

Vectra[®] 3

Quantitative Pathology Imaging System

User's Manual



Vectra 3.0.7
Publication Date: August 27, 2019

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This manual describes system operation using Vectra software.

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Table of Contents

1 Preface	5
2 Vectra Overview	8
3 Getting Familiar with Vectra 3.0	11
3.1 Starting the Vectra 3.0 System.....	12
3.2 Starting the Vectra Software.....	14
3.3 Loading Slides onto the Vectra 3.0 System.....	15
3.3.1 Loading Slides on the Vectra 3.0, 200-Slide System	15
Loading Slides into the Slide Loader.....	15
Loading Slides onto the Vectra 3.0, 200-Slide Stage Manually	16
3.3.2 Loading Slides on the Vectra 3.0, 6-Slide Stage	17
3.4 Overview of the Vectra Home Page.....	18
3.4.1 Check Dashboard	18
3.4.2 Edit Protocol	19
3.4.3 Scan Slides	19
3.4.4 Launch Phenochart	19
3.4.5 Gear Menu	19
4 System Dashboard	20
4.1 Use of the Dashboard.....	20
4.2 Set Nominal Focus.....	20
4.3 Reference Images.....	22
4.3.1 Brightfield Reference Images	22
4.3.2 Fluorescence Reference Images	25
5 Creating and Editing Studies and Protocols	27
5.1 Study and Protocol Creation.....	27
5.2 Brightfield Studies and Protocols.....	28
5.3 Acquiring Brightfield Sample Regions.....	31
5.4 Fluorescence Studies and Protocols.....	33
5.4.1 Creating a Fluorescence Protocol	33
5.5 Setting Exposure Times for MSI Regions.....	42
5.6 Acquiring Fluorescent Sample Regions.....	44
5.7 Automated MSI Field Selection.....	47
6 Performing Whole Slide Scans using Vectra	48

6.1	Whole Slide Scanning on the Vectra 3.0, 6-Slide System.....	48
6.2	Whole Slide Scanning on the Vectra 3.0, 200-Slide System.....	53
6.3	Selecting MSI Fields for Acquisition.....	56
6.3.1	Using Phenochart to select MSI Fields for acquisition	56
6.3.2	Using Vectra to select MSI Fields for acquisition	56
7	Advanced Automated Workflows	58
8	Hardware Overview and Adjustments	60
8.1	Hardware Descriptions.....	60
8.2	Eyepiece Adjustment.....	71
8.3	Cleaning the Vectra Hardware.....	72
9	System Specifications	73
9.1	Site and Environmental Requirements	73
10	Hardware Setup Program	75
11	Frequently Asked Questions	81
12	Troubleshooting	83
13	Appendix A: Supported Slide Formats & Dimensions	86
14	Appendix B: Akoya Biosciences TIFF Specification	88
15	Appendix C: Typical Scan & MSI Field Acquisition Times	94
16	Appendix D: Filter Cube Recommendations when using Opal Reagents	95
17	Appendix E: Compensation Calculations	96
18	Appendix F: Arc-lamp Fluorescence Illuminator User Information	97
19	Appendix G: Epi-Filter Wedge Correction	107
20	Appendix H: Software EULA	109
	Index	117

1 Preface

About This Manual

This manual describes the use and functionality of the Akoya Biosciences Vectra® 3.0 Automated Quantitative Pathology Imaging System. Operating instructions, functional descriptions, troubleshooting, illustrations, and other relevant information are contained in this manual.

Design Change Disclaimer

Due to design changes and product improvements, information in this manual is subject to change without notice. Akoya Biosciences reserves the right to change product design at any time without notice to anyone, which may subsequently affect the content of this manual. Akoya Biosciences makes every reasonable effort to ensure that this User's Manual is up to date and corresponds with the shipped Vectra 3.0.

Reproduction Disclaimer

This User's Manual is solely for the use of the owner and operator of the Vectra 3.0. Any reproduction of this publication in part or in whole without the express written consent of Akoya Biosciences is strictly prohibited. Neither may this publication be made available for electronic download without the express written consent of Akoya Biosciences.

Technical Support

If you experience any difficulty operating or maintaining the Vectra 3.0, please contact your Akoya representative. Office hours are 8:00 a.m. to 8:00 p.m. (Eastern Standard Time), Monday through Friday.

- Telephone: +1 855-896-8401
- Fax: +1 855-404-0061
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- Email (Outside the US): CustomerCareEMEA@akoyabio.com
- Website: <http://www.akoyabio.com>

CE Testing and Certification

The Vectra 3.0, 6 Slide Instrument has been tested by an independent CE testing facility, and bears the appropriate CE mark.











Note: Changes or modifications to this equipment not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Remarque: Tout changement ou modification apporté à cet instrument non expressément approuvé par l'entité responsable de la conformité peut annuler l'autorisation d'opérer l'appareil accordée à l'utilisateur.

Contact Akoya Biosciences [Technical Support](#) for more information.

Table of Symbols

The table below contains symbols that identify particularly important information and alert you to the presence of hazards. These symbols may appear in this manual and/or on the product it describes.

	Warning: Read instructions to determine possible hazard. Caution: Read these operating instructions fully before use, and pay particular attention to sections containing this symbol.
	Warning: Surface may be Hot.
	Warning: UV Output.
	Danger: Electrical Shock hazard.
	AC Input
	DC Input
	USB Port
	WEEE symbol. Do not dispose of as unsorted municipal waste. Contact your Akoya Biosciences Service Engineer for more information.

Operator and Equipment Safety

It is the responsibility of the purchaser to ensure that all persons who operate the Vectra 3.0 are aware of the following cautionary statements. As with any scientific instrument, there are important safety considerations, which are highlighted throughout this User's Manual.

General Cautionary Statements

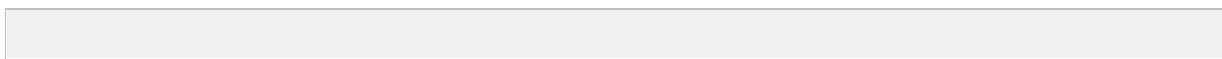
READ AND UNDERSTAND THIS USER'S MANUAL BEFORE ATTEMPTING TO OPERATE, TROUBLESHOOT, OR MAINTAIN THE VECTRA 3.0. READING THIS MANUAL FIRST MAKES IT EASIER AND SAFER TO OPERATE AND MAINTAIN THE SYSTEM.

- The Vectra system contains moving parts that can cause user injury. Always keep your fingers, long hair, loose clothing, dangling jewelry, etc., away from all moving parts to avoid personal injury and possible system damage.
- Use only as specified by the operating instructions. Otherwise, the built-in protections may be impaired.

- Do not operate the system if there has been a malfunction of the motorized stage or slide loading components, as this could lead to equipment damage or physical injury. Contact Akoya Biosciences for assistance.
- Operate the system on a flat, stable surface.
- Do not drop the Vectra imaging module or any other system components.
- Do not expose the imaging module to prolonged heat above 40°C.
- Do not operate the system in an environment with explosive or flammable gases.
- Do not operate the system in places where it may be splashed with liquid.
- Use only the power supply cord set provided with the Vectra system. If the correct cord set for the location was not provided, please contact Akoya Biosciences for a replacement. Do not use power supply cords with inadequate ratings.
- Some cables and adapters supplied with the system have proprietary specifications. Do not connect components supplied by Akoya Biosciences using unqualified cables or adapters. Doing so could result in damage, and voids the Warranty. Contact Akoya Biosciences for a replacement if necessary.
- Use only a properly grounded power outlet when connecting the system to power.
- Place equipment and devices in a manner such that the power switches and disconnecting devices are accessible at all times.
- Follow the recommended maintenance procedures to help ensure optimal performance.



Caution: Installing, servicing, and moving the Vectra system should be performed by Akoya Biosciences or PerkinElmer authorized and trained personnel only. Power must be disconnected from the system before servicing.



2 Vectra Overview

The topics in this section of the User's Manual provide general information about the Vectra® 3.0 Automated Quantitative Pathology Imaging System. It is important to read and understand the [Operator and Equipment Safety](#)^[6] section before using the system. The [Hardware Description](#)^[60] provides a description of each of the hardware components that constitute the Vectra system. If you are imaging fluorescence slides, please review the [Fluorescence Illuminator Safety information](#)^[97] so that you are familiar with the fluorescence illuminator and its safety precautions.

Akoya's Vectra 3.0 is a multi-modal, automated imaging system for scanning tissue sections and microarrays (TMAs), and acquiring multispectral (MSI) images in regions of interest. It supports workflows including whole slide scanning, annotation and review, with a simple, intuitive interface. It includes the following software:

- **Vectra:** Operator-centric software for performing whole slide scans and acquiring MSI regions of interest. Vectra runs on the workstation connected to the Vectra 3.0 instrument. It can automatically select regions for MSI acquisition using inForm® Tissue Finder™ software, for fully automated operation.
- **Phenochart™:** Operator-centric whole-slide contextual viewer program for annotating slide scans performed by Vectra. Regions of interest can be highlighted for review or multispectral acquisition. Phenochart can also be used to enable automated selection of MSI fields by Vectra with the aid of inForm Tissue Finder. Phenochart is freely distributed and can be used by multiple users who wish to view or review slide scans taken by Vectra.
- **inForm® Tissue Finder™:** Analytical software typically used for tissue classification and training, phenotypic and protein expression measurements, and data export. It can be run from the same computer that is controlling the Vectra instrument, or on a separate computer. Additional inForm software 'seats' beyond the two seats that come with each Vectra 3.0 system are available for purchase.

Vectra 3.0 is part of Akoya's Phenoptics™ pathology workflow solution, leveraging Akoya's Opal™ multiplexed fluorescent immunohistochemistry reagent kits and inForm's spectral analysis and classifier training capabilities. Vectra and Phenochart operate in tandem, using Vectra for whole slide scanning and multispectral field acquisition and Phenochart for Viewing and Annotation of the scans. inForm is used for analysis of the multispectral fields.

Phenochart allows the operators to view the whole slide (zoom, pan, etc.), and make decisions (annotations) on next steps for the sample. Annotations in Phenochart are also used to record the workflow actions for each slide scan. Annotations include reviewer requested MSI fields, automated (inForm) field requests, and reviewer edits, approvals, and rejections. The annotation file is a fully auditable transaction log.

Vectra 3.0 workflows range from simple two-step procedures (e.g. acquire whole slide scan and review) to automated acquisition of regions of interest selected by the operator or Vectra 3.0 itself.

As an example, a fluorescence workflow might include the following steps:

- Stain tissue with Akoya Biosciences Opal fluorescent IHC reagents
- Acquire whole slide fluorescent imagery using Vectra
- Review the whole slide imagery with Phenochart, and annotate regions of interest for MSI analysis
- Acquire the MSI regions with Vectra
- Use inForm to phenotype cells and measure protein expression levels in the acquired MSI regions.

In projects involving many samples, one can automate the region selection in Phenochart, or in Vectra, using learn-by-example machine learning based on training by an expert operator.

Two Vectra 3.0 models are available: a 6-slide model and 200-slide model, both of which incorporate automated microscope slide stages. The 6-slide Vectra 3.0 automatically scans up to six slides at a time. The 200-slide model accepts up to four 50-slide cassettes and automates slide handling to enable walkaway scanning of up to 200 slides at a time.



Figure 1. Vectra 3.0, 200-Slide Imaging System

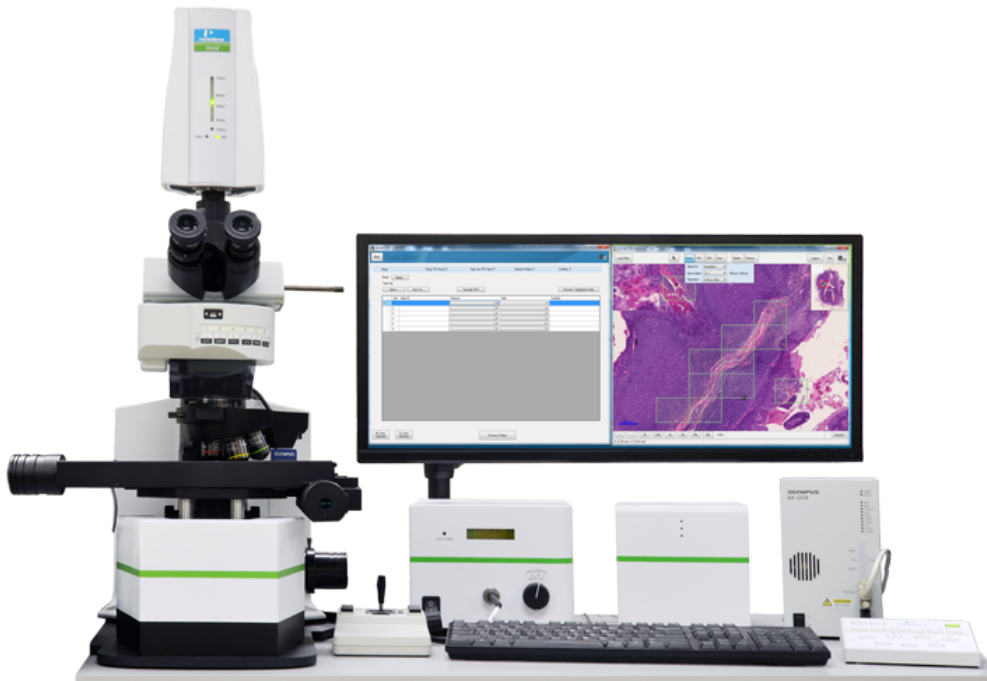


Figure 2. Vectra 3.0, 6-Slide Imaging System

The system is for Research Use Only and not for use in diagnostic procedures.

Examples of Vectra Applications

- Whole slide scanning of tissue samples stained with H&E and conventional IHC stains
- Whole slide scanning and multispectral interrogation of tissue samples stained with Akoya Biosciences Opal™ reagents and markers related to cancer immunology such as CD4, CD8, CD20, PD-1, PD-L1, cytokeratin
- Simultaneous imaging of IHC staining of p21, p27 and Ki67 in psoriatic skin sections
- Imaging of DAPI, Ki67, CD3, CD20, IgD and CD68 labeled with QDot® probes in lymph node germinal center
- Multicolor staining of ER, PR, HER1 and HER2 and hematoxylin in a single breast tissue section
- Co-localization analysis of D2-40, SMA, CD34 and CD105 in tonsil sections
- Simultaneous analysis of distribution of CD3, CD1, cytokeratin and hematoxylin in metastatic lymph nodes
- Automatic identification and analysis of necrosis in liver tissues
- Automatic identification and counting of mitoses
- Multicolor staining of hematoxylin and Schiff's reagent in glomerular tufts of kidney
- Multicolor staining of DNA with DAPI smooth muscle-myosin heavy chain (SM-MHC) with Cy2
- Smooth muscle α -actin (SMA) with Cy®3 in muscles

3 Getting Familiar with Vectra 3.0

Training is provided by Akoya Biosciences personnel after the Vectra 3.0 Quantitative Pathology Imaging System is installed. Akoya personnel will show you how to use the Vectra and will explain its features.

The Vectra instrument computer comes with Vectra and Phenochart installed and ready to use.

Your Vectra 3.0 system also includes two seats of inForm, Akoya's advanced image analysis program. The inForm installer is pre-loaded on the Vectra instrument computer by Akoya, but you must install it and approve its end-user license agreement (EULA) in order to use it. Instructions for licensing and operating inForm are included in the inForm manual, and can also be read via its help menu.

The Phenochart program is free and can be run on any computer that meets the minimum requirements. To download Phenochart, please visit <https://www.akoyabio.com/phenopticstm/software>.

3.1 Starting the Vectra 3.0 System

This section describes how to power up the system and launch the Vectra software.

It is important to read and understand the [Operator and Equipment Safety](#)^[6] section before using the system. If you are not familiar with the Vectra system hardware, the [Hardware Descriptions](#)^[60] section provides a description of each hardware components in the Vectra system. If you are using the fluorescence illuminator, review the [Fluorescence Illuminator Safety](#)^[97] section first so you are familiar with the fluorescence illuminator and its safety precautions.

If you are viewing or reviewing images on a remote computer, consult the Help guide associated with Phenochart.

Powering on the Hardware Components

1. Power up the computer and allow Windows® to start. Wait for the imaging module to initialize (the lights on the front panel will stop blinking).

NOTE: In North America, all components associated with Vectra 3.0 should turn on when the computer turns on, except the Fluorescence Illuminator. Next, skip to step 6.

Outside North America, please follow steps 2-5 before moving on to Step 6:

2. Turn on the [Microscope Module Controller Box](#)^[67] (Olympus BX-UCB) to turn on power to the microscope.
3. Turn on the [Vectra Stage Controller Module](#)^[69] to provide power to the XY automated microscope stage.
4. For 200-slide models, make sure that the slide loader arm is in a safe position. The loader arm should not be close to, or in contact with, the microscope stage or any of the slide cassettes. Do not start the Vectra software until the loader arm is in one of the safe positions shown below. **IMPORTANT:** The arm can be manually retracted and moved in the rotary direction **ONLY** when the power is off.

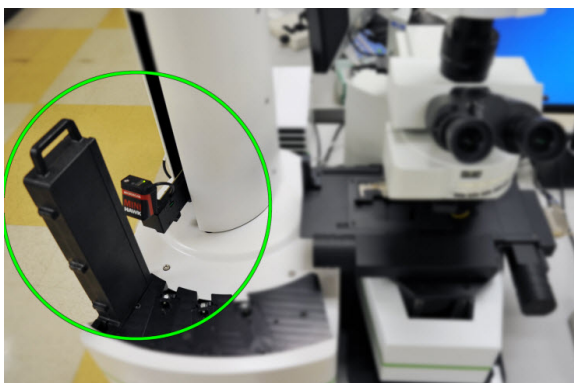


Figure 3. Loader Arm Facing a Slide Cassette

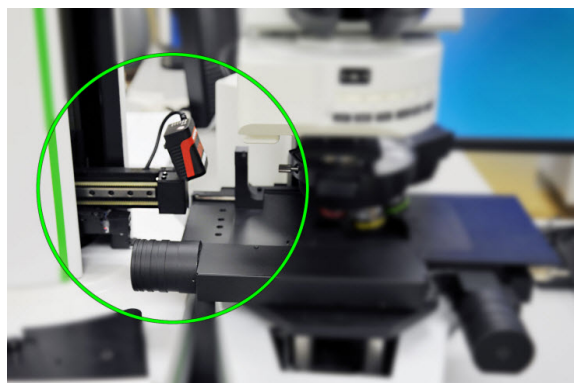


Figure 4. Loader Arm Facing the Stage

5. For 200-slide systems, turn on the Vectra Slide Loader. The switch is located on the back of the unit, toward the left. See [Vectra Slide Loader](#)^[64] to see the rear connectors and power switch.
6. If you intend to perform fluorescence imaging, turn on the LED Illuminator using the power switch located in the back of the LED illuminator unit.



Figure 5 LED Illuminator Power Switch



*Figure 6. LED
on-off switch*

3.2 Starting the Vectra Software

The Vectra software requires an activated license to run. The software was installed on the Vectra computer and activated at the factory. If you need to reinstall or reactivate the Vectra software, contact your Field Application Specialist. Vectra licenses are not recoverable. If the Vectra seat license is damaged or inadvertently lost (e.g. the hard drive crashes), contact [Technical Support](#)⁵ for assistance. Please have the Vectra instrument serial number (5 digits in length) and/or the Vectra 3.0 license number (a pattern of 10-3-7 alphanumeric characters) ready.

IMPORTANT: The Vectra 3.0 instrument must be connected and powered up in order to run the Vectra software successfully.

1. Make sure the computer and Vectra system hardware components are turned on and ready.
2. Double-click the Vectra icon on the Windows desktop.
OR
Select **Start > All Programs > Akoya > Vectra3.0 > Vectra**.

The system initializes all hardware including the motorized XY microscope stage, the imaging module, and the Vectra slide loader (200-Slide model). The system may cycle through its objective lenses and epi-filters, ending with the low power (4x) objective. Hardware initialization takes approximately 30 seconds for the 6-slide system, and 90 seconds for the 200-slide system.

After initialization, the Vectra Whole Slide Home Page appears on the screen.

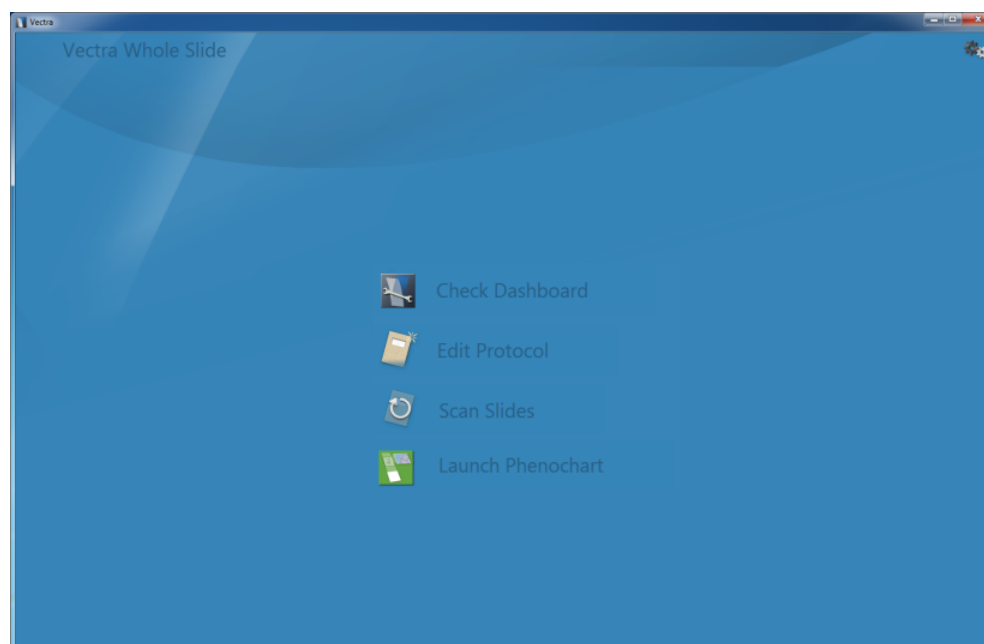


Figure 7. Vectra Whole Slide Home Page

3.3 Loading Slides onto the Vectra 3.0 System

This section describes how to load the slides into the cassettes of the Vectra 3.0, 200-slide or into the stage insert slots of the Vectra 3.0, 6-slide system.

3.3.1 Loading Slides on the Vectra 3.0, 200-Slide System

The Vectra 3.0, 200-slide model features an automated slide-cassette system. Up to 4 slide cassettes that hold up to 50 slides each can be placed onto the slide loader mechanism.

3.3.1.1 Loading Slides into the Slide Loader

Refer to [Appendix A: Supported Slide Formats & Dimensions](#)^[86] for more information on the requirements for slides used with Vectra 3.0. **Warning:** Use only slides that are free of debris, fingerprints and dust. Coverslips and labels must not overhang the edge of the slides. Do not use broken or damaged slides, or slides with broken or damaged coverslips. Slide edges and surfaces must be clean and free of any adhesive. A single slide-label may be applied at one end of the slide. If necessary, it is preferred that multiple labels be applied on top of one another. Once scanning begins, never interfere in any way with the slides or the cassettes. Keep fingers clear of all moving parts to avoid injury.

1. Make sure the Vectra Slide Loader is turned on. (The switch is located on the back of the unit, toward the left. See [Vectra Slide Loader](#)^[64].)
2. You may load up to 50 slides in each cassette. Load slides into cassettes so that the labels are facing upward and outward. Slides can be loaded into any position in the cassette; they do not have to be in contiguous locations (see Figure 8).

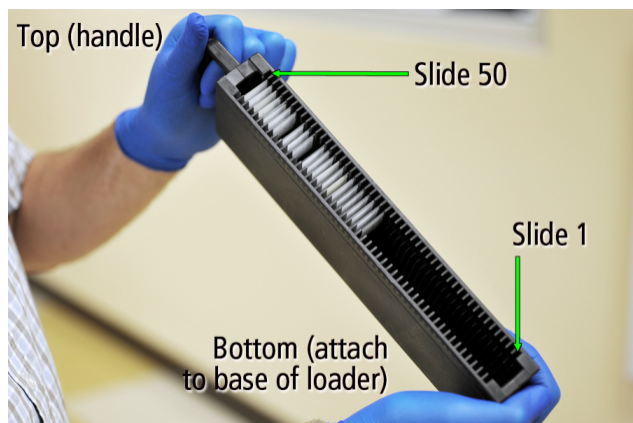


Figure 8. Slides loaded into a cassette

3. Mount the cassette(s) onto the Vectra Slide Loader by setting the rear bottom corner into position first. Then press the front down until it clicks into position (see Figure 9).
4. Mount up to four cassettes, with up to 50 slides in each cassette. The Vectra software automatically recognizes the installed position of each cassette and, after the loader arm passes over the length of each cassette, the presence or absence of each slide.
5. Once each cassette is mounted, verify that all the slides are pushed all the way to the back of the cassette.



Figure 9. Mounting Slide Cassettes to the Slide Loader

3.3.1.2 Loading Slides onto the Vectra 3.0, 200-Slide Stage Manually

At times, it is useful to manually load a single slide onto the stage. This is particularly useful when optimizing exposure times for fluorescent samples and acquiring fluorescent single-stained control samples. Move the stage using the [Stage Control Joystick](#)⁶³ to the left as far as possible and as close as possible to you. The holder will open and you can place the slide onto the stage.

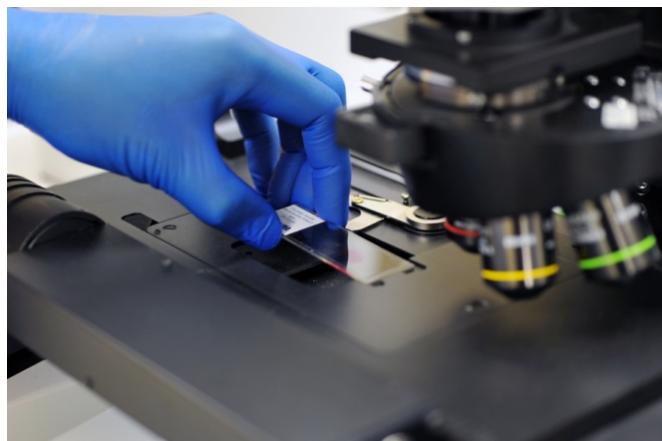


Figure 10. Placing a single slide onto the Vectra 3.0, 200-slide system

3.3.2 Loading Slides on the Vectra 3.0, 6-Slide Stage

1. Slot 1 is located at the far left of the slide holder and slot 6 is at the far right. Using the [Stage Control Joystick](#)⁶³, move the 6-slide stage as far left and front as possible. In this position, slots 1–4 can be easily populated with slides. Prior to populating a slot with a microscope slide, pull back the silver tab associated with the slot by using your thumb and the groove in the tab. Position the slide into the slot, gently pushing it up against the left and front most walls of the slot, then gently release the tab.



