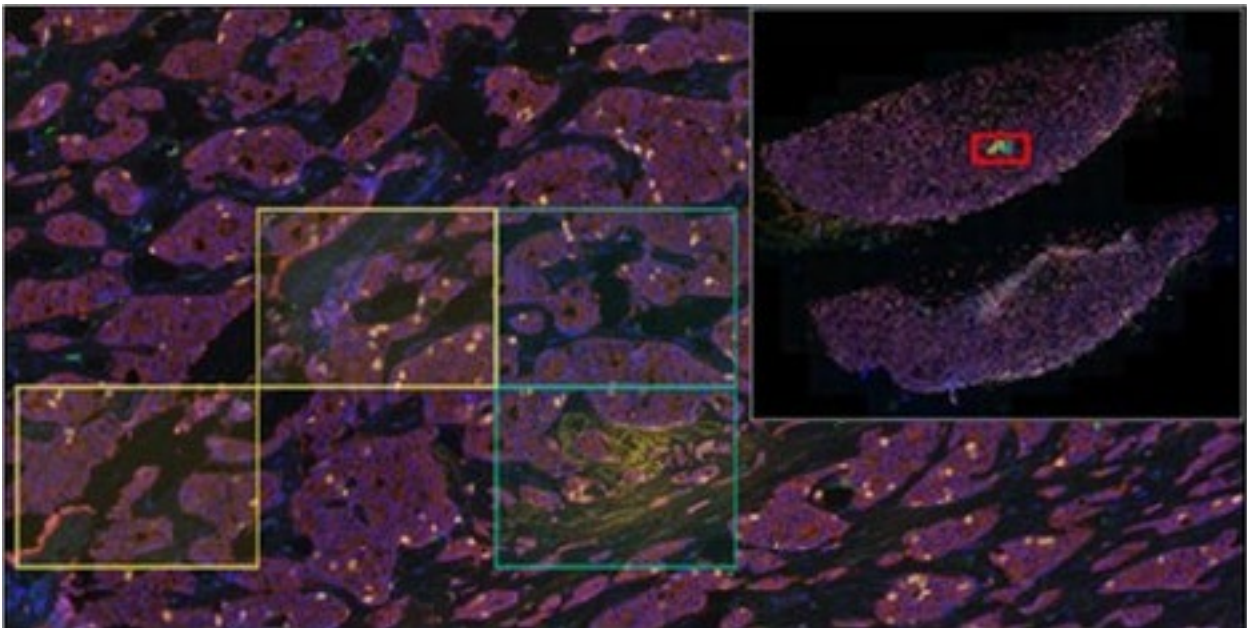


PhenoChart User Guide

WHOLE SLIDE CONTEXTUAL VIEWER FOR ANNOTATION & REVIEW

2.2.0



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Chapter 1: Overview

Phenochart™ is a slide viewer software for scans created by the PhenolMager® HT and PhenolMager® Fusion Quantitative Pathology Imaging Systems that enables users to:

- View and navigate around whole slide images.
- Select areas of interest for further review, multispectral field imaging, or analysis using inForm® Tissue Finder™.
- Identify cores for imaging or analysis.

Phenochart is freely available and can be installed on multiple computers.

Phenochart is for Research Use Only. Phenochart is not for use in diagnostic procedures.

Chapter 2: Loading Slides

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2.1 SELECT A SLIDE

The Slide Browser allows for selection and loading of slides. A list of available studies and the slides within them will be displayed.

1. Click the Load Slide button in the upper left to bring up the Slide Browser. (See Figure 2.1.)

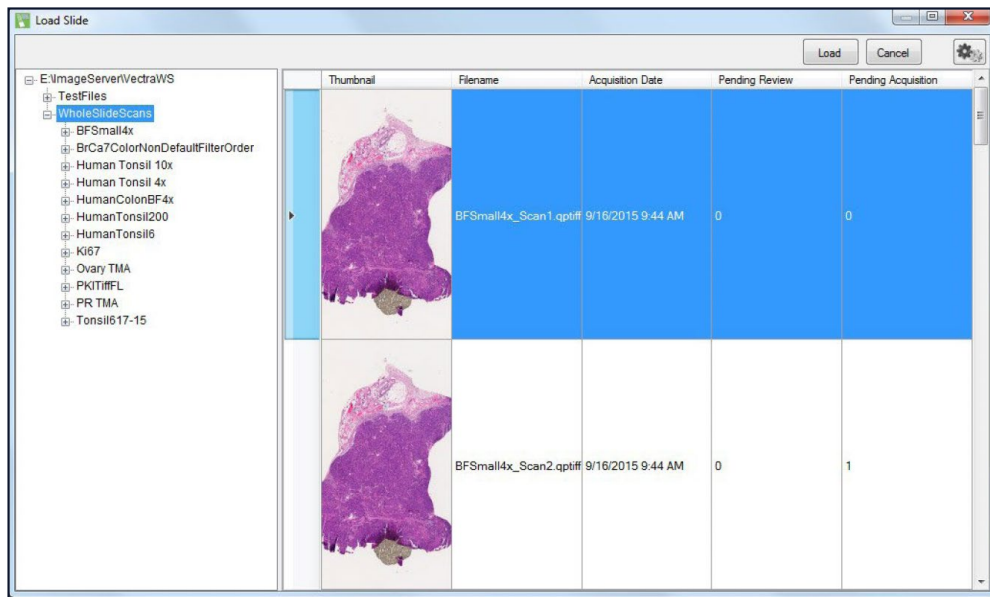


Figure 2.1. Slide Browser.

The list of studies is on the left. Expand/collapse the file hierarchy to view slides within a study.

2. Select a study on the left; the right side shows the scans in that study. Select a slide within a study, and the right side will show scans of that slide only.

Click a column header to sort by it. This may help locate a slide with a specific name, a slide acquired on a certain date, etc.

Double-click any scan on the right-hand side to load that scan or highlight the scan and click Load. Click Cancel to close the Slide Browser without loading anything.

By default, the browser will show the slide thumbnail, a summary column, and columns showing the number of annotations pending acquisition or review.

NOTE *The thumbnail can be used for qualitative sample identification but should not be used to assess relative brightness of different images.*

Other columns can be added. Select Set Column Visibility from the browser's Gear menu to select which columns are shown in the table. (See Figure 2.2.)

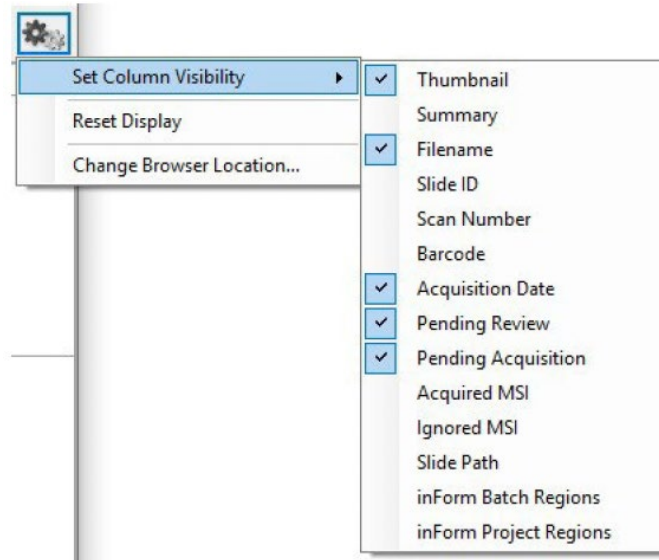


Figure 2.2. Gear menu.

Resize the columns by dragging the column dividers (e.g., to make the thumbnail bigger). Select Reset Display from the browser's Gear menu at any time to restore the original column sizes.

2.2 INITIALIZE THE BROWSER

The first time the Slide Browser is launched, navigate to the Phenolmager® HT or Fusion data. Locate the data by selecting an example scan within it. PhenoChart obtains the example by prompting to open a QTIFF file (.qptiff). (See Figure 2.3.)

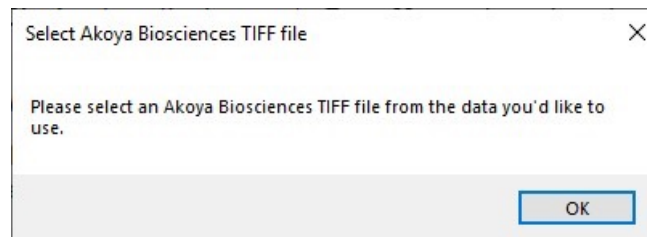


Figure 2.3. Select QTIFF file window.

The next time Load Slide is selected, Slide Browser will be useable.

NOTE *This prompt is displayed the first time the Slide Browser is loaded or if the browser is reset in the menu.*

To change which data the browser points to, use the browser's Gear menu. (See Figure 2.4.)

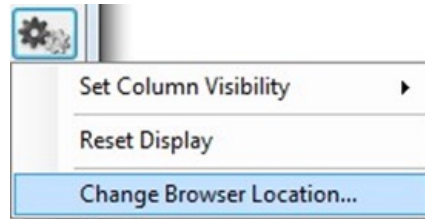


Figure 2.4. Gear menu > Change Browser Location...

Phenochart will again ask for an example QTIFF file (.qptiff). This provides access to data that may have been archived or reside in a different location.

2.3 PHENOIMAGER HT AND FUSION DATA

These systems store scan data by study. (See Figure 2.5.)

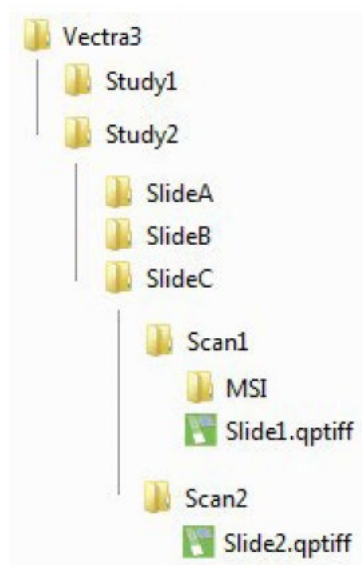


Figure 2.5. Phenochart HT and Fusion studies.

A study is a group of slides that belong together; this could be an actual study (like Ki67 markers in breast cancer), all slides from one customer, etc.

Each study will contain one or more slides. Each slide can be scanned more than once if needed. Any acquired Multispectral fields from a slide scan will be stored within the scan folder.

Chapter 3: Viewing Slides

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3.1 NAVIGATION

The Navigation functions allow for movement around the slide and to view the image in more detail. This may be useful to review the image for focus quality or exposure or to confirm the correct area of the slide was captured by the scan.

The Navigation Tool is the arrow button located in the toolbar at the top of the screen. (See Figure 3.1.)



Figure 3.1. Navigator tool.

When first launching PhenoChart, this button is selected as the default mouse setting. It allows click-and-drag to move the image on the display or to use the mouse wheel to zoom in and out on the image.

The Navigator Window is the small image of the slide located at the top-right of the screen. (See Figure 3.2. and Figure 3.3.)

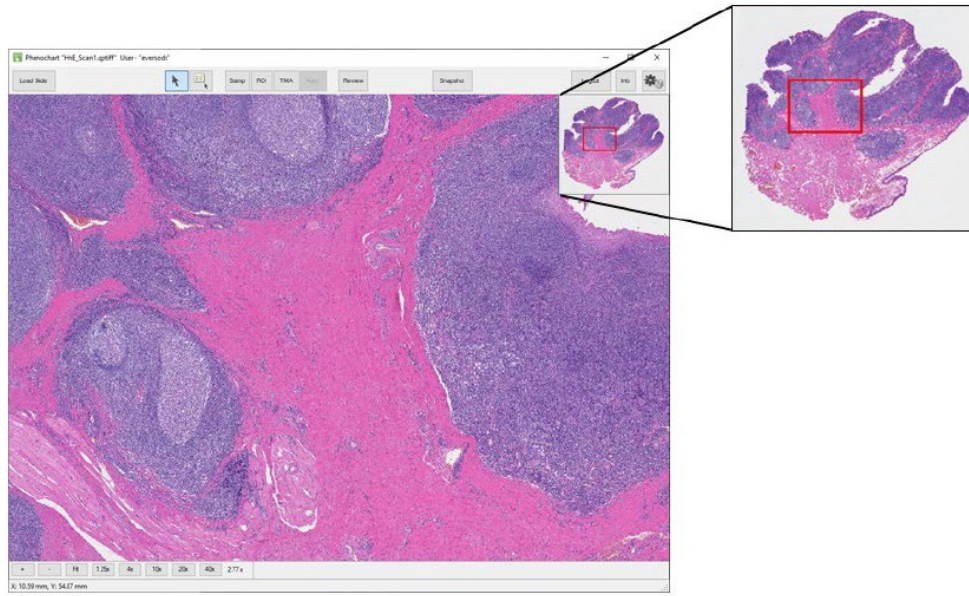


Figure 3.2. Navigator window.

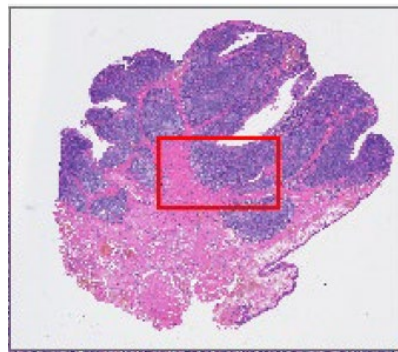


Figure 3.3. Navigator window inset.

The Navigator Window contains a small image of the entire slide and a red box within that image. The red box shows the portion of the slide that is being displayed on the main image on the screen. Click-and-drag the red box in the Navigator Window to change which area of the slide to display.

NOTE *The main image will not update until the mouse is released once the box is moved to the desired location.*

Alternatively, click on any area of the image in the Navigator Window and the red box—and the main image on the screen—will move to that section of the slide.

The Navigator Window is displayed on the screen by default. However, this window may be hidden at any time by deselecting Show Navigator in the Gear menu at the top right of the screen. (See Figure 3.4.)

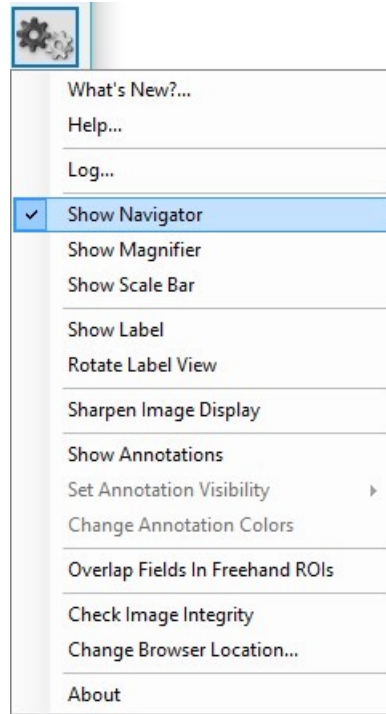


Figure 3.4. Gear menu > Show Navigator.

To redisplay the Navigator Window, select Show Navigator again in the Gear menu. This Navigator preference (show vs. hide) is saved across sessions.

Zoom in and out on the slide using one of three methods. When zooming in and out, the red box in the Navigator Window will change in size to continually identify which portion of the slide is being displayed.

There are three ways to zoom in and out on the slide:

- Select from the pre-defined resolutions (1.25x, 4x, 10x, 20x, and 40x), listed on the Navigation Toolbar at the lower left of the screen. (See Figure 3.5.)



