IONPATH

MIBItracker User Guide

v1.2.3

Contents

MIBItracker Basics

MIBItracker is a web application used to store, manage, and visualize image data produced by the Ionpath MIBIscope. Users interact with the MIBItracker database via a web browser available on any operating system. It is also possible to interact with the MIBItracker through its REST API.

An individual account will be created for each user by the MIBItracker administrator. Each account is accessible through a unique password set by the user when the account is created. Access to data and images is controlled based on this user account.

MIBItracker Login

To login to MIBItracker, first ensure that your computer is connected to the internet.

1. In your web browser navigate to https://*yoursite*.ionpath.com.

   A unique address is provided for each institution.

2. On the login page, type in your email address and password, then click **Submit**. MIBItracker starts on the **Image Set** tab by default.

For security purposes MIBItracker will:

1. Block you for 5 minutes after three attempts to login with the wrong password.
2. Prevent you from re-using your last four passwords when resetting your password.

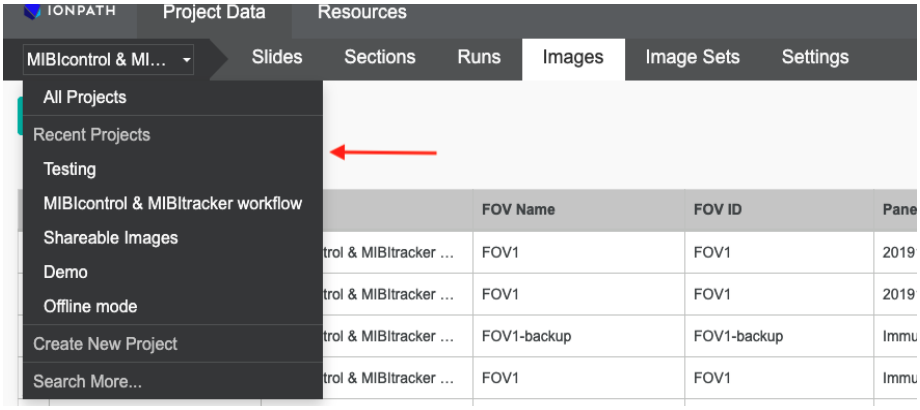
Note: For optimum performance use Chrome, Firefox, or Microsoft Edge v79 and higher.

User Interface

The MIBItracker interface is composed of **Project Data** and **Resources** in the main menu and their respective data in the submenus. Project Data contains **Slides**, **Sections**, **Runs**, **Images**, and **Image Sets and Settings.** Resources contain **Panels**, **Conjugates**, **Tissues,** and **Instruments.**

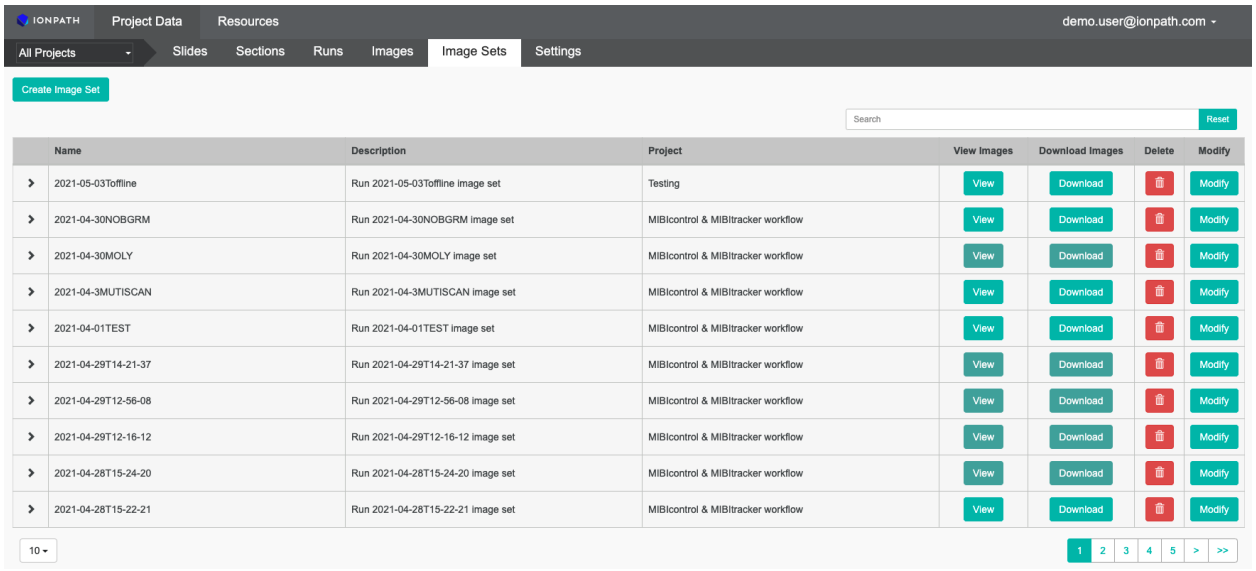
The data in the submenus are represented as searchable tables. The columns in the table are sortable, and clicking on a row of a table navigates to the relevant details page. Individual image fields-of-view (FOVs) collected from the MIBIscope instrument are presented using an interface that allows users to fully customize their display while the underlying image data remains unaltered. Controls enable changing which channels are displayed, the overlay pseudo-colors, and the relative display intensity.

Projects Dropdown



Use this dropdown to filter the Project Data display by **All Projects** or a by specific project. You can also create and search projects from here.

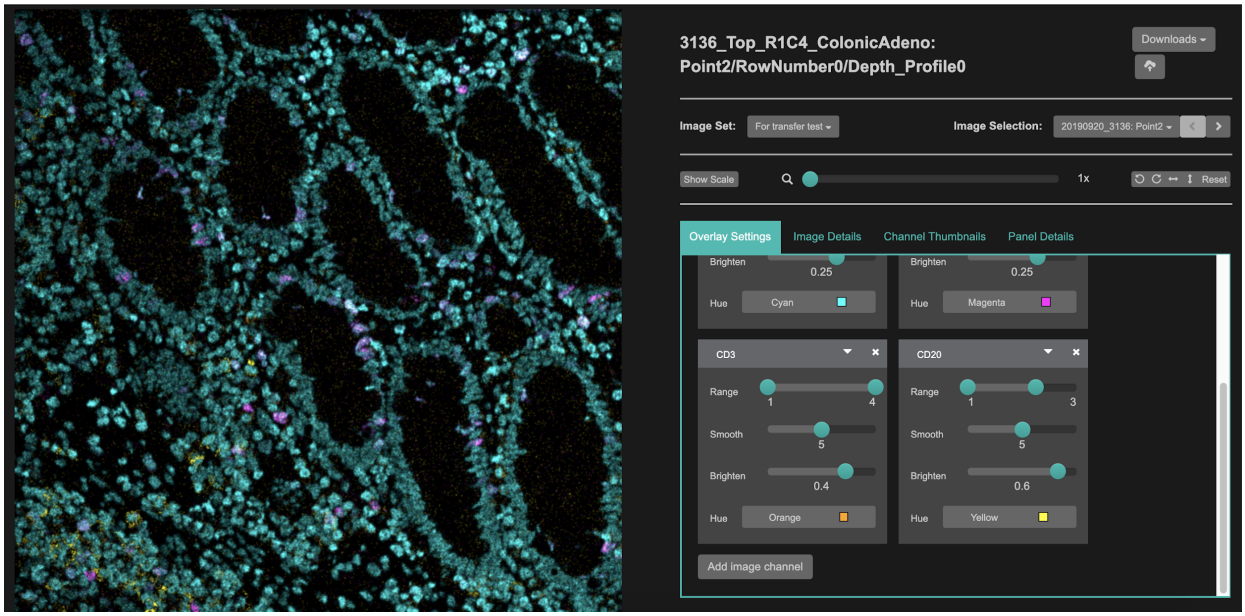
Data Table Display Mode



By default, tables display up to ten rows of information corresponding to the selected tab. You can change the number of rows displayed in the table to 25 or 50 using the drop-down menu below and to the left of each table. Navigate the rows by clicking on the desired page number below and to the right of a table. You can go to the next or previous page in the table by clicking on **<** or **>**, respectively. Click on **<<** or **>>** to jump directly to the first or last page in the list, respectively. The rows in a table can be sorted in a different order by clicking on the column headers. The order can be reversed by clicking on the same column a second time.

Use the **Search** box to find specific row items. You can search on terms present in any of the columns displayed in the table. Click **Reset** to revert to the default display mode.

Overlay Display Mode



The **Overlay** display mode is used to review images representing the quantitative data acquired using the MIBIscope instrument. The display of each image or image set can be customized. Users can select the channels to be overlaid, their relative intensity, pixel smoothing, and channel pseudo-color. All brightness, level, and orientation adjustments will not modify the underlying raw data and are fully reversible. Display settings will be held constant when navigating to another image to enable direct comparison, but they also may be further customized. Visual settings may also be saved for future use. Refer to the Create Image Sets section and the Overlay Display and Control section to learn more about image sets and their visual settings.

Images can be exported as MIBItiff files, which can then be analyzed using compatible image analysis software packages such as Halo, Fiji, and VisioPharm.

Data Management

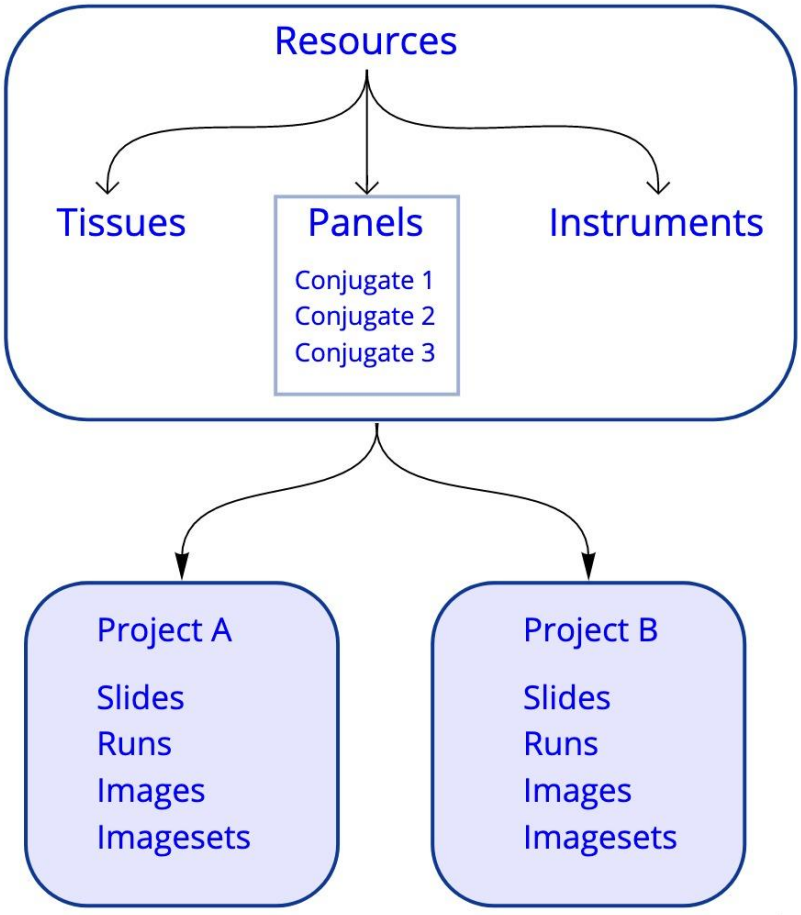
Introduction to Resources and Projects

MIBItracker data is organized into Resources and Projects. These groupings are used to control user access to different types of data.

Resources consist of panels, conjugates, tissues, and instruments. Users are assigned a Resource access level of Admin, Delete, Modify, Create, Read, or Limited. These permissions

apply to all Resources in the database. An Admin must assign an access level when creating a new user but can change it at any point.

Projects consist of slides, runs, images, and image sets. Any user can create a Project except for Limited View users. Limited View users may be granted view access to Projects and they are restricted to view only the data within the Projects. Each Project's Admin can assign users Admin, Edit, or View access to that specific Project.



For both Resources and Projects, each user level includes all the levels below it. For example, Resource Modify users also have access to create and read Resources, and a Project Admin can also View and Edit data in that same Project.

A difference between Resources and Projects is that each user has a single access level to Resources, whereas a user may have Admin access to one Project and View access to another. This enables the same set of panels, conjugates, tissues, and instruments to be used across all Projects, but to retain controlled access to sample and image data within Projects. Please refer to Table 1 and Table 2 below for information on the actions that can be performed by different access levels to Resources and Projects.

Resource Access Levels

Panels, conjugates, tissues, and instruments fall under Resources and are available to be used in any Project. Below is a table showing the actions that can be performed based on resource access levels.