Figure 11. Slide stage positioned front left



Figure 12. Pull back tab, position slide, release tab

2. Using the joystick, move the 6-slide stage to the right until slots 5 and 6 are free and clear of the objectives and are accessible. Populate slots 5 and 6 with microscope slides using the method defined in step 1 above.

3.4 Overview of the Vectra Home Page

Welcome to the Vectra Home Page. From this page, you can link to necessary pages to maintain and run Vectra 3.0.

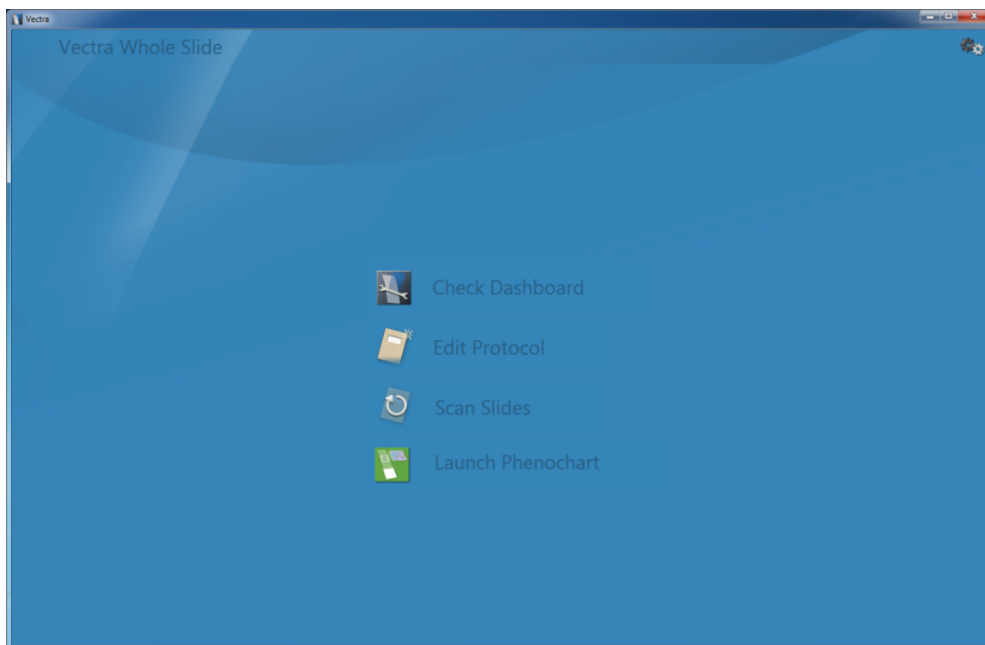


Figure 13. Vectra Whole Slide Home Page

The Home Page contains buttons that link to the following pages:

- [Check Dashboard](#)^[18] (brings you to the System Dashboard page) - Set nominal focus, interrogate disk space, acquire references, and know the stage position.
- [Edit Protocol](#)^[19] - Create or load brightfield and fluorescent protocols and studies.
- [Scan Slides](#)^[19] - Select studies and create task lists.
- [Launch Phenochart](#)^[19] - Launches the Phenochart viewing software.
- [Gear Menu](#)^[19] (in the upper right) - alternate method to get to the above pages, as well as to check your version number or perform other functions, including launching online help.

Below is a description of each page.

3.4.1 Check Dashboard

The Dashboard is used to check status of your system prior to imaging. It includes the following:

- [Setting Nominal Focus](#)^[20] - sets the focus for the 4X objective with a test slide.
- Disk space – checks the available space on the disk drive where images will be stored.
- [Acquire References](#)^[22] - acquire brightfield references and fluorescent references.

For detailed information on how to use the System Dashboard, please see [System Dashboard](#)^[20].

3.4.2 Edit Protocol

The Edit Protocol page is used by the operator to define protocols.

A protocol describes how a sample is to be imaged, including the imaging mode (Brightfield or Fluorescence), and the spatial resolution (magnification) for the whole slide scan and for multispectral regions of interest. For fluorescent imaging, it also describes the exposure times, and what bands to image and use for focusing.

For detailed information on how to create and edit protocols and studies please see [Creating and Editing Studies and Protocols](#)^[27].

3.4.3 Scan Slides

The Scan Slides page is used to actually scan slides, or to prepare task lists of slides to be scanned.

For detailed information on how to perform whole slide scans, see [Performing Whole Slide Scans using Vectra](#)^[48].

3.4.4 Launch Phenochart

This button is used to launch the Phenochart program.

For detailed information on how to use Phenochart, see the *Phenochart User's Manual* or online Help from within the Phenochart program.

3.4.5 Gear Menu

The Gear menu in the upper right hand corner of the Home Page is used by the operator to:

- Find out What's New in the current installed version
- Launch the online Help system
- Open the Vectra software Log for reference or troubleshooting
- Open the About window to view the current software version
- View contact information for Akoya Biosciences technical support

4 System Dashboard

This section describes the tools found in the System Dashboard, and how to use them for trouble-free operation.

4.1 Use of the Dashboard

Akoya Biosciences recommends that references be taken every 50 hours of system operation, to keep drift and aging effects negligible.


The sections below contain detailed descriptions of the System Dashboard controls.

4.2 Set Nominal Focus

During scanning or MSI acquisition, the Vectra system automatically focuses on your samples. The Nominal Focus Position is the starting position it uses when focusing on slides. The system can accommodate actual sample locations that vary by up to 100 microns from this point. If you need to scan a batch of slides that is exceptionally thick or thin, set the Nominal Focus Position using one of these slides.

The Nominal Focus Position is a global setting that is used across all protocols, and unless unusual slides are being processed, should not vary.

To set the Nominal Focus Position:

1. Load a brightfield slide onto the microscope stage – an H&E or other strongly stained sample works well. The glass thickness of this slide should be similar to that of typical slides to be scanned.
2. Start the Vectra software.
3. Click the **Check Dashboard** button  to open the [System Dashboard](#) ^[20].
4. Make sure the [trinoc](#) ^[61] is in the camera-only position.
5. Click the **Set Nominal Focus** button. The microscope light turns on, the live stream displays in the System Dashboard window, and the Set Nominal Focus window displays as shown in Figure 14.

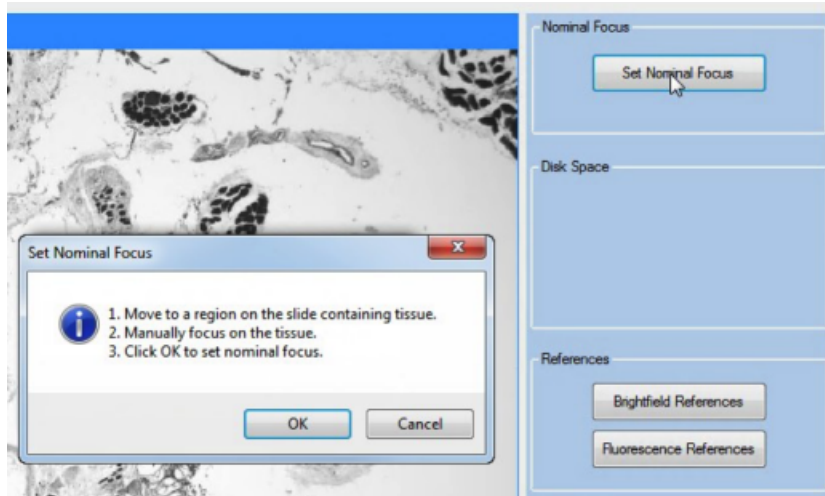


Figure 14. Set Nominal Focus Instructions

6. Use the joystick to manually move to a region on the slide that contains tissue.



Figure 15. Move to a region with tissue using the Stage Control Joystick.

7. Use the joystick to focus on the tissue using the Live View in the System Dashboard.
8. When the image is in focus, click the **OK** button in the Set Nominal Focus window to save the current position as the Nominal Focus Position. The Live Stream turns off.
9. Click the **Back** button on the System Dashboard to return to the Vectra main window.

4.3 Reference Images

The Vectra system requires use of 'reference' images for flat-fielding, and background subtraction to reduce or eliminate non-uniformities caused by uneven illumination or dust and debris in the light path in the optical system.

NOTE: We recommend that your lab decide on a procedure that specifies when new references should be taken.

Taking new reference images takes approximately 5 minutes. To take reference images, you need a suitable brightfield slide and a Vectra Compensation Slide (see Figure 16). The Vectra Compensation Slide is blue with no sample or cover slip attached. Use a typical brightfield slide with tissue and a cover slip attached for a brightfield reference slide. Make sure that there is a clean (empty) section of the brightfield slide available for reference imaging purposes.

Use caution when cleaning your reference slides. Do not use Kimwipes on the Vectra Compensation Slide because their abrasiveness can scratch the more delicate surface of that slide. Instead, use compressed air, keeping the nozzle away from the slide and tilting it to minimize the danger of particles being blown against the surface.

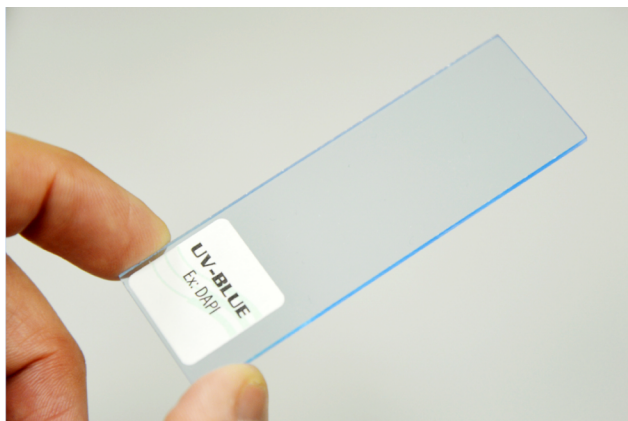



Figure 16. Fluorescence Compensation Slide

4.3.1 Brightfield Reference Images

To take the Brightfield reference images:

1. Start the Vectra software.
2. Click the **Check Dashboard** button  on the [Vectra Homepage](#)^[18] to open the [Dashboard](#)^[20].
3. Click the **Brightfield References** button. The Brightfield Reference Images window opens as shown in Figure 17. The Brightfield Reference Images window allows you to view any existing reference images or acquire new reference images.

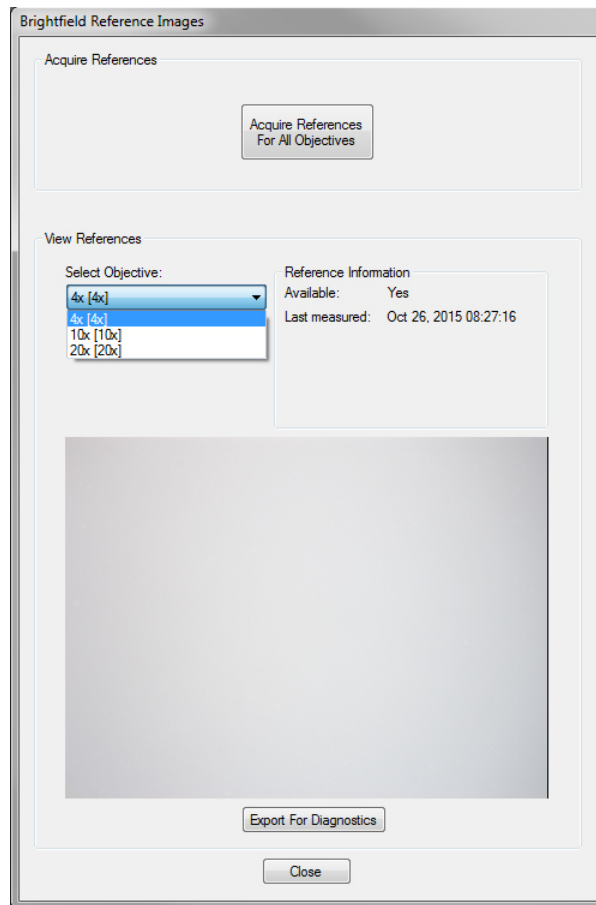


Figure 17. Brightfield Reference Images Window

4. Click the **Acquire References For All Objectives** button.
5. Place a prepared brightfield slide with tissue onto the stage (see [Loading Slides onto the Stage](#)¹⁵ for instructions).
6. Use the [Stage Control Joystick](#)⁶³ to move to an area on the slide with tissue and focus on the tissue.
7. Use the Stage Control Joystick to move to a clean (empty) area of the slide.
8. Make sure that the [trinocular](#)⁶¹ is set to the camera-only setting.
9. Click the **OK** button. The Vectra system acquires and saves the brightfield reference images to disk, going to each objective in turn. During acquisition of the brightfield reference images, a progress bar displays. Clicking the **Cancel** button will result in the current references remaining unchanged or new (first-time) references not being saved.

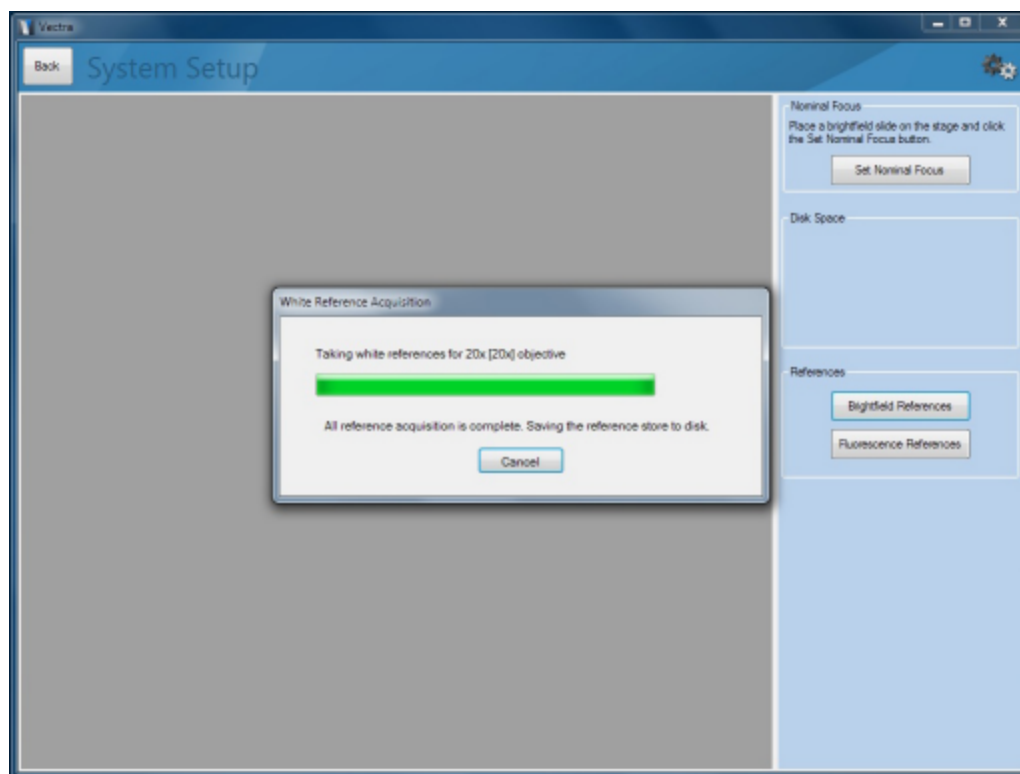


Figure 18. Brightfield Reference Progress Bar

To view existing reference images, you can select an objective in the **Select Objective** drop-down list. The reference image displays at the bottom of the window. The list of objectives depends on the objectives installed on the Vectra system. If instructed by Akoya, you can add or remove objectives using the Vectra [Hardware Setup Program](#)^[75].


The **Export for Diagnostics** button is used to export a copy of a brightfield reference image as a TIFF file (*.TIF) for troubleshooting purposes.

The **Available** text box displays Yes if a reference image is available for the selected objective or No if a reference image is not available. If a reference image is available, the **Reference Information** displays the date and time when the reference image was taken. If no references are available, you must take reference images.

10. Click the **Close** button to close the Brightfield Reference Images window.

4.3.2 Fluorescence Reference Images

To take the Fluorescence reference images:

1. Click the **Set Up System** button  on the [Vectra Homepage](#)¹⁸ to open the [System Dashboard](#)²⁰.
2. Click the **Fluorescence References** button to initiate taking the fluorescence references. The Fluorescence Reference Images window opens as shown in Figure 19.

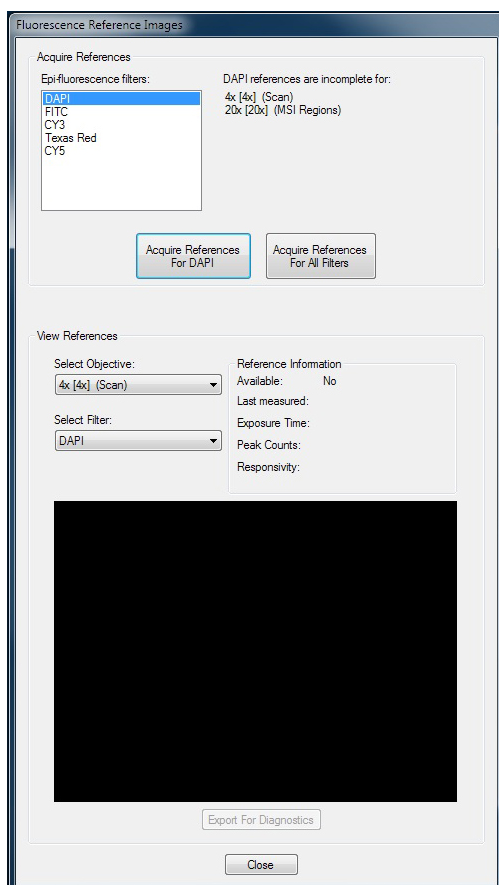


Figure 19. Fluorescence Reference Images Window

3. To acquire references for a specific epi-fluorescence filter:
 - a. Select the desired epi-fluorescence filter. Fluorescence reference images for scans are taken with the LCTF out. Fluorescence reference images for MSI regions are taken with the LCTF in, and for whole-slide scans they are taken with the LCTF out. Some objectives are only used for one purpose or the other, and accordingly their references are only taken for one LCTF configuration.
 - b. Click the **Acquire References for [selected filter]** button.
4. To acquire references for all epi-fluorescence filters:
 - a. Click the **Acquire References for All Filters** button.

5. Place the blue Vectra Compensation Slide onto the stage (see [Hardware Setup](#)^[75] for instructions).
6. Use the Vectra Stage Control Joystick to move to a clean (empty) area of the slide.
7. Make sure the [trinoc](#)^[61] is set to the camera-only position.
8. Click the **OK** button.
9. The Vectra will move to the edge of the reference slide and detects its height, to set proper focus for the rest of the measurement. Then it moves each combination of objective and epi-fluorescence filter into place in turn, acquires the fluorescence reference images, and saves the images to disk. Clicking the **Cancel** button results in the current references remaining unchanged or new (first-time) references not being saved. The Success window opens when the reference image acquisition is complete.

To view existing reference images, select an objective and an epi-fluorescence filter in the drop-down lists. The reference image displays at the bottom of the window. The lists of objectives and filters depend on the objectives and filters installed on the Vectra system. If instructed by Akoya, you can add or remove objectives and filters using the Vectra [Hardware Setup Program](#)^[75].

The **Available** text box displays Yes if a reference image is available for the selected objective or No if a reference image is not available. If a reference image is available, the **Reference Information** displays the date and time when the reference image was taken, the exposure time (in ms), peak counts, and the responsivity for fluorescence. If no references are available, you must take reference images.

10. The **Export for Diagnostics** button saves each reference image as a TIFF (.TIF) image file for troubleshooting purposes.

5 Creating and Editing Studies and Protocols

Vectra 3 stores scan data by 'Study'.

- **Study:** A group of slides that belong together. This could be an actual experimental study (e.g. Ki67 markers in breast cancer tissue), all slides from one source, etc. Each study contains one or more slides. Each slide may be scanned more than once, if needed. The default location within the Vectra 'Data Hive' is D:\Data\Vectra3\[Study] where [Study] is the operator-named study. Any acquired MSI Fields from a particular slide scan will be stored within the scan folder located within the study folder.
- **Protocol:** Operator-defined method that defines the imaging mode, the pixel resolution (determined by the objective selected), filter cubes, exposure times, etc. that will be used during whole slide acquisition. Protocols have the file extension ".vws" and are saved in the location D:\Data\Vectra3\[Study].
- **Task List:** Operator-defined list that identifies which slides to scan, which protocols to use, and what tasks are to be performed. Task lists have the file extension ".csv" and are saved in the location D:\Data\Vectra3\[Study].

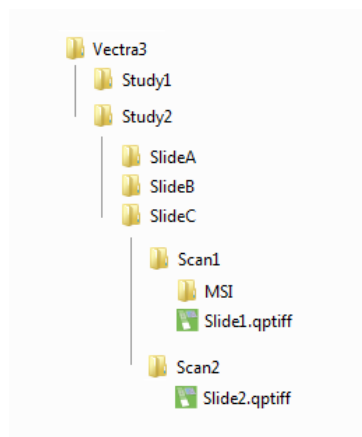


Figure 20. Vectra 3 data is stored in D:\Data\Vectra3. Folders are created in this location for each study. Protocol (.vws) and task list (.csv) files are also stored in this location.

Whole-slide scans and supporting imagery acquired from specific slides are saved to slide-specific subfolders located within the folder for the study they belong to. More details about imagery are provided throughout this section and in [Performing Whole Slide Scans using Vectra](#)^[48].

5.1 Study and Protocol Creation

Prior to creating a study or protocol, the operator should have an understanding of how the slide was stained (chromogenic or fluorescent dyes).

This section is organized by protocol type: either Brightfield or Fluorescence. If you are scanning brightfield slides, proceed to the section [Brightfield Studies and Protocols](#)^[28]. If you are scanning fluorescent slides, proceed to the section [Fluorescence Studies and Protocols](#)^[33].

5.2 Brightfield Studies and Protocols

Brightfield studies are used to acquire imagery from slides stained with H&E or conventional IHC methods.

1. From the Vectra Home Page, select **Edit Protocol**.
2. Click **New** or **Load** and the following window will appear.

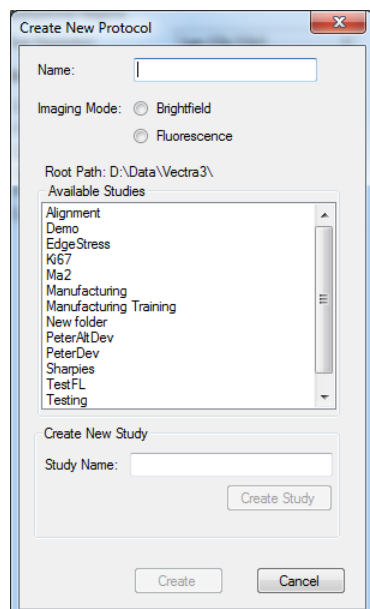


Figure 21. Create New Protocol Window

- a. Enter a Protocol name.
- b. Choose the Imaging Mode by selecting the **Brightfield** radio button.
- c. Create or select a previously created Study.
 - i. To create a study, enter your Study Name in the **Study Name** text box underneath the Create New Study title. Click the **Create Study** button which will push the study to the Available Studies list above. Click on the study in the Available Studies list to highlight/select the study.
 - ii. To select a previously created study, click on the study in the Available Studies list. This will highlight the study.
- d. Click the **Create** button to create the protocol in the specified study.

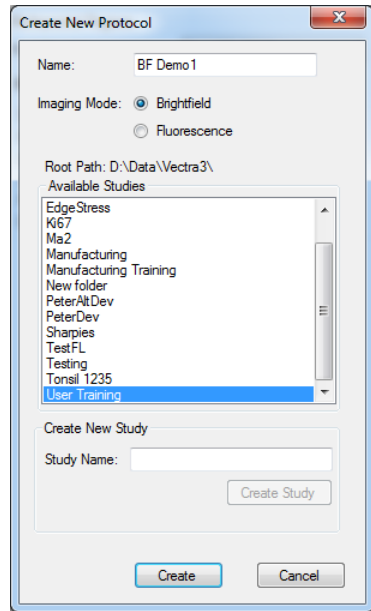


Figure 22. Create a new protocol, and create a new study or select an available study

- After you have created your brightfield protocol and assigned it to a study, the Edit Protocol screen will appear. This will allow you to add specific details to the protocol.

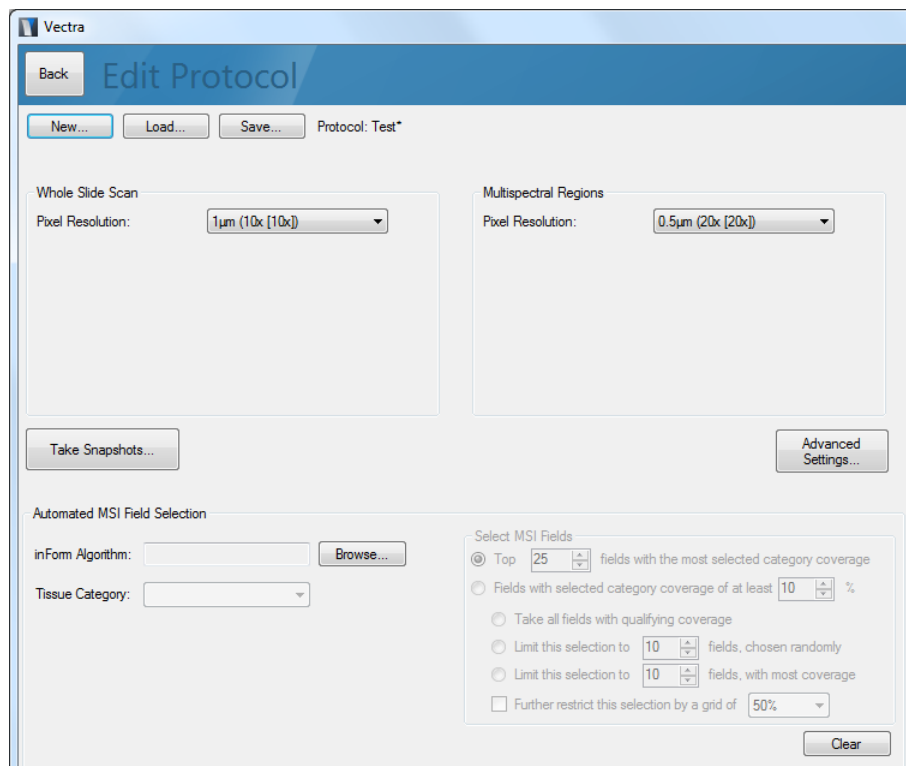


Figure 23. Edit Protocol window where you can add specific details to the protocol

- Under Whole Slide Scan, choose the **Pixel Resolution** that you want to use to image the whole slide (resolutions corresponding to 4X or 10X magnifications are available).

