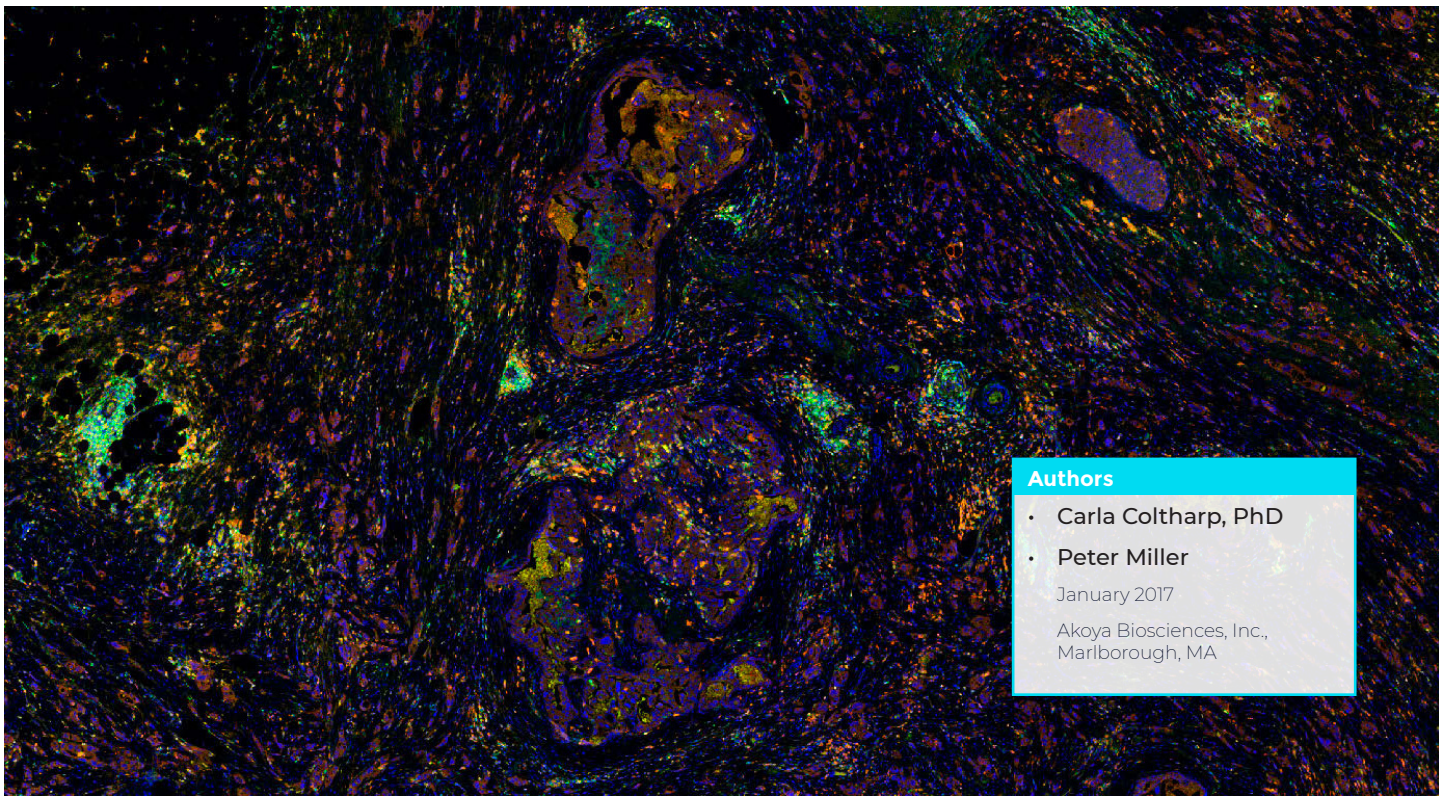
Automated Multiplex Biomarker Staining and Imaging

VECTRA POLARIS MULTISPECTRAL IMAGING AND WHOLE SLIDE SCANNING



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INTRODUCTION

Unraveling the complexity of the tumor microenvironment requires highly multiplexed assays that probe a variety of tumor and immunological biomarkers. Vectra® Multispectral Imaging Systems facilitate these assays with reliable detection up to six tissue biomarkers and a counterstain within the same sample.

