Notice

This manual is published by Akoya Biosciences, Inc. The information in this document is subject to change without notice and should not be construed as a commitment by Akoya Biosciences, Inc. Akoya assumes no responsibility for any errors that may appear in this document. This manual is believed to be complete and accurate at the time of publication. In no event shall Akoya Biosciences be liable for incidental or consequential damages in connection with or arising from the use of this manual.

This manual describes how to use Phenochart software.

For more information contact:

Akoya Biosciences, Inc.
68 Elm Street, Hopkinton, MA, 01748, USA

Phone: +1 855-896-8401
Fax: +1 855-404-0061
Email: CustomerCare@akoyabio.com
Email (outside the US): CustomerCareEMEA@akoyabio.com
Web site: http://www.akoyabio.com
# Table of Contents

1 Overview......................................................................................................................... 5

2 Loading Slides.................................................................................................................. 6

3 Viewing Slides................................................................................................................... 10
   3.1 Navigation.................................................................................................................... 10
   3.2 Magnifier...................................................................................................................... 13
   3.3 Scale Bar...................................................................................................................... 14
   3.4 Label............................................................................................................................ 15
   3.5 Status Bar..................................................................................................................... 17
   3.6 Intensity Cursor.......................................................................................................... 17
   3.7 Slide Info...................................................................................................................... 18
   3.8 Image Sharpening....................................................................................................... 20
   3.9 Image Integrity............................................................................................................ 20
   3.10 Fluorescence Layer Toolbar.................................................................................... 21
   3.11 Fluorescence Composite Views.............................................................................. 23
   3.12 Legend........................................................................................................................ 25
   3.13 Whole Slide Multispectral Unmixing with MOTIF Scans.................................... 26
   3.14 Simulated Brightfield Views (Pathology Views)....................................................... 26
   3.15 Snapshots.................................................................................................................. 30

4 Annotations...................................................................................................................... 31
   4.1 Stamps......................................................................................................................... 33
   4.2 ROI (Region of Interest).......................................................................................... 34
   4.3 TMA............................................................................................................................ 38
   4.4 Automatically Generated.......................................................................................... 42
      4.4.1 Training an inForm Classifier............................................................................ 44
   4.5 Annotation Menu Options....................................................................................... 46
   4.6 Review........................................................................................................................ 49

5 Appendix A: Support....................................................................................................... 50

6 Appendix B: Akoya QPTIFF Specification..................................................................... 52

7 Appendix C: EULA............................................................................................................ 59

8 Contacting Akoya Biosciences...................................................................................... 63
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.

![Slide Browser](image)

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:
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 QPTIFF file (.qptiff).

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:
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](image)

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.
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:
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:
   
   ![Pre-defined Resolutions](image)

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](image)

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:
3.3 Scale Bar

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

![Scale Bar Image]

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:
3.4 Label

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

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.
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:
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](image)

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](image)

**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.

![Intensity Cursor with Normalized Counts](image)

**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:

![Slide Info Window](image)

**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 section for information about Annotation History.
3.8 Image Sharpening

QPTIFF 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:

![Gear Menu](image)

Figure 23. Gear Menu > Sharpen Image Display

3.9 Image Integrity

QPTIFF 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.
3.10 Fluorescence Layer Toolbar

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

![Fluorescence Layer Toolbar](image)

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.

![Layer Toolbar Filter Buttons for CODEX Imagery](image)

**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.

![Layer Toolbar Filter Buttons for Vectra Imagery](image)

**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.

![Layer Color](image)

**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.

![Hatch Pattern](image)

**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:

![Layer Editor](image)

**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.

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.
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.

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.

![Save Changed Views](image)

**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:

![Legend](image)

**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 de-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 MOTIF multispectral slide scans.

![Figure 36. Unmixing Button](image)

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](image)

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

![Snapshot Editor Window](image)

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**, **Scale Bar**, **Legend**, or **Annotations**. 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, drawing ROIs, finding TMA cores, and automatically generating fields through inForm.

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 for how to change the colors, and see Annotation Visibility 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.

![Login Button](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.

![Figure 44. Slide Info > Annotation History Tab](image)

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:

![Stamp Editor](image)

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](#) 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](image)

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. 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.

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.
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.
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](image)

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](image)

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 click Add Missing Core. Select the missing core label from the drop-down list and click the OK button 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, 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.
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.

![Figure 57. Auto Editor Window](image)

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.

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:

- **Fields with selected category coverage of at least X%**

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:
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:

---

*Figure 64. Gear Menu - Set Annotation Visibility*
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.

