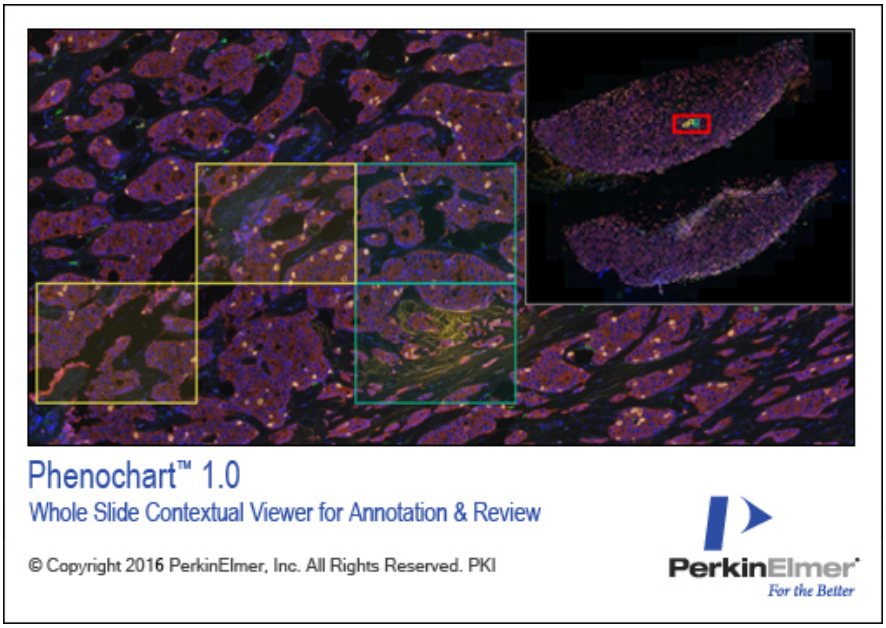
Phenochart™

Whole Slide Contextual Viewer
for Annotation & Review

User's Manual



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This manual describes how to use Phenochart version 1.0.2 software.

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1 Overview

Phenochart is a slide viewer for scans created by the Vectra 3 Quantitative Pathology Imaging System.

Phenochart allows you to:

- View and navigate around whole slide images
- Select areas of interest for further review and multispectral imaging
- Identify TMA cores for imaging

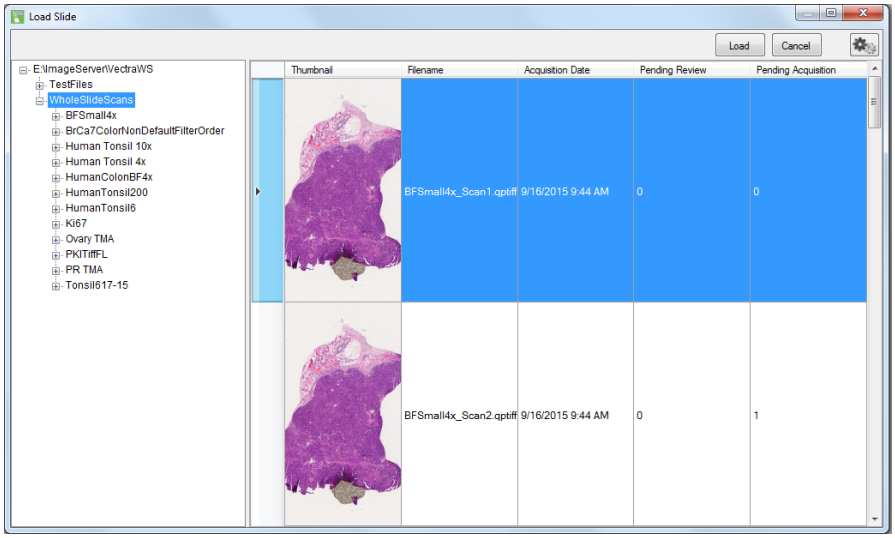
Phenochart is freely available and can be installed on multiple machines at your site.

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

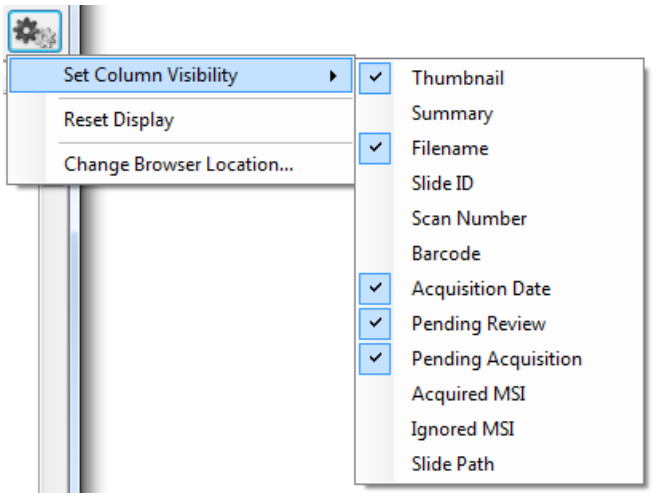


The list of studies is on the left. You can browse deeper to see slides within a study.

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.

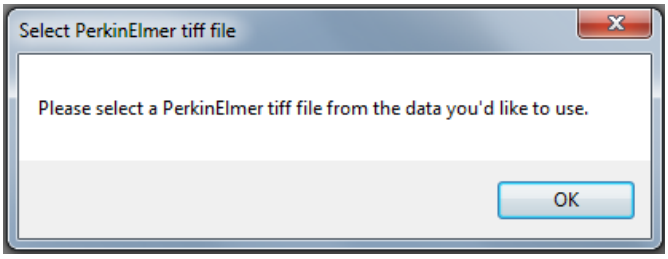
By default, the browser will show the slide thumbnail, a summary column, and columns showing the number of annotations pending acquisition or review. You can add other columns. Select 'Set Column Visibility' from the browser's Gear Menu to select which columns are shown in the table:



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:

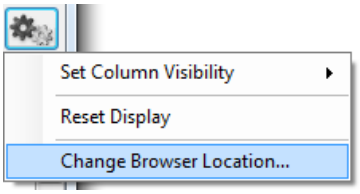
The very first time you launch the Slide Browser, you may need to locate your Vectra Data. You locate the Vectra Data by selecting an example scan within it. Phenochart obtains the example by asking you to open a PerkinElmer TIFF file (.qptiff).



The next time you click **Load**, 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.)

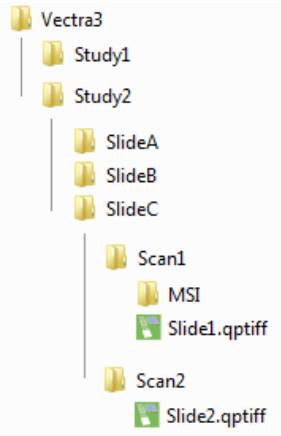
If you need to change which data the browser looks at, use the browser's Gear Menu to change the location:



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

Vectra Data:

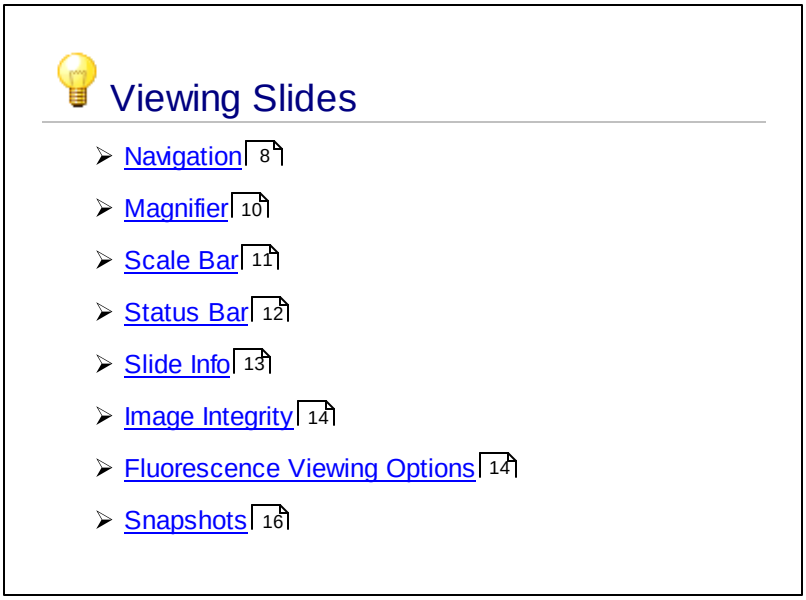
Vectra 3 stores scan data by ‘study.’



