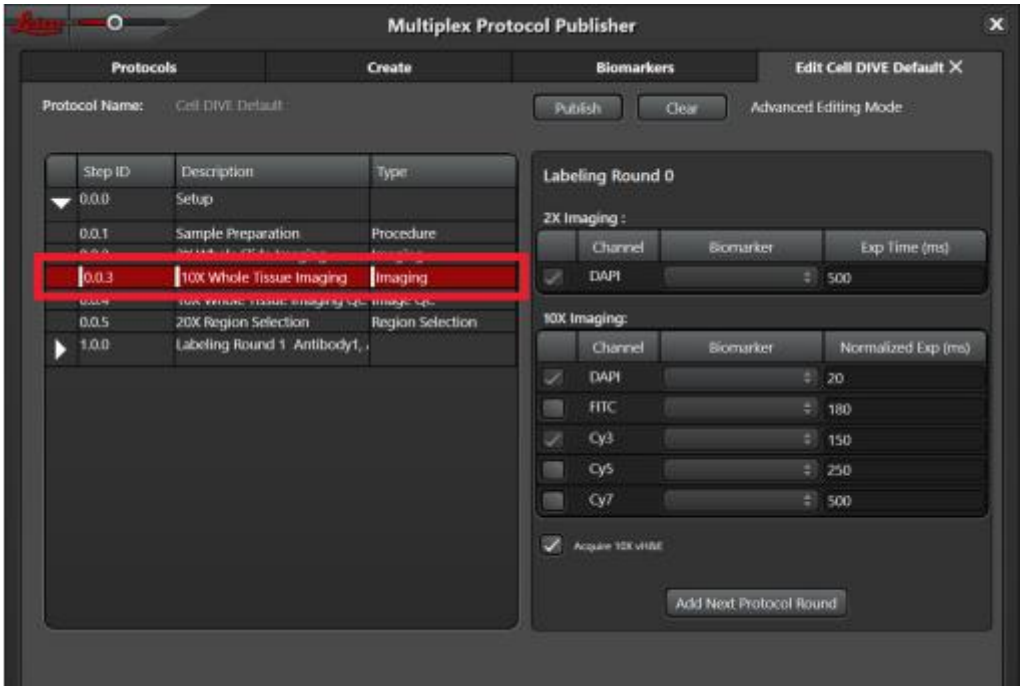
1. **10X Overview.** Regions will be selected in the Cell DIVE Viewer from 10X images. With this option, 2X whole slide imaging is performed first, followed by 10X whole tissue imaging.
2. **2X DAPI Overview.** Regions will be selected directly from the 2X whole slide overview in the Cell DIVE Acquisition software. This option skips 10X imaging entirely. Only select this option if regions of interest can be confidently identified from a 2X DAPI only image.

iii. Special options

1. **Image Only.** Removes laboratory (Biomarker Labeling, Dye Inactivation) and QC steps from the workflow. This feature is intended to be used with automation systems that require less user intervention throughout the course of the protocol.
2. **Biomarker Only.** When selected, this option will create a protocol that has QC steps only for Biomarker Imaging and no laboratory steps. Each 20x round will contain only three steps: AF imaging, biomarker imaging, and biomarker imaging QC. This is intended for use with automation systems that perform the laboratory steps for a complete round without user intervention.

f. Click **Create Setup Round**.

g. In the Protocol Navigation table, click the arrow next to the 0.0.0 Setup step to expand the individual steps in Round 0. Click any of the 0.0.x steps to view the Labeling Round 0 exposure table.



h. Edit the Labelling Round 0 exposure table to define how imaging will be performed for the 2X and 10X imaging steps:

i. **Select channels to image.** Select the check box next to each channel that should be imaged. DAPI 2X and DAPI must always be imaged and Cy3 must be imaged if a 10X vH&E image will be acquired (defined in 0.0.0 Setup).

ii. **Enter normalized exposure time.** Default Normalized Exp (ms) values will populate for all channels. Adjust as needed based upon the expected brightness of the sample.

iii. **Acquire 10X vH&E.** When selected, a virtual H&E (vH&E) image will be created during the 10X whole tissue imaging step. If visualizing tissue characteristics are required for region selection, a 10X vH&E must be acquired. Note: vH&E images are created from the signal in the DAPI and Cy3 channels. When this option is selected, the DAPI and Cy3 channels will be turned on for 10X imaging and cannot be turned off.

i. When the Labelling Round 0 exposure table is complete, click the arrow next to the 1.0.0 Labelling Round 1 step to view the Labelling Round 1 exposure table.

j. Edit the Labeling Round 1 exposure table to define how imaging will be performed for the first 20X imaging round:

i. **Select channels to image.** Select the check box next to each channel that should be imaged. DAPI must always be imaged and Cy3 must be imaged if a 20X vH&E image will be acquired.

ii. **Select biomarker.** For each channel that will be acquired (except DAPI), select a name from the Biomarker drop-down list.

iii. **Enter normalized exposure time.** Default Normalized Exp (ms) values will populate for all channels. Adjust as needed based upon the expected brightness of biomarker staining.