![Review Window](image)

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.

![Checkmark](image)

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.

![X](image)

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.

![Gear Menu - Log...](image)

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
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

<table>
<thead>
<tr>
<th>Description</th>
<th>RGB/mono</th>
<th>Title/Strip</th>
<th>Resolution</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline image</td>
<td>Varies</td>
<td>Varies</td>
<td>Full</td>
<td>Tiled if &gt; 2K x 2K RGB for BF, else mono</td>
</tr>
<tr>
<td>More full-resolution images</td>
<td>Mono</td>
<td>Varies</td>
<td>Full</td>
<td>If N &gt; 1</td>
</tr>
<tr>
<td>Thumbnail</td>
<td>RGB</td>
<td>Stripped</td>
<td>~500 x 500</td>
<td></td>
</tr>
<tr>
<td>Half-resolution images</td>
<td>Varies</td>
<td>Varies</td>
<td>Half</td>
<td>Only if baseline is tiled</td>
</tr>
<tr>
<td>Quarter, eighth, etc.</td>
<td>Varies</td>
<td>Varies</td>
<td>Quarter, eighth, etc.</td>
<td>Continues until 2K x 2K or smaller</td>
</tr>
<tr>
<td>Macro (overview) image of whole slide</td>
<td>RGB</td>
<td>Stripped</td>
<td>~2000 x 4000</td>
<td>Required for whole-slide scans</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>TIFF Tag</th>
<th>Optional?</th>
<th>Description of contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Software</td>
<td></td>
<td>Starts with “PerkinElmer-QPI”</td>
</tr>
<tr>
<td>ImageDescription</td>
<td></td>
<td>Further metadata in XML format (see next section)</td>
</tr>
<tr>
<td>ImageWidth</td>
<td></td>
<td>Width of the image in pixels</td>
</tr>
<tr>
<td>ImageLength</td>
<td></td>
<td>Height of the image in pixels</td>
</tr>
<tr>
<td>ResolutionUnit</td>
<td></td>
<td>Unit used for resolution and position (see below)</td>
</tr>
<tr>
<td>XResolution</td>
<td></td>
<td>Pixel X resolution (see below)</td>
</tr>
<tr>
<td>YResolution</td>
<td></td>
<td>Pixel Y resolution (see below)</td>
</tr>
<tr>
<td>XPosition</td>
<td>Y</td>
<td>Sample X location in ResolutionUnits. This is ULHC location except for Macro image which reports its image center.</td>
</tr>
<tr>
<td>YPosition</td>
<td>Y</td>
<td>Sample Y location in ResolutionUnits. This is ULHC location except for Macro image which reports its image center.</td>
</tr>
<tr>
<td>SampleFormat</td>
<td></td>
<td>Integer (1) for BF, FL; or float (3) for extracted components</td>
</tr>
<tr>
<td>SMinSampleValue</td>
<td></td>
<td>Minimum signal value in the image</td>
</tr>
<tr>
<td>SMaxSampleValue</td>
<td></td>
<td>Maximum signal value in the image</td>
</tr>
<tr>
<td>BitsPerSample</td>
<td></td>
<td>8 (FL); 8, 8, 8 (RGB); or 32 (unmixed component)</td>
</tr>
<tr>
<td>SamplesPerPixel</td>
<td></td>
<td>1 (FL or unmixed component) or 3 (RGB)</td>
</tr>
<tr>
<td>NewSubfileType</td>
<td></td>
<td>0 for full-resolution images, 1 for reduced res images</td>
</tr>
<tr>
<td>TileWidth</td>
<td>Y</td>
<td>Tile width (512) if tiled format is used</td>
</tr>
<tr>
<td>TileLength</td>
<td>Y</td>
<td>Tile height (512) if tiled format is used</td>
</tr>
<tr>
<td>TileOffsets</td>
<td>Y</td>
<td>List of tile offsets, if tiled format is used</td>
</tr>
<tr>
<td>TileByteCounts</td>
<td>Y</td>
<td>Size of each (compressed) tile, if tiled format is used</td>
</tr>
<tr>
<td>StripOffsets</td>
<td>Y</td>
<td>List of strip offsets, if tiled format is not used</td>
</tr>
<tr>
<td>RowsPerStrip</td>
<td>Y</td>
<td>Number of rows per strip, if tiled format is not used</td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>Tag</th>
<th>Optional?</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>DescriptionVersion</td>
<td></td>
<td>Version of the image description field itself, a single number. This document describes version 2 of the field.</td>
</tr>
<tr>
<td>AcquisitionSoftware</td>
<td></td>
<td>Software used to acquire the image</td>
</tr>
<tr>
<td>Identifier</td>
<td></td>
<td>GUID in string format. This is an identifier for the image file itself.</td>
</tr>
<tr>
<td>SlideID</td>
<td>Y</td>
<td>ID of the slide that this image was taken from.</td>
</tr>
<tr>
<td>Barcode</td>
<td>Y</td>
<td>Barcode text of the slide this image was taken from.</td>
</tr>
<tr>
<td>ComputerName</td>
<td>Y</td>
<td>Name of the computer on which the slide was scanned.</td>
</tr>
<tr>
<td>ImageType</td>
<td></td>
<td>A string identifying the type of image within the file (Table 1), with the following values:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• FullResolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• ReducedResolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Thumbnail</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Overview</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Label</td>
</tr>
<tr>
<td>IsUnmixedComponent</td>
<td></td>
<td>“True” for unmixed multispectral images, otherwise “False”.</td>
</tr>
<tr>
<td>ExposureTime</td>
<td></td>
<td>Exposure time as an integer number of microseconds.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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).</td>
</tr>
<tr>
<td>Name</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
</tbody>
</table>
| SignalUnits           | A byte $\text{www tttt}$ where the $\text{tttt}$ nibble indicates the signal unit type from the following:  
|                       |   - raw counts  
|                       |   - normalized (counts/second/gain/full-scale/binning)  
|                       |   - OD (optical density)  
