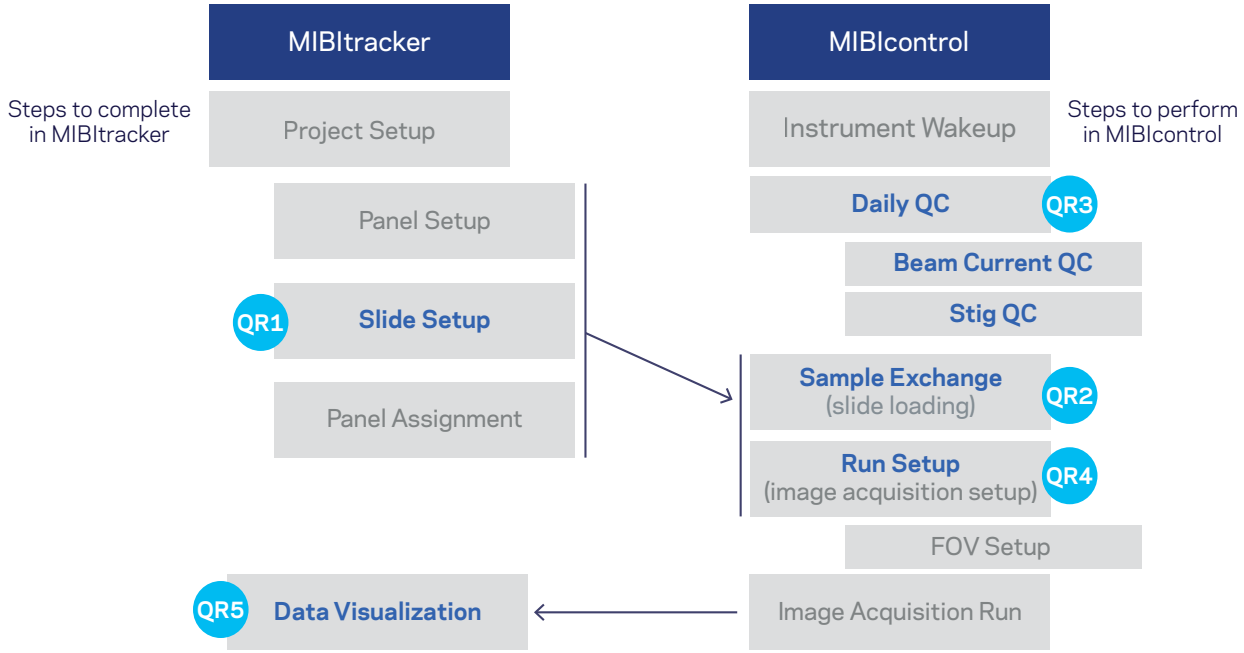
MIBIscope™ Quick Start Guide

Main Steps of Operation



Questions: support@ionpath.com

IONPATH

QUICK REFERENCE 1

MIBItracker | Slide Setup

Slide Setup

Each slide that you will scan with the MIBIscope first needs to be defined and set up in MIBItracker.

1. On the **Slides** page, click **Accession New Slide**. Fill in any details of your slide (Figure 1.1, A). Optionally, enter a name or unique ID for your slide in the **Slide Name/External ID** field (Figure 1.1, B).

   TIP: Enter a name or ID that matches your slide label.

   NOTE: When loading your slide into the MIBIscope, this field can be used to select your slide during the **Sample Exchange** step. The slide names will appear in the **Slide** dropdown, along with the **MIBItracker assigned ID**.

2. Define sections of your slide by clicking **Add Section** (Figure 1.1, C). Then in the table, fill in the information for each section you add. See Figure 1.2 for an example of a slide with more than one tissue section. Click **Submit** to save the new slide/sections.

   NOTE: When loading your slide into the MIBIscope, this field can be used to select your slide during the **Sample Exchange** step. The slide names will appear in the **Slide** dropdown, along with the **MIBItracker assigned ID**.

   IMPORTANT: In the next step, you will assign an **Antibody Panel** (on the **Panels** page) to each defined section. Make sure a panel is assigned to each section before starting a run in MIBIcontrol.

3. Go to the **Panels** page (under **Resources**) and click on the desired panel from the list. If the **Panel** you need is not yet listed, click the **Create New Panel** link at the top right to create a new panel.

   NOTE: The **Antibody Panels** are typically defined during the initial **Project** setup steps. Once you have set up your panels, they will show up in the choices in the dropdown for the Panels column.

4. Once you select your **Panel** from the list, the details of that panel will display. Click on the **Sections** (Figure 1.3, D) tab to see the list of sections that have been linked to this panel.

5. Click **Edit Section Assignment** (Figure 1.3, E) and select the new sections you defined in Step 2 to link to this panel. Click **Submit** to save.

6. You can now proceed to **MIBIcontrol** to set up your run (see QR 4 – Run Setup) and acquire images of this slide.

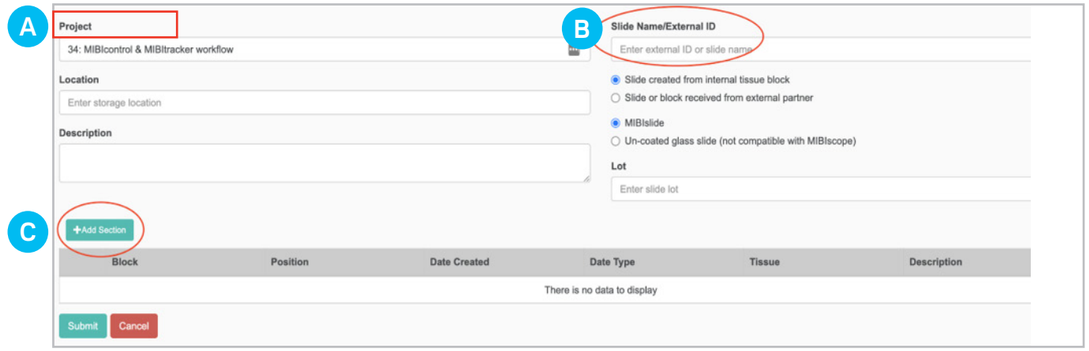


FIGURE 1.1 Slide setup.

