Vectra Polaris™
User Manual

PerkinElmer®
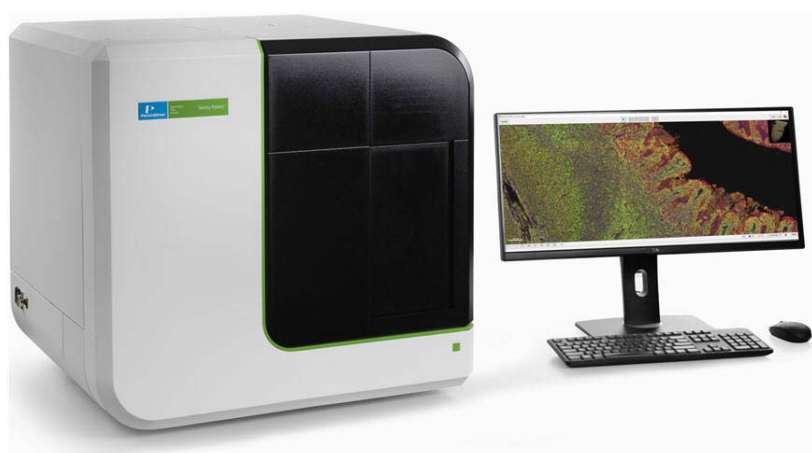
Preface

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US Patents 5,892,612; 5,953,087; 7,655,898; and patents pending.

Content

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Proper Equipment Operation

WARNINGS

- *To reduce the risk of electric shock, do not remove the instrument panels. No user serviceable parts are inside. Refer to qualified service personnel if help is required.*
- *Use this product only in the manner described in this manual. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.*

AVERTISSEMENTS

- *Pour réduire le risque de choc électrique, ne pas retirer le couvercle. Ce produit ne contient aucune pièce pouvant être réparée par l'utilisateur. Au besoin, confier l'appareil à un réparateur qualifié.*
- *Ce produit ne doit être utilisé que comme décrit dans ce manuel. Si cet appareil est utilisé d'une manière autre que celle spécifiée par le fabricant, la protection fournie par l'appareil peut être entravée.*

Contact Us

If you have a question about a product that is not answered in this manual or online Help, or if you need assistance regarding this product, please contact the PerkinElmer Technical Support Center from 8:00 A.M. to 8:00 P.M., Eastern Time, Monday through Friday:

Phone: (US Toll Free): **800-762-4000**
            (Worldwide): **+1 203-925-4602**

Fax: **+1 203-944-4904**

Email: global.techsupport@perkinelmer.com
Internet: www.perkinelmer.com

Before you call, have the following information available for the technical representative:

- Product serial number
- Software version (found by choosing About from the main Help menu)
- If applicable, the *error number* shown on the product's LCD display, in the software, or in the log file.

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CE

This device complies with all CE rules and requirements.

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.

Table of Symbols

Table 1 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.

Table 1. Important Symbols

Symbol Symbole	Description Description
	DANGER: An imminently hazardous situation, which, if not avoided, will result in death or serious injury. **DANGER:** Situation présentant un danger imminent qui, s'il n'est pas éliminé, peut entraîner des blessures graves, voire la mort.
	WARNING: Caution, risk of danger. Refer to the User's documentation. **AVERTISSEMENT:** Attention, danger potentiel. Se reporter à la documentation de l'utilisateur.
	NOTE: A cautionary statement; an operating tip or maintenance suggestion; may result in instrument damage if not followed. **REMARQUE:** Énoncé indiquant une précaution à prendre, un conseil de fonctionnement ou une suggestion d'entretien; son non-respect peut provoquer des dommages à l'instrument.
	Hazardous voltage; risk of shock injury. Tension dangereuse; risque de blessure par électrocution.

Table 1. Important Symbols (Continued)

Symbol Symbole	Description Description
	Crush hazard. Risk of body parts, hair, jewelry, or clothing getting caught in a moving part. Danger d'écrasement. Faire attention que les parties corporelles, les cheveux, les bijoux ou les vêtements ne soient pas pris dans une pièce mobile.
	Risk of fire. Risque d'incendie.
	Risk of explosion. Risque d'explosion.
	Lifting hazard. May result in injury. Levage dangereux. Peut entraîner des blessures.
	Protective ground symbol. Symbole de terre de protection.
	Ground symbol. Symbole de terre.
	Fuse. Fusible.
	Alternating current. Courant alternatif.
	On (supply). Marche (alimentation).
	Off (supply). Arrêt (alimentation).
CE	CE compliance mark. Marque de conformité CE.
	WEEE symbol. Do not dispose of as unsorted municipal waste. See the PerkinElmer website (www.perkinelmer.com) for more information.
HI-POT	Signifies that the unit has passed safety tests for grounding, power line transience, and current leakage. Signifie que l'appareil a réussi les tests de sécurité pour la mise à la terre, le courant transitoire de ligne d'alimentation et la perte de courant.
	Input. Entrée.

Table 1. Important Symbols (Continued)

Symbol Symbole	Description Description
⊖→	Output. Sortie.
Equipment labels are color coded: Les étiquettes de l'appareil sont codées couleur:	**Yellow** Caution, risk of danger **Red** Stop **Blue** Mandatory action **Green** Safe condition or information **Jaune** Attention, danger potentiel **Rouge** Arrêter **Bleu** Intervention obligatoire **Vert** Condition sûre ou informations de sécurité

Instrument Safety

The following safety information about the Vectra Polaris is included in this documentation. Read and review all safety information before operating the Vectra Polaris.

Required Training

Ensure that all personnel involved with the operation of the instrument have:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.

WARNING

Use this product only in the manner described in this manual. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Electrical Safety

The Vectra Polaris is powered by a 100-120VAC/200-240VAC, 50-60Hz (±10%) input power supply.

The wall outlet or the power cable connector on the left side of the instrument should be accessible after the system's installation, to enable trained service personnel to safely disconnect power from the system during servicing.

WARNING

Do not operate the system in an environment with explosive or flammable gases.

WARNING

- *DO NOT remove instrument covers. There are no user serviceable parts inside. The covers are intended to be removed only by qualified PerkinElmer service personnel; they are not intended to be removed during operation or for maintenance by users. Contact PerkinElmer technical support if help is required (see page 3).*
- *Do not operate the system if there has been a malfunction of the system door or slide loading components. Contact PerkinElmer technical support if help is required (see page 3).*
- *Do not operate the system in places where it may be splashed with liquid.*

Power Cord Selection

Contact PerkinElmer Technical Support (see page 3) to order replacement power cords.

WARNING

- *Use only the power supply cord set provided with the Vectra Polaris system. If the correct cord set for the location was not provided, contact PerkinElmer Technical Support (see page 3) for a replacement. Do not use power supply cords with inadequate ratings.*
- *Use only a properly grounded power outlet when connecting the system to power.*

Fuses

Contact PerkinElmer Technical Support (see page 3) to order replacement fuses.

WARNING

Do not attempt to replace the fuses. Only qualified PerkinElmer service personnel can replace the fuses.

Cables and Adapters

Some cables and adapters supplied with the system have proprietary specifications.

WARNING

Do not connect components supplied by PerkinElmer using unqualified cables or adapters. Contact PerkinElmer technical support (see page 3) to order replacement cables and adapters.

Mechanical Safety

WARNING

Instrument components may move during operation. Always keep body parts, hair, jewelry, and clothing away from the instrument during operation.

Procedures which could result in injury may be performed only by operators who have been warned of the potential hazards and have received adequate training in performing the procedures in the safest possible manner.

System Safety

WARNING

Lifting Hazard. Do not move the Vectra Polaris instrument. Installing, servicing, and moving the Vectra Polaris instrument should be performed only by qualified PerkinElmer service personnel. Contact PerkinElmer technical support if help is required (see page 3).

Eye Safety

WARNING

⚠️

Bright Light Hazard. The interior of the Vectra Polaris system includes a barcode reader with an LED Light. To prevent eye injury, do not stare directly into the light source in the interior of the instrument.

Table of Contents

Introduction

This manual describes the use and functionality of the Vectra Polaris Automated Quantitative Pathology Imaging System. It includes operating instructions, functional descriptions, troubleshooting, illustrations, and other relevant information.

This section of the manual contains the following topics:

- Intended Use
- Principles of Operation
- Theory of Imaging
- Whole Slide Scanning
- Multispectral Imaging

Intended Use

The Vectra Polaris is a multimodal digital pathology instrument that integrates both multispectral analysis and automated slide scanning that allows researchers to visualize, analyze, quantify and phenotype immune cells in situ in FFPE tissue sections and TMAs.

This fully automated, high throughput multispectral imaging system is combined with true brightfield and fluorescent whole slide scanning functionality. These whole slide scans are fast and efficient, comparable to standalone systems. Vectra Polaris also allows for on-the-fly, continuous slide loading to support the demands of high throughput research studies.

NOTE

[!]

PerkinElmer's Vectra Polaris Quantitative Pathology Imaging System is for research use only. Not for use in diagnostic procedures.

Principles of Operation

PerkinElmer's Vectra Polaris is an automated imaging system for performing whole slide scans of tissue sections and microarrays (TMAs), and for acquiring multispectral (MSI) regions of interest. The system has been optimized to image samples stained with PerkinElmer's Opal multiplexed fluorescent immunohistochemistry reagent kits and is also compatible with typical brightfield staining reagents.

The Vectra Polaris is configured to store and inventory up to 20 slide carriers, each holding up to 4 tissue slides, for a total of 80 slides.

Figure 1. The Vectra Polaris System

The Vectra Polaris has been designed to expand and support the workflow defined in PerkinElmer's Vectra 3:

- True whole slide scanning of slides at 1.0 um/pix, 0.5 um/pix, and 0.25um/pix
- Review and annotation of whole slides scans for MSI acquisition
- Acquisition of MSI regions
- Analysis of MSI regions including protein expression and phenotyping

Principles of Operation (Continued)

To implement the full capabilities of the instrument and workflow, the Vectra Polaris system includes the following PerkinElmer software:

- **Vectra Polaris**: Operator-centric software for performing whole slide scans and acquiring MSI regions of interest. The Vectra Polaris software runs on the workstation connected to the Vectra Polaris instrument.

- **Phenochart**: Whole-slide viewer and annotator of fluorescent and brightfield scans acquired by the Vectra Polaris. Phenochart allows the user 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. Phenochart is freely distributed and can be used by multiple users who wish to view or review slide scans taken by the Vectra Polaris.

- **inForm Tissue Finder**: Software typically used for the analysis of MSI images. inForm supports features such as tissue classification and training, cell phenotyping, protein expression measurements, and data export. It can be run on the Vectra Polaris computer and other Microsoft Windows 10 computers. Additional inForm software "seats" beyond the two seats that come with each Vectra Polaris system are available for purchase.

Vectra Polaris 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 user or the Vectra Polaris itself. An example fluorescence workflow might include the following steps:

1   Stain tissue with PerkinElmer Opal fluorescent IHC reagents.

2   Acquire whole slide fluorescent imagery using the Vectra Polaris.

3   Review the whole slide imagery with Phenochart and annotate regions of interest for MSI analysis.

4   Acquire the MSI regions with Vectra Polaris.

5   Use inForm to phenotype cells and measure protein expression levels in the acquired MSI regions.

Example Applications

Some examples of Vectra Polaris applications include:

- Whole slide scanning and multispectral interrogation of tissue samples and microarrays stained with Opal reagent kits
- Whole slide scanning of tissue samples stained with H&E and conventional IHC stains
- Phenotypic analysis and protein expression of immune and cancer cells in the context of the tumor microenvironment.

Theory of Imaging

This section introduces some important concepts used by PerkinElmer's Vectra Polaris imaging systems, including:

- Light
- Human Perception of Light Intensity and Color
- Light Absorbance and Reflection
- Fluorescence
- Multispectral Imaging

Light

For purposes of this discussion, light refers to the part of the electromagnetic spectrum that can be seen by the human eye and the nearby ultraviolet and infrared wavelengths. While the physical description of light can be highly complex, we will focus on these wavelengths of light and how they interact with physical and biological materials.
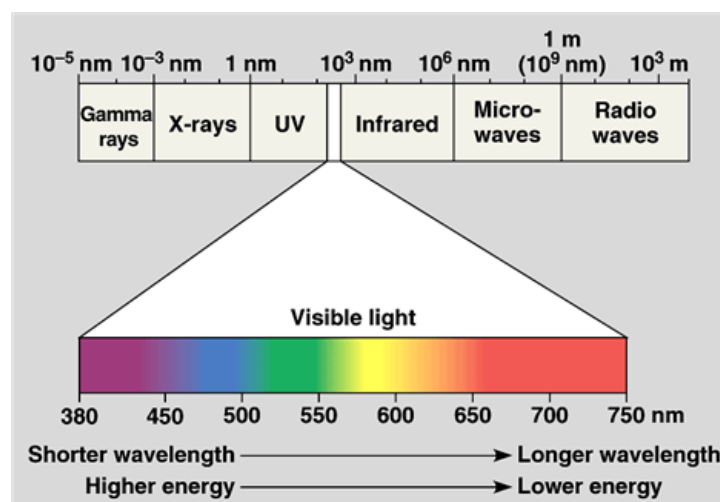
Figure 2. The Electromagnetic Spectrum

Human Perception of Light Intensity and Color

Response to Illumination

The human eye is a highly adaptive light detector. It is significantly more sensitive in low light than in bright light. When light levels change, it takes some time for the eye to fully adjust. This is the reason people need to "dark adapt" in a darkened room before they are able to observe weak fluorescence through a microscope.

Humans can see both in very dark and very bright settings. Because the eye is so adaptable to various lighting conditions, humans are unable to quantify absolute levels of light. In any given situation, the eye has a limited ability to discriminate levels of illumination. US Department of Defense research indicates that most humans can only distinguish approximately 30 to 35 levels of gray, ranging from black to white.

The eye's response to illumination is not linear. It more closely approximates a logarithmic function. The result is that the human eye cannot see small proportional changes in brightness.

Contrasting the eye with conventional microscope imagery, any sensor that has 8-bit resolution can detect 256 levels of gray. As the number of bits of resolution increase, the number of gray levels also increases. A 12-bit sensor yields 4096 levels of gray. Digital electronic sensors are linear in response to light levels.

Ability to Distinguish Colors

While the eye is relatively poor at discriminating intensity, it is very good at distinguishing colors. Most individuals are able to discriminate thousands of colors. However, no two individuals see a given color in exactly the same way. The eye contains three different types of color sensors, also known as cone cells. While the arrangement of cone cells is generally standard from person to person, the ratio of each type of cone cell varies, as does their actual physical arrangement within the eye. These minor variations (along with the brain's interpretation of the color) lead to the differences in perceived color between individuals.

Light Absorbance and Reflection

We perceive objects based on the way they transmit, absorb, and/or reflect light.

Absorbance and **reflection** work in tandem. **Absorbance** refers to the wavelengths of light that are 'taken in by' the objects. This means that an object that we perceive as red has absorbed all visible wavelengths of light except red. The red wavelengths are **reflected** back to the eye of the observer.

Transmittance refers to light emitting objects such as light sources, and fluorescing or phosphorescing objects. An object we would perceive as red in transmission is one that transmits primarily red wavelengths, while absorbing or reflecting other wavelengths.