Figure 3.5. Pre-defined resolutions.

- Use the +/- buttons on the Navigation Toolbar.
- Use the mouse wheel to zoom in and out on the slide. When using the Navigation Toolbar to zoom, the zoom is centered on the field of view. When using the mouse wheel, the zoom is centered on the mouse cursor. At all times the text to the right of the 40x button will update to show the current resolution.

At any time, select Fit (also located on the Navigation Toolbar) to fit the entire scanned image to the application window.

NOTE No matter what resolution was used to scan a slide, when the slide is loaded, it will display to “fit” the screen size.

3.2 MAGNIFIER

The Magnifier is the window in the upper left corner of the screen. (See Figure 3.6 and Figure 3.7.)

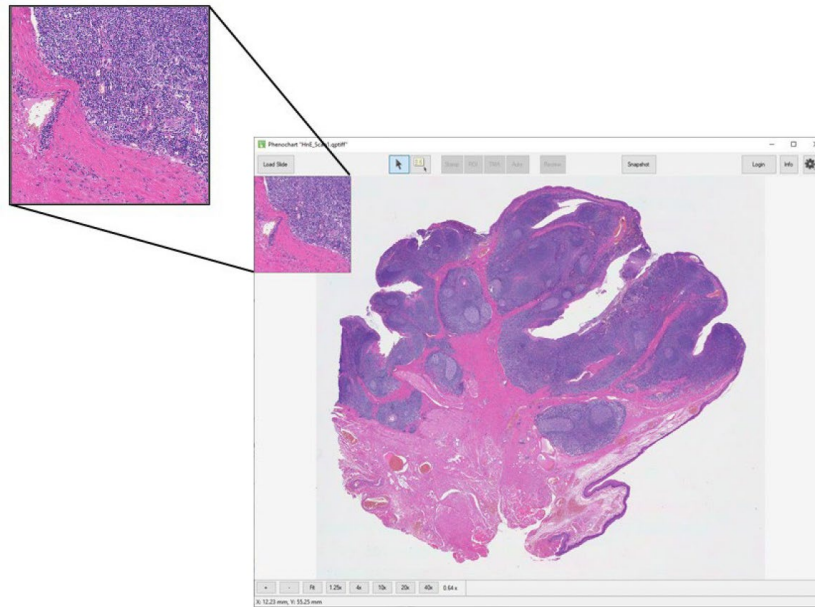


Figure 3.6. Magnifier window.

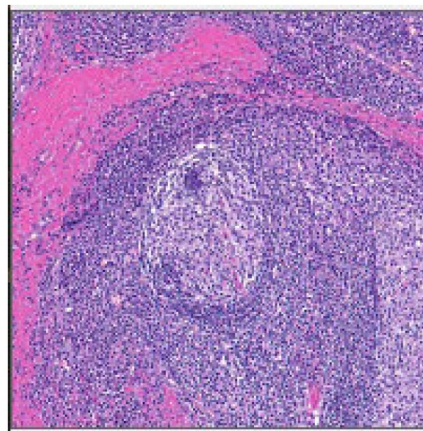


Figure 3.7. Magnifier window inset.

The Magnifier shows a magnified (zoomed in) view of the slide centered on the cursor. This allows for quick inspection of individual regions in more detail without changing the overall zoom.

The Magnifier window is displayed on the screen by default. This window can be hidden by deselecting Show Magnifier in the Gear menu at the top right of the screen. (See Figure 3.8.)

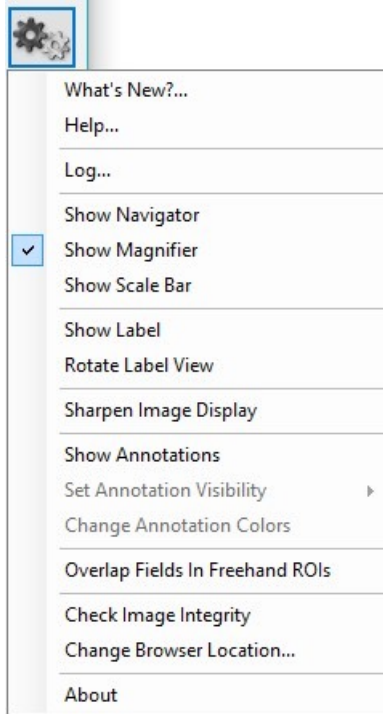


Figure 3.8. Gear menu > Show Magnifier.

3.3 SCALE BAR

The Scale Bar is located at the lower left corner of the screen. (See Figure 3.9. and Figure 3.10.)

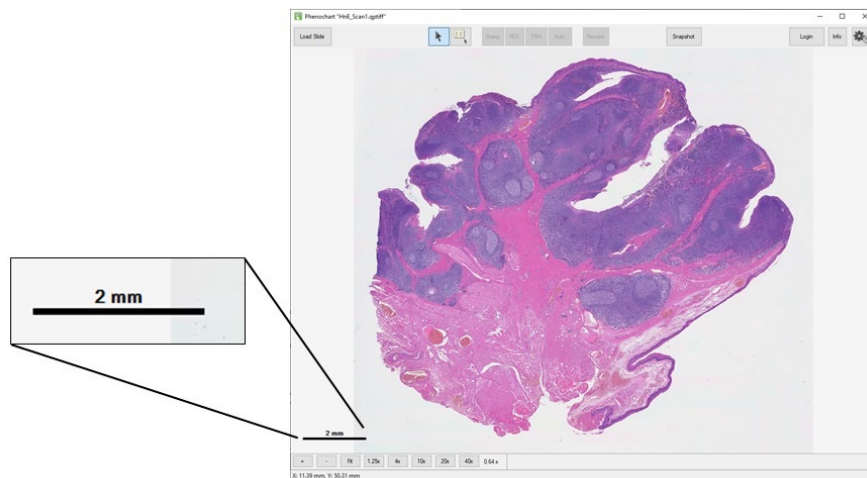


Figure 3.9. Image with Scale Bar.



Figure 3.10. Scale Bar inset.

The Scale Bar is a reference scale showing the actual size of tissue features in the image. The length of the bar will change with zooming in and out on the slide and the units will change from microns to millimeters depending on the magnification.

The Scale Bar is displayed on the screen by default. It can be hidden by deselecting Show Scale Bar in the Gear menu at the top right of the screen. (See Figure 3.11.)

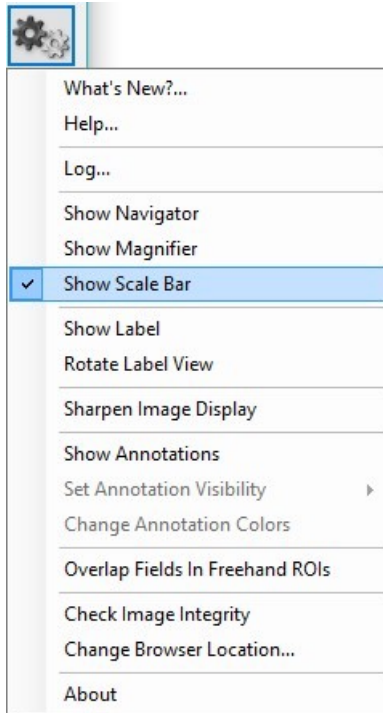


Figure 3.11. Gear menu > Show Scale Bar.

3.4 LABEL

The Label view is located at the lower right corner of the screen. (See Figure 3.12. and Figure 3.13.)

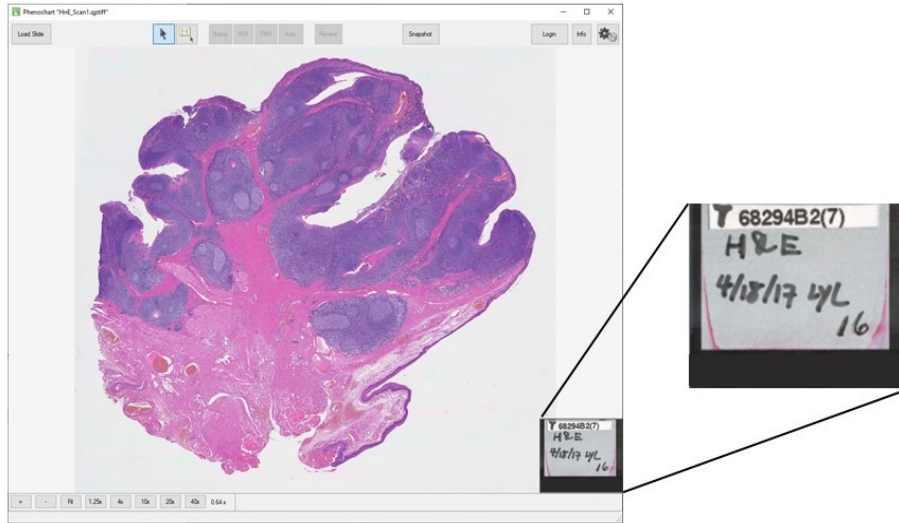


Figure 3.12. Label view.

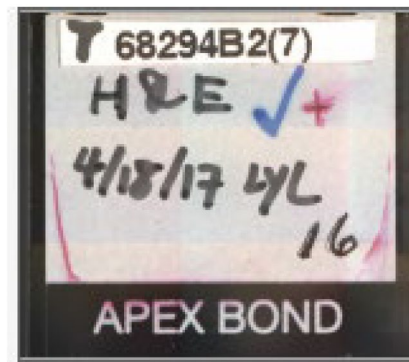


Figure 3.13. Label view inset.

For Phenolmager HT and Fusion imagery, the Label view displays an image of the slide's label. If necessary, rotate the image by clicking on it or by selecting Rotate Label View in the Gear menu. (See Figure 3.14.)

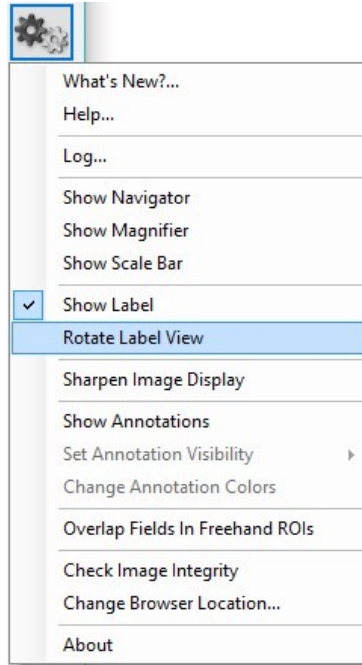


Figure 3.14. Gear menu > Rotate Label View.

The Label view is displayed on the screen by default. It can be hidden at any time by deselecting the Show Label in the Gear menu at the top right of the screen. (See Figure 3.15.)

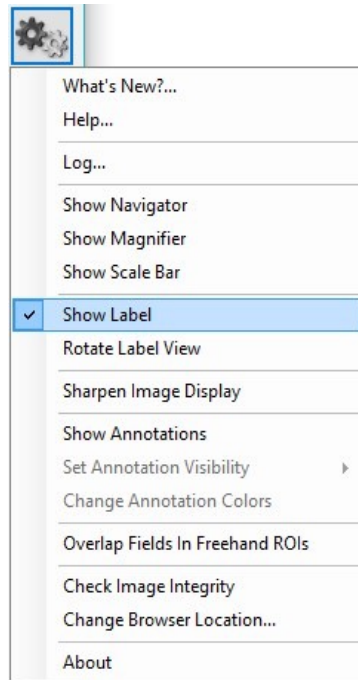


Figure 3.15. Gear menu > Show Label.

3.5 STATUS BAR

The Status Bar is located at the bottom of the screen. (See Figure 3.16 and Figure 3.17.)

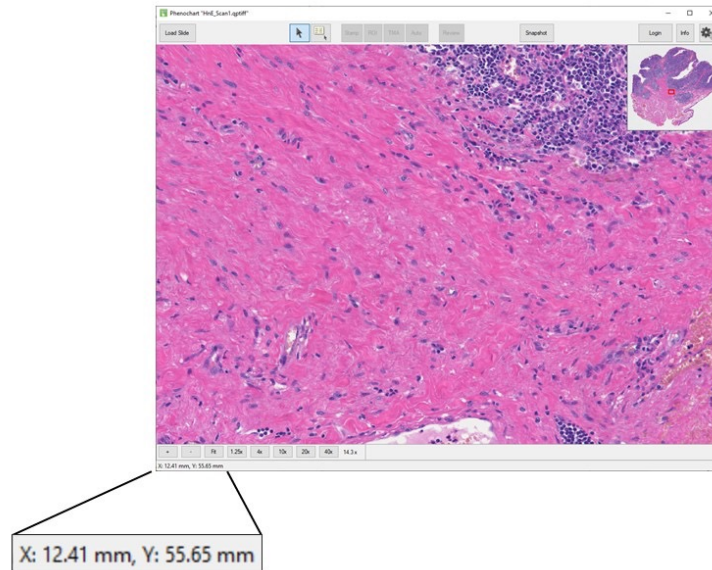


Figure 3.16. Image with Status Bar.



Figure 3.17. Status Bar inset.

The Status Bar shows the current position of the cursor on the slide scan.

The coordinates are shown in millimeters and are updated in real time as the cursor moves on the screen. The origin is the upper left-hand corner of the slide as it sits on the stage. This physical location will differ depending on the system hardware.

3.6 INTENSITY CURSOR

The Intensity Cursor enables viewing of the coordinate and intensity values in a panel at the location of the mouse cursor. (See Figure 3.18.)



Figure 3.18. Intensity Cursor.

Intensity Values Displays

- **Brightfield imagery:** the cursor shows standard RGB values ranging from 0-255.
- **Fluorescence PhenoImager HT or Fusion imagery (without unmixing):** the cursor

shows one value for each filter band. The value is in the range from 0-255 for standard 8-bit images. For Phenolmager HT images in Extended Range format, the reported values are equivalent to what a standard image would show but can exceed 255 to take advantage of the extended range. When this occurs, an "above target" message will be displayed at the bottom of the cursor and a "+" will be displayed next to the channel with value above 255. If the pixel at the current position of the cursor is saturated, in the panel the text of the component name and its count value will display red followed by a red asterisk. Components are sorted by increasing emission wavelength.

- **Unmixed Fluorescence imagery or PhenoCycler® imagery:** the cursor shows normalized counts for each component. The normalization takes into account exposure times. This means that the values are comparable between scans acquired with different exposure times. The same normalization is performed by default in inForm software. Components are sorted by increasing emission wavelength unless acquired with the PhenoCycler platform, in which case components are sorted alphabetically.

NOTE *The "above target" and "saturated" messages shown for images without unmixing are not shown for all unmixed image formats. To check for saturation, review the images without unmixing.*

3.7 SLIDE INFO

Get more information about the slide by clicking the Info button. The Info button is located in the toolbar at the top of the screen. (See Figure 3.19.)