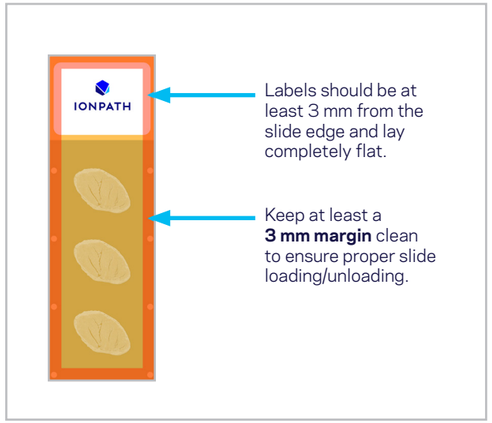


FIGURE 1.2 MIBItracker slide with more than one tissue section. **NOTE:** Please ensure that tissue, pap pen, or any other material is not placed in the area designated red on the slide (a boundary of 3 mm around the slide edges). Failure to keep this region clean could result in improper sample loading/unloading.

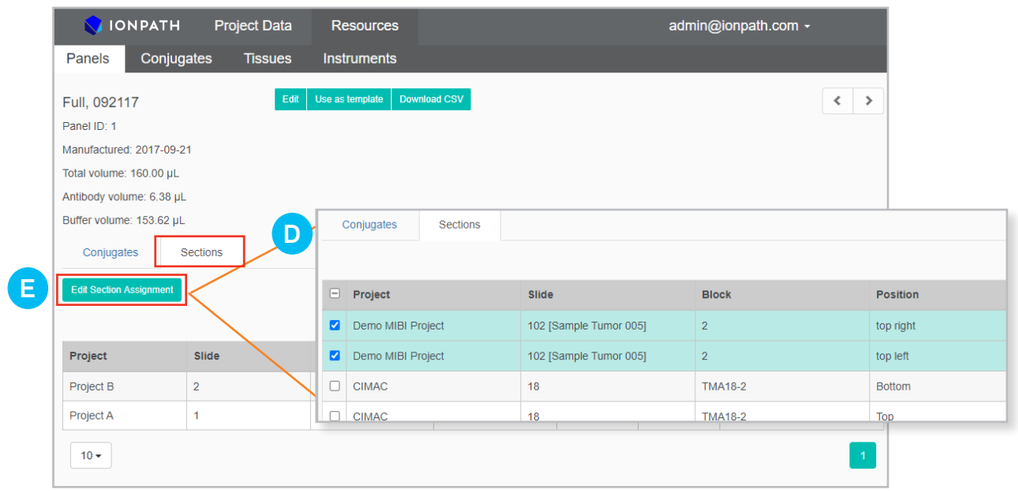


FIGURE 1.3 Linking panels to slide sections.

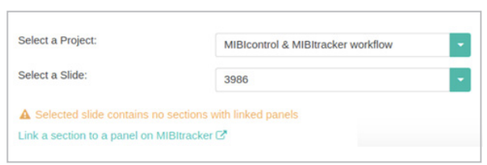
QUICK REFERENCE 2

MIBIcontrol | Sample Exchange

Loading your slide

1. Wake up the instrument if needed.
2. On the **action** menu, click **Exchange Sample** (Figure 2.1, A) to initiate the sample exchange procedure.
3. Click **Continue** on the prompt (Figure 2.1, B) to proceed. When the instrument is ready to load the new slide, a second prompt will appear. Click **Continue** on the second prompt (Figure 2.2) to open the sample exchange door.

   NOTES:
   - It may take 3-5 minutes for the door to open while the instrument moves the slide tray and vents the chamber.
   - If you wish **to cancel** the sample exchange, click the x in the upper right hand corner.
   - To minimize the possibility of contamination of the sample chamber, we recommend having your slide ready and completing the slide exchange and loading (steps 4-6) **within 30 seconds**. The instrument will sound a warning chime while the chamber door is open.
4. Remove the previous slide (if any) from the **unloading slot** (Figure 2.3, C).
5. Place your slide into the **loading slot** (Figure 2.3, D) with the tissue sample facing up and the slide orientation as shown in the image (Figure 2.3).
6. After loading your slide, click **Continue**. The door will close and an **optical image** of the slide will be displayed.
7. Verify that the slide shown in the **Optical Image** (Figure 2.4, E) pane is the correct slide and is in the correct orientation (Figure 2.4).
8. In the **Select a Project** box (Figure 2.4, F), type or select the project to which the slide belongs.
9. In the **Select a Slide** box (Figure 2.4, G), type or select the slide either by its **Slide Name/External ID** or by the **MIBItracker assigned Slide ID**.
10. After designating the **Project** and the **Slide**, click **Continue**. The instrument will evacuate the load dock and then will be ready for the **Setup Run** steps. (This may take 3-5 minutes).



NOTE: A warning appears when a slide does not have any tissue sections defined for it. Click the link to MIBItracker, define sections of your slide, and assign panels to each section, then return.