In brightfield light microscopy we observe light that passes through a specimen. Except for a few pigments and inclusions, biological specimens are essentially invisible. To impart contrast, we employ some absorbing dye, or specific optical arrangement. It is this need for contrast that led to the initial development of biological stains and stain protocols and subsequently to phase contrast and other optical contrast enhancing techniques.

Optical Density (OD) is used to measure the interaction of light with absorbing materials. The science of absorbing spectroscopy is based on the Beer-Lambert law. When absorbing images collected in brightfield are converted to OD images, the information contained in each pixel is quantitative, as to the amount of absorbing material present. PerkinElmer's brightfield multispectral imagery is automatically converted to optical density at acquisition time, enabling quantitative analysis.

Fluorescence

Many biological and natural materials give off light of a particular color when exposed to light of another color. This property is a type of luminescence. There are two types of **luminescence**:

- **Fluorescence** refers to luminescence that occurs when the light is emitted rapidly after illumination (around one-millionth of a second).

- If the light emission takes longer than one-millionth of a second, the luminescence is called **phosphorescence**.

Materials that exhibit fluorescence have proven extremely useful as labels or indicators in many biological systems.

Fluorescence light emission is different than light absorption. Each fluorescent molecule generates light. Fluorescent light can be measured quantitatively because it does not interact with other materials. While it would seem that fluorescence could be measured more accurately than absorbed light, there are a number of factors that complicate such measurements. For example, light scatters, it is affected by the local environment (such as pH), and the measurements can be affected by surrounding molecules.

Stokes Shift

When you excite a specimen with a particular (shorter) wavelength (such as blue), the specimen then shines in a different (longer) wavelength (such as red, orange, or yellow). The difference between the wavelength of the (shorter) exciting light and the wavelength of the (longer) emitted light is called the **Stokes Shift**, which is based on **Stokes Law.**

The wavelength or color you use to excite the specimen (i.e. the 'excitation light') and the color the specimen glows (i.e. the 'emission light') depend on the dye involved. For any given fluorescence dye, there will be a range of excitation wavelengths that will excite fluorescence. This range of excitation wavelengths is known as the absorption spectrum. Each dye also emits across a range of wavelengths, known as the emission spectrum. The figure below contains an example of excitation and emission spectra, showing Stokes Shift and the overlap of the spectra.
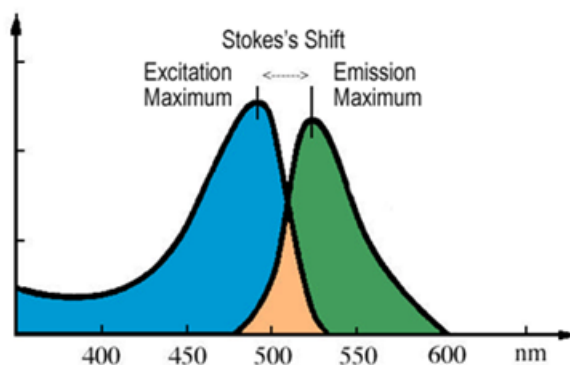
Figure 3. Stokes Shift

In addition, many biological materials are naturally fluorescent. This is known as autofluorescence. In particular, many vitamins, some hormones, and a variety of biological enzymes and structural proteins are naturally fluorescent. These materials often fluoresce strongly enough to interfere with specific fluorescence labeling studies.

Photobleaching

Because dyes can be damaged by intense light, reducing the emission signal ('photobleaching'), it is important to limit the time they are exposed to excitation light or to bright light during routine handling. Usually, blue or UV light is the most damaging. The Vectra Polaris uses an electronically-gated excitation source synchronized with its camera so the sample is only exposed to light while the camera is taking an image. Also, the Polaris front door is made of a translucent plastic that absorbs harsh blue and UV light.

When using Vectra Polaris, avoid spending a long time in the Protocol Exposures Editor while fixed on any one spot of the sample, since it takes a live image stream.

These steps enable repeated measurements with minimal effect on the sample.

Filter Sets for Conventional vs. Multispectral Imaging

Filters used for conventional fluorescence imaging are often designed so they only transmit a very narrow range of wavelengths of light. Limiting the measurement to wavelength bands where the dye is inherently most responsive helps distinguish the desired dye from other dyes or background signals in the sample. In this way, it is possible to image several dyes, provided that their spectra are separated rather than overlapping. Based on the properties of common dyes, this puts an upper limit of ~4 on how many dyes can be imaged in any one sample.

An alternative approach is to image the sample multispectrally. In this case, a broad emission filter is used, and a tunable filter is engaged in the imaging path. The instrument takes pictures at several wavelengths within the emission band, so maps out the full shape of the dye response. This enables analysis software to identify what dye(s) are present, in what amounts, in each pixel, by spectral decomposition ("unmixing"). It also enables identifying, and removing, contributions from autofluorescence.

These two approaches are illustrated in Figure 4 and Figure 5.
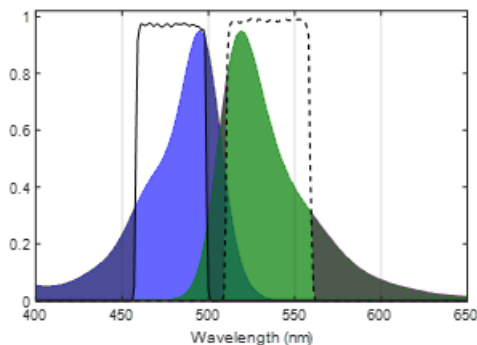
Figure 4. Narrowband excitation (solid line) and emission (dashed line) filter for conventional imaging.
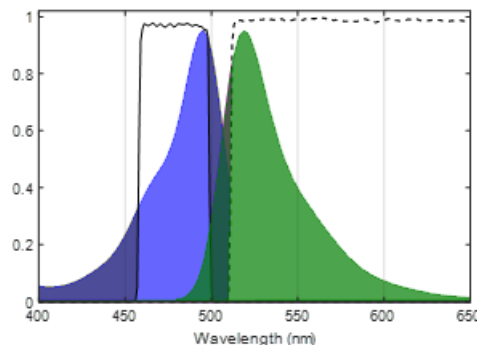
Figure 5. Narrowband excitation (solid line) and long-pass emission filter (dashed line) for multispectral imaging.

Ideally, the excitation filter would match the excitation maximum of the fluorescence label being used, and the emission filter would include the emission maximum. In practical terms, the filter maxima may be slightly different from the ideal case, due to limitations of filter manufacturing and because for many dyes the Stokes shift is small, so the maxima are quite close to one another.

To image multiple dyes conventionally, one selects dyes that have very distinct excitation and/or emission response, and selects filters that are narrow enough to predominantly transmit the signal of only one dye at a time (Figure 6 and Figure 7).
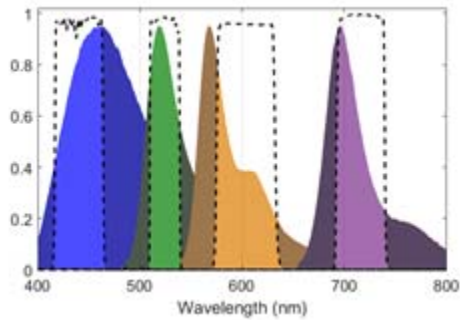
Figure 6. Imaging four dyes by conventional fluorescence methods.
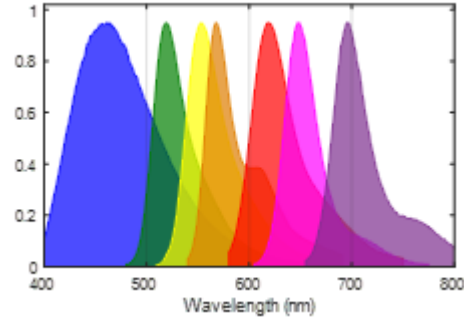
Figure 7. Seven dyes is too many to image by conventional methods.

This shows the emission spectra for four dyes (DAPI, FITC, Cy3, and Cy5.5) that are spectrally fairly distinct, and can be separated by conventional imaging. This works because there is only a little overlap between adjacent dyes. Excitation filters and dye response are omitted for clarity, but are similarly separable.

This approach breaks down when more dyes are present, or when it is important to account for the effects of autofluorescence. Figure 4b shows the spectra of 7 dyes, and with this many dyes there is no way to isolate their signals using conventional imaging techniques, or to account for autofluorescence.

Multispectral Analysis

The Vectra Polaris imaging system offers a unique solution to the problem of separating the signals from highly multiplexed samples. Multispectral analysis is based on the fact that all fluorescent materials produce a unique spectral emission. If you excite a material and examine the emitted fluorescence over a range of wavelengths, the resulting emission intensities can generate an "emission spectrum". This spectrum is different for each specific fluorescent material. For many fluorescent labels of biological interest, the emission spectra overlap, and may be further obscured by autofluorescence from the specimen. Multispectral imaging provides a way to distinguish between many overlapping emission spectra within the same area, overcoming the limitations of conventional filter-based imaging. With the additional information provided by the LCTF during MSI imaging the system can distinguish between dyes with fully overlapping spectra within a single channel ().
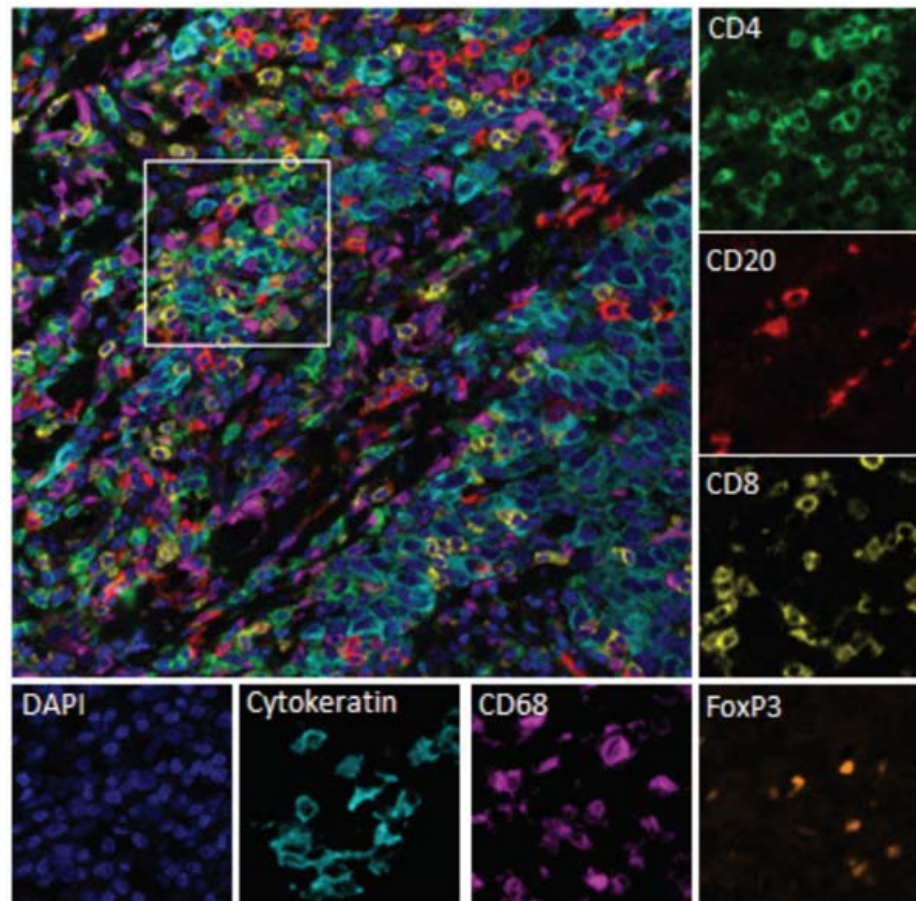
Figure 8. Unmixed multispectral image of human breast cancer tissue stained against CD4 (Green), CD20 (Red), CD8 (Yellow), FoxP3 (Orange), CD68 (Purple), Cytokeratin (Light Blue), and DAPI (Dark Blue) using Opal reagents.

In general, multispectral analysis generates the spectral curves for the various fluorescent dyes or materials in a specimen. In addition, it generates a spectral curve for the autofluorescence that almost always is present to some degree. Using spectral analysis algorithms, the contribution of the individual fluorescence spectra are separated. The result is a set of images representing each spectrum that contributes to the final image 90 (Figure 9).
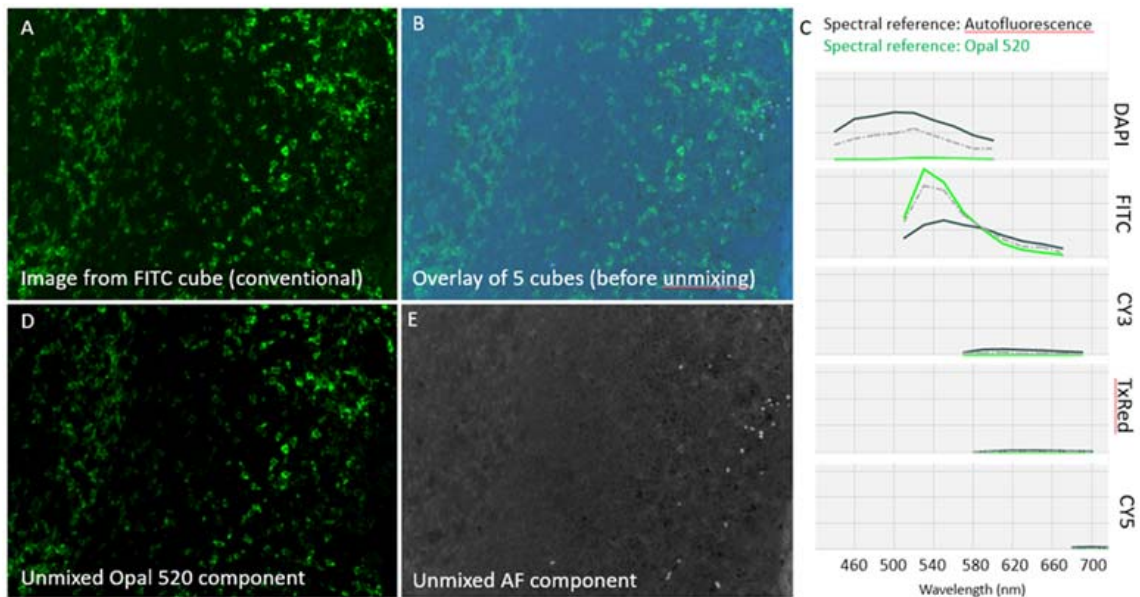
Figure 9. Removing auto-fluorescence with multispectral imaging. A) Conventional fluorescence image of tonsil tissue stained against CD4 with Opal 520 dye. B) Overlay of all 35 layers of a multispectral image acquired using five filter cubes. C) Emission spectra of pure autofluorescence (black line), pure Opal 520 (green line), and the mixture observed in 'B' (gray dashed line). The spectral references are used to 'unmix' the contribution of autofluorescence and Opal 520 at each pixel. D) Image of unmixed Opal 520 signal extracted from 'B' with > 10-fold higher signal-to-background compared to 'A' because the autofluorescence contribution has been separated into the component image in 'E'.

