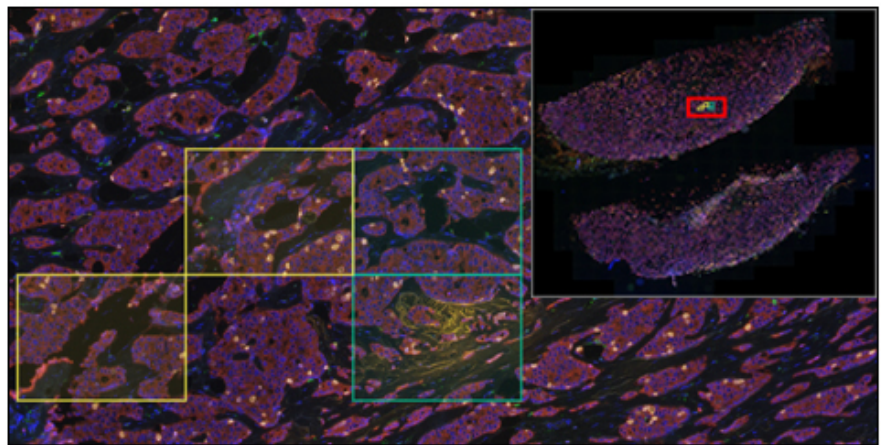


# Phenochart™

Whole Slide Contextual Viewer  
for Annotation & Review

## User's Manual



**Phenochart™ 1.1 with MOTiF™**

Whole Slide Contextual Viewer  
for Annotation and Review

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Phenochart 1.0.12  
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This manual describes how to use Phenochart software.

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# 1 Overview

Phenochart is a slide viewer for scans created by the following Akoya instruments:

- Vectra® 3 and Vectra® Polaris™ Quantitative Pathology Imaging Systems
- CODEX® System

With Vectra imagery, Phenochart enables you to:

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

With CODEX imagery, Phenochart enables you to:

- View and navigate around whole slide images
- Annotate areas of interest for downstream analysis using inForm®
- Identify cores for analysis using inForm®

Phenochart is freely available and can be installed on multiple machines at your site.

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

## 2 Loading Slides

The Slide Browser allows you to select and load slides. It shows a list of available studies and the slides within them.

### To select a slide:

Click the **Load Slide** button in the upper left to bring up the Slide Browser.

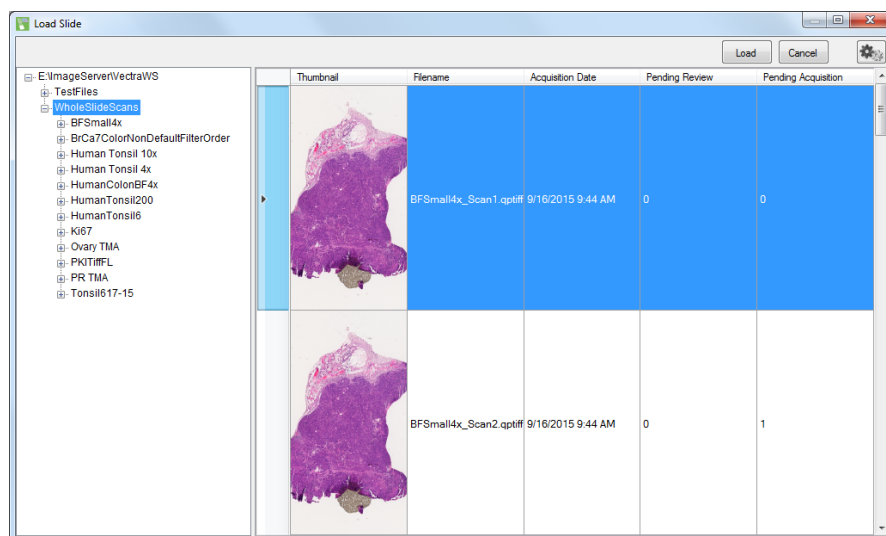


Figure 1. Slide Browser

The list of Vectra studies is on the left. You can browse deeper to see slides within a study.

When you select a study on the left, the right side shows the scans in that study. If you select a slide within a study, the right side will show scans of that slide only. Click a column header to sort by it. This may help you find 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. You can also highlight the scan on the right hand side 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. You can add other columns. Select **Set Column Visibility** from the browser's **Gear Menu** to select which columns are shown in the table:

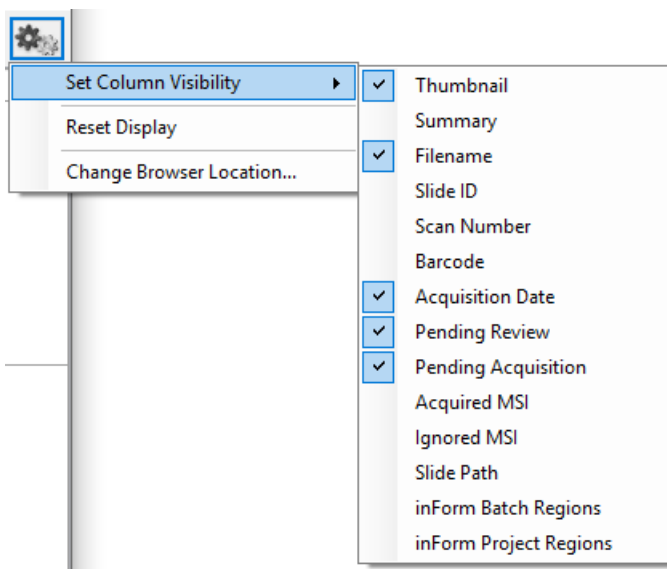


Figure 2. Gear Menu

You can also resize the columns by dragging the column dividers, (i.e. if you want to make the thumbnail bigger). Select **Reset Display** from the browser's **Gear Menu** at any time to restore the original column sizes.

#### To initialize the browser:

The very first time you launch the Slide Browser, you may need to locate your Vectra™ Data. You locate the Vectra Data by selecting an example scan within it. Phenochart™ obtains the example by asking you to open a QTIFF file (.qtiff).

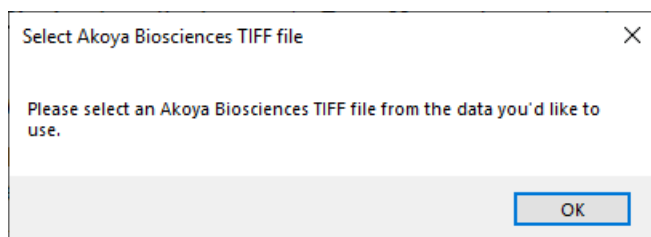


Figure 3. Select QTIFF File Window

The next time you click **Load Slide**, you will be able to use the Slide Browser.

**NOTE:** You will only see this prompt the very first time you load the Slide Browser or if you reset the browser in the menu.

If you need to change which data the browser looks at, use the browser's Gear Menu to change the location:

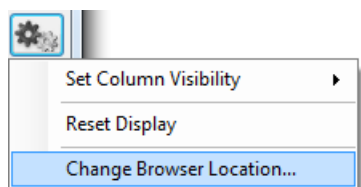
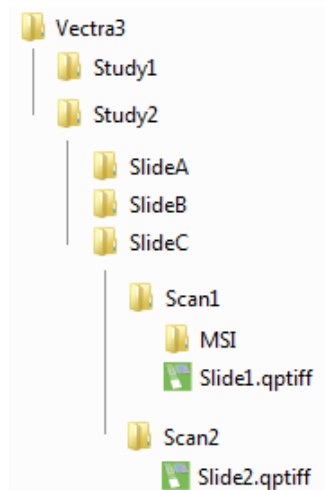


Figure 4. Gear Menu > Change Browser Location...

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

### **Vectra Data:**

Vectra systems store scan data by **study**.



*Figure 5. Vectra 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.

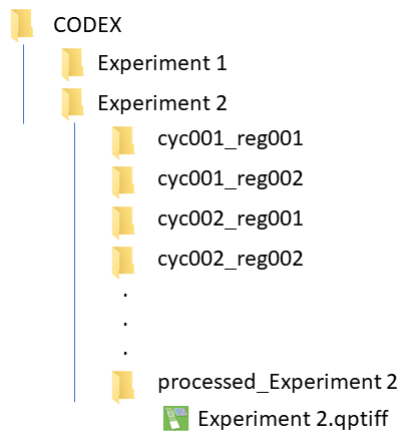
Each study will contain one or more slides. Each slide can be scanned more than once (if needed). Any acquired Multispectral fields from a slide scan will be stored within the scan folder.

### **CODEX Data:**

CODEX systems store scan data by **experiment**, and stores scan data by datasets.

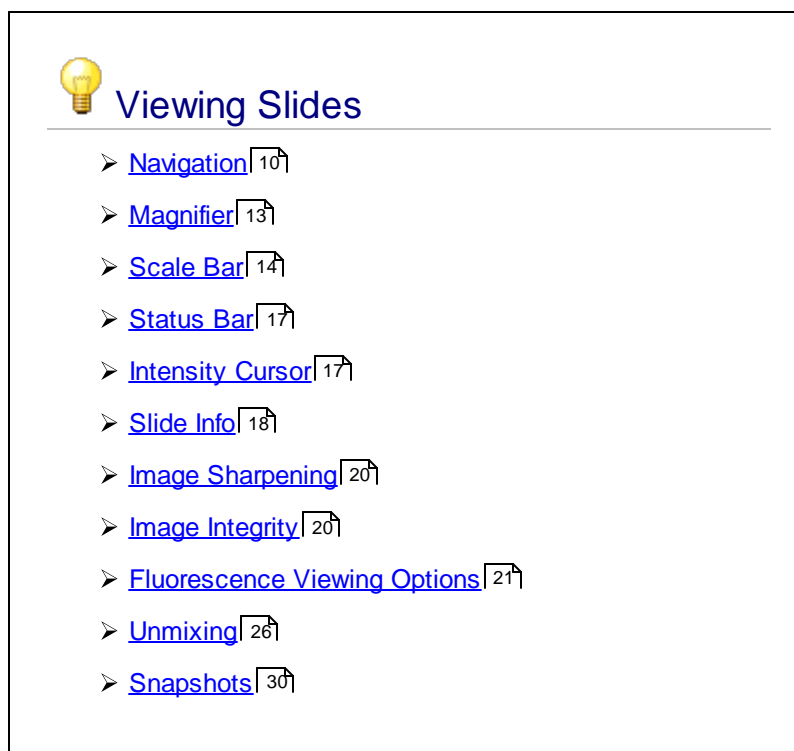
The CODEX System reveals tissue markers cyclically, generating N images per experiment. A CODEX experiment is a group of cycle scans that belong together. In addition, there is a folder containing a processed image rendering all marker data into a single image that can be opened in Phenochart and inForm®.

Each experiment folder will contain data for only a single coverslip.



*Figure 6. CODEX Experiments*

## 3 Viewing Slides



### 3.1 Navigation

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

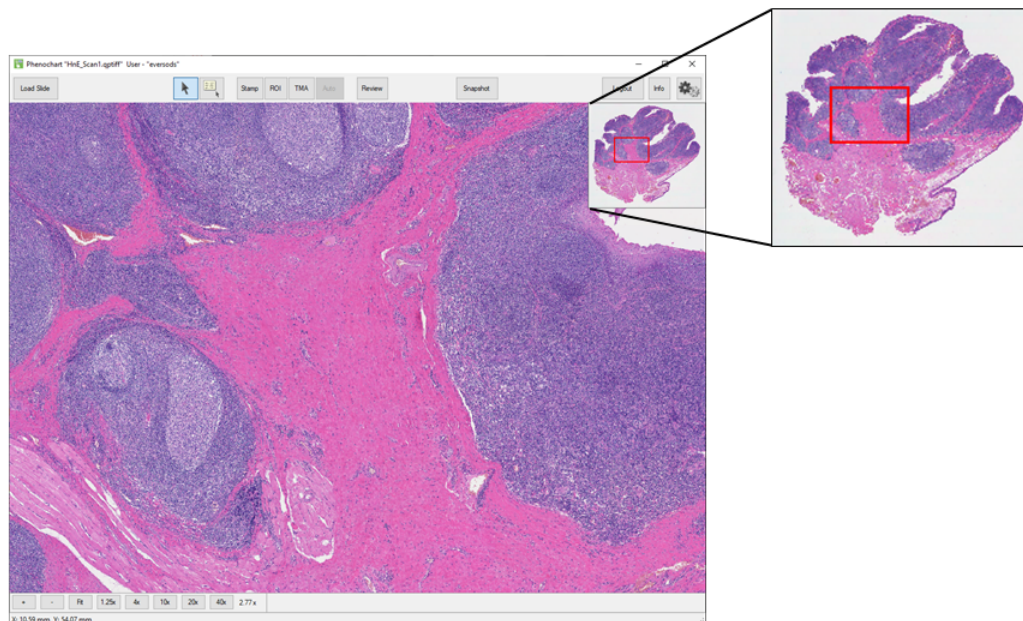
The **Navigation Tool** is the arrow button located in the toolbar at the top of the screen:



*Figure 7. Navigator Tool*

When you first launch Phenochart, this button is selected as the default setting for your mouse. It allows you to click and drag to move the image on your display or to use the mouse wheel to zoom in and out on the image.