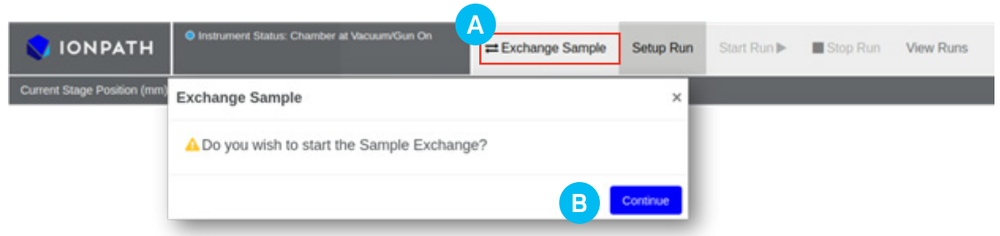


FIGURE 2.1 **Exchange Sample** menu tab.

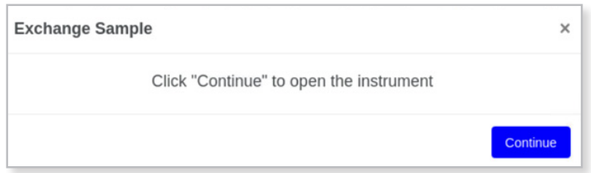


FIGURE 2.2 Open sample exchange door.

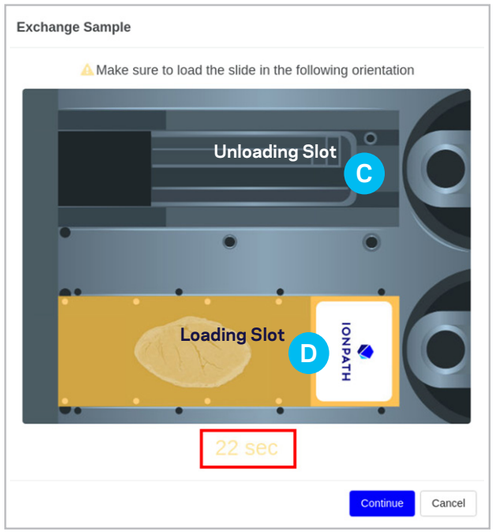


FIGURE 2.3 View of loading slot.

NOTE: Have your slide ready. Complete the slide exchange and loading **within 30 seconds**. The instrument will sound a warning chime while the chamber door is open.

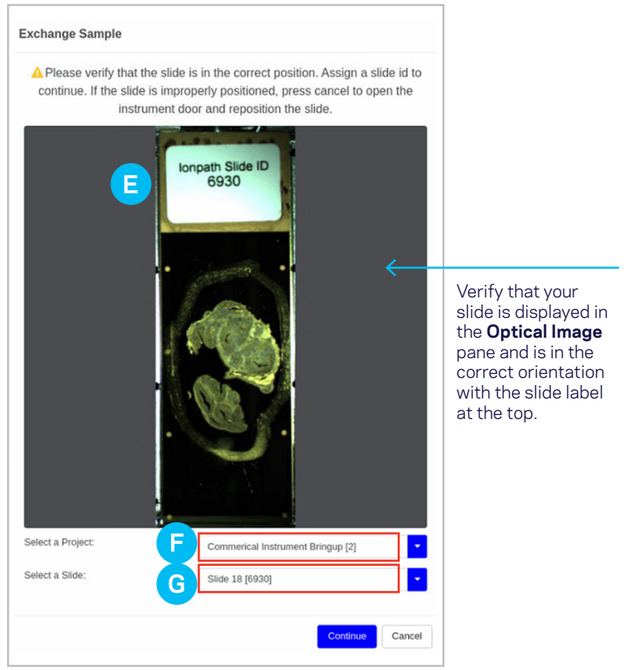


FIGURE 2.4 View of slide in optical image pane.

QUICK REFERENCE 3

MIBIcontrol | Daily QC

Beam Current QC

1. Wake up the instrument if needed.

   NOTE: It is OK to proceed with the beam current QC, but before beginning image acquisition, we recommend waiting one hour after waking up the instrument to allow the beam focus to stabilize.

2. On the **Target Selection** dropdown (Figure 3.1, C), select **Faraday Cup** to move the stage to the Faraday Cup target.

3. Select **SED** on the **Mode** dropdown (Figure 3.1, A). An image of the Faraday Cup will be displayed on the right; adjust the coarse **Gain** slider in order to visualize the image.

4. Select the **Crosshair** checkbox under the **SED** image to verify that the Faraday Cup is centered under the beam.

5. Click **Jog Stage** (Figure 3.2, D) and use the **Arrows** to center the Faraday Cup if needed.

6. Select **Spot** in the **Mode** dropdown. A running average of the **Sample Current** will be displayed, along with the **Lens 1** value for the currently selected imaging preset.

7. On the **Imaging Mode** dropdown (Figure 3.1, B), select **Coarse**.

8. Note the displayed **Sample Current** (nA) and ensure it is within the recommended range listed below. If not, adjust the **Lens 1** value so the **Sample Current** falls within the recommended range. Record the value in the **MIBIscope Parameters Log** spreadsheet.

BEAM PRESET	RECOMMENDED CURRENT (nA)
Coarse	9.5 ± 0.2
Fine	5.5 ± 0.2
Super Fine	2.5 ± 0.2
QC-100	50 (pA) ± 10 (pA)

9. Repeat steps 7–8 for the **Fine** and **Superfine** imaging modes.

   NOTE: If Lens 1 needs to be adjusted by more than 10% to achieve the target **Sample Current**, please contact Ionpath support.