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.

3 Viewing Slides



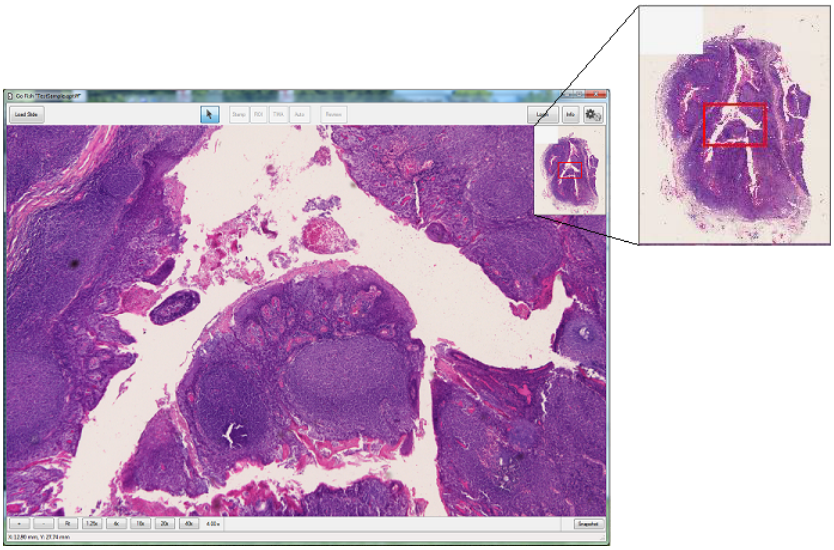
3.1 Navigation

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:

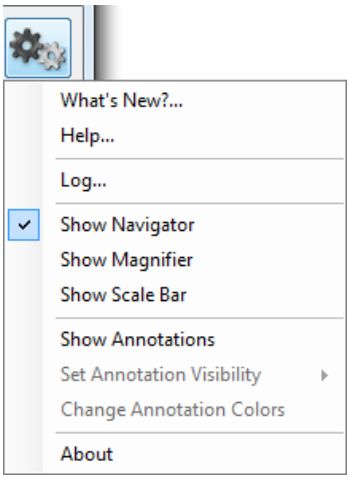
When you first launch Phenochart, this button is selected as the default setting for your mouse. It allows you to click and drag to move the image on your display or to use the mouse wheel to zoom in and out on the image.

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



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 unchecking **Show Navigator** in the Gear Menu at the top right of your screen:



To redisplay the Navigator Window, check **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:

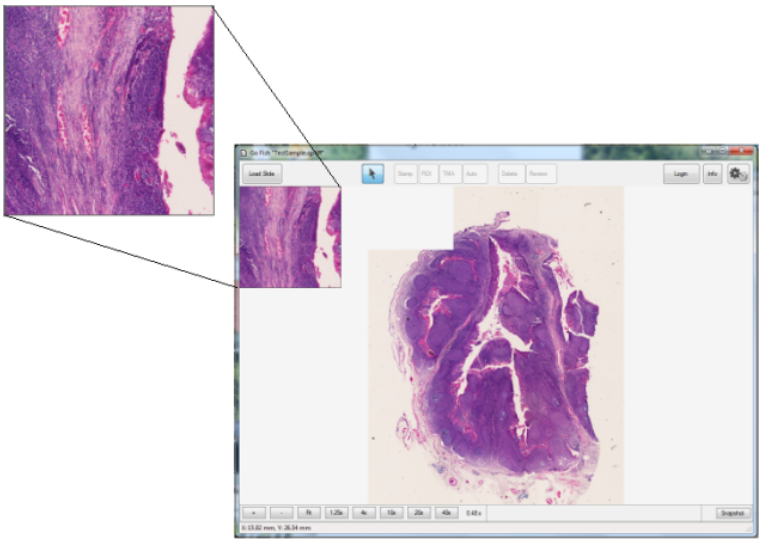
2. Use the +/- buttons on the Navigation Toolbar.

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: In general, the Vectra 3 scanning software will scan at either 4x or 10x. However, whenever you load a slide, it is shown 'fit' to your screen).

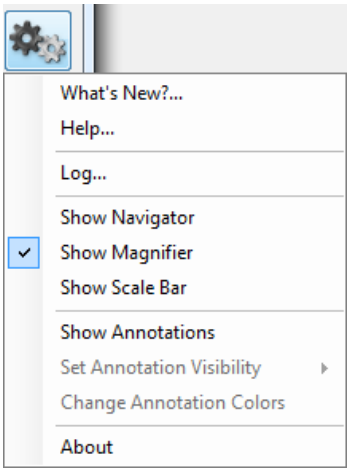
3.2 Magnifier

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



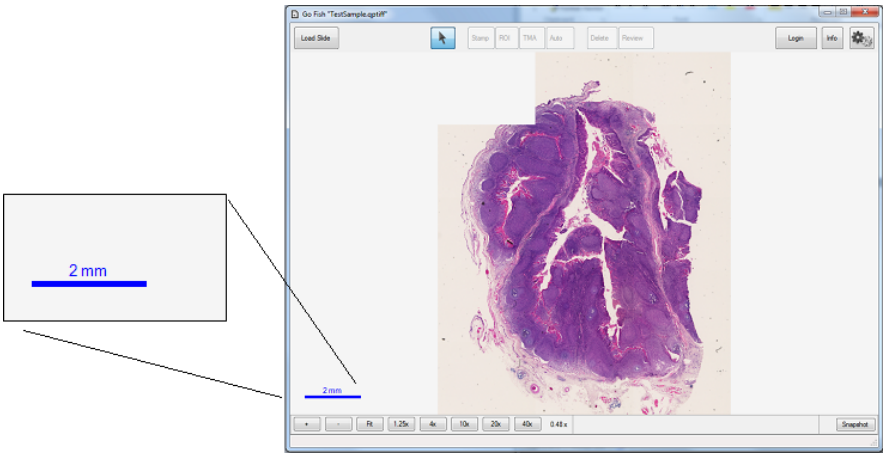
The Magnifier shows a magnified (zoomed in) view of the slide centered on your cursor. This lets you quickly inspect individual regions in more detail without changing the overall zoom.

