**Quantitative Pathology Imaging and Analysis**

**Key Features:**
- Visualizes, analyzes, quantifies and phenotypes immune cells *in situ* in solid tissues
- Intuitive workflow optimized for the research pathologist
- Pathology Views renders immunofluorescence images as H&E, DAB and hematoxylin
- Multiplexed biomarker analysis of tumors and tumor microenvironment *in situ*
- Compact workstation for fluorescence and brightfield
- Supports PerkinElmer’s Cancer Immunology Research Workflow Solution (staining, imaging and analysis)

**Visualize, Analyze, Quantify and Phenotype Immune Cells *in situ***

The Mantra™ quantitative pathology workstation, with inForm® image analysis software, enables easy visualization, quantification and phenotyping of multiple types of immune cells simultaneously in intact FFPE tissue sections for cancer immunology research.

Mantra is a compact workstation where a researcher can phenotype immune cells *in situ* in solid tumors using multiplexed biomarkers. Unlike existing flow cytometry and next generation sequencing methods, which can phenotype and quantify immune cells in homogenized samples, Mantra similarly does this using images of FFPE tissue sections, while maintaining tissue architecture and morphology. This provides spatial information which could lead to a better understanding of the role and types of immune cells within both the tumor and the tumor microenvironment so that new cancer immunotherapy treatments may be identified and researched.

The Mantra is an integrated workstation incorporating multispectral imaging technology, novel image acquisition and inForm analysis software. It can be used with a variety of stains including PerkinElmer’s Opal™ reagent kits. The Mantra system is a part of PerkinElmer’s cancer immunology research portfolio which includes staining reagents, imaging systems, and image analysis software.
Workflow Solution for Phenotyping Immune Cells in situ using Mantra

The Mantra workstation is a part of PerkinElmer’s quantitative pathology solution for cancer immunology research that includes multiplexed staining reagents and methodologies, instrumentation with unique channel-separation technology, and advanced image analysis software that can be trained to phenotype cells within the tumor microenvironment.

Stain

*Reveals Multiple Tissue Biomarkers in Context*

The Mantra can be used with a variety of biomarker reagents for multiplexed imaging. PerkinElmer’s cancer immunology workflow solution has been validated using Opal methodology. The Opal approach enables the specific staining of multiple tissue biomarkers reaching up to 6-plex and beyond in a single tissue section.

It provides researchers with tools to simultaneously interrogate multiple pathways while retaining context provided by tissue images. This approach provides information that is not available from alternative techniques like flow cytometry or analysis of single markers in serial sections. The method is compatible with the standard IHC workflow in your lab and is amenable to automation.

Opal reagents kits use sequential covalent labeling followed by removal of antibodies. Removal of antibodies has minimal impact on the signal and clears the tissue to be probed with another primary antibody without fear of cross-reactivity.

Image and Visualize

*Visualize Immune Cells in situ*

The Mantra workstation is an easy-to-use and compact instrument for the visualization of immune cells in situ within FFPE tissue sections. This multispectral imaging system can seamlessly transfer data, following algorithm development, from image to analysis, performing automatic phenotyping without user intervention. This enables immediate visualization of immune cells in the tumor and tumor microenvironment, thereby accelerating your research.

Features of the Mantra Workstation:
- Images and separates up to eight labels simultaneously enabling more robust biomarker quantification to support multiplexed analysis
- Intuitive Mantra Snap software for integrated data transfer to inForm analysis software saving time
- Reduces autofluorescence through powerful multispectral imaging technology
- Pathology Views™ for visualization of fluorescence imagery in traditional brightfield mode (H&E, DAB and hematoxylin)
- Automated objective and filter cube changing for quicker data results
- Small footprint that fits on any benchtop
- Expanded NIR imaging range to 750 nm for use with dyes such as Alexa Fluor 750 and Cy7

![Figure 1. PerkinElmer’s integrated cancer immunology research workflow solution.](image1)

![Figure 2. Opal schematic (left). Breast cancer tissue using Opal reagent kits. Blockade marker 1 overlaid on DAPI (A), CD8 signal overlaid on DAPI (B), CK signal overlaid on DAPI (C) and composite image of all markers overlaid onto DAPI (D). Additional markers where used, FOXP3, and blockade 2 & 3 (data not shown).](image2)

![Figure 3. Mantra Workstation.](image3)

![Figure 4. Tonsil stained with marker and Alexa Fluor 750 reporter dye (20x objective). Sample courtesy of Radu Stan, MD, PhD, DHMC.](image4)
Quantify and Analyze

Accurately quantify biomarkers in tissue sections with inForm Tissue Finder™, our patented automated image analysis software. It automates the detection and segmentation of specific tissue types using patented user-trainable algorithms that can recognize morphological patterns. inForm Tissue Finder includes per-cell phenotyping functionality to differentiate marked cell types (e.g. T-cells, macrophages) within a segmented area (e.g. tumor versus stroma). Mantra comes with two seats of inForm Tissue Finder.