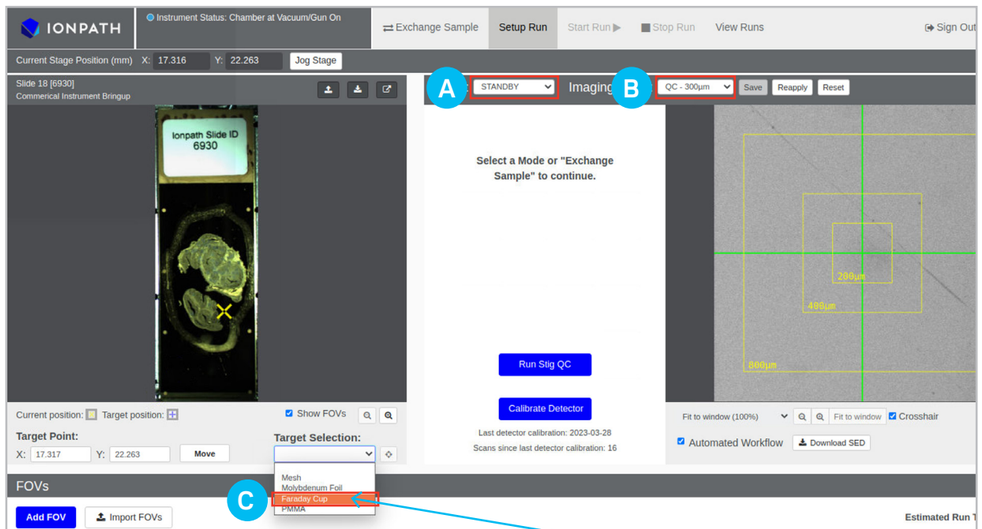


FIGURE 3.1 For Beam QC setup.

Select **Faraday Cup** for **Target Selection**.

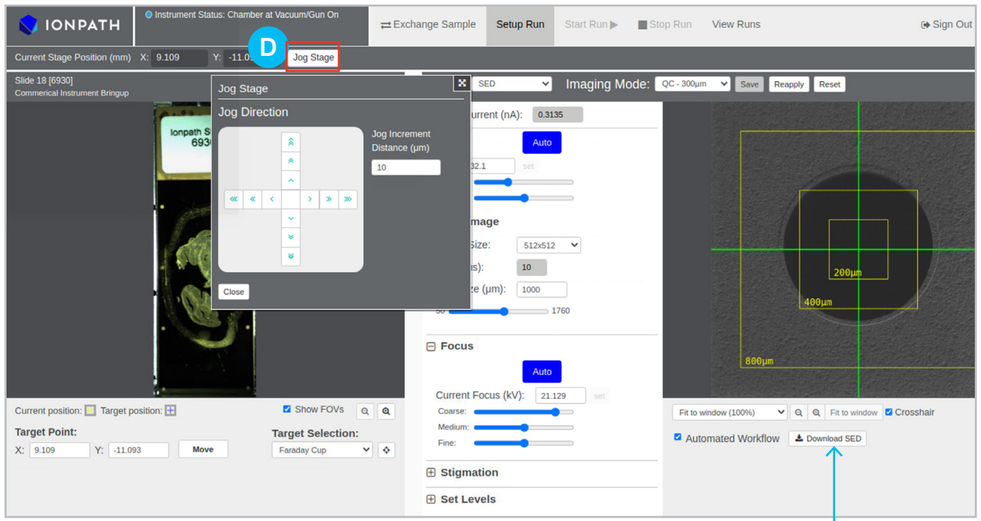


FIGURE 3.2 Faraday cup in SED mode.

Ensure the crosshair is in the center of the Faraday Cup.

Stig QC

The **Stig QC** function automates **Autostigmation** by performing the function for all four Imaging Modes to be used during run setup and image acquisition (Coarse, Fine, Superfine, QC-300).

1. Enter **Standby** mode.
2. Click **Run Stig QC** (Figure 3.3, A).
3. On the dialog that appears, click **Start** (Figure 3.5).
4. The MIBIscope will navigate to an unused area on the Mesh target and run Autostigmation for all Imaging Modes to be used during run setup and image acquisition.
5. When prompted, assess the results for the **Superfine Imaging Mode** (see the Manual tuning of beam optics (focus and stigmation) section of the MIBIscope User Guide for guidance in assessing the stigmation in a SED image):
    a. If the stigmation is acceptable click **Accept** (Figure 3.4) to save the optimized stigmation parameters to the Imaging Mode.
    b. If the stigmation does not appear to be optimized for the Imaging Mode, click **Cancel** and follow the steps in the Manual tuning of beam optics (focus and stigmation) section of the MIBIscope User Guide to manually tune the stigmation. Once completed, you can retry the Autostigmation feature.
6. Repeat Step 5, when prompted, for the **Fine**, **Coarse**, and **QC-300** Imaging Modes.

Once completed, MIBIcontrol will display the timestamp of the last successful Stig QC below the Run Stig QC button.

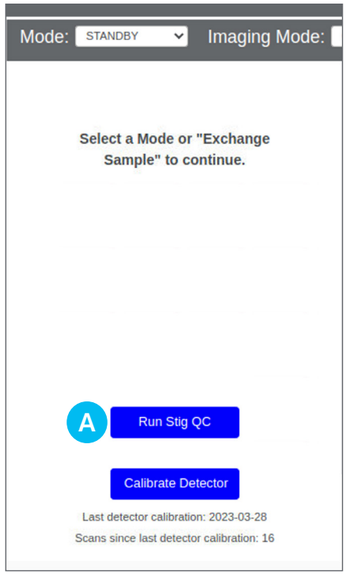


FIGURE 3.3 Starting Stig QC.