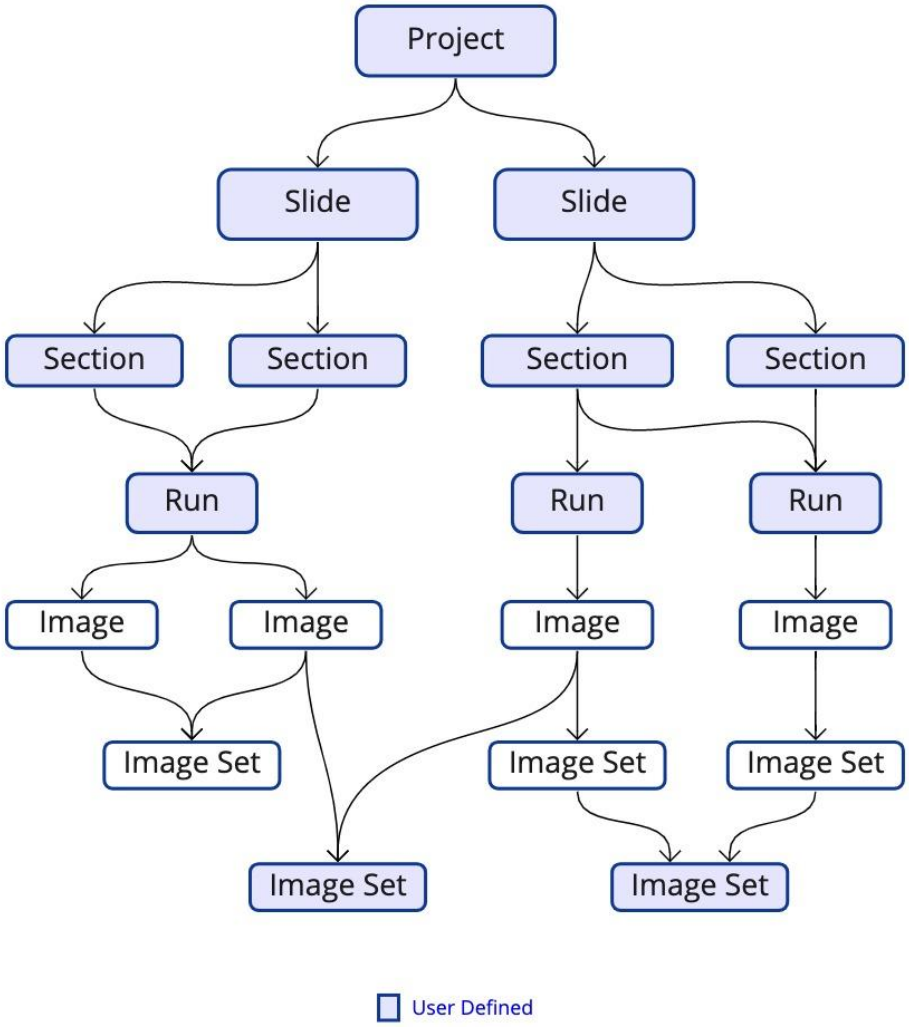
Table 1

Actions	Limited	Read	Create	Modify	Delete	Admin
View Resources associated with images*	x	x	x	x	x	x
View any Resource		x	x	x	x	x
Create any Resource			x	x	x	x
Modify any Resource				x	x	x
Delete any Resource					x	x
Manage users' Resource access						x
Create a Project		x	x	x	x	x

* Limited users can only see the Resources of images in Projects to which they have Project View access.

Project Access Levels

Projects allow you to organize your data and control who has access to it. The diagram below depicts the relationship between Projects, Slides, Runs, and Images.



When a user creates a Project, they are able to add team members by assigning them one of the following access levels: **Admin, Edit, View**. All users will default to None unless they are specifically added to the Project. Limited View users can only be added with View access to a Project. They cannot edit or administer a Project.

The user who created the Project will default to the Project's Admin. They are able to add other users at Admin level as well. A user cannot change their own access level even if they are an Admin, but another Admin can. Below is a table detailing the permissions associated with each Project access level.

Table 2

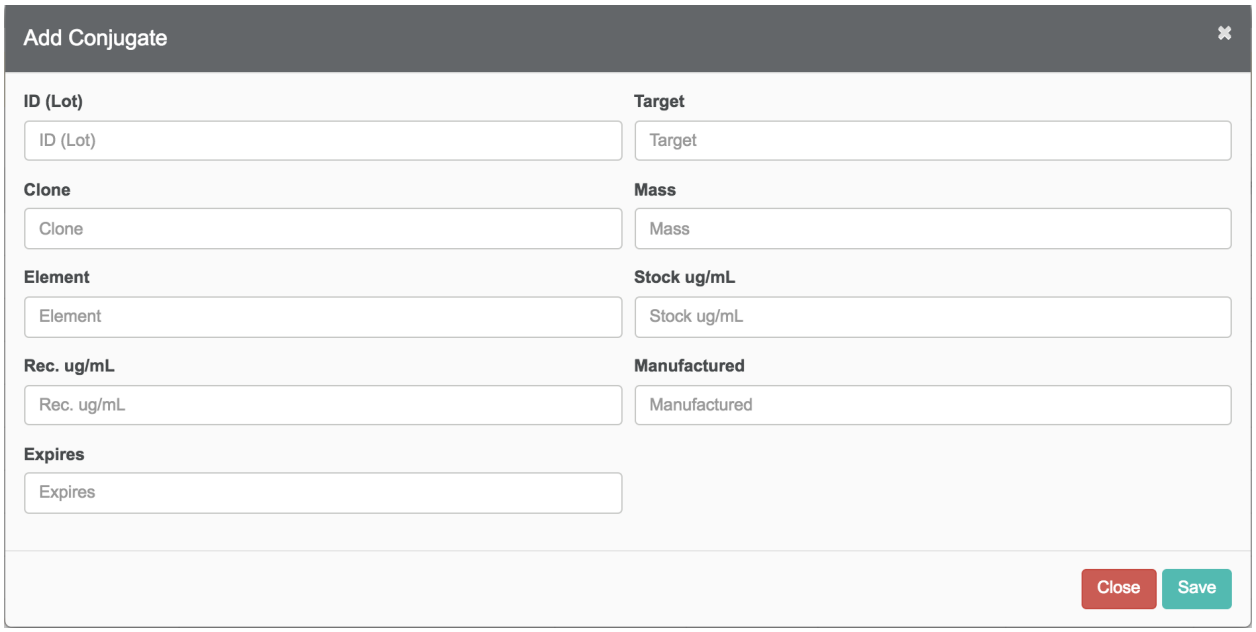
Actions	None	View (Limited)*	View (Full)	Edit	Admin
See that the Project or any of its data exists		x	x	x	x
View images/image sets in the Project, including Resources visible in image details		x	x	x	x
View details of slides, sections, and runs in the Project			x	x	x
Add, edit, and remove slides, runs, images, and image sets in the Project				x	x
View, edit, and remove users in the Project*					x
Duplicate the Project settings including its users					x
Make a Project public to all logged-in users					x
Enable/disable images from the Project to be included in image sets in other Projects					x

*When users with Limited access to Resources are granted View access to a Project, they can see all its images including the summary info about that image's run and slide as part of the image details, but they cannot search or select runs or slides outside of the image context.

Resources Setup

Catalog Antibody Conjugates

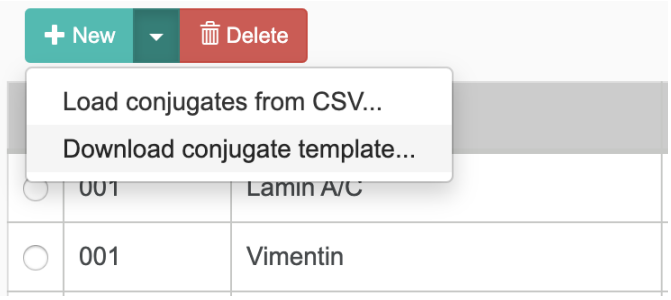
1. On the **Resources** menu, click **Conjugates** to go to the Conjugates page.
2. Click **+New** to add a new conjugate to the list.



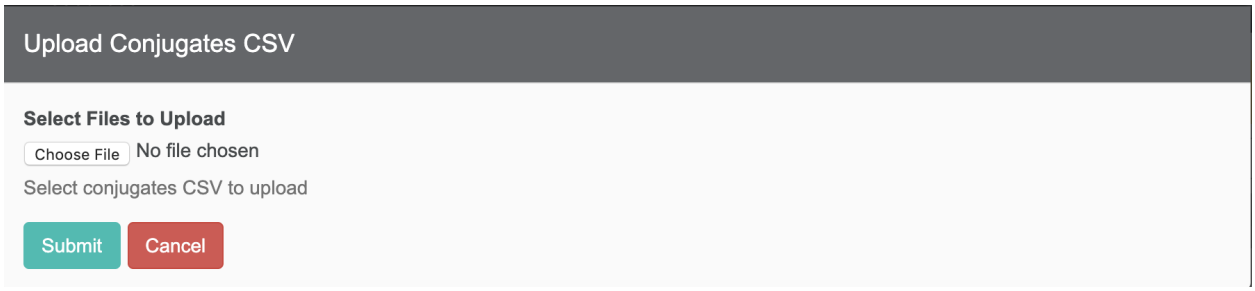
3. Click **New** to add a conjugate, a pop-up will appear. In the **ID (Lot),** box type the lot number of the new conjugate.
4. In the **Target** field, type the antigen name.
5. In the **Clone** field, type the antibody clone.
6. In the **Mass** field, type the isotope-specific mass number for the metal label.
7. In the **Element** field, type the metal tag's chemical symbol (the 1-2 letter abbreviation, for example Au for gold).
8. Optionally, in the **Stock µg/mL** field, enter the concentration of the conjugate stock solution.
9. Optionally, in the **Rec. (recommended concentration) µg/mL** field, enter the recommended staining concentration for the conjugate.
10. Optionally, in the **Manufactured** field, enter the date on which the antibody was conjugated to the metal tag.
11. Optionally, in the **Expires** field, enter the date on which the conjugate expires.

    Note: This information can be found in the Antibody Information Sheet of IONpath MIBI reagents.

Alternatively, conjugates can be added using a CSV file.



1. Click on the dropdown arrow next to the **New** button.
2. If you do not have the conjugates template then download it from the **Download conjugate template** option here, and enter all the details for the list of conjugates.
3. Save the CSV file, and click **Load conjugates from CSV.**

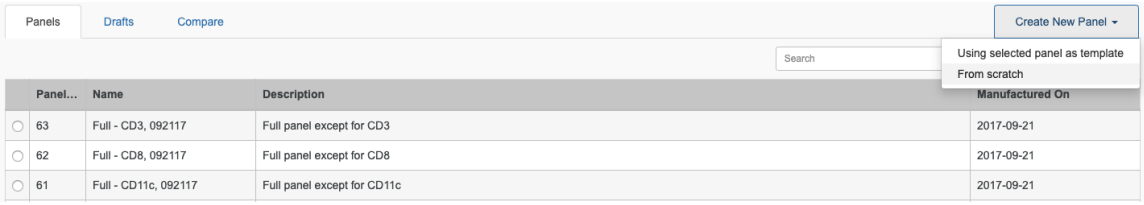


4. Click **Submit.** The conjugates will be populated in the table under the Conjugates page.

Panels

Create a Panel

1. On the **Resources** menu, click **Panels** to go to the Panels page.
2. Click **Create New Panel**. Then select **From scratch** option from the dropdown.



Alternatively, select the radio button within the first column of an existing panel in the **Panels** list, then click **Create New Panel,** and then click **Using selected panel as template** to pre-select all conjugates in the template panel. The selections can then be modified by clearing the checkboxes of unwanted conjugate options from the template panel or by selecting additional conjugates listed on the Conjugates page.

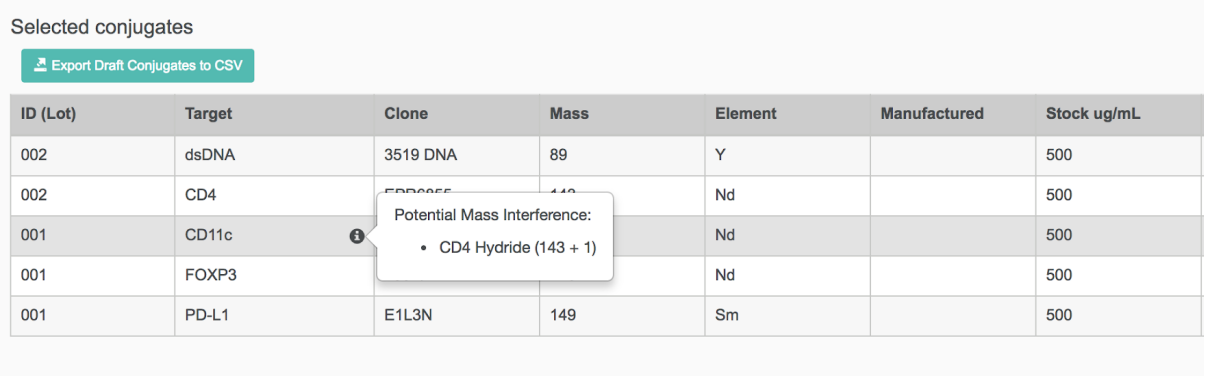
3. On the panel template page, in the **Panel Name** box, type the new panel name.