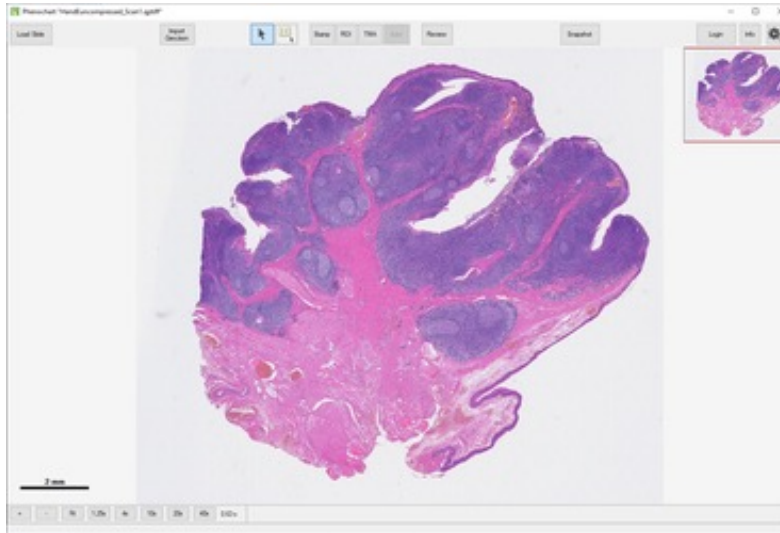


Figure 3.19. Slide Info window.

Click the Info button to open the Slide Info dialog. This dialog shows detailed information about the slide, how it was scanned, and properties of the resulting scan file. It also shows a color overview image of the slide and the label, if available. (See Figure 3.20.)

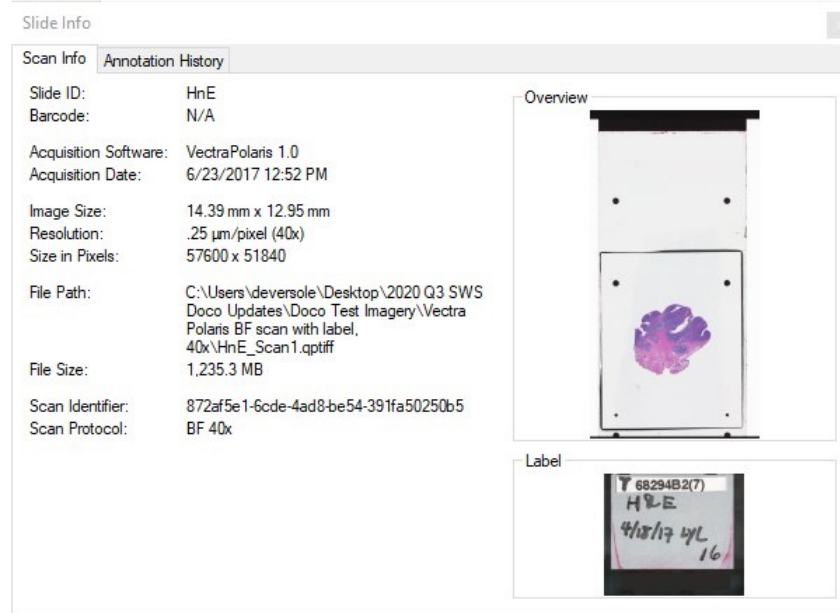


Figure 3.20. Slide Info – Scan Info tab.

Fluorescence imagery detailed information also includes filter band names and corresponding exposure times. Filter bands are listed in acquisition order.

NOTE For information about Annotation History see [Chapter 4 Annotations](#).

3.8 IMAGE SHARPENING

Brightfield QPTIFF files acquired with PhenoImager HT or Fusion may be JPEG compressed to reduce storage size. It is possible to visually enhance these images with image sharpening. This setting only affects the display of the image; the data values remain unchanged.

Images are displayed with sharpening applied by default. This can be turned off by deselecting Sharpen Image Display in the Gear menu at the top-right of the screen. (See Figure 3.21.)

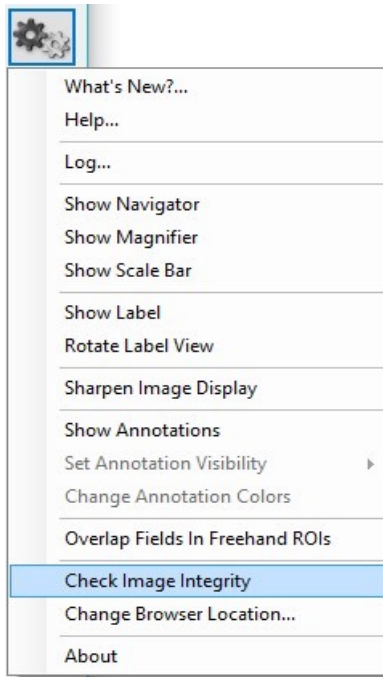


Figure 3.21. Gear menu > Sharpen Image Display.

3.9 IMAGE INTEGRITY

QTIFF files are tamper evident. Validate them to confirm that the image data and metadata have not been altered.

Select Check Image Integrity from the Gear menu at the top right of the screen. The software will perform the integrity check and report the results. (See Figure 3.22.)

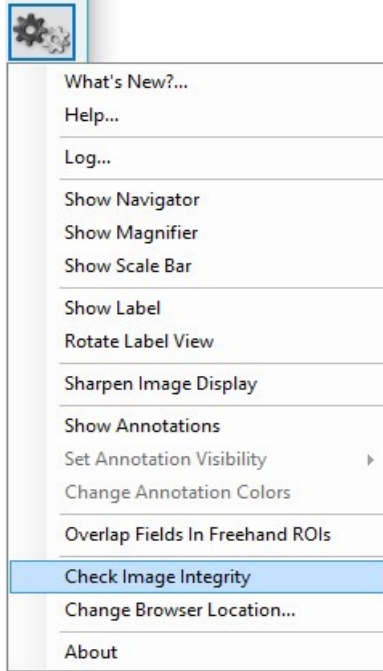


Figure 3.22. Gear menu > Check Image Integrity.

3.10 FLUORESCENCE LAYER TOOLBAR

The Fluorescence Layer Toolbar is located at the bottom of the screen. (See Figure 3.23.)

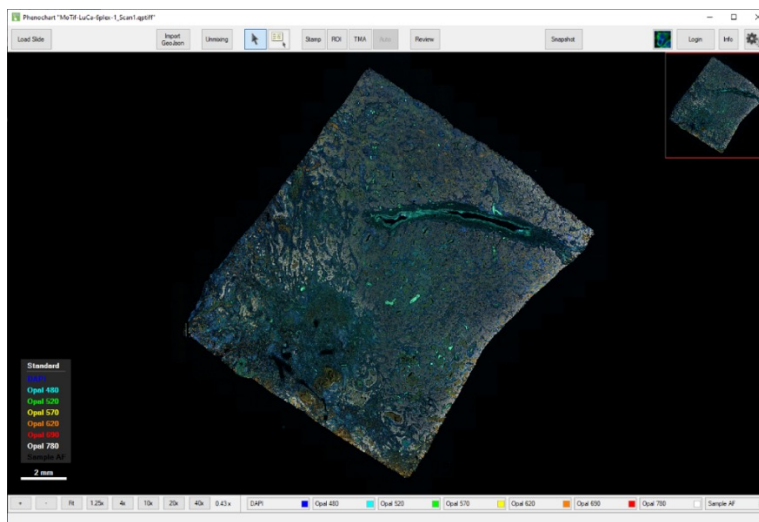


Figure 3.23. Fluorescence Layer toolbar.

The toolbar is only visible for fluorescent slides. Use the toolbar to toggle between which fluorescence channels are shown and adjust their brightness or color.

For Fluorescent imagery, the Layer Toolbar will show one button for each component layer. (See Figure 3.24.)



Figure 3.24. Layer Toolbar filter buttons for fluorescent imagery.

Each Layer Button is labeled with the common name for that component, which is either a filter name or marker name. Components are sorted by increasing emission wavelength unless acquired with the PhenoCycler platform, in which case components are sorted alphabetically. Each layer’s color is determined by the color selected in the scan protocol. (See Figure 3.25.)

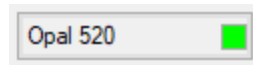


Figure 3.25. Layer Color.

Turn the signal layer on and off by clicking the Layer Button. When the button is On, it shows the display color on the image. When the button is Off, it is drawn with a hatch pattern. (See Figure 3.26.)



Figure 3.26. Hatch Pattern.

Change the display color and signal intensity for any layer. Right-click on any Layer Button to open the Layer Editor. (See Figure 3.27.)

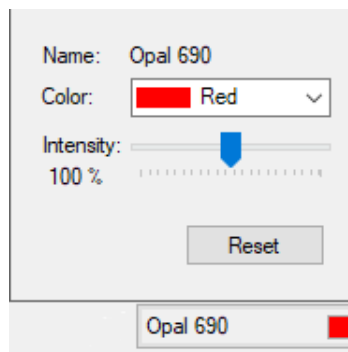


Figure 3.27. Layer Editor.

Change the color of the layer by selecting any of the preset colors or choose Custom to create a custom color.

Change the intensity, which will increase or decrease the apparent brightness for signals from that filter. The default intensity is 100%.

Click Reset at any time to return to the color and intensity default settings.

The Layer Editor will close when the mouse is moved off the editor.

3.11 FLUORESCENCE COMPOSITE VIEWS

Fluorescence Composite Views are intended to simplify visualization of fluorescence slides displaying large numbers of markers.

When an image is first opened, the scan will display the Standard view, which includes all components designated in the scan protocol.

3.11.1 Editing Composite Views

The Composite Views editor allows one to select which components to display. Editing existing views, creating new views, and selecting which components to display per view are all possible. The Composite Views editor is only accessible for fluorescent slides.

To access the Composite Views Editor, click the Fluorescence mode button located in the upper right-hand corner of the screen (to the left of the Login button) and click Edit. (See Figure 3.28.)

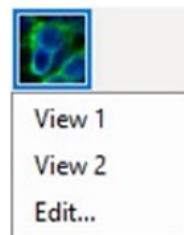


Figure 3.28. Composite View selection.

Select a composite view in the Views list on the left-hand side. Click Rename to change the name of this view.

To change which components are displayed, select individual components from the Available Components list displayed on the right half of the window. Each view can display up to 8 components. Change the color of each component by selecting any of the preset colors or choose Custom to create a custom color. (See Figure 3.29.)

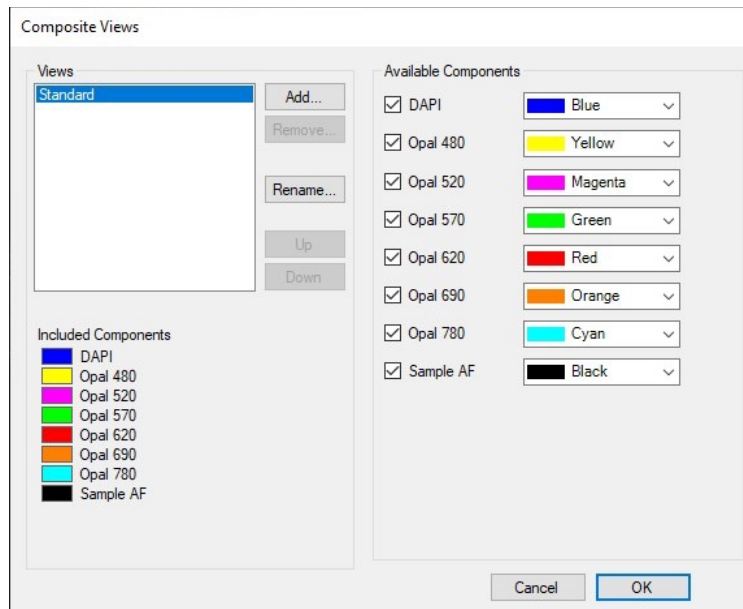


Figure 3.29. Composite Views Editor.

Click Add to create additional Views for display. The software will prompt to name the view. After adding a name, select OK. Change the view name later by selecting the view from the Views list and clicking Rename. The new view will automatically select the first 8 components. Components are sorted by increasing emission wavelength unless acquired with the PhenoCycler platform, in which case components are sorted alphabetically. To change components, first click the checked boxes next to the name of the components to be removed and then select the new components from the Available Components list. Assign each component a desired color. There is no limit to the number of views that can be created.

The display order of all Views can be changed. To change the location of a view, select the desired view and click the Up and Down buttons.

To permanently remove a view, select the desired view and click the Remove button. To prevent accidental deletion of a view, the software will prompt to click the OK button in the Removing Composite View confirmation before a view is permanently deleted.

Viewing Composite Views

To toggle between Views, click the Fluorescence mode button located in the upper right-hand corner of the screen (left of the Login button). A drop-down list of all Views created will display. Select the view name to be displayed. (See Figure 3.30.)

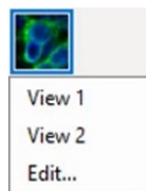


Figure 3.30. Composite View Selection.

Toggle between views by using the up and down keyboard arrows.

3.11.2 Saving Composite Views

Save composite view settings when closing the current slide or loading another slide. Select the Yes button in the Saved Changed Views Window when prompted to save the composite view setting. (See Figure 3.31.)

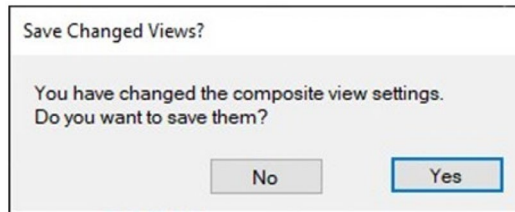


Figure 3.31. Save Changed Views.

3.12 LEGEND

The Legend is located at the lower left corner of the screen. (See Figure 3.32 and Figure 3.33.)

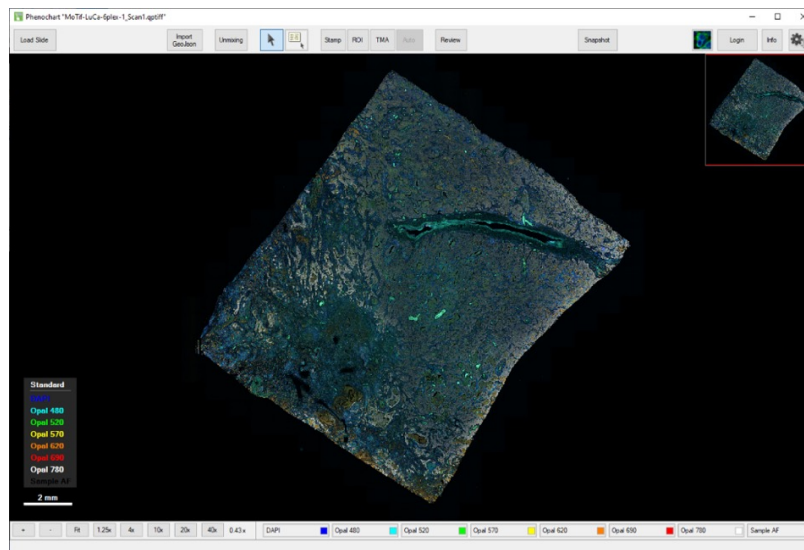


Figure 3.32. Image with legend.

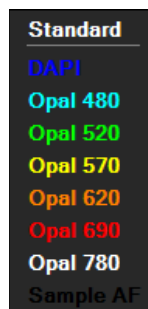


Figure 3.33. Legend inset.

The Legend shows which components are included within the selected Composite View. Components are sorted by increasing emission wavelength unless acquired with the PhenoCycler platform, in which case components are sorted alphabetically. The Legend is displayed on the screen by default. Hide it at any time by deselecting Show Legend in the Gear menu at the top right of the screen. The Legend is only available when viewing in Fluorescence mode.

3.13 WHOLE SLIDE MULTISPECTRAL UNMIXING

Fluorescent scans acquired with a multispectral slide scan protocol on the PhenolMager HT or Fusion can be unmixed by PhenoChart 1.0.10 or later and inForm 2.4.3 or later. The Unmixing button will only be displayed for these multispectral slide scans. (See Figure 3.34.)



