

Mantra™ | From Biomarker Discovery to Assay Validation and Beyond

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Introduction

Over the past several years, multiplex immunofluorescence (mIF) has played a vital role in elucidating novel immune- tumor interactions and identifying targets of interest for drug discovery and development, as well as predictive biomarkers for patient stratification. In cancer research, advancing our understanding of the underlying mechanisms driving disease progression is key to developing new therapeutic regimens and improving patient outcomes. As researchers continue to push the boundaries of mIF, it is critical that the corresponding technology expand to meet their needs.

As an integral part of Akoya's Phenoptics 2.0™ workflow, the Mantra Quantitative Pathology Workstation is the ideal candidate for digital pathology users who are transitioning from conventional immunohistochemistry to multispectral imaging. The Mantra provides an excellent foundation for delving into the world of multiplex assay development and biomarker discovery. Similar to its high-throughput counterpart, the Vectra® Polaris™, the compact Mantra comes equipped to visualize and analyze tissue architecture and morphology in formalin-fixed, paraffin-embedded (FFPE) tissue, and phenotypes and quantifies immune and tumor cells in situ.

Upgrade and Unlock the Full Potential of the Mantra

The Mantra upgrade includes a few notable instrument updates, including the replacement of the halogen lamp for a 6-channel LED illuminator. With an expected life of >20,000 hours, the LED illuminator will not require annual replacement compared to its halogen counterpart. More importantly, LED's do not exhibit the characteristic dimming over time, typical of halogen lamps, thus reducing spectral unmixing variability and the constant demand to create new library slides to compensate for light excitation discrepancies. Furthermore, LED lights utilize 75% less energy compared to halogens and do not produce excessive heat.

The Mantra Upgrade Kit (CLS152356) includes:

- 6-channel LED illuminator
- Light Guide (connection cable)
- Adaptor
- Texas Red/mCherry Excitation Filter
- Opal 780 Filter Cube
- Opal 480 Filter Cube

Building upon our earlier launch of Phenoptics 2.0, we are excited to introduce several new upgrades to the Mantra, including a 6-channel LED illumination source (Figure 1), two new filter cubes to detect Opal™ Polaris 480 and 780 (Figure 2), and a new excitation filter for Texas Red/mCherry. These upgrades now reveal the Mantra's full capabilities, enabling users to increase their multiplex panel to 9-colors and allowing for greater interrogation of their tissue samples like never before.



FIGURE 1. The 6-channel LED illuminator. The device has a life of >20,000 hours and provides a consistent excitation light source that will help to reduce variability caused by halogen lamp dimming over time. Two power settings are available depending on your workflow needs.

In conjunction with the light upgrade are the introduction of two new narrowband filter cubes to detect the Opal Polaris 480 and Opal Polaris 780 fluorophores, as well as a new Texas Red/mCherry excitation filter. With the inclusion of these components, the Mantra is now able to support 8-plex, 9-color assays, promoting deeper investigation of the underlying biology driving disease progression and hypothesis-driven biomarker discovery (Figure 3). Users will be able to identify more than 500 cell phenotypes, observe multiple cell-to-cell interactions, and unveil greater morphological details and spatial complexities than ever before.

Moreover, upgraded Mantras will now have comparable specifications to the Vectra Polaris. With the launch of Phenoptics 2.0, MOTIF™



FIGURE 2. Two new filter cubes unlock the potential to conduct 8-plex, 9-color assays using our Opal Polaris 480 and Polaris 780 fluorophores.

technology was introduced exclusively for the Vectra Polaris, which refers to automated whole slide rapid scanning of the FFPE slide using multi, narrowband filters, as well as an improved streamlined data analysis workflow. Because the Mantra is manual and only acquires multispectral fields of view using a liquid crystal tunable filter, the automated

MOTiF whole slide scan technology cannot be adopted, however, with the upgrade, users will be able to develop multiplex assay panels on the Mantra which can then be readily transitioned to the Vectra Polaris. This facilitates a swifter workflow, freeing up the Vectra Polaris for more high-throughput projects.