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



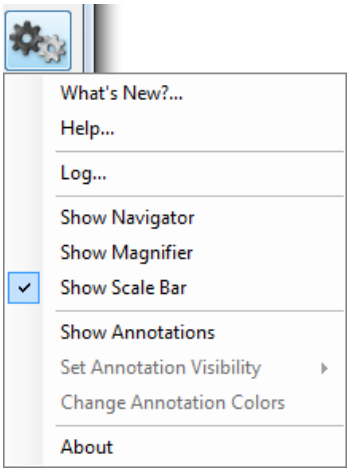
3.3 Scale Bar

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



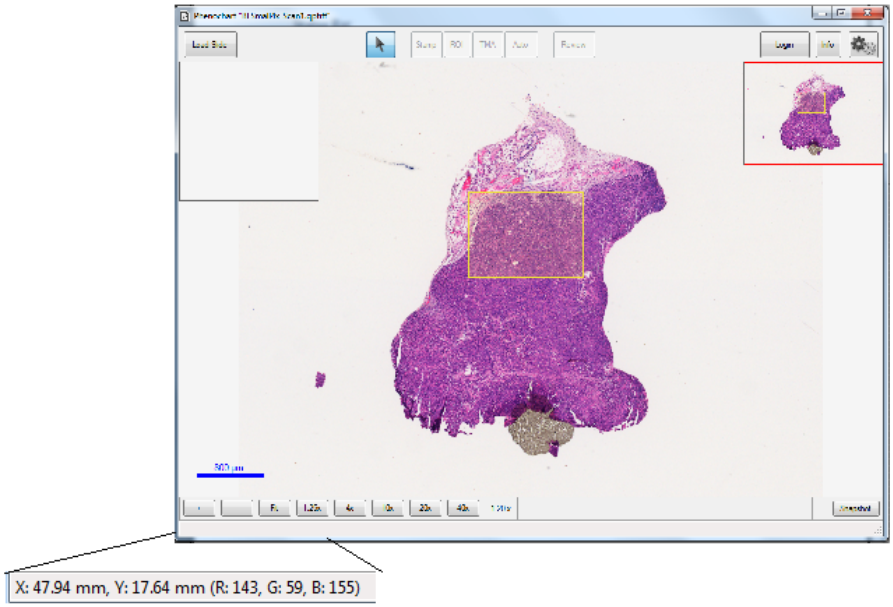
The Scale Bar is a reference scale showing the actual size of tissue features in the image. The length of the bar will change as you zoom in and out on your slide, and the units will change from microns to millimeters depending on your magnification.

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



3.4 Status Bar

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

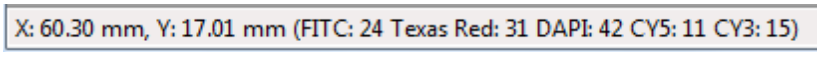


The Status Bar shows the current position of your cursor on the slide scan.

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 hardware.

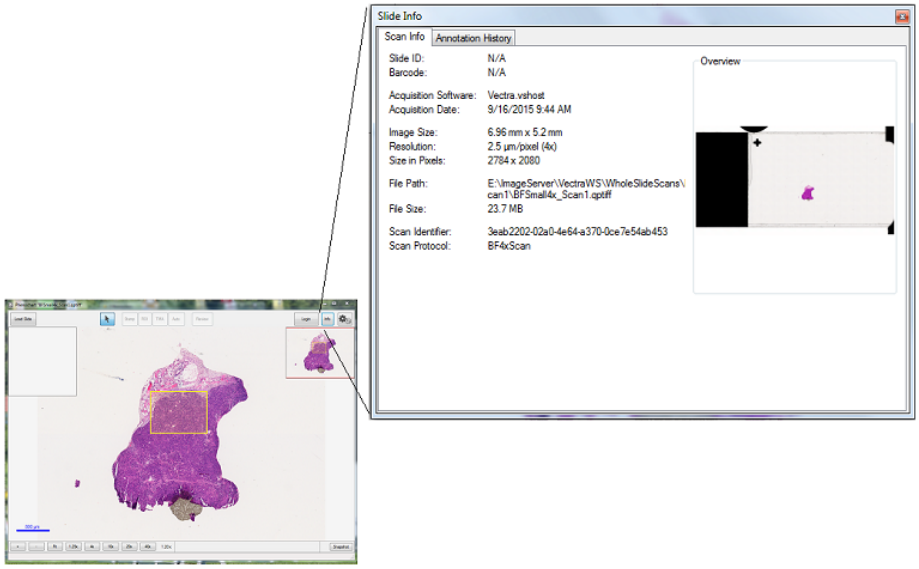
The Status Bar also shows intensity values. For brightfield images, the Status Bar shows standard RGB values ranging from 0-255:

In fluorescence, one intensity value for each filter is shown, also ranging from 0-255:

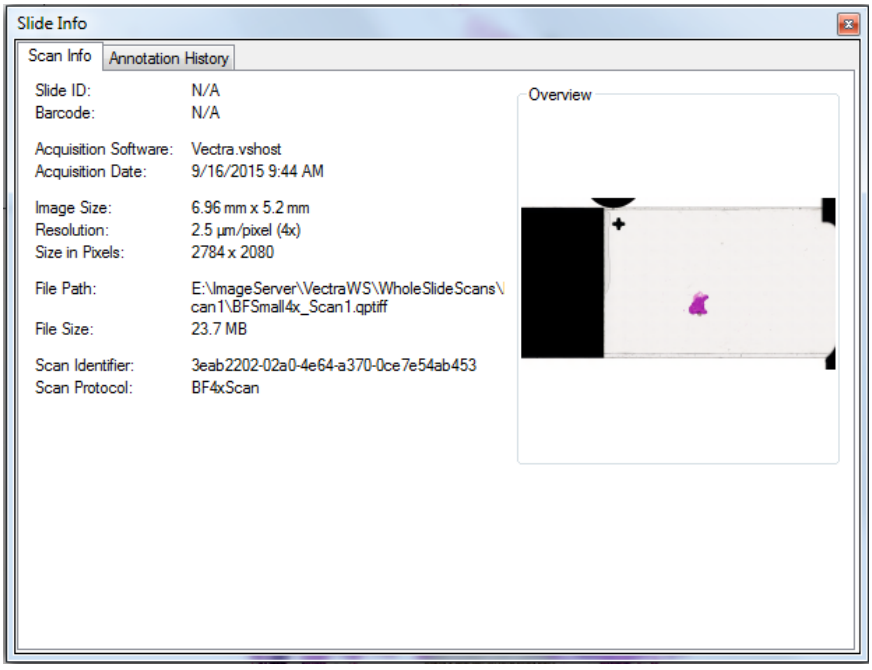


3.5 Slide Info

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:



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.

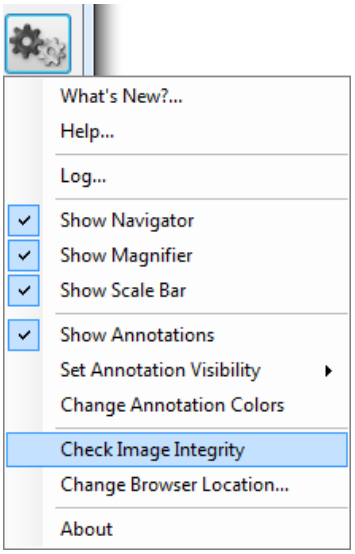


(Note: See the Annotations 17 section for information about Annotation History.)

3.6 Image Integrity

PerkinElmer TIFF files are tamper-evident. You can validate them to confirm that the image data and metadata have not been altered.

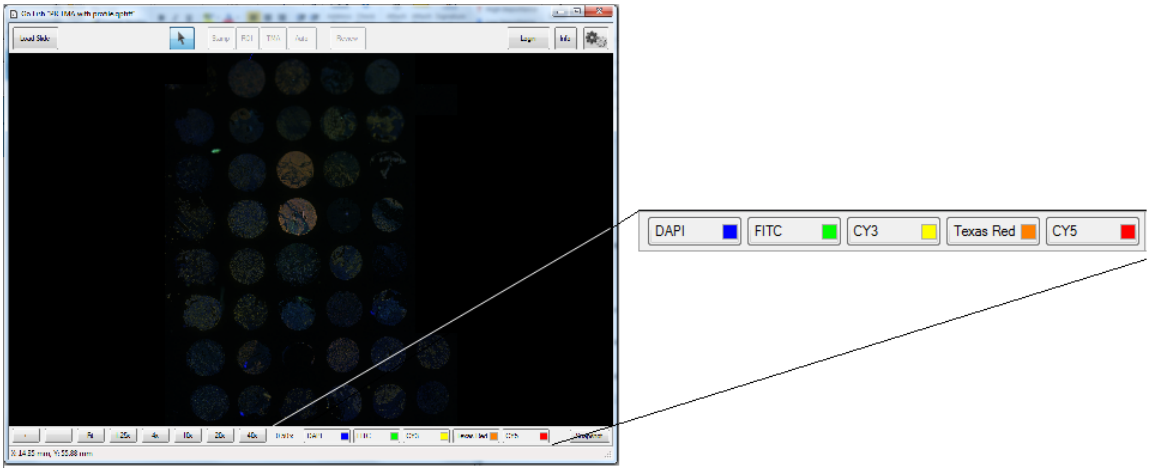
Select **Check Image Integrity** from the Gear Menu at the top right of your screen. The software will perform the integrity check and report the results.



