

## **Vectra Polaris Brightfield 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 project

There are three stages to each project:

- 1) H&E, immunohistochemical stains, or special stains: These can be done in your laboratory, or as fee-for-service at the Core.
  - a. 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).
- 2) Brightfield 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 and excess mounting solution. Marks on the coverslip over the tissue may interfere with focusing.
- 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
  - a. In most cases, we do not recommend circling tissue for brightfield scanning. Improper circling technique may interfere with scanning.
  - b. Circling may be helpful to avoid tissue that you do not want scanned (e.g. control TMA cores)
- 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
- 5) 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 an additional form describing your slides.
- 2) 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).
- 3) Submitting your slides
  - a. Please double-check that slides are clean, and meet conditions described in the “Preparing Slides” section
  - b. Please submit your slides in the EXACT order you would like them to be scanned, starting at slot 1 in your slide box.
  - c. Please group slides to be used for setting exposures together, preferably at the beginning of the slide set
  - d. Your slides should be returned in the order they are received; however, other aspects of slide organization (gaps between slide groups, direction of the slide labels in the box) will NOT be preserved
  - e. Please provide a hard drive for fluorescent scans

### Using Scan Files

The Vectra Polaris generates qTIFF 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.

TMPSR 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 signals in Polaris scans. This software is also capable of several analyses, including signal quantitation 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. For brightfield analyses, we have found that other software such as Qupath freeware may be preferable to InForm, as these can perform similar analyses without requiring the additional steps described below.

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 (Research Histology Core), please submit additional slides needed to generate a spectral library.
  - a. Unstained slide for autofluorescence
  - b. For each stain/chromogen, provide one slide stained with only that single stain (if you have multiple pieces of tissue on the slide, do not apply a different marker to each tissue piece)
- 2) A 2<sup>nd</sup> round of scanning will be required
- 3) 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