The **Navigator Window** is the small image of your slide located at the top right of the screen.



*Figure 8. Navigator Window*

The Navigator Window contains a small image of the entire slide and a red box within that image. The red box shows you what part of the slide is being displayed on the main image on your screen. You can 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 you release the mouse once you have moved the box to your desired location). Or, 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 your screen by default. However, you can hide this window at any time by de-selecting **Show Navigator** in the Gear Menu at the top right of your screen:

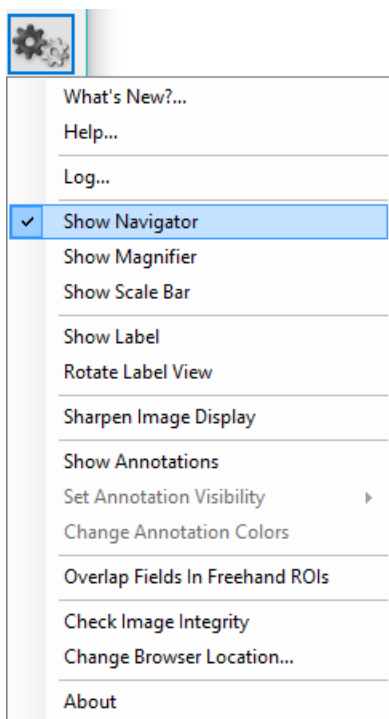


Figure 9. Gear Menu > Show Navigator

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

You can zoom in and out on the slide. As you zoom 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:

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



Figure 10. Pre-defined Resolutions

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

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

**NOTE:** No matter what resolution a slide has been scanned at, when you load a slide, it is shown 'fit' to your screen.



## 3.2 Magnifier

The Magnifier is the window in the upper left corner of your screen:

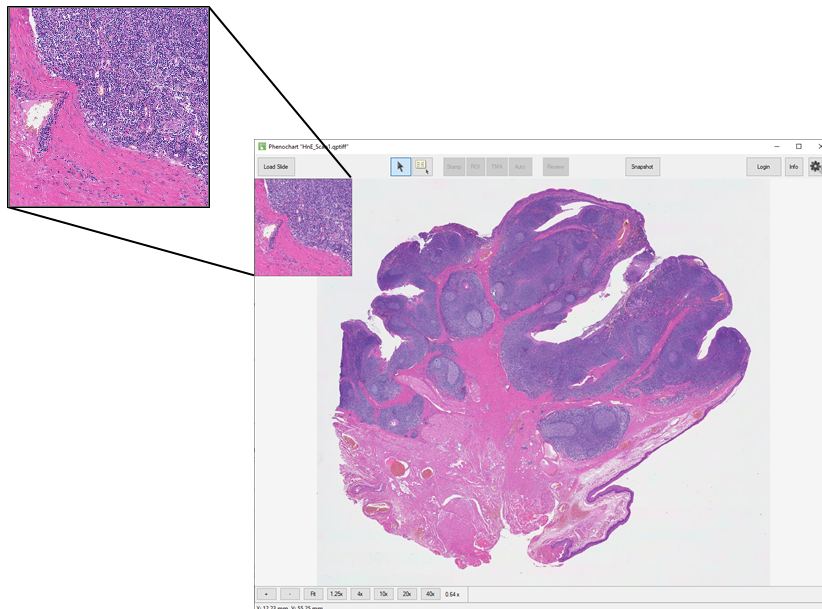


Figure 11. Magnifier

The Magnifier shows a magnified (zoomed in) view of the slide centered on your cursor. This lets you quickly inspect individual regions in more detail without changing the overall zoom.

The Magnifier window is displayed on your screen by default. You can hide this window by de-selecting **Show Magnifier** in the Gear Menu at the top right of your screen:

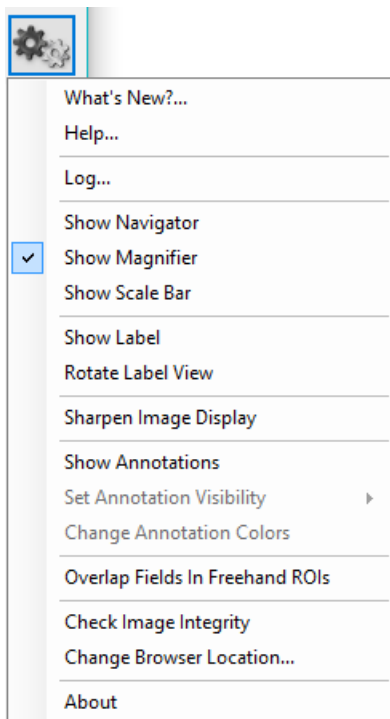


Figure 12. Gear Menu > Show Magnifier

### 3.3 Scale Bar

The **Scale Bar** is located at the lower left corner of your screen:

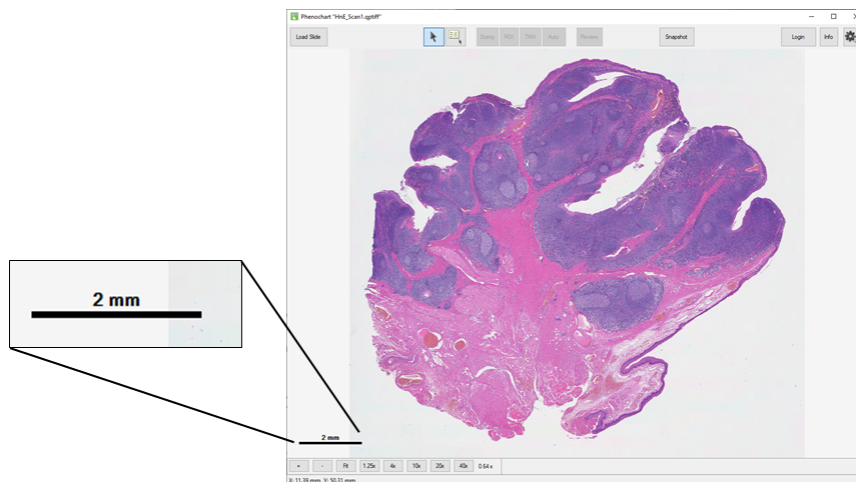


Figure 13. Scale Bar

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

The Scale Bar is displayed on your screen by default. You can hide it by de-selecting **Show Scale Bar** in the Gear Menu at the top right of your screen:

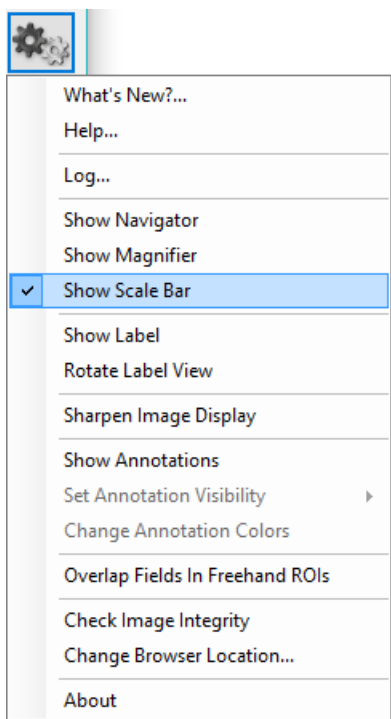


Figure 14. Gear Menu > Show Scale Bar

### 3.4 Label

The Label view is located at the lower right corner of your screen:

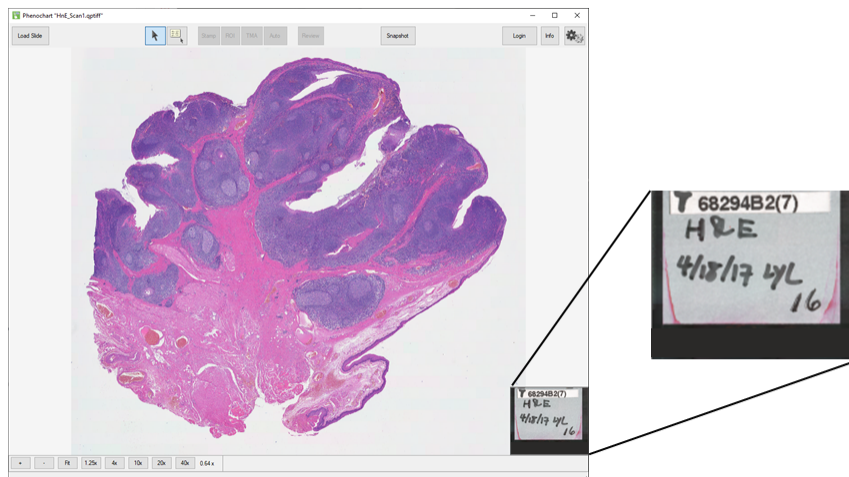


Figure 15. Label View

For Vectra imagery, the Label view displays an image of the slide's label. If necessary, you can rotate the image by clicking on it, or by selecting **Rotate Label View** in the Gear Menu.

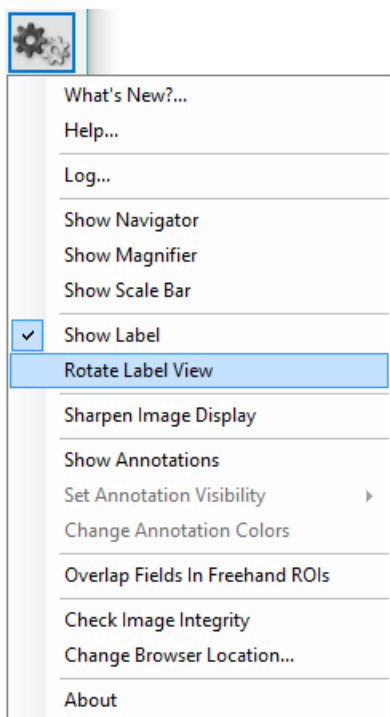


Figure 16. Gear Menu > Rotate Label View

The Label view is displayed on your screen by default. You can hide it at any time by de-selecting **Show Label** in the Gear Menu at the top right of your screen:

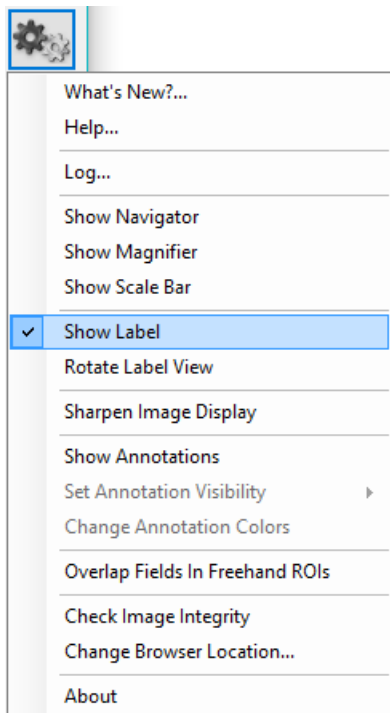


Figure 17. Gear Menu > Show Label

CODEX imagery does not contain a label image. If displayed, the Label view for a CODEX image will display the statement 'No label image available'.

## 3.5 Status Bar

The Status Bar is located at the bottom of your screen.

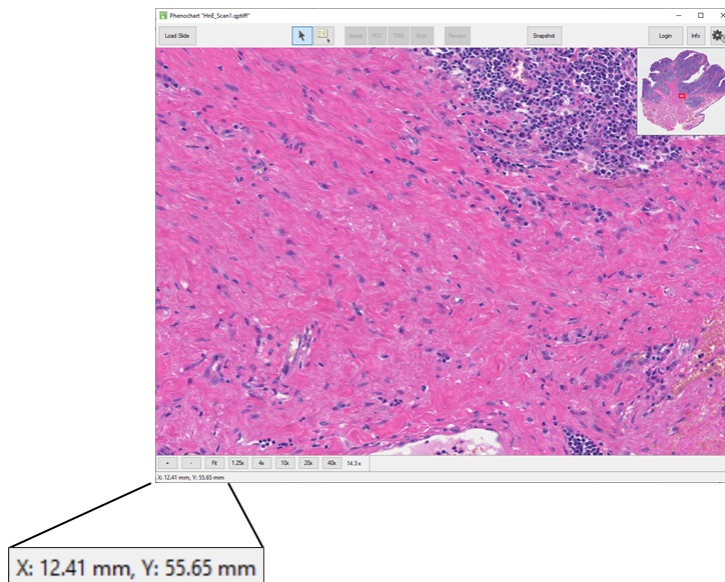


Figure 18. Status Bar

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

The coordinates are shown in millimeters and are updated in real time as you move your cursor 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 your Vectra system hardware.