|                       |   - dark-corrected counts  
|                       | and the $\text{wwww}$ nibble indicates how the signal is weighted across the spectral bands (or colors):  
|                       |   - average across all bands  
|                       |   - total summed signal across all bands  
|                       |   - peak signal in highest-valued band  
|                       | Thus, for example, a value of 68 (hex 44) encodes OD units with peak-signal weighting.                                                     |
| Name                  | Band name for FL whole slide scans, component name for extracted components. Not present for RGB images                                               |
| Color                 | 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          | Instrument responsivity, if available, for FL whole-slide and unmixed images. See below for details.                                            |
| Objective             | Objective name, if known, otherwise not present.                                                                                           |
| ScanProfile           | 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          | Name of the acquisition protocol.                                                                                                           |
| ValidationCode        | Used for internal data integrity checks – readers can ignore this.                                                                           |
| ImagingCycle          | Zero-based acquisition cycle for CODEX imagery                                                                                               |
| Biomarker             | String indicating target such as CD8, Ki67, etc.                                                                                              |
| ScaleFactor           | 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. |
| AutofluorescenceSubtracte d | String containing ‘true’ or ‘false’, indicating whether this dataset has been subtraction-corrected by a background (autofluorescence) image. Treated as ‘false’ by default. |
| LampType              | String with the FL lamp type  
|                       |   - Unknown (default)  
|                       |   - PriorLumen200  
|                       |   - PriorLumen220  
|                       |   - XCiteMultiBandLed  
|                       |   - PriorMimicLed  
|                       |   - SolaLed  
|                       |   - Colibri7                                                                                                                                  |
| InstrumentType        | String with the instrument type, one of the following:  
|                       |   - Unknown (default)  
|                       |   - Keyence700  
|                       |   - Keyence800
<table>
<thead>
<tr>
<th>Field</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CameraType</td>
<td>String with the camera sensor type, one of the following: SonyICX285AL, HamamatsuC11440, IMX265, IMX421</td>
</tr>
<tr>
<td>ExcitationFilter</td>
<td>Description of excitation filter with sub-elements listed below. If present, all its sub-elements must be provided.</td>
</tr>
<tr>
<td>Name</td>
<td>String with the filter public name</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>String with the manufacturer or OEM supplier</td>
</tr>
<tr>
<td>PartNo</td>
<td>String with the manufacturer part number</td>
</tr>
<tr>
<td>Bands</td>
<td>Collection of bands each as described by sub-elements below</td>
</tr>
<tr>
<td>Band</td>
<td>Description of a band with sub-elements noted below</td>
</tr>
<tr>
<td>Name</td>
<td>String with the band name</td>
</tr>
<tr>
<td>Cuton</td>
<td>Cuton wavelength of this band in nm</td>
</tr>
<tr>
<td>Cutoff</td>
<td>Cutoff wavelength of this band in nm</td>
</tr>
<tr>
<td>Active</td>
<td>True if this band is excited, False if not</td>
</tr>
<tr>
<td>EmissionFilter</td>
<td>Description of emission filter with sub-elements listed below. If present, all its sub-elements must be provided.</td>
</tr>
<tr>
<td>Name</td>
<td>String with the filter public name</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>String with the manufacturer or OEM supplier</td>
</tr>
<tr>
<td>PartNo</td>
<td>String with the manufacturer part number</td>
</tr>
<tr>
<td>Bands</td>
<td>Collection of bands each as described by sub-elements below</td>
</tr>
<tr>
<td>Band</td>
<td>Description of a band with sub-elements noted below</td>
</tr>
<tr>
<td>Name</td>
<td>String with the band name</td>
</tr>
<tr>
<td>Cuton</td>
<td>Cuton wavelength of this band in nm</td>
</tr>
<tr>
<td>Cutoff</td>
<td>Cutoff wavelength of this band in nm</td>
</tr>
<tr>
<td>CameraSettings</td>
<td>Description of the camera settings used for this image with sub-elements listed below. If present, all its sub-elements must be provided</td>
</tr>
<tr>
<td>Gain</td>
<td>Gain factor, such as 1, 3, etc.</td>
</tr>
<tr>
<td>Binning</td>
<td>Binning extent, such as 1 for full-resolution, 2 for 2x2 binning</td>
</tr>
<tr>
<td>OffsetCounts</td>
<td>Offset count setting for the camera</td>
</tr>
<tr>
<td>BitDepth</td>
<td>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)</td>
</tr>
<tr>
<td>Orientation</td>
<td>String containing one of the following values: ‘Normal’ (default), ‘Rotate180’, ‘MirrorV’, ‘MirrorH’</td>
</tr>
<tr>
<td>ROI</td>
<td>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</td>
</tr>
</tbody>
</table>
Appendix B: Akoya QPTIFF Specification