Figure 3.34. Unmixing button (in red highlight box).

PhenolMager HT 2.0 and later also support writing files in an unmixed format, where unmixing is done as part of the scan process. There is no option to unmix these within PhenoChart since they are already unmixed.

Click the Unmixing button to choose between two Unmixing Views:

- **None:** The image is shown without any unmixing applied. This is the default.
- **Opal + AF:** The image is shown as unmixed components using synthetic Opal® spectra. By using a representative autofluorescence (AF) from an unstained human tonsil section, PhenoChart can provide a preview of signal and autofluorescence isolation. Unmixing does not alter the underlying data in any way. (See Figure 3.35.)

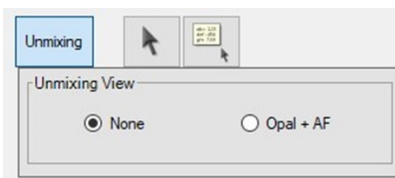


Figure 3.35. Unmixing View editor.

Multispectral scans that have been unmixed are displayed in Fluorescence mode against a black background.

After being opened, the Unmixing View Editor will remain open until another button on the toolbar is clicked. This allows toggling between the original and unmixed views as the slide is navigated.

3.14 SIMULATED BRIGHTFIELD VIEWS (PATHOLOGY VIEWS)

Simulated Brightfield mode (Pathology Views™) renders fluorescence images in a manner that is familiar to anyone trained in histopathology, displaying them as simulated hemotoxylin and eosin (H&E) images or as simulated DAB/Hematoxylin images.

Simulated Brightfield mode is available for use with fluorescent scans acquired with a multispectral slide scan protocol on the PhenolMager HT or PhenolMager Fusion that have been unmixed or for PhenoCycler®-Fusion scans. (See Figure 3.36.)

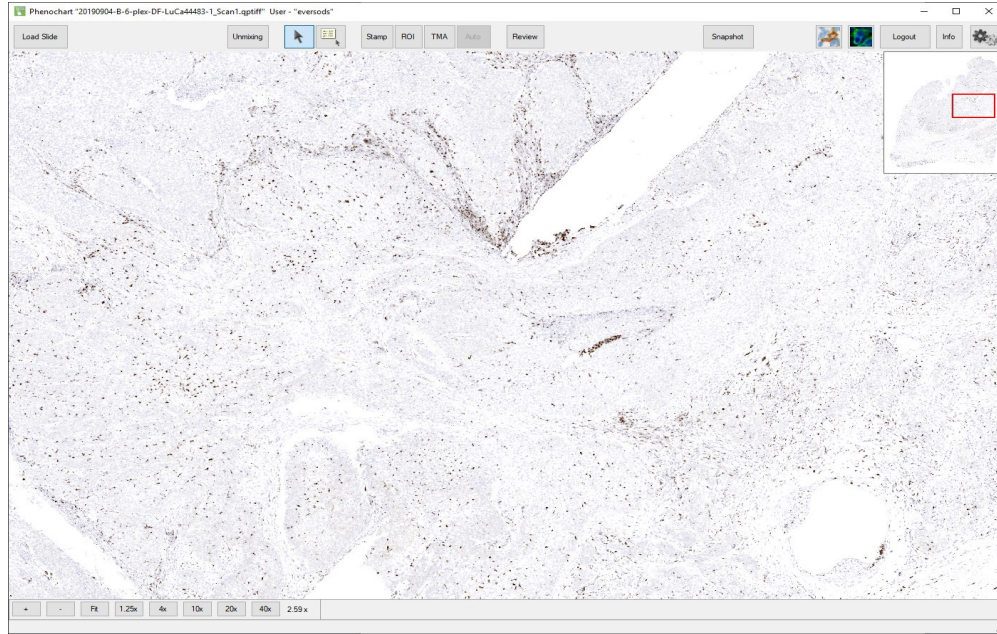


Figure 3.36. Simulated Brightfield Mode (Pathology Views).

Simulated H&E: The autofluorescence component is displayed as a simulated Eosin stain with Hematoxylin counterstain simulated from the DAPI signal against a white background.

Simulated DAB/Hematoxylin: Individual components are displayed as a simulated DAB stain with Hematoxylin counterstain simulated from the DAPI signal against a white background.

To toggle between components, click the Brightfield mode button located in the upper right-hand corner of the screen to the left of the Fluorescence mode button. A drop-down list of all available components will display. Select the view name to be displayed. (See Figure 3.37.)



Figure 3.37. Component selection for Pathology Views.

Toggle from one component to the next by using the up and down keyboard arrows.

Change the colors used in Brightfield mode by selecting Change Brightfield Colors in the Gear menu. (See Figure 3.38.)

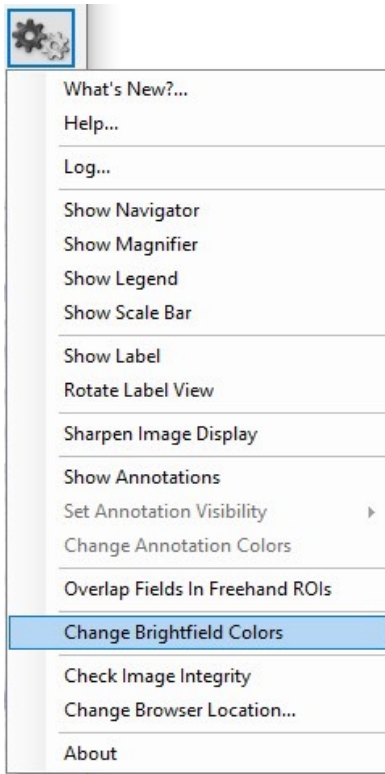


Figure 3.38. Gear menu > Change Brightfield Colors.

Select the desired colors in the Select Brightfield Rendering Colors dialog. Click Reset to set them back to the default. (See Figure 3.39.)



Figure 3.39. Select Brightfield Rendering Colors.

To toggle the display between fluorescence and brightfield modes, use the fluorescence and brightfield buttons located in the upper right-hand corner of the screen to the left of the Login button.

3.15 SNAPSHOTS

The Snapshot button allows a picture of the main display area to be saved. These images can be used in publications or to share screenshots with colleagues.

Click the Snapshot button in the upper right corner of the application window to show the

Snapshot Editor. (See Figure 3.40 and Figure 3.41.)

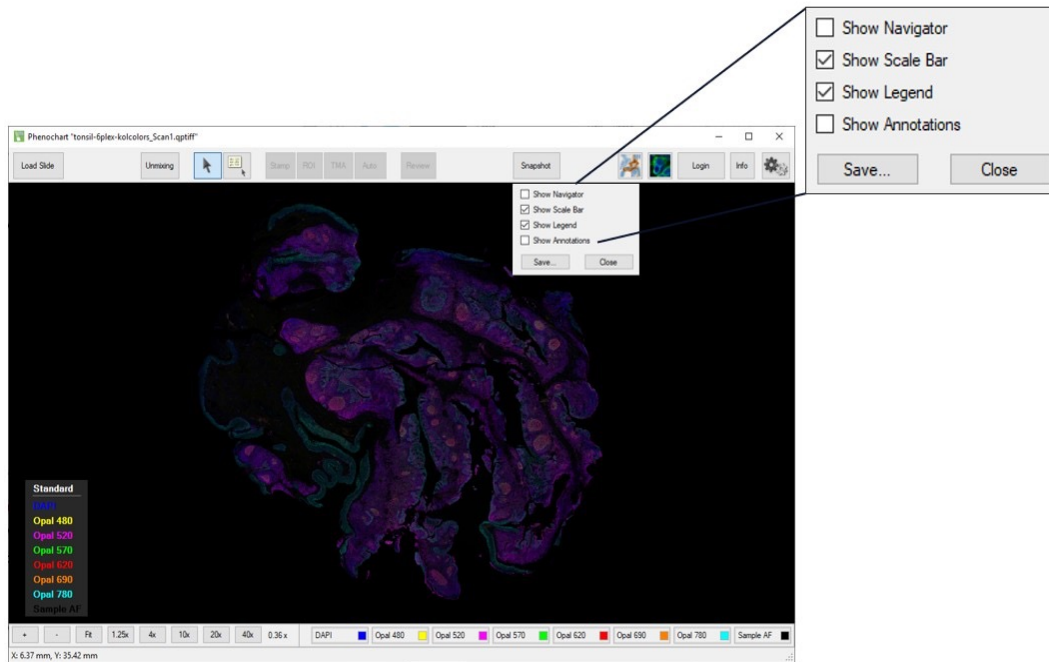


Figure 3.40. Snapshot Editor window.

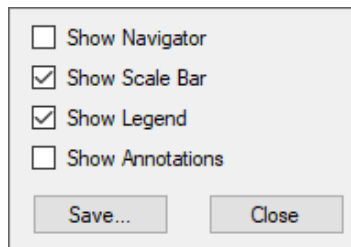


Figure 3.41. Snapshot Editor window inset.

Adjust the location and zoom for the slide using the navigation tools. Use the options in the Snapshot Editor to add or remove overlays of the Navigator Window, Scale Bar, Legend, or Annotations. These options affect only the saved Snapshot, not the main window settings.

NOTE *When PhenoChart is first launched, the Scale Bar overlay is turned on for snapshots. During the session, the software will retain the overlay selections from the last saved snapshot.*

When the image is ready, click the Save button in the Snapshot Editor. Navigate to different parts of the slide to take multiple snapshots. Once all the snapshots have been saved, click the Close button to close the Snapshot Editor.

Chapter 4: Annotations

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An annotation is created when an area of interest is marked on a slide for further multispectral imaging by Phenolmager HT or for analysis in inForm.

There are five ways to create annotations:

- Stamping fields in Phenochart.
- Drawing regions of interest (ROIs) in Phenochart.
- Importing ROIs from GeoJson files.
- Finding TMA cores in Phenochart.
- Automatically generating fields through inForm. (This option is only available for slides scanned with the legacy Vectra 3 system.)

States within each type of annotation:

- **Pending Acquisition** - This annotation has been identified for multispectral field imaging but has not yet been acquired.
- **Pending Review** - A reviewer must accept this annotation before multispectral fields can be acquired.
- **Acquired** - Multispectral fields were taken for this annotation.
- **Acquisition Failed** - This annotation was slated for the acquisition of multispectral fields, but the acquisition failed to complete correctly.
- **Rejected** - A reviewer rejected this annotation; fields will not be acquired there.
- **Ignored** - This annotation was ignored. It will not be considered for multispectral field acquisition or direct analysis in inForm.
- **Deleted** - A user deleted this annotation. If there was multispectral field imagery associated with this annotation, it was also deleted.
- **Selected For inForm Projects** - Scan imagery from this annotation will be loaded into inForm when the qptiff is opened in inForm. This option is only available for slides scanned with Phenolmager HT or Fusion.
- **Selected For inForm Batch** - Scan imagery from this annotation will be loaded into inForm when the qptiff is selected for batch processing. This option is only available for slides scanned with Phenolmager HT or Fusion.
- **Send to inForm** - This annotation was sent to inForm for rudimentary analysis or inForm classifier training. This option is only available for slides scanned with the legacy Vectra 3 system.

Annotations are color coded according to their state. See [Annotation Colors](#) for how to change the colors. See [Annotation Visibility](#) for how to show/hide types of annotations. Annotations can also be assigned descriptive tags. See [Tagging Annotations](#) for how to assign tags.

To create or review annotations, log in by clicking Login and entering a username. When the work is completed, click the Logout button to log out. (See Figure 4.1.)

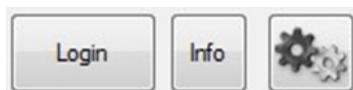


Figure 4.1. Login button.

When logged in, right-click on any annotation to ignore or delete it.

If multispectral fields have already been acquired at your annotation, double-click the annotation to open the acquired fields in inForm.

When the Phenolmager HT is acquiring multispectral fields for a slide, the annotations for the slide are locked. Additions or changes to the annotations are not permitted until Phenolmager HT finishes acquiring fields.

Annotations slated for multispectral field acquisition that were not acquired correctly will be marked.

Acquisition Failed: To re-acquire an individual annotation, right-click on that field and select Re-acquire. This will set the state back to Acquire. Select Re-acquire All if there were multiple failed acquisitions. This will set all fields that failed back to the Acquire state so they can be re-acquired.

The history of a scan's annotations is available in the Slide Info dialog. Click the Info button and click the Annotation History tab to view the log of annotations and actions. The log records each annotation action and the associated username. The annotation ID groups actions for each annotation together.

The annotation log includes a tamper-evident hash code. If a log file is altered, PhenoChart will give an error and refuse to open the file. (See Figure 4.2.)

ID	Type	Date	User	Action
1	Stamp	11/10/2015 9:48 AM	user	Created
1	Stamp	11/10/2015 9:48 AM	user	Pending Acquisition
2	Stamp	11/10/2015 9:48 AM	user	Created
2	Stamp	11/10/2015 9:48 AM	user	Pending Acquisition
3	Stamp	11/10/2015 9:48 AM	user	Created
3	Stamp	11/10/2015 9:48 AM	user	Pending Review
3	Stamp	11/10/2015 9:48 AM	user	Pending Acquisition
3	Stamp	11/10/2015 9:48 AM	anotherUser	ReviewAccepted
4	Stamp	11/10/2015 9:48 AM	user	Created
4	Stamp	11/10/2015 9:48 AM	user	Pending Review
4	Stamp	11/10/2015 9:48 AM	user	Pending Acquisition
4	Stamp	11/10/2015 9:48 AM	anotherUser	Rejected

Figure 4.2. Slide Info > Annotation History tab.

The columns can be sorted by annotation, date, or user to see the annotation history.

4.1 STAMPING FIELDS IN PHENOCHART

Use Stamp annotations to select specific rectangular areas of interest. The user must be logged in to create stamp annotations. Click the Stamp button to open the stamp editor. (See Figure 4.3.)

Figure 4.3. Stamp editor.

Five kinds of stamps:

- **Choose Acquisition** to take multispectral fields of the stamp area the next time this slide is on the PhenoMager HT.
- **Choose Review** to create stamps that must be reviewed before they can be acquired. PhenoMager HT will only acquire multispectral fields of accepted stamp annotations.
- **Choose Push to inForm** to launch the selected stamp area into inForm. This option will only allow stamps at the scan's native resolution and is only available for slides scanned with legacy Vectra 3 system, and when inForm 3.0.0 or higher is installed on the computer. For more information, see [Chapter 4.5.1 Training an inForm Classifier for Field Selection](#).

- **Choose inForm Projects** to select stamp areas for analysis in inForm. Load the qptiff into inForm 3.0 or later to analyze these fields. These stamps can only be made at native resolution. This option is available for slides scanned with Phenolmager HT or Fusion.
- **Choose inForm Batch** to select stamp areas for batch analysis in inForm. Batch process the qptiff in inForm 3.0 or later to analyze these fields. These stamps can only be made at native resolution. This option is available for slides scanned with Phenolmager HT or Fusion.

Select the stamp size in camera fields; this determines how many fields will be tiled together to make the image. The size of the resulting image will be shown in microns.

Select the resolution to use when acquiring the fields.

NOTE For legacy Vectra 3 systems, only 0.25 micron/pixel (40x) fields are acquired if a 40x objective is installed.