4. In the **Date Manufactured** box, select the date on which the panel will be created.
5. Optionally, in the **Description** box, enter a description of the panel.
6. Optionally, click **Add staining batch** to create an additional panel list with different conjugates.

   This function can be used for staining with antibodies that require different incubation times or conditions. Click **Add staining batch** again to add additional staining batches. Click **Remove** to remove any of the additional staining batch lists.
7. On the **Staining Batch 1** pane, in the **Select rows to include in the stain** list by clicking anywhere within the row to be added to the panel.

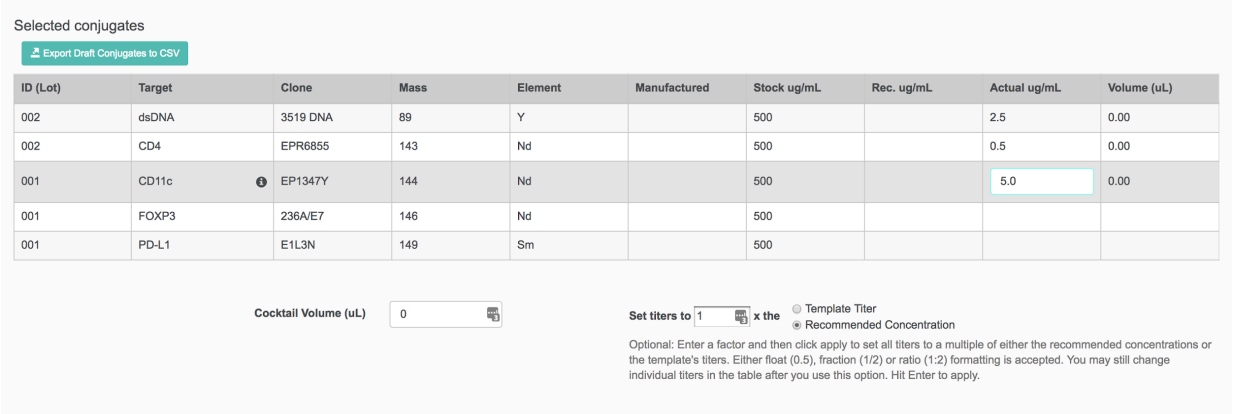
   Use the search function to rapidly parse the conjugates list for specific ones to be included in the new panel. For example, search for “E1L3N” to restrict the table to all PD-L1 [E1L3N] conjugates in the conjugates list.

   Note: Multiple lots may exist for a single clone.



   The **Selected conjugates** pane lists the conjugates selected above. Conjugate selections with potential mass interferences are tagged with ℹ. Point to the ℹ for additional detail about the possible mass interference(s). To learn more about mass interferences refer to the MIBI/O User Guide available under the About Page in MIBItracker.

   Repeat the selection process for each of the additional staining batches.



8. On the **Selected Conjugates** pane, in the **Actual µg/mL** column, enter the antibody concentration in micrograms per milliliter to be used in the staining cocktail for each of the conjugates, and then press the return key.
9. In the **Cocktail Volume (µL)** box, enter the desired total cocktail volume.

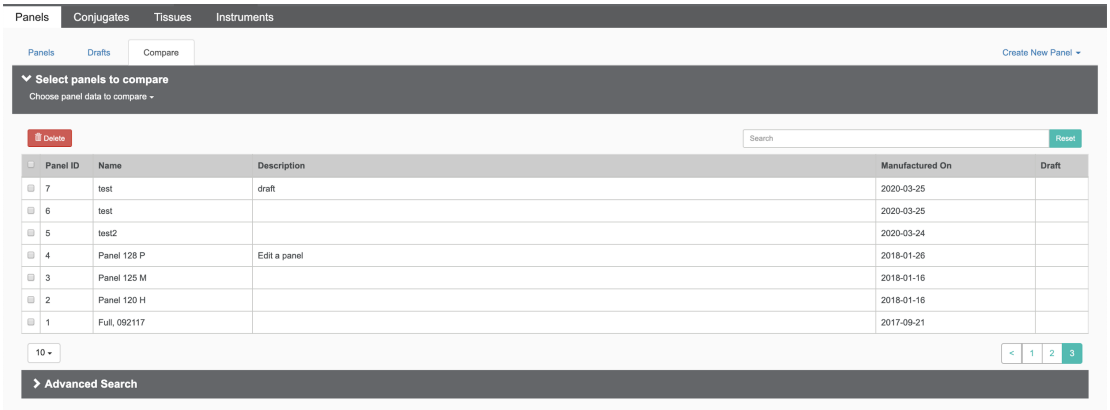
   On the **Selected Conjugates** pane, the required volume for each conjugate is then calculated in the **Volume (µL)** column.

   Alternatively, if the conjugates include a recommended staining concentration in the **Rec. µg/mL** column, or if you are creating a panel based on an existing panel template, you can enter a multiplication factor in the **Set titers to** box to populate the **Cocktail Volume (µL)**. Either float (0.5), fraction (1/2), or ratio (1:2) formatting is accepted. Select **Template Titer** to multiply the template panel titers, or select **Recommended Concentration** to use the **Rec. µg/mL** values, and then press the return key to apply. You can still change individual **Actual µg/mL** values in the table after using this option prior to clicking the Submit button.
10. Click **Export Draft Conjugates to CSV** to save a spreadsheet for the panel being created in a comma-separated values text file format.

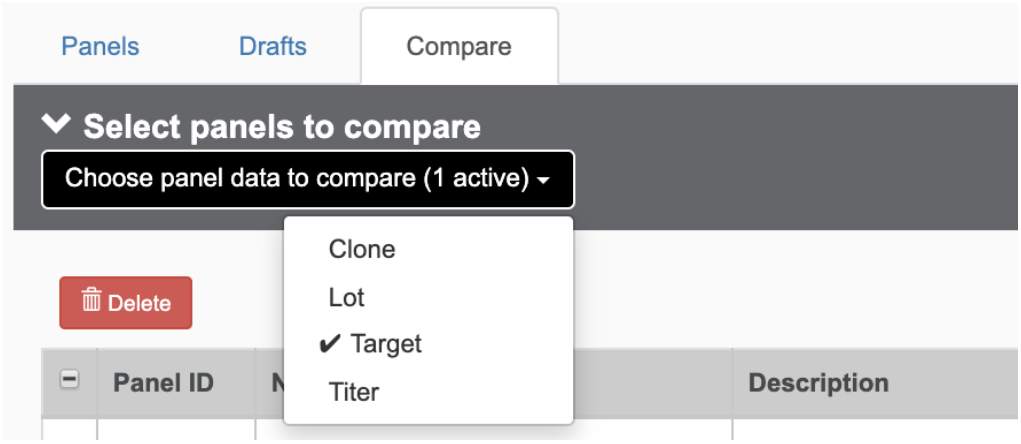
    The volume per conjugate values in the exported spreadsheet can then be used in the lab to manufacture the antibody panel.
11. Click the **Submit** button to complete Panel creation, or use the **Save Draft** option anytime during this panel creation process to save your work. All the draft panels are listed under the Drafts tab under the **Panels** page.

Compare Panels

1. On the Panels page click on the **Compare** tab.

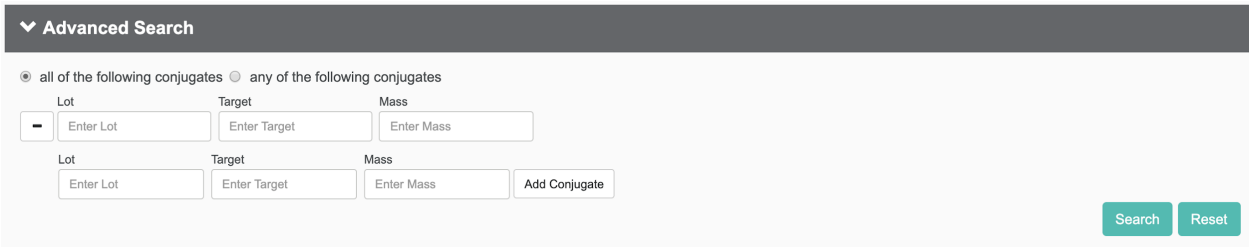


2. Select the panels from the list of panels to be compared. Draft panels are also listed here.
3. As you select you can see the panels being populated below listed by target and by their respective mass and element.
4. Optionally, you can include one or more of the data points, listed under the **Choose panel data to compare** dropdown, to be included in the panels to be compared.



Advanced Search

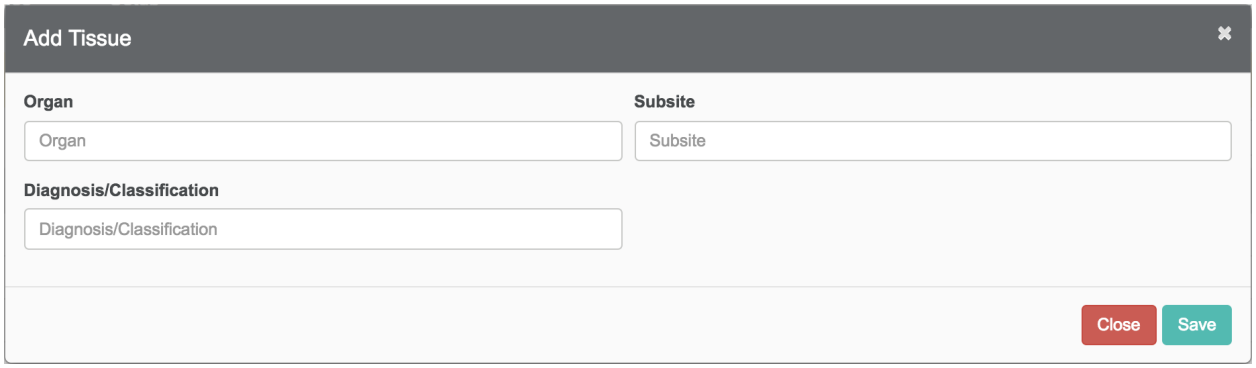
1. At the bottom of the **Panels** page, click on the **Advanced Search** dropdown.
2. Enter the conjugate's **Lot** number, **Target,** or **Mass.**
3. Optionally, click the **Add Conjugate** button to add one or more conjugate to this search, or remove a conjugate by clicking on − button.
4. By default, **all of the following conjugates** is selected. Optionally, select **any of the following conjugates** option.



5. **Search** will return a list of panels with specified conjugate(s), and **Reset** will clear the Advanced Search form.

Create Tissue List

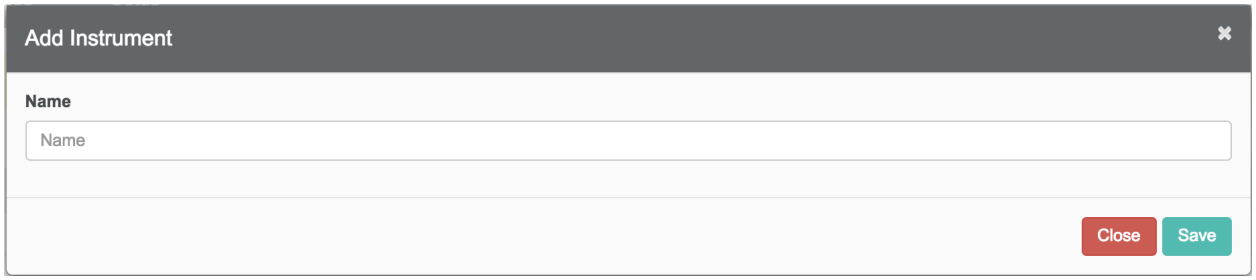
1. On the **Resources** menu, click **Tissues** to go to the Tissues page.
2. Click **+New** to add a new tissue type to the list.



3. On the **Add Tissue** dialog box, in the **Organ** field, enter the organ from which the tissue has been sampled.
4. Optionally, in the **Subsite** field, enter the subsite location of the new tissue sample type.
5. Optionally, in the **Diagnosis/Classification** field, enter a diagnosis and/or classification for the new tissue type.
6. Click **Save**.

Create Instrument List

1. On the **Setup** menu, click **Instruments** to go to the Instruments page.
2. Click **+New** to add a new instrument name to the list.