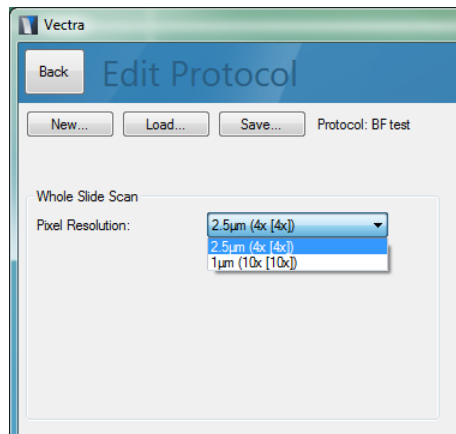


Figure 24. Choose the pixel resolution desired for your whole slide scan.

- Under Multispectral Regions, choose the **Pixel Resolution** that you want to use to image the MSI regions (resolutions corresponding to 10X, 20X, or 40X (if present) magnifications are available).

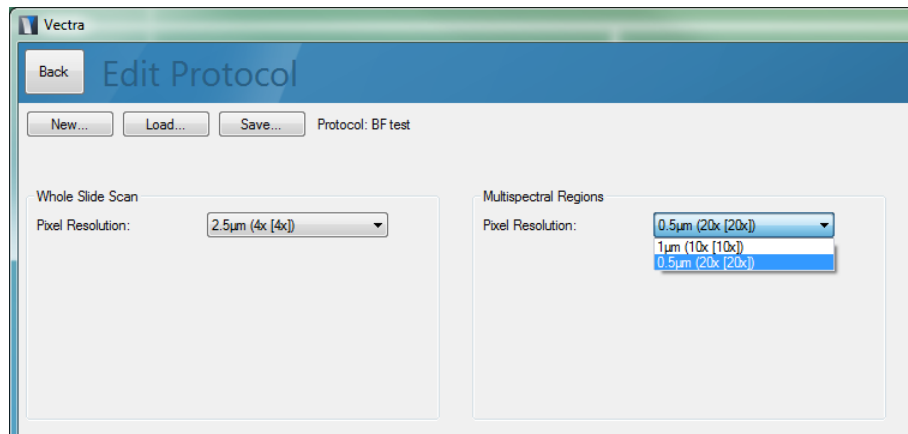


Figure 25. Choose the pixel resolution desired for your multispectral regions.

- You can access advanced settings by clicking the **Advanced Settings** button, located below the Multispectral Regions section in the Edit Protocol window.
 - If Vectra is having difficulty finding your entire tissue sample, check the **Scan within the entire coverslip region** checkbox. This will increase scan time and file size, but will enable you to complete scanning of difficult samples.
 - Click the **Save** button. The Save Protocol window opens.
 - Select the study name that you created from the Available Studies list. The Protocol Name that you created will populate the Protocol Name box.
- NOTE:** If needed, you can change the study name or Protocol name at this step. This will create either a new folder structure or protocol file.
- Click **Save** to save the protocol.

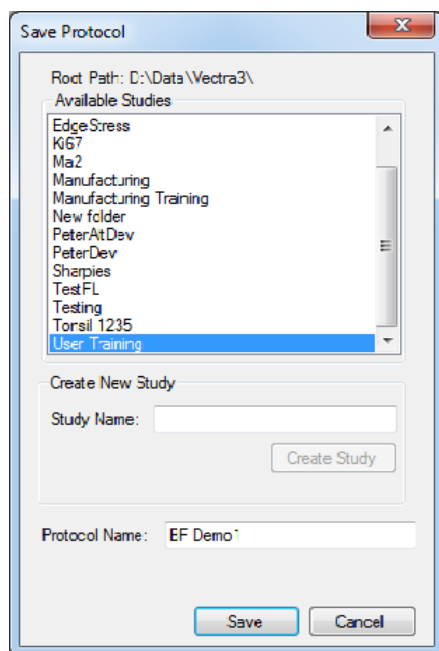


Figure 26. Save your brightfield protocol in the Save Protocol window

11. Previous protocols can be loaded using the **Load** button on the Edit Protocol Page (see above).

Vectra 3.0 can be configured so that MSI Fields are selected automatically. This is described in section [Advanced Automated Workflows](#) ⁵⁸.

5.3 Acquiring Brightfield Sample Regions

The Take Snapshots feature allows you to acquire sample color imagery or multispectral regions without scanning the entire slide. This may be particularly useful for spectral library development. (See the inForm User's Manual for more information.)

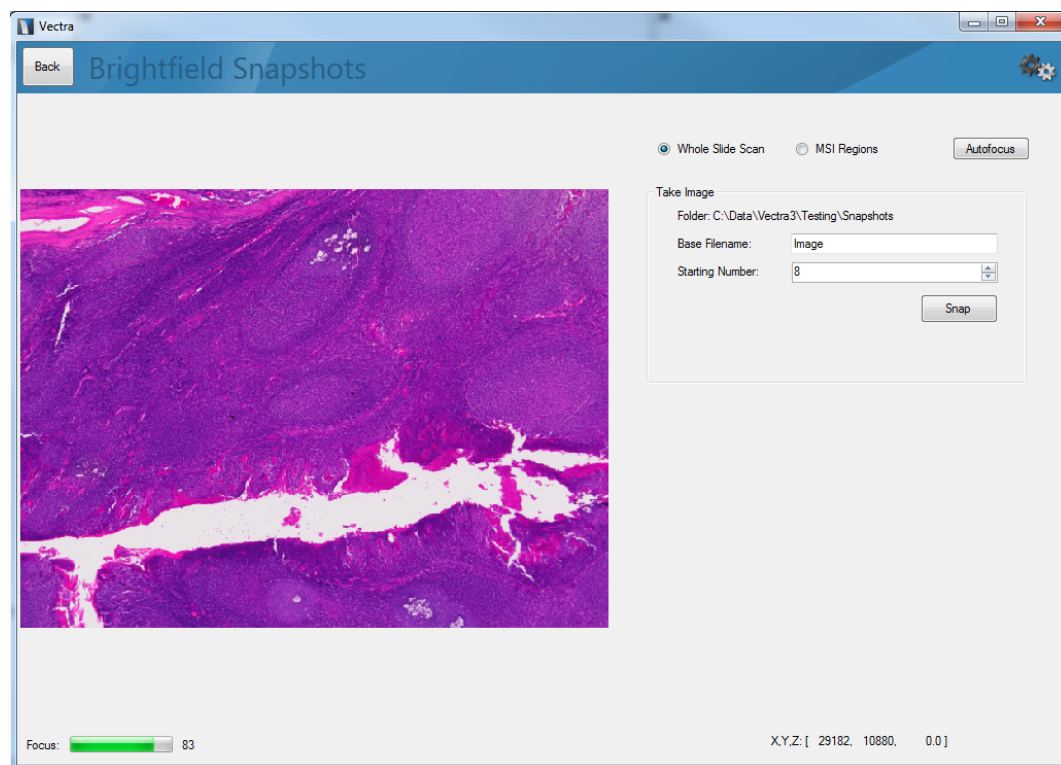


Figure 27. Brightfield Snapshots window

1. Click the **Take Snapshots** button to open the Brightfield Snapshots window.
2. Select whether you would prefer to use the Whole Slide Scan objective or the MSI objective.
3. Using the joystick, drive to an area of interest.
4. Focus on the sample using the Autofocus button or focus wheel.
5. In the Take Image group, enter a name in the Base Filename box.
 - a. The Starting Number will default to 1 if there is no image with this base filename or the next available number if the base filename is already being used.
 - b. The folder label shows where the images will be saved. They are saved in a Snapshots folder within the study folder associated with this protocol.
6. Click the **Snap** button to take a picture.
7. For spectral controls, it is recommended to capture images from at least two regions in each control. The Starting Number will automatically increase when you take an image. Move to a new region expressing that control, check that the image is in focus, and click the **Snap** button again.
8. Click the **Back** button to return to the Protocol Editor.

5.4 Fluorescence Studies and Protocols

Fluorescence studies and protocols are used to acquire imagery from slides stained with fluorescent IHC components.

In order to generate a fluorescence protocols, you should have one or more sample slides available for setting exposure times.

Often, one is interested in developing a protocol to use for scanning whole slides, and taking MSI fields of interesting regions. In this case, you will need slides that include positive expression in all markers of interest, so you can set exposure times that are suitable for the range of expression the protocol will be used for.

Sometimes, your goal is simply to image a few MSI fields on a few specific slides for the purposes of assay development or spectral library creation. In this case, you just need the slides of interest.

5.4.1 Creating a Fluorescence Protocol

1. From the Vectra Home Page, select **Edit Protocol**.
2. Click **New** or **Load** and the following window will appear.

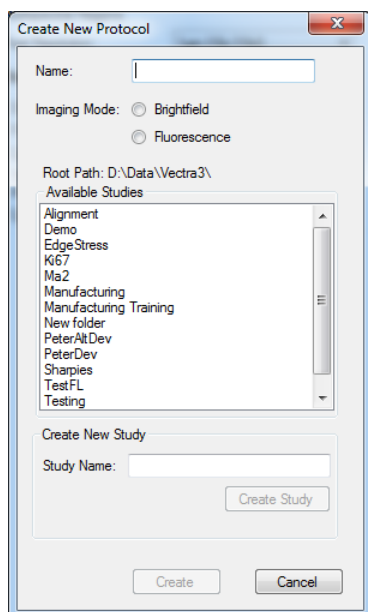


Figure 28. Create New Protocol Window

- a. Enter a Protocol name.
- b. Choose the Imaging Mode by selecting the **Fluorescence** radio button.
- c. Create or select a previously created Study.
 - i. To create a study, enter your Study Name in the **Study Name** text box underneath the Create New Study title. Click the **Create Study** button which will push the study to the Available Studies list above. Click on the study in the Available Studies list to highlight/select the study.

- ii. To select a previously created study, click on the study in the Available Studies list. This will highlight the study.
- d. Click the **Create** button to create the protocol in the specified study.

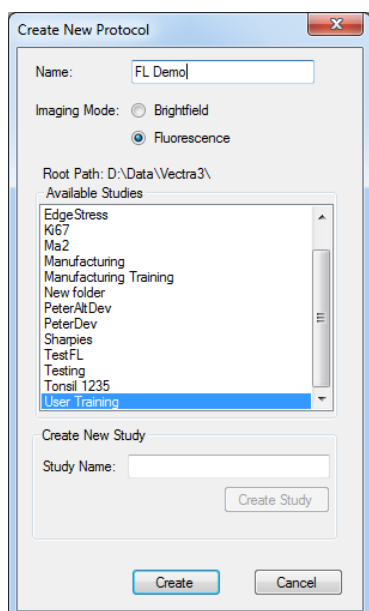


Figure 29. Create a new protocol and create and/or select an available study

3. After you have created your fluorescence protocol and assigned it to a study, the Edit Protocol screen (for fluorescence protocols) will appear.

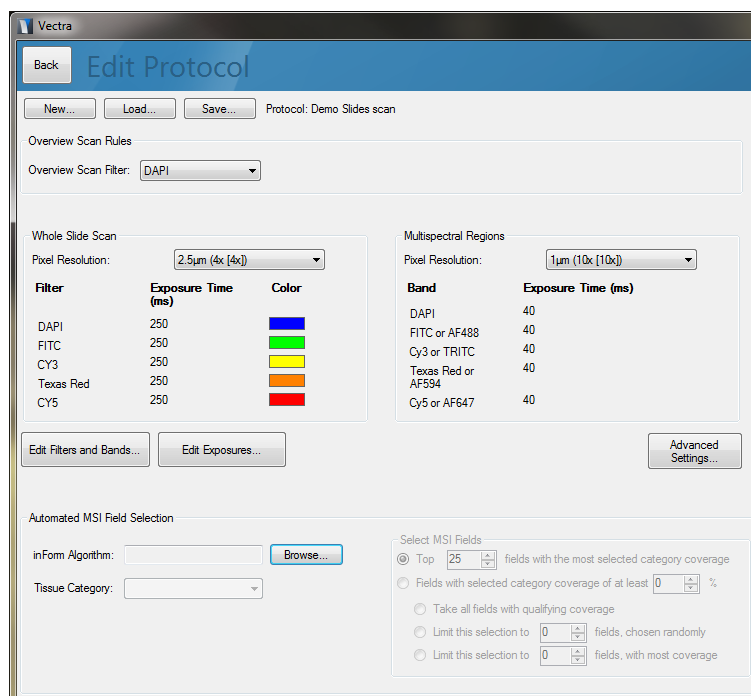


Figure 30. Edit Protocol window where you can add specific details to the protocol

4. In the Overview Scan Rules section, select the desired filter that will be used to help find tissue on the slide. This typically will be the DAPI filter, as the DAPI nuclear counterstain should be ubiquitous throughout the sample. If there is no counterstain, then choose a filter that spectrally aligns with the most abundant fluorophore(s) staining the sample.

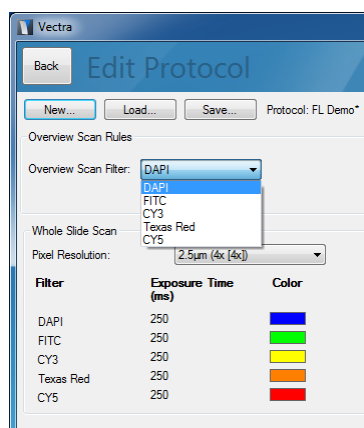


Figure 31. Select your desired Overview Scan Filter under Overview Scan Rules

5. In the Whole Slide Scan section, choose the **Pixel Resolution** to be used while imaging the slide. The pixel resolution for the whole slide scan must be either 2.5 µm (4X) or 1 µm (10X).

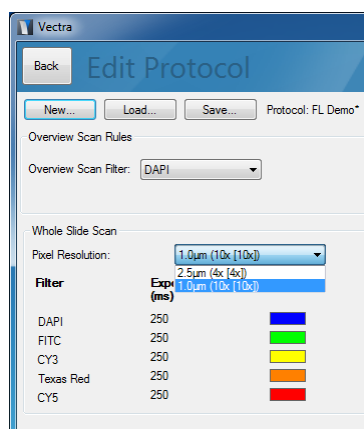


Figure 32. Choose the pixel resolution to be used while imaging the slide under Whole Slide Scan

Changing the pixel resolution will invalidate your exposures.

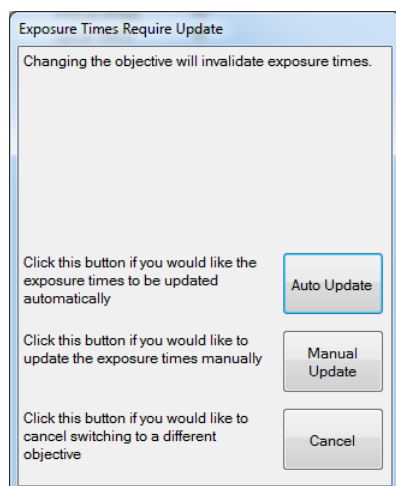


Figure 33. Warning window appears if you change the pixel resolution.

The **Auto Update** button will scale the exposure time to match the difference in the light gathering capability of the original and selected objective. The scale factor when switching from 1µm (10X objective) to 2.5µm (4X objective) is 1/6th the exposure time. When switching back to 1µm (10X objective) scaling is inverted (6 times the exposure time).

The **Manual Update** button will NOT scale the exposure time automatically. If the Manual Update button is selected, the Vectra software maintains the exposure time of the previous objective (the process for determining optimal exposure times is shown later in this section). For now, continue with creating the protocol; optimizing exposure time will be discussed elsewhere.

The **Cancel** button leaves you at your current pixel resolution.

- Under Multispectral Regions, choose the **Pixel Resolution** to be used to image the multispectral (MSI) regions (10X, 20X, or 40X if present). Acquiring MSI regions using Vectra and the Phenochart viewing program will be discussed in [Selecting MSI Fields for Acquisition](#)^[56]. To understand how to use Phenochart to choose MSI regions to acquire, please reference the *Phenochart User's Manual*.

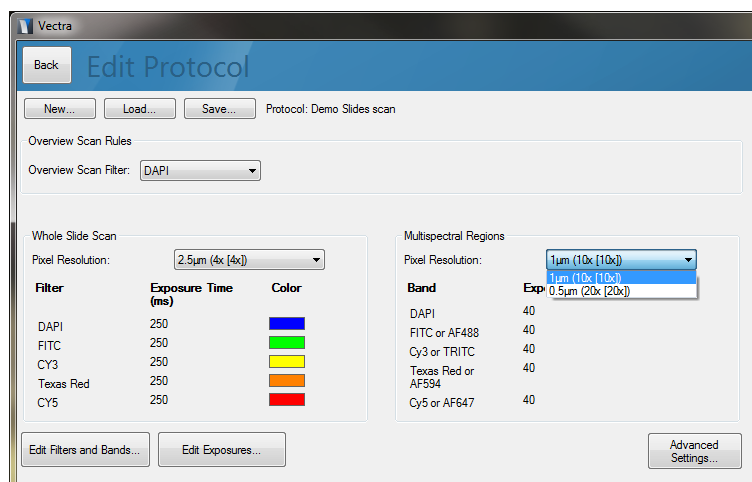


Figure 34. Choose the pixel resolution to be used for Multispectral Regions

7. If the Multispectral Regions resolution is changed, the same **Exposure Times Require Update** box shown in Figure 33 will appear and you will be required to choose how to adjust the exposure times.
8. It is highly recommended to maintain the default configuration for the whole slide filter cubes (and associated colors) and multispectral region bands. However, the **Edit Filters and Bands** button allows the operator to add or remove fluorescence filter cubes used for whole slide scanning and multispectral region bands for MSI acquisition. The colors associated with each filter can also be modified/selected (these colors will be used by Phenochart to display the different image planes and can also be changed, and the images updated in real time, when viewing the sample in Phenochart).

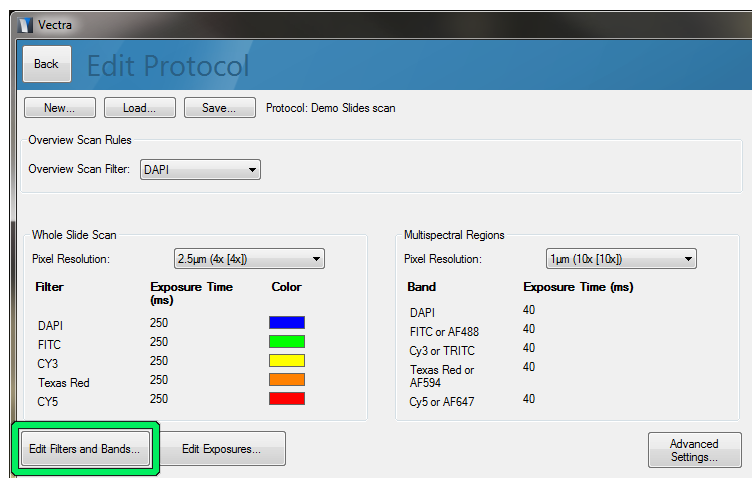


Figure 35. The Edit Filters and Bands button lets you to add or remove fluorescence filter cubes for whole slide scanning and multispectral region bands for multispectral acquisition.

The Edit Filters and Bands window opens.

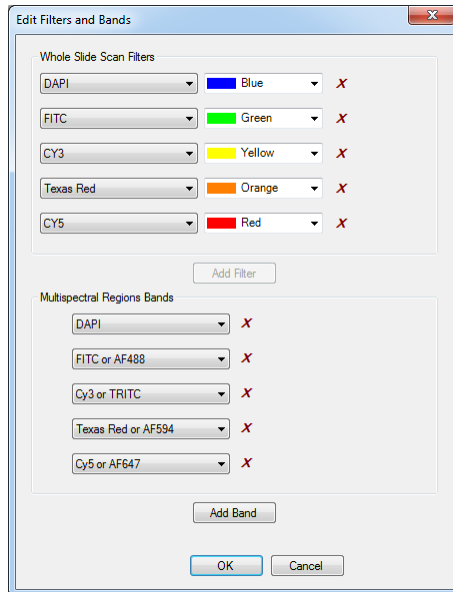


Figure 36. In the Edit Filters and Bands window, choose from drop-down menus or click on the red 'x' next to a filter or band to delete them. You can also choose which colors are associated with each filter.

- a. To remove a filter or region band, click the red **X** next to the filter or band.
 - b. To add a new band, click the **Add filter** or **Add Band** button.
 - c. Click **OK** when finished.
9. You can access advanced settings by clicking on the **Advanced Settings** button, located below the Multispectral Regions section in the Edit Protocol window.
 - o You can specify which filter will be used for setting focus during whole slide scans and for MSI imaging. The drop-down menu contains all the bands included for whole slide scanning (see [Epi-Filters](#))^[76].
 - o Normally, DAPI is the right choice but another band may make sense if there is a different counterstain. For good results, the sample needs to have strong signal in this band throughout its extent.
 - o We highly discourage deactivating the **Use Saturation Protection** setting. The default setting is ON. This prevents you from overexposure when acquiring multi-spectral imagery.
 - o If Vectra is having difficulty scanning your fluorescent sample, and you are unable to mark up the slide, check the **Scan within the entire coverslip region** checkbox. This will greatly increase scan time and file size, but will enable you to complete scanning of difficult samples.

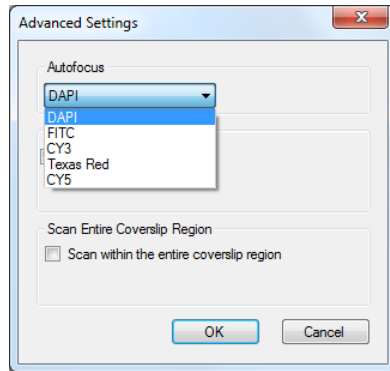


Figure 37. Select the band that will be used for focusing in the Advanced Settings window.

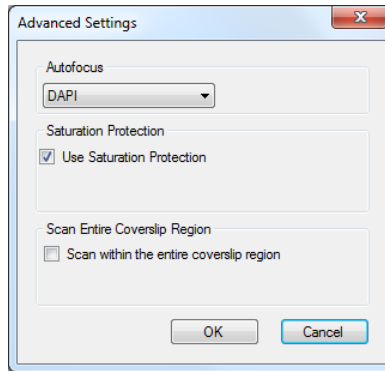


Figure 38. The Saturation Protection feature is ON by default.

10. Set the exposure time for each filter cube associated with the fluorescence whole slide scan:
 - a. Click the **Edit Exposures** button.

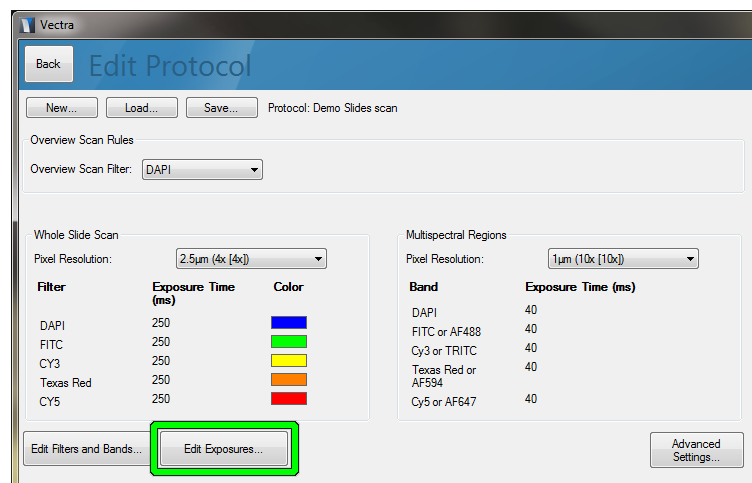


Figure 39. Edit Exposures lets you set the exposure times for your filter cubes

The following screen will appear:

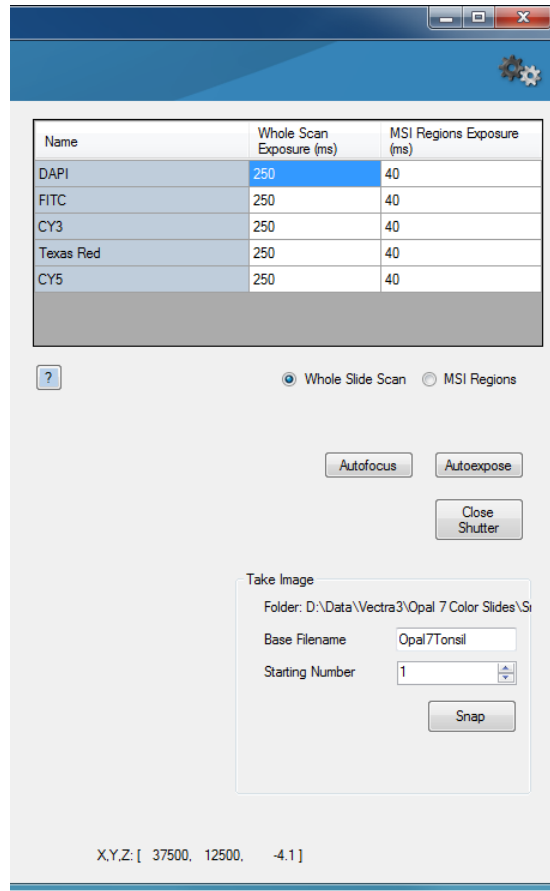


Figure 40. You can set your exposure times using the Autoexpose button or directly enter a value in milliseconds into a highlighted box.

Note: You must click on the **Open Shutter** button in order to enable the **Autoexpose** button. It is good practice to keep the shutter closed when not viewing or imaging in order to reduce the amount of photobleaching that occurs when the tissue is exposed to high-energy light.

- b. If you are new to Vectra 3.0, click the **Instructions (?)** button. Use the instructions on the screen (Figure 41) for guidance. More detailed instructions are provided in the next steps:

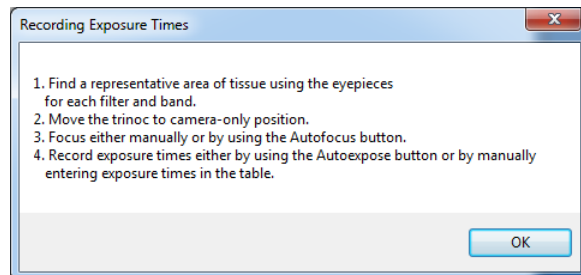
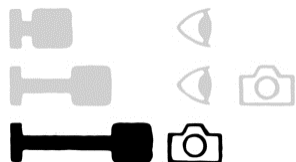


Figure 41. You can manually enter exposure times or click the Autoexpose button, as explained in the Instructions window. However, more detailed instructions are contained within this user's manual.