Whole Slide Scanning

Vectra Polaris scans slides using the following process:

1 Color Overview:

Vectra Polaris takes a low power color overview of all four slides in each carrier, including the label for each slide. This initial step is performed regardless of whether you are using a fluorescence or brightfield protocol and is used to identify the presence of slides in the carrier and capture their labels.

2 Coverslip Finding:

For each slide, Vectra Polaris will then find the coverslip using this overview scan. The coverslip defines the potential scan area.

For fluorescence protocols, you can further restrict this area by making a closed loop with a red, green, or blue Sharpie® marker. If closed-loop markup is present, the system will only scan within the loop. This is useful if your tissue is faintly stained, punctate, or if you have highly fluorescing non-tissue material (PAP pen, for example.)

For brightfield protocols, the coverslip will define the potential scan area. Closed-loop markup is not available for brightfield scans.

3 Slide Height Finding:

Vectra Polaris engages specialized height-sensing optics to measure the top of the coverslip at up to 9 locations. This gives an initial focus estimate based on the expected coverslip thickness.

4 Fluorescence Overview (Fluorescence protocols only):

If you are using a fluorescence protocol for this slide, Vectra Polaris will take another overview within the coverslip (or closed-loop markup), this time in fluorescence.

5 Tissue Finding:

Using the corresponding overview, Vectra Polaris will automatically detect the sample on the slide. The resulting area will be scanned. If requested within the protocol, Vectra Polaris will scan the entire area within the coverslip (or closed-loop markup).

6   Focus Finding:

Vectra Polaris will measure focus at multiple points on the tissue to determine best focus. It uses the sample map from the previous step to choose the measurement grid, and continues until the grid is fully measured.

If the measured tissue height is irregular, Vectra Polaris increases the grid density and takes more readings until it finds the readings are regular at the newly finer scale.

All focus measurements include a dust-rejection algorithm, and an overall consistency check is applied as well, to further reduce the likelihood of dust-induced focus errors.

7   Scanning:

Vectra Polaris then scans the slide.

Brightfield scans are conventional color scans that have been color and background corrected.

Fluorescence scans are multi-layered, with one layer for each filter you chose. To avoid photobleaching, the system uses a pulsed LED so the sample is only exposed to light during the time that the camera is taking a picture.

When scans are complete, they can be opened in Phenochart, the whole slide viewing application.

NOTES

!

Some dyes narrowly express in a single filter. Other dyes may express in multiple filters and may appear in more than one layer in a Vectra Polaris fluorescence scan. For example, Opal 570 will have signal in both Cy3 and TexasRed filters. If your sample is highly multiplexed, multiple dyes may appear in the same channel.

Multispectral Imaging

Once Vectra Polaris has completed a whole slide scan of the tissue, individual regions can be selected for multispectral imaging.

Multispectral imagery is acquired using the following process:

1   Selection of Multispectral Regions:

Regions for multispectral imaging are selected on a previously scanned slide. Using Phenochart, you can select individual fields or regions of interest. If desired, you can also select fields using a trained inForm algorithm. See the Phenochart documentation for more information.

When you configure that slide for MSI acquisition, Vectra Polaris will perform the following actions:

2   Color Overview:

Vectra Polaris takes a low power color overview of all four slides in each carrier, including the label for each slide. This initial step is performed regardless of whether you are using a fluorescence or brightfield protocol and is used to identify the presence of slides in the carrier.

3   Slide Registration:

Using the above overview along with the slide's original overview, Vectra Polaris accounts for any shift or rotation of the slide to ensure that the multispectral region locations are accurate. The slide edges are used to account for any rotation or horizontal shift. The coverslip edges to account for vertical shifting.

4   Slide Height Finding:

Vectra Polaris engages specialized height-sensing optics to measure the top of the coverslip at up to 9 locations. This gives an initial focus estimate based on the expected coverslip thickness.

5   Acquisition of Multispectral Regions:

Vectra Polaris will then travel to each multispectral region site, autofocus, correct for exposure if requested, and acquire the multispectral image.

Multispectral imagery can then be viewed and analyzed in inForm.

Hardware Reference

This section identifies and describes the Vectra Polaris system hardware. It also lists the Vectra Polaris technical specifications.

WARNING

Lifting Hazard. Do not move the Vectra Polaris instrument. Installing, servicing, and moving the Vectra Polaris instrument should be performed only by qualified PerkinElmer service personnel. Contact PerkinElmer technical support if help is required (see page 3).

This section contains the following information:

- Front View hardware components (page 30)
- Top View hardware components (page 32)
- Right-Side View hardware components (page 33)
- Left-Side Connectors (page 34)
- Slide Carrier Hotel (page 35)
- Slide Carrier (page 37)
- System Computer and Monitor (page 39)
- Specifications (page 40)

Front View

Figure 10 shows the front view of the Vectra Polaris instrument with the system door *open*. The parts identified are described below the figure.

Figure 10. Vectra Polaris Front View - System Door Open

Status Progress Display

Each light represents an individual slide and indicates the progress of the slide currently being scanned.

Slide Carrier Hotel

Houses up to 20 slide carriers. See Slide Carrier Hotel on page 35 for more detailed information.

Door Sensor

Opens or closes the System Door when a hand is placed in front of the sensor.

Figure 11 shows the front view of the Vectra Polaris instrument with the system door *closed*. The parts identified are described below the figure.
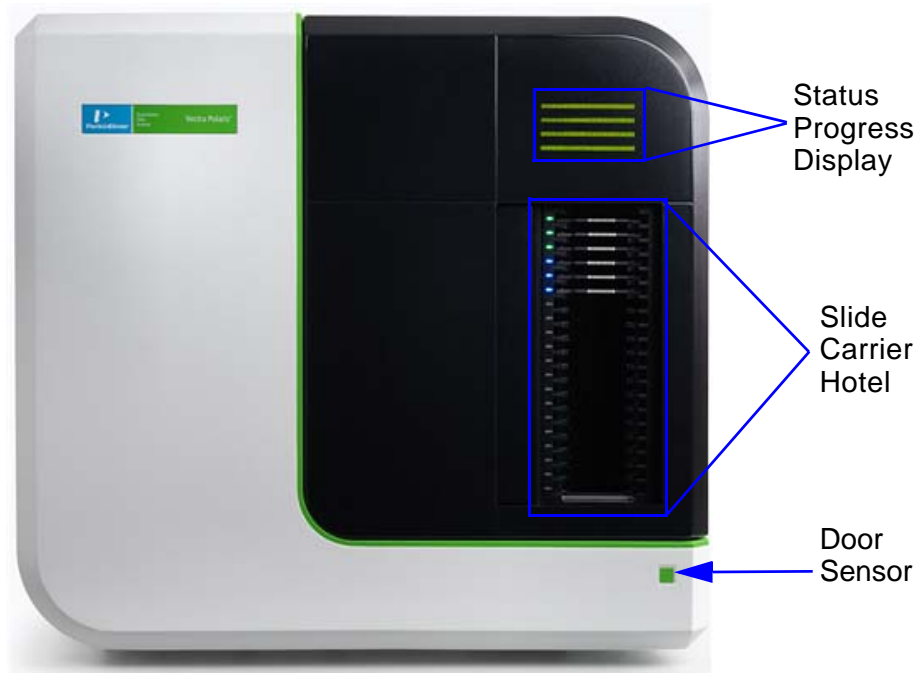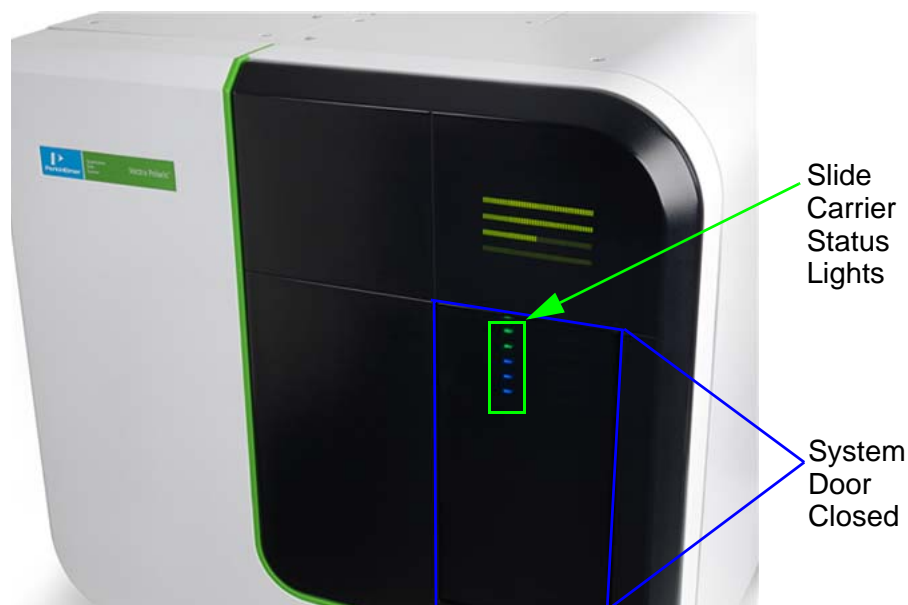

Figure 11. Vectra Polaris Front View - System Door Closed

Slide Carrier Status Lights

Each slide carrier status light represents an individual slide carrier and indicates the processing status of each Slide Carrier. See Table 1 on page 36 for the color codes of the slide carrier status lights.

System Door

When closed, covers the Slide Carrier Hotel and slide processing can occur. When open, reveals the Slide Carrier Hotel.

WARNING

Do not operate the system if there has been a malfunction of the system door or slide loading components. Contact PerkinElmer technical support if help is required (see page 3).

Top View

Figure 12 shows the top view of the Vectra Polaris instrument. The part identified is described below the figure.
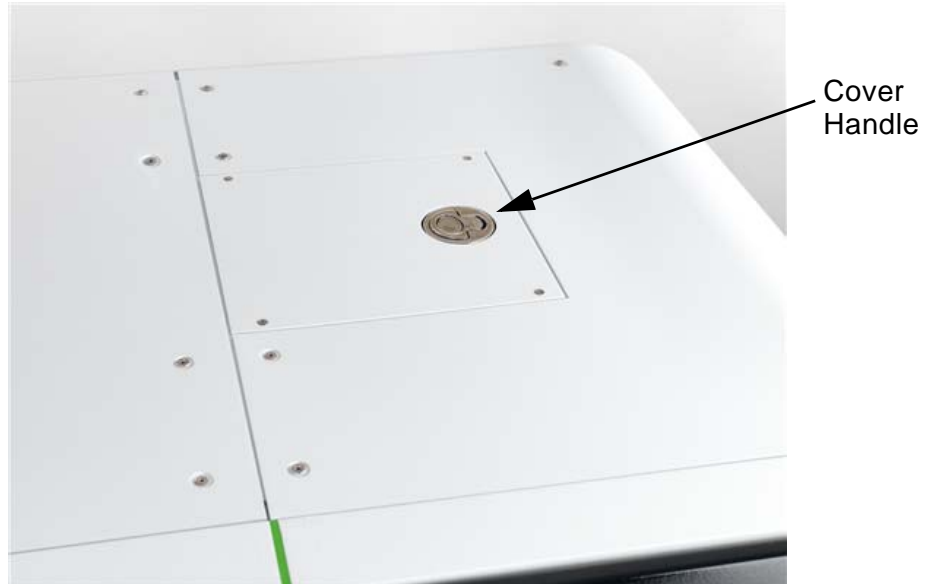
Figure 12. Vectra Polaris Top View

Cover Handle - Used by only service personnel to remove instrument covers during service and installation.

WARNING

DO NOT remove instrument covers. There are no user serviceable parts inside. The covers are intended to be removed by qualified service personnel only; they are not intended to be removed during operation or for maintenance by users. Contact PerkinElmer Technical Support if help is required (see page 3).

Right-Side View

Figure 13 shows the right-side of the Vectra Polaris instrument. The part identified is described below the figure.
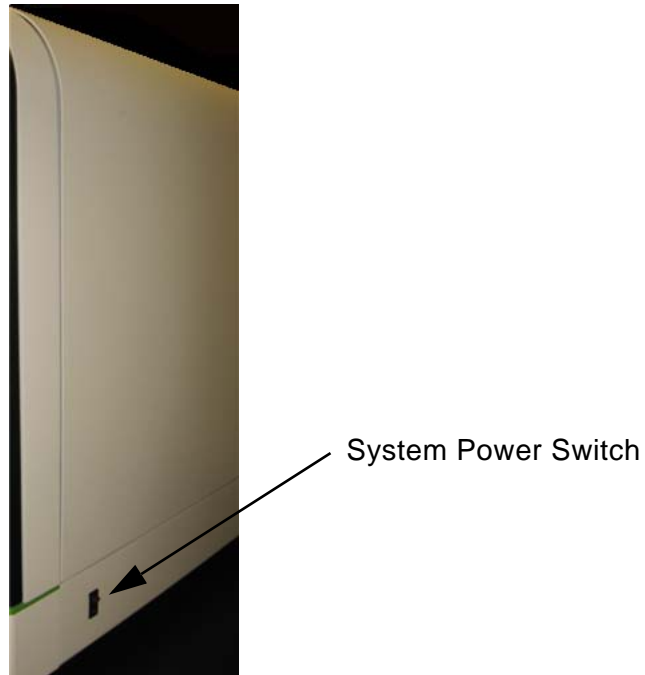

Figure 13. Vectra Polaris Right-Side View

System Power Switch

Turns the Vectra Polaris instrument ON (I) or OFF(O).

Left-Side Connectors

Figure 14 identifies the connectors on the bottom left-side of the Vectra Polaris instrument. The connectors identified are described below the figure.

Figure 14. Vectra Polaris Left-Side Connectors

Power Connector - Connects to the power cord to provide power to the Vectra Polaris instrument.

WARNING

- *Use only the power supply cord set provided with the Vectra Polaris system. If the correct cord set for the location was not provided, contact PerkinElmer Technical Support (see page 3) for a replacement.*

- *Use only a properly grounded power outlet when connecting the system to power.*

USB 2.0 Connector- Connects a USB 2.0 cable to a USB 2.0 port on the System Computer Connectors.

USB 3.0 Hub Connector- Connects a USB 3.0 HUB to a USB 3.0 port on the System Computer Connectors.

Slide Carrier Hotel

The Slide Carrier Hotel houses up to 20 Slide Carriers and is visible when the System Door opens. Figure 15 shows a closeup of the Slide Carrier Hotel and identifies its components. The components are described below the figure.