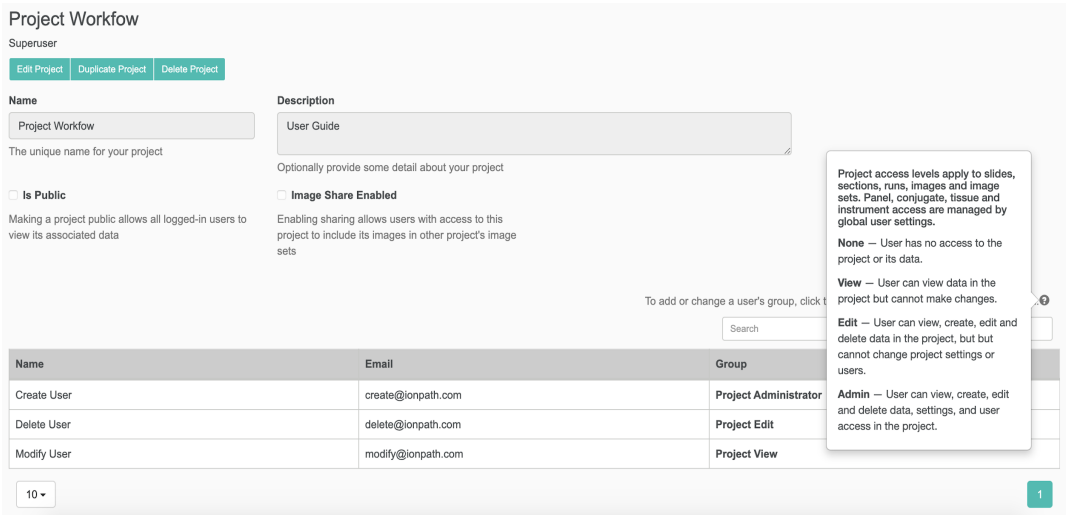


3. On the **Add Row** dialog box, in the **Name** field, enter the new instrument name.
4. Click **Save**.

Project Setup

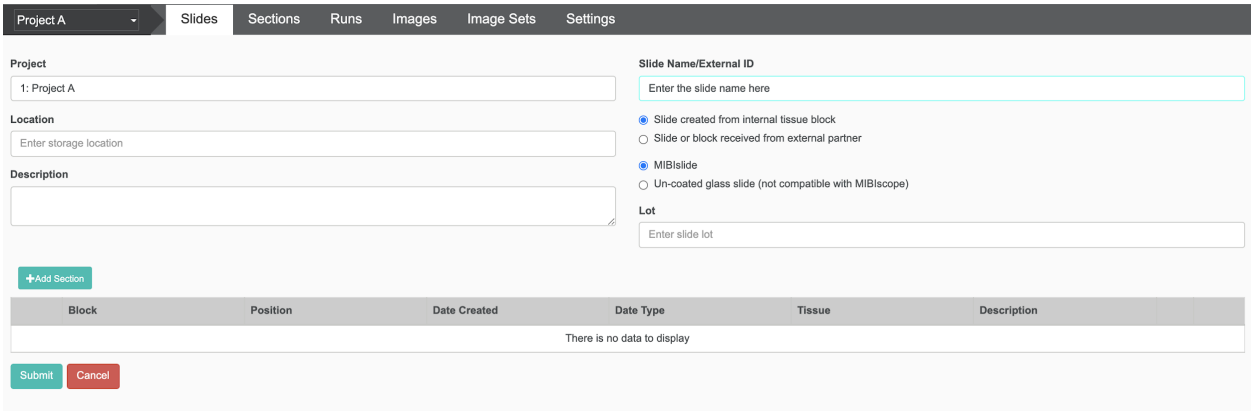
Create a New Project

1. Go to the dropdown under the IONpath logo on the Project Data tab, and choose Create New Project. Alternatively, select Settings in the submenu, and click the Create New Project button.
2. Enter a unique name and description for the Project, and assign permission levels for each user. Hit Submit. The new Project is now created.



Accession slides

1. On the **Project Data** menu, click **Slides** to go to the Slides page.
2. Click **Accession New Slide** to create a new slide.



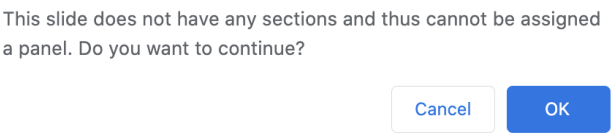
3. In the **Project** box select a project to which the slide is to be assigned. If the Project dropdown filter is set to a project, this field will default to that project. Click in the **Project** box to bring up a list of projects available. Alternatively, start typing to reduce the number of options with type-ahead searching.
4. Optionally, in the **Location** box, enter a storage location for the slide.
5. Optionally, in the **Description** box, enter a description of the slide for later reference.
6. Optionally, in the **Slide Name/External ID** box, enter an ID/slide label in addition to the MIBItracker-assigned ID.

   Note: When setting up a run at the MIBIscope using MIBIcontrol, this field will be available/searchable during sample exchange under the **Slide** dropdown, along with the MIBItracker assigned ID.
7. By default, a newly added slide is designated as a slide created from internal tissue block.

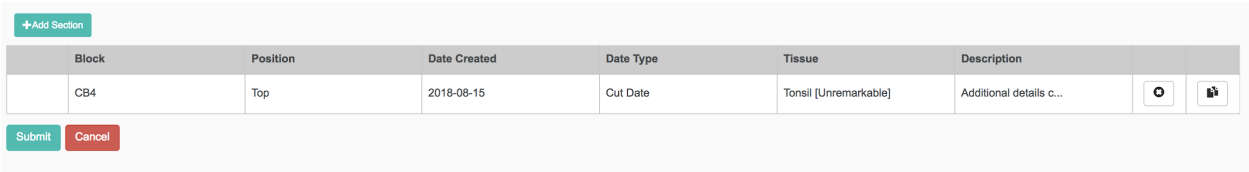
   Alternatively, click on **Slide received from an external partner** for slides originating outside of your institution/lab. This will add a Source field which can then be optionally populated with the relevant information.
8. By default, a newly added slide is designated as a MIBIscope-compatible MIBIslide.

   Alternatively, click on the **Un-coated glass slide** for standard glass microscope slides. This option is useful for cataloging H&E and/or standard IHC slides.
9. In the **Lot** box, enter the lot number of the gold-coated slide, which is printed on the packaging.

   Note: The Lot box is only available when the Gold-coated slide option is selected in step 7.
10. Click **+Add Section** to begin assigning the first tissue section to the new slide. When attempting to submit a slide without a section MIBItraker will display the following warning. This warning can be dismissed to continue to create a slide.

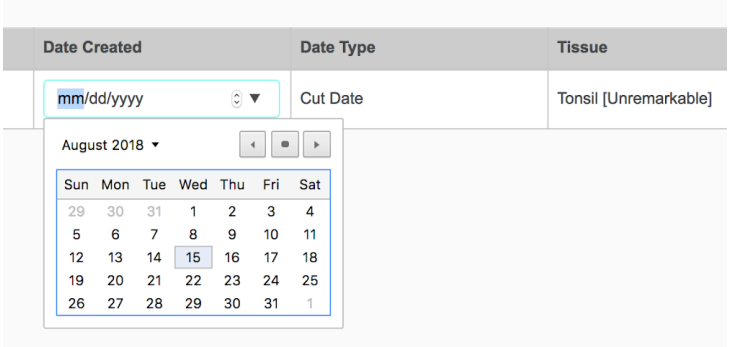


Note: Make sure to add a section for a slide. This is necessary for a panel to be assigned to a section. This will enable the successful operation of the MIBIscope and automatically upload the correct data to MIBItracker after analysis.

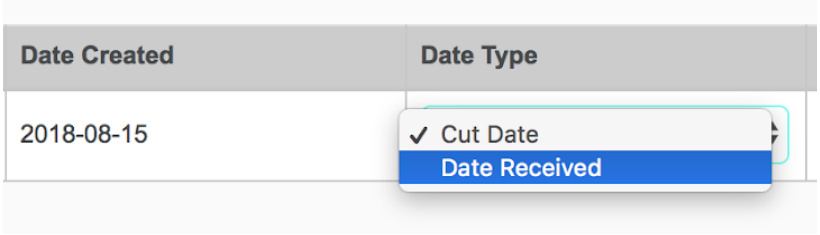


11. On the sections table, select the cell under the **Block** column header, enter an identifier for the block from which the section has been cut, and then press the return key.
12. Select the cell under the **Position** column header, enter the relative location of the section on the slide for multi-section slides, and then press the return key.

    We recommend using a consistent nomenclature across slides, projects, and users. For example, use ‘Top’, ‘Middle’, and ‘Bottom’, with a reference point such as the label agreed upon by all users.



13. Click the cell under the **Date Created** column header, and then select the date on which the slide was created using the calendar dialog.



14. Click the cell under the **Date Type** column header to define what the creation date entered in step 12 represents.

The **Cut Date** option is recommended for slides created internally, while Date Received can be used for slides sourced externally.

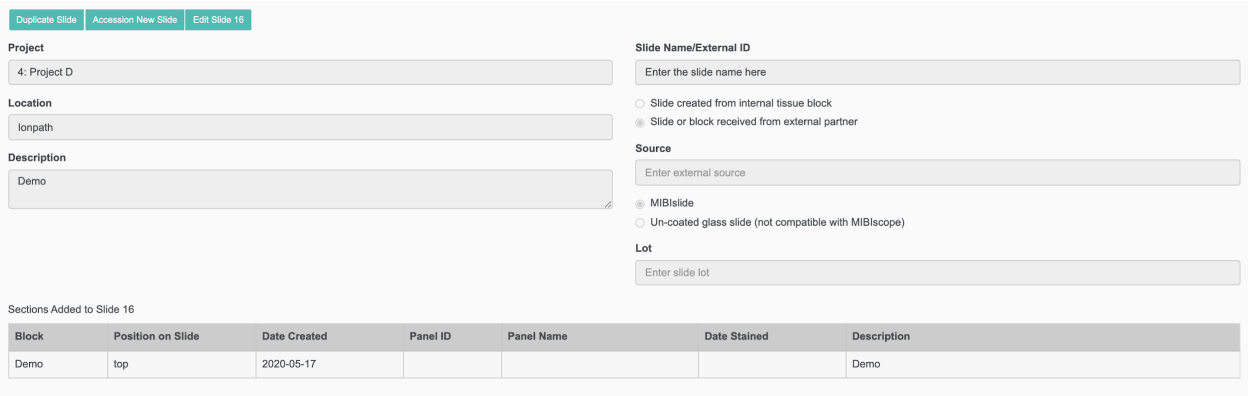
15. Select the cell under the **Tissue** column header, enter the organ or tissue type the section represents, and then press the return key.

    For multi-tissue TMA sections, this field should be left blank, and the tissue type specified at the FOV/image level.

16. Optionally, select the cell under the **Description** column header, enter any additional information related to the section, and then press the return key.

17. Click **+Add Section** to assign an additional tissue section to the slide, and repeat steps 10 through 16 for each additional section to be added to the slide

18. Once all sections have been added to the slide, click **Submit** to add the new slide ID to the MIBItracker database.

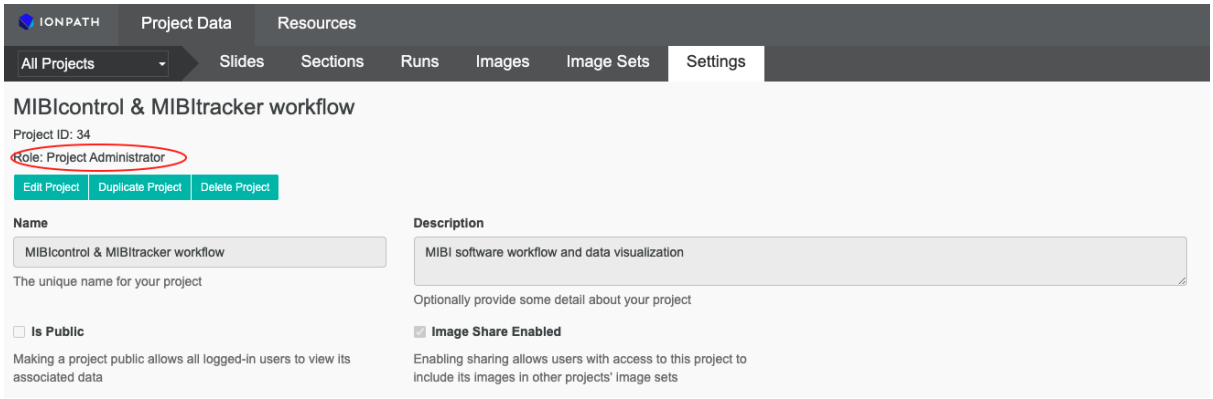


Once the slide has been added to the MIBItracker database the slide details page appears with options to Duplicate Slide, Accession New Slide, and Edit Slide #.

