

# Detecting Protein Biomarkers in Tissue with the Hyperion Imaging System

## Frequently Asked Questions

### APPLICATIONS

#### How many markers can be detected using the Hyperion Imaging System?

The Hyperion™ Imaging System is capable of simultaneous detection of 4 to 37 protein markers at subcellular resolution.

#### What samples can I use with the Hyperion Imaging System?

The Hyperion Imaging System can image biological samples including frozen or formalin-fixed, paraffin-embedded (FFPE) tissue sections or cell smears (liquid biopsies) deposited onto glass slides.

#### What is the resolution of the Hyperion Imaging System?

The Hyperion Imaging System directs a laser beam focused at 1  $\mu\text{m}$  to sample proteins stained with metal-tagged antibodies and directs these metal tags to analysis by inductively coupled plasma time-of-flight (TOF) technology, the basis of CyTOF® mass cytometers. A discrete signal from each ionized metal tag is detected based on differences in mass instead of wavelength, and at 1 Da resolution with minimal background.

#### Can I upgrade my Helios to a Hyperion Imaging System?

If you are a Helios™ customer, then an upgrade to the Hyperion Imaging System will offer imaging capabilities. Customers with CyTOF 1 or CyTOF 2 systems will need to trade in or

upgrade to a Helios to perform the Hyperion Imaging System upgrade. Contact your area specialist for this upgrade.

## **If I upgrade my Helios to a Hyperion Imaging System, can I still use my mass cytometer to analyze cells in suspension?**

The Hyperion Imaging System allows you to switch between suspension mode and imaging mode. For more details contact your regional sales team.

## **If I find an antibody in the current Maxpar catalog that I want to use for suspension cells, will it work with my tissue?**

Some antibodies in the Maxpar® catalog for CyTOF and Helios instruments may work for Imaging Mass Cytometry™ on frozen tissues but few are likely to work on FFPE sections. This is due to the fact that epitopes recognized by antibodies selected for flow applications are often preserved in frozen sections, but rarely in formalin-fixed sections.

## **WORKFLOWS**

### **Have you tested these antibodies using automated staining protocols?**

Fluidigm has not tested the antibodies using automated staining protocols, but some of our customers are experimenting with these. Contact Technical Support to discuss your specific questions.

### **Have you verified antibody performance with shorter staining times?**

Some of these clones have been found to give appropriate staining at room temperature with up to 2 hours of staining. Incubation at room temperature can lead to increased background for some antibodies. However, we have not systematically tested this option and recommend that customers test this with their own tissues and include the appropriate controls.

### **Do you recommend alternate methods for heat-mediated antigen retrieval, such as pressure cookers or microwaves?**

Our protocol utilizes a simple, easy-to-replicate heating block approach. However, individual customers have successfully adopted alternative methods using pressure cookers or microwaves. There is a significant amount of variability among these alternative methods, and therefore it is difficult to guarantee reproducible results. Fluidigm has not yet validated these approaches.

## CHEMISTRY

### **Does Fluidigm manufacture its own antibodies?**

Fluidigm sources purified antibodies from reputable vendors with established expertise in manufacturing and verifying antibodies for tissue imaging applications. Fluidigm does not manufacture its own antibodies.

### **What if I have my own antibodies that I want to use?**

If a desired antibody is not yet present in our catalog, you can conjugate it yourself using our Maxpar antibody labeling kits, or you can use our custom antibody conjugation services. For more information, contact your sales representative.

## CONSUMABLES

### **What antibodies are available for the Hyperion Imaging System?**

Fluidigm has developed 60+ pathologist-verified metal-conjugated antibodies optimized for use with the Hyperion Imaging System. Our initial focus has been to develop antibodies primarily used for immunotherapy and oncology Imaging Mass Cytometry applications.

### **When will more antibodies be available for the Hyperion Imaging System?**

We will release additional antibodies in 2017 shortly after the launch of the system, and we will continue to offer an ever-growing list of antibodies for Imaging Mass Cytometry optimized for use with the Hyperion Imaging System.

### **What ranges of tissues and dilutions have been tested for each antibody?**

Each antibody has a unique testing plan that includes at least one positive and one negative control tissue, typically chosen on recommendation from the original antibody vendor. Many clones have been screened against other tissues, including tumor samples. The positive control tissue staining is demonstrated along with a recommended dilution factor on the individual technical data sheet. For greater technical details on each individual clone, contact Technical Support.

## How do you perform quality control?

Fluidigm conjugates the metal tag to the sourced antibodies and performs quality control verification on the Hyperion Imaging System for correct labeling of the expected cell types in tissues appropriate for the clones.

### For technical support visit [fluidigm.com/support](https://fluidigm.com/support).

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | [techsupport@fluidigm.com](mailto:techsupport@fluidigm.com) Latin America +1 650 266 6100 | [techsupportlatam@fluidigm.com](mailto:techsupportlatam@fluidigm.com)  
Europe/Middle East/Africa/Russia +44 1223 859941 | [techsupporteurope@fluidigm.com](mailto:techsupporteurope@fluidigm.com) China (excluding Hong Kong) +86 21 3255 8368 | [techsupportchina@fluidigm.com](mailto:techsupportchina@fluidigm.com)  
Japan +81 3 3662 2150 | [techsupportjapan@fluidigm.com](mailto:techsupportjapan@fluidigm.com) All other Asian countries/India/Australia +1 650 266 6100 | [techsupportasia@fluidigm.com](mailto:techsupportasia@fluidigm.com)

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