The new Vectra Polaris™ Automated Quantitative Pathology Imaging System builds upon the proven multispectral capabilities of the Vectra portfolio within a highly-efficient new design. Here, we highlight features achieved by the new design, including quantitative multi-spectral imaging, seamless image tiling, and minimal photobleaching. Additionally, we discuss a new capability introduced in the Vectra Polaris – precision whole slide scanning in fluorescence and brightfield for quantitative whole-tissue analysis.

METHODS

Formalin-fixed paraffin-embedded samples of normal tonsil, primary tumors, and tissue microarrays (TMAs) were stained using Opal™ Multiplex IHC Detection Kits. Stained slides were imaged with either a Vectra 3 or a Vectra Polaris Automated Quantitative Pathology Imaging System, as noted. Multispectral images were analyzed with inForm® software.

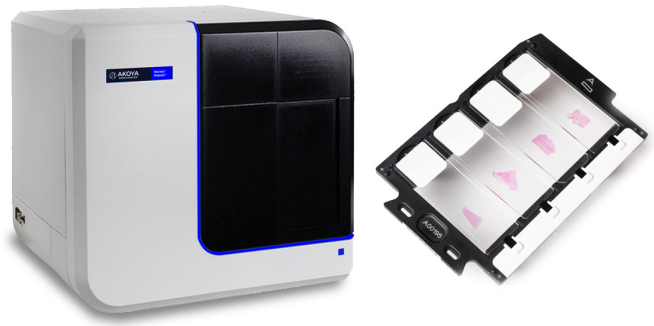

FIGURE 1. Left, Vectra Polaris fully-enclosed multispectral imaging system. Right, slide carrier used with the Vectra Polaris.

RESULTS

Vectra's proven multispectral imaging capabilities

The fully-enclosed Vectra Polaris design includes a highly-efficient optical path that incorporates the proven quantitative, multispectral capabilities of Vectra 3 (Fig. 2).

FIGURE 2

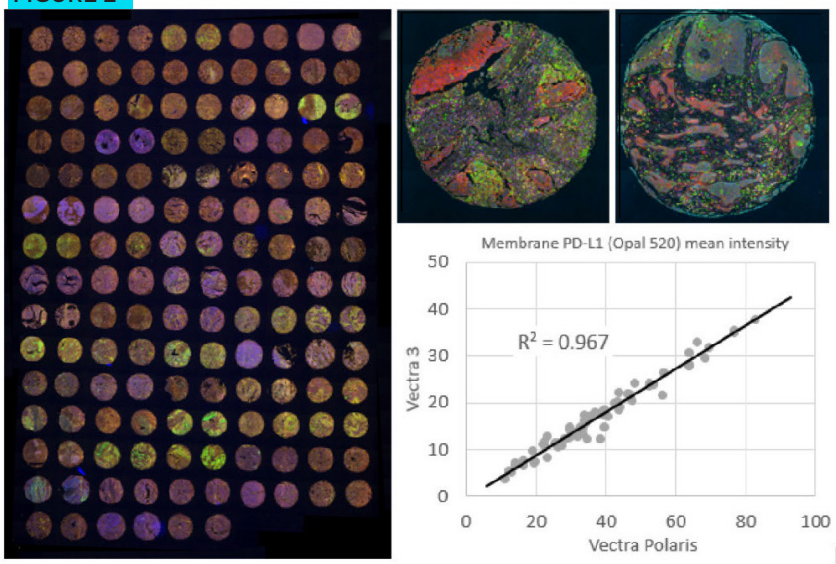

FIGURE 2. Left: Vectra Polaris whole slide scan of an Opal-stained TMA provided by John Cogswell and Darren Locke of Bristol-Myers Squibb (Lawrenceville, NJ). Right-top: example unmixed multispectral images of two cores from the array on the left, acquired on Vectra Polaris. Markers include PD-L1 (Opal 520, red), CD8 (Opal 540, yellow), CD68 (Opal 570, green), PD-1 (Opal 620, magenta), FoxP3 (Opal 650, orange), Cytokeratin (Opal 690, cyan), and DAPI counterstain (blue). Right-bottom Scatterplot showing high correlation between average PD-L1 expression measured from Vectra Polaris vs. Vectra 3 multi-spectral images. Each point represents the average PD-L1 intensity within the top 20 cells of the same TMA core, imaged on Vectra 3 vs. Vectra Polaris.