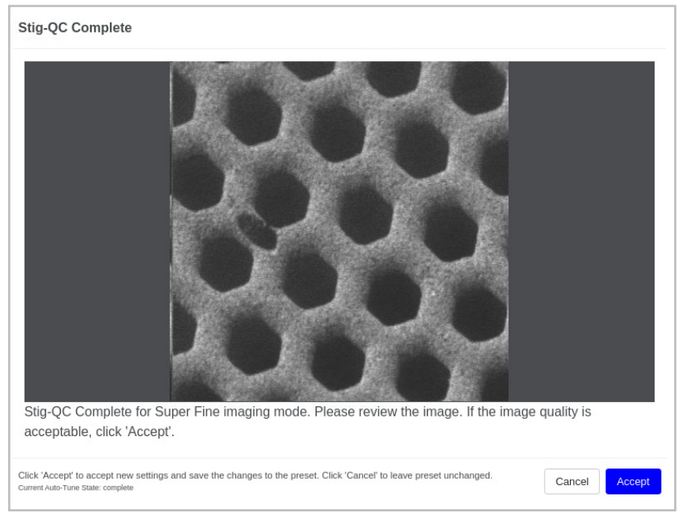


FIGURE 3.4 Stig QC results for verification.

FIGURE 3.5 Stig QC confirmation dialog box.

QUICK REFERENCE 4

MIBIcontrol | Run Setup

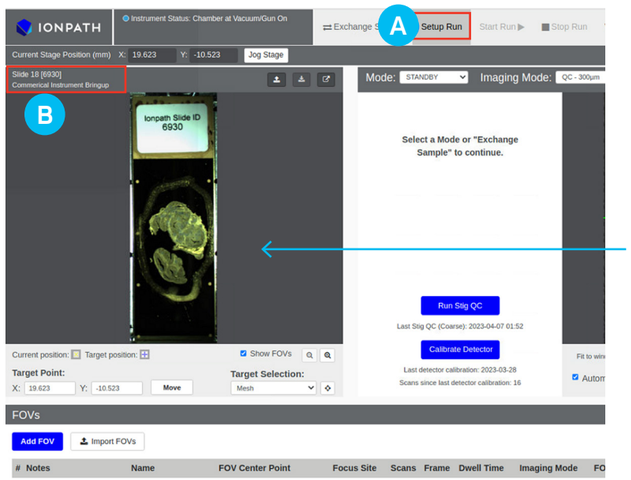
By default, **Automated Workflow** is enabled for a new run, which enables **Autogain**, **Autofocus**, and **Autostig** for unattended runs up to 24 hours. The following steps assume Automated Workflow is enabled. For more information on Automated Workflow or for runs using **Standard Workflow**, please refer to the MIBIscope User Guide.

Starting the run setup

1. Wake up the instrument if needed.

   NOTE: Before beginning image acquisition, we recommend waiting **one hour** after waking up the instrument to allow the beam focus to stabilize.

2. Perform the **Daily Quality Control** checks (see QR 3 – Daily QC).
3. Navigate to the **Setup Run** (Figure 4.1, A) page.
4. In **Standby** mode, verify that the correct slide appears in the **Optical Image** (Figure 4.1, B) pane.
5. Perform a **Sample Exchange** if needed (see QR 2 – Sample Exchange).

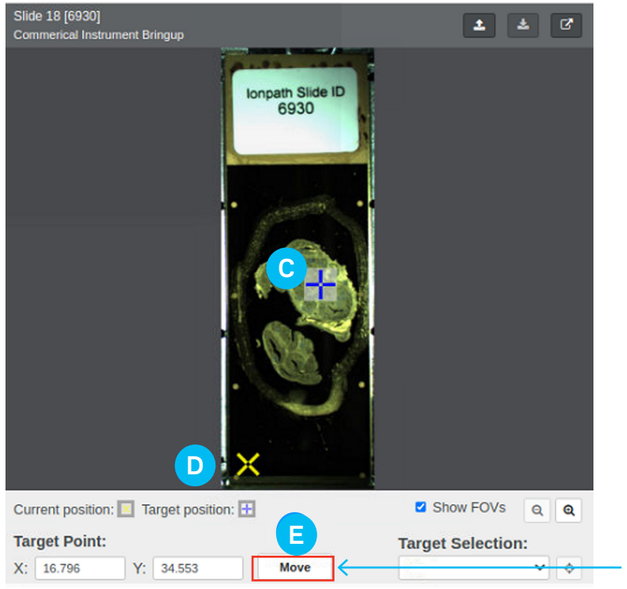


While in **Standby** mode, verify that your slide is displayed here.

FIGURE 4.1 Setup run.

Navigating to a Field of View (FOV)

1. Click on an area of the tissue sample on the slide displayed in the **Optical Image** pane.
2. A ✚ (Figure 4.2, C) will appear to indicate the target stage position.
3. Click **Move** (Figure 4.2, E) to center the beam over the area of interest.
4. The current stage position indicator **X** (Figure 4.2, D) moves to overlap with the target position.
5. The stage can also be navigated by clicking a point in the **SED image**, while in SED imaging mode. In standby mode, the last captured SED image is displayed and the move point and click navigation via SED image control is disabled.
6. On the SED image pane, select the **Crosshairs** checkbox to mark the center of the FOV. Additionally, boxes corresponding to 200 x 200, 400 x 400, and 800 x 800 µm will be overlaid on the SED image.