Features of inForm Tissue Finder Software:
• User-trained feature recognition algorithms enables automatic identification of specific tissue types based on tissue morphology
• Quantitative per-cell analysis of biomarker expression in tissue sections and TMA's
• Separation of weakly expressing and overlapping markers
• Cellular analysis of H&E, IHC and immunofluorescence in FFPE tissues
• Per-cell phenotyping to differentiate cell types
• Pathology Views: creates brightfield pathology (H&E, DAB and hematoxylin) renderings from the original fluorescence image
• Automatic behind-the-scenes processing of Mantra data while continuing to capture new images

Figure 5. Helper T-cells and cytotoxic T-cells in breast cancer: CD4+ T-cells green, CD8+ T-cells in stroma (left). CD4+ T-cells green, CD8+ T-cells in tumor (right).

Figure 6. Pathology View rendering of original fluorescence image, seen as if it were in standard brightfield mode, providing a familiar view for a pathologist used to seeing H&E, DAB and hematoxylin.

Visualize immune cell phenotypes in the context of your tissue

Many types of immune cells play a key role in cancer tumor growth. Until recently it was difficult, if not impossible, to phenotype immune cells in solid tumors while maintaining cellular spatial relationships and morphological context. Both flow cytometry and next-gen sequencing can phenotype cells, but only in disaggregated tissues, and standard pathological analyses can deliver morphology without being able to analyze complex phenotypes.

PerkinElmer’s in situ multiplexed biomarker staining methods, imaging systems, and image analysis software enable multiple cell phenotypes to be studied simultaneously in solid tumors and their microenvironment. This capability enables the quantification of cancer-immune interactions to help researchers reveal disease mechanisms that might be in play. This can help with discovering and validating biomarkers, providing better data to support research into potential subpopulation stratification methodologies.

Figure 7. Breast cancer tissue showing presence of helper and cytotoxic T-cells in and out of tumor and stroma using inForm software. Upper left: Unmixed composite image with cytokeratin (cyan), killer T-cells (purple), helper T-cells (green), B-cells (red), PD-L1 (orange), FOXP3 (yellow). Bottom right: Segmented cell and tissue phenotypes and counts. Upper right: Color key for phenotyped cells. Lower right: Cell count by phenotype.

Work performed in collaboration with Dr. Beth Mittendorf at MD Anderson.
**Table 1. Mantra Specifications**

<table>
<thead>
<tr>
<th><strong>Mantra Instrument</strong></th>
<th>Imaging Modes</th>
<th>Brightfield and fluorescence, multispectral and color imaging</th>
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<tbody>
<tr>
<td></td>
<td>Microscope</td>
<td>Customized research-grade microscope with:</td>
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<tr>
<td></td>
<td></td>
<td>- 8-position motorized epi-fluorescence filter turret</td>
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<tr>
<td></td>
<td></td>
<td>- 7-position motorized objective nosepiece</td>
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<tr>
<td></td>
<td></td>
<td>- Active management of intensity per-objective in brightfield</td>
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<tr>
<td></td>
<td></td>
<td>- Integrated ergonomic controls on microscope base</td>
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<tr>
<td></td>
<td>Camera Type</td>
<td>Multispectral 12-bit camera using cooled scientific-grade CCD</td>
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<tr>
<td></td>
<td>Camera Spectral Range</td>
<td>440 - 720 nm for brightfield imaging and visible fluorescent dyes</td>
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<tr>
<td></td>
<td></td>
<td>770 nm for NIR dyes such as Cy7, Alexa Fluor 750 and similar</td>
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<tr>
<td></td>
<td>Objectives (Apochromatic)</td>
<td>4X / 0.16 NA, 10X / 0.40 NA, 20X / 0.75 NA, 40X / 0.95 NA optional</td>
</tr>
<tr>
<td></td>
<td>Pixel Resolution</td>
<td>0.25 micron (optional 40X), 0.50 micron (20X), 1.0 micron (10X), 2.5 micron (4X)</td>
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<tr>
<td></td>
<td>Filter Cubes</td>
<td>DAPI, FITC, Cy3, Texas Red, Cy5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cy7, Quad-band (DAPI / FITC / TRITC / Cy5) optional for eyepiece visualization</td>
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<tr>
<td></td>
<td>Fluorescence Illumination</td>
<td>Metal halide 200W lamp with liquid light-guide, 2000 hour bulb life</td>
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<tr>
<td></td>
<td>Brightfield Illumination</td>
<td>High-output, color-balanced white LED</td>
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</tbody>
</table>

**Physical Dimensions**

| Width x Height x Depth | 163 x 76 x 56 cm (Mantra workstation including: monitor, computer, keyboard/mouse, fluorescence illuminator) |
| Width x Height x Depth | 145 x 76 x 56 cm (as above without computer) |
| Weight                | 24 kg (Mantra only) |

**Computer (Minimum Specifications)**

| General               | Quad-core, 16 GB RAM, i5 processors, mini-tower style |
| Hard Disk Drives (2)  | 500 GB for operating system and programs, 1 TB for data storage |
| Operating System      | Windows 7/64 |
| Interfaces            | 1 Gigabit Ethernet, USB |
| Monitor               | 29" flat-panel, 2560 x 1280 pixel resolution |

**Software**

| Acquisition Software  | Mantra Snap (1 seat) |
| Image Analysis Software | inform Tissue Finder (2 seats) |

**Power Requirements**

| Mantra Instrument - AC Input Power | 100 - 230 VAC, 1.0A, 50/60 Hz |
| Fluorescence Illuminator - Input Power | 100 - 240 VAC, 2.0A, 50/60 Hz |

For research use only. Not for use in diagnostic procedures.

For more information, please visit www.perkinelmer.com/Mantra

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