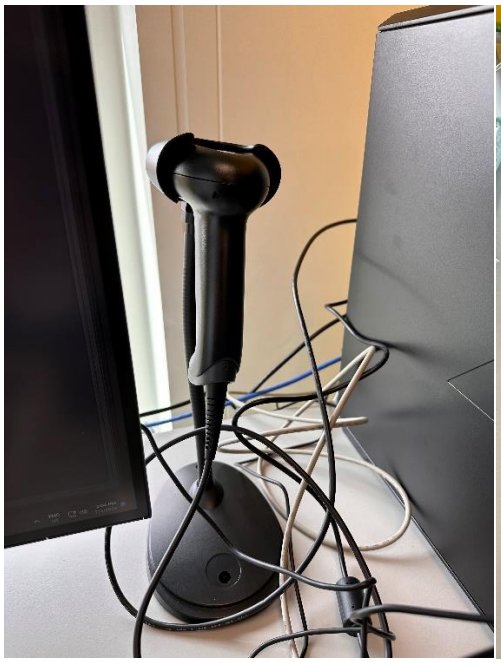
iv. **Create 20X vH&E.** When selected, a vH&E image will be created during the imaging round. This option is only available in Round 1. Note: vH&E images are created from the signal in the DAPI and Cy3 channels. When this option is selected, the DAPI and Cy3 channels will be turned on for imaging and cannot be turned off.

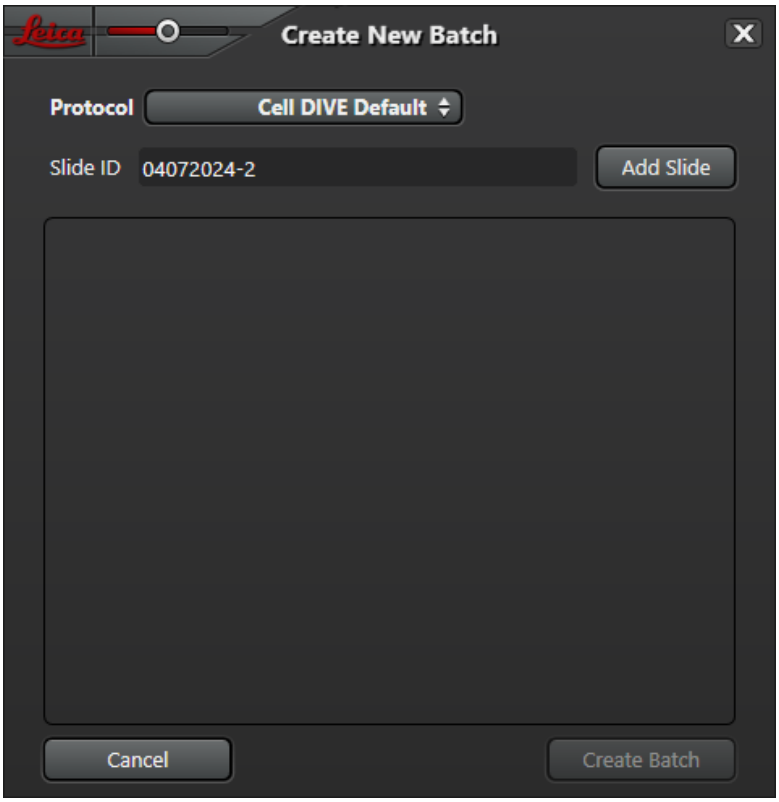
i. **Acquire AF image.** For the first round, the Acquire AF Image check box is selected by default and cannot be changed. However, this option may be deselected in subsequent imaging rounds to disable the acquisition of a new AF image for that round. This feature is included as a time saving option to avoid needing to acquire a new AF image for every round. If Acquire AF Image is enabled, the imaging round will consist of AF imaging and Biomarker imaging and the AF images from that round will be used for AF removal of the biomarker images. If Acquire AF Image is disabled, then AF imaging will be skipped for that round and only biomarker imaging will be performed. The most recently acquired AF images will be used for AF removal.

ii. **Dynamically set exposure times.** Select the check box to enter Slide Preview mode for each slide in the study prior to proceeding with 20X biomarker imaging. In Slide Preview mode, you can explore the sample manually to examine staining quality, check pre-defined exposure conditions, and change Normalized Exp (ms) or Biomarker column values. Note: If Normalized Exp (ms) values are changed in Slide Preview mode and “Acquire Imaging Round” is selected, the new exposure time values will be used for the current slide only. If changes to exposure time values must be made for all slides in the round, exit Slide Preview mode, verify all slides in the batch are at the same protocol step, and edit the protocol in the Protocol Publisher.