- c. Place your fluorescently stained multiplexed sample onto the stage.
- d. Begin with setting the DAPI exposure time.
 - i. Click the box next to DAPI and below Whole Scan Exposure.
 - ii. If there is no image on the screen, click the **Open Shutter** button to expose the sample to excitation light. Using the stage control joystick and the live view on the screen, drive the microscope stage to a location on the slide that contains DAPI signal and focus on the sample.
 - iii. Confirm that the trinocular is set to the camera only position and confirm that the sample is in reasonable focus on the screen. Fine-tune your focus if necessary.



- iv. Click the **Autoexpose** button.
- v. Click the **Autofocus** button.
- vi. Click the **Autoexpose** button again. The exposure time, in milliseconds (ms), will be set so that the focused DAPI signal is bright, but not overexposed (see images in Figure 43).

Name	Whole Slide Scan Exposure (ms)	MSI Regions Exposure (ms)
DAPI	70.14	40
FITC	250	40
CY3	250	40
Texas Red	250	40
CY5	250	40

☒ Whole Slide Scan
 ☐ MSI Regions

Figure 42. The exposure time that was determined by the system when you choose the autoexpose option appears in the highlighted box, in this case for DAPI. You can also manually type in an exposure time yourself.

The new DAPI exposure time is stored in the protocol and will be used when acquiring images with the DAPI filter during whole slide acquisition.

Figure 43 is an example of an overexposed and a properly exposed tissue sample emitting DAPI from the nucleus. Overexposed images contain saturated pixels that cannot be quantitatively analyzed. They display as bright white pixels on the Vectra live view.

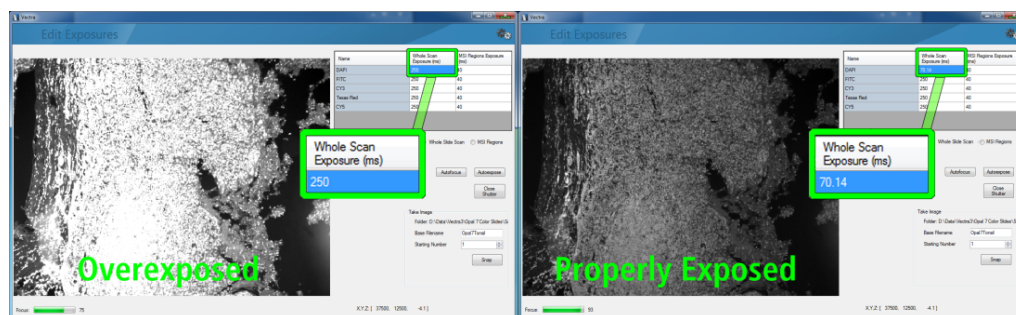


Figure 43. Here is an example of an overexposed tissue sample (left) and properly exposed tissue sample (right). Bright white pixels may be overexposed and data is lost, jeopardizing your quantitative data.

- e. Repeat for remaining filters.

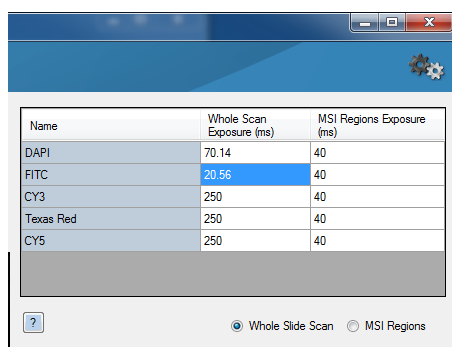


Figure 44. Repeat setting your exposures for each filter cube, in this case for FITC.

5.5 Setting Exposure Times for MSI Regions

This step should be performed if multispectral imaging (MSI) will be performed with this protocol.

You can acquire MSI images from within the exposure-setting dialog, either to test the proposed settings, or to acquire imagery for analysis when only a few fields are needed.

This situation arises during assay development or when making a spectral library from monoplex sample; in such cases there are only a few slides to be imaged, and one only needs a few fields from each slide.

In this case, there is no need to set exposure time based on finding slides with strong expression in all bands; all that matters is to get proper exposure on the samples and regions of interest.

The procedure is similar to that used for setting the whole slide exposures, except you will also use the MSI Regions radio button. A typical workflow will include acquiring a whole slide scan as well as MSI fields. To do this, exposure times must be set for each image type. Follow the steps below.

1. Select the **Whole Slide Scan** radio button.
2. Click in the DAPI exposure time box in the **Whole Scan Exposure (ms)** column.
3. Click the **Open Shutter** button to expose the sample to excitation light. Using the joystick, drive to a location on the slide that contains DAPI signal and get the sample in reasonable focus.
4. Check that the trinocular is in the camera-only position and that the sample is in reasonable focus.

5. Click the **Autoexpose** button.
6. Click the **Autofocus** button.
7. Click the **Autoexpose** button again. The exposure time, in milliseconds (ms), will be set so that the focused DAPI signal is bright, but not overexposed (see Figure 45).

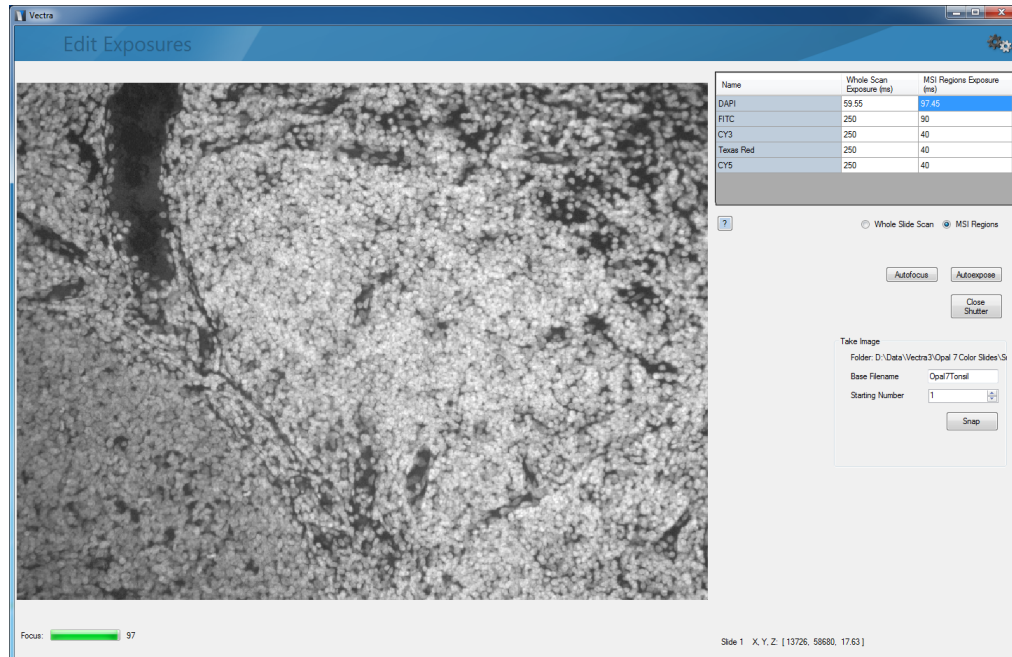


Figure 45. The exposure time found by the system when you click the Autoexpose button appears in the highlighted box, in this case for DAPI. You can also manually type in an exposure time yourself.

8. Select the **MSI Regions** radio button.
9. Click in the DAPI exposure time box in the **MSI Regions Exposure (ms)** column.
10. Click the Open Shutter button to expose the sample to excitation light. Using the joystick, drive to a location on the slide that contains DAPI signal and get the sample in reasonable focus.
11. Check that the trinocular is in the camera-only position and that the sample is in reasonable focus.
12. Click the **Autoexpose** button.
13. Click the **Autofocus** button.
14. Click the **Autoexpose** button again. The exposure time, in milliseconds (ms), will be set so that the focused DAPI signal is bright, but not overexposed.
15. Repeat the above steps for the band associated with the FITC filter cube. If you intend to use this protocol for imaging sample with strong expression, move to a location on the tissue where there is strong expression of the targets labeled in this band first.

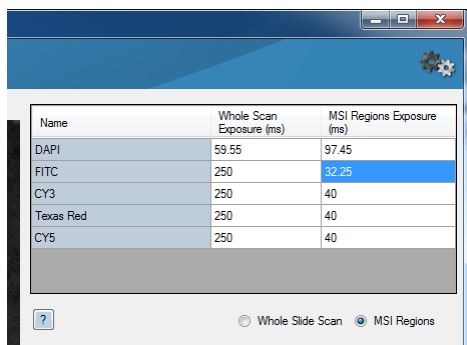


Figure 46. Repeat setting exposures for the band associated with each filter cube in turn, in this case for FITC.

16. Repeat for remaining filters.
17. When this is done, click the **Close Shutter** button to minimize photobleaching.

5.6 Acquiring Fluorescent Sample Regions

This is an easy way to take the images needed for spectral library development (see the *inForm User's Manual* for reference), or simply to check protocol settings you have made.

If you have not already done so, place the single-stained slide(s) that you intend to image onto the stage of the Vectra 3.0 system.

In the example shown below, a DAPI monoplex slide will be used as a sample. For spectral library development, this procedure would be repeated for each fluorophore (using its single-stained control slide) and an unstained autofluorescent witness slide.

1. The Edit Exposures page should be open at this time. If not, browse to this window from the Edit Protocol page by clicking the **Edit Exposures** button.
2. Click in the DAPI **MSI Regions Exposure** box.
3. Using the joystick, drive to a location on the slide that contains DAPI signal and get the sample in reasonable focus
4. Make sure the trinocular is set to the camera only position.

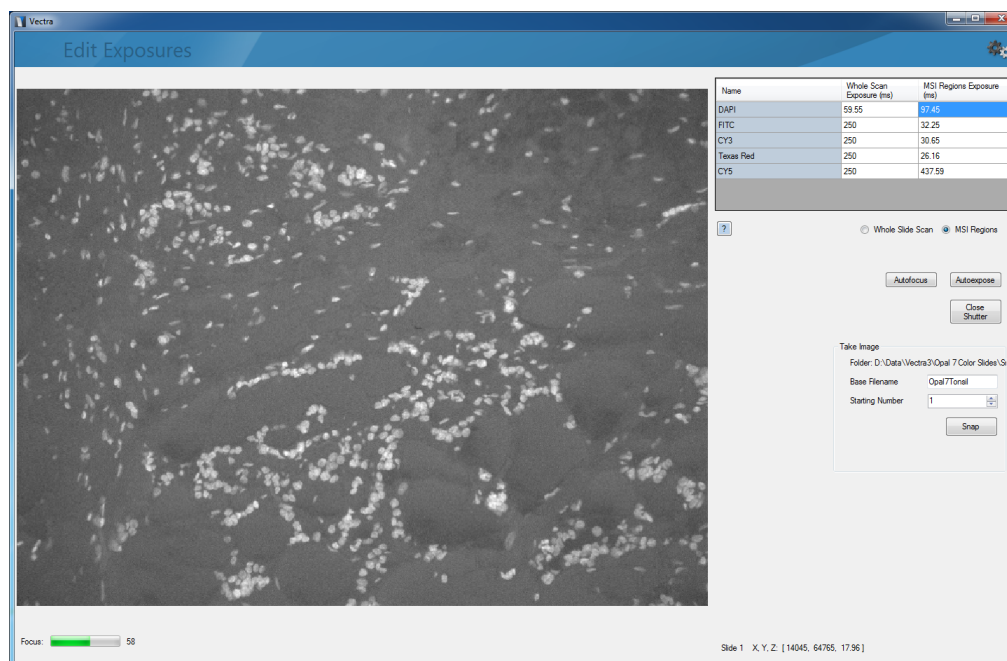


Figure 47. Acquire MSI regions.

5. Get the image in focus using either the autofocus button or the focus wheel on the joystick.
 6. In the Take Image group, enter a name in the **Base Filename** box. For the DAPI monoplex example, a useful name might be something like "DAPI Control".
 - The Starting Number will default to 1 if there is no image with this base filename or the next available number if the base filename is already being used.
 - The folder label shows where the images will be saved. They are saved in a Snapshots folder within the study folder associated with this protocol.
 7. Click the **Snap** button.
 8. For spectral controls, it is recommended to capture images from at least two regions in each control. The Starting Number will automatically increase when you take an image. Move to a new region expressing that control, check that the image is in focus, and click the **Snap** button again.
 9. Even for samples containing just one dye, the Vectra 3.0 system will acquire data across all MSI region bands. This information will be used by the inForm software for spectrally unmixing during analysis.
 10. Repeat this for each single-stained control slide and the unstained autofluorescent witness slide:
- NOTE:** To visualize a dye of interest, click on the row associated with a filter cube that the dye emits in. For Akoya Biosciences Opal fluorophores, use an exposure time of 150 ms for filter cubes where the fluorophores are non-expressing. See [Appendix D: Filter Cube Recommendations when using Opal Reagents](#)^[95] to see which filters Opal fluorophores emit in.
11. Click the **Back** button to return to the Protocol Editor.

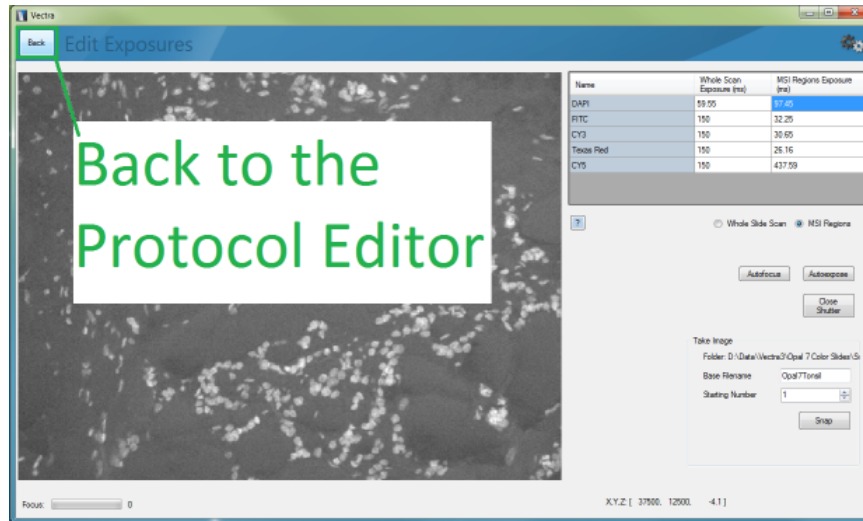


Figure 48. The Back button (highlighted in green) returns you to the Protocol Editor.

12. Click the **Save** button. The Save Protocol window opens.
 13. Select the study name that you created from the Available Studies list. The Protocol Name that you created will populate the Protocol Name box.
- NOTE:** If needed, you can change the study name or Protocol name at this step. This will create either a new study folder structure or a new protocol file in the current study file, respectively.
14. Click **Save** to save the protocol.
 15. Existing protocols can be loaded using the **Load** button on the Edit Protocol Page (see above).

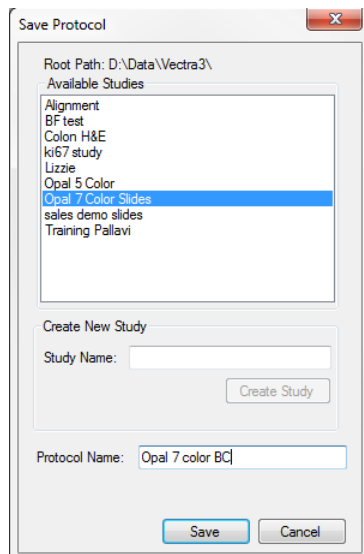


Figure 49. Save your fluorescence protocol in the Save Protocol window

5.7 Automated MSI Field Selection

Vectra 3.0 can be configured so that MSI Fields are selected automatically. This is described in [Advanced Automated Workflows](#)⁵⁸.

6 Performing Whole Slide Scans using Vectra

Once Protocols have been created, Vectra can perform whole slide scanning.

This requires telling Vectra which slides are to be scanned, what protocol(s) are to be used, and the tasks to be performed for each slide. Collectively, this set of information is termed a task list.

Task lists are used to help organize complex and/or repetitive scanning, and greatly reduce the amount of manual entry needed. Once a task list has been entered in Vectra, it can be saved to a .csv file for reuse, or editing. Task lists can then be loaded from disk so you need not re-enter this information. Or, you can create a task list from scratch, if you know the slides to be scanned. Finally, you can load task lists that have only partial information, such as only the slide location and name, but not the protocol or bar code ID.

Overall, the use of task lists enables you to take advantage of Excel or other tools to import your slide data into the Vectra system, and to prepare your work ahead of time; then load them into Vectra when you are in the lab.

This section is divided into two subsections based on the Vectra 3.0 model in your lab.

6.1 Whole Slide Scanning on the Vectra 3.0, 6-Slide System

1. Populate the slots of the 6-slide stage holder with slides that will be whole slide scanned.
2. From the Vectra Home Page, click on **Scan Slides**.

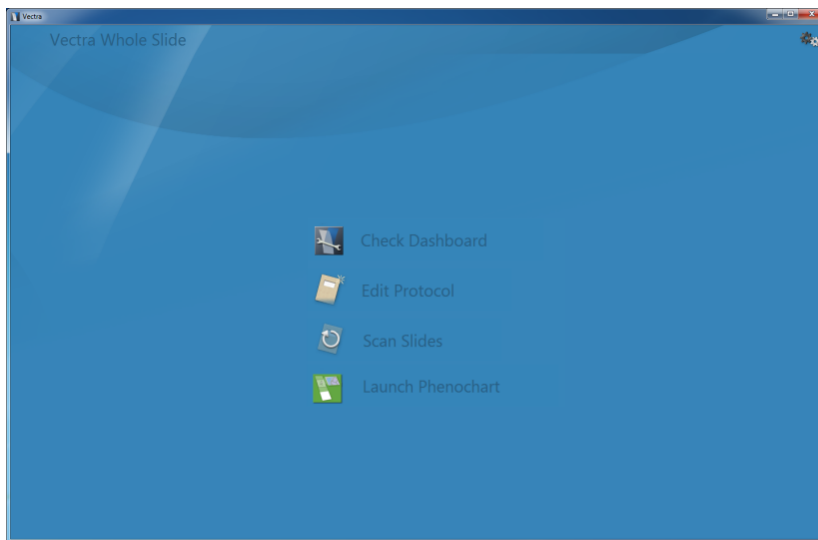


Figure 50. Vectra Whole Slide Home Page

3. Click the Study **Select** button and load the study you previously created.

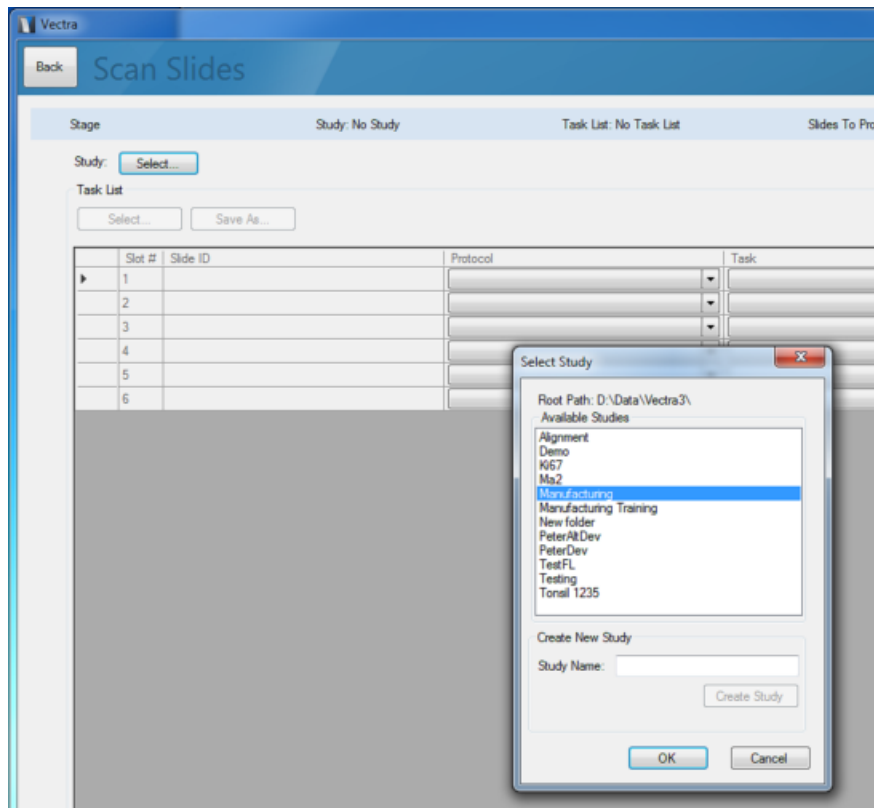


Figure 51. Clicking the Study: Select button brings up a window where you either create a new study, or select and then load a study that you previously created.

4. Click the Task List **Select** button to create and name a task list. Type a Task List name in the **Name** box or select one that you created earlier and click the **Create Task List** button. Click **OK**.

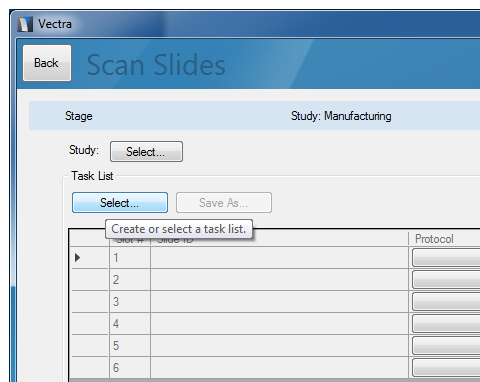


Figure 52. Clicking the Task List: Select button brings up a window where you can create or select and then load a previously created task list (shown in the figure below).

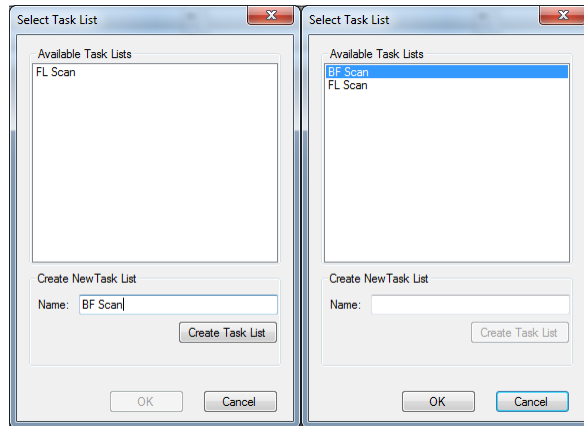


Figure 53. The Select Task List window where you can create a new task list (left) or select and then load a previously created task list (right).

5. Populate the slide IDs for each slot (1–6) that contains a slide. Slide IDs should be used to describe/identify the uniqueness of the slide. For each slide ID, a folder will be created with the same name in the “Study” folder that you named earlier.

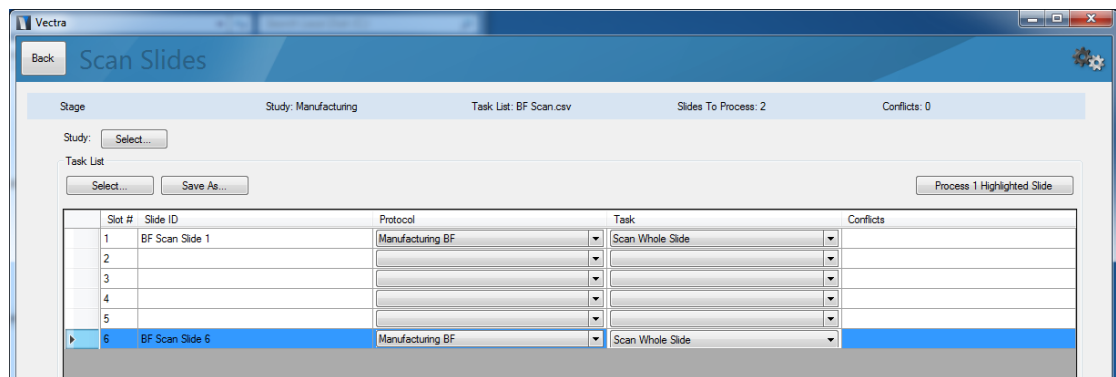


Figure 54. Populate the slide IDs for each slot that is occupied by a slide

6. For each slot number populated with a slide, choose a previously created Protocol. Only protocols associated with the current study will appear in the drop down menu.
7. For each slot number populated with a slide, choose a task from a Vectra defined list (Figure 55). Note: Depending on where you are in the workflow, some or all of the options below may/may not be available to you:
 - **Acquire MSI Fields:** Acquire selected MSI Fields.
 - **Ignore Slide:** Do not perform any operations on this slide.
 - **Scan Whole Slide:** Scan whole slide using the associated protocol for that slide.
 - **Rescan Whole Slide:** Rescan the slide.
 - **Scan and Select Fields:** Scan whole slide using the associated protocol with inForm selecting MSI regions for review.
 - **Scan and Acquire Fields:** Scan whole slide using the associated protocol and automatically acquire MSI regions of interest based on inForm classifier.

Protocol	Task	Conflicts
Manufacturing BF	Scan Whole Slide	
Manufacturing BF		Missing task
	Ignore Slide	
	Scan Whole Slide	

Figure 55. You can choose among several tasks in the Task column for each slide. Specific tasks will appear as options, based on where you are in the workflow.

- Messages may appear in the Conflicts column if you have made drop-down menu selections in the Protocol and Task columns that conflict in some way; for example, selecting a magnification that is not valid for the annotations made to a particular slide scan.

The screenshot shows the Vectra Scan Slides window. At the top, it displays 'Study: PeterDev', 'Task List: BFitress.csv', 'Slides To Process: 0', and 'Conflicts: 4'. Below this is a 'Task List' section with a table. The table has columns for Slot #, Slide ID, Protocol, Task, and Conflicts. Row 2 is highlighted in blue. The Conflicts column for row 2 contains the message 'There are no pending annotations'. Row 3 has 'Missing task', row 4 has 'Missing task', and row 5 has 'Missing task'. Row 6 has 'Ignore Slide'.

Slot #	Slide ID	Protocol	Task	Conflicts
1	Orthotic Liver	BF10xScan20xMSI	Ignore Slide	
2	PhenoTonal	BF10xScan20xMSI	Acquire MSI Fields	There are no pending annotations
3	Viral Pneumonia	BF10xScan20xMSIwithAuto		Missing task
4	Cardiac Muscle	BF10xScan20xMSI		Missing task
5	Pheno TonalA	BF10xScan20xMSI		Missing task
6	Tiny TMA	BF10xScan20xMSI	Ignore Slide	

Figure 56. If there are conflicts with any of the selections that you make, you will see a message in the Conflicts column to help you resolve the issue.