Project Workflow

Manage Project Settings

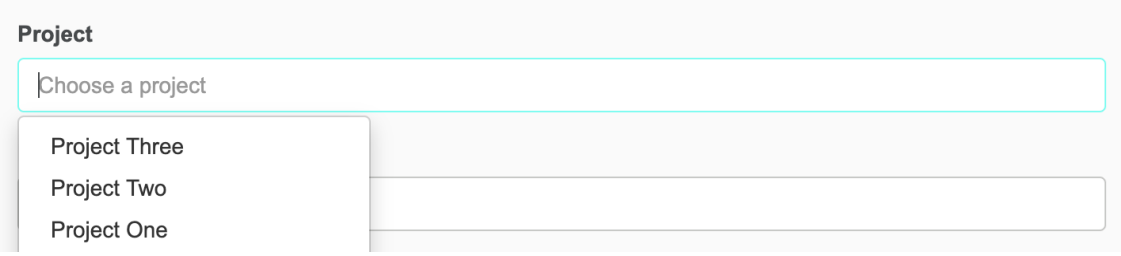
1. On the project details page, the logged-in user will see their access level under the Project name on the upper left.



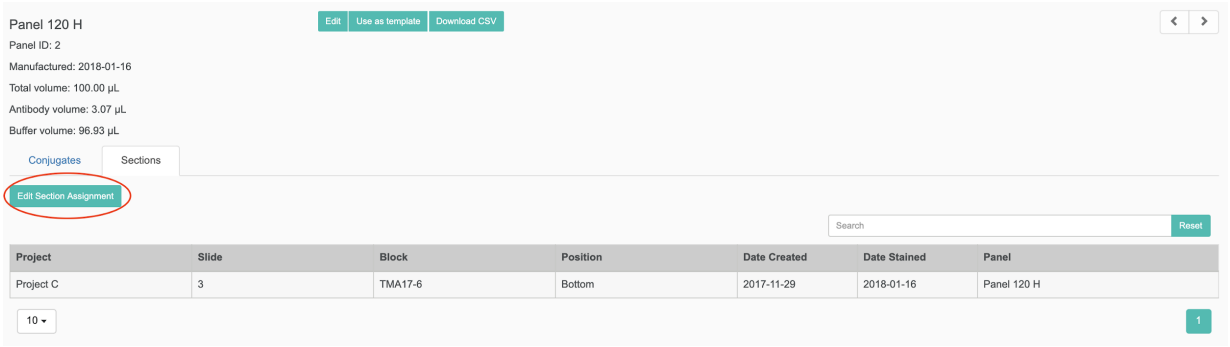
2. In **View** mode, a Project Admin will only see the users who have access listed in the table. In **Edit** and **Create** mode they will see and be able to choose from all available users. Only Project admins can access Edit mode in existing Projects.
3. In **Edit** and **Create** mode, Project admins can enable **Is Public** option for a Project which means all the data including the runs, images, and image sets associated with that Project will be available for any logged-in user who has access to MIBItracker regardless of their permission level.
4. In **Edit** and **Create** mode, Project admins can enable the **Image Share** option which allows users with Edit or Admin access to create image sets from this Project to be shared with another Project.
5. **Duplicate Project** will create a new project including users at the same access level as the previous Project as long as the logged-in user had admin access to the original Project. Otherwise project users are not copied over in the duplication process. The new Project can be edited, and **Submit** will create it.

Assign Slides

1. Make sure to add a slide (**Project Data** menu → **Slides** → **Accession New Slide**) and associate it with a Project. The Project dropdown list will only show those Projects for which you have Edit or Admin access.



2. Assign a panel to the section(s) on the slide (**Resources menu** → **Panels** → Choose a Panel → **Sections** → **Edit Section Assignment).** The list of sections is filtered by the Projects to which you have admin and edit access.



Add a Run

1. Go to the Data menu and select **Runs**.
2. Click **Create a New Run**. Upload the **Run XML/JSON** file.

   Note: If you are using a commercial MIBIcope the FOVs are automatically uploaded to MIBItracker as long as the slide is associated with a project and has a panel assigned to it. In an event where these uploads fail, use a FOV **JSON** file (available in the local directory) to create a Run, and upload the images. You only need to do this for one FOV in the run, after which you can upload additional TIFFs to that run.

   To learn more about run setup in MIBIcontrol, please refer to the MIBIscope User Guide.
3. In the **Project** field select a project to which the run is to be assigned. Click in the **Project** box to bring up a list of projects available. Alternatively, start typing to reduce the number of options with type-ahead searching.
4. The slides will now be filtered to only those in the selected Project. If you do not see the correct slide, you need to either move that slide to this Project, or select another Project for this run.
5. In the **Instrument** field select the instrument with which the Images were acquired.
6. In the **FOV Size** field enter the size of the field of view acquired.

   Note: This field is not applicable for commercial MIBIscope users.
7. If the **Run XML/JSON** is uploaded the **Label** field will be prepopulated. Change this to use a unique label that is different than the default label provided by the run settings file.
8. **Submit** will create this run.
9. A new page will appear where **Section**, **Tissue SED** image, and **Description** can be added to each FOV in the run.

Edit a Run

Users with Admin and Edit access to a project can edit an existing run.

1. Go to **Project Data** and select **Runs.**
2. From the list of runs select the run to be edited.
3. On the run details page, click **Edit** dropdown, and select **Edit Run.**
    a. The editable fields are **Project, Slides, Instrument, Label, and Description**
    b. Click **Submit.**
4. On the run details page, click **Edit** dropdown, and select **Edit Images.**
    a. The editable fields are **Section, Tissue, Description,** and **SED** Image.
    b. Click **Update.**

Download

Here the files associated with a run can be downloaded.

Run metadata CSV — Details related to Run setup which includes the FOV size, Frame size, Tissue type, etc.

Ion counts CSV — Contains target, mass, and their corresponding ion counts.

Run settings file — XML that has all the run parameters.

Upload Images to an Existing Run

Images processed using MIBI/O can be added to existing **Runs** in MIBItracker. This is useful especially when the images have undergone some processing such as filtering, isobaric correction, or using MIBI/O.

Note: To learn more about MIBI/O go to the About page on MIBItracker to find the links to download the MIBI/O application and the user guide.

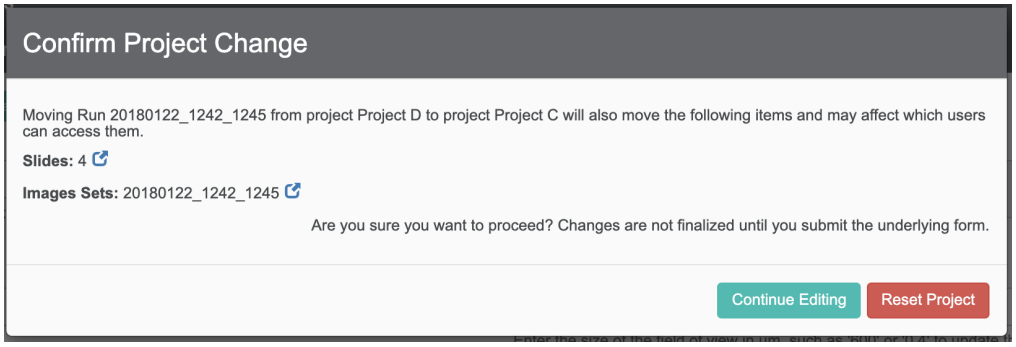
1. Go to **Project Data,** and choose **Runs**.
2. Select the run to which the images need to be added.
3. Select **Upload Images** to choose the tiffs images to be uploaded.
4. Select **Upload Images** to upload the selected images.
5. Images that were mass corrected and/or filtered using MIB/O will be added here with updated FOV-IDs such as Point 1-Filtered.

    Note: Alternatively, images can be uploaded directly from **MIBI/O**. To learn more about this, refer to the MIBI/O User Guide. This feature is available on **MIBI/O v1.10** and above.

Move Runs, Slides, or Image Sets from One Project to Another

To move Runs or Slides from one project to another project.

1. Select the item to be moved, and click **Edit Slide** or **Edit Run**.
2. Click on the **Project** field to choose the destination project from the dropdown.
3. A warning is displayed with the associated runs, slides, and image sets affected by this move. You may continue to edit the item or cancel the move after viewing this warning.
4. Click **Submit** to finalize the move**.**



To move Image sets.

1. On the **Image Sets** page, select the image set to be moved to another project.
2. Click on the **Project field** to choose the destination project from the dropdown.
3. Hit **Enter** on the keyboard**.** A warning is displayed with the associated runs, slides, and image sets affected by this move. You may continue or cancel the move after viewing this warning.
4. Click **Submit Changes** to finalize the move**.**

Image Sets

View Image Sets

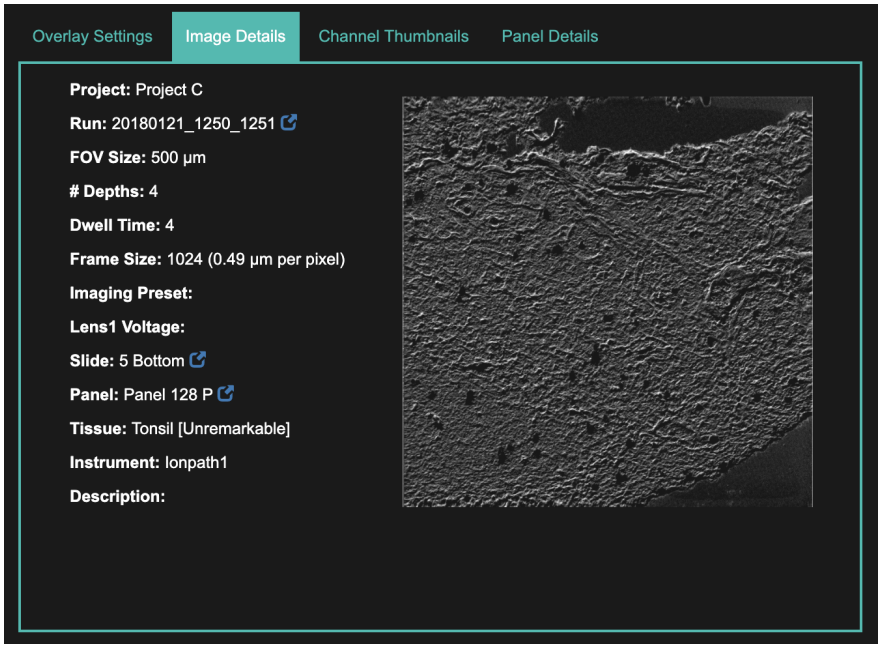
An image set containing all images in a run is automatically created when the run images are uploaded.

1. On the **Project Data** menu, click **Image Sets** to go to the Image Sets page.

2. Click **View** to go to the Overlay Image Display page for the first image in the selected image set.

Field of View Information

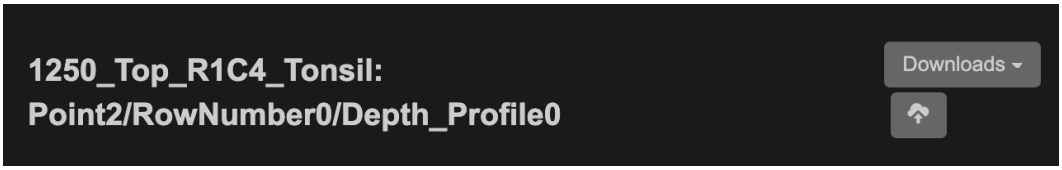
Information for an image can be found in the **Image Details** tab right next to the Overlay Settings. It shows several important properties pertaining to the current FOV.



The **Run**, **Slide**, and **Section** from which the current FOV was acquired, as well as the **Panel** name with which it was stained, are linked to the detailed view of those database items. Click next to the label to open a new tab with the respective detail view.

The **Project** name, **FOV Size** in microns, the **Frame Size** in pixels, and details pertaining to the image acquisition conditions, such as the number of **Depths** (scans) imaged, the ion beam **Dwell Time** per depth in milliseconds, and the **Imaging Preset** used, are also displayed.

The secondary electron detection (SED) image thumbnail is displayed if available. The **SED image** gives the user a general indication of the morphology present in each FOV.



In the upper-right corner of the FOV information pane, click **Downloads** to download a multi-layer TIFF file for the current FOV for image analysis.

A notification banner will be displayed while the images are being prepared for download. Once the compressed folder with the files is ready, the notification banner will contain a **Download** button.

Click to upload image segmentation or results mask images in PNG format. Once uploaded, the masks will be selectable as a channel selection.