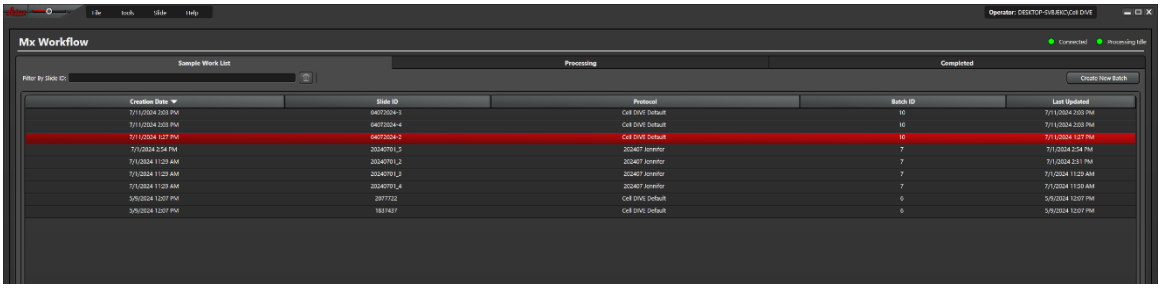
k. When the Labelling Round 1 exposure table is complete, click Add Next Protocol Round to add another imaging round to the protocol.
l. Repeat Steps 9 and 10 for all imaging rounds required for the study.
m. Click Publish to publish the new protocol.

4. Type or scan the slide's barcode into **slide ID** and click **Add slide.** Alternatively, scan the barcodes for each slide with the handheld reader. Repeat to add additional slides.

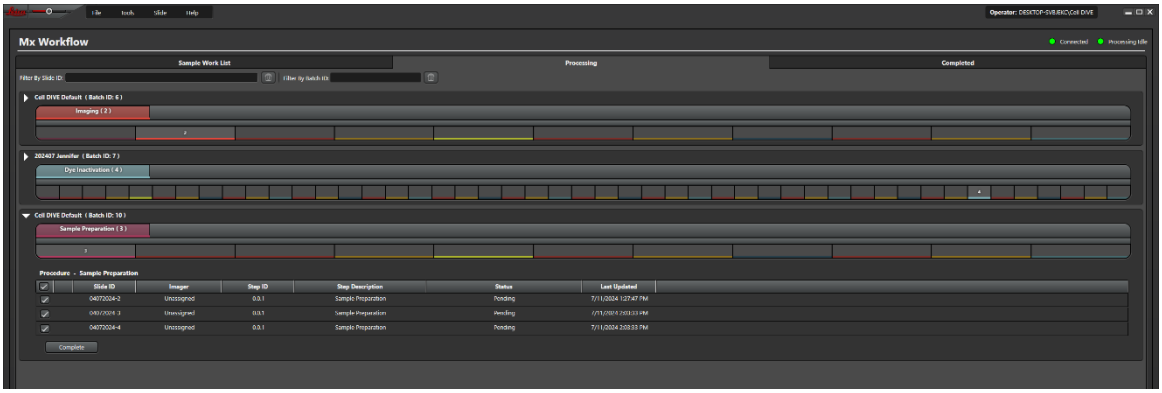




5. Once all slides have been entered, click **Create Batch**. Slides are assigned a Batch ID and Test Status as “Processing” in the sample work list tab.

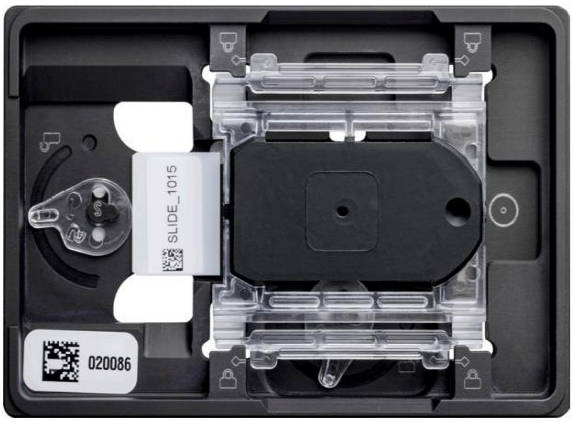


6. Click on the Processing tab. The status and workflow for each slide and batch are displayed. Click the arrow the arrow at the upper-left corner of the batch tile to view the slide details. Click the check box next to each slide the click **Complete**.