ADVANCED FEATURES FOR IMPROVED ACCURACY AND RELIABILITY

Precise stage movement generates seamless tiling (Fig. 3).

FIGURE 3

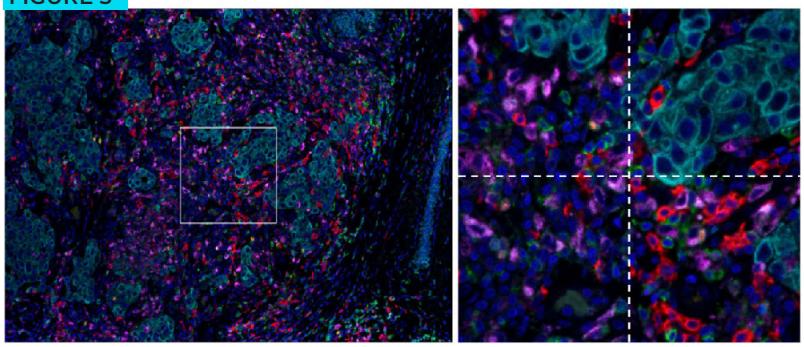

FIGURE 3. Left: Tiled 2 x 2 MSI field of human breast cancer tissue stained with the Opal 7-color Multiplex IHC Detection Kit. Right: Zoomed in view of the center of the 2 x 2 field with dashed outlines of image seams. Cells lying across seams show no tiling-related distortion.

Filter cube calibration eliminates artifacts caused by filter imperfections (Fig. 4).

FIGURE 4

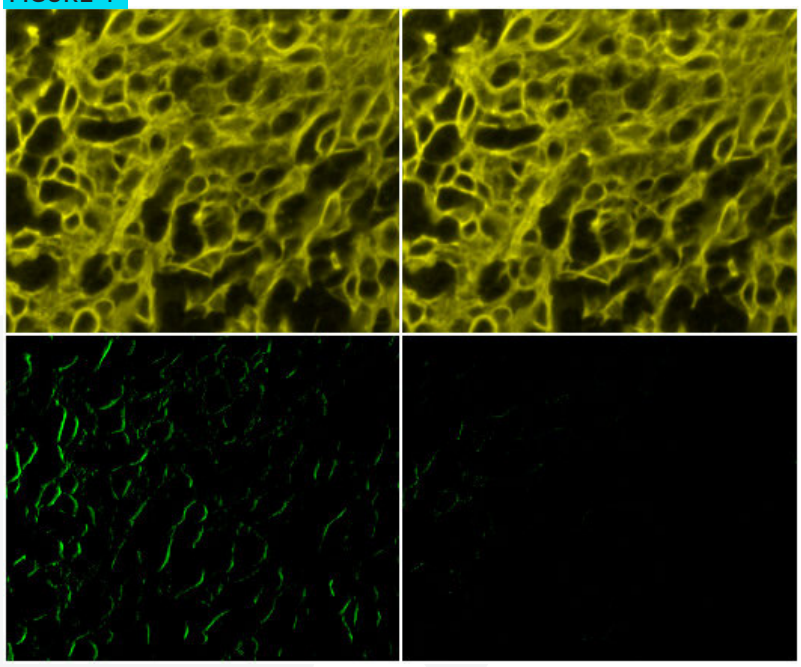

FIGURE 4. Multispectral images of a tonsil tissue section stained against cytokeratin with Opal 540, acquired on Vectra Polaris. When imaged without calibration for filter-related wedge artifacts (left), the unmixed image may show cross-talk artifacts (left, bottom) due to small misalignments between different filter cubes. After applying the Vectra Polaris filter calibration (right), wedge-related artifacts are dramatically reduced. Stained tissue provided by John Cogswell and Darren Locke of Bristol-Myers Squibb (Lawrenceville, NJ).