Overlay Display and Controls

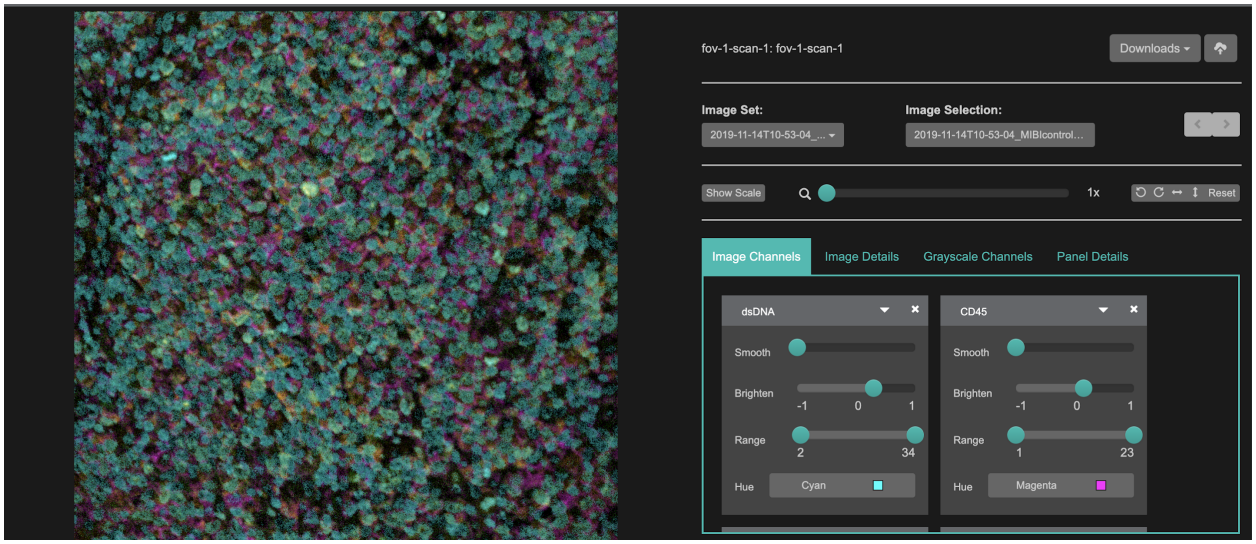
Image sets are used to group FOVs acquired in the same run or related images from separate runs. The image overlay view displays a single FOV at a time. An image set containing all images in a run is automatically created when run images are uploaded. The run image sets are visible to all users who can access that Project.

The **Image Set** field lists additional image sets to which the current FOV belongs. Select a different set to load it.

The image set controls on the right of the panel are used to switch among the FOVs that are part of the same image set while maintaining consistent overlay control settings to enable visually comparing the images in terms of relative intensity.

From the dropdown select another FOV that is part of the current image set to load its overlay view. Alternatively, click › to load the next FOV in the image set, or click ‹ to load the previous FOV in the image set.

Overlay Display

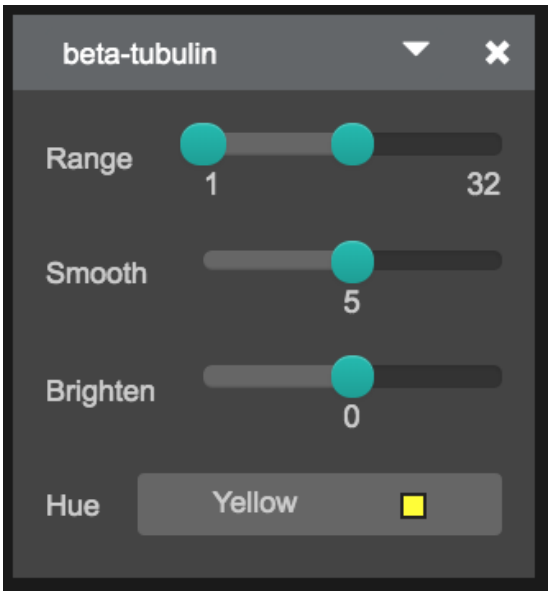


Click **Show Scale** to display a scale bar in the lower left corner of the overlay image. The scale is automatically adjusted based on the zoom level applied to the image.

The overlay image can be zoomed using either the scroll bar below the image or by using the mouse wheel/laptop touchpad pinching gesture. Click **Reset** to show the entire FOV.

Right-click the overlay image to **Save** or **Copy** the displayed image.

Click ↺ to rotate the image counterclockwise, or ↻ to rotate clockwise. Click ↔ to mirror the image on its horizontal axis or ↕ to mirror the image on its vertical axis. Click **Reset** to revert to the original image orientation.



Overlay Settings

Use the channel drop-down menu to select an available channel to be displayed on the overlay view. The channel names are defined from the panel associated with each imported image set. To add more channels to the overlay image, click **Add image channel**. Up to nine channels and masks can be displayed concurrently.

Range

Set a minimum and maximum count value to display, where pixel counts outside of this range are capped at the threshold value. Raising the minimum value of the range sets all pixels with counts below it to zero. Lowering the maximum value of the range fully saturates all pixels with counts above it.

The default values below the slider display range from one to the maximum per pixel value in the FOV displayed. When moving to a different FOV within the same image set, the selected minimum and maximum values will remain fixed. However, the newly displayed FOV may have a higher maximum count value, and thus the available selection range may increase.

Note: The intensity values are independent across channels.

Smooth

Apply a smoothing filter to the channel image. Drag the slider to the right to increase the radius of the filter. This setting can produce more visually appealing illustration images.

Brighten

The **Brighten** slider is set to zero by default. Increase the intensity of the displayed channel by dragging the slider to the right toward a setting value of 1. Decrease the intensity of the displayed channel by dragging the slider to the left toward a setting value of -1. The setting values displayed below the slider indicate the available setting range and not the currently selected value. A value of -1 subtracts the maximum value possible from all pixels; a value of 1 fully saturates all nonzero pixels.

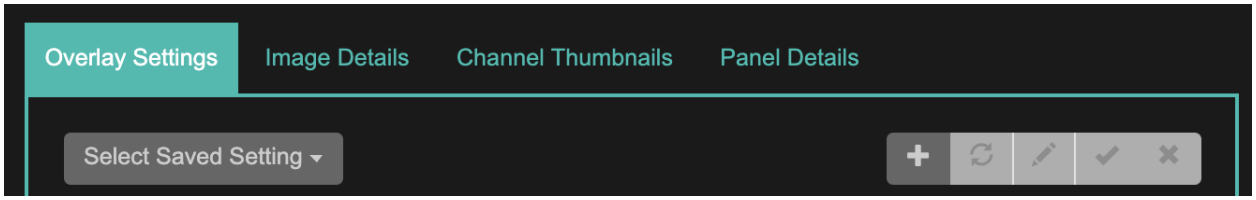
Hue

Select a color to be assigned to the selected channel in the overlay.

Note: The raw data is a grayscale map of pixel intensities.

Visual Settings

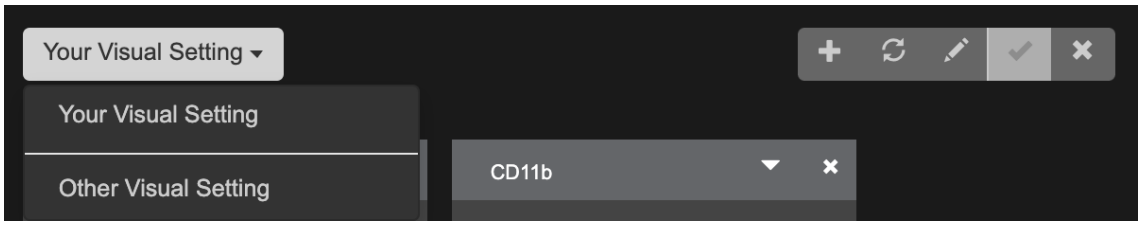
The selection of Image Channels and their settings can be changed and saved for an image set. When the FOVs are viewed these saved Visual Settings will be available across the Image Set.



Visual Settings can be created, modified, and deleted based on the user's access level to a

project. Resource users with access to the project can create, modify, and delete their saved visual settings. The settings they create are available to everyone with access to the project. Limited users with access to a project and resource users viewing a public project may also create additional settings for their individual use, but these are not visible to other users.

Select  to enter the name for a setting and click **Submit**. This visual setting will now be available in the **Select Saved Setting** dropdown. Select  to reload the selected setting. Select  to edit the current setting. Select  to update the changes to the current setting. Select  to delete the current setting.



Visual settings created by a logged in user appears above the line, and settings created by other project members appear below the line.

Download Image Sets

1. On the **Project Data** menu, click **Image Sets** to go to the Image Sets page.
2. Click **Download** to create a compressed .zip file containing the multi-layer TIFF files for all images in the selected image set.

   A notification banner will be displayed while the images are being prepared for download. This may take up to several minutes for large image sets. Once the compressed folder with the TIFF files is ready, the notification banner will contain a **Download** button. You can navigate to other pages while the images are being prepared for download.
3. Click **Download,** and select the destination folder for the compressed .zip file.

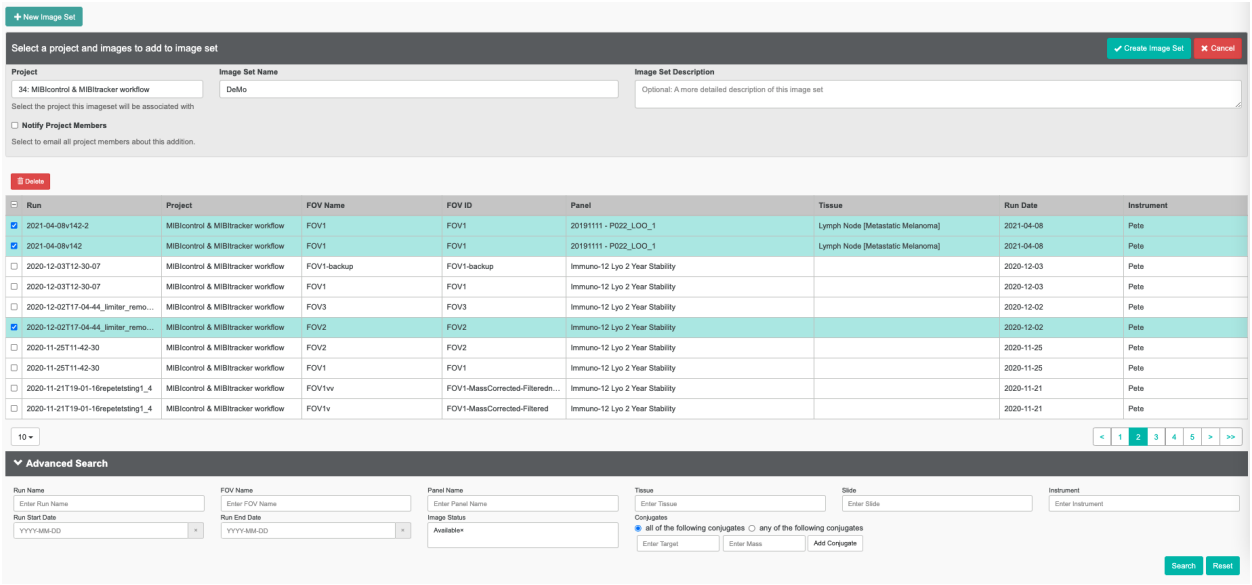
Create Image Sets

Image Sets are visible to users who have permission to view a Project.

On the **Project Data** menu, click **Images** to go to the Images page.

Alternatively, on the Image Sets page, click **Create Image Set** to go to the Images page.

1. Click **+New Image Set** to go to the image selection page.



2. Choose a project from **Project dropdown** to list its images.
3. In the **Image Set Name** box, type in a name for the new image set.
4. Optionally, in the **Image Set Description** box, type in a description or other pertinent details.
   This field can be useful when sharing custom image sets with another user to indicate what is being shared and why.
5. Enable **Notify Project Members** to notify the project members once this image set is created.
6. Select the checkbox in the first column of the image list for each image to be added to the image set, and then click **Create Image Set**.

Share Images

Granting Access via the Project

If you want to share all images in the Project with another user, any Project admin can simply add them as a View user to the Project. As outlined in Table 2, this will either allow them to see all Project data, unless they are a Limited Resource user in which case only the images and their details are accessible.

Granting Access via Creating Image Sets in Another Project

If you want to share only a subset of the images from a Project, or do not want to grant full access to slides and runs in the Project, then there is an option to accomplish this by creating an image set containing these images in another Project. This requires that the original Project in which the images are saved has Image Sharing enabled. Only Project admins can change this setting.

For Projects with Image Sharing Enabled

1. Create a new Project through which you want to share the images, and grant the relevant users View access.
2. Go to **Image Set,** under the **Data menu.**
3. Click **Create Image Set**. Select the Project you've designated, and fill in the name and description.
4. Select the images from the original Project that you wish to include.
5. Once the images are chosen click **Create Imageset**.
6. This image set will now appear under your new Project.