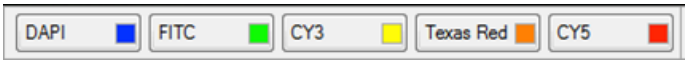
3.7 Fluorescence Viewing Options

Fluorescence viewing options allow you to see how your fluorescent slide was scanned.

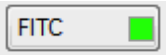
The **Fluorescence Layer Toolbar** is located in the bottom right of your screen:



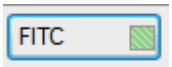
The toolbar is only visible for fluorescent slides. When you load a fluorescent slide, the Layer Toolbar will show one button for each filter used during the scan. This enables you to toggle which fluorescence channels are shown, and adjust their brightness or color if you want.



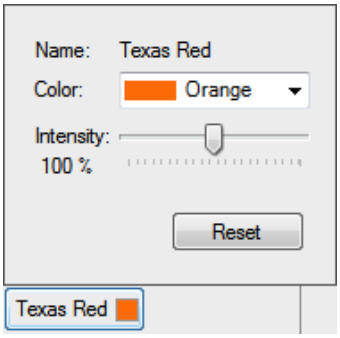
Each **Layer Button** is labeled with the common name for that filter. Each layer's color is determined by the color selected in the Vectra scan protocol.



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.



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

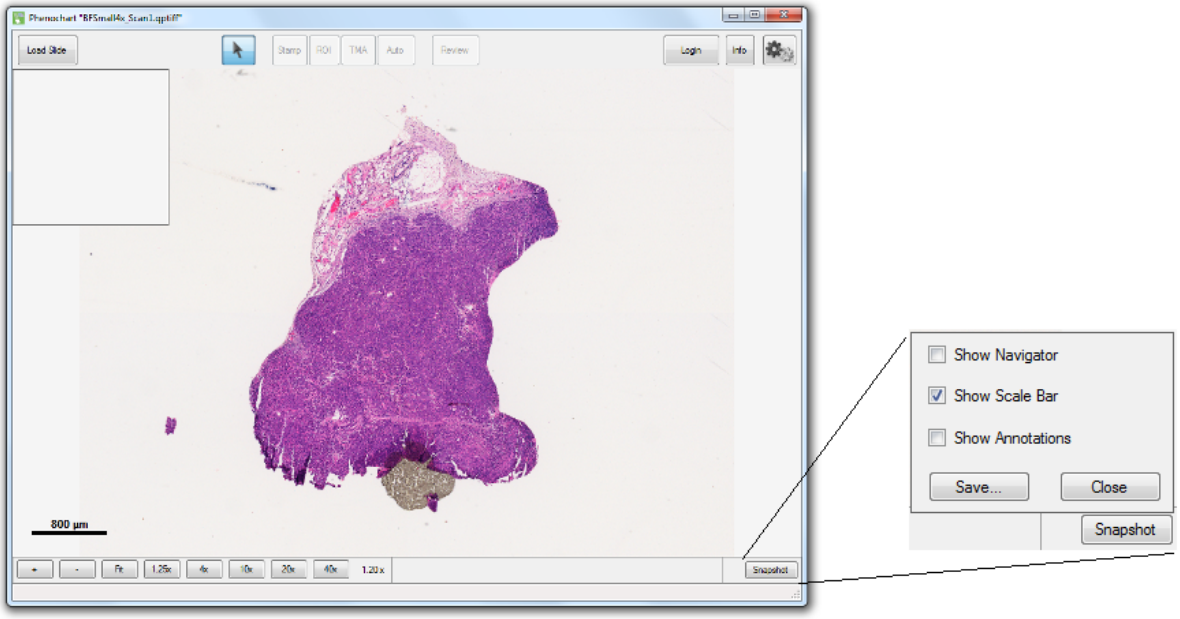
Click **Reset** at any time to return to the color and intensity default settings.

The Layer Editor will close when you move your mouse off the editor.

3.8 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 lower right corner of your application window. It will show the Snapshot Editor.



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 Scale Bar 11, Navigator Window 8, or Annotations 17. 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. There are several types of annotations: stamped fields 19, TMA cores 22, and inForm automatically generated fields 25.

There are different states within each type of annotation.

- **Pending Acquisition:** This annotation has been identified for multispectral imaging, but has not yet been acquired.
- **Pending Review:** A reviewer must accept this annotation before it can be imaged.
- **Acquired:** Multispectral imagery was taken for this annotation.
- **Acquisition Failed:** This annotation was slated for multispectral imaging, but the acquisition failed to complete correctly.
- **Rejected:** A reviewer rejected this annotation; it will not be acquired.
- **Ignored:** This annotation was ignored. It will not be considered for multispectral imaging.
- **Deleted:** A user deleted this annotation. If there was multispectral imagery associated with this annotation, it was also deleted.
- **Sent to inForm:** This annotation was sent to inForm for rudimentary analysis or inForm classifier training.

Annotations are color coded according to their state. See Annotation Colors 30 for how to change the colors, and see Annotation Visibility 29 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 same button to Logout.

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

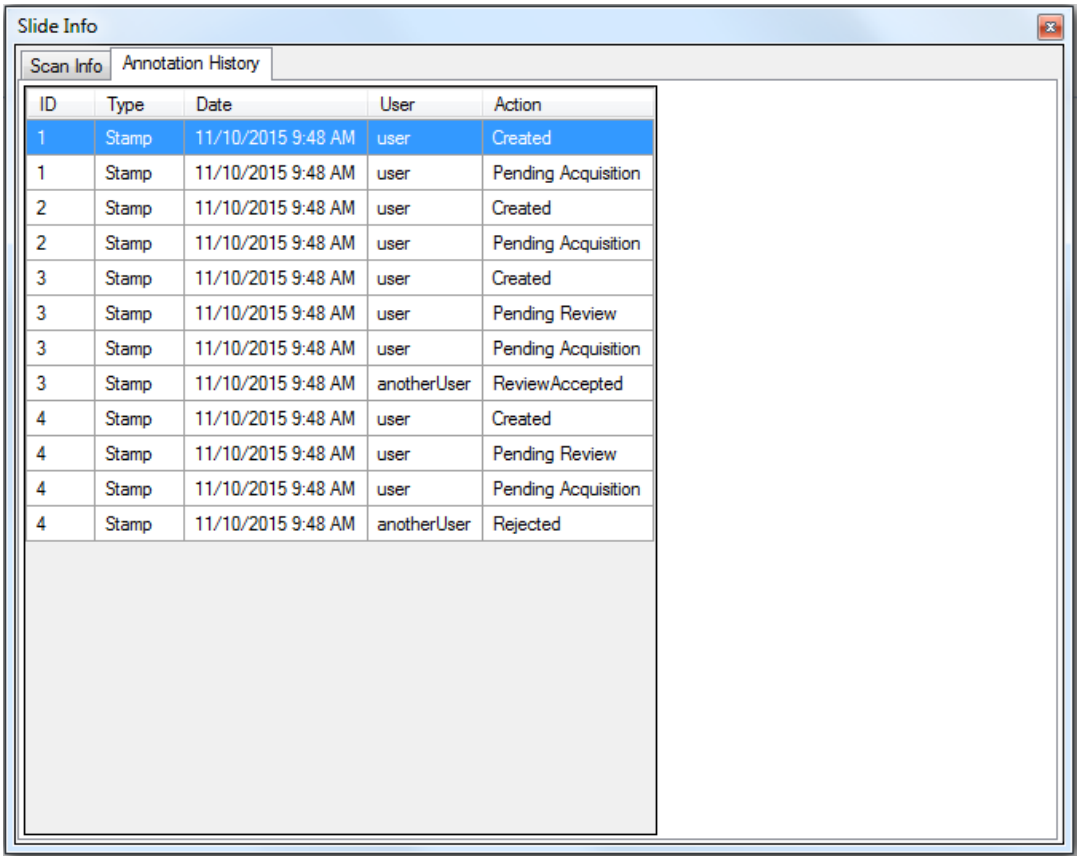
If your annotation has already been acquired, double-click that annotation to open the multispectral imagery in inForm.