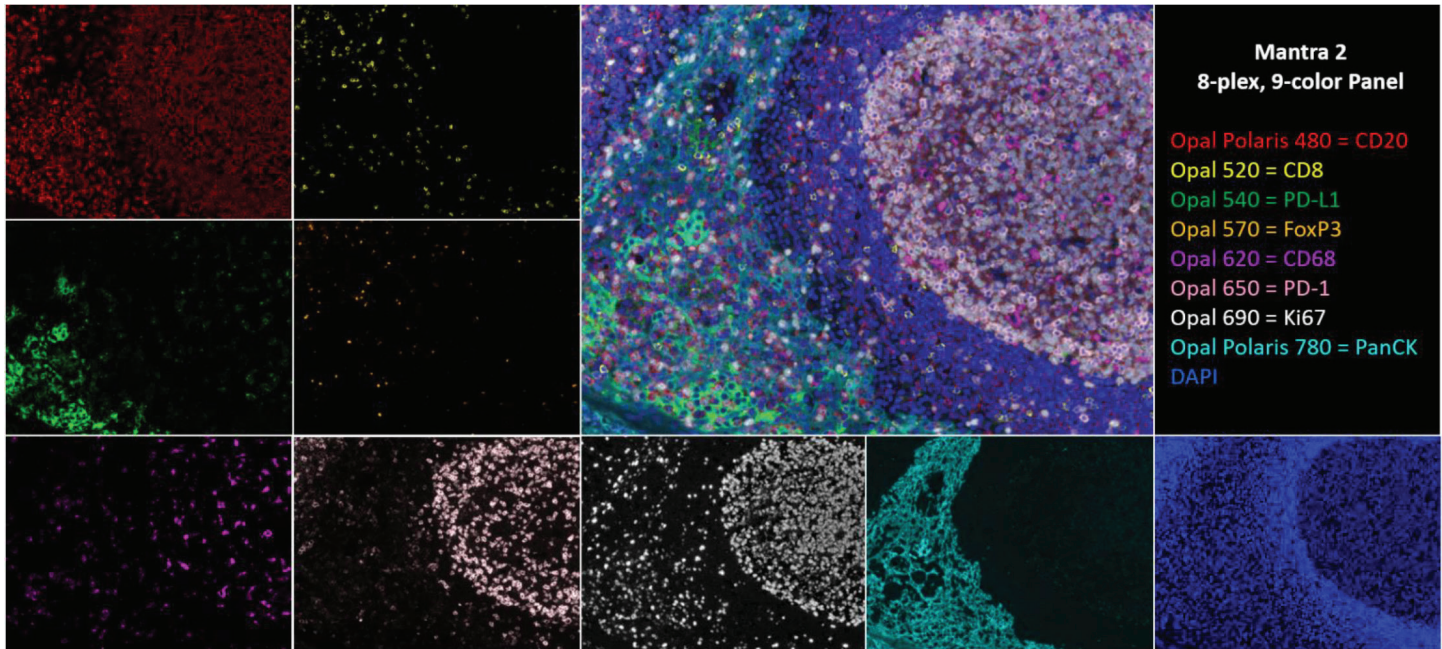


FIGURE 3 The Mantra Upgrade (6-channel LED illuminator along with two new filter cubes and excitation filter) expands your multiplexing capabilities and allows for greater interrogation of tissue samples. 8-plex, 9-color tonsil composite image with respective channel monoplex images: Opal Polaris 480 = CD20 (red), Opal 520 = CD8 (yellow), Opal 540 = PD-L1 (green), Opal 570 = FoxP3 (orange), Opal 620 = CD68 (purple), Opal 650 = PD-1 (pink), Opal 690 = Ki67 (white), Opal Polaris 780 = PanCK (teal), and DAPI counterstain (blue).

