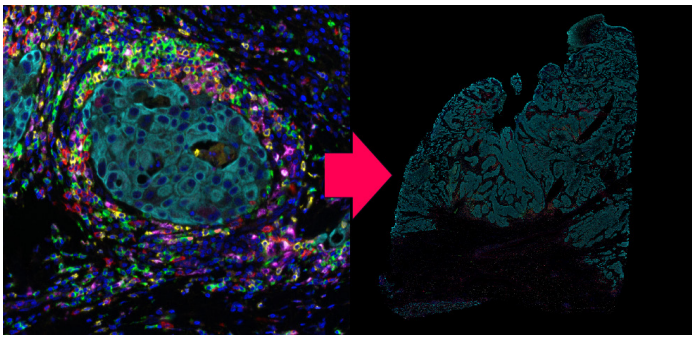
Field of View to Whole Slide Multispectral Imaging

Converting your Classic Opal Assays to the MOTiF Workflow

WELCOME TO THE WORLD OF WHOLE SLIDE MULTISPECTRAL IMAGING!



From unmixed multispectral field of view to unmixed multispectral whole slide imaging and analysis

Introduction

The MOTiF workflow is an exciting upgrade encompassing the Vectra Polaris Imaging System and two new Opal fluorophores that facilitate a greater understanding of biology driving disease states. Here, we are excited to showcase the two newest Opal fluorophores: Opal Polaris 480 and Opal Polaris 780. These fluorophores come standard as part of our new 7-Color Opal Polaris Multiplex kits including the MOTiF PD-1/PD-L1 Panel Kits for Lung Cancer or Melanoma, or may be purchased individually to use in conjunction with the existing 7-Color Opal Detection kits for 8-plex, 9-color assays. In addition, the Vectra Polaris imaging platform allows researchers to conduct rapid 7-color whole slide multispectral imaging, with the additional capability of capturing up to eight IHC targets of interest plus DAPI nuclear stain. With these upgrades, researchers will be able to identify more cellular phenotypes of interest, assess their functional states, measure spatial relationships, and see both whole-slide architecture as well as cell-to-cell interactions.

MOTiF™ technology provides:

- 7-color Whole Slide Multispectral Imaging (MSI) in less than 10 minutes
- Utilizing signal unmixing, multispectral imaging enables the identification and downstream quantification of multiple overlapping biomarkers (up to 8) without the interference of autofluorescence.
- Whole Slide MSI with the capability of unmixing all the spectra and autofluorescence in a live unmixing preview in Phenochart
- Simplified whole slide workflow that allows for importing of annotations from scanned slides in Phenochart directly into inForm, bypassing the .im3 acquisition
- 8-plex, 9-color multiplexing opportunity with Opal Polaris 480 and Polaris 780 fluorophores

Fluorophore	Opal Multicolor IHC Kits			Excitation (nm)	Emission (nm)	Part#
	4-color	7-color	Polaris 7-color			
Spectral DAPI	✓	✓	✓	368	461	FP1490
Opal Polaris 480			✓	450	500	FP1500001KT
Opal 520	✓	✓	✓	494	525	FP1487001KT
Opal 540		✓		523	536	FP1494001KT
Opal 570	✓	✓	✓	550	570	FP1488001KT
Opal 620		✓	✓	588	616	FP1495001KT
Opal 650		✓		627	650	FP1496001KT
Opal 690	✓	✓	✓	676	694	FP1497001KT
Opal Polaris 780			✓	750	770	FP1501001KT

The main advantage of MOTiF is that now the whole slide has been imaged multispectrally, so you don't have to go back and select specific fields of interest, reducing sampling bias or data loss.

Classic Opal 7-Color (.im3)	Intensity in New Relative to Classic	New Opal Polaris 7-Color MOTiF (.qptiff MSI)
Opal 520	➔100%➔	Opal 520
Opal 540	➔180%➔	Opal Polaris 480
Opal 570	➔50%➔	Opal 570
Opal 620	➔70%➔	Opal 620
Opal 650	➔2%➔	Opal Polaris 780
Opal 690	➔90%➔	Opal 690

This table will help researchers develop strategies to reoptimize their previous protocols based on relative intensities of dyes in MOTiF scanning. With established protocols, you may need to change your antibody-fluorophore pairings and/or order of staining. Use this as your guide whether you are switching from the traditional Opal workflow or developing a new panel.

Opal users can easily integrate the Opal Polaris 480 fluorophore into their already established protocols for formalin fixed paraffin embedded tissue. Staining with Opal Polaris 480 follows similar steps in the workflow schematic to Opal fluorophores 520, 540, 570, 620, 650, and 690, as the Opal signal will not be detrimentally affected by heat treatment for antibody removal. Inclusion of Opal Polaris 780 is a two-step process that is necessary to complete after all other Opal fluorophores have been used. Opal Polaris 780 utilizes an antibody-based staining step for further amplification, and heating of the slide cannot be performed after it has been applied to the marker of interest.

Of note:

1. Opal reagents come as a lyophilized powder that must be reconstituted to a stock solution using 75uL of DMSO, provided in the kit (Opal 780 is reconstituted in 300uL diH2O).
2. Subsets of the MOTiF panels (like the four-color kits) can also be used in the whole slide workflow, as long as there's no Opal 540 or Opal 650 on your sample.
3. Rare vs. Abundant markers: Low expressers should be assigned to brighter fluorophores, while more abundant markers should be allotted to dimmer fluorophores (see table to the right). For example, pair a high expressor with a low signal intensity Opal (PanCk with 780) and vice versa (PD-1 with 480).

Opal Fluorophore	Brightness Ranking (Vectra Polaris 9-color)
Opal Polaris 480	Highest
Opal 520	Highest
Opal 540	Medium
Opal 570	Medium
Opal 620	Medium
Opal 650	Highest
Opal 690	Low
Opal Polaris 780	Lowest

Don't miss a single cell with our MOTiF Open Detection Kits for manual or automated use and Immuno-Oncology Ready-to-Use Panel Kits. Order online today at www.akoyabio.com.

Product #	Name	Size
NEL861001KT	Opal Polaris 7 Color Manual IHC Detection Kit	50 Slides
NEL871001KT	Opal Polaris 7 Color Automation IHC Detection	50 Slides
OP-000001	MOTiF PD-1/PD-L1 Panel: Auto Lung Cancer	50 Slides
OP-000003	MOTiF PD-1/PD-L1 Panel: Auto Melanoma	50 Slides

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