- When completed, you can click the **Save As** button underneath Task List to Save the Task List to the Study. This Task List can be opened again in future sessions. The task list is also editable outside of Vectra using a spreadsheet-based software package such as Excel. Files generated in Excel should be saved as .csv files into the Study folder.

Flexibility of Task Lists: Task Lists that are edited outside of Vectra must have a column header named "Slot". In addition, it is useful to have at least one of the following: Slide ID, Protocol, or Task. These do not have to be in the order in which they appear on the Vectra Edit Protocol page. You can even have columns with other names. For example, you might create a task list from a previously created Microsoft Excel spreadsheet with column headers named Slide ID, Slot, Patient Name, and Blood Type. It is okay to have Slot not appear to the far left, and the columns under headers Patient Name and Blood Type will be ignored.

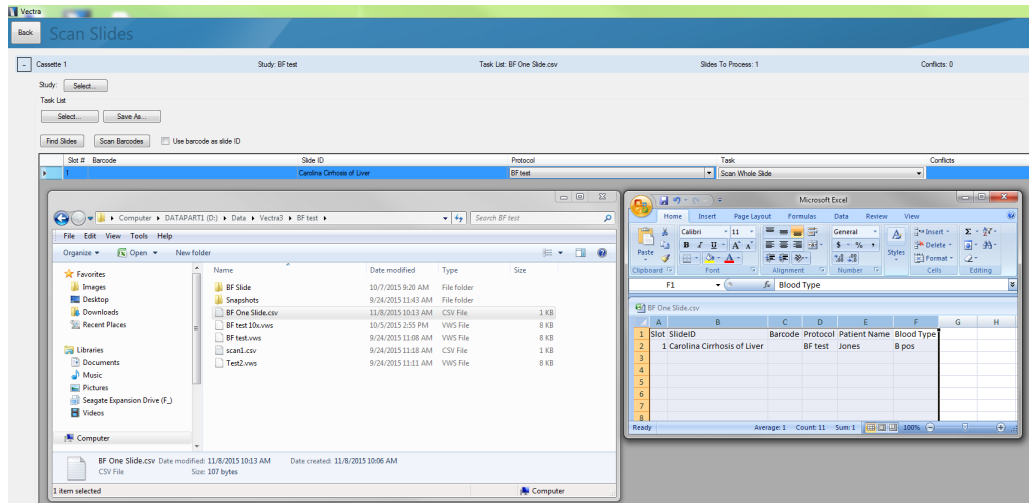


Figure 56. You can edit a saved Task List (.csv file), in this example with Microsoft Excel. Notice that the information from the Excel Slot and Protocol columns correctly populated the Slot # and Protocol columns in the Vectra Task List interface in the Scan Slides page; in addition, notice that the Patient Name and Blood Type columns were ignored.

10. Once your task list is finalized, click the **Process # Slides** button at the bottom of screen. If you prefer to perform a test scan, highlight one or more rows and click the **Process # Highlighted Slides** button on the upper left of the screen.

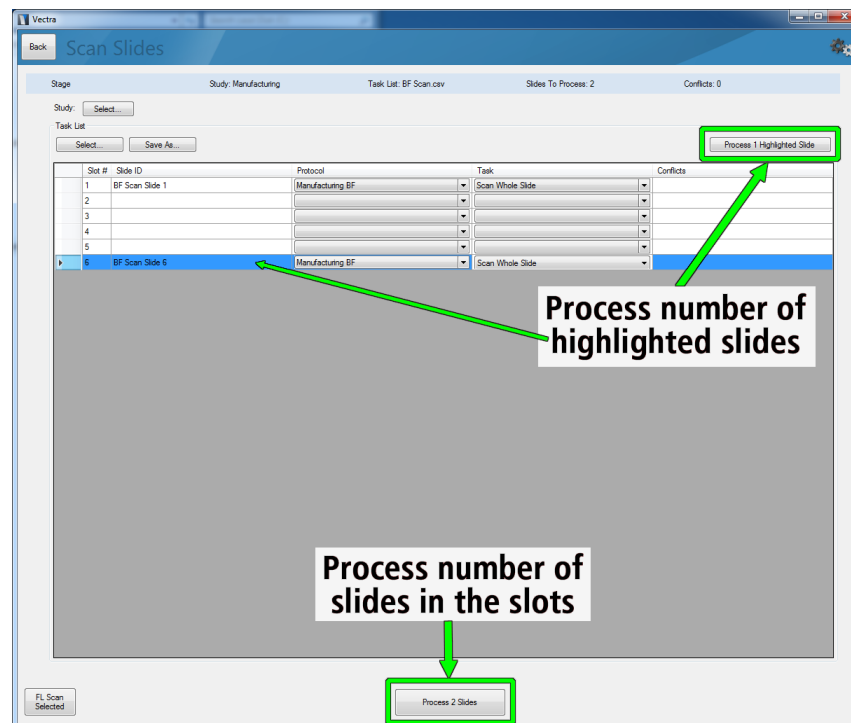


Figure 57. The 'Process # Highlighted Slide(s)' button will process slides that you have highlighted (top). The 'Process # Slides' button will process all the slides in the slots for which you have provided information (bottom).

6.2 Whole Slide Scanning on the Vectra 3.0, 200-Slide System

- 1. Populate the slots of the 50-slide cassettes with slides that will be scanned.
- 2. From the Vectra Home Page, click on **Scan Slides**.

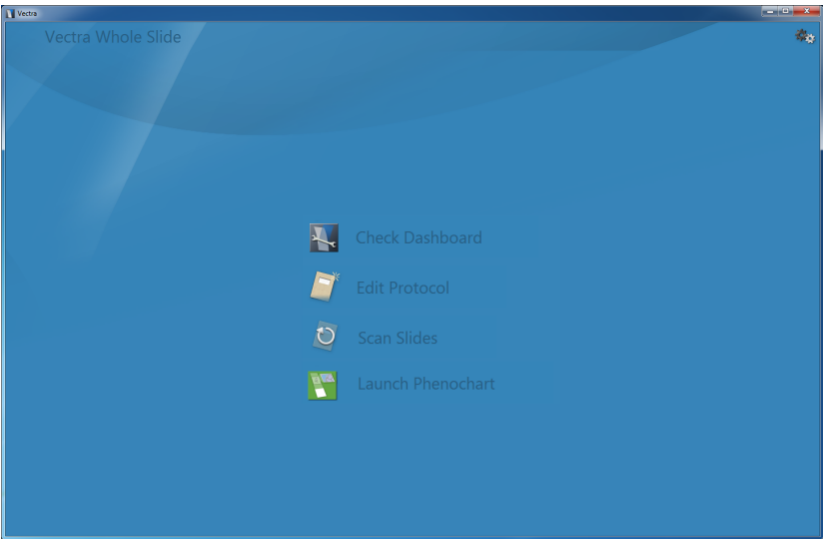


Figure 58. Vectra Whole Slide Home Page

- 3. Expand Cassette 1 by clicking on the + sign next to cassette 1.

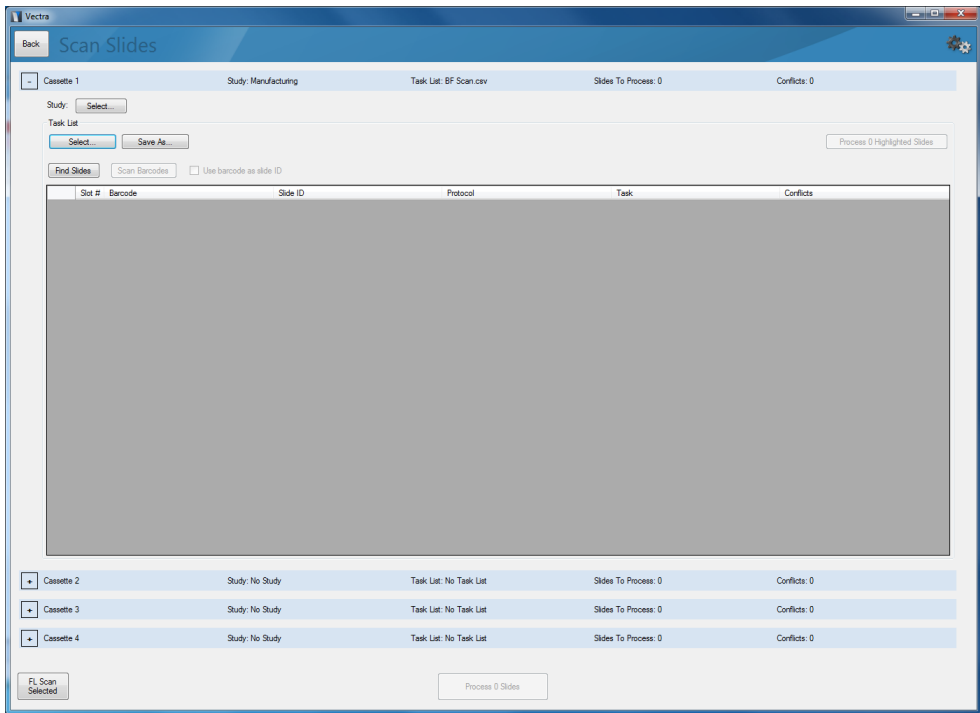


Figure 59. Clicking on the '+' button next to a cassette expands it so that you can see all of the slides for which you have provided information. If you have not yet clicked the Find Slides button, the area will be blank.

- 4. Click the **Study Select** button and load the study you previously created.

5. Click the **Task List Select** button to create and name a task list. Type a Task List name in the **Name** box and click the **Create Task List** button. Click **OK** (see Figure 60).

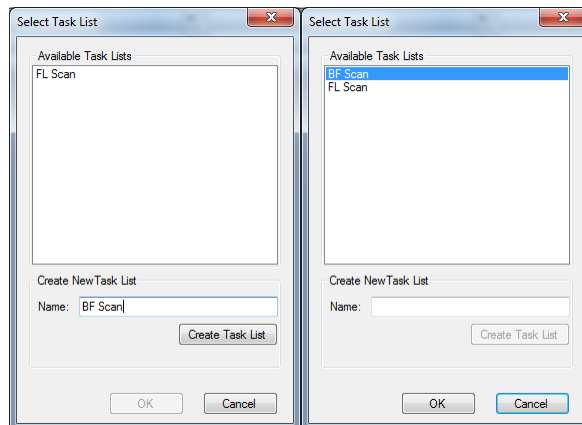


Figure 60. The **Select Task List** window where you can create a new task list (left) or select and then load a previously created task list (right).

6. Click on the **Find Slides** button. This will cause Vectra to automatically find slides and associate them with slots as they are labeled on the cassette (slot 1 is at the bottom of the cassette and slot 50 is at the top of the cassette).
7. If some or all of your slides contain barcodes, click the **Scan Barcodes** button to populate the Barcode field for each slide with the barcode. If a slide does not have a bar code, this field will remain blank. If you would like to use barcodes as the Slide ID, you can check the **Use barcodes as Slide ID** box.
8. Populate the slot IDs with slide names. Slide IDs should be used to identify and describe the uniqueness of each slide. For each slide ID, a folder will be created with the same name under the "Study" folder.
9. For each slide, choose a previously created Protocol. Only protocols associated with the current study will appear in the drop down menu.
10. For each slide, choose a task from a Vectra defined list (Figure 61). Note: Depending on where you are in the workflow, some or all of the options below may/may not be available to you:
 - **Acquire MSI fields:** Acquire MSI Fields that have previously been selected in Phenochart.
 - **Ignore Slide:** Do not perform any operations on this slide.
 - **Scan Whole Slide:** Scan whole slide using the associated protocol for that slide.
 - **Rescan Whole Slide:** If the slide has already been scanned, and you'd like to rescan it, you can choose options whose names start with 'Rescan'.
 - **Scan and Select Fields:** Scan whole slide using the associated protocol with inForm selecting MSI regions for review.
 - **Scan and Acquire Fields:** Scan whole slide using the associated protocol and automatically acquire MSI regions of interest based on training classifier.

11. When completed, you can click the **Save As** button underneath Task List to Save the Task List to the Study. This Task List can be opened again in future sessions. As noted above, the task list is editable outside of Vectra using a spreadsheet-based software package such as Excel. Files generated in Excel should be saved as .csv files into the Study folder, and can then be read into Vectra.
12. Repeat the steps above for cassettes 2, 3, and 4 if they are populated with slides.
13. Once your task list is finalized, click the **Process # Slides** button at the bottom of the screen. If you prefer to perform a test scan, highlight one or more rows and click the **Process # Highlighted Slides** button on the upper right of the screen.

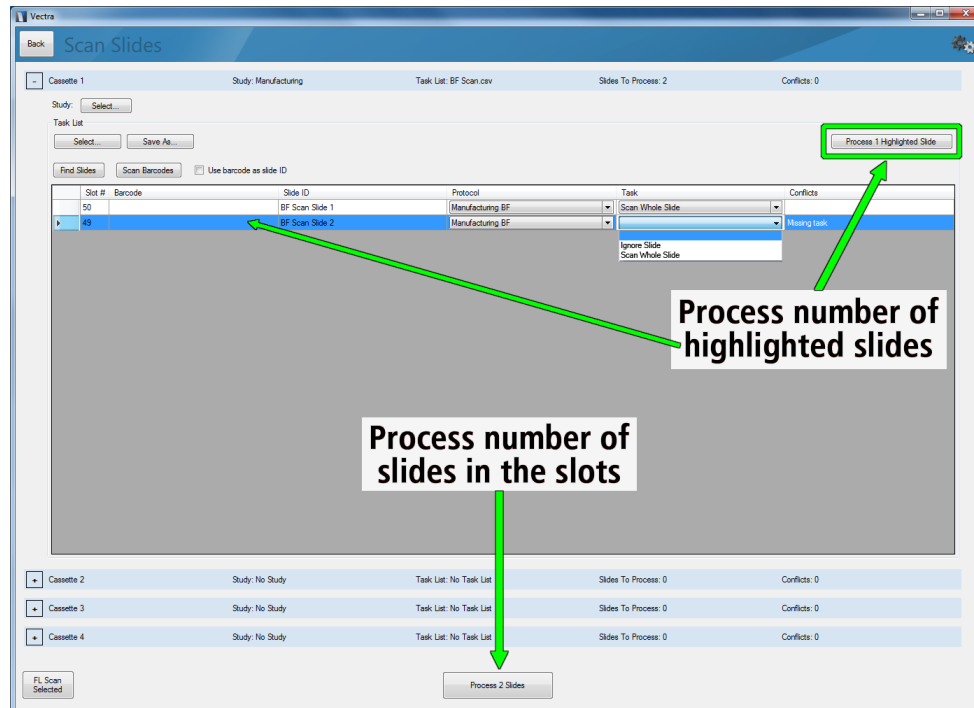


Figure 61. The 'Process # Highlighted Slide(s)' button will process slides that you have highlighted in a particular cassette (top). The 'Process # Slides' button will process all the slides in the slots for which you have provided information across all loaded cassettes (bottom).

6.3 Selecting MSI Fields for Acquisition

Once the whole slide scan(s) have been completed, the resultant files can be opened, reviewed, and annotated in Phenochart. These files can be found in this location: “D:\Data\Vectra3\[Study]\[Slide]\Scan[n]”, where [Study] is the name you gave to the study, [Slide] is the name you gave for the particular slide, and [n] is the scan number. Whole slide scan files are saved with a “.qptiff” file extension.

Typically the whole slide scan will be reviewed and regions of interest will be selected. These regions of interest will ultimately be imaged as MSI regions via Vectra.

6.3.1 Using Phenochart to select MSI Fields for acquisition

MSI Field selection can be done using the tools within Phenochart. For a description of how to annotate regions using Phenochart, please refer to the *Phenochart User's Manual*.

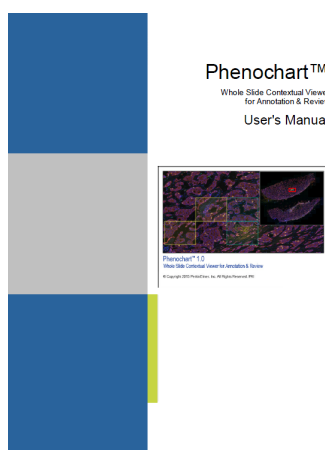


Figure 62. Phenochart User's Manual Cover

6.3.2 Using Vectra to select MSI Fields for acquisition

Selection can also be done automatically through Vectra using a selected inForm algorithm.

Vectra 3.0 can be configured so that MSI Fields are selected automatically, based on the criteria below. An existing inForm algorithm must be chosen using the Browse button, and a tissue category (associated with the particular algorithm) is specified using a drop-down menu (see Figure 63).

- Number of fields with the most selected category coverage
- Fields with the selected category coverage equal to or greater than a specified percentage
 - All fields with qualifying coverage
 - Number of fields with qualifying coverage, selected randomly
 - Number of fields with qualifying coverage with the most coverage

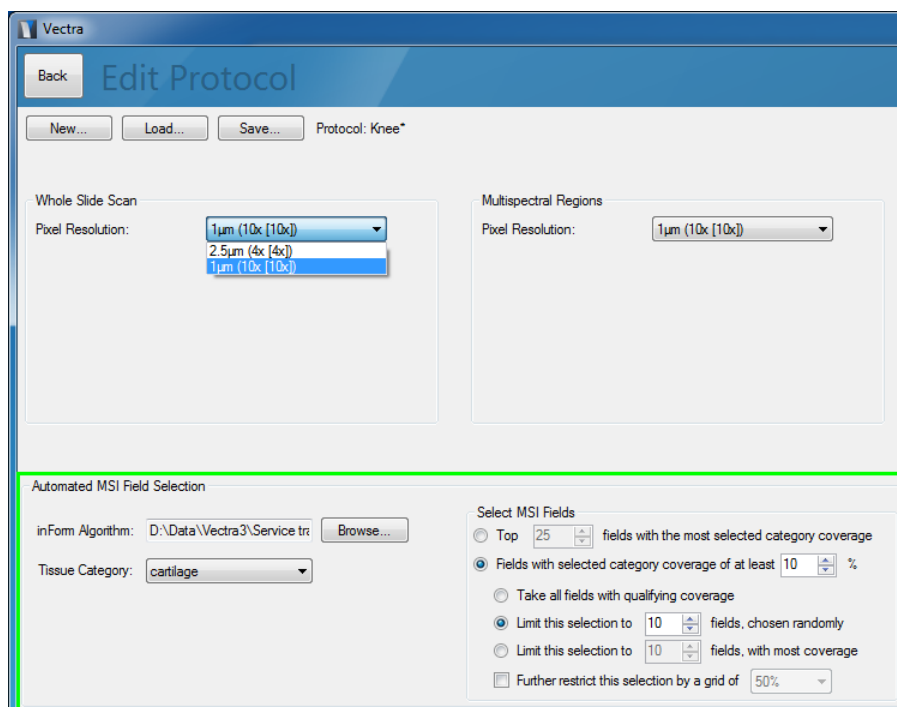


Figure 63. Automated MSI Field Selection options, located at the bottom of the Edit Protocol window.

Using this feature requires familiarity with the Phenochart and inForm software programs. Using these, you can create and save an inForm algorithm that can be selected using the inForm Algorithm **Browse** button. The **Tissue Category** drop-down menu is populated by the tissue categories that were defined in the inForm software and saved with the algorithm. See [Advanced Automated Workflows](#)^[58] for more detail.

7 Advanced Automated Workflows

The Vectra program allows the use of advanced workflows such as automatically selecting fields for review or multispectral acquisition based on criteria that you specify. These selections may be made from a scan that has been classified using a previously saved inForm algorithm. See the Phenochart and inForm user's manuals for more detail on how to do so.

Automated MSI Region Selection

Here is an example showing how you can set up and perform an automated MSI region selection.

1. Perform a whole slide scan in Vectra.
2. Use the Phenochart program to select and 'push' a region to the inForm analysis program (version 2.2 or later). **IMPORTANT:** The inForm program must be installed on your computer for this feature to work.
3. inForm will automatically open and load the region.
4. Develop and save an algorithm in the inForm program. We recommend that you save your algorithm file (.ifp) or project file (.ifr) to your particular study folder for convenience. Note that older algorithms not based on whole-slide scans cannot be used.
5. In the Vectra **Edit Protocol** page, create a new protocol and select a Pixel Resolution that matches your whole slide scan and the desired pixel resolution for the multispectral regions.
6. In the **Automated MSI Field Selection** area at the bottom of the Vectra Edit Protocol page:
 - a. Click the **Browse** button and choose the algorithm or project that you saved earlier.
 - b. Choose a **Tissue Category** based on a category that you saved as part of the algorithm.
 - c. Now make your selections and enter your desired numerical values in order to define your criteria for MSI fields:
 - i. Top [number] fields with the most selected category coverage
 - ii. Fields with selected category coverage of at least [number] percent
 1. Of the above fields, take all with qualifying coverage
 2. Of the above fields, limit to [number] fields, chosen randomly
 3. Of the above fields, limit to [number] fields, with the most coverage
 4. You can also further restrict the above selections by a grid of [number] percent.

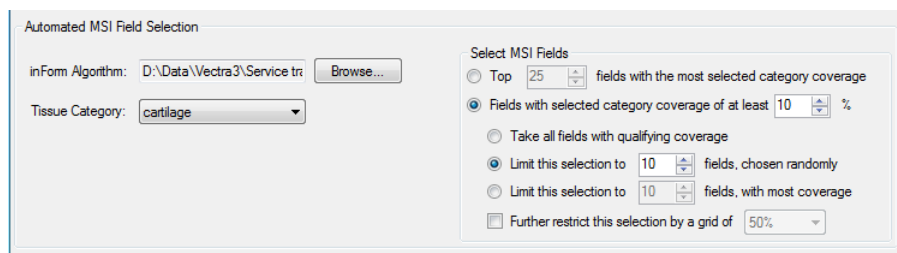


Figure 64. Selecting a previously saved inForm Algorithm and Tissue Category enables the automated selection of MSI Fields.

7. Save your protocol.
8. Go to the **Scan Slides** page, select your Study, and then select or save your Task List.
9. If you have not already done so, enter the relevant information in **Slide ID** and select from the drop-down-menu options in the **Protocol** (the one that you just saved) and **Task** columns. If there are any conflicts, as previously described in the section [Performing Whole Slide Scans using Vectra](#)⁴⁸, resolve them.
 - a. Note that you will have several options available to you in the Task column, such as ignoring the slide, scanning or rescanning the slide, etc. In this case, you should choose either **Rescan and Select Fields** for review or **Rescan and Acquire Fields** for MSI Field acquisition.

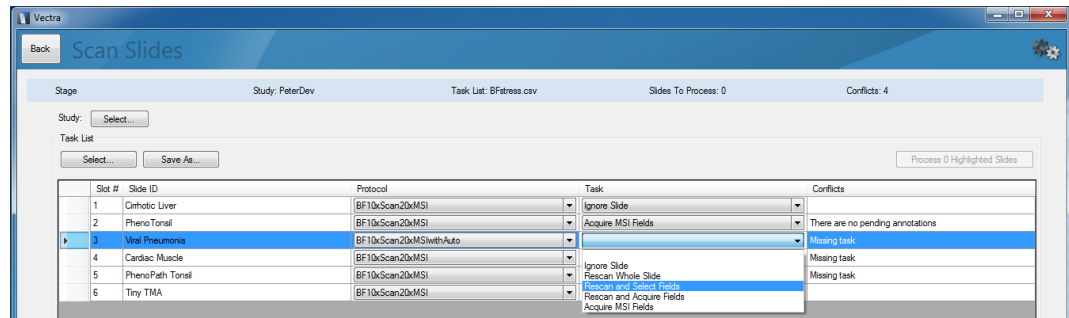


Figure 65. If you select a Protocol that had Automated MSI Field Selections criteria specified, then you can choose, in this case, to rescan the slide and select MSI fields that meet the criteria. As you can see in this example, you could also choose to ignore the slide, rescan the whole slide, rescan the whole slide and acquire the MSI fields that meet the criteria, or simply acquire MSI fields that meet the criteria.

10. If there are no conflicts, click the **Process # Highlighted Slides** button or the **Process # Slides** button to initiate the task(s) you want accomplished.

Using this workflow can result in significant time savings because this optimizes the number of MSI Fields to exactly what you want.

8 Hardware Overview and Adjustments

There are two models of Vectra 3.0, one with a 6-slide capacity and the other with a 200-slide capacity. The models share much of the same hardware, but differ in some important respects that are noted in this section.

8.1 Hardware Descriptions

The Vectra3.0 hardware consists of the components identified in the figures below. Each component is described in detail in this section.

Vectra Imaging Module

The Vectra Imaging Module contains a cooled, scientific-grade camera system and Akoya's solid-state tunable imaging filter. A linear green LED display on the front panel indicates the current wavelength and initialization status, and 3 additional LEDs located below the linear LED display indicate camera and imaging mode status.

- High-resolution, scientific-grade CCD imaging sensor
- Solid-state liquid crystal (LC) wavelength tuning element
- Spectrally optimized lens and internal optics
- Industry-standard C-mount (compatible with 1x C-mount camera tube)

Power: 5V Power Cable from Wall Adapter Power Supply (100- 240VAC, 50/60 Hz, 0.5A)

Fuses: No fuses.



Figure 66. Vectra Imaging Module (camera) front

Rear Connectors

USB Port: connects to a USB Port on the [computer](#)⁷⁰ (via the supplied shielded USB Type A to Type B cable).

Power: connects to the power source.



Figure 67. Vectra Imaging Module (camera) Rear Connectors

Olympus BX-51WI Microscope

A multi-modal microscope suitable for brightfield and fluorescence microscopy.

Includes 4x 10x, and 20x objectives, standard. A 40X objective is optional.

Focus Knob: Allows coarse and fine focus of the slide on the microscope stage.

Eyepieces: Allow you to view the slide directly.

Epi-Fluorescence Filter Turret: a motorized turret containing the epi-fluorescence filters.

IMPORTANT: Do not move the epi-fluorescence filters from their designated and marked positions unless directed to do so by Akoya Biosciences.

Objective Turret: a motorized turret containing the microscope objectives.

IMPORTANT: Do not move the objectives from their designated and marked positions unless directed to do so by Akoya Biosciences.

Power: Control cable (low voltage) to [Microscope Module Controller Box](#) ⁶⁷ 36 pin Hirose rectangular connector.

Fuses: No fuses.