## 3.6 Intensity Cursor

The **Intensity Cursor** enables you to see coordinate and intensity values in a panel at the location of the mouse cursor.



Figure 19. Intensity Cursor

Intensity Values Displays:

- Brightfield Vectra imagery: the cursor shows standard RGB values ranging from 0-255.
- Fluorescence Vectra imagery (without unmixing): the cursor shows one value for each filter, also from 0-255.
- Unmixed Fluorescence Vectra imagery: the cursor shows normalized counts for each unmixed component. The normalization takes in 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. See the [Unmixing](#) section of the manual for more information.

- CODEX imagery - the cursor shows normalized counts for all available components.

If the pixel at the current position of your cursor is saturated, in the panel the text of the component name and its normalized count value will display red followed by a red asterisk.

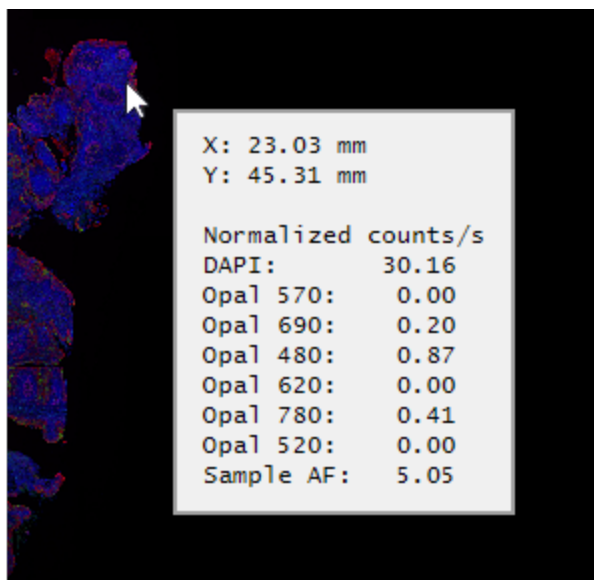


Figure 20. Intensity Cursor with Normalized Counts.

### 3.7 Slide Info

You can get more information about your slide by clicking the **Info** button. The **Info** button is located in the toolbar at the top of the screen:

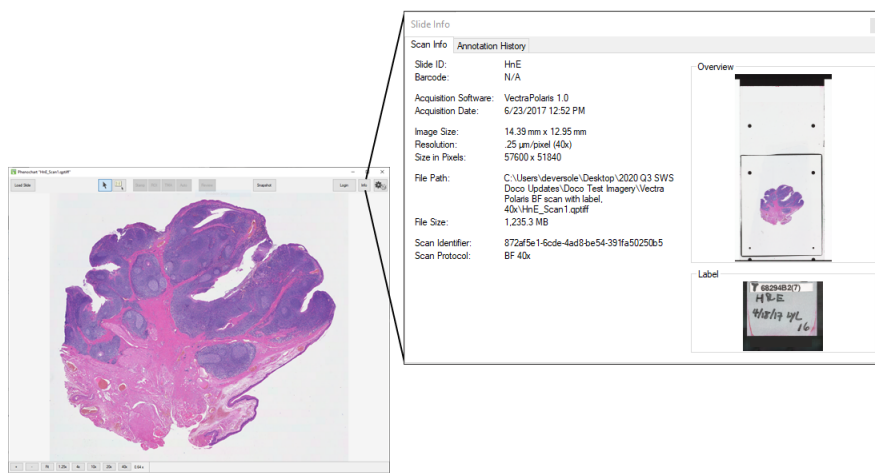


Figure 21. Slide Info Window

Click the **Info** button to open the Slide Info dialog. This dialog shows detailed information about your 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.

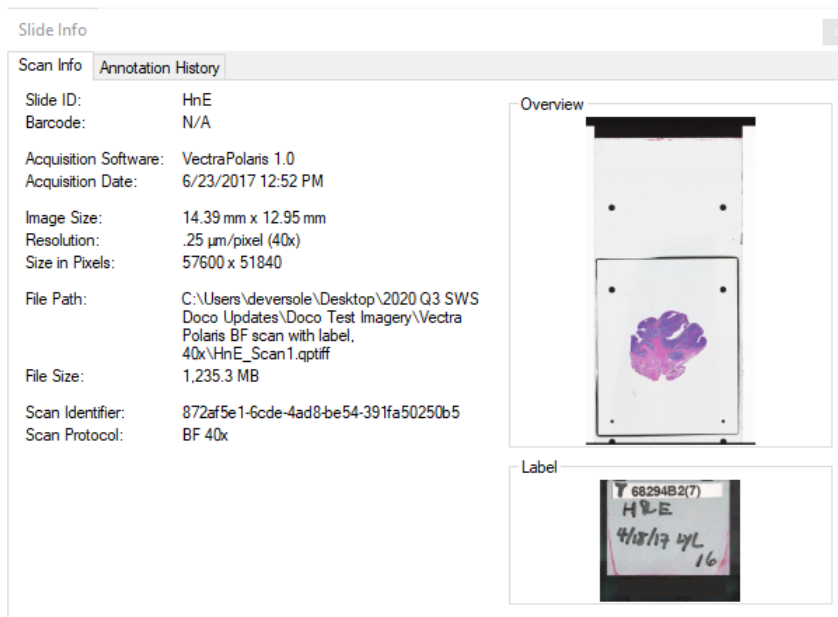


Figure 22. Slide Info - Scan Info Tab

Fluorescence Vectra imagery detailed information also includes filter names and corresponding exposure times.

CODEX imagery detailed information will include marker name and corresponding exposure times. The overview image of the slide will display the DAPI stain. CODEX imagery does not include a Label image.

**NOTE:** See the [Annotations](#) <sup>314</sup> section for information about Annotation History.

## 3.8 Image Sharpening

QTIFF files acquired with Vectra™ Polaris 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. You can turn this off by de-selecting **Sharpen Image Display** in the Gear Menu at the top right of your screen:

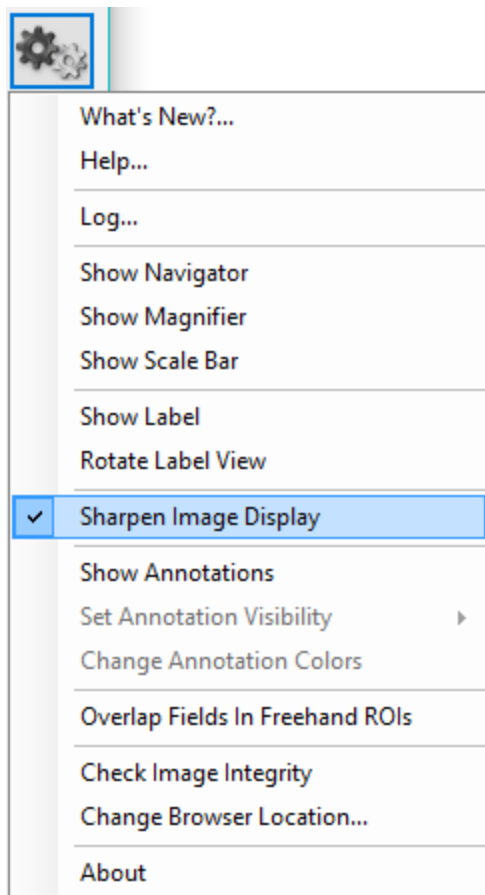


Figure 23. Gear Menu > Sharpen Image Display

## 3.9 Image Integrity

QTIFF files are tamper-evident. You can 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 your screen. The software will perform the integrity check and report the results.



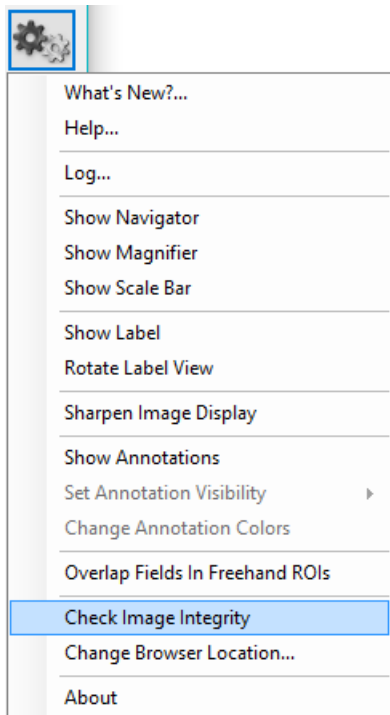


Figure 24. Gear Menu > Check Image Integrity

### 3.10 Fluorescence Layer Toolbar

The **Fluorescence Layer Toolbar** is located in the bottom right of your screen:

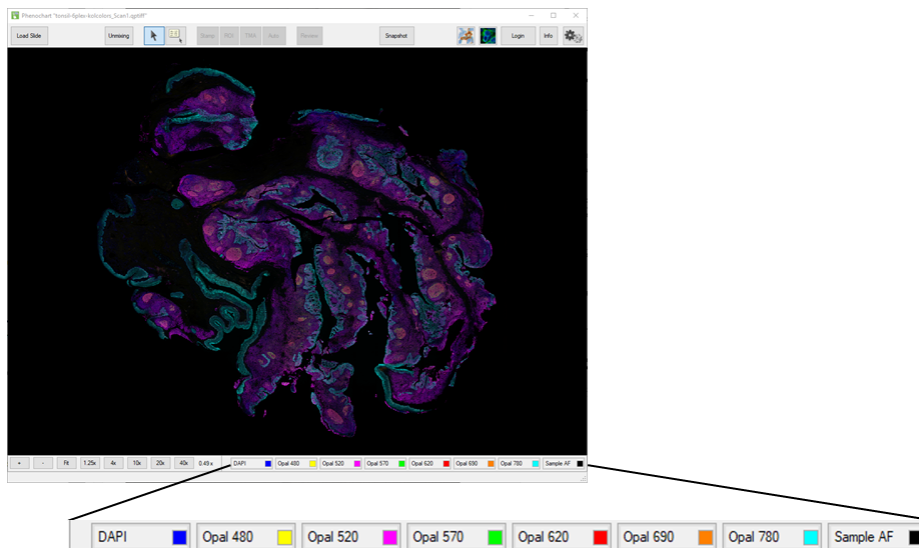


Figure 25. Fluorescence Layer Toolbar

The toolbar is only visible for fluorescent slides. The toolbar enables you to toggle which fluorescence channels are shown, and adjust their brightness or color if you want.

For CODEX imagery, the Layer Toolbar will show one button for each marker.

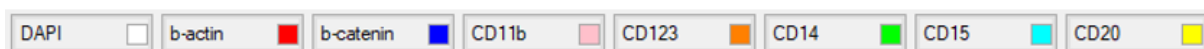


Figure 26. Layer Toolbar Filter Buttons for CODEX Imagery

For Fluorescent Vectra Imagery, the Layer Toolbar will show one button for each filter used during the scan.



Figure 27. Layer Toolbar Filter Buttons for Vectra Imagery

Each **Layer Button** is labeled with the common name for that filter or assigned marker for Vectra and CODEX imagery, respectively. Each layer's color is determined by the color selected in the scan protocol.



Figure 28. Layer Color

You can turn the signal layer on and off by clicking the **Layer Button**. When the button is 'on,' it shows the display color on your image. When the button is 'off,' it is drawn with a 'hatch' pattern.

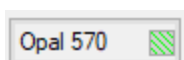


Figure 29. Hatch Pattern

You can change the display color and signal intensity for any layer. Right-click on any **Layer Button** to open the Layer Editor:

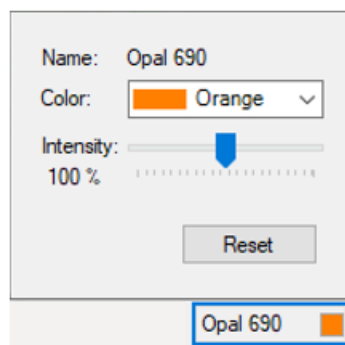


Figure 30. Layer Editor

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

You can also 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 you move your mouse off the editor.

## 3.11 Fluorescence Composite Views

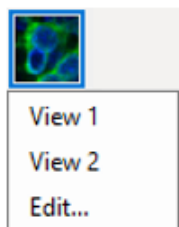
Fluorescence **Composite Views** are intended to simplify visualization of fluorescence slides displaying large numbers of markers.

When an image is first opened, the scan will display the first 8 components designated in the scan protocol. This is the default for both CODEX and Vectra imagery.

### Editing Composite Views

The **Composite Views** editor allows you to select which components you would like to display. You can edit existing views, create new views, and select which components to display per view. The Composite Views editor is only accessible for fluorescent slides and will automatically display when opening a CODEX® image for the first time.