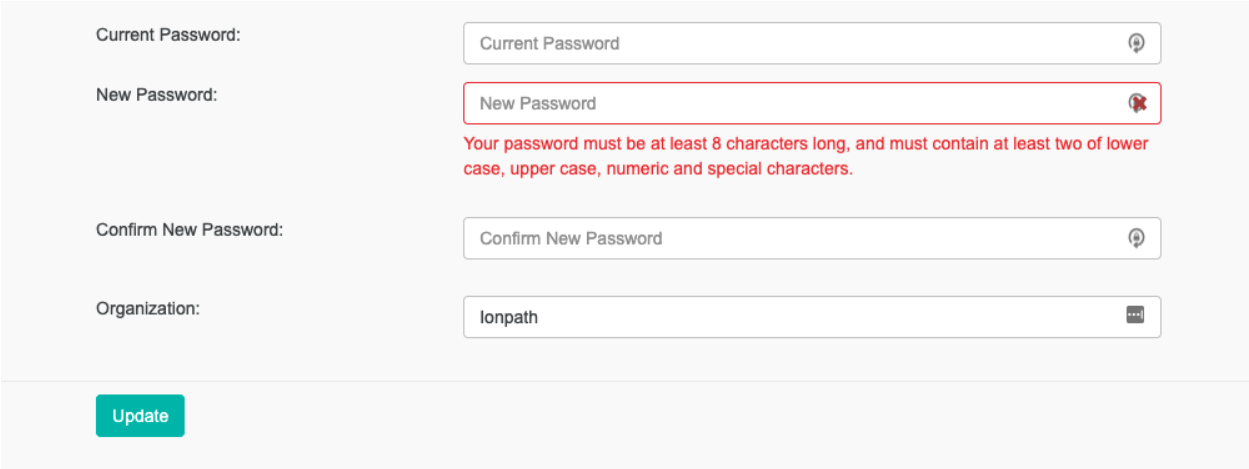
If an admin of the original Project disables sharing, the images from that Project will be removed from image sets in other Projects.

User Name

The user name menu in the upper right corner of MIBItracker contains a link to the **Profile** and **About** pages. The **Logout** menu item can be used to sign out of MIBItracker.

Profile Page

Use the profile page to change your password.



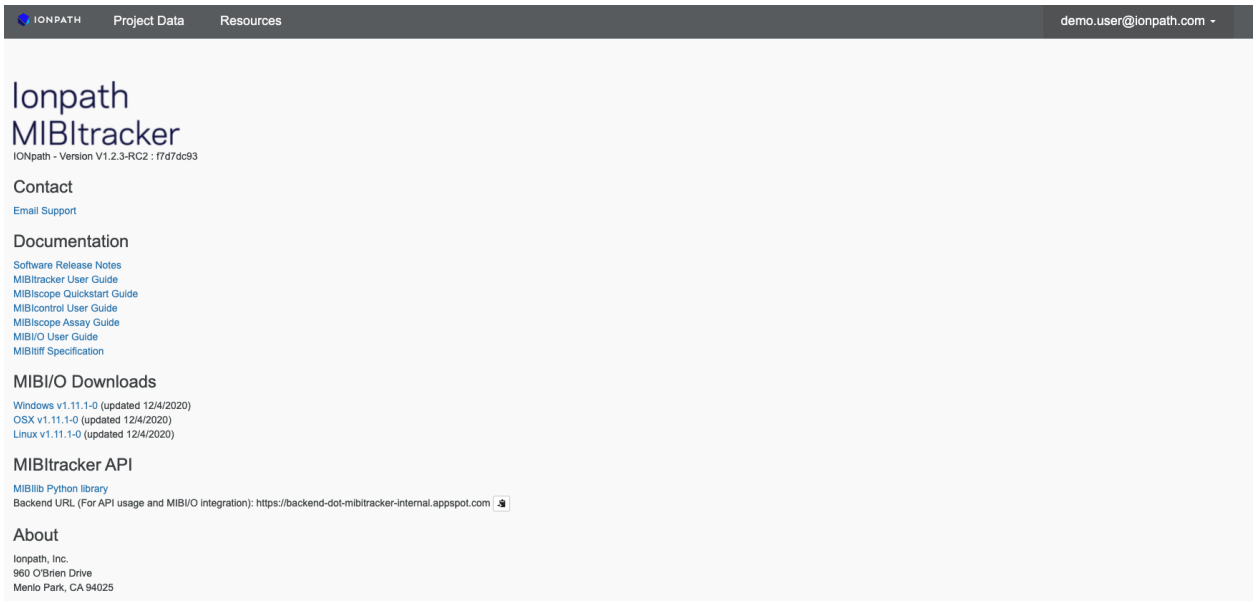
1. In the **Current Password** field, type in your current password.
2. In the **New Password** field, type in the new password that you want to set.
   Note: Passwords must be at least 8 characters long with 2 complex cases.
3. In the **Confirm New Password** field, re-type the password that you want to change to, and then click **Update**.
   The password entered into the **Confirm New Password** field must match the one entered into the **New Password** field.

4. Optionally, in the **Organization** field, type in the name of your organization.

About Page

The **About** page lists the current MIBItracker version and a link to download the current MIBI/O software. It also displays the link that needs to be copied and pasted to the *MIBItrackerURL* field under MIBI/O settings. This is essential to enable the upload of images from MIBI/O to MIBItracker.

It also lists the support information and links to download a copy of the MIBItracker and MIBI/O user manual.

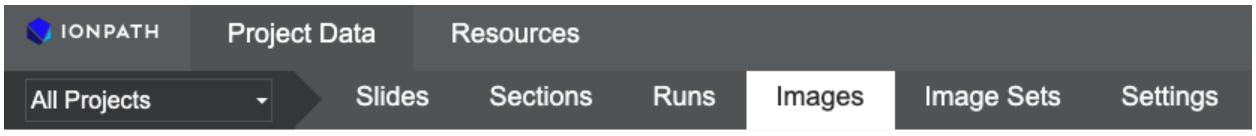


MIBItracker Pages

The MIBItracker navigation bar contains links to all database lists. Each of the pages is described in detail in this section. On most lists, click on a row to access a detailed view of the listed item.

Note: Some functions and buttons may be unavailable depending on your user account privilege level.

Project Data



The Project Data menu contains links to the Project Settings, Image Sets, Runs, Slides, Images, and Sections.

Projects Page

The **Projects** page lists all projects to which slides, sections, and panels can be assigned. There is a search bar on the top where the projects can be searched based on Name, ID, Created By, and Last Modified.

ID

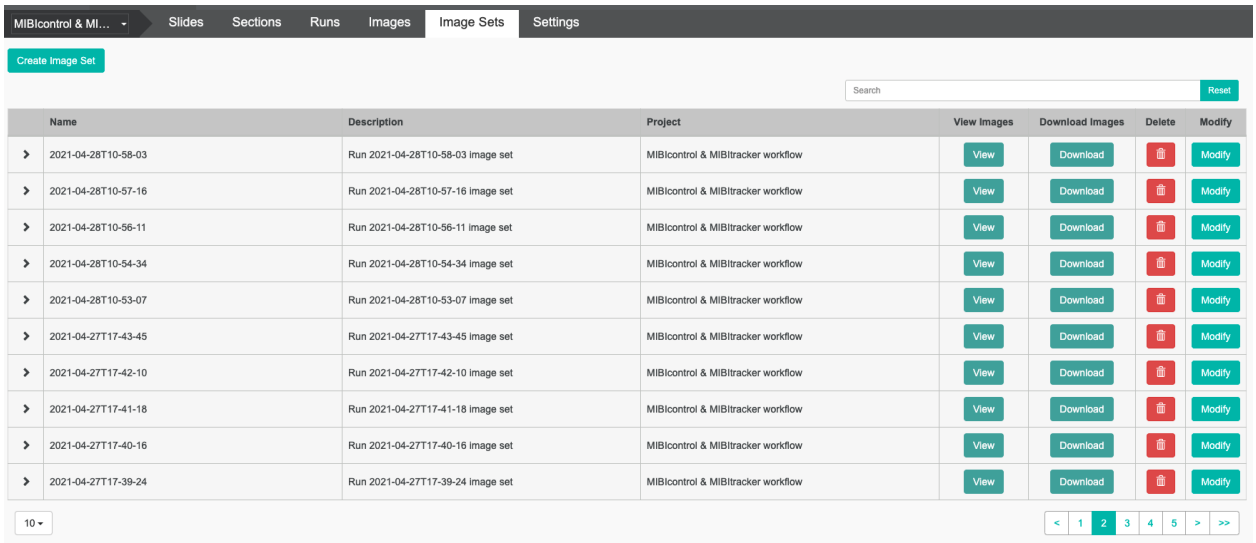
The **ID** field is automatically generated by the database when a new project is created. It can be used for anonymized labeling of the slides belonging to a given project.

Name

The Name field is the label given by the user to a project.

Image Sets Page

The **Image Sets** page lists all available image sets. An image set is created automatically for all FOVs in a run when it is uploaded to MIBItracker.



The image sets list contains the following columns, and can be sorted on any of them by clicking on the column header. Click **View** to open the image set in **Overlay** view. Click **Download** to download a zip file containing a multi-layer TIFF for each image in the image set. It may take some time for the TIFF download to prepare, but you may continue to browse to other MIBItracker pages during the preparation without interrupting the process. A notification banner will let you know when the images are ready to download.

Name

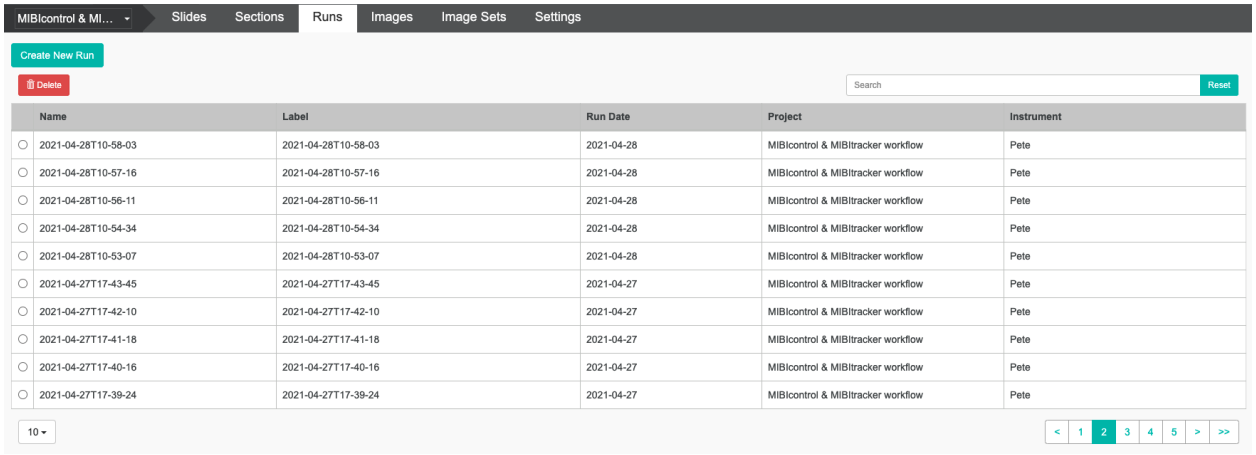
The **Name** field is either automatically generated by the database using the run name from which the images in the set have been imported, or it may be manually assigned by the user when creating custom image sets.

Description

The **Description** field is either automatically generated by the database using the run name from which the images in the set have been imported, or it may be manually assigned by the user when creating custom image sets.

Runs Page

The **Runs** page lists the acquisition sessions uploaded to MIBItracker and allows for creating new acquisition runs.



The runs list contains the following columns and can be sorted by any of them by clicking on the column header. Click on a row to display, edit, or download information specific to a single run. Please refer to the Upload and Manage Runs section for more information on how to edit a run.

ID

The **ID** column displays the acquisition run name in which the image has been acquired. A run is a single MIBIscope acquisition session comprising one or two slides. The run name is generated automatically from the *.xml* file name.

Name

The **Name** is the custom label assigned by the user. It may be different from the run .xml file name.

Run Date

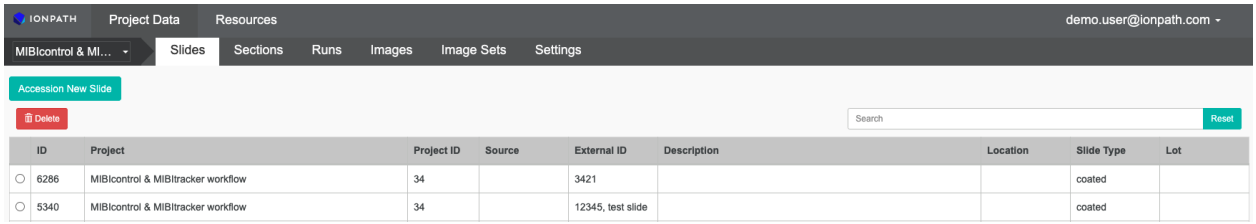
The **Run Date** is the date on which an image has been acquired. The images are sorted on the run date by default, with the most recently acquired images listed first. The date is automatically extracted from the run .xml file.