Trinocular and Optical Path Switch

The Trinocular is located on top of the microscope and directs the image of the slide to the camera port, eyepieces, or both. The Optical Path Switch is located on the right side near the eyepiece and camera port. The Optical Path Switch should direct all the light to the camera port during Fluorescence imaging.

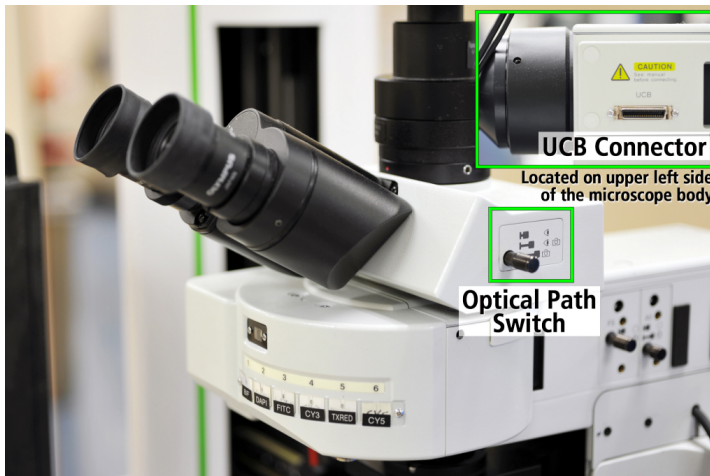


Figure 68. Microscope trinocular with the optical path switch identified. The UCB connector is located on the other side of the microscope body (inset in upper right).

Connectors

UCB Connector: connects to the RFAA/RLAA/NP connector on the [Microscope Module Controller Box](#)⁶⁷.

Microscope Stage

The 200-Slide Stage holds one slide under the microscope objectives and moves in the X and Y directions to position the slide. The slides are loaded onto the stage by the Vectra Slide Loader. The Microscope Stage is controlled manually by the Stage Control Joystick or automatically by the Vectra software.

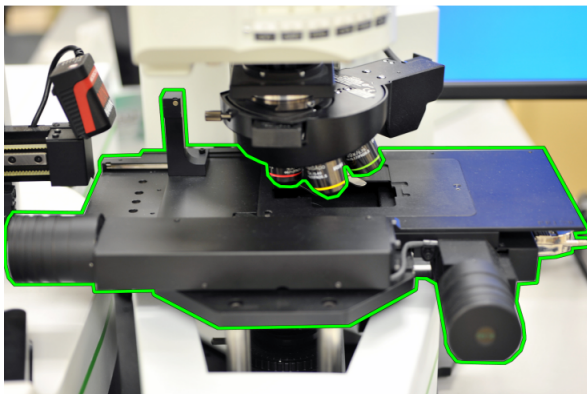


Figure 69. Microscope Stage for the Vectra 3.0, 200-slide model

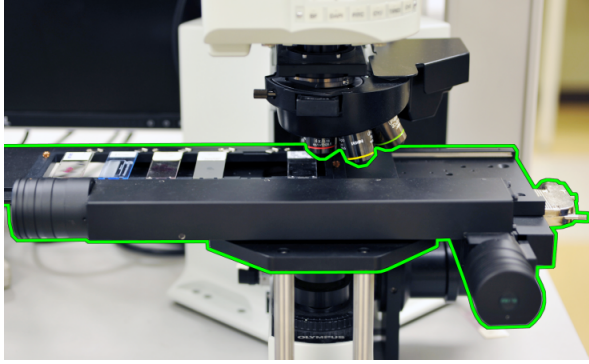


Figure 70. Microscope Stage for the Vectra 3.0, 6-slide model

The gray Encoder Cable connects to the Stage Connector on the [Vectra Slide Loader](#)^[64].

The control cable connects to the DB25 Stage connector on the [Stage Controller Module](#)^[69].

The 6-Slide Microscope Stage holds 6 slides and moves in the X and Y directions to position each of the six slides under the microscope objectives. The slides are loaded onto the stage manually. The Microscope Stage is controlled manually by the [Stage Control Joystick](#)^[63] or automatically by the Vectra software.

The Microscope Stages can only be changed by trained Akoya Biosciences Service personnel.

Stage Control Joystick

The Stage Control Joystick is used to manually control the X/Y/Z position of the stage. This module connects to the 8-pin DIN Joystick connector on the [Stage Controller Module](#)^[69], which serves as the interface for the joystick between the computer and the microscope stage.

On top there is a joystick to control the X-axis and Y-axis of the stage, and on the right side there is a knob to allow manual focusing via control of the Z-axis. The further the joystick is deflected from the center, the faster the stage will move. Sliding tensioners are located adjacent to the joystick to allow adjustment of the feel of the joystick.

Flanking the joystick are two large buttons to control the speed of movement. Pressing the button on the left once reduces the speed of the stage to 50% of maximum. Pressing the button a second time reduces the speed of the stage to 25% of maximum. And pressing the button a third time returns the speed to 100%. The button on the right side provides the same function for the focus knob on the right side of the controller.



Figure 71. Stage Control Joystick

Vectra 200 Slide Loader

The automated slide loader comes with the Vectra 3.0, 200-slide model. Slides are housed in up to 4 removable slide cassettes. Each cassette has a capacity of 50 slides for a total on-board capacity of 200 slides.

The slide loader is used to move both tissue section and tissue microarray (TMA) slides to and from the microscope stage. When batch scanning slides with tissue sections, the loader automatically moves slides to the microscope stage for scanning. When batch scanning TMA slides, you must manually pre-scan each slide to locate the TMA cores. The Vectra software also enables you to load and scan slides one at a time.

AC Power: 100-240VAC, 50/60 Hz, 2A, Standard IEC620 connector

Fuse: 2A, 250VAC

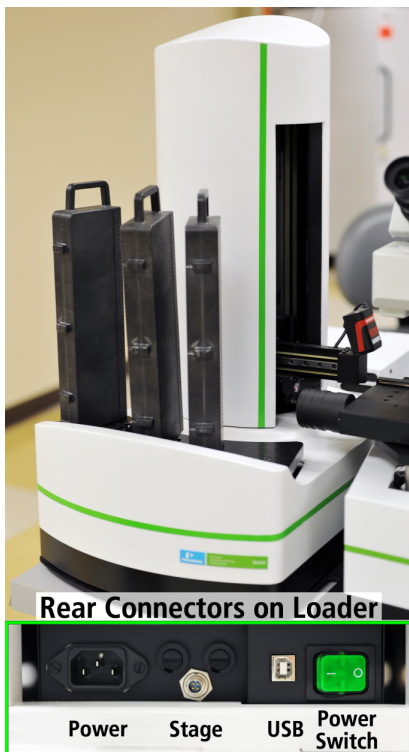


Figure 72. The motorized 200-Slide Loader that comes with the Vectra 3.0, 200-slide model.

Rear Connectors

Power Cable Connector: connects to the power source.

Stage Connector: connects to the [Microscope Stage](#)^[61] (low voltage encoder cable).

USB Connector: connects to a USB Port on the [computer](#)^[70] (USB Type B).

Power Switch: turns the Slide Loader On (I) or Off (O).

Six Slide Stage

The Six-Slide stage adapter holds up to 6 slides on the motorized stage (Slot 1 on the left, Slot 6 on the right). Slides are manually loaded into the Slide Holder, and then automatically scanned with the Vectra software.

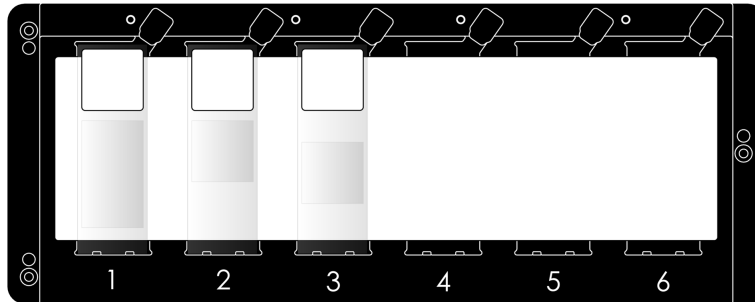


Figure 73. Six-slide insert that is attached to the motorized microscope stage of the Vectra 3.0, 6-slide model. Three slides are shown here loaded into Slots 1, 2, and 3. The total capacity is 6 slides at once.

LED Fluorescence Illuminator

The LED Fluorescence Illuminator is used for fluorescence epi-illumination. The illuminator uses 4 different LEDs to excite fluorescently-labeled tissue sections. The liquid light guide transfers the fluorescent excitation light to the microscope.



Figure 74. LED Fluorescence Illuminator

100W Tungsten Halogen Lamp

The Tungsten Halogen Lamp is used for brightfield trans-illumination. The Tungsten Halogen Lamp connects via cable to the right Lamp Connector on the back of the Microscope Module Controller Box (Olympus BX-UCB) cable, also pictured is the extension cable.

Cable to [Olympus BX-UCB Controller](#)⁶⁷: 4 pin Hirose HR5 circular



Figure 75. 100W Tungsten Halogen Lamp

Stage Focus Motor

The Stage Focus Motor mounts onto the stage focus knob on the right side of the microscope body and is used to focus the microscope using the controls in the Vectra software. The Stage Focus Motor cable connects to the DB15 Focus connector on the [Stage Controller Module](#) ^[69].



Figure 76. Stage Focus Motor

BX Motor Controller Handset

The BX Motor Controller Handset is used to make filter and objective turret selections when the Vectra software is not open. The BX Motor Controller Handset cable connects to the 20-pin Hirose HS Connector on the [Microscope Module Controller Box](#) ^[67].

This unit is automatically disabled when you start the Vectra software.



Figure 77. BX Motor Controller Handset

Microscope Module Controller Box

The Olympus BX-UCB Control Box is the microscope controller. It is the interface by which the computer controls the Olympus BX microscope.

Cables:

- Control cable (low voltage) to [Olympus BX-51WI microscope](#)^[61], 36-pin Hirose rectangular connector.
- RS-232 communications cable (low voltage) to the [computer](#)^[70], DB9 connector.
- Lamp power cable to 100Watt [Tungsten Halogen Lamp](#)^[65], 4 pin circular Hirose HR5 connector.
- Control cable (low voltage) to the [BX Motor Controller Handset](#)^[66], 20-pin Hirose rectangular connector.

AC Power: 100-240VAC, 50/60 Hz, 3.5A/1.5A, standard IEC320 connector

Fuses: No fuses



Figure 78. Microscope
Module Controller Box

Front Connectors

RS232C Connector: connects to an RS-232 port on the computer.

HS Connector: connects to the [BX Motor Controller Handset](#)^[66].

Power Switch: turns the [Microscope Module Controller Box](#)^[67] On (I) or Off (O).



Figure 79. Olympus BX-UCB
Front Connectors

Rear Connectors

UCB Connector – connects to the [microscope](#)^[67].

Right Lamp Connector - connects to the [Tungsten Halogen Lamp](#)^[65].

Power Cable Connector - connects to the power source.

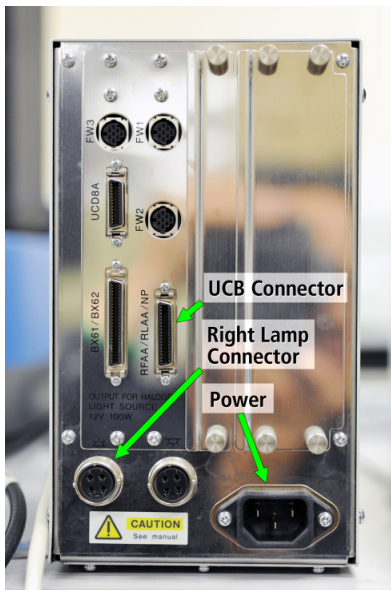


Figure 80. Olympus BX-UCB
Rear Connectors

Stage Controller Module

The Stage Controller module drives the XY stage as well as the Z focus drive. It is the interface for the Stage Control Joystick to drive the XY stage.

Power: 100-240VAC, 50.60Hz, 150Watts, standard IEC320 connector

Fuses: (2) 250VAC, 2A



Figure 81. Vectra Stage Controller Module

Rear Connectors

Focus Connector - connects to the [Stage Focus Motor](#)^[66].

Stage Connector - connects to the [Microscope Stage](#)^[61] (DB25).

USB Connector - connects to a USB Port on the [computer](#)^[70] (USB Type B connector).

Joystick Connector - connects to the [Stage Control Joystick](#)^[63].

Power Cable Connector - connects to the power source.

Power Switch - turns the Stage Controller Module On (I) or Off (O).

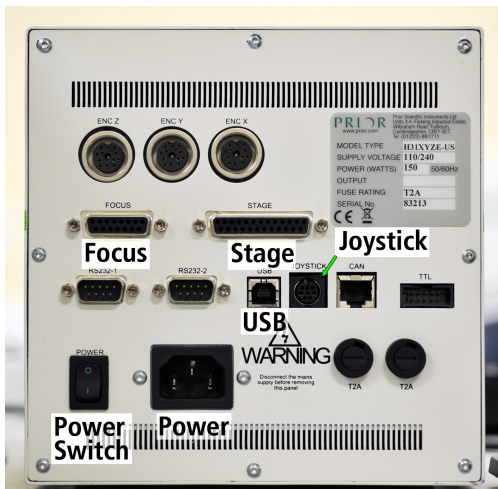


Figure 82. Vectra Stage Controller Module Rear Connectors

Vectra Computer

The Vectra computer is included with the Vectra 3.0 instrument. It has the Vectra, Phenochart, and inForm software installed and activated at the factory. Also included are a wireless keyboard and mouse.



Figure 83. Vectra Computer

Widescreen Monitor

This ultra-widescreen color monitor enables side-by-side viewing of both the Vectra and Phenochart program windows. It comes with an articulated arm that clamps to your benchtop that allows the monitor to be positioned to your liking.

Power: 100-240VAC, 50.60Hz, 150Watts, DVI-D, DisplayPort, and HDMI connectors



Figure 84. Vectra ultra-widescreen monitor

8.2 Eyepiece Adjustment

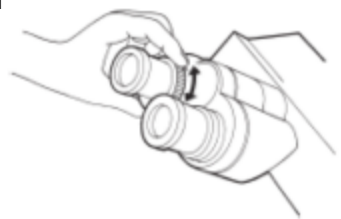
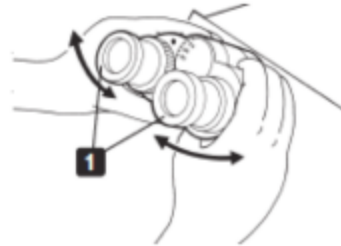
The Vectra 3.0 camera adapter is adjusted by the Akoya Biosciences Service technician during installation to maintain proper focus on the specimen when the image is displayed on the screen and when you are looking through the eyepieces, even with different objectives - although there may be some small variation. This means that the setup is 'parfocal'. If the focus is significantly different, contact Akoya [Technical Support](#) ⁵.

To optimize your comfort while using the Vectra 3.0 instrument hardware, there are adjustments available to suit your needs and preferences while using the microscopy controls. Each user can make adjustments to the following settings without negatively affecting the functionality of the Vectra 3.0 system.

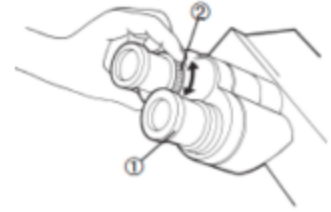
Vectra Eyepieces

To adjust the Vectra eyepieces:

1. Pull or push the trinocular Optical Path Switch into the trinocular to send the light to the eyepieces or to both the eyepieces and to the camera. Akoya Biosciences recommends leaving the switch in the camera-only setting. Inadvertently leaving the switch in the other settings can result in longer exposure times or error conditions.
2. While looking through the eyepieces with 50% or 100% of the light sent to the eyepieces, adjust the interpupillary distance between the two eyepieces until you achieve binocular vision with both your left and right fields of view coinciding completely.
3. Set the objective to 20X (or 40X if your Vectra 3.0 is equipped with a 40X objective) with 50% or 100% of the light sent to the Vectra camera. Focus on a detailed specimen on a slide on the slide stage. Then with 50% or 100% of the light sent to the eyepieces, adjust the diopter settings in the eyepieces until the image as seen through the eyepieces is equally sharp.



Normally the image on the computer monitor will be as sharp as the image seen through the eyepieces, but if it is not, and you prefer to fine-tune the eyepieces and then make adjustments to the focus while looking at the monitor before taking pictures, perform the following routine:



1. Set the diopter adjustment rings on both sides to scale '0'.
2. Engage a high-power objective (20X or 40X) in the light path, look into the right eyepiece with your right eye, and rotate the coarse and fine adjustment knobs to bring the specimen into focus. Do not use an oil-immersion objective.
3. Engage a low-power objective (10X or so) in the light path, rotate the right diopter adjustment ring to bring the specimen into focus. Do not touch the coarse and fine adjustment knobs during the focusing.
4. Looking into the left eyepiece with your left eye, rotate the left diopter adjustment ring to bring the specimen in focus. The above procedure adjusts the diopter with reference to the right eye, but it is also possible to adjust with reference to the left eye. In this case, follow the above procedure by inverting 'right' and 'left.'

The Vectra eyepieces are equipped with rubber eyeshades that can be folded down for users with eyeglasses or folded up to prevent extraneous light from entering the eyepieces and eyes.



8.3 Cleaning the Vectra Hardware

Stage


You may clean the Slide Insert and Slide Holder with compressed air. You can use a squeeze bulb (air duster) or cans of compressed air (with no additives) available from electronics or office-supply stores. Hold cans of compressed air upright and do not shake, so that freezing cold fluid does not spray out. Isopropyl alcohol (>90% pure) and a lintless wipe can also be used to clean/remove debris that cannot be removed with the compressed air.

Microscope

1. Clean the exterior of the microscope using an air duster and/or microfiber cloth.
2. Clean the microscope eye pieces with an air duster and/or lintless lens tissue that will not scratch the delicate surface of the oculars.

All other critical components are cleaned by Akoya Biosciences Service Engineers during preventative maintenance procedures.

9 System Specifications

Spectral range	440 to 720 nm
Objective lens	4x, 10x, and 20x standard, additional 40X objective optional
Pixel size at sample	0.5 microns/pixel (when using 20x objective)
Fluorescence Illuminator	Universal integral power supply: Input 110-240V, 50/60Hz Use within ambient temperature range: 18-28 °C Required clearance: 4" (100mm) minimum Transfers light to the microscope via liquid light guide
File format	Akoya Biosciences .qptiff format for whole slide scans, proprietary .im3 file format for multispectral data; 24-bit Windows-compatible bitmap for RGB/Mono imagery
Operating system	Windows 10™, 64 bit
RAM	12Gb minimum, 16Gb recommended
Barcode Reader (optional)	<p>Imaging: SXGA/QXGA Symbologies: Data Matrix (ECC 0-200), QR Code, PDF417, Micro PDF417, GS1 Databar (Composite & Stacked), Code 39, Code 128, BC 412, I2 of 5, UPC/EAN, Codabar, Code 93 Light source: High output LEDs</p> 

9.1 Site and Environmental Requirements

Power

Parameter	Specification
Electrical power	100-120 /200-240 VAC, 3A, 50/60 Hz System does not have transient overvoltage protection

Environmental

Parameter	Specification
Operating temperature	59°F to 86°F (15°C to 30°C)
Operating humidity	0% - 50% non-condensing
Storage temperature	59°F to 86°F (15°C to 30°C)

Storage humidity	0% - 70% non-condensing
Altitude	0 - 6561 feet (0 - 2000 meters)
Shipping temperature (24 hours maximum)	14°F to 113°F (-10°C to 45°C)
Pollution degree	2
Indoor Use Only	

10 Hardware Setup Program

When the Vectra system is installed in your laboratory by the Akoya Biosciences Service Engineer, the Vectra Hardware Setup program is used to configure the proper objectives, epi-fluorescence filters, and communication settings.

You should NOT have to change the hardware configuration unless you make changes to the Vectra hardware. This appendix provides an overview of the settings in the Hardware Setup program. You should contact Akoya [Technical Support](#) for instructions on making any hardware changes to the system.

To start the Hardware Setup program, select **Start > All Programs > Akoya > Vectra Hardware Setup**. The **Hardware Setup** window opens.

Objectives

If you add or remove an objective from the Vectra, you must change the settings in the Objectives tab to reflect the current hardware configuration.

The Turret Size defaults to 6 and is not editable. The Vectra hardware features a six- position turret. Objective positions are marked Empty if an objective is not installed.

Use the Available Objectives list to create or edit entries for each of the objectives mounted in the objectives turret.

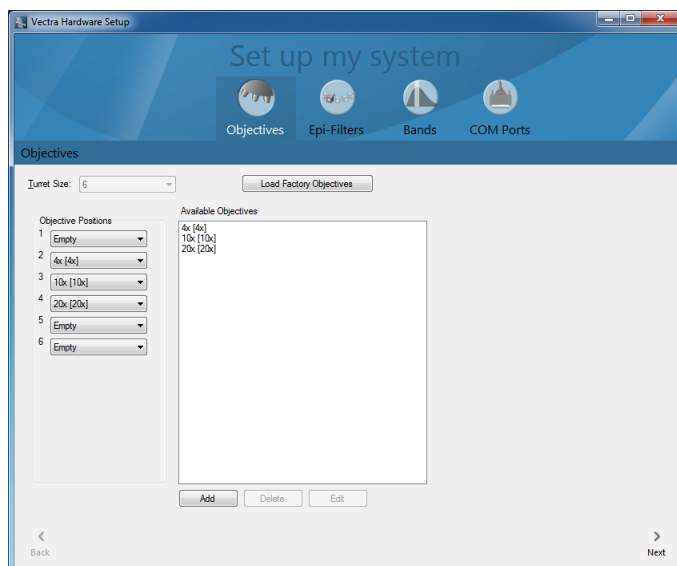


Figure 85. Objectives Tab

- To add entries, click the **Add** button. Enter a short descriptive name and choose the nominal magnification. Click **OK** when finished. The new entry is added to the list.
- To edit the name of any objective, select the objective name and click Edit. You can only edit the objective's name, not its nominal magnification. To change the magnification, delete the entry and create a new entry for the objective.
- To delete an objective from the system, first remove it from any objective position. Then, select the objective name in the list and click Delete. Choose Yes to confirm you want to delete the objective from the system.

Use the Objective Positions boxes (1-6) to arrange the objective entries so they match the locations of the actual objectives mounted in the turret. As you assign objectives to positions, the software prevents you from assigning the same objective to more than one turret position. To change an objective's location, remove it from the current location and then select the new location.

The default objectives and their positions are as follows:

- Position 1 – Empty
- Position 2 – 4X
- Position 3 – 10X
- Position 4 – 20X
- Position 5 – Empty or optional 40X
- Position 6 – Empty

The **Load Factory Objectives** button automatically populates the Available Objectives box with the default factory objectives.

Click the **Next** button or the **Epi-Filters** button to continue with system setup, or close the window if you are finished setting up or making changes to the hardware.

Epi-Filters

If you add or remove an epi- fluorescence filter cube or cubes on the Vectra system, you must change the settings in the Epi-Filters tab to reflect the new hardware configuration. You should contact Akoya [Technical Support](#) ⁵ for instructions on changing or adding hardware.

The Turret Size defaults to 6 and is not editable. The Vectra hardware features a 6-position turret. Epi-Filter positions are marked Empty if an epi-fluorescence filter cube is not installed.

Use the Available Epi-Filters list to create or edit entries for each of the epi-fluorescence filters mounted in the filter turret. Select a filter to display the filter information to the right of the Available Epi-Filters list.

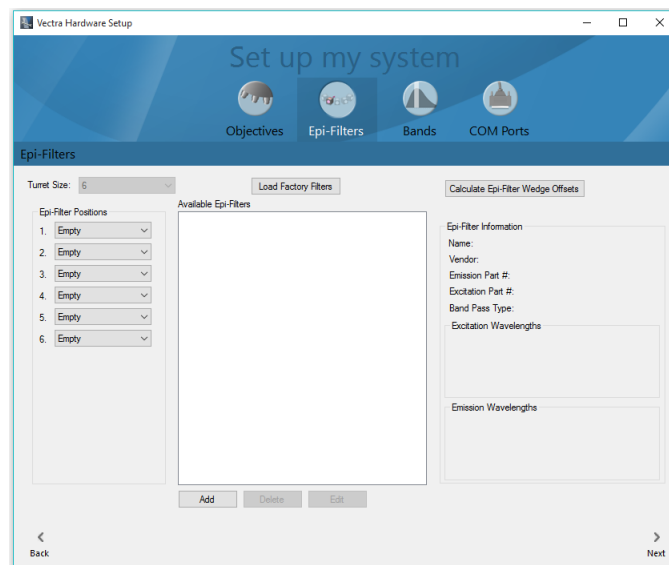


Figure 86. Epi-Filters Tab

- **Add Filter:** To add entries for filters you have purchased separately, click the **Add** button. In the Add Epi-Filter window, enter a short descriptive name. Enter the vendor and vendor part number. Select the Band Pass type, either Single-, Double-, Triple-, or Quad-band pass. Enter the excitation and emission cut-on and cut-off wavelengths, in nanometers. Click **OK** when finished.
 - **Band Name:** When you add a new epi-filter, you are prompted to enter a **Band Name** for each emission cut-on/cut-off range. Every epi-fluorescence filter in the Vectra filter turret must have at least one band associated with it, and this is the filter's default band. Enter a name that includes the filter's specific target and intended wavelength range. See the next section, [Bands](#)^[78], for more about bands.
- **Edit Filter:** Only the Filter Name of custom bands can be edited. The default 'predefined' filters provided with the Vectra system cannot be edited. Select the filter name and click **Edit**. Selecting a predefined filter and clicking the Edit button displays the filter parameters, but the parameters cannot be changed. Existing filter cannot be edited because they may have already been used in experiments, and if the parameters are changed, the current parameters would not agree with the parameters used in the experiment. To change a custom filter, you must delete the filter and then add the filter again with the correct information. Note that deleted filters are permanently deleted and cannot be recovered.
- **Delete Filter:** To delete a filter from the system, first un-mount the filter from its epi-filter position. Then select the filter name in the list and click Delete. You can only delete filters you have created; you cannot delete any of the predefined filters. Choose Yes to confirm you want to delete the filter from the system.

Use the Epi-Filter Positions boxes (1-6) to arrange the epi-filter names so that they match the locations of actual epi-fluorescence filters mounted in the turret, which are as follows:

- Position 1 – Brightfield (no filter cube present)
- Position 2 – DAPI
- Position 3 – FITC
- Position 4 – CY3
- Position 5 – Texas Red
- Position 6 – CY5

The Brightfield filter is permanently assigned to position one; its position cannot be changed. As a filter is assigned to a turret position, the software prevents you from assigning the same filter to any other position. To change a filter's location, remove it from its current position, and then select the new position.