WARNING

Do not operate the system if there has been a malfunction of the system door or slide loading components. Contact PerkinElmer technical support if help is required (see page 3).
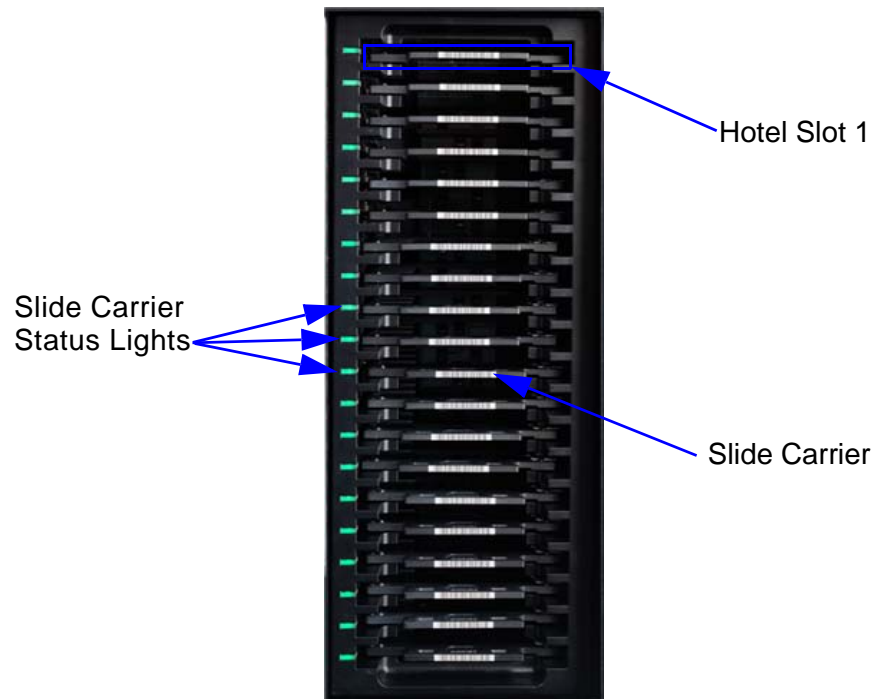

Hotel Slot 1

Slide Carrier Status Lights

Slide Carrier

Figure 15. Slide Carrier Hotel

Slide Carrier Status Lights

Indicates the processing status of each slide carrier. Table 1 on page 36 lists the color codes associated with the slide carrier status lights.

Table 1. Slide Carrier Status Lights - Color Codes

Color	Status
None	Slide carrier hotel is empty.
White	Initial state of slot after slide carrier is inserted and no rules have been applied via software.
Solid Red	Hotel slot is malfunctioning.
Blinking Red	Slide carrier is not completely inserted.
Blue	Awaiting Processing.
Blinking Blue	Prioritized and awaiting processing.
Yellow	Processing instructions are either incomplete or invalid.
Blinking Green	Processing.
Solid Green	Processing Complete.
Orange	Processing complete but a processing error occurred.

Hotel Slot

Each hotel slot holds one Slide Carrier. The hotel slots are numbered 1-20, starting at the top of the hotel.

Slide Carrier

Houses up to four slides. See Slide Carrier on page 37 for more detailed information.

Slide Carrier

Slide Carriers are an integral component of Vectra Polaris. The Slide Carrier holds up to four microscope slides and helps protect slides from damage. The microscope slide positions s are labeled 1 to 4 and correspond to slide identification in the Vectra Polaris software.

Figure 16 shows a closeup of a Slide Carrier and identifies its components. The identified components are described below.
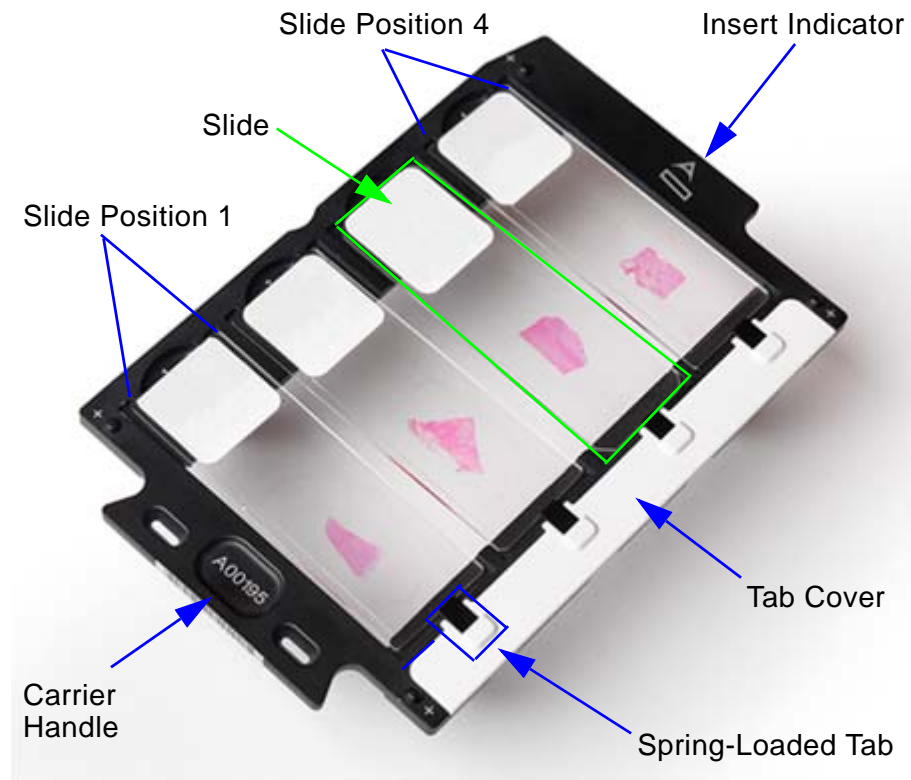
Figure 16. Vectra Polaris Slide Carrier

Slide Position

Each slide position is populated with one slide. Slide position 1 is on the far left; slide position 4 is on the far right.

Slide

Each microscopic slide is manually loaded into the slide positions. The table below identifies the details of the slide format.

Slide Type	Width	Height	Thickness
Metric	25.0 ± 1.0	75.0 ± 1.0	1.00 ± 0.10
English	25.4	76.2	1.00 ± 0.10

Insert Indicator

A Perkin Elmer icon that indicates the side of the slide carrier to be inserted into a Hotel Slot.

Tab Cover

Holds the spring-loaded tabs in place.

Spring-Loaded Tab

When a slide is inserted into a slide slot, it is gently placed up against a spring-loaded tab.

Carrier Handle

The side of the slide carrier to hold when inserting and removing a slide carrier from a Hotel Slot. A unique number is printed on top of the handle for slide carrier identification purposes.

System Computer and Monitor

The Vectra Polaris system includes a widescreen monitor and a computer pre-installed with Vectra Polaris, Phenochart, and inForm software. A wireless keyboard and mouse are also included.

System Computer Connectors

Figure 17 shows the Vectra Polaris computer connectors. The connectors identified are described below the figure.
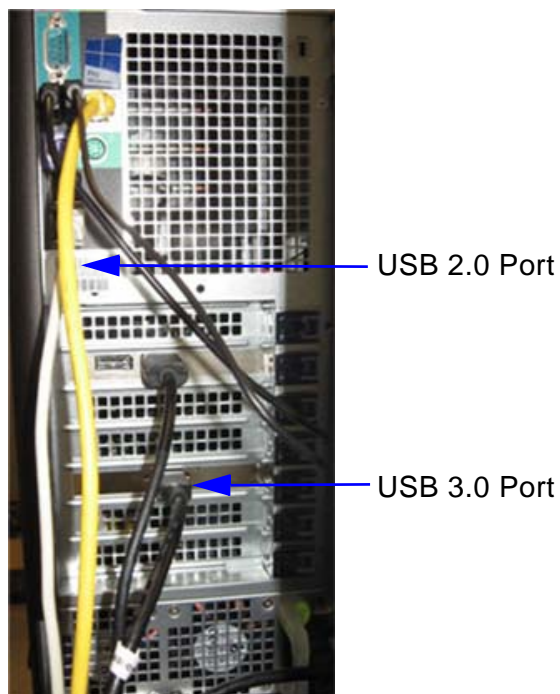

Figure 17. System Computer Connectors

USB 2.0 Port - Connects a USB 2.0 cable to the USB 2.0 connector on the instrument's Left-Side Connectors (see page 34).

USB 3.0 Port - Connects a USB 3.0 HUB to the USB 3.0 HUB connector on the instrument's Left-Side Connectors (see page 34).

Specifications

This section lists the technical specifications for the Vectra Polaris instrument. Technical specifications are subject to change without notice.

NOTE

!

Vectra Polaris is for research use only. Not for use in diagnostic procedures.

System

Size (H x W x L)	28" (72 cm) x 30" (77 cm) x 27.25" (69.2 5cm)
Weight	185 lbs. (84 kg)
Spectral Range	440 nm to 720 nm
Objectives	4x, 10x, and 20x
Pixel Resolution	0.25 um, 0.5 um, or 1.0 um
File Format	PerkinElmer proprietary .qptiff format for whole slide scans, .im3 file format for multispectral data; 24- bit Windows-compatible bitmap for RGB/Mono imagery
Operating System	Windows 10®, 64-bit
RAM	16GB

Environmental

Operating Temperature	59°F to 83°F (18°C to 28°C)
Operating Humidity	10% - 50% non-condensing
Storage Temperature	59°F to 86°F (15°C to 30°C)
Storage Humidity	0% - 80% relative humidity, non-condensing
Altitude	Up to 2000m (6560ft.)
Shipping Temperature (up to 24 hours max)	14°F to 113°F (-10°C to +45°C)
Pollution Degree	2
Indoor Use Only	

Electrical

One properly grounded AC power outlet for the computer, monitor, and instrument must be located within 6 feet (1.8 m) of the location.

Input Voltage	100 to 230 VAC (±10%), 500 W, 50/60 Hz System does not have transient overvoltage protection.
Computer Interface	USB 2.0, USB 3.0
Fuse	4A Littelfuse 250V, 5mm x20mm

Hardware Operation

This section describes how to operate the Vectra Polaris hardware. It is important to read and understand Instrument Safety on page 7 before using the system. If you are not familiar with the Vectra Polaris system hardware, Hardware Reference on page 29 provides a description of each hardware component in the Vectra Polaris system.

This section includes the following procedures:

- System Startup on page 43
- System Shutdown on page 44
- Inspecting Slides and Slide Carriers on page 44
- Loading Slides into the Slide Carriers on page 45
- Loading Slide Carriers into the Slide Carrier Hotel on page 46

System Startup

This section describes how to start the Vectra Polaris system.

Turn on the Vectra Polaris Instrument

To start the Vectra Polaris instrument:

1   If necessary, plug the Vectra Polaris power cord from the Left-Side Connectors into an appropriate power outlet.

WARNING

- *Use only the power supply cord set provided with the Vectra Polaris system. If the correct cord set for the location was not provided, contact PerkinElmer Technical Support (see page 3) for a replacement. Do not use power supply cords with inadequate ratings.*
- *Use only a properly grounded power outlet when connecting the system to power.*
- *The wall outlet or the power cable connector on the left side of the instrument should be accessible after the system's installation, to enable trained service personnel to safely disconnect power from the system during servicing.*

2   Turn on the computer and allow Windows 10 to start.

3   Switch the System Power Switch to the ON (I) position. The Status Progress Display flash green to indicate a quiescent state. The Door Sensor is responsive and functional.

Launch the Vectra Polaris Software

To launch the Vectra Polaris software:

1   Click the Vectra Polaris icon on the Windows 10 desktop.

2   The Vectra Polaris homepage opens (see page 49).

System Shutdown

This section describes how to shut down the Vectra Polaris system.

To shutdown the Vectra Polaris system

1   Exit the Vectra Polaris software. If open, the System Door closes.

2   Select **Shut down** from the Windows Start Menu to power down the computer.

3   Switch the System Power Switch on the right side of the instrument to the OFF (O) position. The status lights turn off and the system shuts down.

Inspecting Slides and Slide Carriers

Before inserting slides into slide carriers, both should be inspected for potential defects. This section describes what to look for when inspecting the slides and slide carriers.

Inspect Slides

- Verify the slides meet the required formats and dimensions. See page 37.
- Do not use broken or damaged slides, or slides with broken or damaged coverslips.
- Use only slides that are free of debris, fingerprints, and dust.

Inspect Slide Carriers

- Verify the Slide Carrier Tab Cover is secure.
- Do not use slide carriers that are warped or bent.
- If any sticky residue is on the carrier handle or outer surfaces, clean before use.

NOTE

See page 81 for slide carrier cleaning instructions.

Loading Slides into the Slide Carriers

After the Slides and Slide Carriers have been successfully inspected, slides can be loaded into the Slide Carriers.

To load a slide into a slide carrier:

1 Place the slide carrier onto a flat surface.

2 Hold the microscope slide by the label end with the coverslip side up.

3 Gently push the opposite end of the slide into the desired Slide Position until the slide is up against the Spring-Loaded Tab.
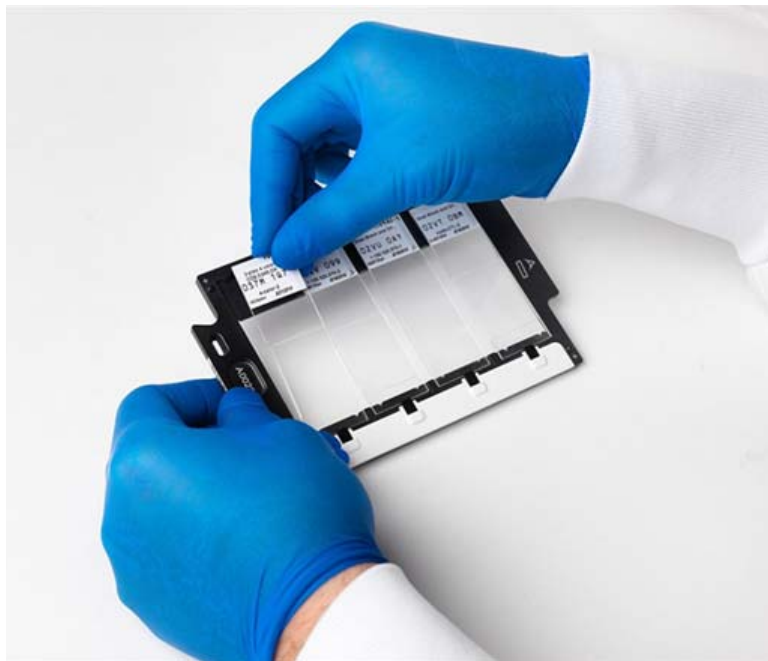

Figure 18. Insert Slide into Slide Carrier

4 Gently lay the label end of the slide completely into the slide position. The tab should push the slide against the opposite wall of the slide position.

5 Load up to four slides into each slide carrier. Slides need not be contiguous.

6 Click **Enter Slide IDs** (see page 74) in the Vectra Polaris software. The Enter Slide IDs dialog opens.

7 Type the applicable slide IDs into the text boxes and click the **OK** button.

8   To load additional slides into another slide carrier, repeat steps 1 to 7 above. Slide carriers may be stacked on top of one another for easy handling and storage (see Figure 19).

Figure 19. Slide Carrier Stack

Loading Slide Carriers into the Slide Carrier Hotel

The Slide Carrier Hotel stores Slide Carriers before and after microscope slide scanning. The slide carrier hotel can store up to 20 slide carriers for a total of 80 slides. The Hotel Slots are numbered 1-20, starting at the top of the hotel.

NOTES

[!]

Slide carriers can be loaded into the slide carrier hotel before or after launching the software.

To load a slide carrier into the slide carrier hotel:

1   Inspect the slide carrier (see page 44).

2   Verify the Vectra Polaris System is on (see page 43).

3   If the System Door is closed, place your hand in front of the Door Sensor to open it. The system doors slides to the left to reveal the slide carrier hotel.

4   Hold the slide carrier by the Carrier Handle with the slide labels facing upwards.

5   Gently insert the end of the slide carrier with the Insert Indicator (▯▷) into a Hotel Slot.

Figure 20. Ends of a Slide Carrier

6   When the Slide Carrier is halfway into the Hotel Slot, the carrier engages a roller and creates a small amount of friction. Push the carrier further into the slot until it is fully seated.