When Vectra is acquiring MSI 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 imaging 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 select 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.

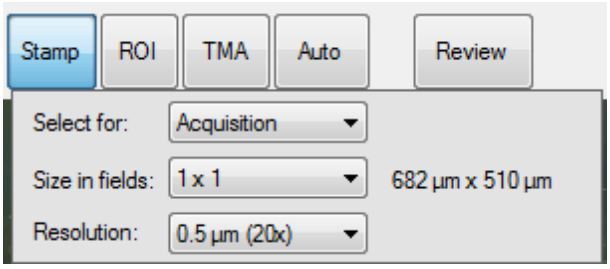
The annotation log includes a tamper-evident hash code. If a log file is altered, Phenochart will give an error and refuse to open the file.



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

4.1 Stamps

Stamp annotations allow 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:



There are three different kinds of stamps: Acquisition, Review, and Push to inForm:

- Choose **Acquisition** to take multispectral imagery of the stamp area(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 imagery 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 if inForm 2.2 or higher is installed on your machine. See Training a Classifier for more information.

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: You can only acquire 0.25 micron/pixel (40x) fields if your Vectra system has a 40x objective.)

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 Control key and clicking on the annotation. The cursor will change to a trashcan with a red X when an annotation can be deleted.

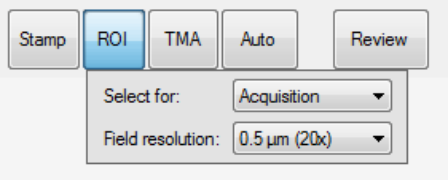
4.2 ROI (Region of Interest)

The ROI (Region of Interest) Tool allows you to draw a region of interest for imaging. It creates stamp annotations corresponding to the region you draw. This may be useful when you want to acquire multispectral imagery for a specific region of your slide.

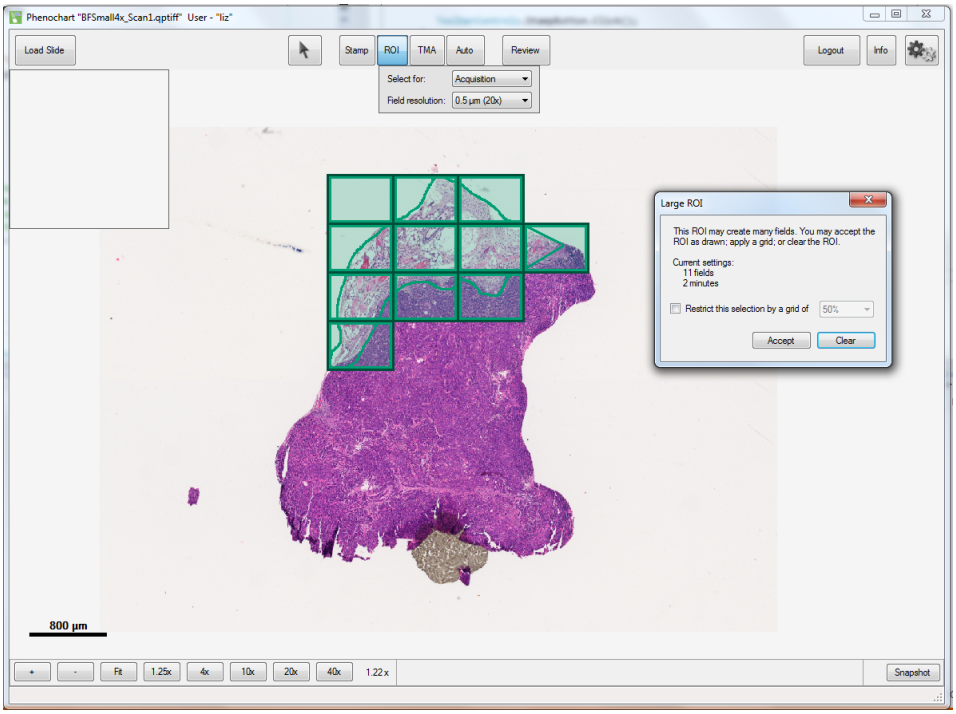
Use the ROI Editor to label the stamp annotations for Acquisition or Review.

- Choose 'Acquisition' to take multispectral imagery 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 imagery of accepted stamps.

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

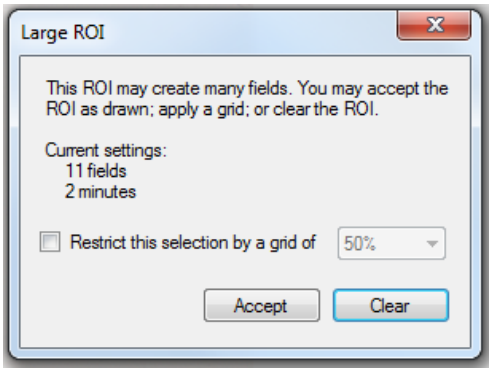


When you have made your selections, draw a region of interest on the slide by clicking and dragging an outline. If you don't close the area, it will be closed for you when you release the mouse button. The software will then create tiled stamp annotations that contain the area you have drawn.



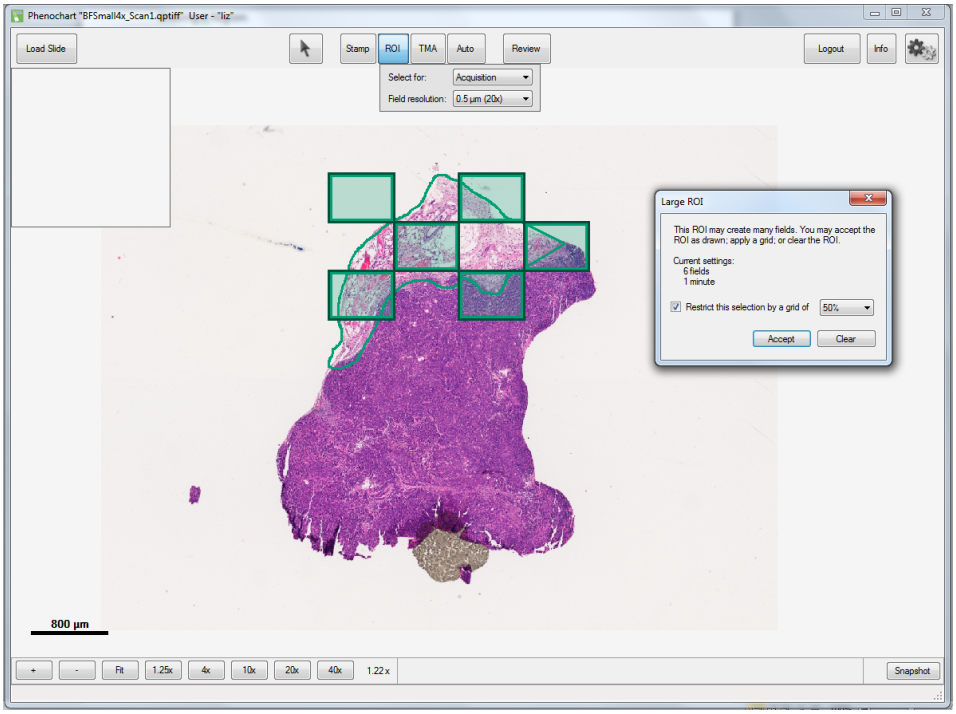
If your region is small enough to fit within a 3x3 stamp, it will be included in a single stamp annotation.