The **Load Factory Filters** button automatically populates the Available Epi-Filters box with the default factory filters.

- **Calculate Epi-Filter Wedge Offsets:** Epi-filters can produce image shifts when filter wedge artifacts bend transmitted light. Even so-called "zero pixel shift" filters can produce subpixel amounts of image shift that may impair image quantification and spectral unmixing.

The **Calculate Epi-Filter Wedge Offsets** feature allows you to measure and record these residual wedge-related image shifts for the filters installed on the Vectra system. Once recorded, these values will be applied as correction offsets to remove image shift from MSIs acquired in the future.

Note that previously acquired MSIs can be corrected using the separate Wedge Calculation and Correction program. See [Appendix G](#)^[107] for more information.

Before computing wedge correction offsets, MSI images must be acquired using a set of single-stained samples (see [Section 5.6](#)^[44] for acquisition instructions). All images in this set should be acquired using the same filters, in the same order. For best results, the signal bands of these single-stained samples should span all filters used to acquire MSI images (see [Appendix D](#)^[95] for signal bands of Opal fluorophores). Autofluorescence samples can be included as well.

To measure and record wedge correction offsets from single-stained images, click the **Calculate Epi-Filter Wedge Offsets** button to launch the Wedge Calculation window.

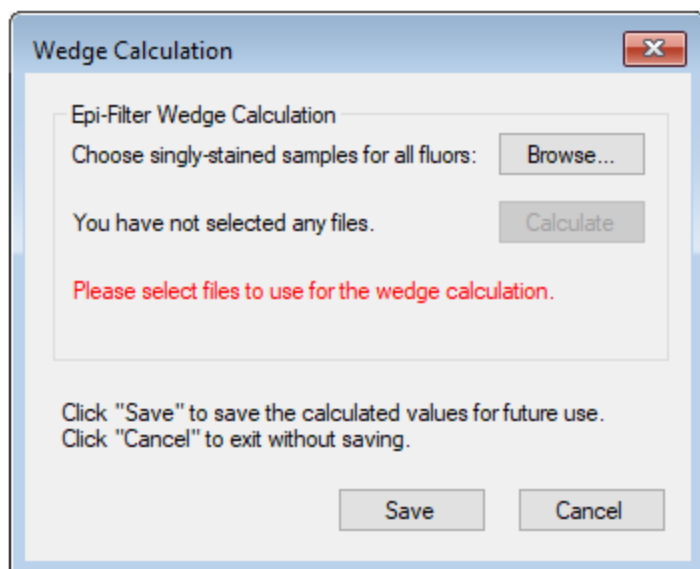


Figure 87. Wedge Calculation Window

- **Choose images:** To select the single-stained images, click the **Browse...** button. Select all MSI imagery corresponding to the acquired single-stained samples. This will activate the **Calculate** button.
- **Calculate offsets:** To measure the wedge correction offsets, click the **Calculate** button. After the calculation is complete, the measured offsets will be displayed in a separate window. Click **OK** to continue.
- **Save offsets:** To save the calculated offsets, click the **Save** button. These offsets will now be applied to all future MSIs acquired on the Vectra system. Clicking the **Cancel** button will leave the recorded wedge offsets in their previous state.

Click the **Next** or **Bands** button to continue with system setup, or close the window if you are finished setting up the hardware.

Bands

You can also set up the bands associated with each epi- fluorescence filter.

The Bands tab lists all the installed epi-fluorescence filters and the Bands created for each filter. Bands specify how Vectra measures light over the filter's range of wavelengths. By adding multiple bands to a filter, you can quickly select how you want to use the filter during a specific protocol (e.g., within a narrow wavelength range, in high definition (10 nm step size) mode, etc.).

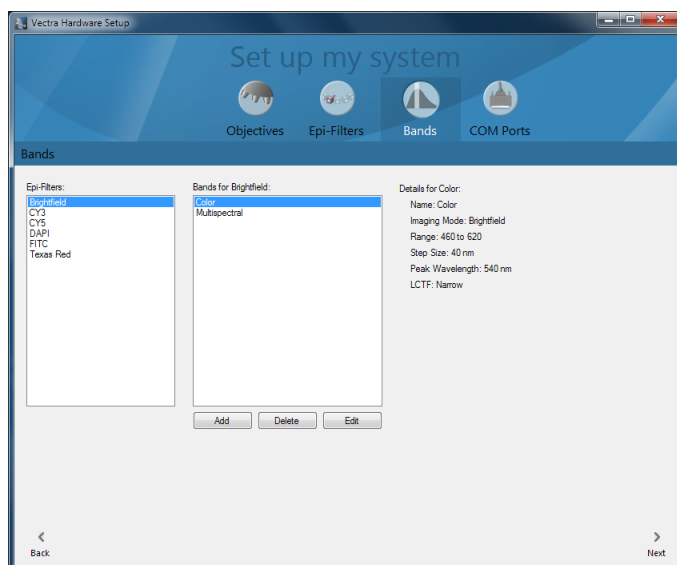


Figure 88. Bands Tab

- **Name:** The name specified for the band when it was created. Factory band names are not editable; you can only edit the names of bands you create.
- **Imaging Mode:** Brightfield for any bands created for the brightfield filter. Fluorescence for bands created for any of the fluorescence filters.
- **Range:** The starting and ending wavelengths specified when the band was created. This range is limited by the emission filter. Click the Edit button to change the values for non-factory bands.
- **Step Size:** The default step size is Normal (20nm). A High-Definition (10nm) step size is also available. You can choose either step size when adding or editing a band. Note that High-Definition bands will increase the file size significantly, and may not improve your data quality.
- **Peak Wavelength:** The peak wavelength is the wavelength that displays in the live view on the monitor and that is used for focusing. Typically, the peak emission is approximately 20nm after the filter's emission cut-on wavelength, so this peak wavelength is used for the live view as well as for focusing. For example, if the epi-filter's emission cut-on wavelength was 633nm (or any value between 630–640nm), the default band's starting wavelength would be 630nm, and its peak wavelength would be 650nm. For any subsequent bands you create, the peak wavelength is 20nm above the band's starting wavelength.
- **LCTF:** The Vectra system always uses a broad bandwidth Liquid Crystal Tunable Filter, so this entry is for display-status purposes only, and cannot be changed.

COM Ports

If instructed, you can change the communications ports used by the Vectra system.

Use this panel to select which COM port connects to each device. Once a COM port is assigned to a device, it is unavailable for selection for the other devices. To reassign a COM port, remove it from the current device and then assign it to the new device.

- The **Scope** COM port provides communication to the microscope hardware.

- The **Stage** COM port provides communication to the Vectra Stage Controller module.
- The **Loader** COM port provides communication with the Slide Loader, if installed.

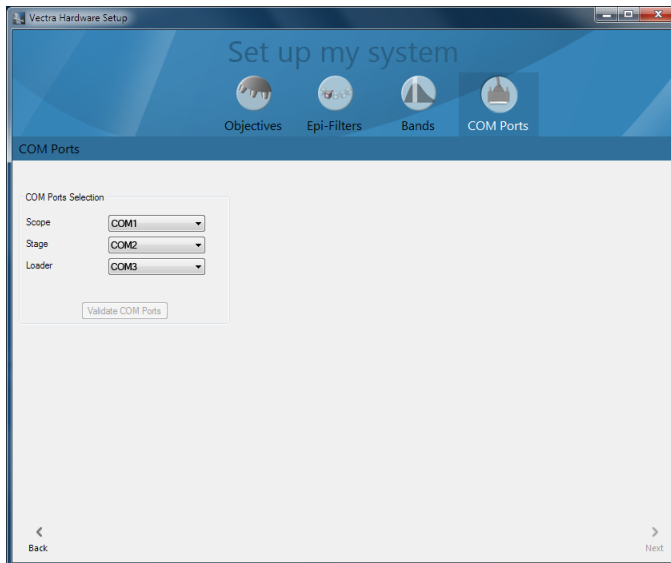


Figure 89. COM Ports Tab

Click the **Validate COM Ports** button to test the selected ports to confirm proper operation (e.g. each COM port is controlling the proper hardware, so that you do not receive any error messages when starting the Vectra software).

11 Frequently Asked Questions

Q: When I am determining optimal exposure times for the whole slide scan and for acquiring MSI regions, why do I need to click the Autoexpose button, click the Autofocus button, and then click on the Autoexpose button again? Why can't I simply click on the Autoexpose button once and then the Autofocus button, or click on the Autofocus button and then the Autoexpose button?

A: You may find that when the starting exposure time is long and the sample is very bright, it may be difficult to obtain sharp focus. In that case, it is helpful to click on the Autoexpose button first. Your exposure time could vary if you are not in sharp focus, click on the Autoexpose button, and then obtain sharp focus, because you may not be seeing the light from the same cells as before.

Q: Can I select any inForm algorithm in order to perform automated MSI Field selection?

A: Algorithms for automated MSI Field selection must be saved from inForm version 2.2 or later.

Q: What should I do if Vectra does not recognize the tissue on my fluorescence slide?

A: Use a permanent lab marker, for example a Sharpie® or VWR® Lab Marker, in red, green, or blue (not black) to encircle the tissue. Vectra will scan everything within the encircled area. You may see traces of the marker in the scan overview, but those traces will not affect the MSI Field images. See [Sample Finding](#)^[84] in the [Troubleshooting](#)^[83] section for more detail.

Q: Are there any exclusions for anti-virus?

A: Please exclude the following directories from anti-virus scanning while the Vectra is in operation:

- The Vectra data directory. By default this is D:\Data\Vectra3, check the Dashboard to see if it has been configured differently.
- The Vectra configuration directory, C:\ProgramData\Akoya\Vectra.
- The Vectra log directory, C:\Users\Public\Akoya\Vectra.

Q: What IT privileges do I need?

A: The user running the Vectra will need read and write privileges to the following directories:

- The Vectra data directory. By default this is D:\Data\Vectra3, check the Dashboard to see if it has been configured differently.
- The Vectra configuration directory, C:\ProgramData\Akoya\Vectra.
- The Vectra log directory, C:\Users\Public\Akoya\Vectra.

Q: Why do I see the brightfield light come on even during scanning of fluorescent slide protocols?

A: Vectra always images the edges of the slide to find its thickness, then takes the color overview image that's shown in the Phenochart info dialog. These steps are done using bright-field illumination at 4x.

Q. When I take MSI fields, does Vectra re-scan the slide?

A: No. Vectra takes 4x images of the slide edge, and one point on the coverslip, for registration. Then it takes the MSI images at the selected locations.

Q. Why is Vectra using the 20x objective during MSI acquisition of 0.25 (40x) regions?

A: The 40x objective has a short working distance (180 microns). So rough focusing is done with the 20x objective, and the 40x is engaged only for fine focusing over a small range. The rough focusing step is skipped for subsequent regions that are near to acquired sites.

12 Troubleshooting

You may receive an error message while in the Scan Slides page: **'Errors occurred while processing slide(s). Please check the conflict tool tip for details'**. Hovering your cursor over a Completion Status Message in the Conflict Column will result in a Tool Tip popping up with more information. In addition, Vectra will write a log of any processing errors to the Study Folder.

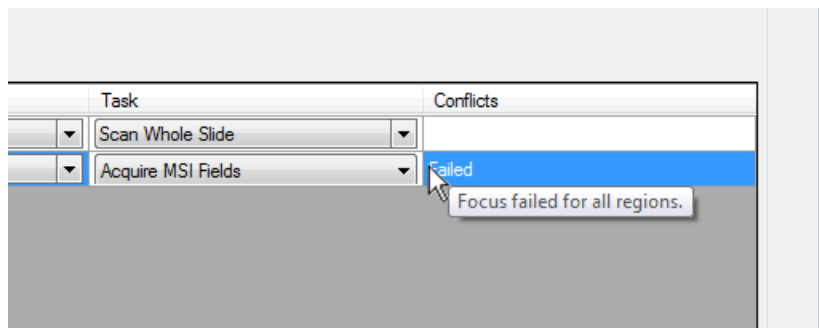


Figure 90. Tool Tips with additional information appear when you hover your cursor over a Completion Status Message in the Conflicts Column on the Scan Slide page.

Registration Issues

You may see a Tool Tip message that Vectra could not register the slide: **'Images taken but slide registration failed'**. When Vectra scans a slide and then later acquires an MSI Field, the slide might have been removed and replaced on the microscope stage prior to the MSI Field acquisition, and an exact registration of the previously scanned image and the newly acquired MSI Field image might not be possible. The MSI Field scan will have been made and the file saved, with no effect on the image quality.

Failure to Focus

You may see one of 4 Tool Tip messages that focus could not be obtained for one or more slides:

'Focus failed for the region' – Vectra tried 1 and it failed.

'Focus failed for all regions'– Vectra tried more than 1 and all failed.

'Focus failed for one of the regions' – Vectra tried more than 1 and 1 failed.

'Focus failed for several regions' – Vectra tried more than 1 and more than 1 failed.

In the Phenochart program, the MSI region(s) will be color-coded as an Acquisition Failed region, by default an orange/brown color. Check for large debris such as dust on the slide, which could confuse the focus-finding algorithm. Clean the slide with compressed air or by drag-wiping with lintless tissue and cleaning solution and try to acquire the image(s) again. You can also increase your exposure time if there is insufficient signal to allow focus to be found.

Sample Finding

You may see a Tool Tip message that no sample material was detected: 'No sample material was detected'. For fluorescence slides, you can try increasing the exposure time if there is insufficient signal. Check that the fluorescence illuminator is ON and set for 10%. You can use a permanent lab marker, for example a Sharpie®, Fisherbrand™, or VWR® Lab Marker, in red, green, or blue (not black) to encircle the tissue. Vectra will scan everything within the encircled area. You may see traces of the marker in the scan overview, but those traces will not affect the MSI Field images.

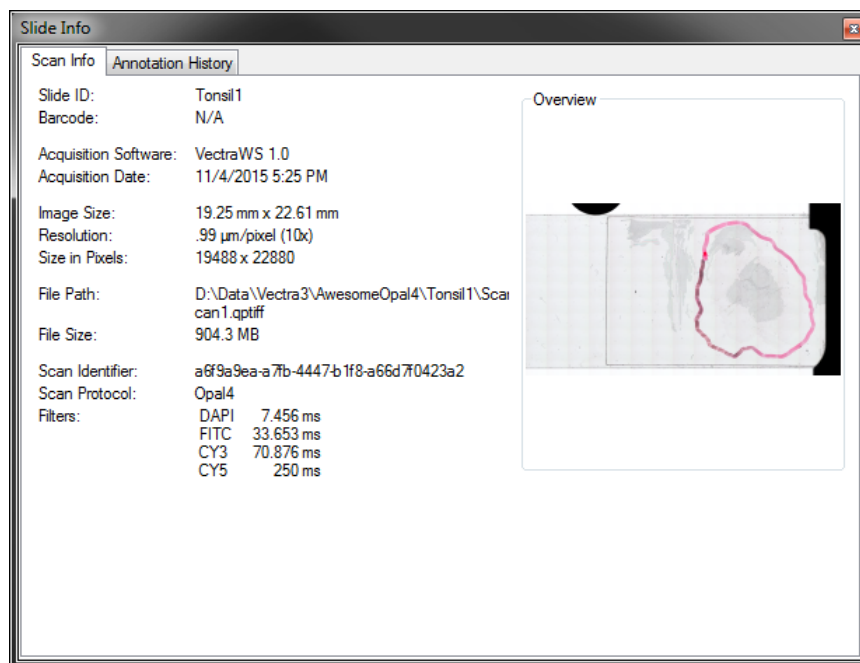


Figure 91. Encircled tissue on a slide seen in the Phenochart overview thumbnail image

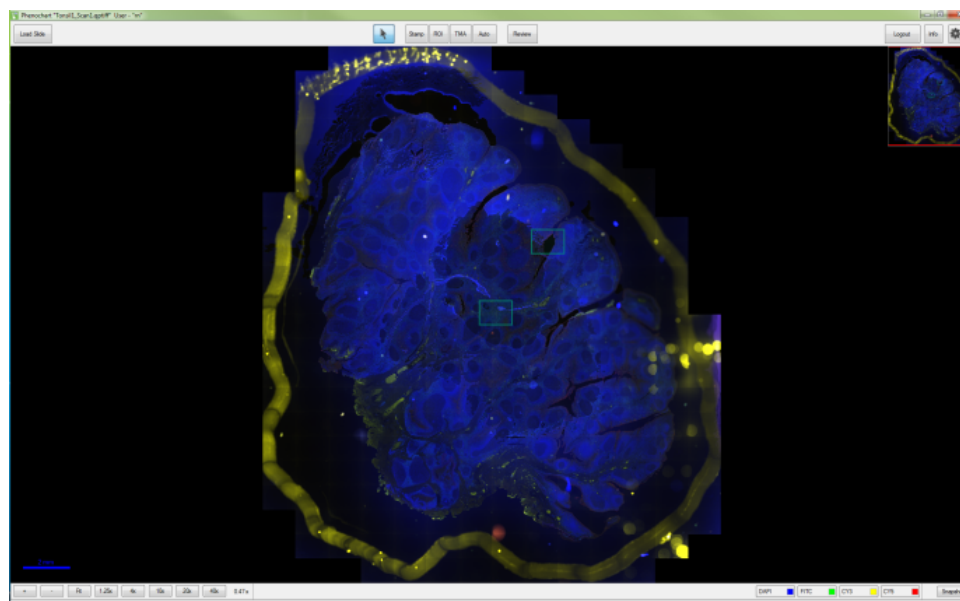


Figure 92. Scan of a slide where a red marker was used to encircle tissue that was initially not recognized.

If your brightfield sample is not found, it is possible that the staining is too faint or perhaps is a color that interferes with the Vectra focus-finding algorithm that allows the system to focus using green light. Very faint methyl green could theoretically cause a problem, although this is extremely rare. In this case, use the lab-marker method described above to allow the Vectra to scan your slide.

You may see a Tool Tip message even after drawing around the tissue with a lab marker: **'Markup was found, but Vectra was unable to identify the scan region(s)'**. Try re-drawing around the tissue to close any gaps in the line or try drawing around the tissue with another color marker.

13 Appendix A: Supported Slide Formats & Dimensions

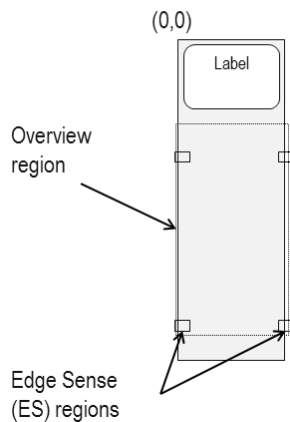
Slide formats supported by Vectra 3

Slide Type	Width	Height	Thickness
Metric	25.0 +/- 1.0	75.0 +/- 1.0	1 +/- 0.10

Scan regions and edge measurement points Vectra 3.0, 6-Slide system

Region	Center (mm)	ULHC (mm)	LRHC (mm)
ES Region 1	[0.95, 28.00]	[-0.80, 26.70]	[2.69, 29.30]
ES Region 2	[0.95, 68.00]	[-0.80, 66.70]	[2.69, 69.30]
ES Region 3	[25.70, 28.00]	[23.95, 26.70]	[27.44, 29.30]
ES Region 4	[25.70, 68.00]	[23.95, 66.70]	[27.44, 69.30]
Overview Region	[13.00, 45.00]	[-0.50, 20.00]	[26.50, 70.0]

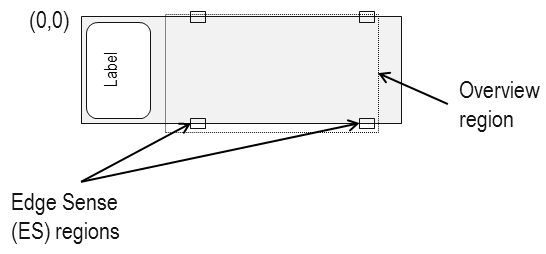
Vectra 6 / LC



Scan regions and edge measurement points Vectra 3.0, 200-Slide system

Region	Center (mm)	ULHC (mm)	LRHC (mm)
ES Region 1	[28, 0.00]	[26.25, -1.30]	[29.74, 1.30]
ES Region 2	[28, 25.40]	[26.25, 24.10]	[29.74, 26.70]
ES Region 3	[65, 0.00]	[63.25, -1.30]	[66.74, 1.30]
ES Region 4	[65, 25.40]	[63.25, 24.10]	[66.74, 26.70]
Overview Region	[45.0, 13.0]	[20.0, -0.50]	[70.0, 26.5]

Vectra 200 / HC



14 Appendix B: Akoya Biosciences TIFF Specification

Background

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

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

Data format

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

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

The highest resolution (baseline) image(s) appear first in the file. For each resolution there are N baseline images where N depends on the contents. For BF images, N=1 and each image is an RGB image. For FL images or unmixed component images, N = number of bands, which is usually > 1, and each image is a grayscale image.

A thumbnail RGB image is provided, and this is a good image to use as an icon in graphical image lists. This comes after the baseline images, meaning it is the second image in BF (RGB) images, and the (N+1)st image for FL images or unmixed component images.

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

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

Overall, the arrangement is:

Table 1. Images

Description	RGB / mono	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	

Description	RGB / mono	Tile / Strip	Resolution	Notes
Half-resolution images	Varies	Varies	Half	Only if baseline is tiled
Quarter, eighth, etc.	Varies	Varies	Quarter, eighth, etc.	Continues until 2K x 2K or smaller
Macro (overview) image of whole slide	RGB	Stripped	~2000 x 4000	Required for whole-slide scans Optional for simple RGB images and extracted components
Label image	RGB	Stripped	~500 x 500	Optional, whole-slide scans

Detection

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

Metadata

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

Table 2. TIFF tags

TIFF Tag	Optional	Description of contents
Software		Starts with “PerkinElmer-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.

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

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

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

Image Description contents

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

Table 3. Image Description tags

Tag	Optional	Contents
DescriptionVersion		Version of the image description field itself, a single number. This document describes version 2 of the field.
AcquisitionSoftware		Software used to acquire the image.
Identifier		GUID in string format. This is an identifier for the image file itself.
SlideID	Y	ID of the slide that this image was taken from.
Barcode	Y	Barcode text of the slide this image was taken from.
ComputerName	Y	Name of the computer on which the slide was scanned.
ImageType		A string identifying the type of image within the file (Table 1), with the following values: <ul style="list-style-type: none"> - 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 <i>www ttt</i> where the <i>ttt</i> nibble indicates the signal unit type from the following: <ul style="list-style-type: none"> 0 – raw counts 1 – normalized (counts/second/gain/full-scale/binning) 4 – OD (optical density) 5 – dark-corrected counts and the <i>www</i> nibble indicates how the signal is weighted across the spectral bands (or colors): <ul style="list-style-type: none"> 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 name for FL whole slide scans, component name for extracted components. Not present for RGB images.

Tag	Optional	Contents
Color	Y	Color to use when rendering this band, as decimal r,g,b byte triplet. Present for FL whole-slide scans or extracted components. Not present for RGB images.
Responsivity	Y	Instrument responsivity, if available, for FL whole-slide and unmixed images. See below for details.
Objective	Y	Objective name, if known, otherwise not present.
ScanProfile	Y	Element containing scan and/or 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.
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.

Instrument Responsivity

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

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

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

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

Each <Filter> or <Band> tag describes the instrument responsivity for acquisitions using that filter or band. The contents of the <Filter> and <Band> tags are described below.

Table 4. Contents of Filter and Band Tags

Tag	Contents
Name	The name of the filter (whole-slide image) or band (component image).
Response	The instrument response to the reference artifact, normalized for exposure. This is raw counts / (2bit depth x exposure time x gain x binning area), where bit depth is the bit depth of the imagery, exposure time is in seconds, gain is the gain setting of the camera, and binning area is 1 for 1x1, 4 for 2x2, etc.

Tag	Contents
Date	The date and time of the reference image in UTC, ISO 8601 format.
FilterID	Detailed description of the acquisition filter.

Sample ImageDescription

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

```
<?xml version="1.0" encoding="utf-8"?>
< PerkinElmer-QPI-ImageDescription >
  <DescriptionVersion>1</DescriptionVersion>
  <AcquisitionSoftware>VectraScan 1.0.0</AcquisitionSoftware>
  <ImageType>FullResolution</ImageType>
  <Identifier>AABED946-BB58-44FB-95B3-48E177E3BB83</Identifier>
  <IsUnmixedComponent>False</IsUnmixedComponent>
  <ExposureTime>50</ExposureTime>
  <SignalUnits>64</SignalUnits>
  <Name>DAPI</Name>
  <Color>0,0,255</Color>
  <Responsivity>
    <Filter>
      <Name>DAPI</Name>
      <Response>30.7</Response>
      <Date>2015-10-22T13:10:18.0618849Z</Date>
      <FilterID>Semrock:FF02-409/LP-25 Emission / Semrock:FF01-377/50-25 Excitation</FilterID>
    </Filter>
  </Responsivity>
  <Objective>4x</Objective>
  <ScanProfile><!-- this will be a serialized scan protocol. It is valid XML but otherwise opaque -->
</ScanProfile>
  <ValidationCode>4281fff86778db65892c05151d5de738d</ValidationCode>
</ PerkinElmer-QPI-ImageDescription >
```

15 Appendix C: Typical Scan & MSI Field Acquisition Times

Acquire Whole Slide Scan	Time (min)	File Size (MB)
Brightfield, 10×	7:30	500
Brightfield, 4×	6:30	130
Fluorescence, 10×	12:00	1000
Fluorescence, 4×	7:30	220

Note: Fluorescence data is for a 5-band scan. Using fewer bands saves approximately 60 seconds and 180 MB per band (10×) or 30 seconds and 40 MB per band (4×).

Acquire MSI Regions	Fluorescence		Brightfield	
	Time (sec)	File Size (MB)	Time (sec)	File Size (MB)
1x1 Field Region	30	90	12	76
2x2 Field Region	120	350	40	280
3x3 Field Region	260	800	90	500

Acquire TMA Cores	Fluorescence		Brightfield	
	Time (sec)	File Size (MB)	Time (sec)	File Size (MB)
0.6 mm	60	90	24	76
0.8 mm	60	160	24	134
1.0 mm	120	248	48	210
1.2 mm	180	358	72	300
1.5 mm	270	560	110	470
2.0 mm	270	810	110	684
2.2 mm	270	810	110	684
2.5 mm	270	810	110	684

16 Appendix D: Filter Cube Recommendations when using Opal Reagents

If you are using your Vectra 3 system with Akoya's Opal multiplex IHC detection kits or with individual Opal fluorophores, please refer to the table below for exposure time recommendations for each filter cube.