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X origin of ROI. Left-most pixel in sensor array is 0.</td>
</tr>
<tr>
<td>Y</td>
<td>Y origin of ROI. Top-most pixel in sensor array is 0.</td>
</tr>
<tr>
<td>Width</td>
<td>Width of ROI</td>
</tr>
<tr>
<td>Height</td>
<td>Height of ROI</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Tag</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>The name of the filter (whole-slide image) or band (component image)</td>
</tr>
<tr>
<td>Response</td>
<td>The instrument response to the reference artifact, normalized for exposure. This is raw counts / (2&lt;sup&gt;bit depth&lt;/sup&gt; × 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.</td>
</tr>
<tr>
<td>Date</td>
<td>The date and time of the reference image in UTC, ISO 8601 format.</td>
</tr>
</tbody>
</table>
| 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
<?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>
  </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").

AKOYA IS WILLING TO LICENSE THE SOFTWARE TO YOU ONLY UPON THE CONDITION THAT YOU ACCEPT ALL OF THE TERMS CONTAINED IN THIS AGREEMENT. PLEASE READ THE TERMS CAREFULLY BEFORE USING THE SOFTWARE, AS INSTALLING AND/OR USING THE SOFTWARE WILL INDICATE YOUR AGREEMENT TO THEM. IF YOU DO NOT AGREE TO ALL OF THE TERMS AND CONDITIONS IN THIS AGREEMENT, AKOYA IS NOT WILLING TO LICENSE THE SOFTWARE TO YOU, AND YOU (1) MAY NOT USE THE SOFTWARE AND (2) MUST CONTACT AKOYA WITHIN THREE (3) DAYS OF RECEIPT TO ARRANGE FOR THE RETURN OF THE SOFTWARE. BY INSTALLING AND/OR USING THE SOFTWARE, YOU ALSO REPRESENT AND WARRANT THAT YOU HAVE THE AUTHORITY TO AGREE TO AND BE Bound BY THE TERMS OF THIS AGREEMENT.

1. Single Use License. The Software is licensed to you and not sold, and you acknowledge that there is no transfer of title or ownership of the Software are to you. Subject to the terms and conditions of this Agreement and your payment of the license fees for the Software, Akoya hereby grants to you a restricted, non-exclusive, non-transferable, non-assignable, non-sublicensable and revocable single-use license to use, for your internal research purposes only, the executable code version of the Software and the accompanying documentation in hard copy or electronic format. Akoya grants no other licenses hereunder, except for the Research Use Only Phenochart Software set forth above. Nothing in this Agreement shall be construed as a general internal use license. For the avoidance of doubt, no right to use Phenochart in Diagnostic Applications is granted under this Agreement. Akoya RESERVES ALL RIGHTS NOT EXPRESSLY GRANTED BY THIS AGREEMENT.