2X whole slide imaging and tissue selection

Sample carriers:



ClickWell

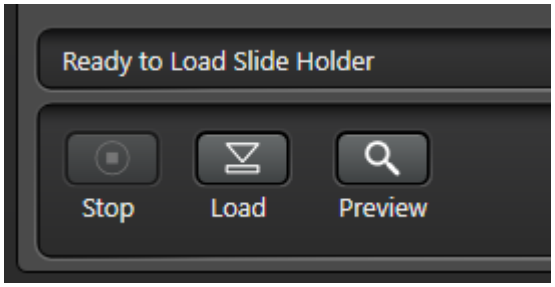
Dual slide cassette

1. Load slides into ClickWell
    a. Rotate both cams to the unlock position. Place the slide into the base with the barcode and tissue side facing up and the barcode towards the short side cam (S cam).
    b. Rotate the long side cam (L cam) to push the slide gently against the two long-side contact points. Do not tighten (left). Rotate the S cam firmly to seat the slide against the short side contact point (right).
    c. Tighten the L cam to lock the slide into position.
    d. Orient the insert so the port is aligned with the port orientation symbol on the base.
    e. Squeeze the insert wings together and place the insert on top of the slide. At this point, the insert will engage partially, but will not lock.
    f. Push down and out on the top-left and bottom-left insert wings. When properly locked, an audible "click" will be heard from both corners. Repeat on the right side.
    g. Check that all four lock indicators on the insert wings (one in each corner) line up with the corresponding lines on the base to verify that the insert is installed securely. Verify that the four lock indicators are aligned with the lines on the insert wings carefully, as leakage can occur if the insert is not properly seated.
    h. If slides will be imaged immediately, proceed to Imaging setup. Otherwise, store slides in the ClickWell for up to two weeks at 4°C in ClickWell storage mounting medium in a light-protected environment. If slides will not be imaged within two weeks, change mounting media every two weeks to prevent dehydration and crystallization of the media or coverslip the slides to protect the tissue.
2. Load the ClickWell into the Cell DIVE imager with the barcode on the cassette positioned to the lower left.

ClickWell

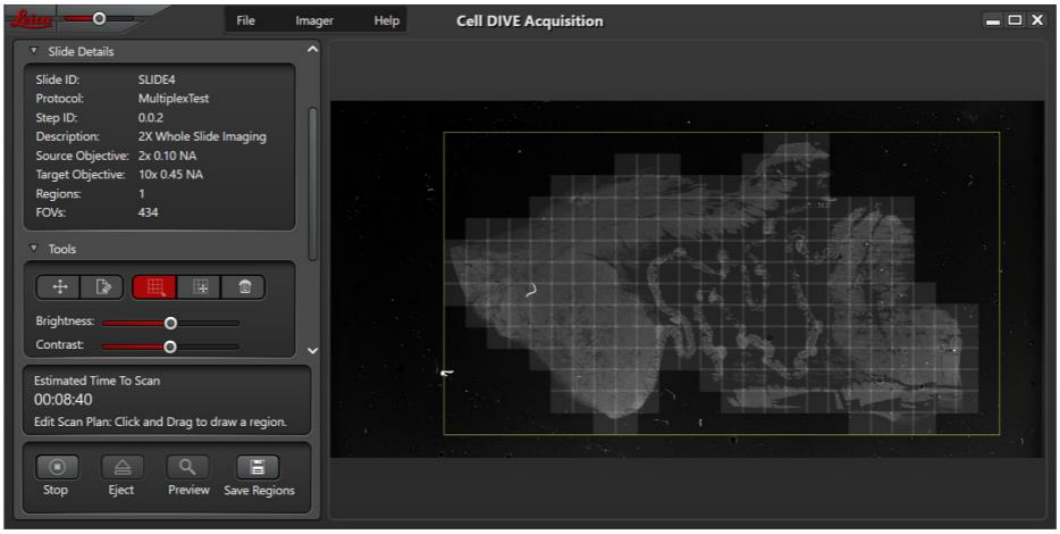
Dual slide cassette

3. Click **Load** to close the sample door the Cell DIVE Acquisition software.



4. In the Cell DIVE Acquisition software, click **Eject** if the door to the imager is closed.

5. If the tilt check fails, remove the slide holder from the imager, reseat the slides, and try again.
6. When the 2X imaging is completed, the system Cell DIVE acquisition displays the DAPI overview. Use the tools to identify the tissue location on the slide:
    a. Zoom with mouse wheel
    b. Pan with click and drag
    c. Use **Brightness** and **Contrast** as needed
    d. Identify a retangular area with **Draw region**
    e. Use the edit region tools to modify the rectangular areas as needed: use the **Move Region** tool to adjust the position of an existing region; use the **Delete Region** tool to delete an existing region.
    f. Use **Edit Region** tool to deselect any FOV that do not contain tissue.