Filter	DAPI	FITC	Cy3	Texas Red	Cy5
Dye					
DAPI					
Opal 520					
Opal 540					
Opal 570					
Opal 620					
Opal 650					
Opal 670					
Opal 690					

Key

						Signal band for this dye, set exposure per sample
						Non-signal band, use 150 milliseconds exposure for MSI

17 Appendix E: Compensation Calculations

Brightfield

The sample images are normalized (divided) by the reference images on a pixel-by-pixel, wavelength-by-wavelength basis. So a given sample pixel is normalized by that same pixel, at that same wavelength, in the reference image. This yields the sample transmission T , which is in the range 0 – 1.

For MSI images, the OD is calculated – this is based on $\text{Log}_{10}(T)$.

For simple color images like the LP mosaic, the transmission is mapped onto the 0 – 255 signal levels.

Fluorescence

The sample images are normalized by a shading pattern derived from the reference images. The shading pattern is applied on a pixel-by-pixel basis, with one pattern per epi-filter. The shading pattern is simply the reference image, divided by the mean intensity in the center of the image. Thus it represents the spatial variation across the image, with a value of 1 in the center (on average), and it typically decreases going toward the edges or corner of the image. Here 'center of the image' means the central $\frac{1}{4}$ in X and Y, hence the central $\frac{1}{16}$ th of the image area.

This arrangement seeks to put signals from all parts of the image on an equivalent basis, namely the signal strength that would have been measured if they were in the center of the field.

While the exact shape of the shading pattern varies per instrument, the overall effect is to increase the signal near the image edges, and to do little or nothing to the signal from the center of the image.

18 Appendix F: Arc-lamp Fluorescence Illuminator User Information

Fluorescence Illuminator Safety

Before using the illuminator, please read all warnings and operating/safety instructions in this User's Manual. Keep this manual in a safe place for future reference.

- Do not use the illuminator for purposes other than its intended use. Doing so could cause damage to the unit and/or personal injury, and may void the warranty.
- Do not expose the illuminator to water, moisture, extreme heat or cold, or open flames.
- Do not allow objects to fall on or liquids to spill on the illuminator.
- Use only the power supply cord set provided with the illuminator. If the correct cord set for the location was not provided, please contact Akoya Biosciences for a replacement. Do not use power supply cords with inadequate ratings.
- Connect the AC power cord only to the designated power sources as marked on the illuminator. Make sure the power cord is located so that it is not subject to damage.
- Always make sure the illuminator is disconnected from power before installing the bulb, connecting components, or cleaning the exterior surfaces.
- Do not in any way attempt to tamper with or alter the illuminator; doing so voids the warranty, and may damage the system. This product does not contain consumer serviceable components other than the replaceable lamp and the fuses. Contact Akoya Biosciences for any other required service or repairs.
- Ensure that the cooling vents in the controller case are not blocked.



Warnings:

- Hg-LAMP CONTAINS MERCURY. Handle, maintain, and dispose of the lamp in accordance with local Disposal Laws.
- Before replacing a fuse, DISCONNECT THE ILLUMINATOR FROM THE POWER SUPPLY.
- Eye damage may result from directly viewing the light produced by the lamp used in the illuminator.
- Always make sure the light guide is properly attached to the illuminator and inserted into the collimator, and that the collimator is firmly attached to the microscope before turning on the power to the unit.
- The illuminator has built-in protection features to avoid unintentional exposure to UV radiation. Do not attempt to defeat the protection features.



Caution:

- Never look into the emitting end of a light guide. The light could severely damage the cornea and retina of the eye if the light is observed directly.

- Appropriate eye shielding must be used at all times; clothing should be used to protect exposed skin.
- Never place the end of an emitting light guide near skin as this may result in burning and damage to the skin.
- Never place the end of an emitting light guide near a flammable substrate, as sufficient power is emitted from the light guide to ignite flammable substances.
- When turned on, the illuminator should be attended at all times by a qualified operator. Do not leave the illumination lamp on and unattended longer than the amount of time required to complete operation.

Setting the FL Illuminator to 10%

If you intend to perform fluorescence imaging, verify the Fluorescence Illuminator is connected to the rear of the Vectra microscope module by a liquid light guide. Verify the illuminator's power cable is connected to a power outlet. Switch the power switch located on the lower left front of the Vectra Fluorescence Illuminator to the ON position. The knob on the front of the illuminator controls how much light from the bulb enters/exits the Liquid Light Guide. Akoya recommends setting the knob on the front of the illuminator to 10% as shown below. Allow 30 minutes for the fluorescence illuminator to reach operational temperature.



Figure 93. Setting the Fluorescence Illuminator to 10%

IMPORTANT: Maintain a minimum of 4 inches (100mm) clearance around the Vectra Fluorescence Illuminator at all times. Equipment and objects should be placed such that the power switch and disconnecting devices are accessible at all times. Although the illuminator will reach 70% output in under 5 minutes, allow 30 minutes for best stability.

WARNING: Do not switch the unit off within 5 minutes of switching it on. Not complying with this warning may result in damage to the bulb. After switching the unit off, allow the bulb to cool for at least 30 minutes before changing the bulb or switching the unit back on. Failure to do so is likely to result in damage to the bulb.

Note: For brightfield imagery, the microscope was "Koehler" aligned during installation and calibration. There should be no need to adjust the condenser.

200 Watt Fluorescence Illuminator

The Fluorescence Illuminator is used for fluorescence epi-illumination. The Fluorescence Illuminator is a 200W module that provides epi-illumination of slides on the Vectra microscope stage. The Fluorescence Illuminator has a manual 6-position shutter, (0, 10, 25, 50, 75, or 100%) using a self-aligning, temperature-controlled 200 Watt metal halide bulb. The liquid light guide transfers the light to the microscope. See [Installing the Bulb](#)^[10] for bulb replacement and operating instructions.



WARNING

- Maintain a minimum of 4" (100mm) clearance around the Fluorescence Illuminator.
- Place equipment such that the power switch and disconnecting devices are accessible at all times.

Power: 100-240VAC, 50/60 Hz, 250 Watts, standard IEC320 connector

Fuses: 5A, 500VAC



Figure 94. 200W Fluorescence Illuminator and liquid light guide with the collimator and adapter attached for the microscope.

Side Connectors

Power Cable Connector - connects to the power source.



Figure 95. 200W Fluorescence Illuminator Power Connector

Arc-lamp versions of the Vectra Fluorescence Illuminator is furnished with a manual 6-position shutter, (0, 10, 25, 50, 75, and 100%). It contains a self-adjusting, temperature controlled 200 Watt metal halide bulb. The bulb is coupled via special optics to the liquid light guide, which transfers the light to the microscope. This appendix applies to the arc-lamp illuminator only, not the LED illuminator.

Specifications

Power	Universal integral power supply: Input 110-240V, 50/60Hz.
Operating environment	Use within ambient temperature range: 65-82 °F (18-28 °C)
Required clearance	4" (100mm) minimum

Liquid Light Guide

The liquid light guide has a limited lifetime, regardless of whether it is in storage or in use. However, the lifetime may vary depending on climate conditions. Cold and humid environments tend to extend lifetime; hot and/or dry environments tend to shorten the lifetime. Even though UV performance does not markedly degrade during usage, it is recommended that the light guide be replaced before its expected lifetime expires. Final degradation is generally caused by the formation of bubbles in the liquid, and optical output may then drop very rapidly.

Liquid light guides have an expected lifetime of 3 years. The suggested replacement interval is every 2-3 years. These figures are based on 73.4 °F (23 °C) and 60% humidity.

If the system is used at ambient temperatures beyond the recommended range, it is likely that bubbles could form inside the liquid. Bubbles may be reabsorbed by the liquid by storing the light guide at room temperature for several days.

Illuminator Hardware Overview

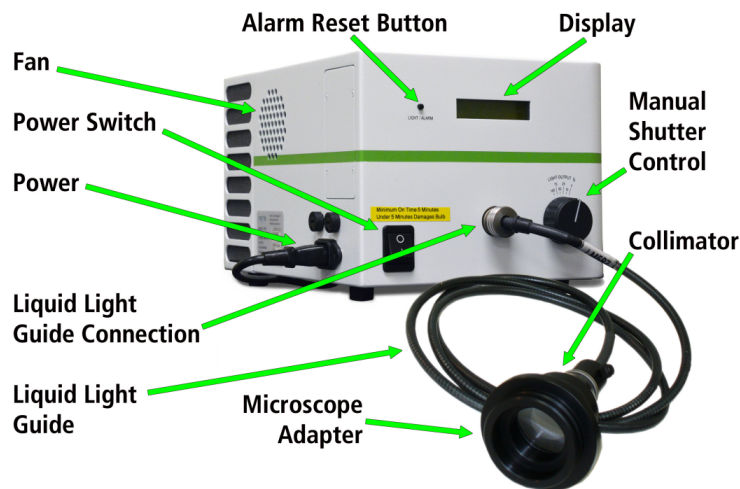


Figure 96. Fluorescence Illuminator

Installing or Replacing the Bulb

Required Equipment:

- Hex Key (supplied with the unit)
- 200 Watt Metal Halide Bulb (P/N 132364)



Warnings:

- Use only the specified 200W Metal Halide bulb. Attempting to install any other bulb may cause damage to the unit.

- **Do not touch the inside of the reflector of the bulb.**
- The bulb is delicate, handle it carefully.

Installation Instructions:

1. Make sure the Illuminator is turned OFF and disconnect the power supply.
2. Wait 30 minutes to allow the bulb to cool after turning the unit off.
3. Lay the Illuminator upside down on a flat, padded surface.
4. Remove the four hex screws and remove the panel, as shown.

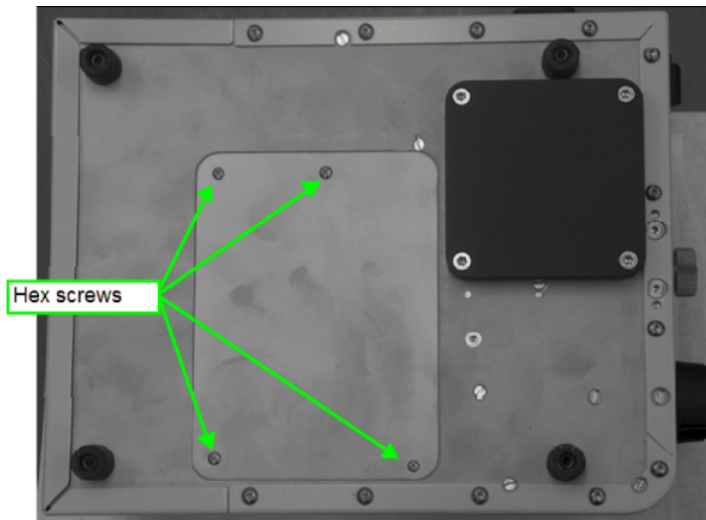


Figure 97. Bulb Housing

5. Carefully remove the bulb from the packaging:
Do not touch the silver surface of the lamp or the PCB on the back of the bulb.
Open the top of the carton, remove the V-shaped cardboard holder, push back the cardboard flaps, and lift the bulb out of the carton.
6. Turn the bulb so that the cables and connector hang down into the opened bulb housing.
7. Plug the brown connector from the bulb into the power socket in the bulb housing. Make sure it is pushed firmly into position.
8. Make sure the bulb is oriented so that the (Hg) label faces upwards. Then place the bottom side of the bulb into the groove in the bottom of the lamp housing.
9. Lift the spring towards the bulb. This pushes the bulb into the correct position. Click the spring all the way into the Lamp Spring Restraints to lock the bulb in place.

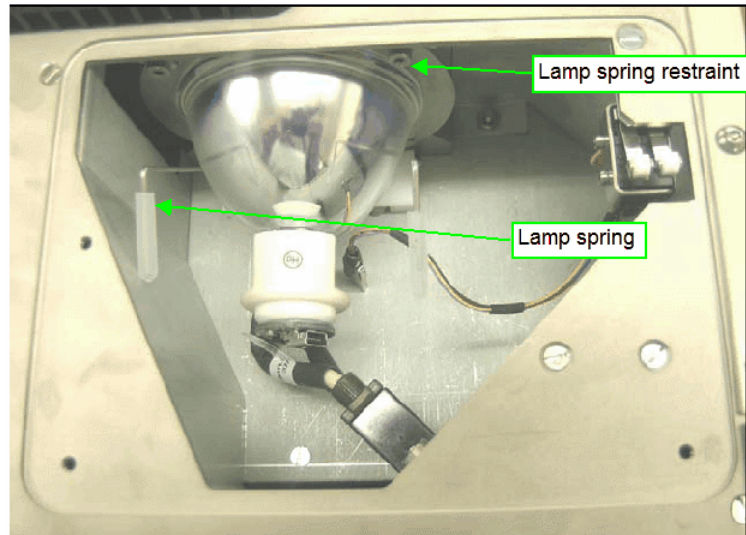


Figure 98. 200W Metal Halide Bulb Installed

10. Slightly rotate the lamp in its holder and set it in the middle of its travel.
11. Plug the data connector into the base of the bulb.

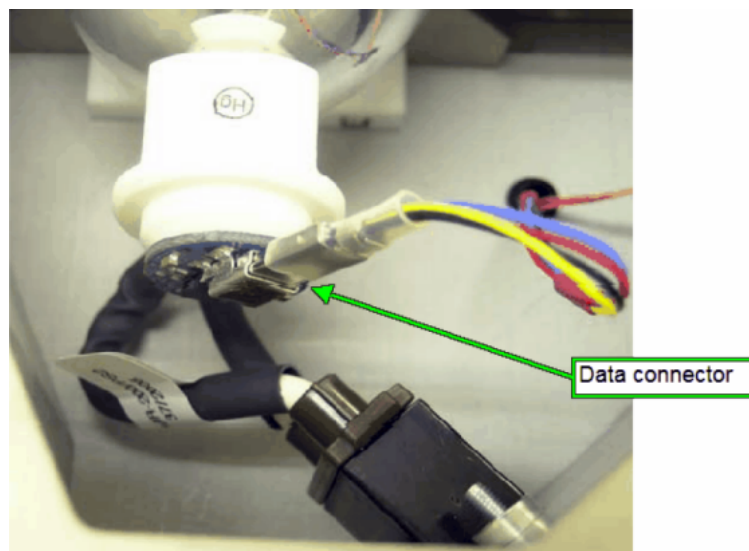


Figure 99. Bulb Data Connection

12. Re-install the bulb housing cover using the four hex screws.

Connecting the Light Guide to the Microscope

1. Position the Fluorescence Illuminator on the bench so that none of the fan vents are obstructed.
2. Unpack the liquid light guide from the foil packaging and remove both plastic caps from the light guide.
3. Unscrew the connector on the front of the Illuminator and fully insert the light guide. (Push the light guide in until it stops.)
4. Tighten the connector until tight to fully secure the light guide to the Illuminator.
5. Loosen the screw on the back of the collimating lens and push the light guide firmly into the hole. Make sure it reaches the end stop, and then tighten the screw.
6. Attach the collimator to the Microscope's fluorescence illumination port.
7. Switch the Illuminator on and adjust the collimator for light evenness:
 - a. Loosen the silver lock ring.
 - b. Unscrew the end of the collimator.
 - c. When light is even, screw the silver lock ring forward, locking the collimator end into place.

Starting the Illuminator



Warnings:

- Make sure that the power switch is in the OFF position when the unit is plugged in. Plugging the unit into a power outlet when the switch is in the ON position can result in damage to the unit.
- Do not power up the Illuminator without the light guide attached to both the Illuminator and the Microscope.
- Only power up the Illuminator when it is installed on a level surface.
- Maintain a minimum of 4" (100mm) clearance around the Fluorescence Illuminator.
- Place equipment such that the power switch and disconnecting devices are accessible at all times.

1. Ensure the light guide is attached to both the Illuminator and the Microscope.
2. Connect the power cable to the Illuminator.
3. Switch the Illuminator power switch ON.
4. Allow 30 minutes for the Illuminator to reach operational temperature.

Warning: Do not switch the unit off within 5 minutes of switching it on. Not complying with this warning may result in lamp damage.

Shutting Off the Illuminator



Warnings: Please note the following, or damage to the bulb could result.

- It is best to leave the lamp on for at least 30 minutes before switching it off. **Never** switch the unit off within 5 minutes of switching it on.
- After switching the unit off, allow the bulb to cool for at least 30 minutes before switching the unit on again or changing the bulb. Failure to do so is likely to result in damage to the bulb.

Switch the power switch to the OFF position.

When to Change the Bulb

The bulb is installed with a timer chip that counts the number of hours the bulb has been turned on. Once the bulb reaches the recommended lifetime of 2000 hours, an audible reminder sounds each time the Illuminator is switched on.

- Replace the bulb, following the instructions in [Installing/Replacing the Bulb](#)¹⁰¹.
- To silence the alarm, press the **Alarm Reset** button located on the left of the display panel on the front of the Illuminator.

Routine Maintenance

The internal dust filters require cleaning every 12 months of use.

1. Make sure the Illuminator is turned OFF and disconnect the power supply. (Wait 30 minutes to allow the bulb to cool after turning the unit off.)
2. Remove the three screws that fasten the rear cover.
3. Lift the rear cover off.
4. Remove the filters.
5. Wash the filters in warm soapy water.
6. Rinse the filters in water and allow them to dry.
7. Reinstall the filters.
8. Reinstall the rear cover ensuring the isolator switches are beneath the cover.
9. Replace and tighten the screws.

Alarms and Warnings

This table lists the warning that might appear on the Illuminator display. The table also gives the recommended solution for each warning.

Message	Alarm Reason	Quiet Alarm	Actions
Bulb Fault 1	Software not recognizing hour count from bulb.	No	Switch off unit. Check bulb for damage and check connections.
Bulb Fault 2	Bulb failure	Yes. Hold Alarm Reset button for 5-10 seconds. Reset to clear alarm.	Switch off unit. Check bulb for damage and check connections.
Over Temp Fault	Bulb over temperature	Yes. Hold Alarm Reset button for 5-10 seconds. Reset to clear alarm.	Check vents at rear of unit. Make sure they are not blocked or covered.
CHANGE BULB	Bulb reached 2000 hour life limit.	Yes. Hold Alarm Reset button for 5-10 seconds.	Change bulb.

19 Appendix G: Epi-Filter Wedge Correction

Introduction

Epi-filters can produce image shifts when filter wedge artifacts bend transmitted light. Even so-called “zero shift” filters can produce subpixel amounts of image shift that may impair image quantification and spectral unmixing.

The Wedge Calculation and Correction program allows you to measure these residual wedge-related image shifts from imagery of single-stained samples then apply the measured shifts as correction offsets to remove image shift from other MSI imagery.

Note that wedge correction offsets are automatically applied at the time of acquisition on the Vectra Polaris, and can optionally be applied at the time of acquisition on Vectra (see [Section 10](#)⁷⁵ for instructions). If you attempt to apply wedge corrections to an image that is already corrected (at acquisition or via the Wedge Calculation and Correction program), the Wedge Calculation and Correction program will apply the net difference between the original correction and the new correction.

Calculate and Apply Epi-Filter Wedge Offsets

Before computing wedge correction offsets, MSI images must be acquired using a set of single-stained samples (see [Section 5.6](#)⁴⁴ or Vectra Polaris Fluorescence Protocols section for acquisition instructions). All images in this set should be acquired using the same filters, in the same order. For best results, the signal bands of these single-stained samples should span all filters to be corrected (see [Appendix D](#)⁹⁵ or Vectra Polaris Fluorescence Protocols section for signal bands of Opal fluorophores). Autofluorescence samples can be included as well.

To measure and apply wedge correction offsets from single-stained images, launch the Wedge Calculation and Correction program located in the **WedgeCorrectionApp** folder within the Vectra installation folder.

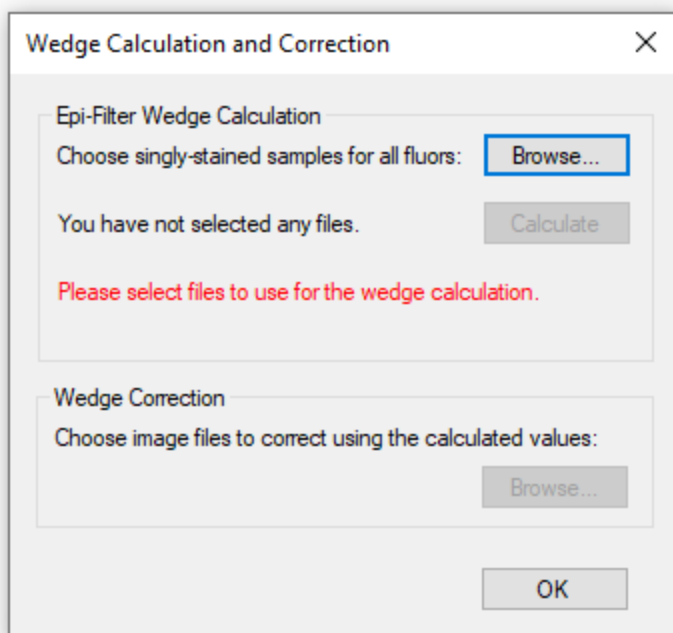


Figure 100. Wedge Calculation and Correction Program

- **Choose single-stained images:** To select the single-stained images to be used for calculation of wedge offsets, click the **Browse...** button under the **Epi-Filter Wedge Calculation** heading. Select all imagery corresponding to the single-stained samples. This will activate the **Calculate** button.
- **Calculate offsets:** To measure the wedge correction offsets, click the **Calculate** button. After the calculation is complete, the measured offsets will be displayed in a separate window. Click **OK** to continue.
- **Apply wedge correction offsets:** To apply the calculated offsets to previously acquired MSI images, click the **Browse...** button under the **Wedge Correction** heading and select one or more MSI files. The original files will be replaced with corrected imagery. A backup of the original, non-corrected image will be saved in the same folder with the suffix “_orig”. When all corrections are complete, a separate window will display a list of the new file names. Click **OK** to close this window.

To correct files from another folder, click the second **Browse...** again. To close the program, click **OK**.

20 Appendix H: Software EULA

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Index

- 1 -

100W Tungsten Halogen Lamp 65

- A -

Acquire References 22, 25
Acquiring Sample Fluorescent Regions 44
Acquiring Sample MSI Regions 31
Adjusting Microscope Eyepieces 71
Advanced Automated Workflows 58
Autofocus Band 31
Automated MSI Field Selection 47
Automated MSI Region Selection 58

- B -

Bands 78
Bar Codes 48, 53
Brightfield Reference Images 22
Bulb Fault Alarm 106
Bulb Installation/Replacement 101
BX Motor Controller Handset 66

- C -

CE Compliance 5
Change Bulb Alarm 106
Check Dashboard 18
Cleaning 72
COM Ports 79
Compensation Calculations
 Brightfield 96
 Fluorescence 96
Computer 70
Creating and Editing Studies and Protocols 27
Creating Brightfield Studies 28
Creating Fluorescence Protocols 33
Customer Care Center 5

- D -

Dimensions
 Slides 86
Disclaimers
 design change 5
 reproduction 5

- E -

Edit Protocol 19
Environmental requirements 73
Epi-Filters 76
 Wedge Correction 107
Errors 83
EULA 109
Exposure Times
 MSI Regions 42
 Whole Slide 33
Eyepiece Adjustment 71

- F -

Filter Cube Recommendations 95
Fluorescence Illuminator 97, 98, 99
 Safety 97
Fluorescence Reference Images 25
Fluorescent Regions
 Acquiring 44
Focus Failed Errors 83
Frequently Asked Questions 81

- G -

Gear Menu 19

- H -

Handset 66
Hardware
 Descriptions 60
 Overview and Adjustments 60
 Setup 75
Hardware Setup Program
 Bands 78

Hardware Setup Program	
COM Ports	79
Epi-Filters	76
Objectives	75
Home Page	18

- I -

Illuminator	
Alarms	106
Routine Maintenance	105
Shutting Off	105
Starting	104
When to Change Bulb	105
Illuminator Hardware Overview	101
inForm Algorithm	56

- J -

Joystick	63
----------	----

- L -

Launch Phenochart	19
Liquid Light Guide	101
Connecting	104
Loading Slides	15
200-Slide	15
6-Slide	17

- M -

Marker	84
Microscope	61
Microscope Eyepiece Adjustment	71
Microscope Module Controller Box	67
Microscope Stage	61
Motor Controller Handset	66
MSI Fields	
Automated Selection	47
Selecting for Acquisition	56
Selecting with Phenochart	56
Selecting with Vectra	56
Typical Acquisition Time	94
MSI Regions	
Acquiring	31

Automated Selection	58
Setting Exposure Times	42

- N -

Nominal Focus	20
---------------	----

- O -

Objectives	75
Olympus BX-51WI Microscope	61
Opal Reagents, Filter Cube	95
Operator Safety	6
Optical Path Switch	61
Outline Sample	84
Over Temp Fault	106
Overview	8

- P -

PerkinElmer Technical Support	5
Phenochart	
Selecting MSI Fields	56
Pixel Resolution	28, 33
Preface	5
Process Slides	
200-Slide	53
6-Slide	48
Protocol	
Selecting 200-Slide	53
Selecting 6-Slide	48
Protocols	
Brightfield	28
Fluorescence	33

- R -

Reference Images	22
Brightfield	22
Fluorescence	25
Registration Issues	83

- S -

Safety symbols	6
Sample Finding	84

Saturation Protection	31
Scan Regions	86
Scan Slides	19
200-Slide	53
6-Slide	48
Selecting MSI Fields	56
Set Nominal Focus	20
Settings	19
Site requirements	73
Six Slide Stage	65
Slide IDs	48, 53
Slide Loader	64
Slides, Supported	86
Software License Agreement	109
Specifications	73, 100
Spectral Library	
Fluorescent Regions	44
MSI Regions	31
Stage	
6-Slide	65
Stage Control Joystick	63
Stage Controller Module	69
Stage Focus Motor	66
Starting	
Vectra Software	14
Vectra System	12
Studies	
Brightfield	28
Fluorescence	33
Supported Slide Formats and Dimensions	86
Symbols	6
System Dashboard	20
System Specifications	73

- T -

Technical Support	5
TIFF Specifcation	88
Times, Typical Scan and Acquisition	94
TMA Cores	
Typical Acquisition Time	94
Trinocular	61
Troubleshooting	83
Turning on the system	12
Typical Scan and MSI Field Acquisition Times	94

- U -

User's manual, about	5
----------------------	---

- V -

Vectra	
Selecting MSI Fields using	56
Vectra 200 Slide Loader	64
Vectra Applications	8
Vectra Home Page	18
Vectra Imaging Module	60

- W -

Warning symbol	6
Whole Slide Scan	48
200-Slide	53
6-Slide	48
Typical Acquisition Time	94
Widescreen Monitor	70