2. Specific Restrictions. You may use the Software only on a single computer. You may make only one (1) copy of the Software, solely for backup purposes, and such copy must reproduce and include all copyright notices indicating Akoya's ownership of the Software. Except as expressly permitted by applicable law or Akoya, you agree that neither you nor a third party acting on your behalf, directly or indirectly, shall: (i) decompile, disassemble or reverse engineer the Software or otherwise attempt to determine the source code of the Software or underlying ideas, techniques or algorithms, including the review of data structures or similar materials produced by the Software; (ii) modify, alter, translate, or create derivative works of the Software; (iii) lease, rent, loan, commercially share or otherwise use the Software in any manner to provide service bureau, commercial time-sharing or other computer services to third parties; (iv) transmit the Software or provide its functionality, in whole or in part, over the Internet or other network; (v) use the Software for any purpose other than solely for your internal research; (vi) use the Software to provide any analytics or diagnostics or otherwise for the benefit of any third party; (vii) sell, distribute, publish, rent, lease, sublicense, assign possession, allow use of or otherwise transfer the Software or any copy of the Software are to a third party; (viii) create any computer software program or documentation which is similar to or competitive with the Software; (ix) encode, disclose or otherwise make available the Software are to any third-party; or (x) remove, modify or obscure any copyright, trademark, or other proprietary rights notice in or on the Software. This Agreement may not be assigned in whole or in part, except that either party may assign this Agreement without the other party's prior written consent in the event of a merger with an affiliate or other reorganization with an affiliate. Any attempted assignment contrary to the terms of this Agreement shall be void.

3. Ownership of Software. As between the parties, Akoya owns all right, title and interest, including all existing or future copyrights, trademarks, tools, know-how, and processes, in and to the Software are and any copies, modifications, alterations or derivative works thereof. You shall have no title to or ownership of the Software are. As between the parties, Akoya shall retain all rights to market, license and/or sell the Software are. The Software are (including accompanying documentation) constitutes the unpublished confidential information and trade secrets of Akoya. You (i) acknowledge, and agree not to contest, Akoya's rights in the Software are; and (ii) agree not to disclose and to take reasonable steps to protect any and all confidential information of Akoya regarding the Software are or that is otherwise disclosed to you in connection with this Agreement. You agree that you are responsible for ensuring your employees and agents are prohibited from taking any action otherwise prohibited to you under this Agreement and to notify Akoya promptly and in writing of the circumstances surrounding any possession, use or knowledge of the Software are or any part thereof known to you which is prohibited hereunder. You recognize that the covenants contained in this Agreement are reasonable and necessary to protect the legitimate interests of Akoya, that Akoya would not have entered into this Agreement in the absence of such covenants, and that your breach or threatened breach of such covenants shall cause Akoya irreparable harm and significant injury, the amount of which shall be extremely difficult to estimate and ascertain, thus making any remedy at law or in damages inadequate. Therefore, you agree that Akoya shall be entitled, without the necessity of posting any bond or security, to the issuance of injunctive relief by any court of competent jurisdiction enjoining any breach or threatened breach of such covenants and for any other relief such court deems appropriate. This right shall be in addition to any other remedy available to Akoya at law or in equity.
4. Termination. The license granted in Section 1 above is effective until terminated. This Agreement is conditioned upon your continued compliance with the terms and conditions hereof and will terminate automatically without notice from Akoya if you fail to comply with any term or condition of this Agreement. Furthermore, Akoya may terminate this Agreement at any time upon thirty (30) days’ notice. Upon termination of this Agreement, your license to use the Software or any part thereof shall immediately terminate, and you shall immediately destroy all copies of the Software (including all accompanying documentation) and any other confidential and proprietary information you have received during or in connection with this Agreement. Termination of this Agreement by Akoya shall not entitle you to a refund of any fees paid for the license of the Software.

5. Limited Warranty. The sole and exclusive warranty applicable to software and firmware products provided by Akoya for use with a processor is as follows: Akoya warrants that such software and firmware shall substantially conform to Akoya’s documentation and program manuals current at the time of shipment to you when properly installed on the processor. The limited warranty set forth above shall not apply if the claimed problem or issue is caused by: the malfunction of computer hardware or software not provided by Akoya; your negligence or fault; your failure to follow the instructions set forth in the documentation; changes in the hardware or other software not authorized by Akoya; modifications or changes to the Software not made or suggested by Akoya; or your failure to implement and maintain a proper backup and recovery system. Your exclusive remedy under this Section 5 shall be, at Akoya’s option, the replacement or correction of the problem or issue causing the non-conformance with the above warranty as promptly as possible following notice of such non-conformity, or a refund of the price paid for the Software.