To access the **Composite Views** Editor with Vectra® Imagery, click the **Fluorescence mode** button located in the upper right-hand corner of your screen (to the left of the Login button) and click **Edit**.



*Figure 31.  
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 you can 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.

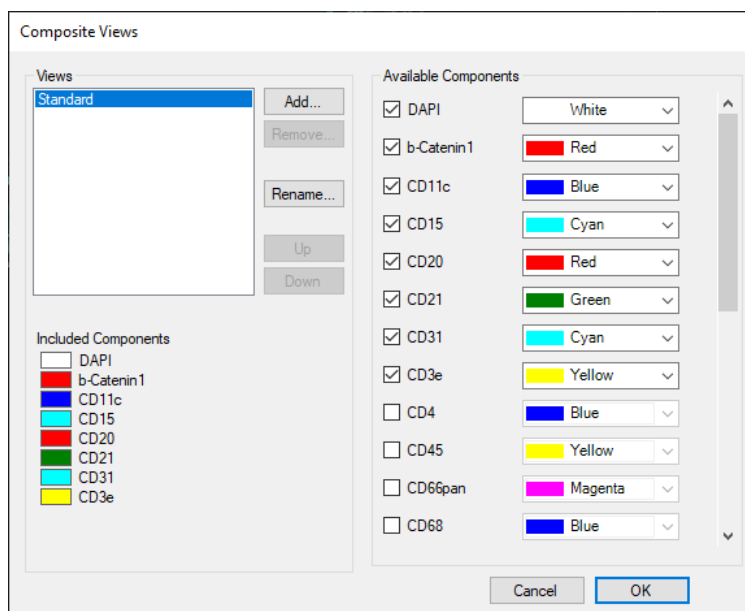


Figure 32. Composite Views Editor

Click **Add** to create additional **Views** for display. You will first be prompted to name your view. After adding a name, select **OK**. You can change the view name at a later time by selecting the view from the **Views** list and clicking **Rename**. The new view will automatically select the first 8 components. To change components, first click the checked boxes next to the name of the components you wish to remove 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, you will be prompted 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 your screen (to the left of the Login button). A drop-down list of all **Views** created will display. Select the view name you want to display.

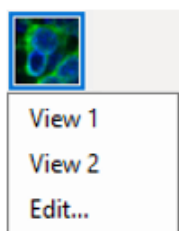


Figure 33.  
Composite View  
Selection

You can also toggle between views by using the up and down keyboard arrows.

## Saving Composite Views

You can save composite view settings when closing the current slide or loading another slide. When prompted, select the **Yes** button in the **Saved Changed Views** Window if you want to save the composite view setting.

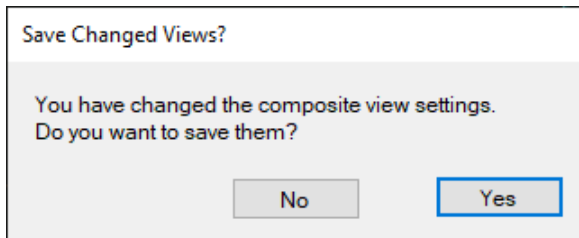


Figure 34. Save Changed Views

Saved Composite View Settings include user generated Views, which signal layers are on and off, layer display color, and layer signal intensity. You will be prompted to save views when closing a slide if you make changes to any of the Composite View Settings.

## 3.12 Legend

The **Legend** is located at the lower left corner of your screen:

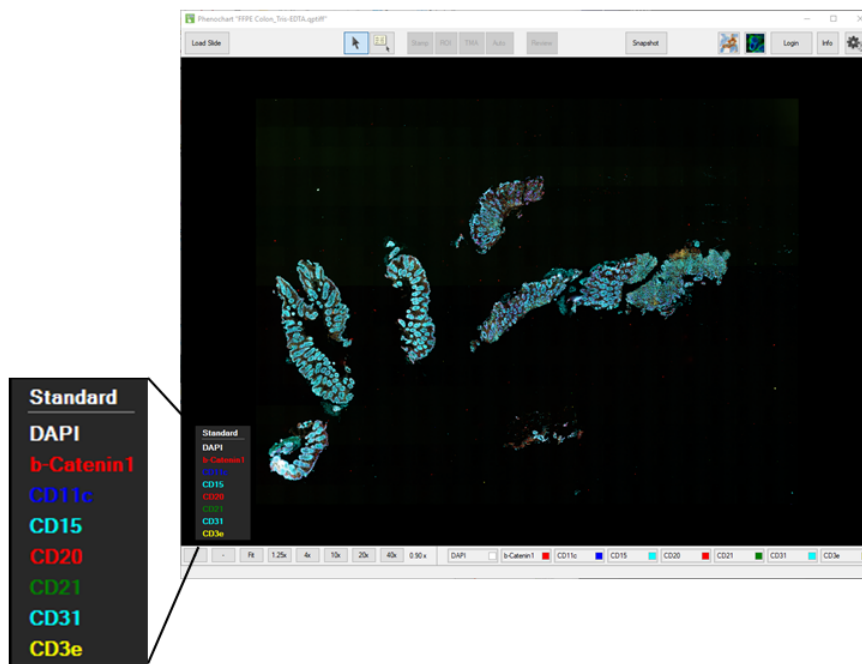


Figure 35. Legend

The **Legend** shows which components are included within the selected Composite View. The Legend is displayed on your screen by default. You can hide it at any time by del-selecting **Show Legend** in the

Gear Menu at the top right of your screen. The Legend is only available when viewing in Fluorescence mode.

### 3.13 Whole Slide Multispectral Unmixing with MOTiF Scans

MOTiF scans acquired with a multispectral slide scan protocol on the Vectra Polaris 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 MOTiFmultispectral slide scans.



Figure 36. Unmixing Button

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 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.

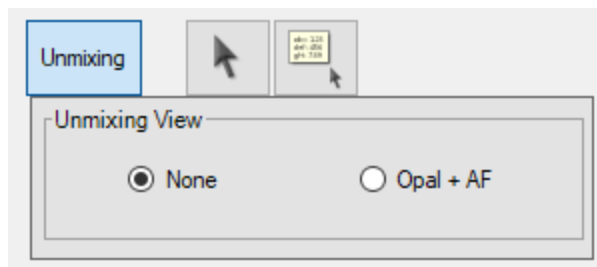


Figure 37. Unmixing View Editor

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

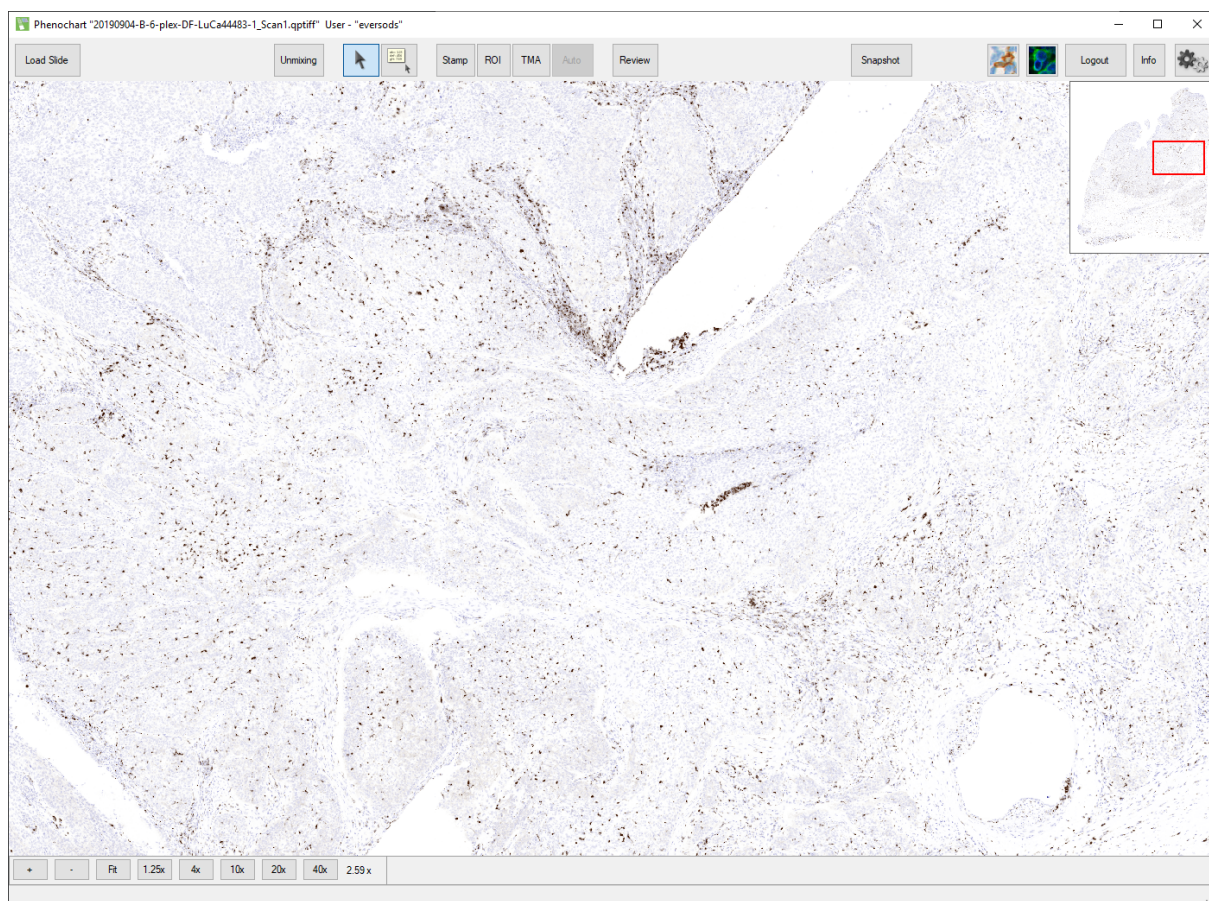
After being opened, the Unmixing View Editor will remain open until you click another button on the toolbar. This allows you to toggle between the original and unmixed views as you navigate the slide.

### 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 H&E images or as simulated DAB/Hematoxylin images.

Simulated Brightfield mode is available for use with:

- Images acquired on the CODEX System
- MOTiF scans acquired with a multispectral slide scan protocol on the Vectra Polaris that have been unmixed

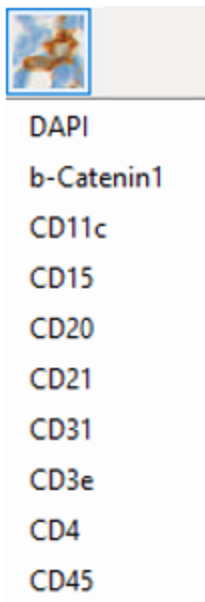


*Figure 38. Simulated Brightfield Mode (Pathology Views)*

**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. CODEX imagery does not include an autofluorescence component and cannot display simulated H&E view

**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 your screen to the left of the Fluorescence mode button. A drop-down list of all available components will display. Select the view name you want to display.



*Figure 39.  
Component  
Selection for  
Pathology Views*

You can also toggle from one component to the next by using the up and down keyboard arrows.

You can change the colors used in Brightfield mode by selecting Change Brightfield Colors in the Gear Menu.



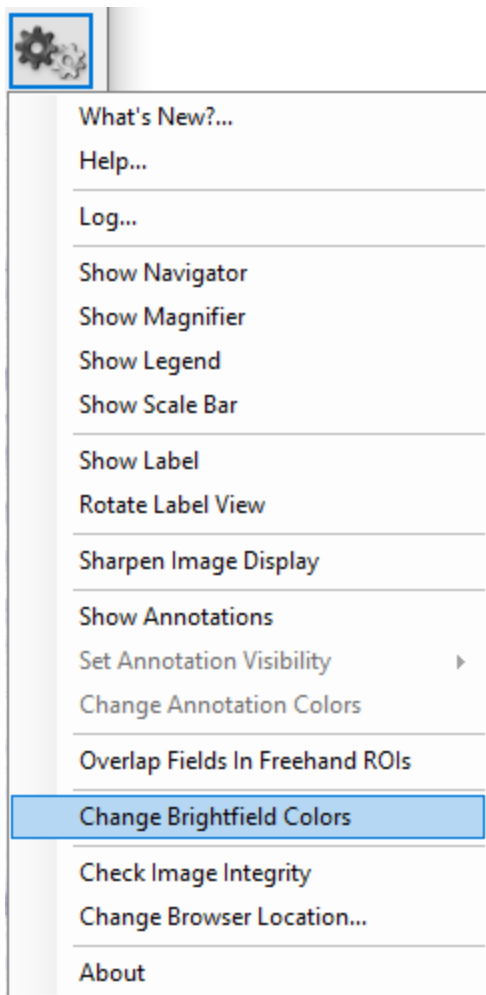


Figure 40. Gear Menu > Change Brightfield Colors

Select your desired colors in the Select Brightfield Rendering Colors dialog. Click Reset if you would like to set them back to the default.