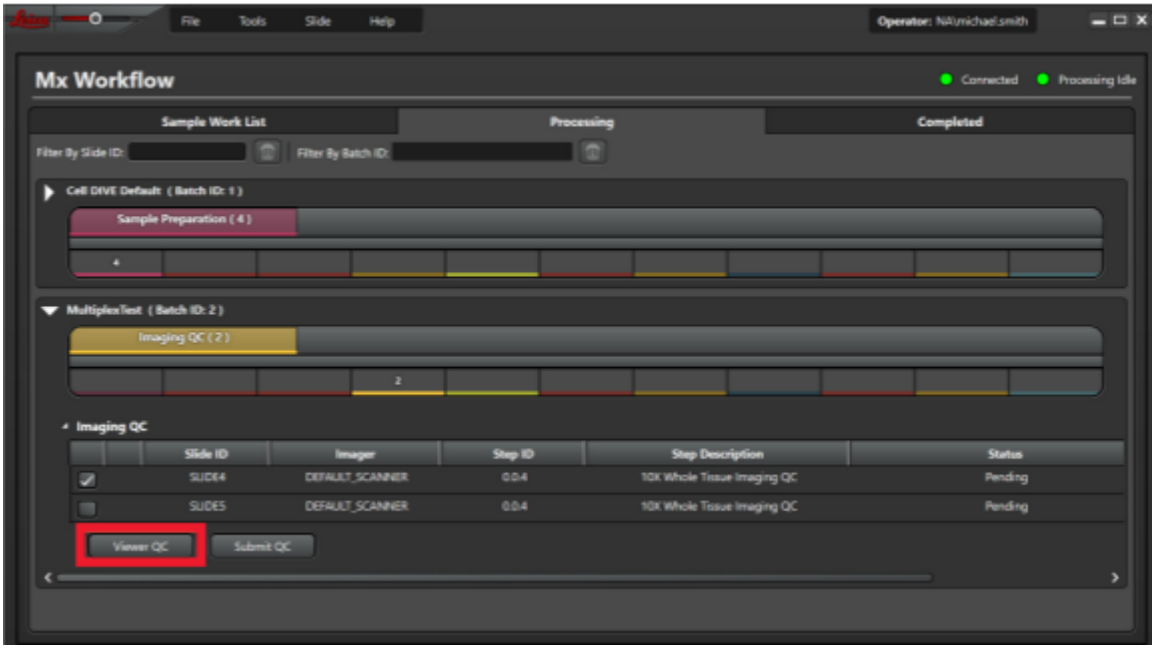


    g. Click **Save Regions**.
    h. Once the tissue location is saved for all slides in the current sample carrier, click **Eject** to open the sample door of the imager.
    i. If 10X whole tissue imaging will be performed (defined in the protocol), do not remove the sample carrier from the imager. Proceed to 10X whole tissue imaging for the current slide or slides. When 2X imaging and tissue selection is done for all slides in the batch, proceed to section 20X autofluorescence imaging.

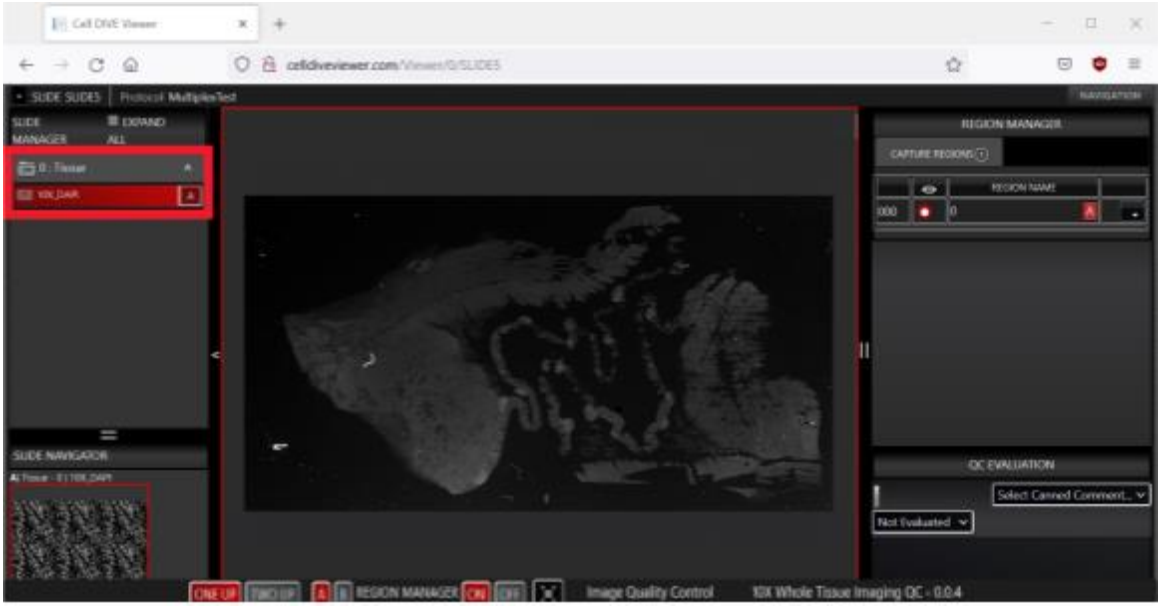
10X Whole Tissue imaging and QC Evaluation

1. In the Cell DIVE Acquisition software, click Load to close the sample door and begin 10X whole tissue imaging.
2. After 10X whole tissue imaging is complete, the images for each region of interest (ROI) are automatically stitched together and the Protocol column on the Processing tab of Mx Workflow displays “10X Whole Tissue Imaging QC” when the slide (or slides) are ready for QC.
3. While 10X images are processing, repeat sections 2X whole slide imaging and tissue selection and 10X whole tissue imaging for additional slides in the batch.