6. No Other Warranties. EXCEPT FOR THE LIMITED WARRANTY STATED IMMEDIATELY ABOVE AND TO THE MAXIMUM EXTENT PERMITTED BY APPLICABLE LAW, THE SOFTWARE IS PROVIDED “AS IS” WITHOUT WARRANTY OF ANY KIND, AND AKOYA EXPRESSLY DISCLAIMS ANY AND ALL IMPLIED WARRANTIES, INCLUDING WITHOUT LIMITATION ANY WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT OF THIRD PARTY RIGHTS. AKOYA DOES NOT REPRESENT OR WARRANT THAT THE RESULTS OR THE USE OF THE SOFTWARE WILL BE CORRECT, ACCURATE OR RELIABLE, WILL MEET YOUR REQUIREMENTS, OR THAT THE SOFTWARE WILL OPERATE UNINTERRUPTED OR ERROR-FREE, OR THAT DEFECTS IN THE SOFTWARE WILL BE CORRECTED. Some jurisdictions do not allow the exclusion of implied warranties, so the above disclaimer may not apply to you. YOU ASSUME ALL RISK ASSOCIATED WITH THE USE, RESULTS, AND PERFORMANCE OF THE SOFTWARE.

7. Limitation of Liability. IN NO EVENT SHALL AKOYA, ITS AFFILIATES OR SUPPLIERS, OR THEIR RESPECTIVE EMPLOYEES, OFFICERS OR AGENTS, BE LIABLE FOR ANY DAMAGES ARISING OUT OF THE DELIVERY, INSTALLATION, PERFORMANCE, OR USE OR INABILITY TO USE THE SOFTWARE, INCLUDING WITHOUT LIMITATION INCIDENTAL, SPECIAL, CONSEQUENTIAL, PUNITIVE, EXEMPLARY, INDIRECT OR DIRECT DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF PROFITS, LOSS OF DATA, RE-RUN TIME, INACCURATE INPUT, WORK DELAYS, BUSINESS INTERRUPTION OR ANY OTHER COMMERCIAL DAMAGES OR LOSSES), WHETHER IN AN ACTION IN CONTRACT, TORT (INCLUDING NEGLIGENCE AND STRICT LIABILITY) OR OTHERWISE, AND EVEN IF AKOYA HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND REGARDLESS OF WHETHER ANY REMEDY FAILS OF ITS ESSENTIAL PURPOSE. AKOYA’S AGGREGATE LIABILITY ARISING OUT OF OR RELATING TO THIS AGREEMENT, FOR ANY AND ALL DAMAGES THAT YOU MIGHT INCUR (REGARDLESS OF THE FORM OF ACTION GIVING RISE TO SUCH LIABILITY, WHETHER IN CONTRACT, TORT OR OTHERWISE) SHALL NOT EXCEED THE PRICE PAID BY YOU FOR THE LICENSE OF THE SOFTWARE. Some jurisdictions do not allow the exclusion or limitation of liability for consequential or incidental damages. In such jurisdictions, our liability is limited to the greatest extent permitted by law, or the amount you paid for your purchase, whichever is less.

8. U.S. Government End Users. The Software qualifies as commercial computer software for purposes of the Federal Acquisition Regulations (FAR) §52.227-19 and the Department of Defense Supplement to the FAR (DFARS) §52.227.7013. If the Software is acquired by a civilian government agency, it is furnished with only the minimum Restricted Rights provided by FAR 52.227-19. If the Software is acquired by a military agency, it is furnished with only the minimum Restricted Rights provided by DFARS 52.227-7013(c)(1)(ii).

9. Miscellaneous. This Agreement contains the entire agreement of the parties with respect to the subject matter hereof and supersedes any proposal or prior agreement, written or oral, and any other communications between the parties relating to the subject matter hereof. No modification or waiver of any provision of this Agreement shall be effective unless in writing and signed by the parties. No delay or failure on the part of any party in exercising any right under this Agreement shall impair any such right or any remedy of such party, nor shall it be construed to be a waiver of any continuing breach or default under this Agreement. No action, regardless of form, arising out of the transactions contemplated herein may be brought by either party more than one (1) year after the cause of action has accrued. In the event any provision of this Agreement is held to be unenforceable, the remaining provisions of this Agreement will remain in full force and effect. This Agreement shall be governed by the laws of the State of Delaware without regard to principles of conflicts of laws. Any disputes relating hereto shall be adjudicated only in any court of competent jurisdiction in the State of Delaware, and you hereby consent to the exclusive jurisdiction of those courts. This Agreement shall not be governed by the United Nations Convention on Contracts for the International Sale of Goods, the application of which is expressly excluded. You may not assign or otherwise transfer this Agreement or any of your rights or obligations therein without the prior written consent of
Akoya. You may not use the Software for any unlawful purpose nor export or re-export the Software except as authorized by law.