Two Power Settings to Meet Your Workflow Needs

At Akoya Biosciences, we strive to ensure that any upgrades to our Phenoptics 2.0 workflow yields greater results with minimal impact on your experiments. To that end, we recognize that Mantra users have different research needs spanning biomarker discovery in preclinical work to multiplex assay development for the clinical setting. Because of this, we are pleased to offer two power setting options for our LED 6-channel illuminator.

The first offering is a low-power option that mimics a new halogen bulb. This setting is ideal for users who want to maintain consistency with previous or ongoing Mantra-based research projects. Our second option is a high-power setting that mimics the Vectra Polaris illumination settings. This option is recommended for users who currently have, or want to have greater compatibility to, a Vectra Polaris. The high-power setting will assist in streamlining the assay multiplex development optimization and transition starting from the Mantra to the Vectra Polaris. Moving forward, all new Mantra’s will come configured with the high-power setting and be branded as the Mantra.

Opal Target Brightness Counts and Intensity Percent Differences to Expedite Your Fluorophore Optimization

It is recommended that this upgrade be conducted between projects, however, that may not always be possible. Displayed below are tables demonstrating individual Opal

fluorophore target brightness (Table 1) as well as fluorophore signal intensity differences for each fluorophore under different Mantra illumination parameters (Table 2 and 3). These tables will provide a starting point for titrating Opal fluorophore concentrations without having to undertake additional laborious experiments for re-optimization of previously developed multiplex panels.

Opal Fluorophore	Target Brightness Counts (Average)
Opal Polaris 480	20
Opal 520	20
Opal 540	20
Opal 570	20
Opal 620	20
Opal 650	20
Opal 690	20
Opal Polaris 780	5

TABLE 1. Target brightness counts for the Mantra with LED.

	Percent Intensity Brightness Relative to Mantra Legacy		
Opal Fluorophore	Mantra Legacy (Halogen)	Mantra (LED, Low Power)	Mantra (LED, High Power)
DAPI	100%	180%	580%
Opal 520	100%	100%	330%
Opal 540	100%	120%	310%
Opal 570	100%	50%	50%
Opal 620	100%	80%	50%
Opal 650	100%	100%	190%
Opal 690	100%	140%	400%

TABLE 2. Percent differences in brightness intensities relative to the Mantra Legacy (halogen with original filters and excitation filter) compared to the Mantra (LED with two power settings and upgraded filter cubes and excitation filter); low to mimic a new halogen bulb, and high to replicate the Vectra Polaris. Users will select which upgrade setting they would prefer based on their current workflow. Halogen lamp age: 350 hours.

	Percent Intensity Brightness Relative to Mantra LED High Power		
Opal Fluorophore	Mantra (LED, High Power)	Vectra Polaris	Mantra (LED, Low Power)
DAPI	100%	80%	30%
Opal Polaris 480	100%	150%	30%
Opal 520	100%	90%	30%
Opal 540	100%	80%	50%
Opal 570	100%	100%	110%
Opal 620	100%	100%	170%
Opal 650	100%	80%	50%
Opal 690	100%	90%	40%
Opal Polaris 780	100%	80%	30%

TABLE 3. Percent differences in brightness intensities relative to the Mantra (LED) with high power settings to the Vectra Polaris, and the Mantra (LED). Mantra's with the complete upgrade, including LED and filter cubes and excitation filter, will produce similar output results to that of the Vectra Polaris. This will help to expedite multiplex assay development and streamline the entire workflow.

UPGRADE YOUR EXPERIENCE TODAY

Ready to advance your Mantra experience? Still have questions? Please feel free to reach out to your Technical Application Scientist or contact Customer Care at customercare@akoyabio.com where we can assist you in all your Mantra needs. This upgrade can only be performed by field service engineers and needs to be scheduled accordingly.

To learn more visit AKOYABIO.COM
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