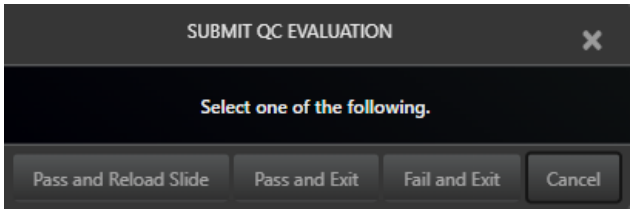
4. In Mx Workflow, select the check box next to the Slide ID, then click **Viewer QC** to open 10X images in the Cell DIVE Viewer (opens in Google Chrome).



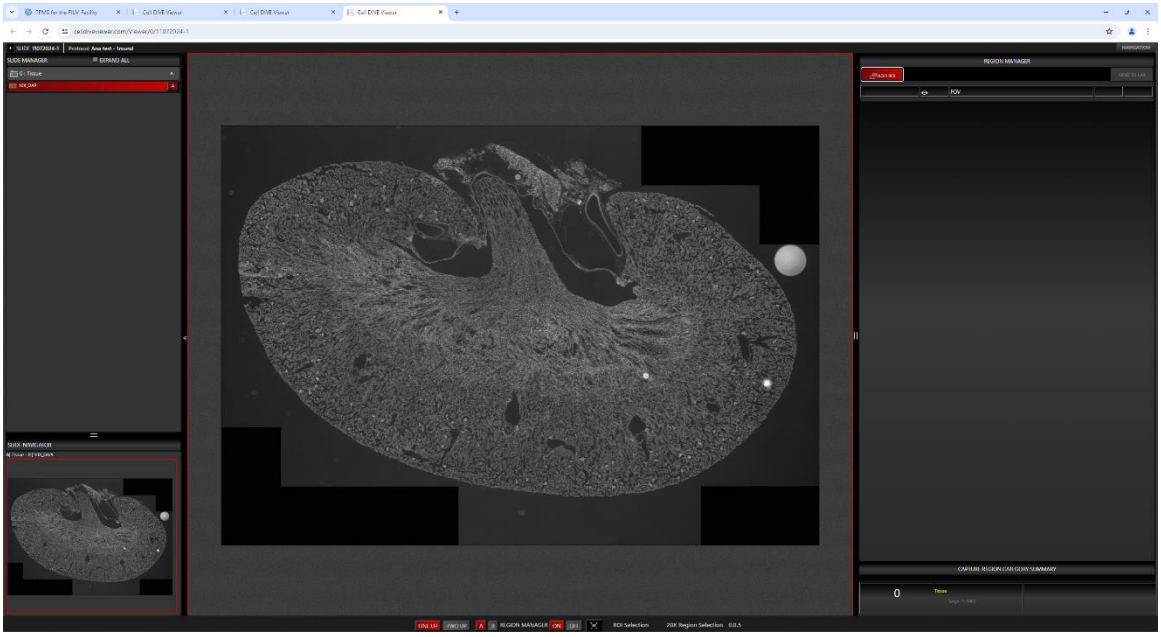
5. Click the **Eye icon** next to the first channel to examine. When prompted, choose to "Set as A" to view the channel on its own or "Blend with A" to blend with the currently displayed image. If the blend option is selected, select the colour and blend.