Click **Move** to center the beam over the area of interest.

FIGURE 4.2 Target Stage position.

Adding FOVs to a run

1. On the **Mode** dropdown (Figure 4.3, A), select **SED**.
2. You can modify the **SED FOV Size** by moving the slider (Figure 4.3, B) in the **SED Image** control pane.
3. The **SED Frame Size** can also be adjusted using the dropdown. We recommend using a 512x512 px frame size for FOV selection.
4. To adjust the **field of view (FOV)** location, click the **Jog Stage** button (Figure 4.3, C).
5. Click the arrows in the pop-up modal to move the FOV to the desired location.
6. Check the box for **Automated Workflow** (Figure 4.3, D) to enable **Autogain**, **Autofocus**, and **Autostigmation** for the run.
7. When ready, click **Add FOV** (Figure 4.3, E).
8. On the FOV detail panel (Figure 4.4) that appears, define the various **acquisition settings** using Table 4.1 “Recommended acquisition settings” (page 15) as a guide. Refer to Table 4.2 for additional guidance on choosing the best settings for your experiment.
9. Select an **Auto-Focus Site**.
   a. The location is defined in the **Selected Auto-Focus Site** pane (Figure 4.4, F). The eight directions indicate the location outside of the FOV area to be scanned in which **Auto-Focus** will be run prior to scanning the FOV. To change the location, select one of the eight directions.
   b. For the first FOV defined in **Automated Workflow**, an **Auto-Focus Site** is required and NW will be selected by default.
   c. For all other FOVs, if the FOV location is within 5 mm of a previous Auto-Focus Site, Auto-Focus is not needed and can be disabled for the FOV by checking **No Auto-Focus** (Figure 4.4, G). If the FOV location is more than 5 mm from a previous Auto-Focus Site, leave No Auto-Focus unchecked and select one of the eight directions.
10. Select the desired **Slide** and **Section** under **Assign Section** (Figure 4.4, H).
11. Click **Confirm**.
12. Repeat steps 4–10 to add additional FOVs.
13. You can **Modify** or **Delete** your FOVs by clicking on the icons (Figure 4.3, I) in the list table. You can also save a list of FOVs as a JSON file using the Export FOVs function (Figure 4.3, J).

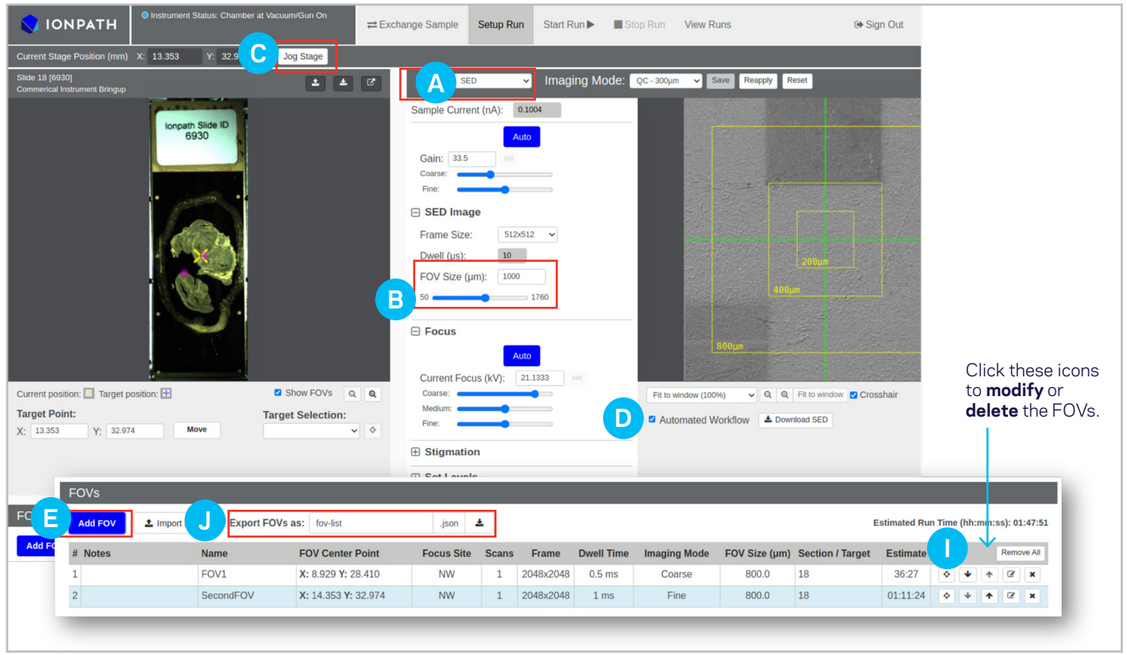


FIGURE 4.3 FOV setup in SED mode.