FIGURE 5

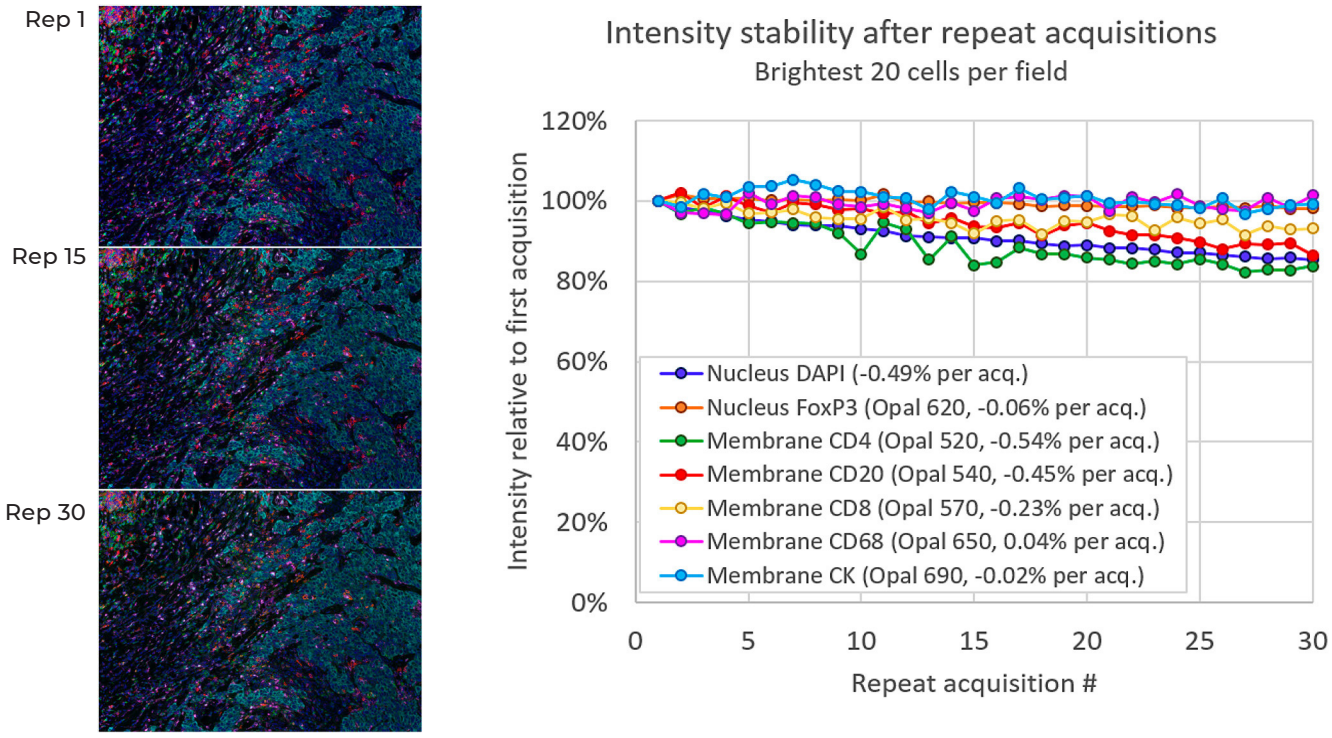

FIGURE 5. Representative unmixed images from repeated acquisitions of the same multispectral field. Images shown at the same contrast; labels indicate repeat number. Right: cell intensities relative to repeat acquisition number. After 30 repeat acquisitions Opal 620, Opal 650, and Opal 690 show negligible photo-bleaching. DAPI, Opal 520, Opal 540, and Opal 570 show minimal 0.2 - 0.5 % signal loss per acquisition.

PRECISION WHOLE-SLIDE SCANNING FOR QUANTITATIVE ANALYSIS

The advanced features of the Vectra Polaris work together to facilitate fast, accurate whole slide scans (Fig. 6).