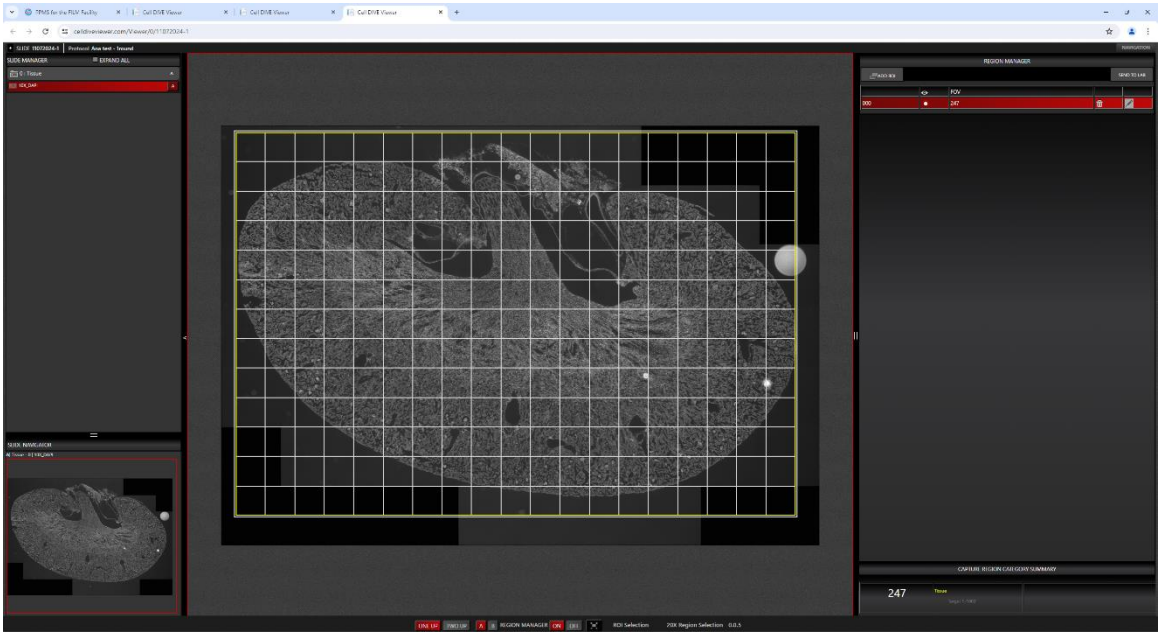
6. Examine each channel and region of interest (ROI) to ensure that:
    a. The entire image is in focus.
    b. The borders between individual fields of view (FOV) are not easily identifiable.
7. After reviewing all regions, click QC Evaluation and select either "Pass and Reload Slide" or "Fail and Exit".



8. In the **Region Manager** section of the window, click **Add ROI.**



9. In the image pane, click once in the image to identify one corner of the ROI. Move the mouse to the opposite corner of the ROI and click once to set the size of the ROI. To edit, use the **Pencil icon** to select or deselect individual FOVs. When region selection is complete, click **Send to Lab**. Repeat Steps 9-10 for all slides in the study that are ready for region selection.



20X autofluorescence imaging

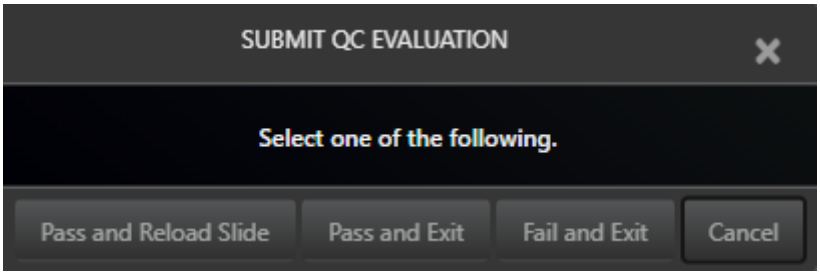
1. Click Load to close the sample door of the imager. 20X autofluorescence imaging will begin. Upon completion, “Scan Completed” will be displayed at the bottom of the Cell DIVE Acquisition software window.
2. Once stitching is completed, on the Processing tab of Mx Workflow, select the check box next to the Slide ID and click **Viewer QC.**
3. **I**n the Cell DIVE Viewer, examine the 20X autofluorescence images to ensure that:
    a. The entire image is in focus.
    b. The borders between individual fields of view are not easily identifiable.
    c. Complete dye inactivation (if applicable).
4. After reviewing all regions, click QC Evaluation and select either “Pass and Reload Slide” or “Fail and Exit”.
5. In the Cell DIVE Acquisition software, click **Eject** to open the sample door and remove the slide from the imager. The slide is ready for Biomarker labelling.
6. Complete this section for each additional slide in the study.