Figure 21. Push the Slide Carrier until Fully Seated

- If the carrier is inserted correctly, a click noise indicates that the carrier has been seated correctly into the slot. The Slide Carrier Status Light (page 36) next to associated Hotel Slot turns white.

- If the carrier is *not* inserted correctly, the Slide Carrier Status Light next to associated the Hotel Slot blinks red until the slide carrier is removed from the slot or is positioned correctly.

7 Continue to load up to 20 slide carriers into their slots.

8 When done, place your hand in front of the Door Sensor to close System Door.

Removing Slide Carriers from the Slide Carrier Hotel

When slide scanning is complete, the Slide Carriers can be removed from the Slide Carrier Hotel.

To remove a slide carrier from the hotel:

1 Pull the Carrier Handle until the slide carrier is free from its Hotel Slot. The Slide Carrier Status Lights next to the Hotel Slot turns off, indicating no slide carrier is inside the Hotel Slot.

2 Lay the slide carrier onto a flat surface.

Removing Slides from the Slide Carriers

After Slide Carriers are removed from the Slide Carrier Hotel, the microscopic slides can be removed from the Slide Carriers.

To remove a slide from a slide carrier:

1 Gently push against the slide label end of the microscope slide using your index finger, compressing the tab on the far end of the slide.

2 Using the same finger, lift the label end of the microscope slide from its slot.

3 Grab the label end of the slide with your thumb and index finger.

4 Remove the remaining end of the slide from the slide carrier.

Software Operation

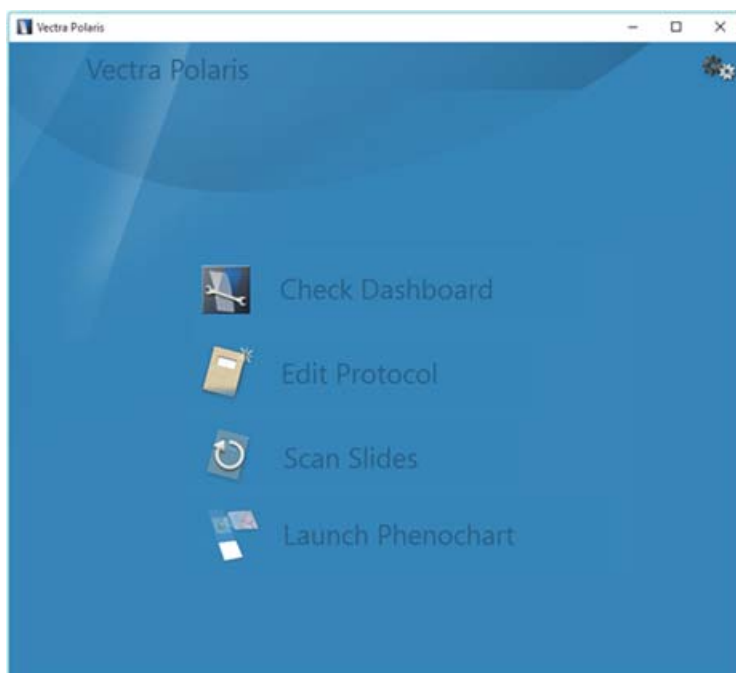This section describes how to operate the Vectra Polaris software.
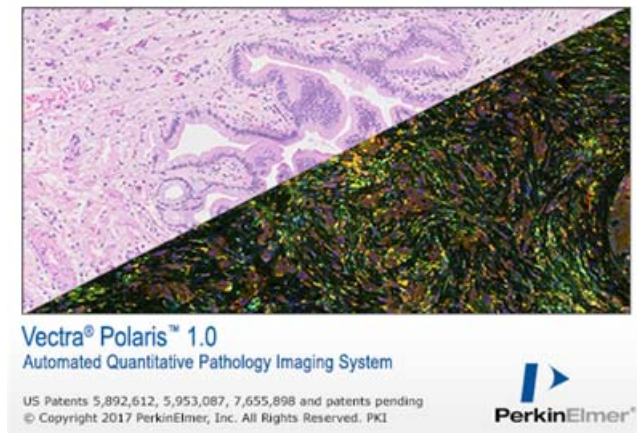
This section includes the following topics:

- Software Overview
- System Dashboard
- Creating and Editing Protocols
- Scanning Slides

Software Overview

The Home Page links to the pages needed to maintain and run Vectra Polaris.


The Home Page buttons link to the following pages:

Check Dashboard - Check your remaining disk space and acquire references.

Edit Protocol - Create or edit brightfield and fluorescent protocols and studies.

Scan Slides - Select the rules to scan your slides and scan your slides based on those rules.

Launch Phenochart - Launch the Phenochart viewing software.

You can also get to those pages by using the Gear Menu (in the upper right):
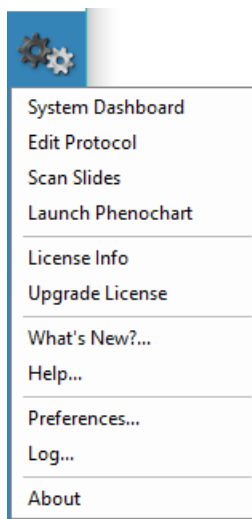
Additionally, you can use the Gear Menu to check your version number or perform other functions, including launching online help.

Below is an overview of each page.

Check Dashboard

Use the Dashboard to check the status of your system prior to imaging. It includes the following:

- Disk space - checks the available space on the disk drive where images will be stored.
- Acquire References - views and acquires brightfield references and fluorescent references.

For detailed information on how to use the System Dashboard, see System Dashboard.

Edit Protocol

Use the Edit Protocol page to define protocols and take snapshots.

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 use for focusing and imaging.

For detailed information on how to create and edit protocols and studies, see Creating and Editing Protocols.

Scan Slides

Use the Scan Slides page declare how slides should be imaged. Slide scanning can be started and stopped from this page.

For detailed information on how to perform whole slide scans, see Scanning Slides.

Launch Phenochart

Use this button 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.

Gear Menu

Use the Gear menu (in the upper right hand corner of the Home Page) to:

- Link to the following pages:
    - Check Dashboard
    - Edit Protocol
    - Scan Slides
    - Launch Phenochart
- Obtain license information and upgrade your license
- Find out What's New in the current installed version
- Launch the online Help system
- Open the Preferences dialog
- Open the Vectra Polaris software **Log** for reference or troubleshooting
- Open the About window to view the current software version
- View contact information for PerkinElmer technical support

System Dashboard

Use the System Dashboard to:

- See the available Disk Space
- View and acquire Brightfield References
- View and acquire Fluorescence References
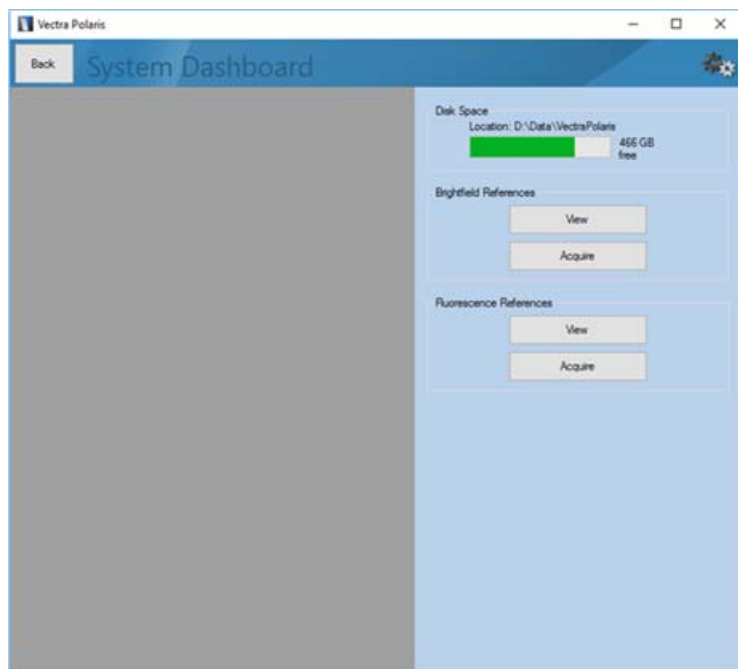
Disk Space

The disk space bar shows where your data is stored and how much space is available on that drive.
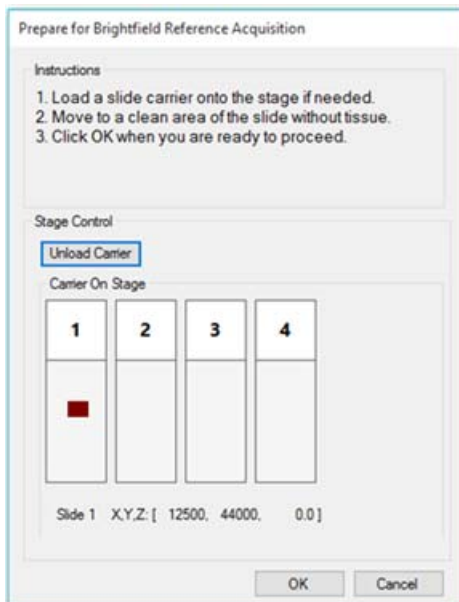
Brightfield References

Click **View** to see reference imagery and information for the label image, brightfield overview, color image and multispectral image.

- **Export For Diagnostics** will save this image to aid in technical support.
- **Show raw images** will display the images without scaling, which is useful for technical support.
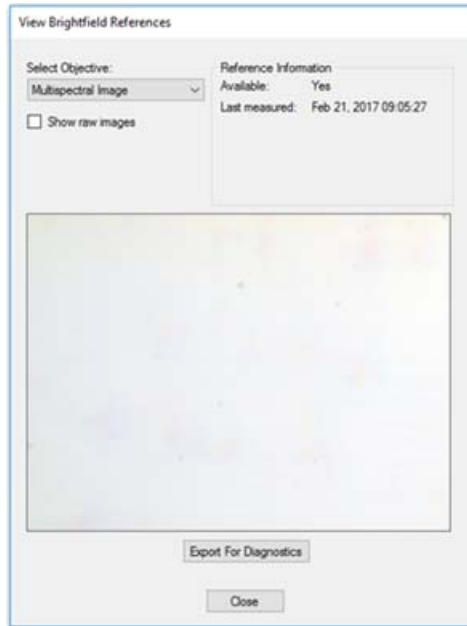
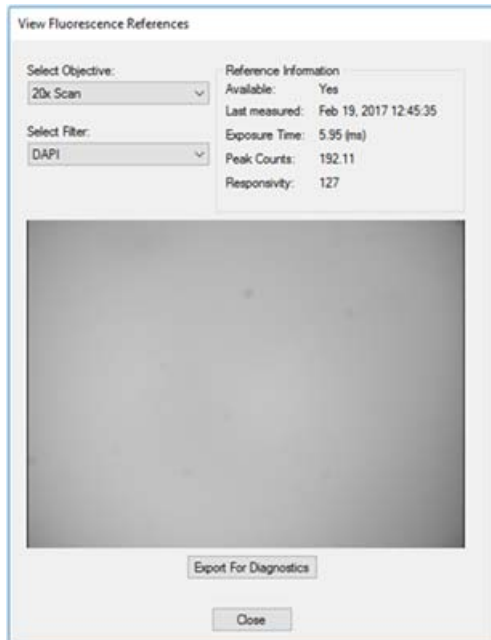Click **Acquire** to take new brightfield references. Use the stage control to:

- Move around to a clean area in the live view (no coverslip lines, tissue, or label) using the stage navigation tool.

- Click within the slide to change positions. You can refine the position by using arrow keys for small movements, and control+arrow for slightly larger movements.

- Click **OK** to take references.

Fluorescence References

Click **View** to see reference imagery and information for the overview, 20x and 40x resolution references for each filter.

- **Export For Diagnostics** will save this image to aid in technical support.

Click **Acquire** to take new fluorescent references. We recommend you take references for all filters at once, but you can take references for a single filter if necessary. Acquiring references for all filters can take over 30 minutes, and the system can be left unattended during this time.
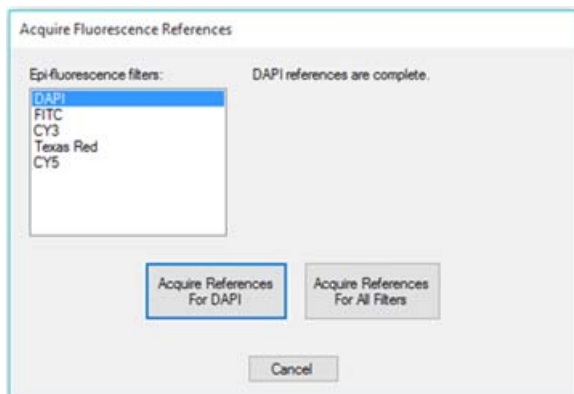
Compensation Information

Brightfield

Acquired images of a sample are normalized (divided) by the reference images on a pixel-by-pixel, wavelength-by-wavelength basis. This yields the sample transmission T, which is in the range 0 - 1. This transmission (T) is then mapped:

- For multispectral images, the Optical Density is calculated - this is based on Log10(T).
- For simple color images, like a whole slide scan, the transmission is mapped from 0 - 255.

Fluorescence Protocols

Acquired images are normalized by a shading pattern derived from the reference images. The shading pattern, which is the reference image divided by the mean intensity in the center, is applied on a pixel-by-pixel basis, with one pattern per epi-filter. Here, center means the central $1/16^{th}$ of the image area. 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.
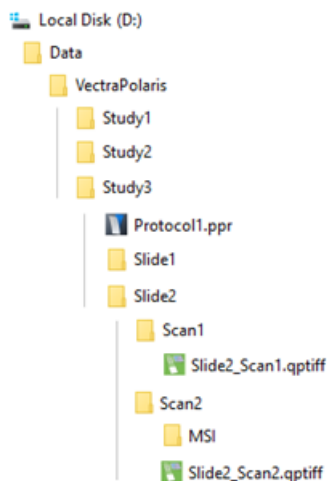
Creating and Editing Protocols

Studies

Vectra Polaris stores scan data by 'Study'.

A **study** is a group of slides that belong together. This could be an experimental study (e.g. Ki67 markers in breast cancer tissue), all slides from one source, or other groupings. Each study contains one or more slides. Each slide may be scanned more than once, if needed.

- The default location for a study is D:\Data\VectraPolaris\[Study] (where [Study] is the name of the study).
- Whole-slide scans and supporting imagery acquired from specific slides are saved to slide-specific subfolders in the main study folder. See the section on Scanning Slides for more details about imagery.
- MSI Fields acquired from a particular slide scan will be stored in an MSI folder within the scan folder.

A **protocol** defines the set of rules to be used during whole slide and multispectral region acquisition, including imaging mode, pixel resolution, filter cubes, exposure times, and other parameters.

- Protocols have the file extension ".ppr" and are saved in D:\Data\VectraPolaris\[Study].

Creating Protocols

Before you create a protocol, it is helpful to know how the slide was stained.

- Brightfield protocols are used to acquire imagery from slides stained with H&E or conventional chromogenic IHC methods.
- Fluorescence protocols are used with PerkinElmer Opal and other fluorescent dyes.