10. TIFF module: This software uses the LibTIFF 4.0.3 library (http://remotesensing.org/libtiff/)
The TIFF module used is Copyright © 1988-1997 Sam Leffler Copyright © 1991-1997 Silicon Graphics, Inc.

Permission to use, copy, modify, distribute, and sell this software and its documentation for any purpose is hereby granted without fee, provided that (1) the above copyright notices and this permission notice appear in all copies of the software and related documentation, and (ii) the names of Sam Leffler and Silicon Graphics may not be used in any advertising or publicity relating to the software without the specific, prior written permission of Sam Leffler and Silicon Graphics.

THE SOFTWARE IS PROVIDED “AS-IS” AND WITHOUT WARRANTY OF ANY KIND, EXPRESS, IMPLIED OR OTHERWISE, INCLUDING WITHOUT LIMITATION, ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.

IN NO EVENT SHALL SAM LEFFLER OR SILICON GRAPHICS BE LIABLE FOR ANY SPECIAL, INCIDENTAL, INDIRECT OR CONSEQUENTIAL DAMAGES OF ANY KIND, OR ANY DAMAGES WHATSOEVER RESULTING FROM LOSS OF USE, DATA OR PROFITS, WHETHER OR NOT ADVISED OF THE POSSIBILITY OF DAMAGE, AND ON ANY THEORY OF LIABILITY, ARISING OUT OF OR IN CONNECTION WITH THE USE OR PERFORMANCE OF THIS SOFTWARE.

11. This software uses the VIGRA 1.6.0 library (http://hci.iwr.uni-heidelberg.de/vigra/)

This software includes machine-executable object code generated by a source language processor from the VIGRA libraries covered by the VIGRA license. These libraries have not been modified by Akoya and all rights are reserved by the copyright holder. This software is provided “as is” without express or implied warranty, and with no claim as to its suitability for any purpose.

The VIGRA library is Copyright © 2008, Ulrich Kothe, Heidelberg Collaboratory for Image Processing

The VIGRA License

=================================

(identical to the MIT X11 License)

Permission is hereby granted, free of charge, to any person obtaining a copy of this software and associated documentation files (the “Software”), to deal in the Software without restriction, including without limitation the rights to use, copy, modify, merge, publish, distribute, sublicense, and/or sell copies of the Software, and to permit persons to whom the Software is furnished to do so, subject to the following conditions:

The above copyright notice and this permission notice shall be included in all copies or substantial portions of the Software.

THE SOFTWARE IS PROVIDED “AS IS”, WITHOUT WARRANTY OF ANY KIND EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO THE WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE AND NONINFRINGEMENT. IN NO EVENT SHALL THE AUTHORS OR COPYRIGHT HOLDERS BE LIABLE FOR ANY CLAIM, DAMAGES OR OTHER LIABILITY, WHETHER IN AN ACTION OF CONTRACT, TORT OR OTHERWISE, ARISING FROM, OUT OF OR IN CONNECTION WITH THE SOFTWARE OR THE USE OR OTHER DEALINGS IN THE SOFTWARE.

12. This software uses the libjpeg-turbo 1.5.13 library (http://libjpeg-turbo.virtualgl.org)

This software makes use of the libjpeg-turbo library. This library has not been modified by Akoya and all rights are reserved by the copyright holder. This software is provided "as is" without express or implied warranty, and with no claim as to its suitability for any purpose. The library is covered by the libjpeg-turbo License. Additionally libjpeg-turbo incorporates parts of libjpeg which is covered by the BSD license.

The libjpeg-turbo license is reproduced below:

Redistribution and use in source and binary forms, with or without modification, are permitted provided that the following conditions are met:

• Redistributions of source code must retain the above copyright notice, this list of conditions and the following disclaimer.
• Redistributions in binary form must reproduce the above copyright notice, this list of conditions and the following disclaimer in the documentation and/or other materials provided with the distribution.
• Neither the name of the libjpeg-turbo Project nor the names of its contributors may be used to endorse or promote products derived from this software without specific prior written permission.
THIS SOFTWARE IS PROVIDED BY THE COPYRIGHT HOLDERS AND CONTRIBUTORS "AS IS", AND ANY EXPRESS OR IMPLIED WARRANTIES, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE ARE DISCLAIMED. IN NO EVENT SHALL THE COPYRIGHT HOLDERS OR CONTRIBUTORS BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO, PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGE.