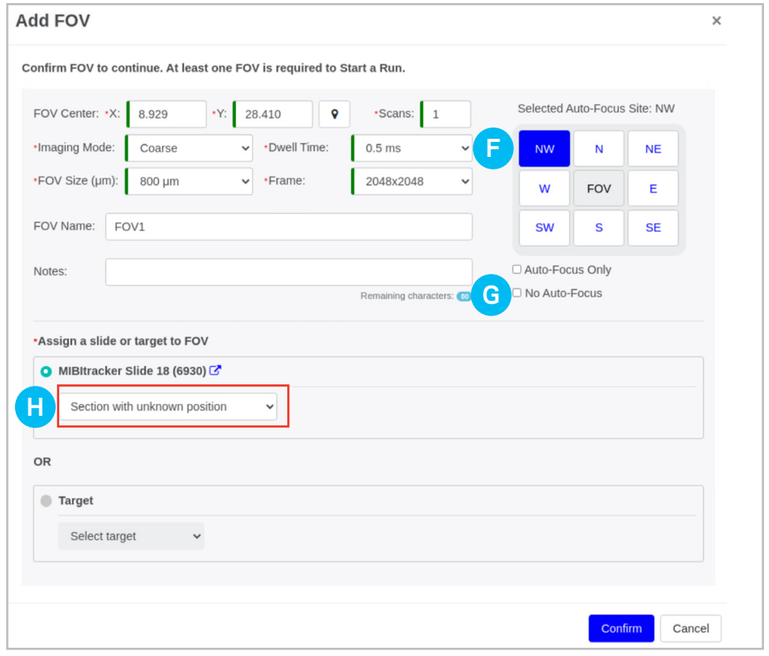


FIGURE 4.4 FOV detail panel.

Enter various settings for each FOV, for example:

- Number of scans
- Frame Size
- FOV Size
- Dwell Time
- Imaging Mode

Starting the run

1. After all FOVs have been added to the run, click **Start Run** to finish configuring the run.
2. In the **Start a Run** dialog (Figure 4.5), customize the name of the run (A).
3. If it has been more than three hours since the last Stig QC, click **Run Stig-QC** to perform Stig QC.
4. If desired, check the Put instrument to sleep after run completes option to have the MIBIscope automatically go to **Sleep** after the run finishes.
5. Click **Start Run** to begin the acquisition.

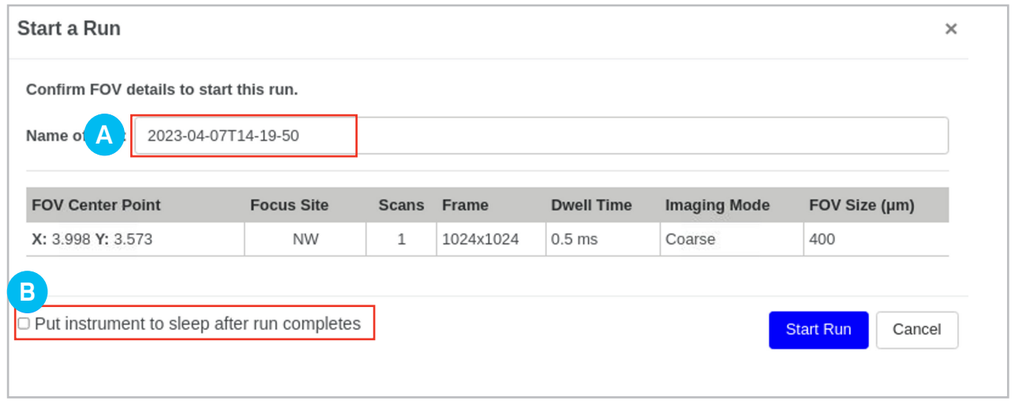


FIGURE 4.5 Starting a run.

Run completion

When the run has finished acquiring, you may either set up a new run or put the instrument to sleep. If the MIBIscope will not be in use for more than four hours, it is recommended to put the instrument to sleep to prevent unnecessary wear to the system.

TABLE 4.1 Recommended acquisition settings

FOV SIZE (μM^2)	FRAME SIZE (px)	RESOLUTION	DWELL TIME (ms)	ACQUISITION TIME (min)
Mo foil - 200x200	128x128	NA	1	0.25
400x400	1024x1024	Coarse	0.25	4
			0.5	9
		Fine	1	17
		Super Fine	2	35
			4	70
800x800	2048x2048	Coarse	0.25	17
			0.5	35
		Fine	1	70
		Super Fine	2	139 (2.33 hr)
			4	282 (4.7 hr)

TABLE 4.2 Guidelines for imaging settings. Choose the best imaging mode and settings suited for your experiment.

IMAGING MODE	DWELL TIME (ms)	EXAMPLE USE CASE
Coarse	0.25	Use this setting to find a region of interest while preserving the tissue or to quickly perform a survey scan.
	0.5	This setting will give approximately the same sensitivity of the Fine mode at a lower spatial resolution. It can reveal low expression markers that might not be completely detectable at 0.25ms dwell time.
Fine	1	Use this mode to acquire FOVs with high sensitivity and spatial resolution sufficient for cell segmentation.
Super Fine	2	This setting will give approximately the same sensitivity of Fine mode with a slightly higher spatial resolution.
	4	This setting offers the highest sensitivity and spatial resolution but with higher sample consumption.

NOTE: Antibody titrations remain valid across the highlighted modes.

QUICK REFERENCE 5

MIBItracker | Data Visualization

Viewing an image

An **image set** is created automatically to contain all FOVs in a run when uploaded to MIBItracker. Refer to the MIBItracker User Guide to learn more about sharing **Projects** and **Images**.

1. On the **Image Sets** page, click **View** to open an image in the **Overlay Viewer**.
2. In the **Overlay Viewer**, select the channels (Figure 5.1) to be displayed and choose a color overlay of your choice to facilitate viewing your multiplexed MIBI image.

OVERLAY VIEWER CONTROLS

Adjust the image using the following overlay viewer controls (Figure 5.1)

A. Download the current **multiplexed MIBItiff** or a **TIFF** or **PNG** of the overlay image here.

B. Navigate to other images in an image set using the right or left arrows or this dropdown menu.

C. Add a visual setting, reload, modify, or delete it from here.

D. Choose from the list of available channels and use the **Range**, **Smooth**, and **Brighten** sliders to make visual adjustments to a channel.