To create a new protocol:

- Select **Edit Protocol** from the Vectra Polaris Home Page.
- Click **New** and the following window will appear:

Create New Protocol   ✕

Protocol Name: [   ]

Imaging Mode: ◯ Brightfield
              ◉ Fluorescence

Please select a study for this protocol

Root Path: C:\Data\VectraPolaris

Available Studies:

Study1
Study2
Study3

Create New Study

Study Name: [   ]

[Create Study]

[Create Protocol]   [Cancel]

- Enter a Protocol name.
- Select **Brightfield** or **Fluorescence** under Imaging Mode.
- Select a previously created Study or create a new Study.
    — To select a previously created study, click on the study in the **Available Studies** list. This will highlight the study.
    — To create a new study, enter the Study Name in the text box and click the **Create Study** button. The new study will be added to the Available Studies list where you can select the study.
- Click the **Create** button to create the protocol in the selected study.

To load an existing protocol, click **Load** and select the protocol from the study folder.

Editing Protocols

After you have created your brightfield or fluorescence protocol and assigned it to a study, use the Edit Protocol screen to add specific details to the protocol. The next sections are organized by two different types of protocols: brightfield and fluorescence. The sections appear as follows:

- Brightfield Protocols
- Brightfield Snapshots
- Fluorescence Protocols
- Fluorescence Exposures

Brightfield Protocols

After you have created your brightfield protocol and assigned it to a study, the Edit Protocol screen (for brightfield protocols) will appear:
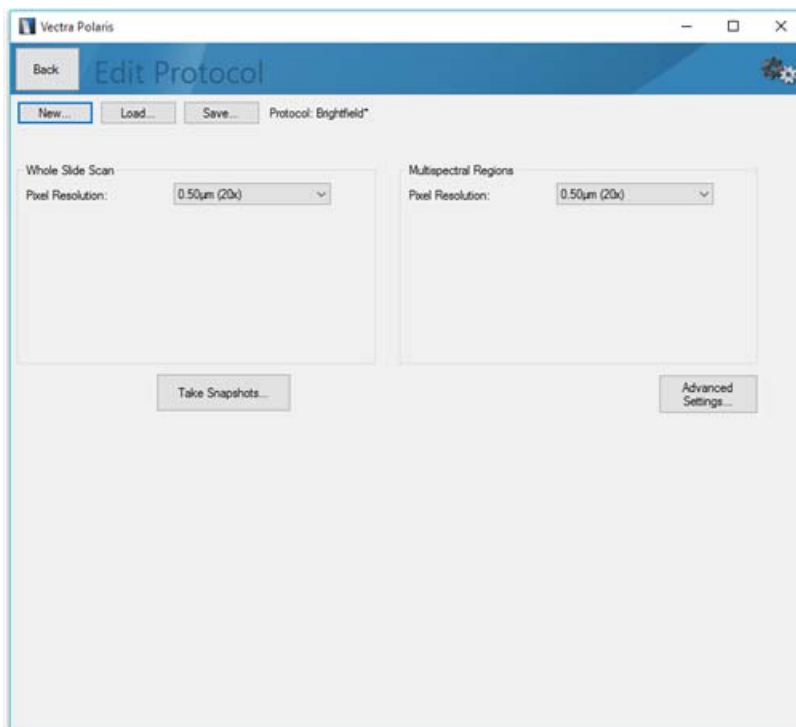
Under Whole Slide Scan, choose the Pixel Resolution that you want to use to image the whole slide. You can choose 1 um per pixel (nominally 10x), 0.5 um per pixel (nominally 20x), or 0.25 um per pixel (nominally 40x).
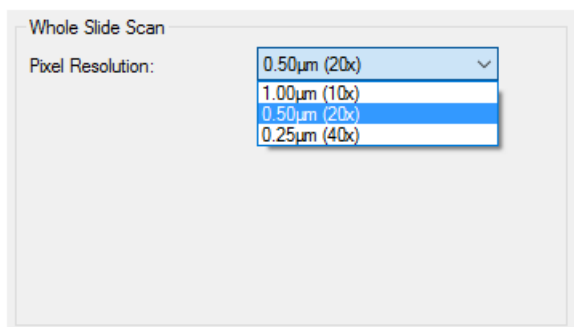
If you plan to take Multispectral Regions, choose the Pixel Resolution that you want to use for imaging. Again, available resolutions are 1 um per pixel (nominally 10x), 0.5 um per pixel (nominally 20x), or 0.25 um per pixel (nominally 40x).
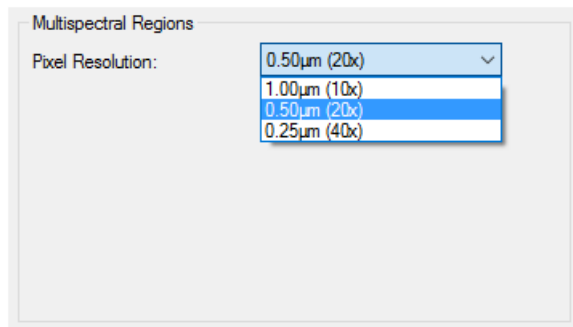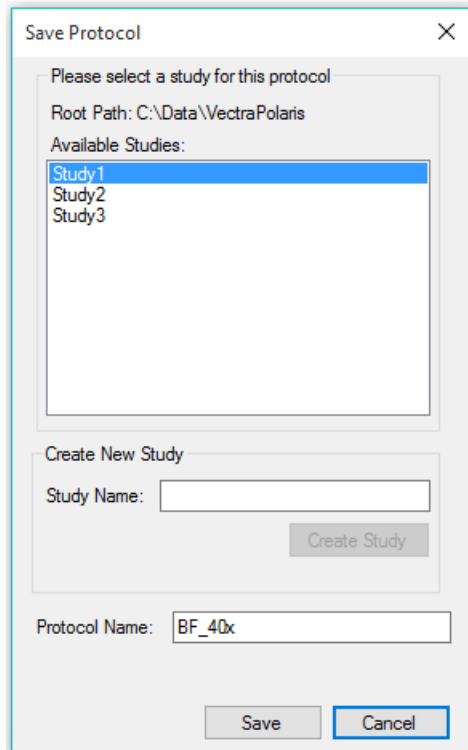
Advanced Settings

There may be situations when you need to fine-tune how Vectra Polaris scans the slides. Click the **Advanced Settings** button to adjust the following functions:

- If Vectra Polaris 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.

- By default, brightfield whole slide scans in Vectra Polaris are .jpg compressed in order to save disk space. You can adjust the image quality as needed; higher quality will result in larger files. If you would like use lossless compression instead, select LZW rather than JPEG.
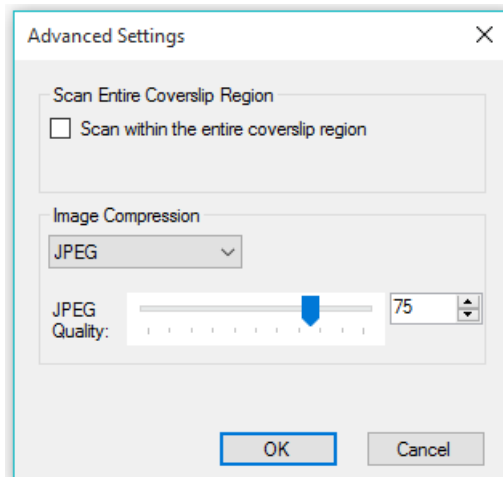
Click **Save** to save the protocol. The study you previously chose will be automatically selected. You can change the protocol name or study if needed.

Brightfield Snapshots

Once you have selected your scan and MSI resolutions, you can take example snapshots of your slides.

Click **Take Snapshots** to load the Brightfield Snapshots editor.
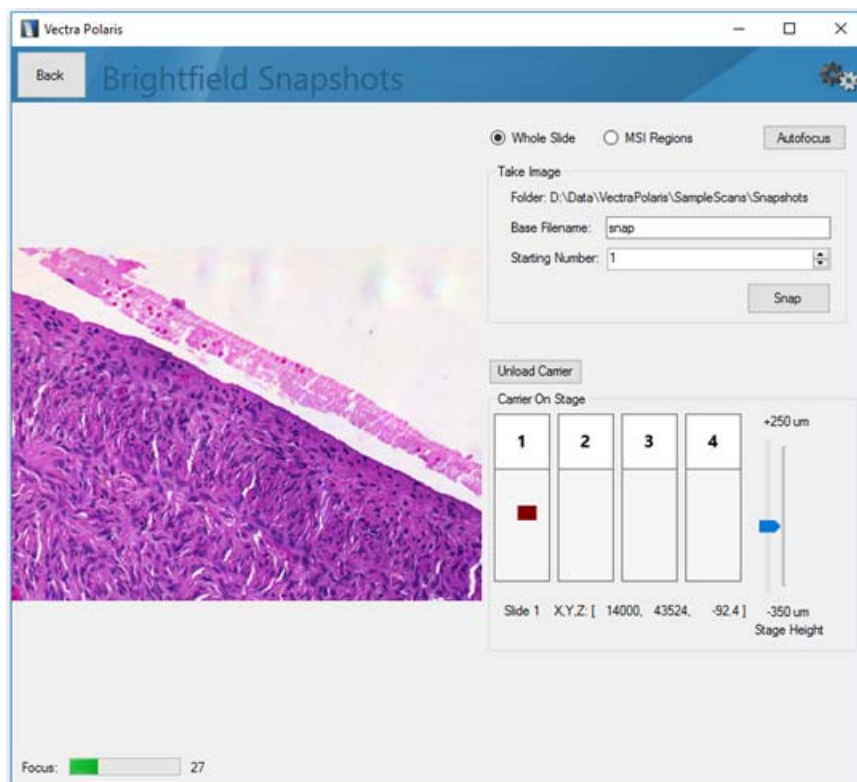
If a carrier is not already on stage, click **Load Carrier** to select a carrier that contains the slides you wish to image.
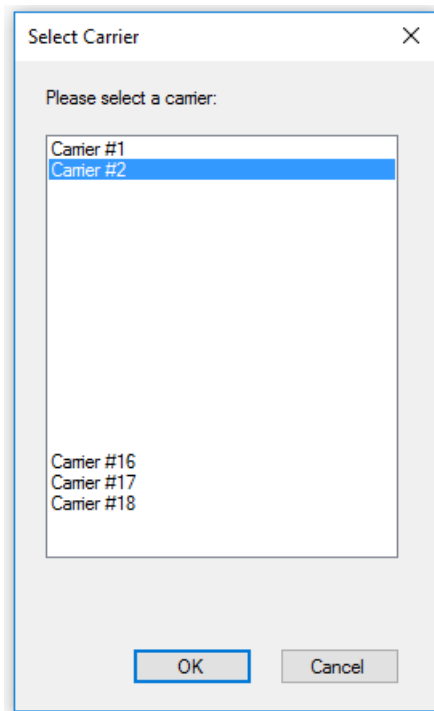
Use the navigation tool to select an area on the slide. Click within the slide to change positions. You can refine the position by using arrow keys for small movements, and control+arrow for slightly larger movements. Click **Autofocus** or use the Stage Height slider to bring the live view into focus.
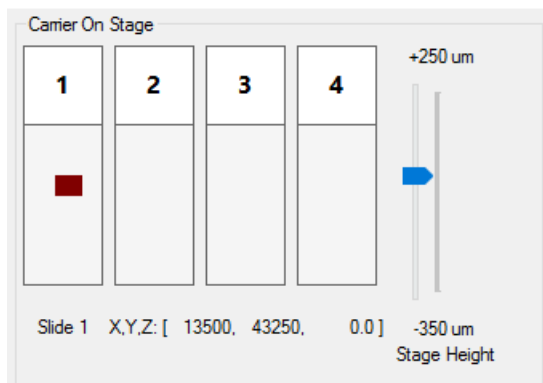
To take a snapshot of your current live view, select either the whole slide or the MSI regions radio button, pick a base file name, and click Snap.
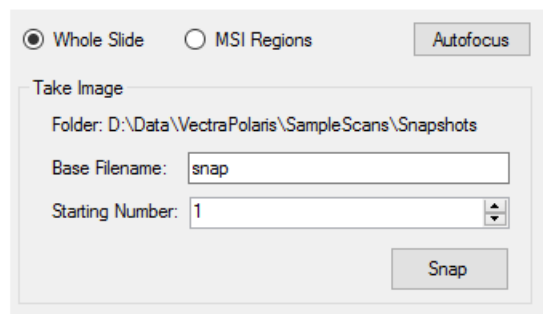
You can navigate to new places, focus as needed, and take as many snapshots as you want. Snapshots will be numbered incrementally.

Click **Back** when you are ready to return to the protocol editor.

Fluorescence Protocols

When creating a fluorescence protocol, you will need slides that include positive expression in all markers of interest in order to set suitable exposure times.

After you have created your fluorescence protocol and assigned it to a study, the Edit Protocol screen (for fluorescence protocols) will appear:
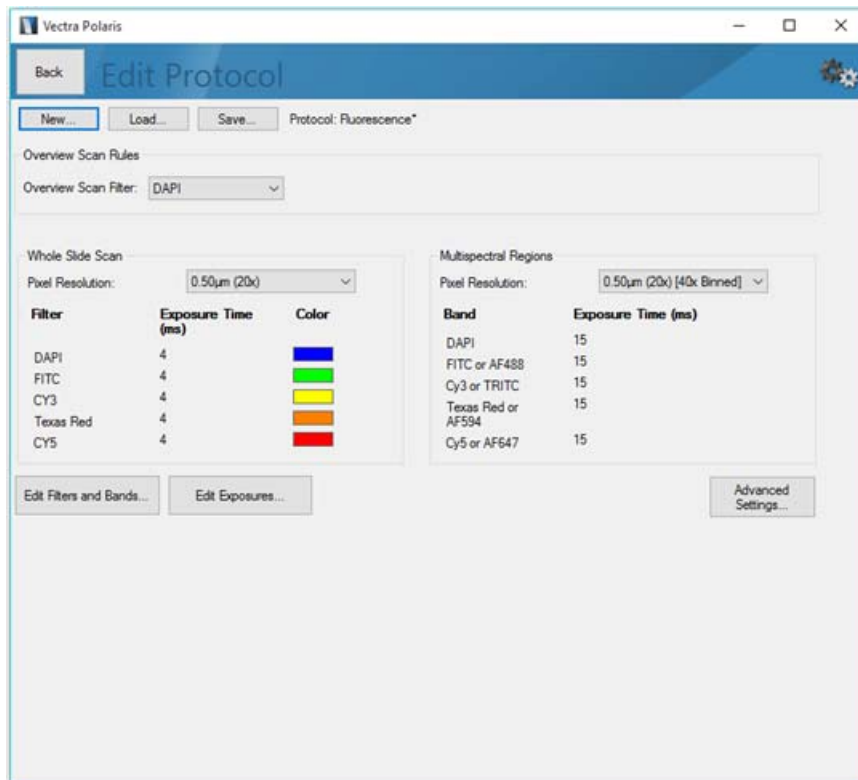
Under Overview Scan Rules, select the desired filter that will be used to help find tissue on the slide. This will typically be your DAPI counterstain. If there is no counterstain, then choose a filter that aligns with the expression of your most common stain or auto-fluorescence.
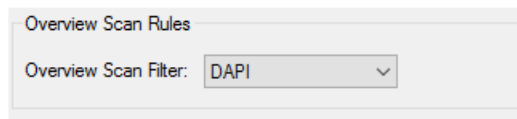
Under Whole Slide Scan, choose the Pixel Resolution to be used while imaging the slide. Options include:

- 1.0 um per pixel (nominally 10x)
- 0.5 um per pixel, (nominally 20x)
- 0.5 um per pixel, (nominally 20x, binned from a 40x acquisition)

• 0.25 um per pixel (nominally 40x).
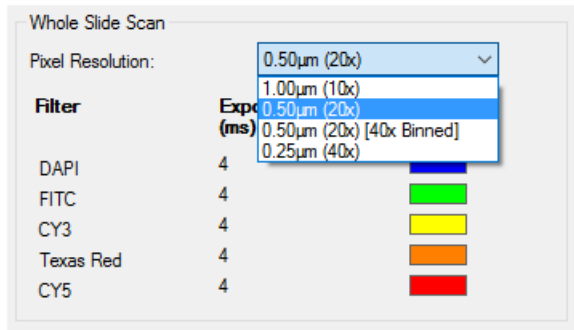
NOTE

Each time you change the pixel resolution, you will need to reset the exposure times.