Biomarker labelling

1. Proceed to biomarker labelling (follow instructions on page 149 from Cell DIVE manual).

20X biomarker imaging

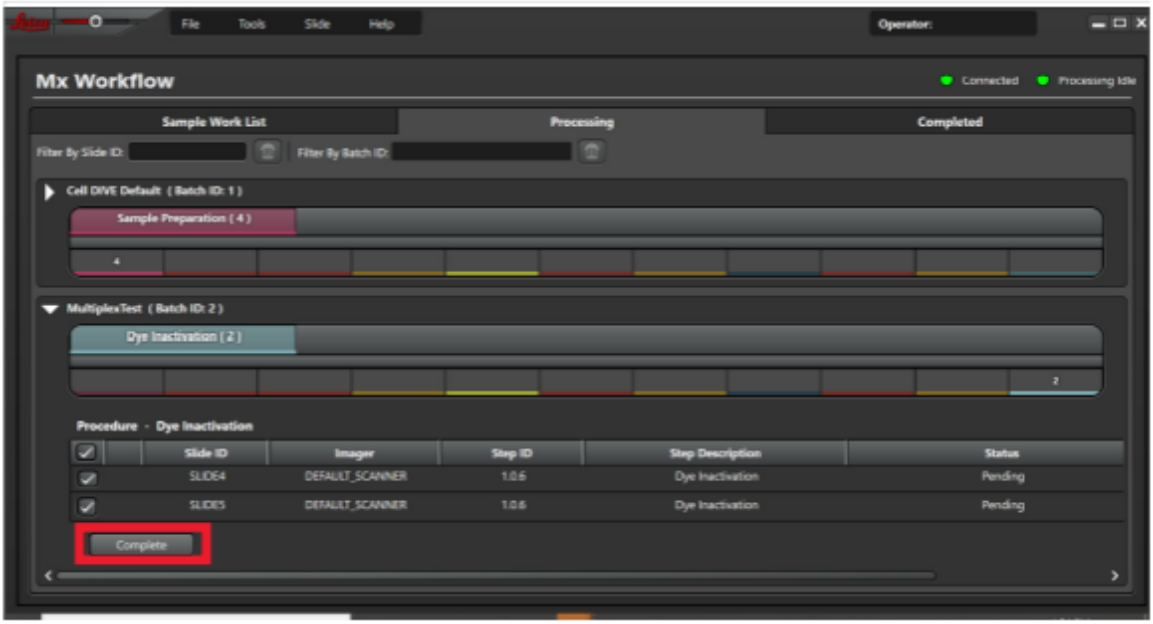
1. When biomarker labelling is complete, on the **Processing tab** of Mx Workflow, select the check box next to each slide then click **Complete**.
2. Click **Load** to close the sample door. Imaging will begin.
3. On the **Processing tab** of Mx Workflow, select the check box next to the Slide ID and click **Viewer QC**.
4. In the Cell DIVE viewer, examine the images to ensure that:
    a. Labelling was successful and there is sufficient signal for each channel imaged
    b. There is no significant loss of tissue
    c. The entire image is in focus
    d. The borders between individual fields of view are not easily identifiable
5. After reviewing all regions, click QC Evaluation and select either “Pass and Reload Slide” or “Fail and Exit”.



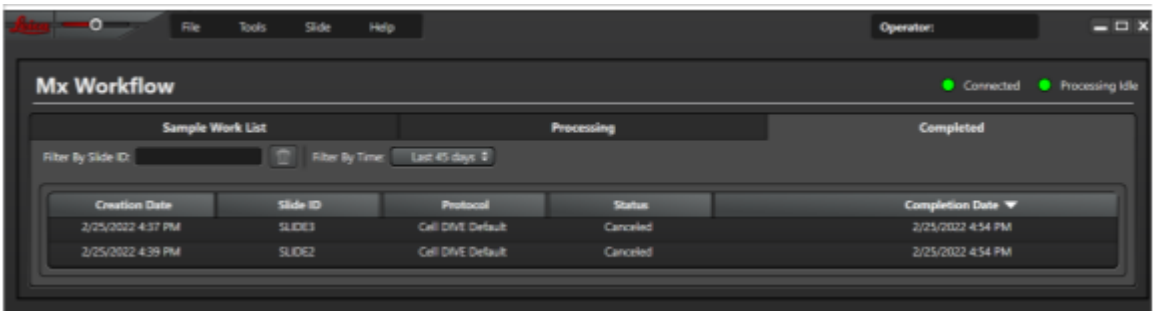
6. In the Cell DIVE Acquisition software, click Eject to open the sample door. Remove the slide(s) from the imager and perform the **dye inactivation** protocol to quench dyes conjugated to the first set of biomarkers (page 164 from Cell DIVE manual).

20X autofluorescence imaging – Round 2

1. When dye inactivation is complete, on the **Processing tab** of Mx Workflow, select the check box next to each slide and then click **Complete.**



2. To complete additional imaging rounds, complete the following steps in order for each round:
    a. 20X autofluorescence imaging
    b. Biomarker labeling
    c. 20X biomarker imaging
    d. Dye inactivation
3. When all imaging rounds are complete, on the **Processing tab** of Mx Workflow, select the check box next to each slide and then click **Complete**. Slides will be added to the **Completed tab**.
4. Slides can be recovered from the **Completed tab** by using the **Slide Rollback Tool** to return the slide to a previous round. This method can be used to add additional imaging rounds to a protocol.



Images

1. Images are stored in D:\Cell DIVE Images
2. Folder names are as follows:

3. Open images with HALO (analysis PC) or QuPath.

SHUT DOWN

1. Review any active slides in the Mx Workflow software to ensure processing tasks are complete.

2. In the Cell DIVE Acquisition software, click File | Power Off Imager. The imager turns off within 45 seconds.

3. Click the “X” in the top right-hand corner of the Cell DIVE Acquisition software windows and the Mx Workflow window to close the software.

4. From the workstation taskbar, click Start | Power | Shut down to turn off the acquisition Workstation.