FIGURE 6

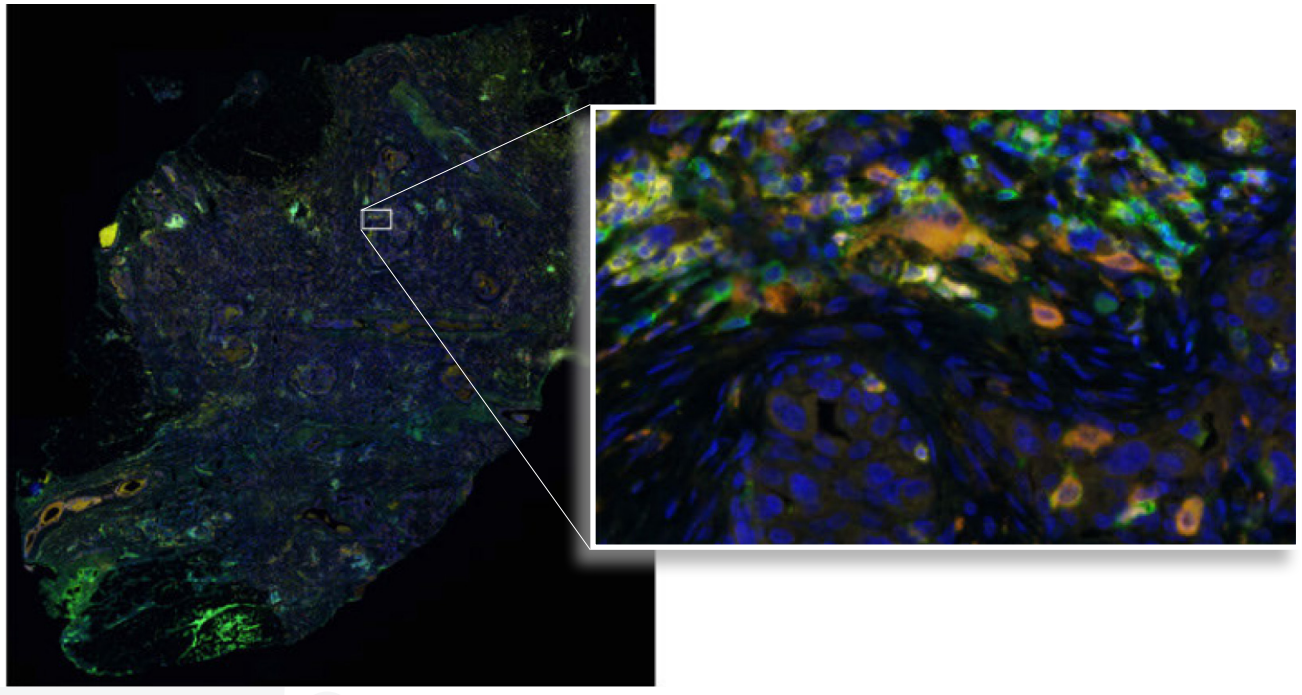

FIGURE 6. Left: 5-color whole slide scan of human breast cancer tissue stained with the Opal IHC fluorophores, acquired on the Vectra Polaris with 0.25 um resolution. Right: zoomed-in image of a small field within the whole slide scan.

TABLE 1. Specifications: Vectra Polaris vs. Vectra 3.

	VECTRA 3	VECTRA POLARIS
Multiplex Markers	•	•
LCTF Camera System	•	•
Brightfield and Fluorescence	•	•
Multichannel FL	•	•
Automated Multichannel FL	•	•
Autofocus	•	•
Operator Driven Imaging	•	•
Automated Single Slide Imaging	•	•
Automate Multislide Imaging	•	•
inForm® Tissue Analysis	•	•
Phenotyper Analysis	•	•
View Whole Slide	•	•
Phenochart Whole Slide Viewer	•	•
TMA Workflow	•	•
Whole slide scan speed	~2 hrs	~10min
Supports MOTiF workflow		•
Slide Capacity	6	80
Continuous Loading (supports higher throughput vs. Vectra 3)		•
Filter Expandability		•
Whole slide Scanning BF and FL 10x-40x		•
Closed System		•
Touchless Automation		•
Patent pending technology for error-free, superior image focusing		•
Photobleaching protection		•

Note: Table 1. has been updated to reflect the Phenoptics 2.0 update in 2018 which built upon the Vectra Polaris' whole slide scanning capability with the launch of the MOTiF workflow.

CONCLUSION

The Vectra Polaris provides improved image quality, scanning reliability, and workflow flexibility for multispectral imaging of up to 6-plex stained tissues. Additional whole-slide scanning capabilities provide additional avenues for quantitative analysis of whole tissue sections.

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