• You can auto-update your exposures. This is recommended as a first step to give a close approximation of your exposure times. We then recommend you use the exposures editor to fine-tune your exposures.

• You can manually update your exposures. In this case, the current exposures remain unchanged and you must use the exposures editor to obtain valid exposures.

• You can cancel. This will leave the pixel resolution unchanged.
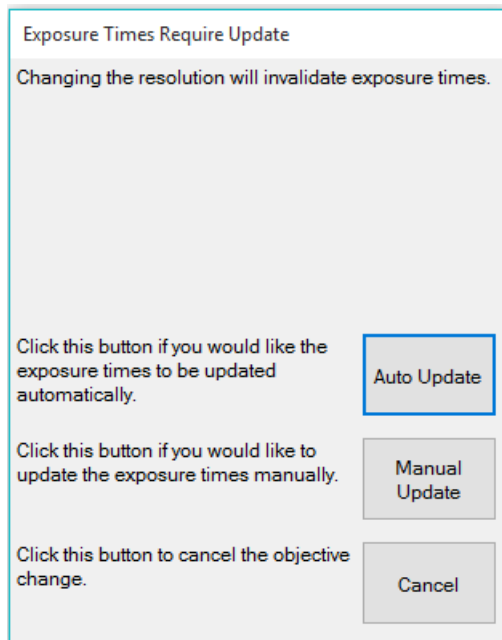
If you plan to take Multispectral Regions, choose the Pixel Resolution that you want to use to image the MSI regions. Available resolutions are 10x, 20x, 20x binned, or 40x.
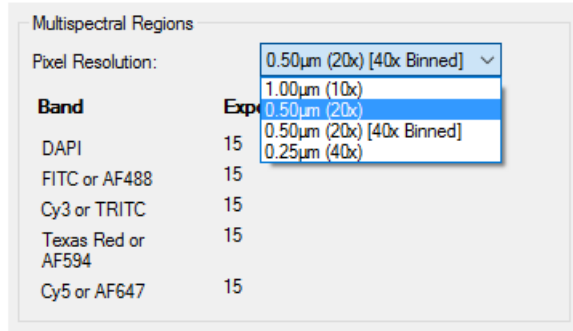
The **Edit Filters and Bands** button allows you to choose which filters you use to take your whole slide scan and which bands you use for multispectral imaging. Imagery for each filter or band will be taken in the order it is shown.
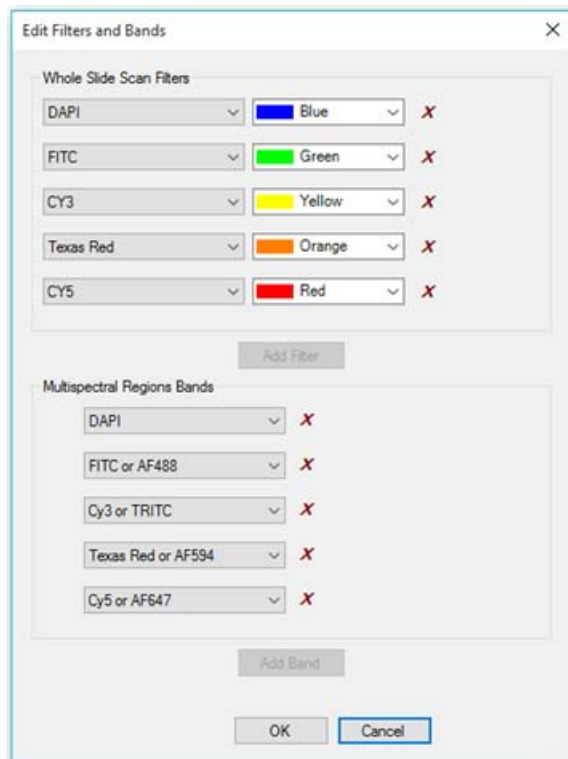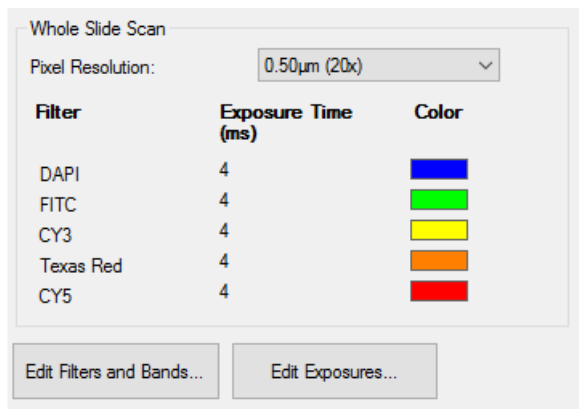
Whole Slide Scan

By default, Vectra Polaris will take a whole slide scan with all five filters. If you would like to save disk space, choose only the filters where fluorescent signals are expressing. You can remove a filter by clicking on the red 'x' next to that filter. Click **Add Filter** to add an additional filter. You can change the colors associated with each filter; these are the colors that Phenochart will use to display the whole slide scan.

Multispectral Imaging

In contrast, in multispectral imaging, we strongly recommend that you maintain the default band configuration. Non-signal bands are integral to multispectral stain extraction and unmixing.

Advanced Settings

There may be situations when you need to fine-tune how Vectra Polaris scans the tissue. Click the **Advanced Settings** button to adjust the following functions.

- You can specify which filter will be used for setting focus during whole slide scans and for MSI imaging. Choose the filter where your counterstain expresses. This is normally DAPI.

- Saturation protection prevents you from overexposure when acquiring multispectral imagery. We highly discourage deactivating the Use Saturation Protection setting.

- If Vectra Polaris 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.

NOTE

If there is Sharpie® markup on your fluorescent slide, Vectra Polaris will use that as the region instead of the coverslip.
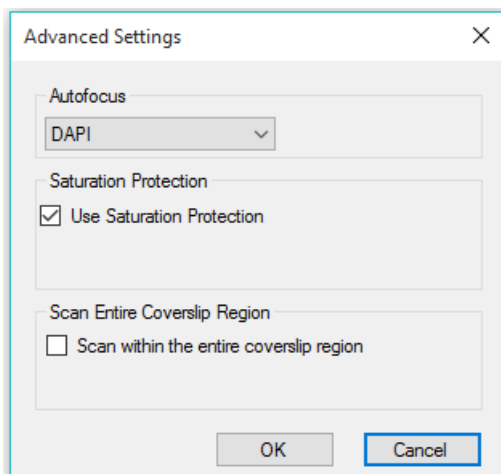
Once you have selected your settings, use the Exposures Editor to set exposures for your protocol.

Filter Cube Recommendations when using PerkinElmer Opal Reagents

If you are using your Vectra Polaris system with PerkinElmer'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
DAPI	BLUE				
Opal 520		GREEN			
Opal 540		GREEN	YELLOW		
Opal 570		GREEN	YELLOW	ORANGE	
Opal 620			YELLOW	ORANGE	
Opal 650				ORANGE	RED
Opal 670				ORANGE	RED
Opal 690					RED

<u>**Key**</u>**:**

- Colors are signal bands: autoexpose or manually set the desired exposure per sample
- Gray is non-signal band: manually set to 20ms exposure for MSI

Fluorescence Exposures

Once you have selected your scan and MSI resolutions, filters, and bands, you need to set exposures. Click **Edit Exposures** in the Fluorescence Protocol Editor to set exposure times.
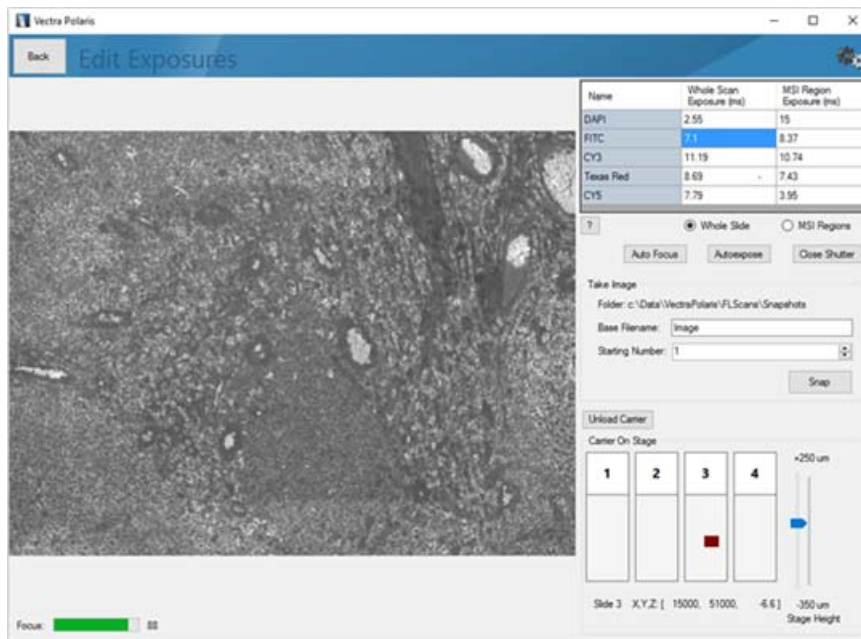
If a carrier is not already on stage, click **Load Carrier** to select a carrier that contains representative slides. Select your carrier from the list and load it to the stage.
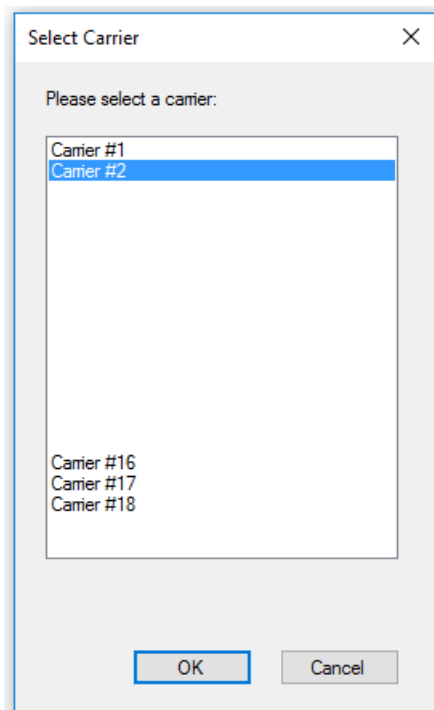
Use the navigation tool to select a slide. You will see a live view of your first filter for your imaging rules. In most cases, this will be DAPI. The highlighted entry in the table identifies which filter or band is currently shown.
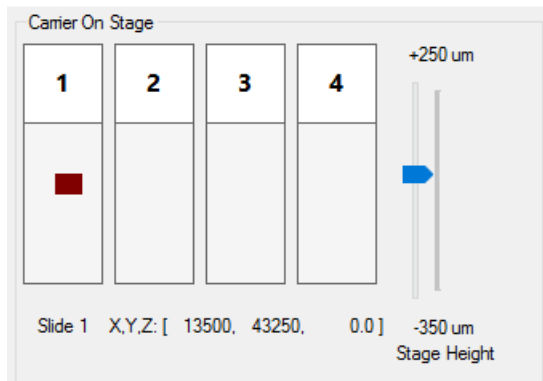
Locate and focus on an area of the slide with good signal expression. Click within the slide to change positions. You can refine the position by using arrow keys for small movements, and control+arrow for slightly larger movements. Use the Stage Height slider or the **Auto Focus** button to bring the live view into focus. Click **Autoexpose** to have the system find the best exposure for that filter / band.

- After auto-exposing, you may want to auto-focus and auto-expose again to refine your focus and exposure estimates.

- You can override the auto-exposure value by typing a value in the highlighted cell. Values must be between 0.1 and 2000 ms.

- If you would like to turn off the fluorescence illumination and live view, click **Close Shutter**. You will need to re-open the shutter to see a live view and set exposures.

If you plan to use this protocol for multispectral region acquisition, you should set the MSI exposures at this time. In most cases, you can use the same area of the slide to set your MSI exposures. Click the MSI regions radio button to set exposures for MSI regions. Focus and click **Autoexpose**.

- As with whole slide scan exposures, after auto-exposing, you may want to auto-focus and auto-expose again to refine your focus and exposure estimates.

- You can override the auto-exposure value by typing a value in the highlighted cell. Values must be between 0.1 and 2000 ms.

- If you would like to turn off the fluorescence illumination and live view, click **Close Shutter**. You will need to re-open the shutter to see a live view and set exposures.

Repeat the steps above for all filters and bands in your protocol. You may need to change locations and/or slides to find the best signals for setting your exposures.

NOTE

if there is an N/A in the table, that filter or band is not in use, so no exposure is needed.

Snapshots

When you have set your exposures, you can take snapshots to see sample imagery of how your exposures perform on a given area of the slide. Select either the **Whole Slide** or the **MSI Regions** radio button, pick a base file name, and click **Snap**. You can navigate to new places and take as many snapshots as you want. They will be numbered incrementally.

Click **Back** when you are ready to return to the protocol editor.

NOTE

The exposures you just set are only valid for the resolutions you picked. If you change your scan or MSI resolution, you will need to revisit the exposures editor to update your exposures.

Scanning Slides

The Carrier

Slides are loaded into Vectra Polaris via carriers, which are stacked into the Slide Carrier Hotel.

The hotel can hold up to 20 carriers, and each carrier can hold up to 4 slides. This means Vectra Polaris can be loaded with up to 80 slides at any given time. Those 80 slides can all be scanned based on the same set of rules, or you can tailor your scan rules to each slide and/or each carrier as needed.
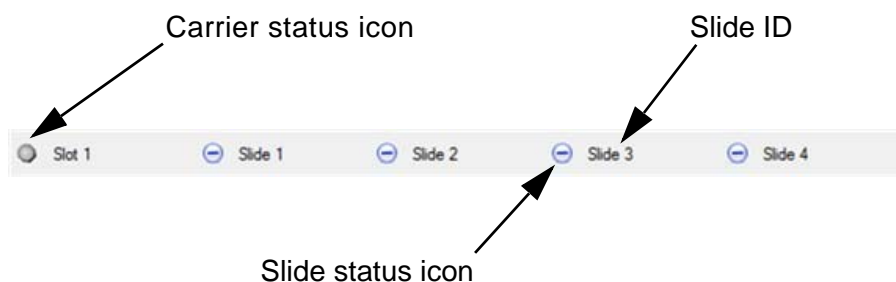
Carrier Status

Within the software, each hotel slot is identified as 'Slot N,' where N is 1-20 running top to bottom. When a slide carrier is loaded into the hotel, an LED light changes color to identify the status of that carrier.