Instrument

The **Instrument** column specifies which instrument was used to acquire the run.

Slides Page

The **Slides** page lists all slides that have been accessioned into the database.



The slides list contains the following columns and can be sorted by any of them by clicking on the column header. Click on a row to display, edit, or download information specific to a single section.

ID

The slide **ID** field is automatically generated by the database when the slide is first created. The slide identification number is useful for anonymized labeling of slides.

Project

The **Project** field is linked to the Projects list and is used to assign the section to a specific project. Each section is linked to a single project only.

Project ID

The **Project ID** field is automatically generated by the database when a new project is created. The project identification number is useful for anonymized labeling of slides.

Source

The **Source** field is used to track external partners from whom the slide has been obtained. This field is only used for externally sourced slides.

External ID

The **External ID** field is used to track the label assigned to the slide by an external provider. This field is optional.

Location

The **Location** field is used to record the storage location of a slide after it has been stained. We recommend storing slides under vacuum.

Slide Type

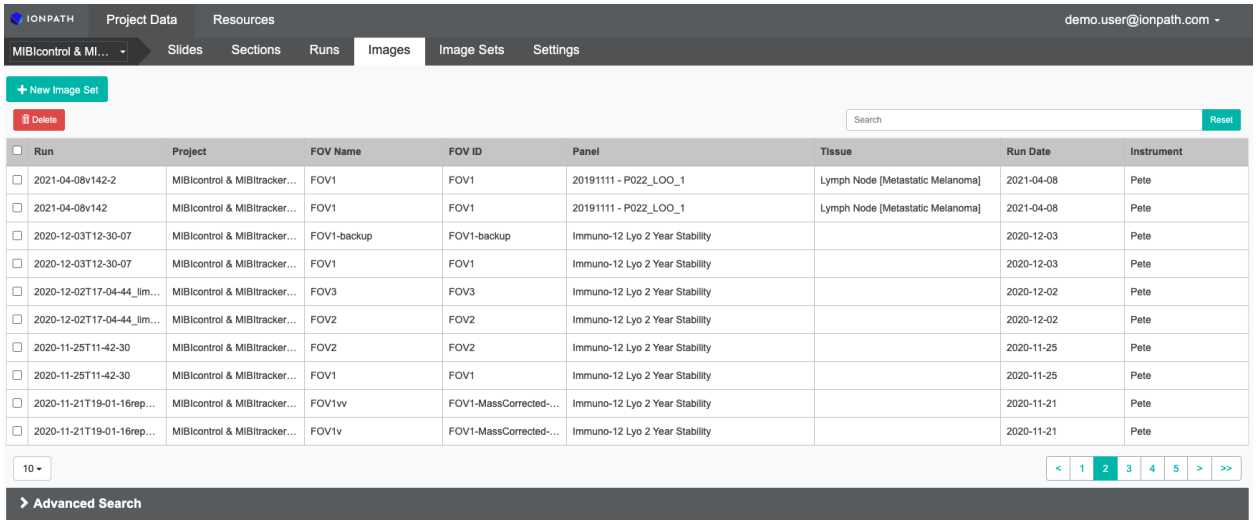
The **Slide Type** field is used to indicate whether the slide is coated with a conductive material for use in the MIBIscope system or uncoated.

Lot

The **Lot** field is used to track the lot number of coated slides. It is not available and will always be blank for uncoated slides.

Images Page

The **Images** page lists all acquired FOVs. Each row of the data table represents a single FOV. Click on any row to go to the overlay display mode for a particular image.



The **Images** list contains the following columns and can be sorted on any of them by clicking on the column header. The **Advanced Search** pane allows searching on one or more of the fields. Click on **Advanced Search** to reveal all searchable fields.

Run

The **Run** column displays the acquisition run in which the image has been acquired. A run is a single MIBIscope acquisition session comprising one or two slides. The run name is generated automatically from the .xml file name.

Point Name

The **Point Name** is a custom label assigned by the user to each image during data acquisition.

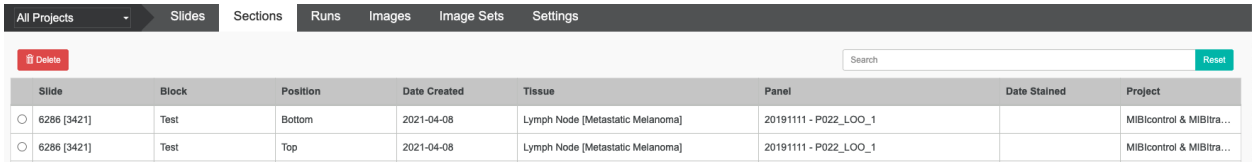
Point ID
The **Point ID** is the label automatically assigned to the image by the MIBIscope software.

Panel
A **Panel** is the group of conjugated antibodies (**Conjugates**) with which a slide and/or section has been stained. The **Conjugates** define the specific channels available for each image.

Tissue
A tissue type defined on the **Tissues** page can be assigned to each section. If an FOV is part of a tissue microarray (TMA) with multiple tissue types, this field may be blank.

Run Date
The **Run Date** is the date on which an image has been acquired. The images are sorted on the run date by default, with the most recently acquired images listed first. The date is automatically extracted from the run .xml file.

Sections Page
The **Sections** page lists all sectioned tissue samples in the database.



The sections list contains the following columns and can be sorted on any of them by clicking on the column header. Click on a row to display, edit, or download information specific to a single section.

Slide
The **Slide** field is linked to the Slides list and indicates the slide on which the section is placed. A section can be linked to one slide only, but one slide can hold multiple sections.

Block
The tissue block from which the section has been cut.

Position
The Position field indicates the location of a section on a slide holding multiple sections. We recommend settling on a standard nomenclature such as top, middle, and bottom, with a standard reference point such as the label.

Date Created
The Date Created field typically refers to the date on which the section has been cut from block, but could also refer to the date a slide was received from an external source.

Tissue

The Tissue field is linked to the Tissues list and is used to describe the section's tissue type. If the section is a tissue microarray composed of multiple tissue types, this field can be left blank.

Panel

The Panel field is linked to the Panels list. It indicates which panel of conjugates was used to stain the section.

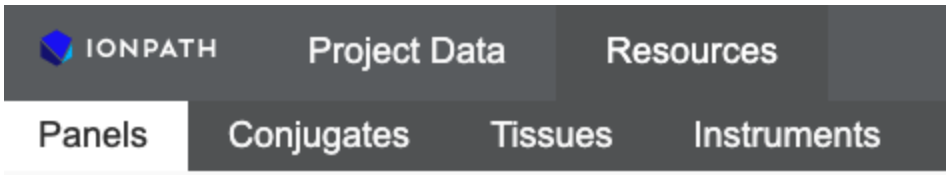
Date Stained

The Date Stained field indicates the date on which the section has been stained. The field can be left blank if a section has not yet been stained.

Project

The Project field is linked to the Projects list and is used to assign the section to a specific project. Each section is linked to a single project only.

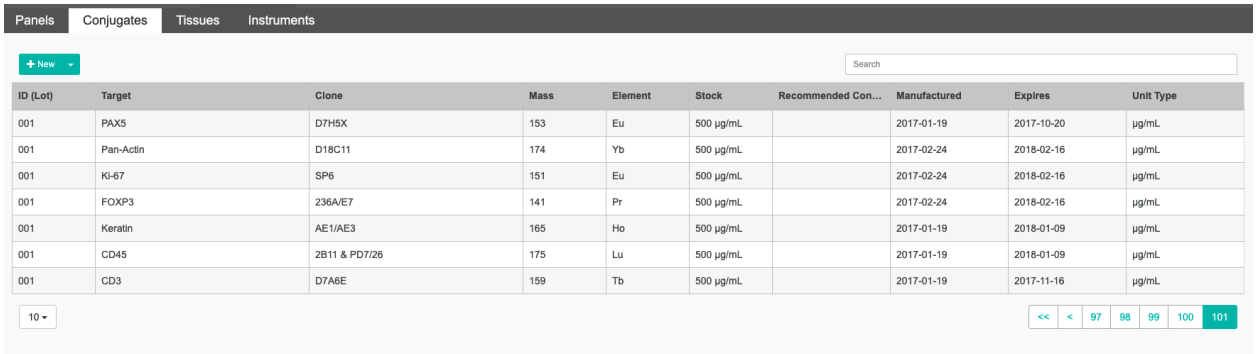
Resources



The Resources menu contains links to the Conjugates, Panels, Tissues, and Instruments pages.

Conjugates Page

The Conjugates page lists all conjugates available for creating staining panels.



The conjugates list is either a simple table listing manual entries or a read-only table, populated from an external database, depending on the application's configuration. MIBItracker can be linked to many popular lab databases such as Odoo or Knack.

The conjugates list contains the following columns and can be sorted on any of them by clicking on the column header. Click on a row to display, edit, or download information specific to a single section.

ID (Lot)
The ID (Lot) field is used to track the lot number of the conjugates, either assigned by the vendor or an in-house tracking of successive conjugations.

Target
The Target field is used to track the biomolecule to which the conjugated antibody binds.

Clone
The Clone field is used to track the clone of monoclonal antibodies.

Mass
The Mass field is used to track the atomic mass of the metal isotope conjugated to the antibody.

Element
The Element field is used to track the metal element conjugated to the antibody.

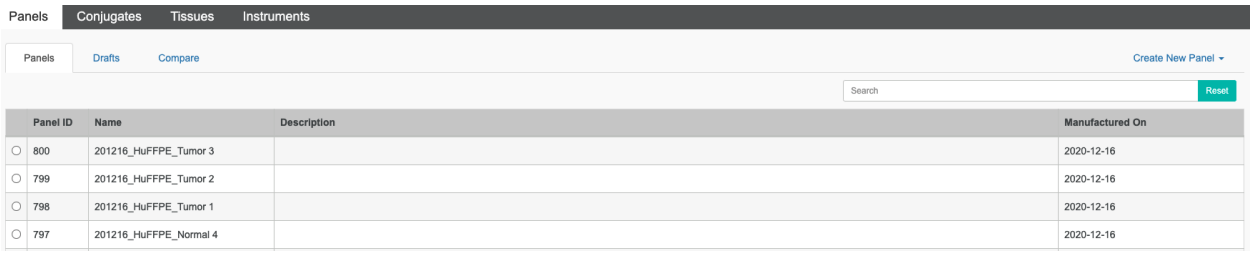
Stock µg/mL
The Stock µg/mL field is used to track the concentration in micrograms per milliliter of the conjugate stock solution.

Rec. µg/mL
The Rec. µg/mL field is used to track the manufacturer or user recommended concentration in micrograms per milliliter of the conjugate in the staining solution.

Manufactured
The Manufactured field is used to track the manufacture/conjugation date of the conjugate.

Expires
The Expires field is used to track the expiration date of a conjugate reagent.

Panels Page
The Panels page lists all conjugates panels used to stain slides. The conjugates listed in a panel define which channels are available to overlay in a FOV.



The panels list contains the columns listed below and can be sorted by any of them by clicking on the column header. Click on a row to display, edit, or assign the panel to sections. You can also download the panel in CSV format for use in the MIBI-TIFF Generator or other databases and applications.

Panel
The Panel field is an identification number automatically generated by the database when the panel is created.

Name
The panel Name field is a custom label for the panel entered by the user when the panel is created.

Description
The optional Description field is used to hold additional information about the panel.

Manufactured On
The Manufactured On column is a required field used to record the date on which the panel was created.

Project
The Project field is linked to the Projects list and is used to assign the panel to a specific project. Each panel is linked to a single project only.

Tissues Page
The Tissues page lists all tissue types that can be assigned to sections. The tissues list contains the following columns and can be sorted on any of them by clicking on the column header. Click on a row to display, edit, or download information specific to a single section.

Organ
The Organ field is used to track the organ from which a tissue sample is extracted.

Subsite
The Subsite field is used to more specifically describe the location from which a tissue sample has been extracted.

Diagnosis/Classification
The Diagnosis/Classification field is used to describe the pathology of the tissue sample.

Instruments Page
The Instruments page lists MIBIscope instruments which can be assigned to each slide, section, or image to denote the specific instrument or instrument version used for acquisition of a given data set.

The instruments list contains a single column and can be sorted by clicking on the column header.

Name

The Name field is the label given to a specific instrument by the user.