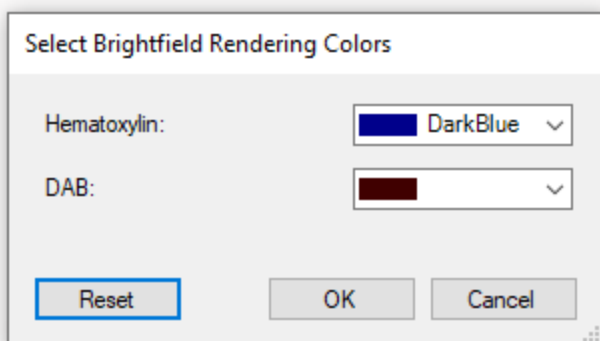


Figure 41 Select Brightfield Rendering Colors

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

## 3.15 Snapshots

The **Snapshot** button allows you to save a picture of the main display area. You can use these images in publications or to share screenshots with colleagues.

Click the **Snapshot** button in the upper right corner of your application window. It will show the Snapshot Editor.

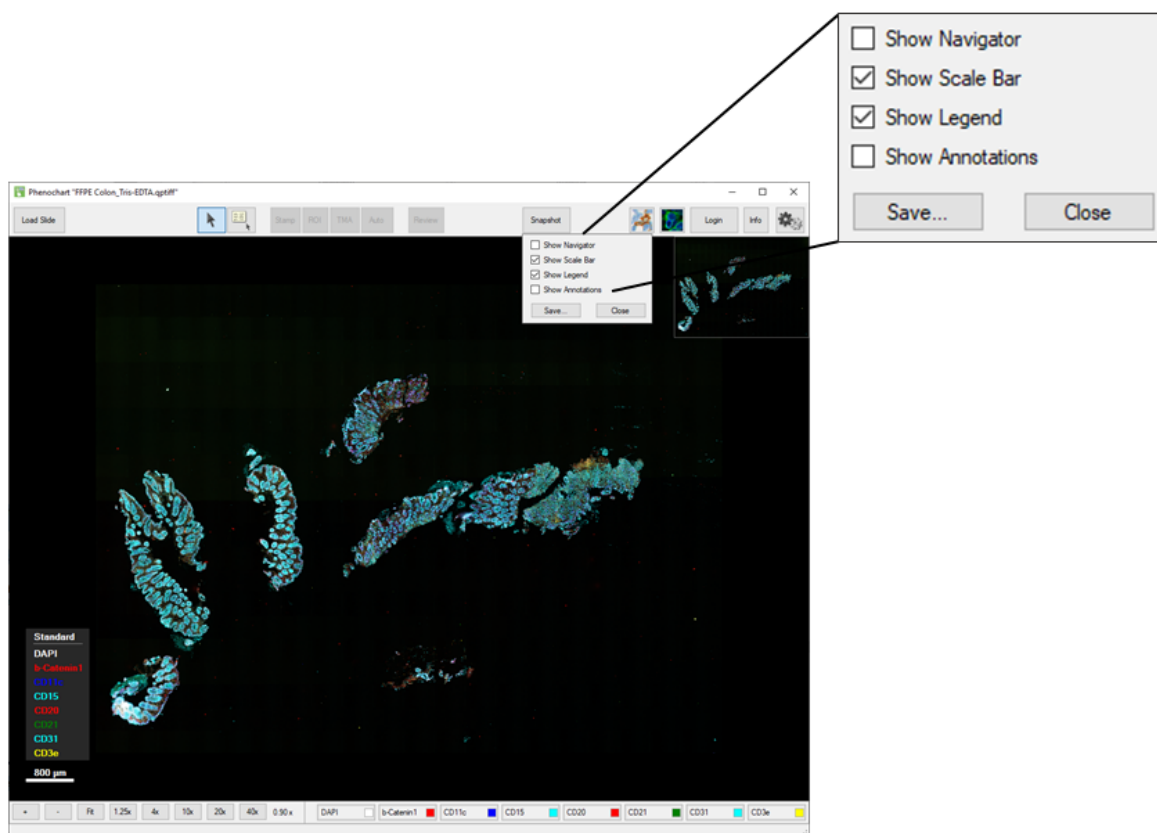


Figure 42. Snapshot Editor Window

Adjust the location and zoom for your slide using the navigation tools. Use the options in the Snapshot Editor to add or remove overlays of the [Navigator Window](#)<sup>[10]</sup>, [Scale Bar](#)<sup>[14]</sup>, [Legend](#)<sup>[25]</sup>, or [Annotations](#)<sup>[31]</sup>. These options affect only the saved Snapshot, not the main window settings.

**NOTE:** When you first launch Phenochart, the Scale Bar overlay is turned on for snapshots. During your session, the software will retain the overlay selections from the last saved snapshot.

When your image is ready, click the **Save** button in the Snapshot Editor. You can navigate to different parts of the slide to take multiple snapshots. When you have saved all your snapshots, click the **Close** button to close the Snapshot Editor.

## 4 Annotations

An annotation is created when you mark an area of interest on a slide for further multispectral imaging by Vectra™ or for analysis in inForm®. There are ways to create annotations: [stamping fields](#)<sup>[33]</sup>, drawing [ROIs](#)<sup>[34]</sup>, finding [TMA cores](#)<sup>[38]</sup>, and [automatically generating fields through inForm](#)<sup>[42]</sup>.

There are different states within each type of annotation.

- **Pending Acquisition** - This annotation has been identified for multispectral field imaging, but has not yet been acquired. This option is only available for slides acquired on a Vectra system.
- **Pending Review** - A reviewer must accept this annotation before it can be multispectral fields can be acquired. This option is only available for slides acquired on a Vectra system.
- **Acquired** - Multispectral fields were taken for this annotation. This option is only available for slides acquired on a Vectra system.
- **Acquisition Failed** - This annotation was slated for the acquisition of multispectral fields, but the acquisition failed to complete correctly. This option is only available for slides acquired on a Vectra system.
- **Rejected** - A reviewer rejected this annotation; fields will not be acquired there. This option is only available for slides acquired on a Vectra system.
- **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 CODEX imagery or slides scanned with Vectra Polaris.
- **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 CODEX imagery or slides scanned with Vectra Polaris.
- **Sent to inForm** - This annotation was sent to inForm for rudimentary analysis or inForm classifier training. This option is only available for slides scanned with Vectra 3.

Annotations are color coded according to their state. See [Annotation Colors](#)<sup>[47]</sup> for how to change the colors, and see [Annotation Visibility](#)<sup>[48]</sup> for how to show or hide types of annotations.

To create or review annotations, you must log in. Click **Login** and enter a username. When you have completed your work, click the **Logout** button to log out.



Figure 43. Login Button

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

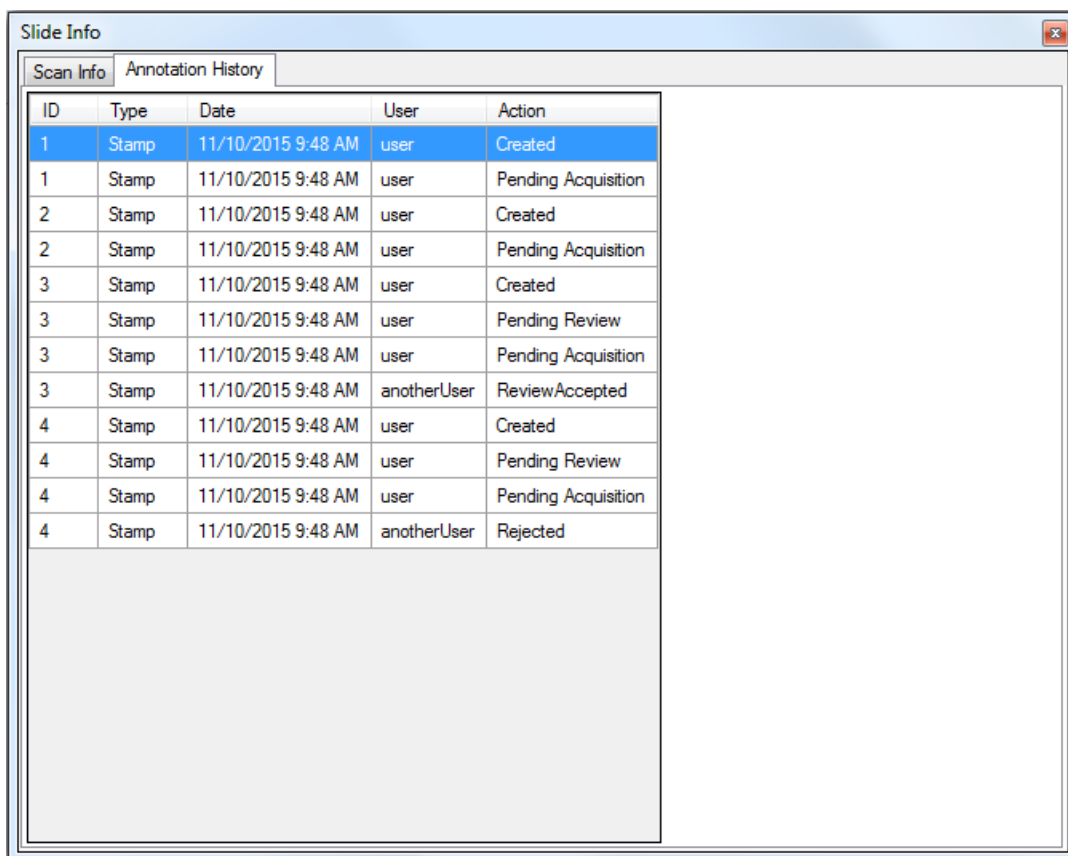
If multispectral fields have already been acquired at your annotation, double-click the annotation to open the acquired fields in inForm.

When the Vectra™ is acquiring multispectral fields for a slide, the annotations for the slide are locked. You will not be able to add or change the annotations until Vectra 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. You can also 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.

You can see the history of a scan's annotations 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 user name. 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.



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 44. Slide Info > Annotation History Tab

You can sort the columns by annotation, date, or user to see the actions taken for each annotation.

## 4.1 Stamps

Stamp annotations allows you to select specific rectangular areas of interest. You must be logged in to create stamp annotations. Click the **Stamp** button to open up the stamp editor:

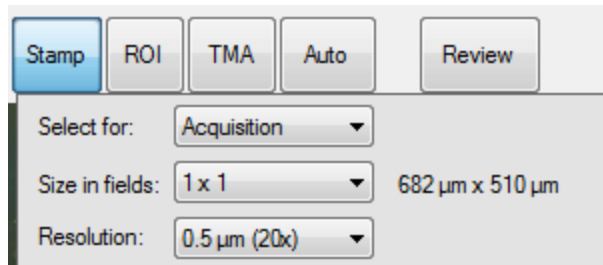


Figure 45. Stamp Editor

There are five different kinds of stamps:

- Choose **Acquisition** to take multispectral fields of the stamp area the next time this slide is on the Vectra™. This option is not available for CODEX imagery.
- Choose **Review** to create stamps that must be reviewed before they can be acquired. Vectra will only acquire multispectral fields of accepted stamp annotations. This option is not available for CODEX imagery.
- 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 Vectra 3, and when inForm 2.2 or higher is installed on your machine. See [Training a Classifier](#)<sup>[44]</sup> for more information.
- Choose **inForm Projects** to select stamp areas for analysis in inForm. Load the qptiff into inForm 2.4.3 or later to analyze these fields. These stamps can only be made at native resolution. This option is available for CODEX imagery and slides scanned with Vectra Polaris.
- Choose **inForm Batch** to select stamp areas for batch analysis in inForm. Batch process the qptiff in inForm 2.4.3 or later to analyze these fields. These stamps can only be made at native resolution. This option is available for CODEX imagery and slides scanned with Vectra Polaris.

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 Vectra 3 systems, you can only acquire 0.25 micron/pixel (40x) fields if you have a 40x objective installed.

Your cursor will show a rectangle which previews the stamp size based on your settings. Click on the slide to create a stamp at that location. You can create multiple stamps. 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 you have selected a resolution in error, you will need to ignore or delete the pending fields. If you wish to acquire fields at multiple resolutions, stamp and acquire the fields at one resolution first. You may then return to stamp and acquire fields at other resolutions.

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

## 4.2 ROI (Region of Interest)

The ROI (Region of Interest) Tool allows you to draw a region of interest to:

- Acquire multispectral images on a Vectra system
- Batch analyze fields using inForm

The ROI (Region of Interest) Tool creates stamp annotations corresponding to the region you draw.