The User Interface will reflect the current state of each slot in the hotel. If there is no carrier present, you will see:

//

Each carrier present in the hotel will be represented as follows:

Carrier status icon   Slide ID

⊖ Slot 1   ⊖ Slide 1   ⊖ Slide 2   ⊖ Slide 3   ⊖ Slide 4

Slide status icon

Each carrier is represented by a carrier status icon, four slide status icons, and four slide IDs.

The carrier status icon corresponds to the hotel LED for that particular carrier. Each possible status is represented by a different color.

When you insert the carrier into the hotel, a white icon ⚪ indicates that the carrier is present but has no rules to use for slide scanning. If the icon is blinking red 🔴, the carrier has not been completely inserted. Try pushing it in further.

Once the carrier has been inserted in the hotel, you can define rules for your scan. The icon will change to blue 🔵 when there is at least one slide ready to be scanned. Carriers that have been prioritized will be blinking blue 🔵.

If no slides on the carrier can be scanned with the current rules, the icon will change to yellow 🟡. This carrier will be ignored until the problem is resolved.

When scanning has started, the icon for the carrier on stage will be blinking green 🟢. When the carrier is returned to the hotel, the icon will either be green 🟢 (for a successful scan) or orange 🟠 (if there was a problem with the scan).

If the icon is solid red 🔴, that hotel slot's sensor is malfunctioning. Do not use this slot.

Slide Status

Each carrier also has a status icon for the 4 slides it contains.

➥ means the slide has complete rules and is ready to be scanned.

🟢 (blinking) means the slide is currently being scanned.

✔ means the slide was scanned successfully.

Other options include ⚠ , which means the slide has rules, but they cannot be used. (They may be incomplete, in which case you will need to edit the rules for that slide)🚧. indicates that the slide failed to scan correctly.

Finally, ⊖ will indicate that the slide is being ignored. This is useful if the carrier has fewer than four slides or if you don't want to process that slide at this time.

Setting Up Scan Rules

Scan rules must be defined for each slide you would like to process. Scan rules are complete when a study, protocol, task, and slide ID have been assigned.

Slide IDs

You must manually enter the ID for each slide in the carrier. You can enter IDs before the carrier is placed into the hotel, or you can enter IDs after the carrier has been placed into the hotel.

If you would like to enter the IDs while the carrier is still in your hand, click **Enter Slide IDs**.

It will bring up this dialog:
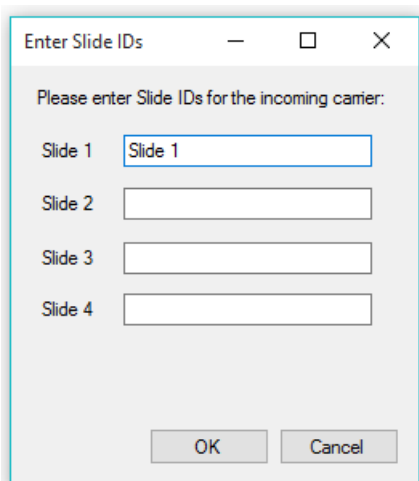
You can then enter between 1-4 slide IDs for your slides and click **OK**. Immediately put the carrier in the hotel, and Vectra Polaris will assign the slide IDs you entered to that carrier.

If your carrier is already present in the hotel, you can edit the carrier to add the slide IDs along with the other scanning rules.

Editing a Single Slide

To edit the scanning rules for a single slide, click the status icon for that slide. It will bring up this editor:

For this slide, enter:

- A task: Choose **Scan** or **Take MSI** (Note: Select **Ignore** if you do not wish to process this particular slide).
- A study: Select a study.
- A protocol: Select any protocol you have saved within the study.
- The Slide ID source: Select manual entry and add your slide ID, if necessary.

Check **Prioritize the containing carrier** if you want this slide's carrier to be scanned at the front of the line.

Editing All Slides Within a Single Carrier

To edit the scanning rules for a single carrier, click the status icon for that carrier. It will bring up this editor:

For each slide, you can enter:

- A task: Choose **Scan**, **Take MSI** or **Ignore**. (Note: select **Ignore** if there is no slide in that particular slot).
- A study: Select a study.
- A protocol: Select any protocol you have saved within the study.
- The Slide ID source: Select manual entry and add your slide ID, if necessary.

Check **Prioritize this carrier** if you want this carrier to be scanned at the front of the line.

Editing Multiple Carriers

To edit multiple carriers, Click **Configure Tasks**.
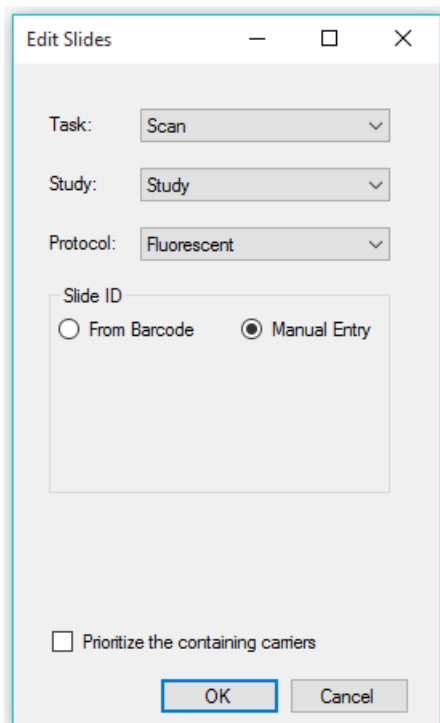
Select the carriers you want to edit. Multiple selection is available.
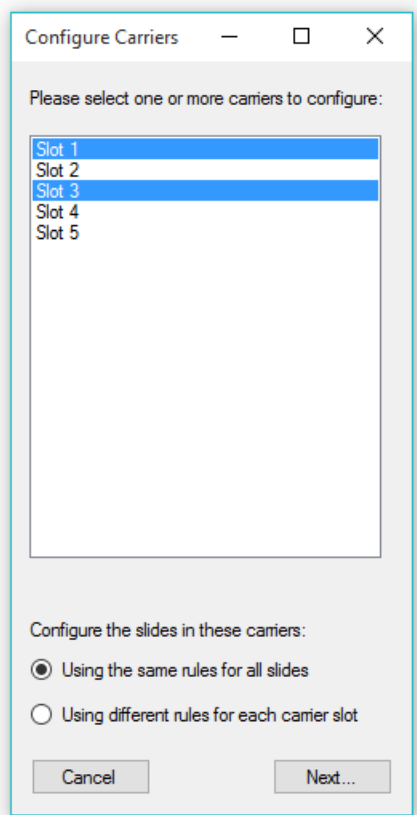
You will also need to select how you want to process your slides.

To process all slides using the same rules, choose **Using the same rules for all slides** and click **Next…** The Edit Slides dialog will appear:

- Select your task, study, and protocol.
- Select manual entry for your slide ID source.
- Check **Prioritize the containing carriers** if you want the selected carriers to be scanned at the front of the line.

NOTE

You will not be able to edit the slide ID, because you are applying rules across multiple slides. If you need to enter slide IDs, you can edit the individual carriers.

To use different processing rules for each carrier position, choose **Using different rules for each carrier slot** and click **Next**… For each position in the carrier, select the task, study, and protocol. Select manual entry for your slide ID source, and prioritize if desired. Again, you will not be able to edit the slide ID, because you are applying rules across multiple slides.
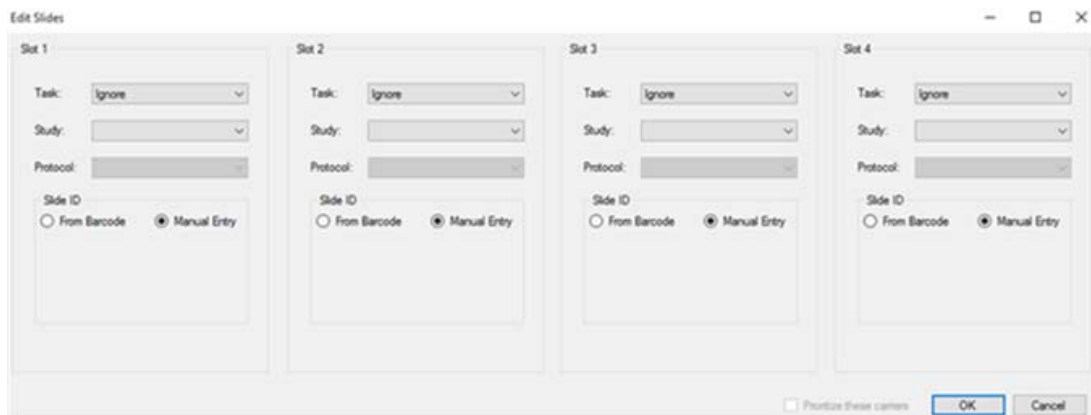
Scanning

The Scan button will enable when at least one carrier is ready to be scanned. Click **Scan** to start scanning. If any carriers have been prioritized, these carriers will be processed first.

Vectra Polaris will report scanning progress for each slide on the progress dialog and on the front panel LEDs.
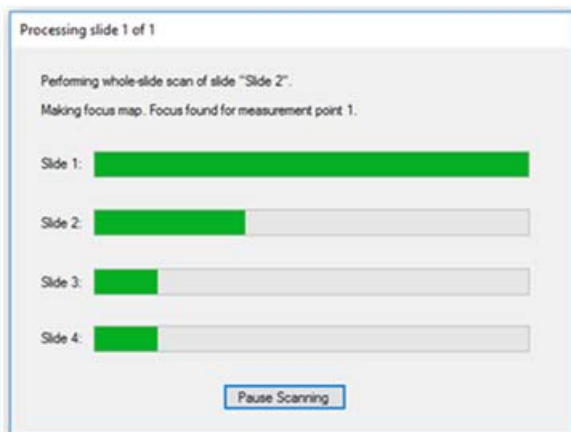
You click **Pause Scanning** to remove completed carriers and add new carriers to be scanned. When you pause a scan, Vectra Polaris will return the current carrier to the hotel. It will then be safe to open the door. You can remove carriers that are completed and replace them with unscanned carriers. Edit those carriers to set them up for scanning, and reprioritize carriers if needed. When you're ready, click **Scan** to resume scanning.

Maintenance

This section includes procedures that are to be performed periodically, either to make the Vectra Polaris run better, or to protect its components from damage.

Cleaning the Instrument Exterior

Clean the Vectra Polaris instrument exterior as necessary.

The non-electrical exterior parts of the Vectra Polaris can be wiped down with a soft cloth using standard laboratory grade cleaning solutions including:

- 70% ethanol
- 10% bleach
- Clidox
- Sporicidin

WARNING

DO NOT spray cleaning solutions directly onto the Vectra Polaris instrument. Sprays and liquids that come into contact with the Vectra Polaris instrument may result in damage to the system or electric shock. Always spray the cleaning solution onto a cloth and then wipe the instrument exterior with the cloth.

Cleaning the Monitor

Clean the monitor as necessary with a soft, lint-free cloth. If needed, dampen the cloth with water or an eyeglass cleaner.

WARNING

DO NOT spray cleaning solutions directly onto the monitor screen. Sprays and liquids that come into contact with the screen may result in damage to the system or electric shock. Always spray the cleaning solution onto a cloth and then wipe the screens with the cloth.

Cleaning the Power and Communication Ports

Dust can be removed from the power and communication ports with a gentle air stream from compressed air cans. DO NOT shake the can before use. Hold can at a distance from the ports to prevent condensation.

WARNING

Turn off the electrical power to the Vectra Polaris system by shutting down the system (see page 44) before cleaning any part of the instrument where electrical or fiber optic cables make connections.

Cleaning the Slide Carriers

It is important to keep carriers free from sticky debris caused by slide labels, tape, or mounting media.

Slide carriers can be cleaned using soap and water with a soft cloth, or using an ultrasonic bath.

Replacing the Fuses

For blown fuses and to order replacement fuses, contact PerkinElmer Technical Support (see page 3).

WARNING

Electric Shock Hazard. DO NOT attempt to replace the fuses. Only qualified PerkinElmer service personnel can replace the fuses.

Appendix A: PerkinElmer TIFF Specification

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

The goal is to use the same syntax and metadata for all these kinds of images, and minimize the semantic distinctions where possible. Specifically, the unmixed MSI images can be considered to be an idealized multiband FL image where signal corresponds to the presence of a stain or fluorescent dye in the sample.

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.

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 A-1. Images

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

Detection

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

Metadata

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

Table A-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)

Table A-2. TIFF tags

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

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

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

Image Description contents

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

Table A-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 S-1), with the following values: • FullResolution • ReducedResolution • Thumbnail • Overview • Label
IsUnmixedComponent		"True" for unmixed multispectral images, otherwise "False".
ExposureTime		Exposure time as an integer number of microseconds. For unmixed images, this is the exposure time for the dominant wavelength band for the component (FL); or the brightest wavelength in the cube (BF).

Table A-3. Image Description tags(Continued)

Tag	Optional	Contents

SignalUnits		A byte *wwww tttt* where the *tttt* nibble indicates the signal unit type from the following:
		
		    0 –raw counts
		
		    1 –normalized (counts/second/gain/full-scale/binning)
		
		    4 –OD (optical density)
		
		    5 –dark-corrected counts
		and the *wwww* nibble indicates how the signal is weighted across the spectral bands (or colors):
		
		    0 –average across all bands
		
		    2 –total summed signal across all bands
		
		    4 –peak signal in highest-valued band
		Thus, for example, a value of 68 (hex 44) encodes OD units with peak-signal weighting.

Name	Y	Band (component) name for FL or unmixed MSI images, not present for RGB images.
Color	Y	Color to use when rendering this band, as decimal r,g,b byte triplet, r FL or unmixed MSI images. Not present for RGB images.
Responsivity	Y	Instrument responsivity, if available, for FL whole-slide and unmixed images. See below for details.
Objective	Y	Objective name, if known, otherwise not present.
ScanProfile	Y	Element containing scan and/or and unmix parameters. It is valid XML whose contents are opaque to most readers. It is only provided on the first (baseline) image, and is omitted from all other IFDs.
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 MSI 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 IM3 file; typically there will be five <Band> tags. The same <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 A-4. Contents of Filter and Band tags

Tag	Contents
Name	The name of the filter (whole-slide image) or MSI band (unmixed image).
Response	The instrument response to the reference artifact, normalized for exposure. This is raw counts / ($2^{\text{bit depth}}$ × exposure time × gain × binning area), where bit depth is the bit depth of the imagery, exposure time is in seconds, gain is the gain setting of the camera, and binning area is 1 for 1×1, 4 for 2×2, etc.
Date	The date and time of the reference image in UTC, ISO 8601 format.
FilterID	Detailed description of the acquisition filter.

Sample ImageDescription

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

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

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Should you have any question concerning this Agreement, you may contact CRI by writing to CRI, 68 Elm St., Hopkinton, MA 01748. You may also call +1-508-435-9500.

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