For larger regions, the system will break the region up into separate stamps. You will then 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.



To reduce the number of fields within your region, you can apply a grid. Use the grid dropdown 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.

You can then 'Accept' to accept the ROI as shown, and the system will add all of the stamps to the list of pending annotations (acquisition or review). Select 'Clear' to remove the ROI, and no fields will be added.



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 Control key down and use your mouse to zoom and pan.

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

4.3 TMA

Tissue Micro-Array (TMA) annotations let you locate TMA cores within whole-slide images and acquire multispectral imagery 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.

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

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

Identify if the TMA cores are for acquisition or review. If you select cores for review, only the accepted cores will be acquired.

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:

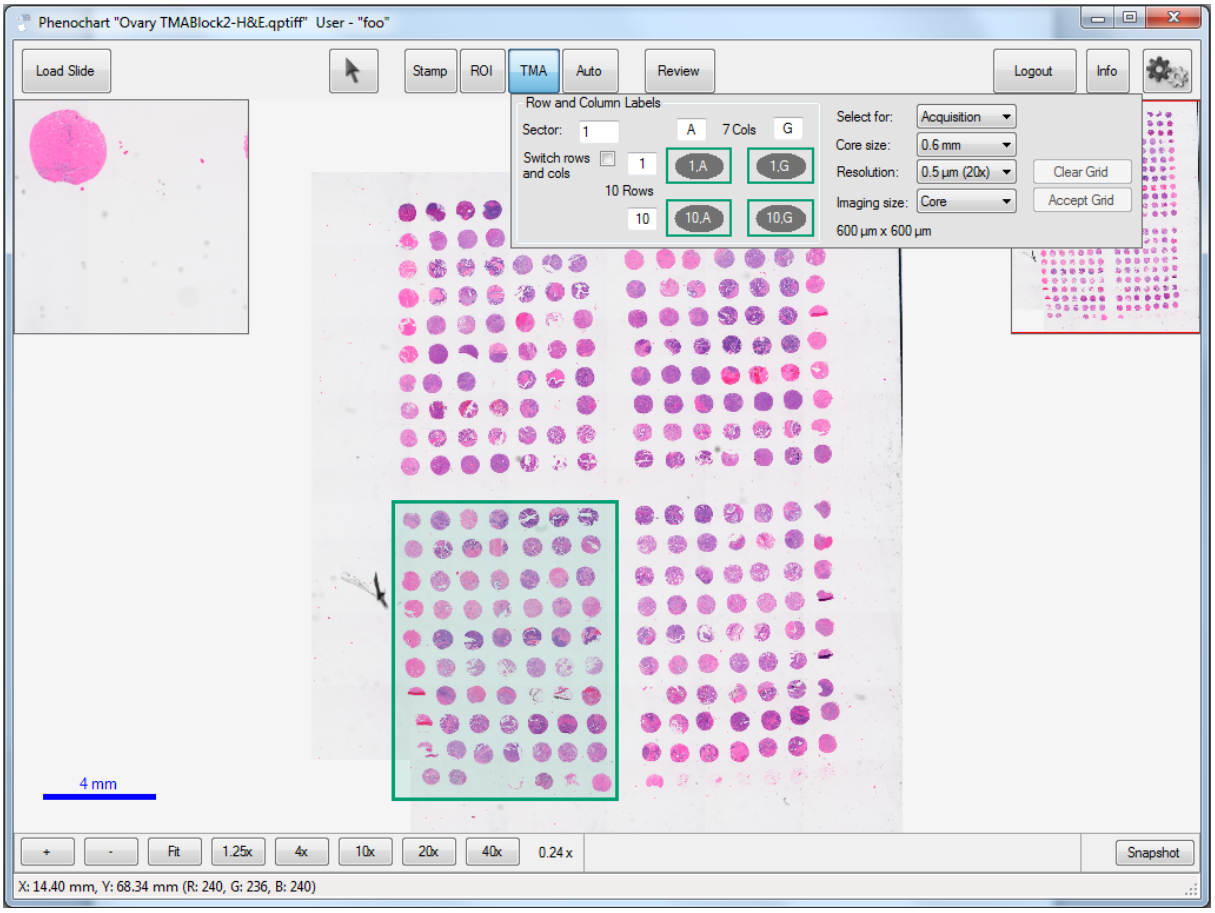
- **Core** will take a square image of exactly Core Size x Core Size microns.
- **Core + 10%** and **Core +20%** will give you square images with a margin to account for slight variations in core size.
- **NxN** fields are rectangular fields as described. This option will enable you to take imagery consistent 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

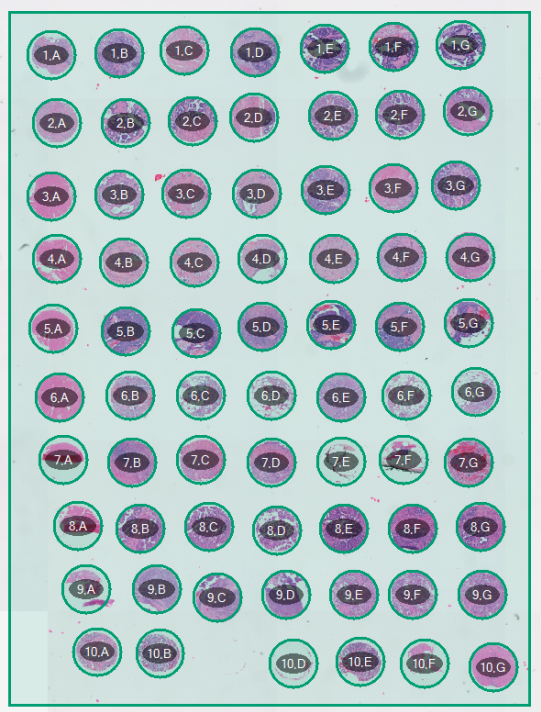
Finding Cores

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 control key down and use your mouse to zoom and pan.

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



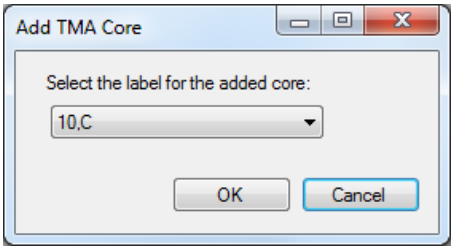
When you release your mouse, Phenochart will find the cores within the rectangle. Each core will be labeled. If the labels aren't visible, zoom in to see them.



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 select 'Add Missing Core'. Select the missing core label from the dropdown and click OK to add the core.



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, control+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.

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

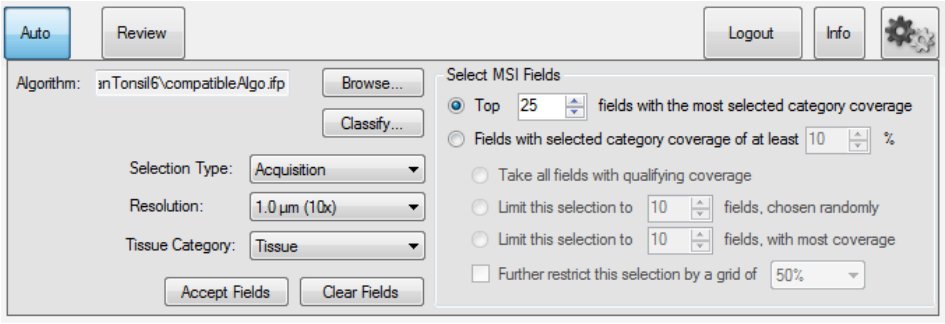
In Fluorescence, only visible layers will be used to find the cores. Turn off any layers that seem to be preventing the core finder from identifying the cores.