The cursor will show a rectangle which previews the stamp size based on the settings. Click on the slide to create a stamp at that location.

Multiple stamps can be created. The preview rectangle will snap to existing stamps when it is close to them. Zoom and pan normally with the mouse or zoom buttons to find areas of interest.

All pending annotations (acquisition or review) must be the same resolution. If a resolution is selected in error, ignore or delete the pending fields. To acquire fields at multiple resolutions, stamp and acquire the fields at one resolution first. Then return to stamp and acquire fields at other resolutions.

If an incorrect annotation is created, delete it by holding down the Ctrl key on the keyboard and clicking on the annotation. The cursor will change to a trashcan with a red X when an annotation can be deleted.

4.2 DRAWING REGIONS OF INTEREST IN PHENOCHART

The Region of Interest (ROIs) tool allows one to draw a region of interest to:

- Acquire multispectral images on a Phenolmager HT.
- Batch analyze fields using inForm.
- Create descriptive annotations that can be tagged.

Use the ROI Editor to label the stamp annotations for Acquisition, Review, inForm Batch, or Description:

- Choose Acquisition to take multispectral fields stamp/s that fill the ROI region the next time this slide is on the Phenolmager HT.
- Choose Review to create stamps that fill the ROI region, which must be reviewed before they can be acquired. Phenolmager HT will only acquire multispectral fields of accepted stamps.
- Choose inForm Batch to create stamps that fill the ROI region for batch analysis in inForm. Batch process the qptiff in inForm 3.0 or later to analyze these fields. These stamps can only be made at native resolution. This option is available for slides scanned with Phenolmager HT or Fusion.
- Choose Description to create an ROI annotation that can be tagged. Descriptive annotations will not be filled with stamps.

The ROI Editor can be used to select the resolution. (See Figure 4.4.)



Figure 4.4. ROI Editor.

When the selections have been made, draw a region of interest on the slide by clicking and dragging an outline. If the area is not closed, it will automatically be closed when the mouse button is released.

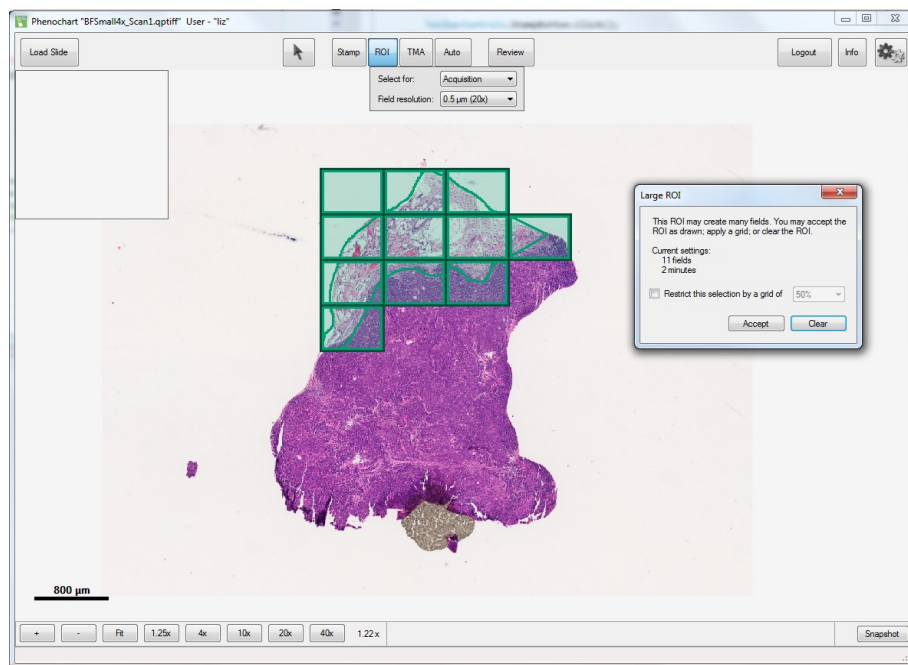


Figure 4.5. Tiled Stamp Annotations.

For Acquisition, Review, and inForm Batch ROIs, the software will then create tiled stamp annotations that contain the area that has been drawn. (See Figure 4.5.) If the region is small enough to fit within a 3x3 stamp, it will be included in a single stamp annotation. For larger regions, the system will break the region up into separate stamps.

If drawing regions for Acquisition or Review, a Confirmation Dialog will appear that displays the number of fields about to be taken. It will also provide an approximate time for the acquisition. The user may want to change the region based on how long it will take to image. (See Figure 4.6.)

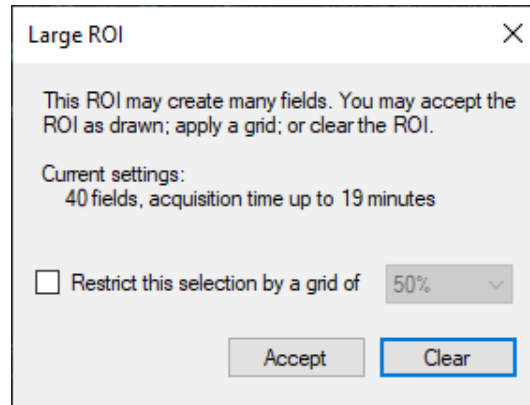


Figure 4.6. Large ROI window.

To reduce the number of fields within the region, apply a subsampled grid. Use the grid drop-down list to choose the percentage of fields for a gridded sample of stamp annotations. As the percentage is changed, the overlay will update to show which stamps will be generated. In addition, the number of fields and approximate time will update accordingly.

By default, fields will abut with no overlap. If overlapping fields are desirable by a small percentage, select **Overlap Fields in Freehand ROIs** in Freehand ROIs from the Gear menu. (See Figure 4.7.)

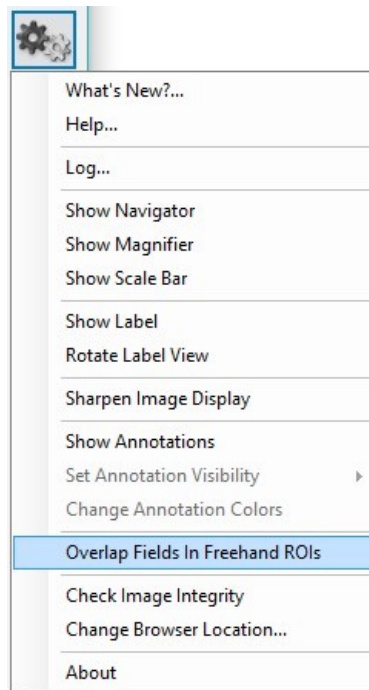


Figure 4.7. Gear menu > Overlap Fields in Freehand ROIs.

Click the **Accept** button to accept the ROI as shown, and the system will add all the stamps to the list of pending annotations (acquisition or review).

Click the **Clear** button to remove the ROI, and no fields will be added. (See Figure 4.8.)

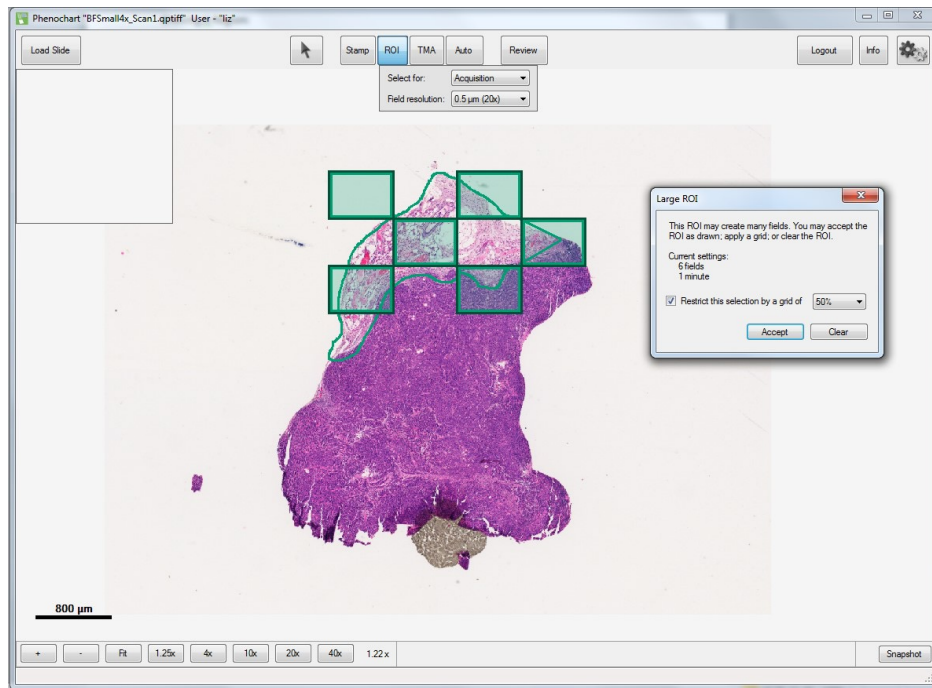


Figure 4.8. Accept ROI.

All pending annotations (acquisition or review) must be the same resolution. If a resolution was selected in error, ignore, or delete the pending stamp annotations. To acquire stamps at multiple resolutions, draw a region of interest and acquire the annotations at one resolution first. Then return to draw regions and acquire annotations at other resolutions.

Navigate to bring the region of interest in to view, hold the Ctrl key down on the keyboard, and use the mouse to zoom and pan.

If an incorrect ROI is created, then right-click on any stamp in the region. If Delete Region is selected, the ROI and any stamps in that region will be deleted. If Delete is selected, only the stamp clicked on will be deleted. (See Figure 4.9.)

Additionally, delete individual stamps within an ROI by holding down the Ctrl key on the keyboard and clicking on the annotation. The cursor will change to a trashcan with a red X when a stamp can be deleted.

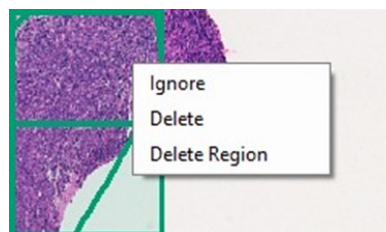


Figure 4.9. ROI right-click menu.

4.3 IMPORTING GEOJSON ROIS

A new feature in Phenochart 2.0.1 is the ability to import ROIs from compatible GeoJson files using the Import GeoJson button (see Figure 4.10.). For more information, contact Akoya Biosciences technical support at support@akoyabio.com. Once imported, annotations from the GeoJson file will be converted into Phenochart ROIs with Description type. (See [Section 4.2: Drawing ROIs in Phenochart.](#))

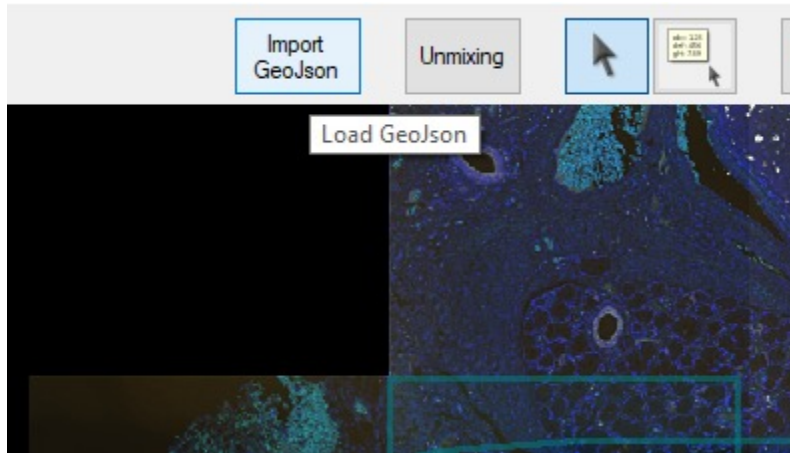


Figure 4.10. Import GeoJson button.

4.4 ANNOTATING TMA CORES IN PHENOCHART

Tissue microarray (TMA) annotations allow the user to locate TMA cores within whole-slide images and analyze or acquire multispectral fields of each TMA core. Use sector labels to identify different sections of cores, as appropriate, and use the row and column labels to match the TMA's numbering scheme. Correct labeling will make it possible to match downstream analysis with the original core information.

TMA Editor

Click the TMA button on the toolbar to open the TMA editor. (See Figure 4.11.)

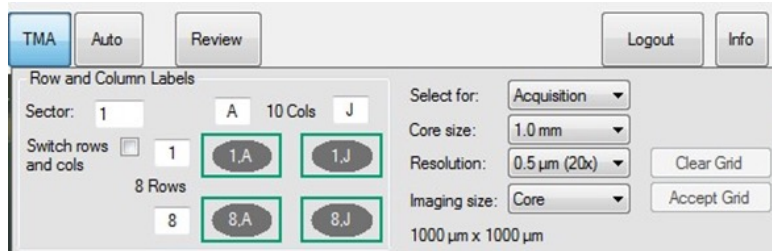


Figure 4.11. TMA Editor.

For best results, select the core finding parameters in the editor before drawing the sector.

Row and Column Labels

Select the Sector number for the TMA sector. Then choose the appropriate labels for the sector's rows and columns. Row and column labels can be either numbers or letters and can go in increasing or decreasing order. The grid will show the labels of the sector's four corners.

Phenochart displays labels as row, column, where rows go across and columns go up and down in the slide view. Check the Switch rows and cols checkbox if columns go across and rows run up and down.

Core Settings

After the labels are correct, select the desired core finding parameters to the right of the labels.

Identify if the TMA cores are for acquisition, review, or inForm Batch. If cores are selected for review, only the accepted cores will be acquired. If cores are selected for inForm Batch, only the scan's native resolution can be used. InForm Batch is only available for slides scanned with Phenolmager HT and Fusion.

Choose the size of the TMA cores. If the exact core size is not listed, choose the next larger one.

Choose the resolution to use when imaging the cores. If planning to image multiple sectors at the same time, the resolution must be the same for all sectors. To choose another resolution, first acquire, delete, or ignore the pending cores.

Choose an imaging size for the cores:

- **Core:** takes a square image of exactly Core Size x Core Size microns.
- **Core + 10% and Core + 20%:** gives square images with a margin to account for slight variations in core size.
- **NxN:** fields are rectangular fields as described. This option enables the software to take imagery consistent with early versions of Phenolmager HT.

NOTE *These fields may take significant extra area around the core and may include neighboring cores.*

Drawing the Sector

Finding Cores

Once the parameters are set, click-and-drag to draw a rectangle around the entire sector. To bring the entire sector in to view, hold the Ctrl key down on the keyboard and use the mouse to zoom and pan.

An example display is illustrated below. (See Figure 4.12.)