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

- Choose **Acquisition** to take multispectral fields of the stamp(s) the next time this slide is on the Vectra™.
- Choose **Review** to create stamps that must be reviewed before they can be acquired. Vectra will only acquire multispectral fields of accepted stamps.
- Choose **inForm Batch** to create stamps for batch analysis in inForm. Batch process the qptiff in inForm 2.4.3 or later to analyze these fields. These stamps can only be made at native resolution. This option is available for CODEX imagery and slides scanned with Vectra Polaris.

You can also use the ROI Editor to select your resolution.



Figure 46. ROI Editor

When you have made your selections, draw a region of interest on the slide by clicking and dragging an outline. If you don't close the area, it will be closed for you when you release the mouse button. The software will then create tiled stamp annotations that contain the area you have drawn.

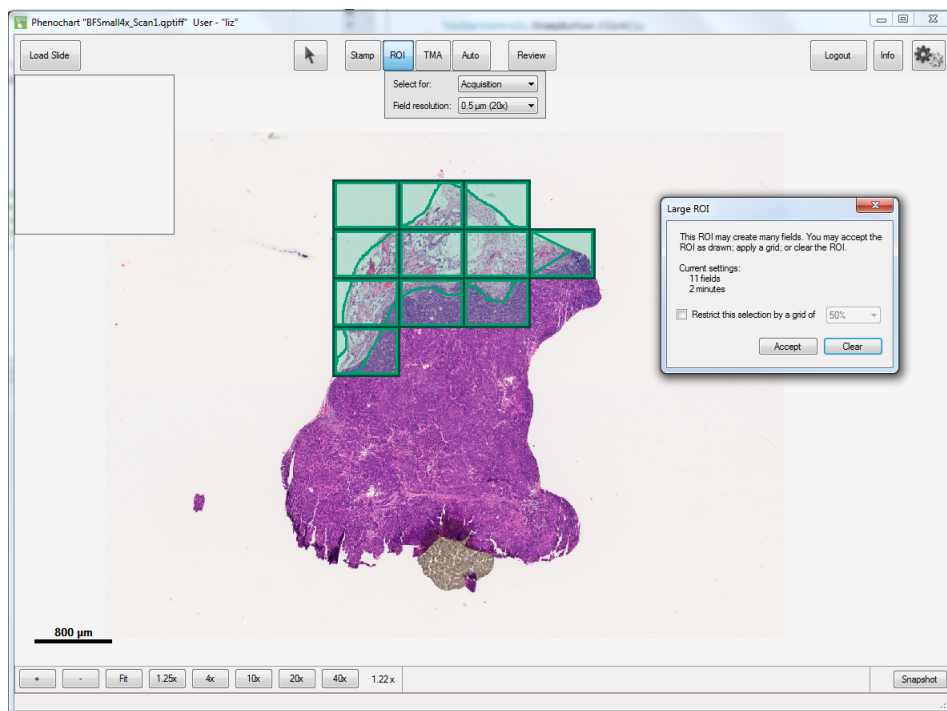


Figure 47. Tiled Stamp Annotations

If your 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 you are drawing regions for Acquisition or Review, you will see a Confirmation Dialog, which will tell you how many fields you are about to take. It will also give you an approximate time for the acquisition. You may want to change your region based on how long it will take to image.

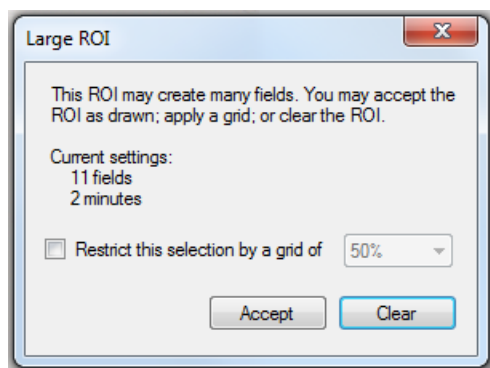


Figure 48. Large ROI Window

To reduce the number of fields within your region, you can apply a grid. Use the grid drop-down list to choose the percentage of fields for a gridded sample of stamp annotations. As you change the percentage, 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 you would like the fields to overlap by a small percentage, select **Overlap Fields in Freehand ROIs** from the Gear Menu.

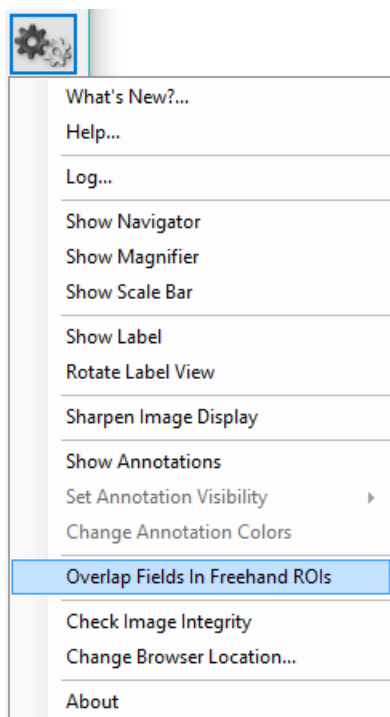


Figure 49. Gear Menu > Overlap Fields In Freehand ROIs

Click the **Accept** button to accept the ROI as shown, and the system will add all of 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.

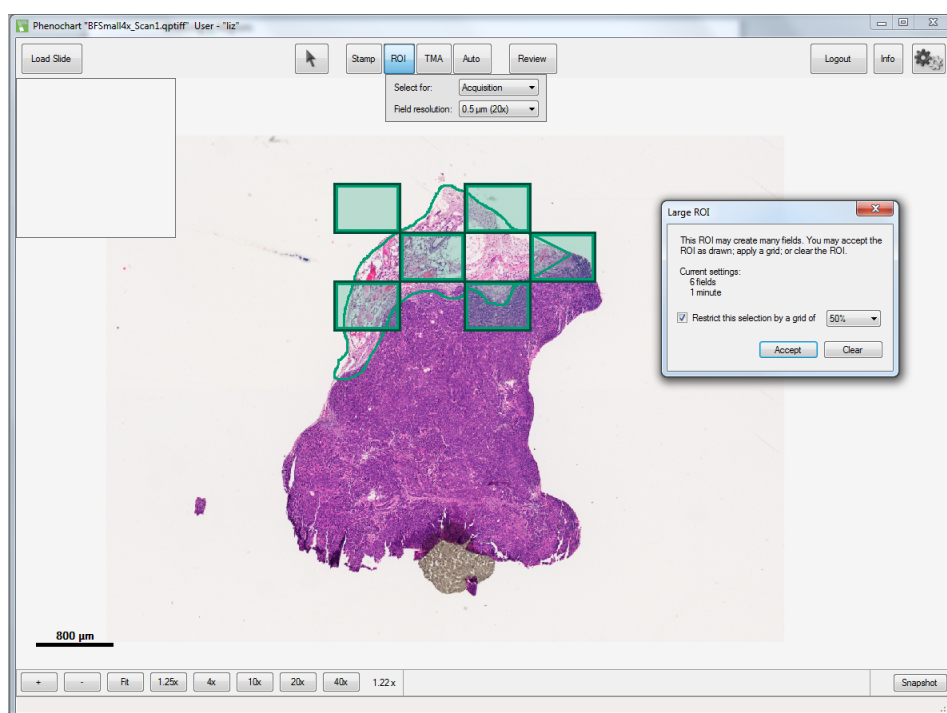


Figure 50. Accept ROI

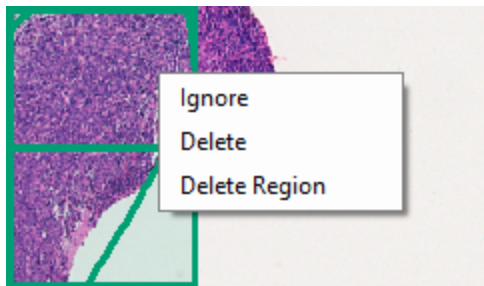


All pending annotations (acquisition or review) must be the same resolution. If you have selected a resolution in error, you will need to ignore or delete the pending stamp annotations. If you wish to acquire stamps at multiple resolutions, draw a region of interest and acquire the annotations at one resolution first. You may then return to draw regions and acquire annotations at other resolutions.

If you need to navigate to bring your region of interest in to view, hold the **Ctrl** key down and use your mouse to zoom and pan.

If you create an incorrect ROI, you can right-click on any stamp in the region. If you then click 'Delete Region', all the stamps in that region will be deleted. If you click 'Delete', only the stamp you clicked on will be deleted.

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



*Figure 51. ROI Right-Click Menu*

## 4.3 TMA

Tissue Micro-Array (TMA) annotations let you locate TMA cores within whole-slide images and analyze or acquire multispectral fields of each TMA core. You can 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.

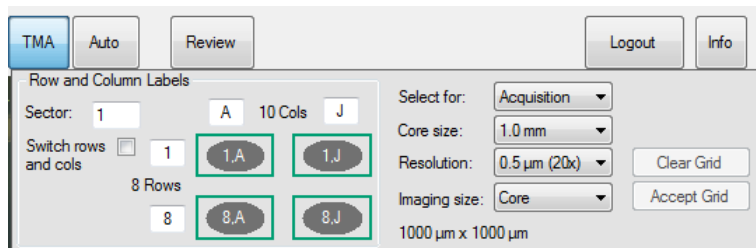


Figure 52. TMA Editor

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

#### Row and Column Labels

Select the **Sector** number for your TMA sector. Then choose the appropriate labels for your 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 you 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 you have columns that go across and rows that go 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 you select cores for review, only the accepted cores will be acquired. If you select cores for inForm Batch, only the scan's native resolution can be used. inForm Batch is only available for slides scanned with Vectra Polaris.

Choose the size of your TMA cores. If the exact core size isn't listed, choose the next larger one.

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

Choose an imaging size for your cores:

- **Core** will take a square image of exactly Core Size x Core Size microns.
- **Core + 10%** and **Core +20%** will give you square images with a margin to account for slight variations in core size.
- **NxN** fields are rectangular fields as described. This option will enable you to take imagery consistent with early versions of Vectra. Note that these fields may take significant extra area around the core, and may include neighboring cores.

## Drawing the Sector

### Finding Cores

Once you have set your parameters, click and drag to draw a rectangle around the entire sector. If you need to navigate to bring your entire sector in to view, hold the **Ctrl** key down and use your mouse to zoom and pan.

While you're drawing, your display will look something like this:

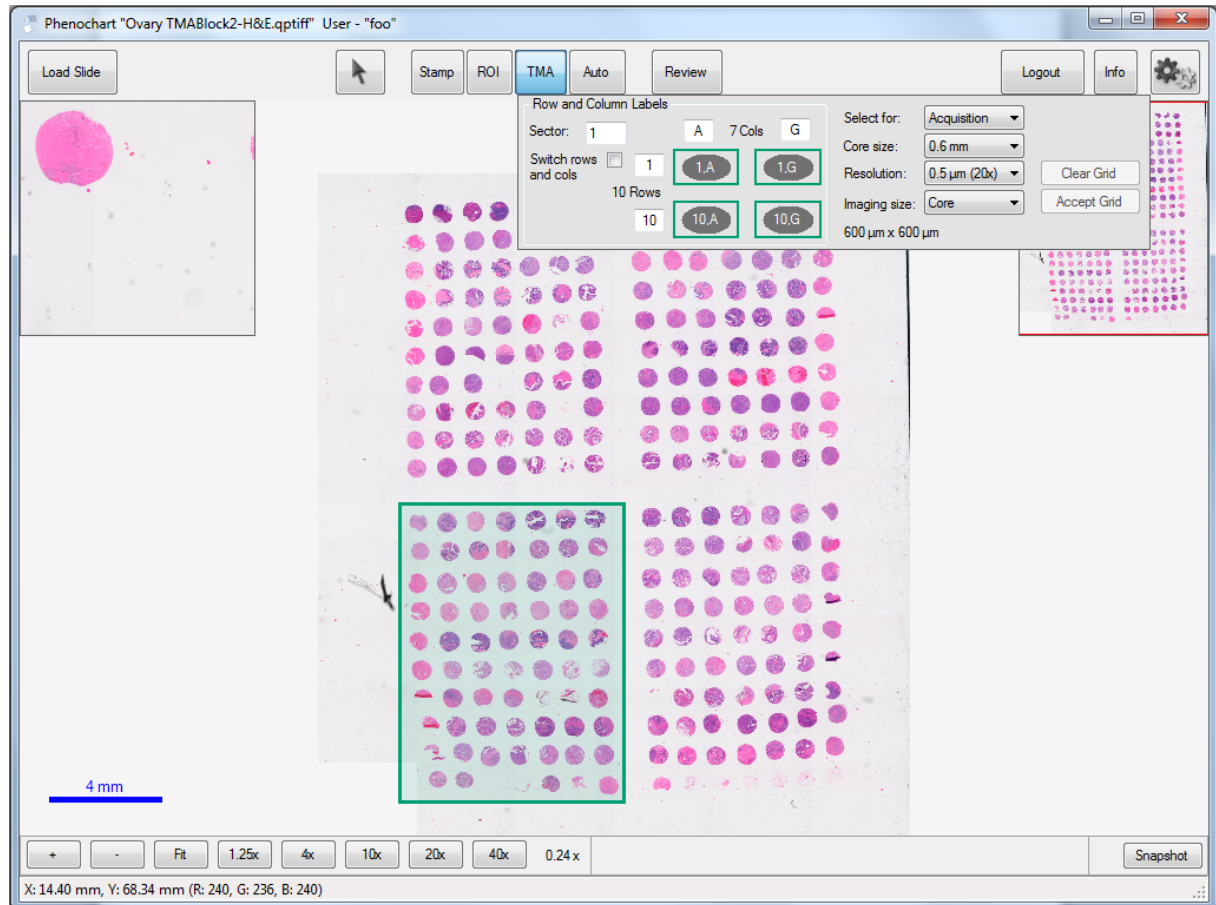


Figure 53. Drawing the Sector Display

When you release your mouse, Phenochart will find the cores within the rectangle. Each core will be labeled. If the labels aren't visible, zoom in to see them.

