

## Vectra Polaris Fluorescence Scanning

### How the Vectra Polaris scanner works

The Vectra Polaris is a multispectral and brightfield whole-slide scanner that is designed to apply pre-set exposures and automated tissue detection across a set of slides, at magnifications of up to 40x. For these reasons:

--Slide and stain quality are critical for successful scans. If the instructions below were not followed and you are not satisfied with the quality of the resulting scan, you will be charged for repeat scans that you request.

--Planning analysis in advance is essential for applying the appropriate staining and scanning approach.

### Planning your immunofluorescence project

There are three stages to each project:

- 1) Immunofluorescence staining: This can be done in your laboratory, or as fee-for-service at the Core.
  - a. If you plan to perform staining in your own laboratory, we can provide immunofluorescence staining protocols optimized for Vectra Polaris scanning. However, the Polaris scanner can accommodate many different staining protocols, as long as quality conditions are met.
  - b. If you are planning to analyze by InForm, we recommend that you prepare additional control slides (a single stained slide for each marker, and an unstained slide for autofluorescence).
  - c. Finally, because the same exposure settings are applied across the entire slide, and multiple coverslips are not compatible with scanner function, please do not use multiple different staining protocols on the same slide.
- 2) Immunofluorescence scanning: Our scanning approach will partly depend on whether InForm analysis will be used. Additional details on scanning are below.
- 3) Analysis:
  - a. Polaris scan files are compatible with most image analysis software. The larger size of whole slide scans can be problematic for some programs.
  - b. There is a freeware slide viewing program designed for the Vectra Polaris (Phenochart).
  - c. We can advise on the best analysis approach for your project.

### Preparing slides for scanning

- 1) Please clean slides to remove fingerprints, markers (other than blue Sharpie to circle tissue), and excess mounting solution. Marks on the coverslip over the tissue may interfere with focusing.
  - a. PAP pen (hydrophobic barrier pen) should be removed prior to coverslipping (dipping slide in xylene for about a minute and then wiping off, or by Q-tip dipped in xylene).
  - b. If hydrophobic barrier can't be removed, then alternatively can try circling with Sharpie inside the borders of the barrier, as described below
- 2) The label for each slide should display a legible, concise, unique name
  - a. Please cover protected health information on the slide label (e.g. patient name on UM Pathology clinical slides) with a sticker or additional label.
- 3) Circling tissue—Although not necessary in many cases, circles can be helpful in identifying a specific tissue section, and avoiding scan failure due to imperfections such as bubbles. However, improper circling technique may prevent successful scanning.

- a. Please use blue Sharpie on the coverslip only. Other markers (PAP pen, other colors of Sharpie, etc.) can interfere with scanning. See 1a for instructions on removing PAP pen.
  - b. Use closed circles
  - c. Do not use concentric circles
  - d. Do not allow circles to stray off the coverslip
  - e. There should be at least a small amount of space between the circle and the tissue, so that the circle does not interfere with focusing
  - f. Please allow at least 4 millimeters between the circle and the edge of the slide farthest from the label (even if this area is coverslipped)—this part of the slide will be blocked by the holder
  - g. Please clean thoroughly before circling, as ethanol cleaning cannot be done after circling without erasing the circle
- 4) Coverslipping
- a. Avoid using more than one coverslip per slide: only one of the coverslips will be scanned, and we will not be able to control which one. We can provide long coverslips if needed.
  - b. Two or more coverslips stacked on top of each other will result in scan failure
  - c. Cracks and chips in the coverslip may interfere with scan function
  - d. Avoid bubbles
- 5) If you have multiple tissue sections on the same slide:
- a. Unless a particular section is circled by blue sharpie, we cannot control which section(s) will be scanned
  - b. As noted above, please do not use multiple staining protocols on the same slide
- 6) Upon request, our staff can assist with improving the condition of your slides, for a fee based on the time required

#### Submitting your order for scanning

- 1) Ordering is through iLabs. There will also be two additional forms describing your staining panel and specific slides.
- 2) Please restrict each iLabs order to a single staining panel.
- 3) Exposures
  - a. The exposure settings for the Polaris will often be different than those for the Mantra or your fluorescence camera
  - b. For scanning, we will use one or two representative slides to generate exposure settings for each marker, that will then be automatically applied across all slides during scanning. Because of this, it is essential for you to identify slides with the strongest staining and weakest positive staining that we can use when setting exposures. Otherwise, overexposure or underexposure can result for some cases.
  - c. You are welcome to provide us with additional optional control slides (not to be scanned) that might be helpful for us when setting the exposures. There will be no charge for these.
  - d. Please let us know if you expect that uniform exposure settings will not work for your sample group (e.g. some samples will be much weaker or stronger for a particular marker), and we will discuss the best scanning strategy.
- 4) Magnification: The default magnification is 20x, which is sufficient for most analyses. 40x magnification scans can be performed with Director approval if needed. (Note that some

journals also include the 10x ocular when reporting magnification—by this approach, the default magnification is 200x).

### Using Scan Files

The Vectra Polaris generates qptIFF files that are compatible with a variety of image viewers that incorporate the BioFormats extension. Older version of some programs may require addition of BioFormats as a plug-in. Please see our “Using Polaris Scan Files” sheet for more details.

The Tissue Core will maintain backups of scan files for a limited period of time. However, you will be responsible for long-term storage of your data.

### InForm Analysis

InForm software performs spectral unmixing to provide maximum resolution between fluorescence signals in Polaris scans. This software is also capable of several analyses, including quantitation and phenotyping, on selected fields of your slide. Because of the complexity of this approach and the logistical considerations associated with the limited number of workstations that have this software, discussion with a Core director is necessary to confirm that this analysis can be used for your project. If you are interested in using InForm software, several additional steps will be required:

- 1) If you are not using stains performed by the TMP Shared Resource, please submit additional slides needed to generate a spectral library.
  - a. Unstained slide for autofluorescence
  - b. For each marker in the panel, provide one slide stained with only that single marker (if you have multiple pieces of tissue on the slide, do not apply a different marker to each tissue piece)
- 2) We recommend using OPAL approach for immunofluorescence staining
- 3) For some projects (especially if an OPAL staining approach was not used), a 2<sup>nd</sup> round of scanning may be required
- 4) Although we currently do not charge for InForm per se, please be aware that you will be charged at the usual rate for the extra scans required for this analysis