4.4 Automatically Generated

Phenochart allows you to automatically select fields of interest using a trained inForm classifier. This will only be available on computers with an activated version of inForm Tissue Finder, 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 slide scan.

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

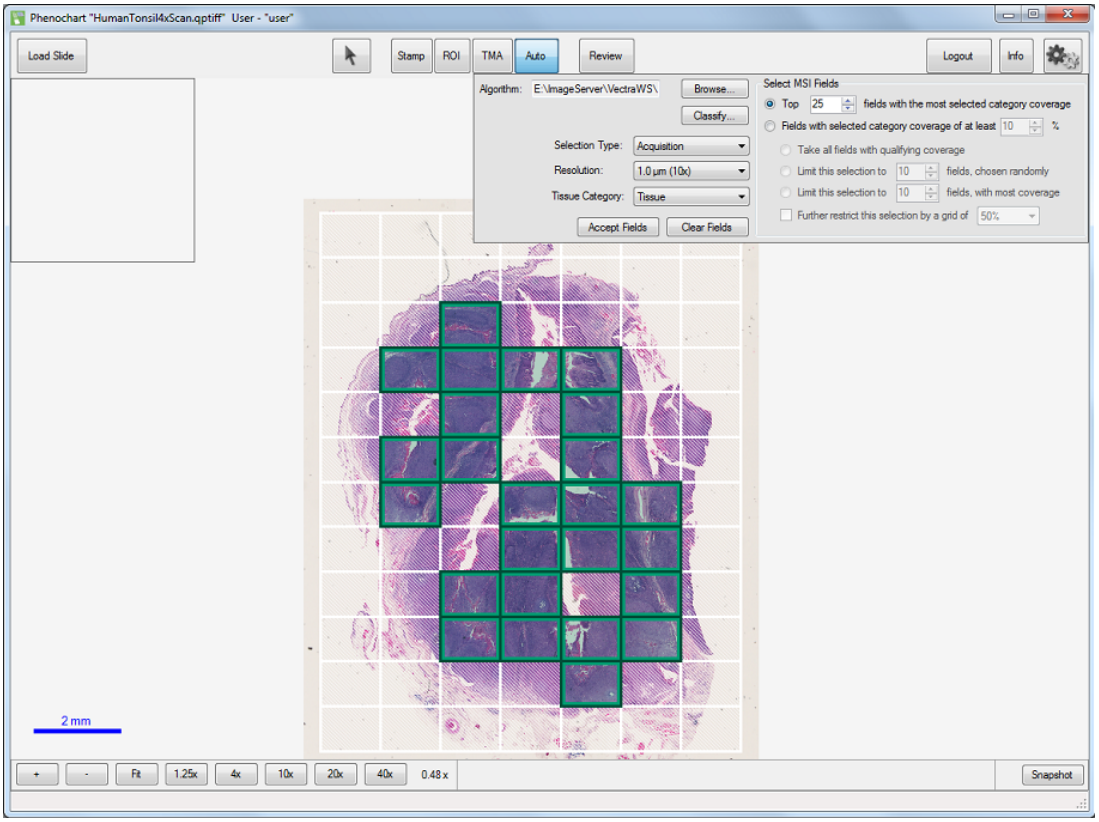


Click the Browse... button to load an inForm algorithm or project that you've trained for this type of slide. If you haven't created an algorithm yet, please see the section Training an inForm Classifier 27.

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.



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

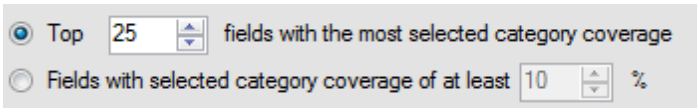
Use **Selection Type** to identify whether the fields are for Acquisition or Review. Fields marked for review must be reviewed and accepted before they can be acquired by Vectra.

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.

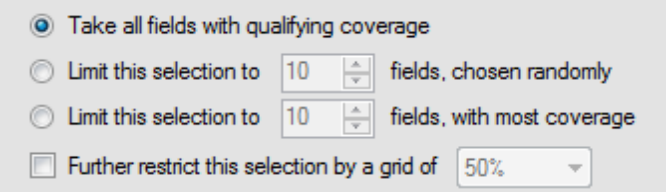
There are two types of strategies:



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 second option ('Fields with selected category coverage of at least X%'), you will also need to choose if you want to take:



- 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.

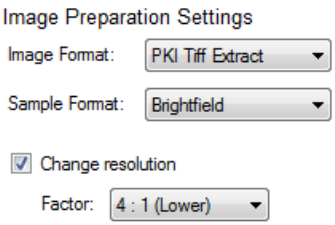
4.4.1 Training an inForm Classifier

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.

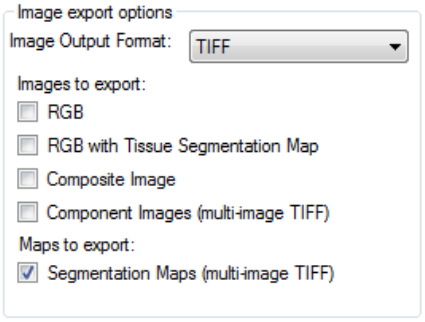
To train a classifier in inForm, select the fields for training on your slide using Phenochart's stamp tool. Within the **Stamp** tool, select **Push to inForm**, and select the fields that represent all tissue categories that you need. inForm will launch automatically and load the fields. You can load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



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)**.

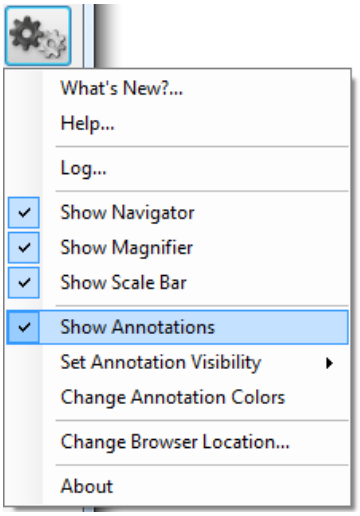


5. Save your project. Saving the project within the study folder will make it easy to locate.

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.

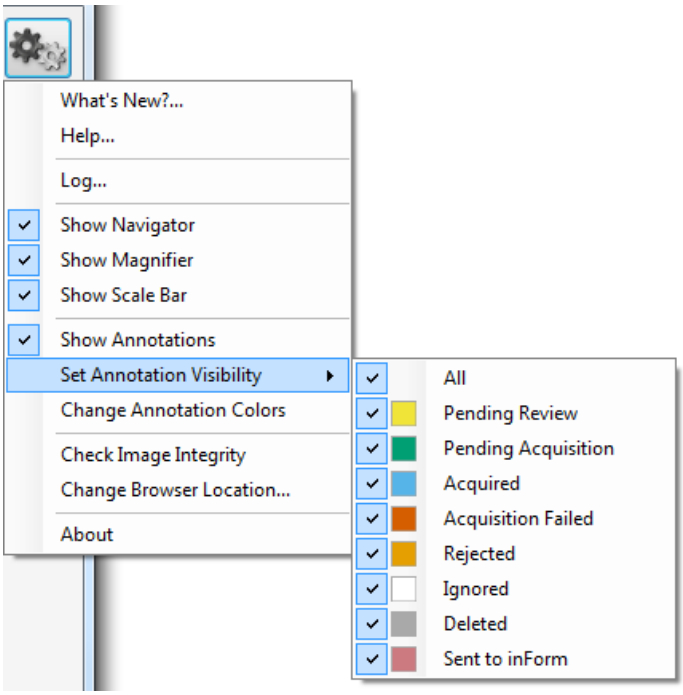
4.5 Annotation Menu Options

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



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:

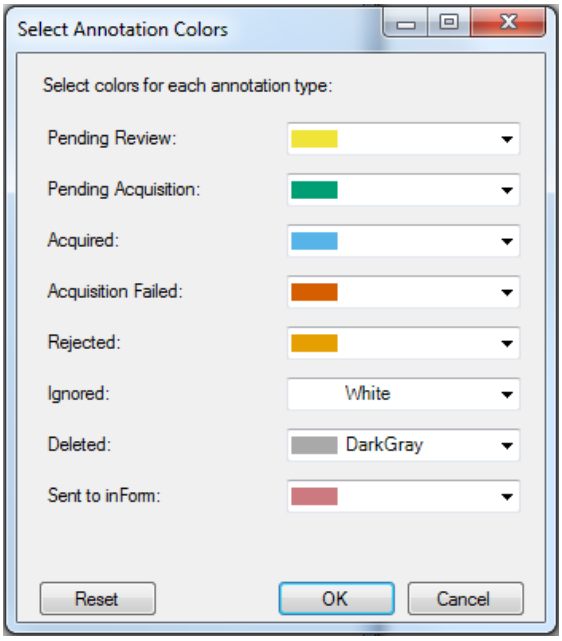


Check an annotation type to make it visible whenever annotations are shown. Uncheck it to hide all annotations of that type. Check **All** to turn all annotations on.

(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:

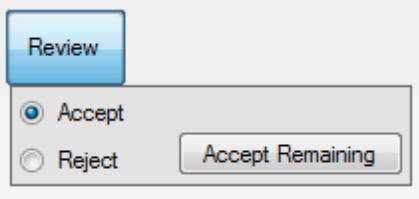
The default annotation colors are chosen to be distinguishable, even by most color-blind people. You can change the colors as needed. Select **Change Annotation Colors** from the Gear Menu to bring up the color changing dialog:



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.



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 unreviewed 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 Contacting PerkinElmer

For more information, contact PerkinElmer or your local authorized PerkinElmer distributor:

PerkinElmer, Inc.
68 Elm Street
Hopkinton, MA, 01748, USA

Technical Support:
Phone: 800-762-4000 or +1 203-925-4602
Fax: +1 203-944-4904

PerkinElmer Web Site:

http://www.perkinelmer.com

Technical Support Email:

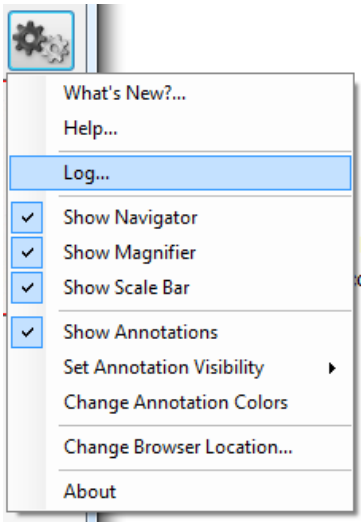
global.techsupport@perkinelmer.com

6 Appendix A: Support

Log File:

When you contact PerkinElmer Technical Support 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.



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

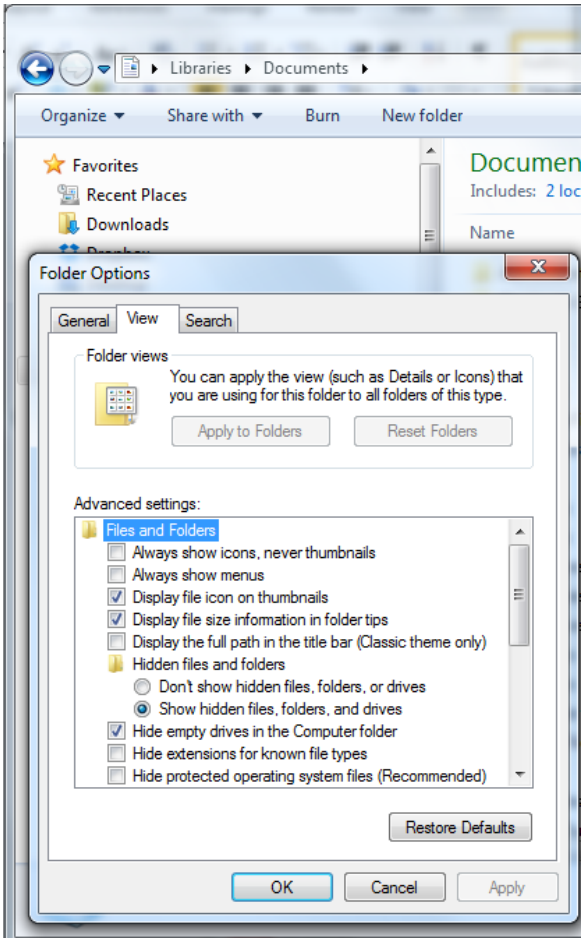
You can either attach the log file or cut and paste the log file contents into your correspondence.

Config File:

In some cases, you may be asked for the Phenochart.config file. You can find this file in C:\ProgramData \PerkinElmer\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. Select the View tab
3. Select 'Show hidden files, folders, or drives' and then click OK


Libraries
Documents
Organize
Share with
Burn
New folder
Favorites
Recent Places
Downloads
Name
Folder Options
General
View
Search
Folder views
You can apply the view (such as Details or Icons) that you are using for this folder to all folders of this type.
Apply to Folders
Reset Folders
Advanced settings:
Files and Folders
Always show icons, never thumbnails
Always show menus
Display file icon on thumbnails
Display file size information in folder tips
Display the full path in the title bar (Classic theme only)
Hidden files and folders
Don't show hidden files, folders, or drives
Show hidden files, folders, and drives
Hide empty drives in the Computer folder
Hide extensions for known file types
Hide protected operating system files (Recommended)
Restore Defaults
OK
Cancel
Apply

7 Appendix B: PerkinElmer TIFF Specification

Background

This describes a TIFF format that PerkinElmer uses for its tissue images. The imagery may be a simple RGB image, a set of unmixed multispectral image (MSI) components, 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, the unmixed MSI images can be considered to be an idealized multiband FL image where signal corresponds to the presence of a stain or fluorescent dye in the sample.

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	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	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

Description	RGB / mono	Tile / Strip	Resolution	Notes
Macro (overview) image of whole slide	RGB	Stripped	~2000 x 4000	Required for whole-slide scans Optional for simple RGB and MSI components
Label image	RGB	Stripped	~500 x 500	Optional, whole-slide scans

Detection

Readers can recognize PerkinElmer tissue images via the contents of the “Software” TIFF tag (see below). The file suffix is .qptiff for whole slide scans.

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-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)
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 unmixed MSI images
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)

TIFF Tag	Optional	Description of contents
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). PKI TIFF pixels 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 PerkinElmer 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

Tag	Optional	Contents
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: - 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).
SignalUnits		A byte *wwww tttt* where the *tttt* nibble indicates the signal unit type from the following: 0 – raw counts 1 – normalized (counts/second/gain/full-scale/binning) 4 – OD (optical density) 5 – dark-corrected counts and the *wwww* nibble indicates how the signal is weighted across the spectral bands (or colors): 0 – average across all bands 2 – total summed signal across all bands 4 – peak signal in highest-valued band Thus, for example, a value of 68 (hex 44) encodes OD units with peak-signal weighting.
Name	Y	Band (component) name for FL or unmixed MSI images, not present for RGB images
Color	Y	Color to use when rendering this band, as decimal r,g,b byte triplet, r FL or unmixed MSI images. Not present for RGB images.
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.

Tag	Optional	Contents
ValidationCode		Used for internal data integrity checks – readers can ignore this.

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.

Sample ImageDescription for the DAPI band of a FL image:

```
<?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>
    <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 >
```

8 Appendix C: EULA

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