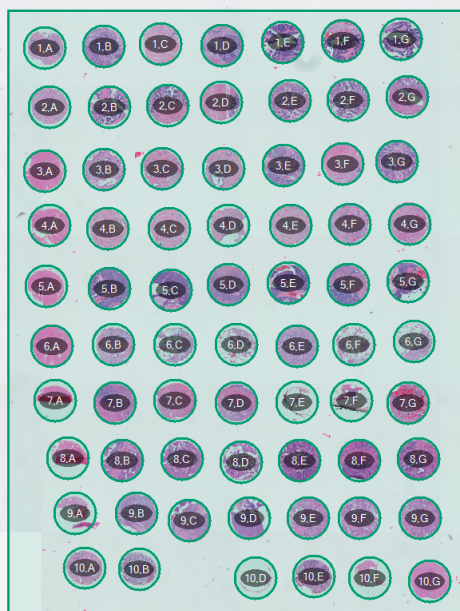


Figure 54. Labeled Cores

## Adjusting the Cores

You can adjust your settings after the cores are found. The software will update immediately to the new settings.

If the core finding missed a core, you can 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.

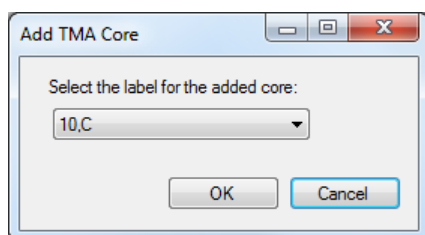


Figure 55. Add TMA Cores Window

There may be cases where you need to further refine the results of the core finding. To tweak the position of a single core annotation, hold the **Ctrl** key + click and drag on the annotation to move it. Be sure your settings are correct before adjusting cores. You will lose the individual core tweaks when you change your settings and the system refinds the cores.

Right-click to ignore a core or to bring back an ignored core. You can also delete a core, or delete the entire sector if necessary.



*Figure 56. TMA Right-Click Menu*

## Accepting the Grid

---

Click **Accept Grid** to complete your TMA annotation. After this, you cannot make any further changes.

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

You can continue to add additional sectors if desired/necessary.

## Common Issues and Resolutions

### Numbering

If the software finds more or fewer cores than you've drawn, you will see a warning in red. You may need to change your row and column labels so the number of cores found matches the number you 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 you have selected, you will see a warning in red. 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 your 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.

## 4.4 Automatically Generated

Phenochart allows you to automatically select fields of interest using a trained inForm<sup>®</sup> classifier. This will only be available on computers with an activated version of inForm<sup>®</sup> Tissue Finder<sup>™</sup>, version 2.2 or higher.

You can use inForm to add fields for acquisition or review, or to preview how inForm will select fields on your slide during a fully automated Vectra<sup>™</sup> slide scan.

Click the **Auto** button to open the editor.

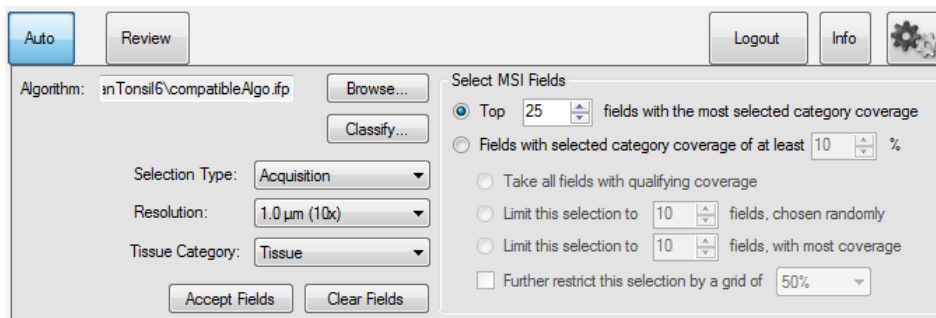


Figure 57. Auto Editor Window

Click the **Browse...** button to load an inForm algorithm or project that you've trained for this type of slide. If you haven't created an algorithm yet, please see the section [Training an inForm Classifier](#)<sup>[44]</sup>.

Phenochart will confirm that the algorithm is valid for your slide. This takes a few seconds.

Click **Classify** to classify the slide once the algorithm is validated. Depending on your algorithm settings and the size of your slide, this can take anywhere from a few to upwards of ten minutes.

Adjust the selection settings when the slide has been classified to see which fields will be chosen.

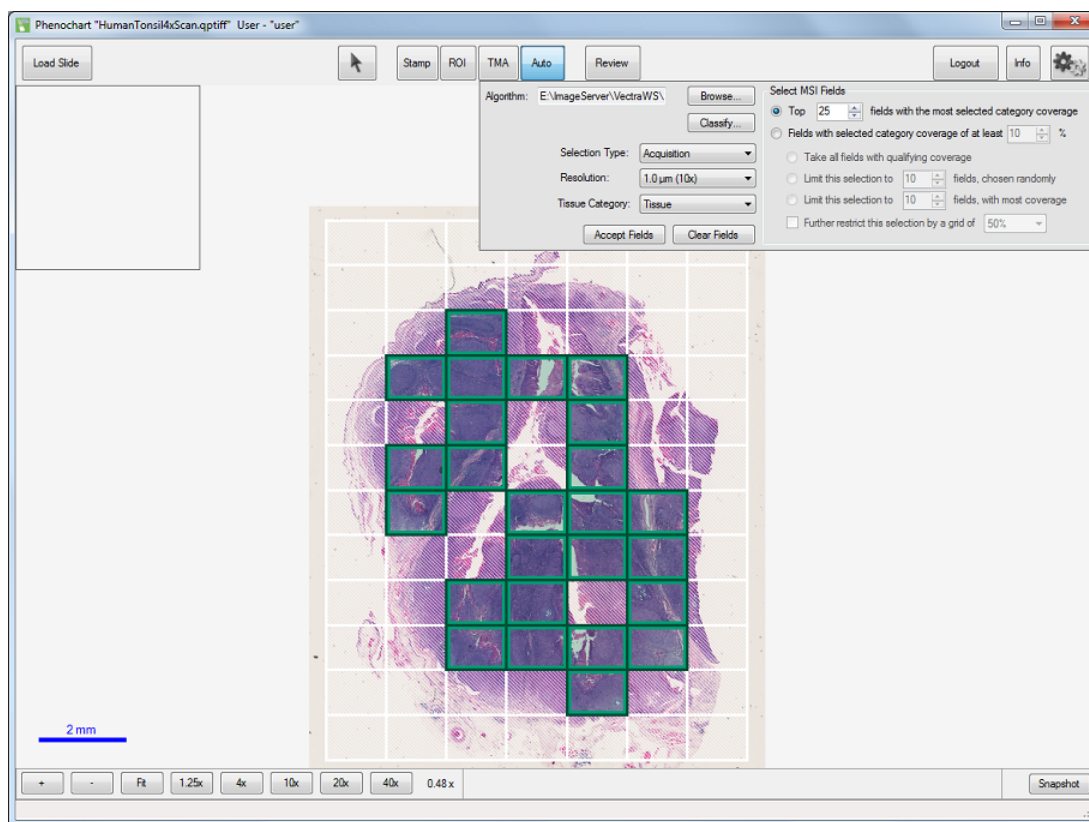


Figure 58. Classified Slide

The fields on the screen update immediately as you change the selection settings.

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 Vectra.

Choose the **Resolution** to be used when acquiring your fields.

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

You will need to determine an MSI (multispectral imaging) Strategy to determine 1) how many fields you will take, and 2) how strong they are in the category of interest. This allows you to get the fields you are most interested in, with the most efficient use of time and disk space.

There are two types of strategies:

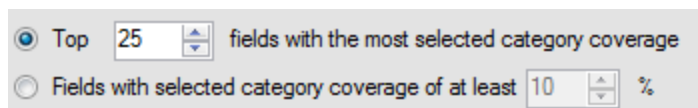


Figure 59. Select MSI Fields - MSI Strategies

You can choose the top fields in your Tissue Category of interest. If you choose this, inForm will select the fields with the greatest coverage of your Category of Interest.

You can also choose from any fields that have at least some coverage of your Tissue Category. If you choose this, 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.

If you choose the **Fields with selected category coverage of at least X%** option, you will need to choose if you want to take:

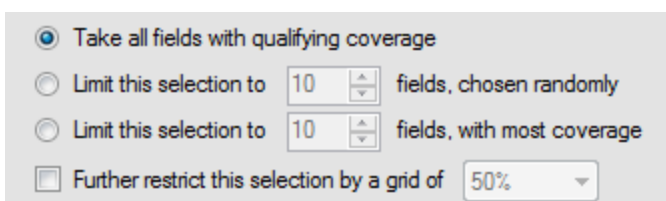


Figure 60. Coverage of % Options

- All the fields that meet the threshold
- A selected number of randomly chosen fields that meet the coverage threshold. (Note: With this option, you may get fewer fields than you asked for.)
- A selected number of fields with the most coverage of the Tissue Category of Interest. These fields must meet the minimum coverage threshold. (Note: Again with this option, you may get fewer fields than you asked for.)
- Using a minimum threshold can result in a significant number of fields. You can further limit the selected fields to a grid by selecting the grid coverage.

As you adjust the settings listed above, your fields will update in real time to show you which fields meet your selection criteria. This is useful for classifier experimentation and testing for fully automated Vectra protocols.

Phenochart will show the fields that inForm has selected as well as the fields that were ignored (because they did not meet the selection criteria). If you would like to override the inForm decision, you can do so by using the right-click menu. The right-click menu gives you the percentage of your class of interest. Toggle to ignore or acquire/review that field.

When you are 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.

#### 4.4.1 Training an inForm Classifier

inForm® Tissue Finder may be used from Phenochart and Vectra 3™ to automatically select Multispectral fields for acquisition or review. 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 and Vectra 3.

To train a classifier in inForm, select the fields for training on your slide using Phenochart's stamp tool. Within the **Stamp** tool, select **Push to inForm**, and select the fields that represent all tissue categories that you need. inForm will launch automatically and load the fields. You can load additional slides and stamp additional fields within Phenochart.

When you have finished selecting fields within Phenochart, switch to inForm to train your classifier:

1. Configure your project to have a Trainable Tissue Finding Step.
2. It is often beneficial to downsample your 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.



Image Preparation Settings

Image Format: PKI Tiff Extract ▼

Sample Format: Brightfield ▼

☒ Change resolution

Factor: 4 : 1 (Lower) ▼

Figure 61. Prepare Images - Downsample

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

Image export options

Image Output Format: TIFF ▼

Images to export:

☐ RGB

☐ RGB with Tissue Segmentation Map

☐ Composite Image

☐ Component Images (multi-image TIFF)

Maps to export:

☒ Segmentation Maps (multi-image TIFF)

Figure 62. Maps To Export - Segmentation Maps

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

Return to Phenochart if you would like to test out your classifier. Click **Auto** to open the automatic field editor, load your project, and click **Classify**. If you feel that the classifier needs more training, you can push additional fields into inForm and continue to train and test until you get satisfactory results.