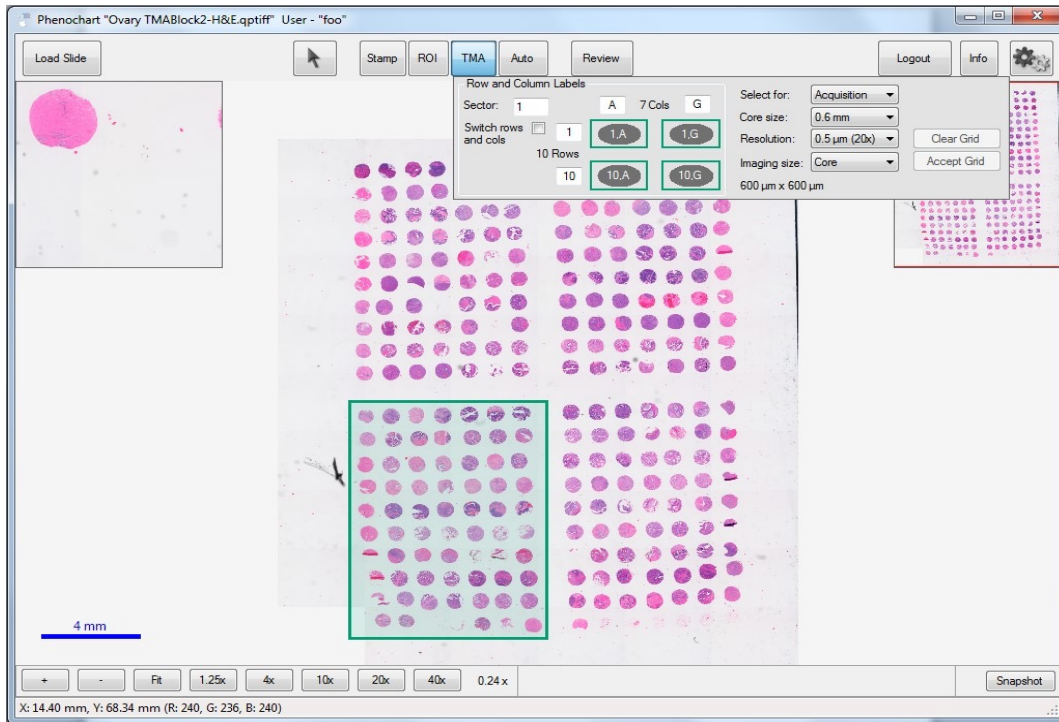


Figure 4.12. Drawing the sector display.

Once the mouse is released, PhenoChart will find the cores within the rectangle. Each core will be labeled. If the labels are not visible, zoom in to see them. (See Figure 4.13.)

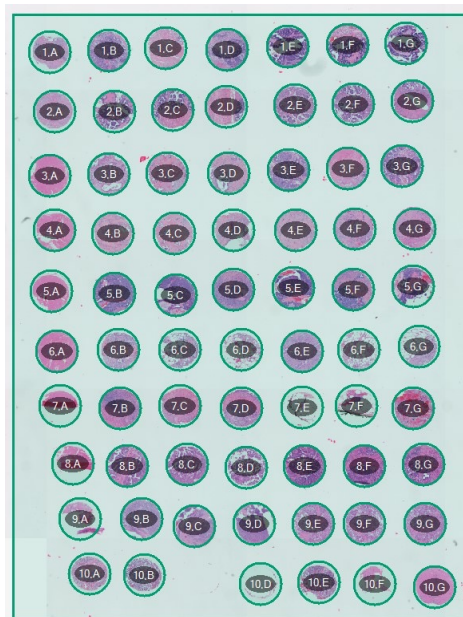


Figure 4.13. Labeled cores.

Adjusting the Cores

Adjust the settings after the cores are found. The software will update immediately to the new settings.

If the core finding missed a core, right-click on the missing core location and click Add Missing Core. Select the missing core label from the drop-down list and click the OK button to add the core. (See Figure 4.14.)

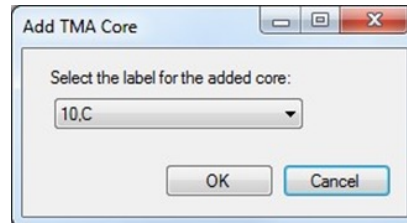


Figure 4.14. Add TMA Cores window.

The following are instructions to further refine the results of the core finding. To tweak the position of a single core annotation, hold the Ctrl key on the keyboard and click-and-drag on the annotation to move it. Be sure the settings are correct before adjusting cores. The individual core tweaks will be lost when the settings are changed, and the system relocates the cores.

Right-click to ignore a core or to bring back an ignored core. Delete a core or delete the entire sector if necessary. (See Figure 4.15.)

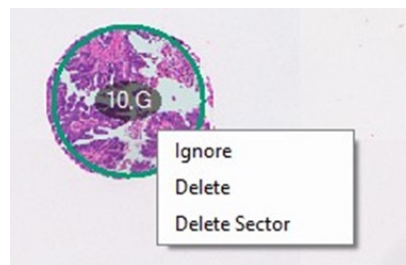


Figure 4.15. TMA right-click menu.

Accepting the Grid

Click Accept Grid to complete the TMA annotation. After this, no further changes can be made.

Click Clear Grid to reset the grid and start over by drawing a new sector rectangle.

Continue to add additional sectors if desired/necessary.

Common Issues and Resolutions

Numbering

If the software finds more or fewer cores than was drawn, a warning in red will appear. It may be necessary to change the row and column labels so the number of cores found matches the number selected.

If the sector, row, and column label of a new core is the same as that of another pending core, or if the software finds more or fewer cores than selected, a warning in red will appear. Change the sector, row, or column labels to correct the conflict.

Core Size

If the core finder provides drastically incorrect results, it usually means the core size is not set correctly. Try adjusting the Core Size so the size of the circles matches the size of the cores.

Fluorescence Layers

In Fluorescence, only visible layers will be used to find the cores. Turn off any layers that seem to be preventing the core finder from identifying the cores.

4.5 AUTOMATICALLY GENERATING FIELDS THROUGH INFORM

For slides scanned with the legacy Vectra 3 system, PhenoChart allows for automatic selection of fields of interest to acquire using a trained inForm classifier. This will only be available on computers with an activated version of inForm Tissue Finder Version 2.2 or higher.

Use inForm to add fields for acquisition or review.

Click the Auto button to open the editor. (See Figure 4.16.)

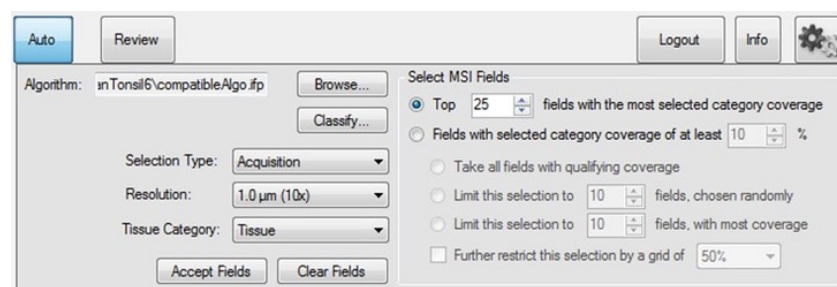


Figure 4.16. Auto Editor window.

Click the Browse button to load an inForm algorithm or project that was trained for this type of slide. If an algorithm was not yet created, see [Section 4.5.1: Training an inForm Classifier for Field Selection](#).

PhenoChart will confirm that the algorithm is valid for the slide. (This takes a few seconds.)

Click Classify to classify the slide once the algorithm is validated. (This takes anywhere from a few minutes up to ten minutes, depending on the algorithm settings and the size of the

slide.)

Adjust the selection settings when the slide has been classified to see which fields will be chosen. (See Figure 4.17.)

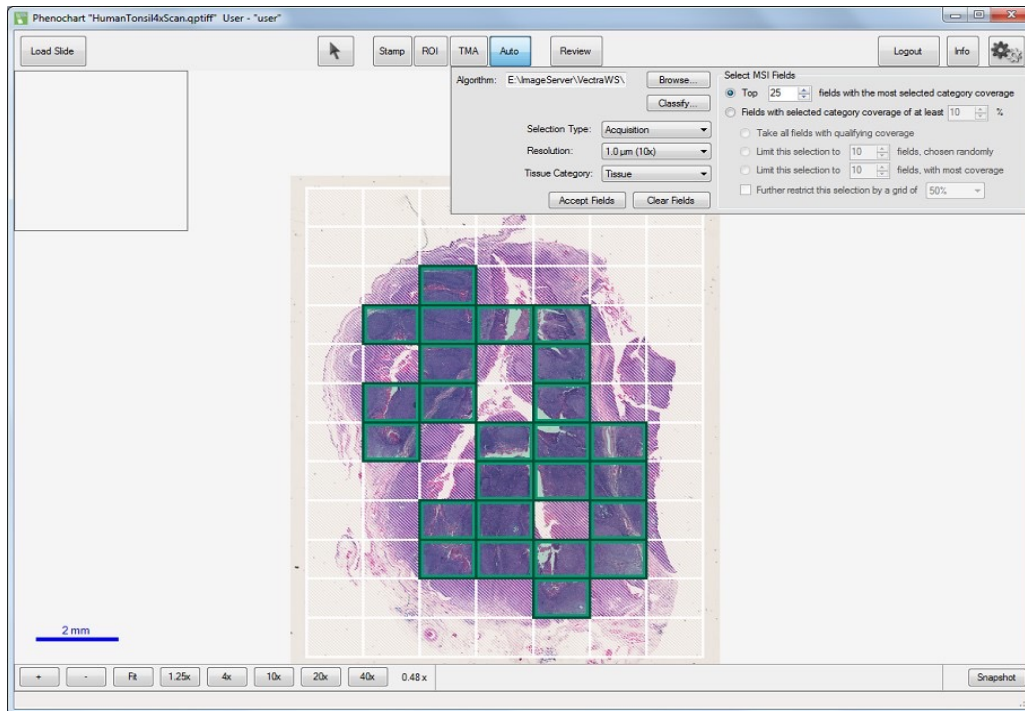


Figure 4.17. Classified Slide.

The fields on the screen update immediately as the selection settings are changed.

Use Selection Type to identify whether the fields are for Acquisition or Review. Fields marked for review must be reviewed and accepted before they can be acquired by PhenoImager HT.

Choose the Resolution to be used when acquiring the fields.

Select the Tissue Category of interest from the Tissue Classifier in the inForm algorithm.

Determine a multispectral imaging (MSI) strategy to ascertain 1) how many fields are taken, and 2) how strong they are in the category of interest. This approach allows the user to get the most interesting fields with the most efficient use of time and disk space.

Two types of MSI Strategies are illustrated in Figure 4.18.

- Choose the top fields with the most selected category coverage.
- Choose fields with selected coverage of a set percentage.

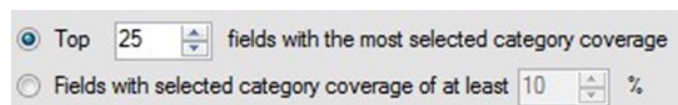


Figure 4.18. Select MSI fields – MSI Strategies.

Choose the top fields in the Tissue Category of interest. If this is selected, inForm will select the fields with the greatest coverage of the Category of Interest.

Choose from any fields that have at least some coverage of the Tissue Category. If this is selected, inForm will limit its selection of fields. Only fields that cover the required percentage (or greater) of the Tissue Category of interest will be considered.

Choosing the Fields with selected category coverage of at least X% option, allows one to choose what to take. (See Figure 4.19.)

- All the fields that meet the threshold.
- A selected number of randomly chosen fields that meet the coverage threshold.

NOTE *With this option, fewer fields than requested may result.*

- A selected number of fields with the most coverage of the Tissue Category of Interest. These fields must meet the minimum coverage threshold.

NOTE *With this option, fewer fields than requested may result.*

- Using a minimum threshold can result in a significant number of fields. Further limit the selected fields to a grid by selecting the grid coverage.

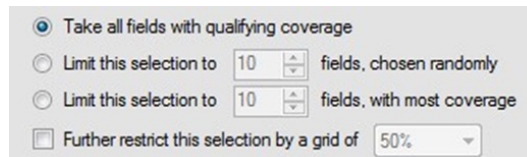


Figure 4.19. Coverage of % options.

As the settings listed above are adjusted, the fields will update in real time to show which fields meet the selection criteria. This is useful for classifier experimentation and testing for fully automated protocols on the legacy Vectra 3 system.

Phenochart will show the fields that inForm has selected as well as the fields that were ignored due to not meeting the selection criteria. To override the inForm decision, use the right-click menu, which provides the percentage of the class of interest. Toggle to ignore or acquire/review that field.

When satisfied with the selected fields, click Accept Fields to make the selection permanent. Click Clear Fields to clear the selection and start over. The right-click menu will also have entries to accept fields and clear fields.

4.5.1 Training an inForm Classifier for Field Selection

inForm Tissue Finder may be used from Phenochart to automatically select Multispectral fields for acquisition by Vectra 3 including an optional review step.

Phenochart is used to select fields for inForm training and to validate the tissue classifier during development. inForm Tissue Finder version 2.2 or higher is required to train classifiers for use with Phenochart.

To train a classifier in inForm, select the fields for training on the slide using the Phenochart

Stamp tool. Within the Stamp tool, select Push to inForm and select the fields that represent all tissue categories that are needed. InForm will launch automatically and load the fields. Load additional slides and stamp additional fields within Phenochart.

Once finished selecting fields within Phenochart, switch to inForm to train the classifier:

1. Configure the project to have a Trainable Tissue Finding Step.
2. It is often beneficial to downsample the imagery. This makes the classification much faster and for 10x scans it can improve the results as well. Within the Prepare Images step, start with a downsampling of 4:1. If the classifier is not performing well, reduce the downsampling to 2:1. (See Figure 4.20.)

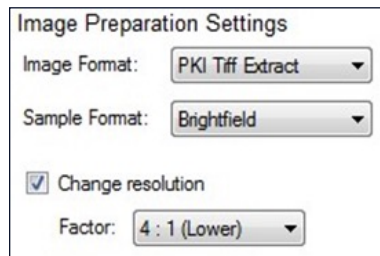


Figure 4.20. Prepare Images downsample.

3. In the Segment Tissue step, select the tissue categories and train the classifier.
4. In the Export step, check the Segmentation Maps (multi-image TIFF). (See Figure 4.21.)

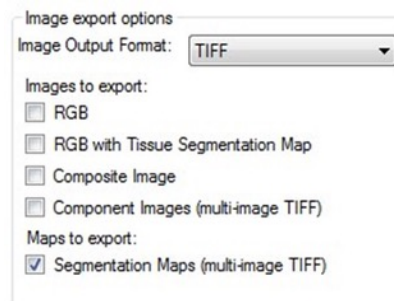


Figure 4.21. Maps to export Segmentation Maps.

5. Save the project. Saving the project within the study folder will make it easy to locate.

Return to Phenochart to test the classifier. Click Auto to open the automatic field editor, load the project, and click Classify. If the classifier needs more training, push additional fields into inForm and continue to train and test until satisfactory results are obtained.

4.6 TAGGING ANNOTATIONS

To add descriptive tags to an annotation, right-click on it to view the list of available tags. For ROI annotations that include stamps, right-click to choose the individual stamp. To instead select the ROI itself, hold down the Shift key on the keyboard while right-clicking.

After right-clicking, select an existing tag from the menu to apply it to the annotation. (See Figure 4.22.) To create a new tag, select New Tag, to enter a custom tag. Custom tags will continue to be available after closing PhenoChart and then re-launching on the same computer.

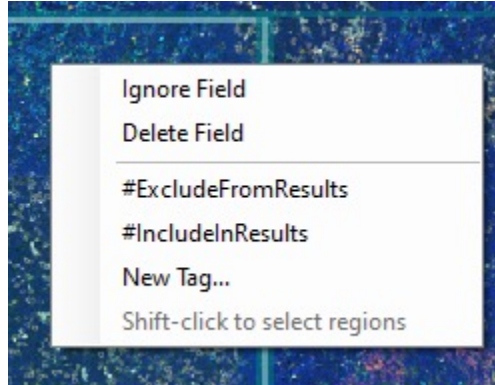


Figure 4.22. Create a New Tag.