The BSD for libjpeg license is reproduced below:

The authors make NO WARRANTY or representation, either express or implied, with respect to this software, its quality, accuracy, merchantability, or fitness for a particular purpose. This software is provided "AS IS", and you, its user, assume the entire risk as to its quality and accuracy.

This software is copyright (C) 1991-2010, Thomas G. Lane, Guido Vollbeding.

All Rights Reserved except as specified below.

Permission is hereby granted to use, copy, modify, and distribute this software (or portions thereof) for any purpose, without fee, subject to these conditions:

(1) If any part of the source code for this software is distributed, then this README file must be included, with this copyright and no-warranty notice unaltered; and any additions, deletions, or changes to the original files must be clearly indicated in accompanying documentation.

(2) If only executable code is distributed, then the accompanying documentation must state that "this software is based in part on the work of the Independent JPEG Group".

(3) Permission for use of this software is granted only if the user accepts full responsibility for any undesirable consequences; the authors accept NO LIABILITY for damages of any kind.

These conditions apply to any software derived from or based on the IJG code, not just to the unmodified library. If you use our work, you ought to acknowledge us.

Permission is NOT granted for the use of any IJG author's name or company name in advertising or publicity relating to this software or products derived from it. This software may be referred to only as "the Independent JPEG Group's software".

We specifically permit and encourage the use of this software as the basis of commercial products, provided that all warranty or liability claims are assumed by the product vendor.

13. This software uses the zlib 1.2.7 library (http://www.zlib.net)

This software makes use of the zlib1 library. This library has not been modified by Akoya and all rights are reserved by the copyright holder. This software is provided "as is" without express or implied warranty, and with no claim as to its suitability for any purpose. The library is covered by the zlib1 License.

The zlib1 license is reproduced below:

(C) 1995-2012 Jean-loup Gailly and Mark Adler

This software is provided 'as-is', without any express or implied warranty. In no event will the authors be held liable for any damages arising from the use of this software.

Permission is granted to anyone to use this software for any purpose, including commercial applications, and to alter it and redistribute it freely, subject to the following restrictions:

1. The origin of this software must not be misrepresented; you must not claim that you wrote the original software. If you use this software in a product, an acknowledgment in the product documentation would be appreciated but is not required.

2. Altered source versions must be plainly marked as such, and must not be misrepresented as being the original software.

3. This notice may not be removed or altered from any source distribution.

Should you have any question concerning this Agreement, you may contact Akoya by writing to Akoya Biosciences, 100 Campus Drive, 6th Floor, Marlborough, MA 01752. You may also call +1 855.896.8401
8 Contacting Akoya Biosciences

For more information, contact Akoya Biosciences or your local authorized distributor:

Akoya Biosciences, Inc.
100 Campus Drive, 6th Floor
Marlborough, MA, 01752, USA

Technical Support:
Phone: +1 855-896-8401
Fax: +1 855-404-0061

Akoya Biosciences Web Site: http://www.akoyabio.com
Technical Support Email: customercare@akoyabio.com
Index

- A -
  Akoya Biosciences Technical Support 63
  Algorithm training 44
  Annotated fields or cores 49
  Annotation 31
  Annotation Colors 46
  Annotation file 31
  Annotation Visibility 46

- C -
  Check Image Integrity 20
  Config File 50
  Contact Akoya 63

- F -
  Fluorescence Layer Toolbar 21

- G -
  Gear Menu 10

- I -
  Image Integrity 20
  Image Sharpening 20
  Instrument Responsivity 52

- L -
  Label View 15
  Layer Button 21
  Layer Editor 21
  Log File 50
  Login 31

- M -
  Magnifier 13

- N -
  Navigation Tool 10
  Navigation Toolbar 10
  Navigator Window 10

- O -
  Overlap Fields in Freehand ROIs 34

- P -
  Phenochart 5

- Q -
  QPTIFF Specification 52

- R -
  Resolution 42
  ROI Editor 34
  ROI Tool 34
  Rotate Label View 15

- S -
  Scale Bar 14
  Selection Type 42
  Sharpen Image Display 20
  Slide Browser 6
  Slide Info 18
  Snapshot button 30
  Snapshot Editor 30
  Software EULA 59
  Stamp Annotations 33
  Status Bar 17
  Study 6
  Support, Technical 63

- T -
  Technical Support 63
  TIFF Specification 52
Tissue Category  42
TMA button    38
TMA editor    38

- V -

Vectra Data    6