E. Add additional **image channels**.

FIGURE 5.1 Overlay viewer controls.

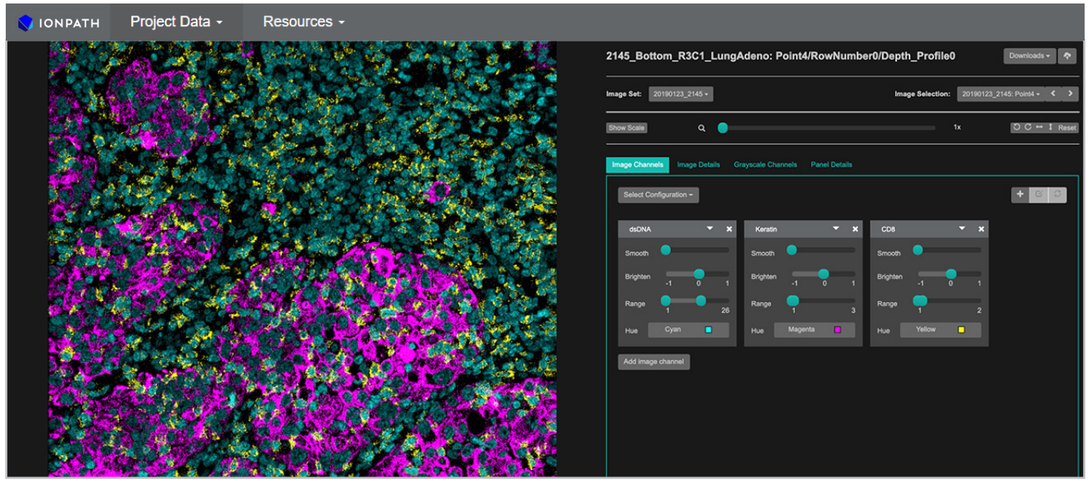


FIGURE 5.2 Image view.

PROJECTS DROPDOWN

When in the **Project Data** mode, the **Projects** menu can be used to filter the data by a specific project or to view data from all projects. You can also **Search** or **Create New Projects** from this menu.

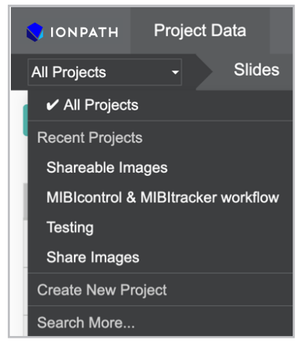


FIGURE 5.3 View of Projects dropdown.

Troubleshooting

Restarting a Failed Run

Starting in MIBIcontrol v1.8.0, when FOVs in a run fail to acquire because the instrument encounters an error or if autofocus failed to determine an optimal focus setting, a button to reload the incomplete FOVs will be displayed in the run history view (View Runs → that failed). Clicking this button will import all FOVs that failed to acquire from the run into a new run. Navigate back to **Setup Run**, confirm that settings are correct, adjust focus site if necessary, and click **Start Run** to acquire the failed FOVs.

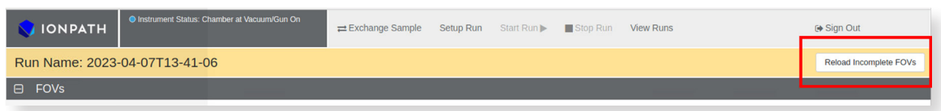


FIGURE 6.1 Restarting after an interrupted run.

Restarting MIBIcontrol

In some cases, MIBIcontrol will need to be reset to resolve a number of software issues. If one has occurred, you may be prompted to restart MIBIcontrol to try to recover the system. In cases where an error modal does not appear, you can initiate a restart by pressing **CTRL + ALT + R**. Click **Restart** in the restart modal to begin the process. This will reset all of the system software and return the instrument to its sleep state. If the error modal continues to appear after the restart, or the observed issue continues, please contact Ionpath support at support@ionpath.com.

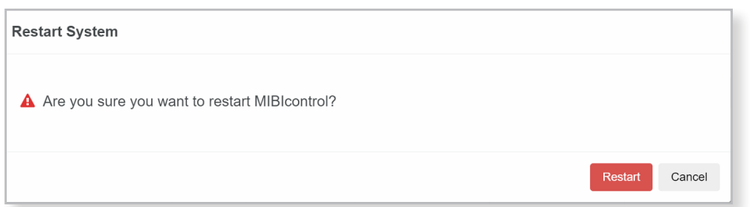


FIGURE 6.2 Restart MIBIcontrol.

Browser refresh

Some software errors can be cleared by simply refreshing the browser page. In these cases, a dialog will appear prompting you to refresh the browser page and also allow you to download a report. You may download the report and send it to support@ionpath.com. To refresh your browser, simply press the **refresh** button. If the error persists after refreshing the page, please contact Ionpath support at support@ionpath.com.

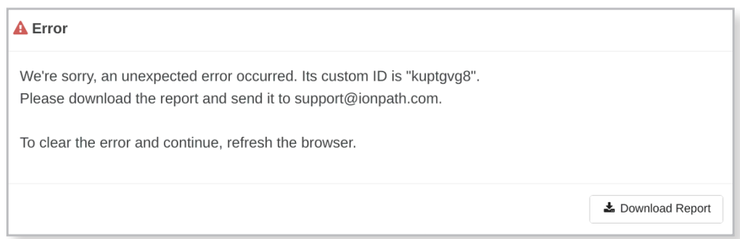


FIGURE 6.3 Refresh browser.

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