Right-click again to apply another tag to the same annotation or to remove a tag from an annotation.

4.7 ANNOTATION MENU OPTIONS

Use the Gear menu to adjust the visibility and color of the annotations. (See Figure 4.23.)

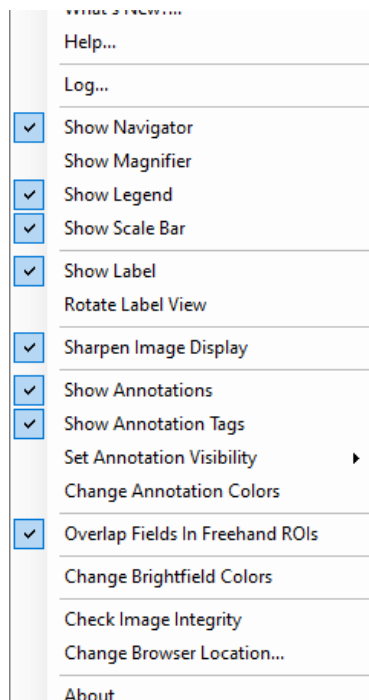


Figure 4.23. Gear menu > Show Annotations.

Annotation Visibility

Check Show Annotations to see that all annotations that have been selected are visible.

NOTE See below for instruction on customizing the visibility settings.

Uncheck Show Annotations to hide all annotations on the slide.

Use the Set Annotation Visibility submenu to customize visibility settings for each annotation type. (See Figure 4.24.)

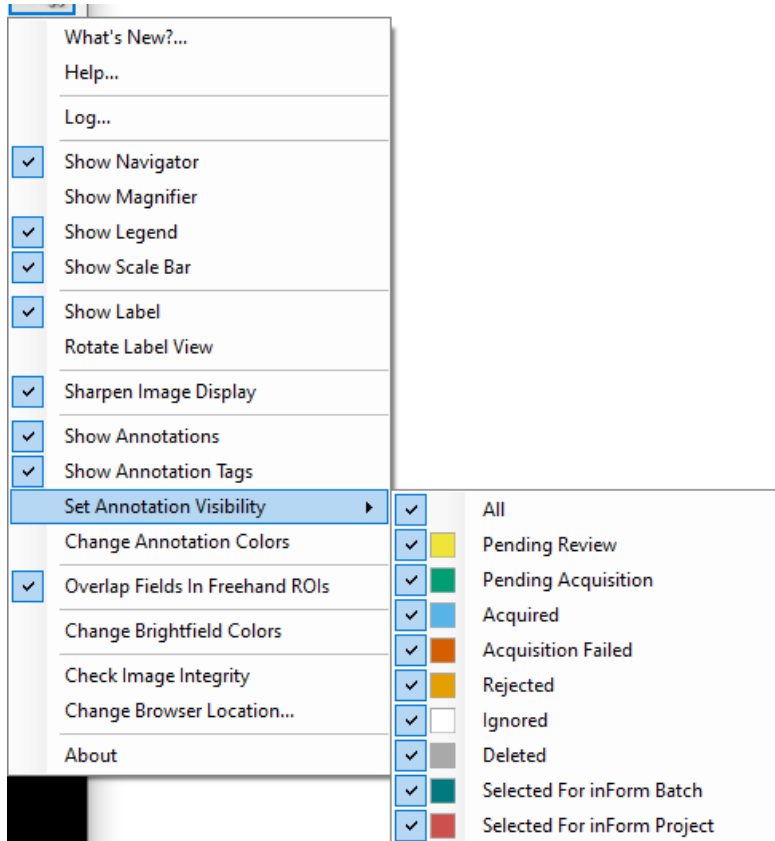


Figure 4.24. Gear menu > Set Annotation Visibility.

Check an annotation type to make it visible whenever annotations are shown. Uncheck it to hide all annotations of that type. Check All to turn all annotations on.

NOTE When first starting PhenoChart, Ignored and Deleted annotations are hidden by default. Any changes that are made to the type of visibility will be remembered for the next session.

Annotation Tag Visibility

Check Show Annotation Tags to see all annotation tags when annotations are visible. Uncheck to hide annotation tags.

Annotation Colors

The default annotation colors are chosen to be distinguishable, even for users that are visually impaired. Change the colors as needed. Select Change Annotation Colors from the Gear menu to bring up the color changing dialog. (See Figure 4.25.)

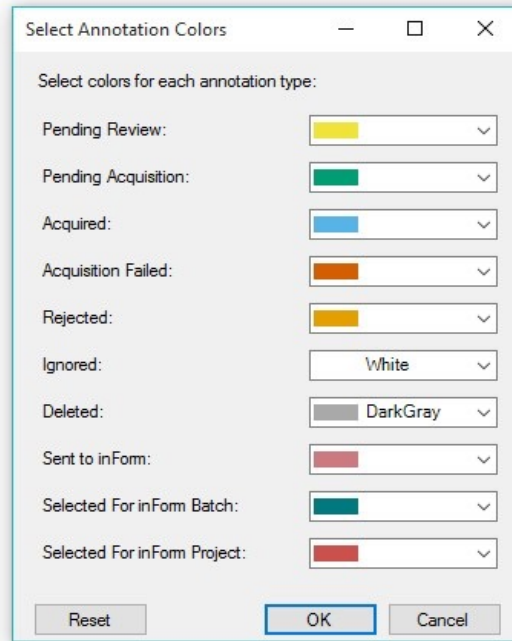


Figure 4.25. Select Annotation Color window.

Change the color by selecting any of the preset colors or choose Custom to create a custom color. Click Reset to restore the factory color-blind-safe defaults. Click OK to save the changes. Cancel will exit without saving the changes. The color settings will be saved across sessions.

4.8 REVIEW

Annotations that have been selected for Review must be accepted before they will be acquired by Phenomager HT. Click the Review button to open the review editor. (See Figure 4.26.)

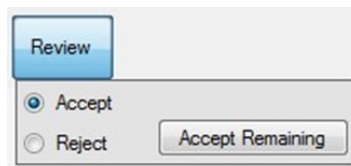


Figure 4.26. Review window.

Choose Accept to accept annotated fields or cores. When moving the mouse over an annotation awaiting review, the cursor will change to a green checkmark. (See Figure 4.27.)



Figure 4.27. Green checkmark.

Click the annotation to accept it. The annotation is now ready for acquisition.

Choose Reject to reject annotations. When moving the mouse over an annotation awaiting review, the cursor will change to a red x. (See Figure 4.28.)



Figure 4.28. Red x symbol.

Click the field to reject it; it will not be acquired.

Click Accept Remaining to accept all remaining un-reviewed fields. A confirmation dialog will appear revealing how many fields are about to be accepted.

Use the right-click menu to Accept or Reject fields and to Accept Remaining. Also use the right-click menu to un-review a field or change the review of a field.

Appendix A: Support

Log File

When contacting Akoya Biosciences Customer Care about a Phenochart issue, the log file will be requested. Including the log file with the initial report will aid in solving the problem.

1. Click the View tab.
2. Select Show hidden files, folders, or drives and then click the OK button.

If the issue has just occurred, select Log... from the Gear menu to obtain the current log file. (See Figure A.1.)

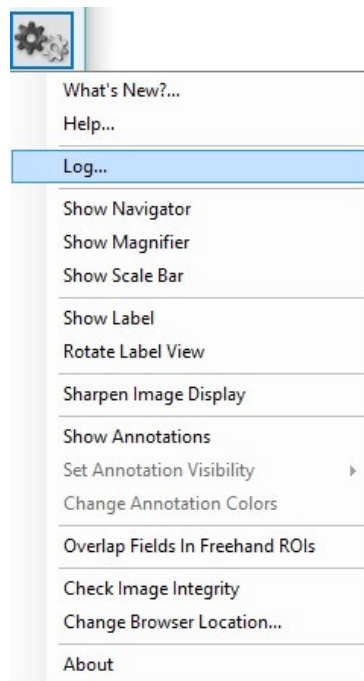


Figure A.1. Gear menu > Log...

If the issue occurred on a previous day, go to C:\Users\Public\Akoya\Phenochart to find the appropriate log file in. Log files are named by date.

The log file can either be attached to the email or cut and paste the log file contents into the correspondence.

Config File

In some cases, the Phenochart.config file may be requested. This file can be found in C:\ProgramData\Akoya\Phenochart\.

By default, this directory is hidden from view. Use the Windows browser to view this folder:

1. Select Organize and then Folder and search options. (See Figure A.2.)

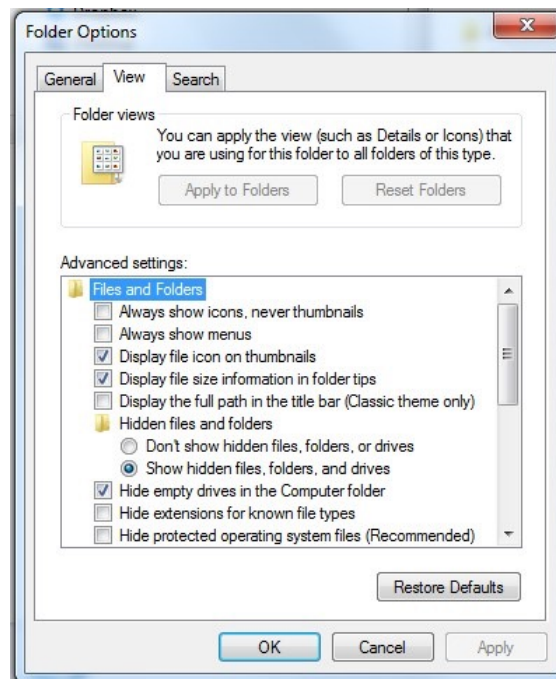


Figure A.2. Folder Options > View > Files and Folders.

Appendix B: Akoya QPTIFF Specification

Background

This describes a TIFF format that Akoya Biosciences uses for its tissue images. The imagery may be a simple RGB image, a set of components extracted from a field or region, or a whole-slide scan. In the latter case, it may be a brightfield (BF) color RGB image or a multiband fluorescence (FL) image.

The goal is to use the same syntax and metadata for all these kinds of images, and minimize the semantic distinctions where possible. Specifically, an extracted component represents signal attributed to a stain or fluorescent dye in a sample. The signal values incorporate image preprocessing such as normalization for exposure time and spectral unmixing when that technique is used.

Data Format

The files are TIFF or BigTIFF images, depending on image size, with multiple images per file.

For images larger than about 2K x 2K pixels, tiled format is used, and the image is provided in several resolutions (pyramidal tiled images). Tile size is 512 x 512 pixels. Images smaller than 2K x 2K are stored in strips rather than in tiles.

The highest resolution (baseline) image(s) appear first in the file. For each resolution there are N baseline images where N depends on the contents. For BF images, N=1 and each image is an RGB image. For FL images or unmixed component images, N = number of bands, which is usually > 1, and each image is a grayscale image. The sample format for grayscale raw images can be unsigned 8-bit or unsigned 16-bit. For grayscale unmixed images, the sample format can be unsigned 16-bit, signed 16-bit, or 32-bit float.

A thumbnail RGB image is provided, and this is a good image to use as an icon in graphical image lists. Note that the thumbnail can be used for qualitative sample identification, but should not be used to assess relative brightness of different images. This comes after the baseline images, meaning it is the second image in BF (RGB) images, and the (N+1)st image for FL images or unmixed component images.

Next come the reduced-resolution images (if present). The pyramid contains enough levels that the image size is no larger than 2K x 2K at the coarsest resolution.

For whole-slide scans, there are two more non-tiled images after these: an optional RGB image of the label, and a macro (low-resolution) RGB image of the whole slide.

Overall, the arrangement is:

Table B.1. Images

Description	RGB/mono	Tile/Strip	Resolution	Notes
Baseline image	Varies	Varies	Full	Tiled if > 2K x 2K RGB for BF, else mono.
More full-resolution images	Mono	Varies	Full	If N > 1.
Thumbnail	RGB	Strip	~500 x 500	
Half-resolution images	Varies	Varies	Half	Only if baseline is tiled.
Quarter, eighth, etc.	Varies	Varies	Quarter, eighth, etc.	Continues until 2K x 2K or smaller
Macro (overview) image of whole slide	RGB	Strip	~2000 x 4000	Required for whole-slide scans. Optional for simple RGB images and extracted components.
Label image	RGB	Strip	~500 x 500	Optional, whole-slide scans.

Detection

Readers can recognize Akoya tissue images via the contents of the “Software” TIFF tag (see below). The file suffix is .qptiff for whole slide scans. Some TIFF tags contain “PerkinElmer” for historical reasons; they remain to retain 3rd party reader compatibility.

Metadata

Metadata is contained in two locations: within standard TIFF tags as listed in the table below, and within the ImageDescription string, using a set of XML tags described below. These are provided for each image (IFD) in the file, and describe that image rather than the baseline image or the scan as a whole. The ScanProfile tag is only provided on the first, baseline image as it may be large.

Table B.2. TIFF tags

TIFF Tag	Optional?	Description of Content
Software		Starts with “PerkinElmer-QPI”
ImageDescription		Further metadata in XML format (see next section)
ImageWidth		Width of the image in pixels
ImageLength		Height of the image in pixels
ResolutionUnit		Unit used for resolution and position (see below)
XResolution		Pixel X resolution (see below)
YResolution		Pixel Y resolution (see below)

TIFF Tag	Optional?	Description of Content
XPosition	Y	Sample X location in ResolutionUnits. This is ULHC location except for Macro image which reports its image center.
YPosition	Y	Sample Y location in ResolutionUnits. This is ULHC location except for Macro image which reports its image center.
SampleFormat		Integer (1) for BF, FL; or float (3) for extracted components
SMinSampleValue		Minimum signal value in the image
SMaxSampleValue		Maximum signal value in the image
BitsPerSample		8 (FL); 8, 8, 8 (RGB); or 32 (unmixed component)
SamplesPerPixel		1 (FL or unmixed component) or 3 (RGB)
NewSubfileType		0 for full-resolution images, 1 for reduced resolution images
TileWidth	Y	Tile width (512) if tiled format is used
TileLength	Y	Tile height (512) if tiled format is used
TileOffsets	Y	List of tile offsets, if tiled format is used
TileByteCounts	Y	Size of each (compressed) tile, if tiled format is used
StripOffsets	Y	List of strip offsets, if tiled format is not used
RowsPerStrip	Y	Number of rows per strip, if tiled format is not used
StripByteCounts	Y	Size of each (compressed) strip, if tiled format is not used
PlanarConfiguration		1 (chunky) for RGB images, 2 (planar) otherwise.
PlanarConfiguration		1 (chunky) for RGB images, 2 (planar) otherwise
PhotometricInterpretation		2 (RGB) for RGB images, 1 (BlackIsZero) otherwise
DateTime		Acquisition time
Compression		May be None, CCITT Group 3, PackBits, LZW, or JPEG
JPEG fields	Y	JPEG fields are defined when JPEG compression is used

ResolutionUnit, XResolution and YResolution are required fields in a valid TIFF file. When the true resolution of the image is known, ResolutionUnit will be 3 (cm) and XResolution and YResolution will be pixels/cm. When the true resolution is not known, ResolutionUnit will be 2 (inch) and XResolution and YResolution will be 96 (pixels/inch). Pixels from Akoya Biosciences instruments are always square so XResolution and YResolution will always have the same value.