## 4.5 Annotation Menu Options

Use the Gear Menu to adjust the visibility and color of your annotations:

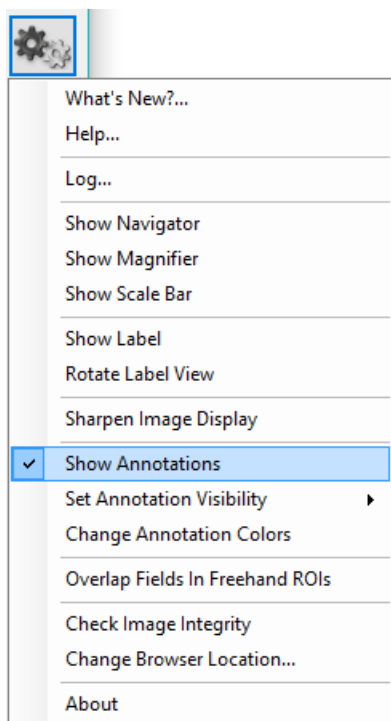


Figure 63. Gear Menu - Show Annotations

Click **Show Annotations** to see all annotations you have selected to be visible. (Note: See below for how to customize your visibility settings). Uncheck **Show Annotations** to hide all annotations on your slide.

Use the **Set Annotation Visibility** submenu to customize visibility settings for each annotation type:

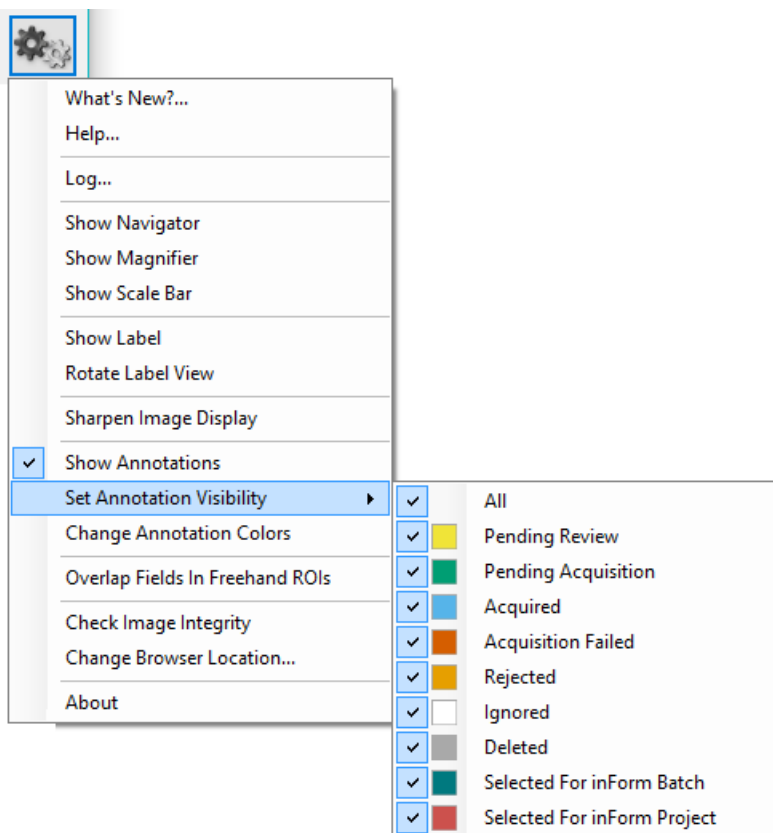


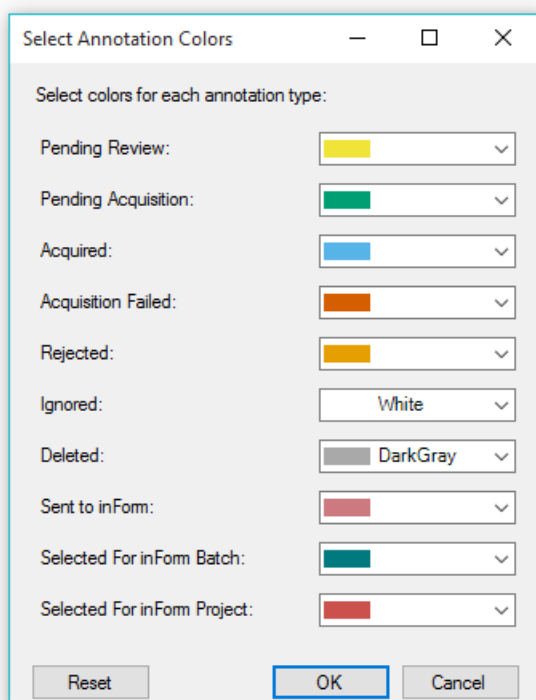
Figure 64. Gear Menu - Set Annotation Visibility

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 you first start Phenochart, Ignored and Deleted annotations are hidden by default. Any changes that you make to the type visibility will be remembered for the next session.

#### Annotation Colors:

The default annotation colors are chosen to be distinguishable, even by most color-blind people. You can change the colors as needed. Select **Change Annotation Colors** from the Gear Menu to bring up the color changing dialog:



*Figure 65. 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 your changes. **Cancel** will exit without saving your changes. Your color settings will be saved across sessions.

## 4.6 Review

Annotations that have been selected for Review must be accepted before they will be acquired by Vectra™. Click the **Review** button to open the review editor.

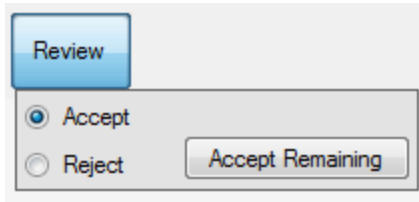


Figure 66. Review Window

Choose **Accept** to accept annotated fields or cores. When you move your mouse over an annotation awaiting review, the cursor will change to a checkmark.



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

Choose **Reject** to reject annotations. When you move your mouse over an annotation awaiting review, the cursor will change to an x.



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

Click **Accept Remaining** to accept all remaining un-reviewed fields. You will get a confirmation dialog telling you how many fields you are about to accept.

You can use the right-click menu to **Accept** or **Reject** fields and to **Accept Remaining**. You can also use the right-click menu to un-review a field or change your review of a field.

## 5 Appendix A: Support

### Log File:

---

When you contact Customer Care for a Phenochart issue, you will be asked for the log file. Including the log file with your initial report will aid in solving the problem.

If the issue has just occurred, select **Log...** from the Gear Menu to get the current log file.

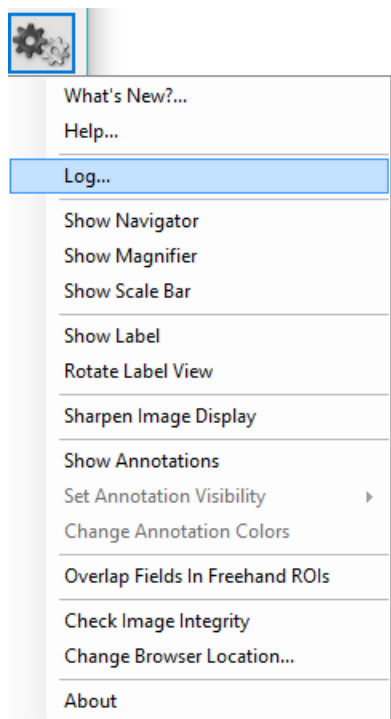


Figure 67. Gear Menu - Log...

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.

You can either attach the log file or cut and paste the log file contents into your correspondence.

### Config File:

---

In some cases, you may be asked for the Phenochart.config file. You can find this file in C:\ProgramData\Akoya\Phenochart\.

By default, this directory is hidden from view. Use your Windows browser if you need to view this folder:

1. Select **Organize** and then **Folder and search options**.
2. Click the **View** tab.
3. Select **Show hidden files, folders, or drives** and then click the **OK** button.

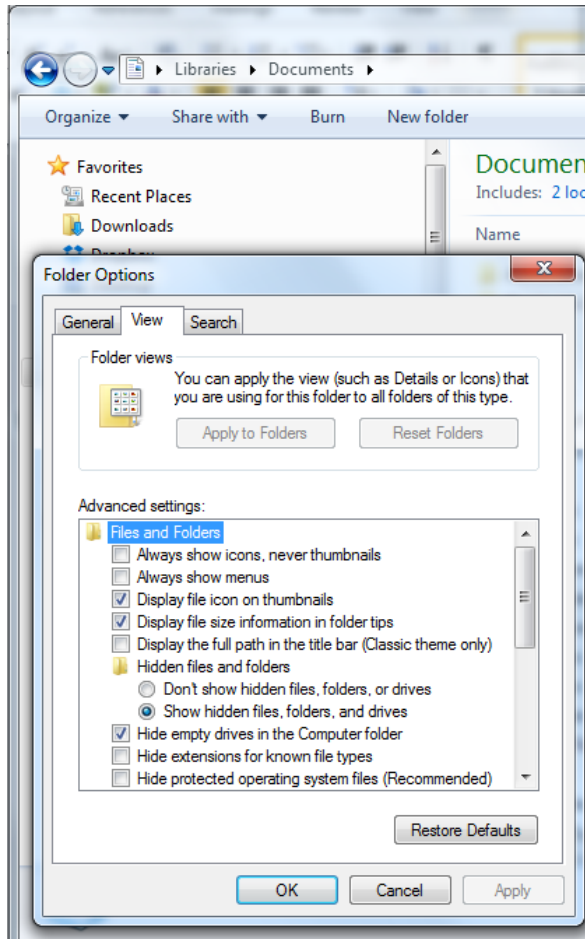


Figure 68. Folder Options - View - Files and Folders

## 6 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 use stripped format.

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.

A thumbnail RGB image is provided, and this is a good image to use as an icon in graphical image lists. 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 1. Images**

Description	RGB/mono	Title/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	Stripped	~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	Stripped	~2000 x 4000	Required for whole-slide scans



				Optional for simple RGB images and extracted components
Label image	RGB	Stripped	~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 2. TIFF tags**

TIFF Tag	Optional?	Description of contents
Software		Starts with “PerkinElmer-QP”
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)
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 res 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
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).

### Image Description 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 the example below.

**Table 3. Image Description tags**

Tag	Optional?	Contents
DescriptionVersion		Version of the image description field itself, a single number. This document describes version 2 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.
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 1), with the following values: <ul style="list-style-type: none"> <li>• FullResolution</li> <li>• ReducedResolution</li> <li>• Thumbnail</li> <li>• Overview</li> <li>• Label</li> </ul>
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).

SignalUnits		<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"> <li>– raw counts</li> <li>– normalized (counts/second/gain/full-scale/binning)</li> <li>– OD (optical density)</li> <li>– dark-corrected counts</li> </ul> <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"> <li>– average across all bands</li> <li>– total summed signal across all bands</li> <li>– peak signal in highest-valued band</li> </ul> <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.
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 example, 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, ScaleFactor would be 1.3201. Default is 1.
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	<p>String with the FL lamp type</p> <ul style="list-style-type: none"> <li>Unknown (default)</li> <li>PriorLumen200</li> <li>PriorLumen220</li> <li>XCiteMultiBandLed</li> <li>PriorMimicLed</li> <li>SolaLed</li> <li>Colibri7</li> </ul>
InstrumentType	Y	<p>String with the instrument type, one of the following:</p> <ul style="list-style-type: none"> <li>Unknown (default)</li> <li>Keyence700</li> <li>Keyence800</li> </ul>

		<ul style="list-style-type: none"> <li>• AxioObserver</li> <li>• Dmi8</li> <li>• Polaris</li> <li>• Vectra3</li> </ul>
CameraType	Y	String with the camera sensor type, one of the following: <ul style="list-style-type: none"> <li>• SonyICX285AL</li> <li>• HamamatsuC11440</li> <li>• IMX265</li> <li>• IMX421</li> </ul>
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 by sub-elements below
Band		Description of a band with sub-elements 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 OEM supplier
PartNo		String with the manufacturer part number
Bands		Collection of bands each as described by sub-elements below
Band		Description of a band with sub-elements noted below
Name		String with the band name
Cuton		Cuton wavelength of this band in nm
Cutoff		Cutoff 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
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"> <li>• 'Normal' (default)</li> <li>• 'Rotate180'</li> <li>• 'MirrorV'</li> <li>• 'MirrorH'</li> </ul>
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. The contents of the <Filter> and <Band> tags are described below.

**Table 4: Contents of Filter and Band tags**

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 / ( $2^{\text{bit depth}}$ × exposure time × gain × 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 1×1, 4 for 2×2, etc.
Date	The date and time of the reference image in UTC, ISO 8601 format.
FilterID	Detailed description of the acquisition filter, as {ExcitationFilter band name}_ {ExcitationFilter manufacturer}:{ExcitationFilter part number} / {EmissionFilter manufacturer}:{EmissionFilter part number}  The {ExcitationFilter band name}_ prefix may be omitted if there is only a single excitation band.

## 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 >

```

## 7 Appendix C: EULA

The following is an agreement (the "Agreement") between you and Akoya Biosciences, Inc., 100 Campus Drive, 6th Floor, Marlborough, MA 01752 ("Akoya") for software known as Research Use Only Phenochart, including any updates, upgrades or enhancement provided by Akoya and any and all accompanying documentation (collectively, the "Software").

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