The TIFF spec is not explicit about the data type and value for SMinSampleValue and SMaxSampleValue; the writer uses the same data type as the image pixels (byte or float).

ImageDescription Contents

The ImageDescription tag contains a string in XML format. The string contains a top-level <PerkinElmer-QPI-ImageDescription> element. Nested within this element are child elements with the tag names and values as listed in the table below. Elements appear in the order listed. Values are stored as text content of the element. Elements are required unless otherwise specified. See Table B.3.

Table B.3. ImageDescription Tags

TIFF Tag	Optional?	Contents
DescriptionVersion		Version of the image description field itself, a single number. This document describes version 5 of the field.
AcquisitionSoftware		Software used to acquire the image
Identifier		GUID in string format. This is an identifier for the image file itself.
SlideID	Y	ID of the slide that this image was taken from.
SampleDescription	Y	Text description of the sample
OperatorName	Y	Name of the operator as reported by the operating system running the acquisition software.
Barcode	Y	Barcode text of the slide this image was taken from.
ComputerName	Y	Name of the computer on which the slide was scanned.
ImageType		A string identifying the type of image within the file (Table B.1), with the following values: <ul style="list-style-type: none"> • FullResolution • ReducedResolution • Thumbnail • Overview • Label
IsUnmixedComponent		“True” for unmixed multispectral images, otherwise “False.”
ExposureTime		Exposure time as an integer number of microseconds. For unmixed images, this is the exposure time for the dominant wavelength band for the component (FL); or the brightest wavelength in the cube (BF).
ExposureTimeArray	Y	Exposure times as an integer number of microseconds. Holds multiple values if there is more than one sample per pixel, as for RGB imagery; otherwise the array has a single element.

TIFF Tag	Optional?	Description of contents
Signal Units		<p>A byte <i>www ttt</i> where the <i>ttt</i> nibble indicates the signal unit type from the following:</p> <ul style="list-style-type: none"> – raw counts – normalized (counts/second/gain/full-scale/ binning) – OD (optical density) – dark-corrected counts <p>and the <i>www</i> nibble indicates how the signal is weighted across the spectral bands (or colors):</p> <ul style="list-style-type: none"> – average across all bands – total summed signal across all bands – peak signal in highest-valued band <p>Thus, for example, a value of 68 (hex 44) encodes OD units with peak-signal weighting.</p>
Name	Y	Band name for FL whole slide scans, component name for extracted components. Not present for RGB images
Color	Y	Color to use when rendering this band, as decimal r,g,b byte triplet. Present for FL whole slide scans or extracted components. Not present for RGB images.
Responsivity	Y	Instrument responsivity, if available, for FL whole-slide and unmixed images. See below for details.
Objective	Y	Objective name, if known, otherwise not present. This does not imply a specific pixel resolution – see the XResolution and YResolution standard TIFF tags instead.
ScanProfile	Y	Element containing scan and/or and unmix parameters. It is valid XML whose contents are opaque to most readers. It is only provided on the first (baseline) image and is omitted from all other IFDs.
ProtocolName	Y	Name of the acquisition protocol.
ValidationCode		Used for internal data integrity checks – readers can ignore this.
ImagingCycle	Y	Zero-based acquisition cycle for CODEX imagery
Biomarker	Y	String indicating target such as CD8, Ki67, etc.
ScaleFactor	Y	After allowing for bit-depth conversion, pixel values in this dataset have been scaled by this factor. For ex-ample, if a 12-bit image is stored in an 8-bit image, and counts were scaled from 4095 to 255, this would be 1; if counts were scaled from 3102 to 255, Scale-Factor would be 1.3201. Default is 1.

TIFF Tag	Optional?	Description of contents
NormalizedUnitsFactor	Y	<p>For unmixed data, multiplying the pixel value by this factor will yield component signal in units of normalized counts.</p> <p>Normalized counts are defined by:</p> $Normalized\ counts = \frac{counts}{[FSC * B^2 * G * E * WDF * SW]}$ <p>Where: <i>FSC</i> is the camera full-scale value in counts <i>B</i> is the binning <i>G</i> is the camera gain <i>E</i> is the exposure time, in seconds <i>WDF</i> is a well-depth factor for the sensor used <i>SW</i> is a spectral weighting factor that accounts for the total energy associated with that component's spectrum across all bands.</p>
AutofluorescenceSubtracted	Y	String containing 'true' or 'false', indicating whether this dataset has been subtraction-corrected by a background (autofluorescence) image. Treated as 'false' by default.
LampType	Y	String with the FL lamp type <ul style="list-style-type: none"> - Unknown (default) - PriorLumen200 - PriorLumen220 - XCiteMultiBandLed - PriorMimicLed - SolaLed - Colibri7
BFLampType	Y	String with the BF lamp type <ul style="list-style-type: none"> - Unknown - FlashLamp - WhiteLED - TricolorLED
InstrumentType	Y	String with the instrument type, one of the following: <ul style="list-style-type: none"> - Unknown (default) - Keyence700 - Keyence800 - AxioObserver - Dmi8 - Polaris - Vectra3

TIFF Tag	Optional?	Description of contents
CameraType	Y	String with the camera sensor type, one of the following: <ul style="list-style-type: none"> - SonyICX285AL - HamamatsuC11440 - IMX265 - IMX421
ExcitationFilter	Y	Description of excitation filter with sub-elements listed below If present, all its sub-elements must be provided.
Name		String with the filter public name
Manufacturer		String with the manufacturer or OEM supplier
PartNo		String with the manufacturer part number
Bands		Collection of bands each as described below
Band		Description of a band with child tags noted below
Name		String with the band name
Cuton		Cuton wavelength of this band in nm
Cutoff		Cutoff wavelength of this band in nm
Active		True if this band is excited, False if not
EmissionFilter	Y	Description of emission filter with sub-elements listed below. If present, all its sub-elements must be provided.
Name		String with the filter public name
Manufacturer		String with the manufacturer or EOM supplier
PartNo		String with the manufacturer's part number
Bands		Collection of bands, each described by sub-elements below
Band		Description of a band with sub-elements noted below
Cuton		Cut-on wavelength of this band in nm
Cutoff		Cut-off wavelength of this band in nm
CameraSettings	Y	Description of the camera settings used for this image with sub-elements listed below. If present, all its sub-elements must be provided.
Gain		Gain factor, such as 1, 3, etc.
Binning		Binning extent, such as 1 for full-resolution, 2 for 2x2 binning

TIFF Tag	Optional?	Description of contents
OffsetCounts		Offset count setting for the camera
BitDepth		Bit resolution used to read out the camera. This may differ from the resolution used to store this image (for example, it may be acquired with 12-bit resolution but saved in a QPTIFF file with 8-bit resolution)
Orientation		String containing one of the following values: <ul style="list-style-type: none"> - 'Normal' (default) - 'Rotate180' - 'MirrorV' - 'MirrorH'
ROI	Y	Description of the sensor ROI contributing to the image. Coordinates are sensor pixels before binning. Even if the CameraSettings are provided, this field is optional
X		X origin of ROI. Left-most pixel in sensor array is 0.
Y		Y origin of ROI. Top-most pixel in sensor array is 0.
Width		Width of ROI
Height		Height of ROI

For whole slide images (BF and FL), SignalUnits will be 64 (hex 40) (raw counts, peak signal). For unmixed images, SignalUnits will reflect the unmix settings.

Instrument Responsivity

The <Responsivity> tag is a container for a list of normalized instrument response values. This tag is present for whole-slide FL images from Vectra Polaris and unmixed FL multispectral images originating from Vectra 3 and Vectra Polaris.

For whole-slide images, the <Responsivity> tag will contain one <Filter> tag. The <Filter> tag contents will be different for each image within the TIFF file, reflecting the filter used to take the image.

For unmixed component images, the <Responsivity> tag will contain one <Band> tag for each band in the original image file. The <Band> tags are repeated for each unmixed component image.

The overview, thumbnail and label images do not have <Responsivity> tags.

Each <Filter> or <Band> tag describes the instrument responsivity for acquisitions using that filter or band. Their contents are described in Table B.4.

Table B.4: Contents of <Filter> or <Band> tags within <Responsivity> tag

Tag	Contents
Name	The name of the filter (whole-slide image) or band (component image).
Response	The instrument response to the reference artifact, normalized for exposure. This is raw counts/ ($2^{\text{bit depth}} \times \text{exposure time} \times \text{gain} \times \text{binning area}$), where bit depth is the bit depth of the imagery, exposure time is in seconds, gain is the gain setting of the camera, and binning area is 1 for 1x1, 4 for 2x2, etc.
Date	The date and time of the reference image in UTC, ISO 8601 format.
FilterID	<p>Detailed description of the acquisition filter, as {ExcitationFilter band name}_ {ExcitationFilter manufacturer}:{ExcitationFilter part number} / {EmissionFilter manufacturer}:{EmissionFilter part number}</p> <p>The {ExcitationFilter band name}_ prefix may be omitted if there is only a single excitation band.</p>

Sample ImageDescription

Sample ImageDescription for the DAPI band of a FL whole-slide image, containing a single <Filter> tag:

```
<?xml version="1.0" encoding="utf-8"?>
< PerkinElmer-QPI-ImageDescription >
  <DescriptionVersion>1</DescriptionVersion>
  <AcquisitionSoftware>VectraScan 1.0.0</AcquisitionSoftware>
  <ImageType>FullResolution</ImageType>
  <Identifier>AABED946-BB58-44FB-95B3-48E177E3BB83</Identifier>
  <IsUnmixedComponent>False</IsUnmixedComponent>
  <ExposureTime>50</ExposureTime>
  <SignalUnits>64</SignalUnits>
  <Name>DAPI</Name>
  <Color>0,0,255</Color>
  <Responsivity>
    <Filter>
      <Name>DAPI</Name>
      <Response>30.7</Response>
      <Date>2015-10-22T13:10:18.0618849Z</Date>
      <FilterID>Semrock:FF02-409/LP-25 Emission / Semrock:FF01-377/50-25
        Excitation</FilterID>
    </Filter>
  </Responsivity>
  <Objective>4x</Objective>
  <ScanProfile><!-- this will be a serialized scan protocol. It is valid XML but otherwise
    opaque -->
  </ScanProfile>
  <ValidationCode>4281ff86778db65892c05151d5de738d</ValidationCode>
</ PerkinElmer-QPI-ImageDescription >
```

Appendix C: Akoya Software & Spectra Compatibility

Some files are read by multiple Akoya acquisition and analysis software programs to facilitate unmixing and analysis workflows. Due to major updates in the combined release of Phenomager HT 2.0.0, Phenochart 2.0.0, and inForm 3.0.0 software, outputs from these versions are not backward compatible with previous versions of software. However, files from previous versions of software are forward compatible. These compatibility considerations are detailed in Table C.1.

Table C.1. Akoya Software Compatibility Considerations

	VectraPolaris 1.x.x	Phenomager HT 2.x.x	Phenochart 1.x.x	Phenochart 2.x.x	inForm 2.x.x	inForm 3.x.x
Scanning protocols created in VectraPolaris 1.x.x can be opened by	YES	YES	N/A	N/A	N/A	N/A
Scanning protocols created in Phenomager HT 2.x.x can be opened by	NO	YES	N/A	N/A	N/A	N/A
Imagery acquired with VectraPolaris 1.x.x can be opened by	N/A	N/A	YES	YES	YES	YES
Imagery acquired with Phenomager HT 2.x.x can be opened by	N/A	N/A	Some*	YES	NO	YES
Annotations created by Phenochart 1.x.x can be opened by	YES	YES	YES	YES	YES	YES
Annotations created by Phenochart 2.x.x can be opened by	NO	YES	NO	YES	NO	YES
Algorithms created by inForm 2.x.x can be opened by	N/A	N/A	N/A	N/A	YES	YES
Algorithms created by inForm 3.x.x can be opened by	N/A	N/A	N/A	N/A	NO	YES
Projects created by inForm 2.x.x can be opened by	N/A	N/A	N/A	N/A	YES	YES**
Projects created by inForm 3.x.x can be opened by	N/A	N/A	N/A	N/A	NO	YES

	VectraPolaris 1.x.x	Phenolmager HT 2.x.x	Phenochart 1.x.x	Phenochart 2.x.x	inForm 2.x.x	inForm 3.x.x
Spectra extracted in inForm 2.x.x can be exported as an unmixing library for HT 2.x.x by	N/A	N/A	N/A	N/A	NO	YES
Spectra extracted in inForm 3.x.x can be exported as an unmixing library for HT 2.x.x by	N/A	N/A	N/A	N/A	NO	YES

*Can be opened, but will not allow for stamping for inForm.

**Background on Projects created by inForm 2.x opening in inForm 3.x. Two considerations:

1. Beginning with inForm 3.0, there are slight changes to cell splitting or segmentation due to the different numerical routines used. Also, the cell numbering can be assigned differently even for the same segmentation results. These do not affect the results in a significant way for normal use, but as a result, the assignment of cell ID #s to cells can differ between 2.x and 3.x. This means that phenotyping training sets from inForm 2.x projects cannot be used for training phenotyping classifiers in 3.x because the training sets consist of tables of phenotypes keyed by cell ID. If the cell ID is wrong, the training data is erroneous so the resulting classifier will not be properly trained. For this reason, inForm 3.x will not bring in training cell ID information for projects from inForm 2.x. It will post this message when this happens: "[Project] was created with inForm 2.8 or earlier, and phenotype training data from 2.8 cannot be imported into inForm 3.0. All cell phenotype training data has been discarded for that reason. The algorithm's phenotype step can be run with its current training, or use inForm 2.8 or earlier to add training cells or revise phenotyping."
2. For projects created in inForm 2.8 with imagery that has 10+ channels (i.e., PhenoCycler imagery), phenotyping computation may become unusably slow in inForm 3.x. It is recommended that inForm 2.8 projects with imagery that has 10+ channels are only run in inForm 2.8. New projects created in inForm 3.0 do not have this issue.

Considerations for Side-by-Side Installations

- Vectra Polaris 1.x and Phenolmager HT 2.x software can be installed side-by-side. If needed, you can keep using Vectra Polaris 1.x for some ongoing studies that use inForm 2.x algorithms.
- Discerning which version is launching will be easy due to differing program names. Phenochart 1.x and 2.x can be installed side-by-side.
- Users often launch Phenochart by double-clicking a QPTIFF. In this case, the launched version might not be obvious but will be easily confirmed via About Box. inForm 2.x and 3.x can be installed side-by-side.
- Users sometimes launch inForm by double-clicking on a project. In this case, the launched version might not be obvious but will be easily confirmed via About Box.

Spectra compatibility considerations are detailed in Table C.2.

Table C.2. Spectra compatibility and incompatibility considerations

	Unmix 8-bit QTIFFs in inForm	Unmix Extended-bit QTIFFs in inForm	Unmix in HT via .umxlib
Synthetic Opal & AP spectra can be applied to	YES	YES	YES
Opal spectra extracted from 8-bit QTIFFs can be applied to	YES	YES	YES
AF spectra extracted from 8-bit QTIFFs can be applied to	YES	NO	YES
Opal spectra extracted from Extended-bit QTIFFs can be applied to	YES	YES	YES
AF spectra extracted from Extended-bit QTIFFs can be applied to	NO	YES	YES

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