Leids Universitair Medisch Centrum

Imaging Mass Cytometry (IMC) enables the simultaneous analysis of >40 parameters in fixed tissue sections using metal-tagged antibodies and routine immunohistochemical procedures. IMC can only be performed using the the **Hyperion™ Imaging** platform.

The Hyperion Imaging platform is composed of two modules: a *Hyperion Tissue Imager* and a *Hyperion CyTOF® system*. The Hyperion Tissue Imager liberates antibodies bound to specific proteins of interest in select regions of fixed tissue sections or cell smears and transports them to the Hyperion CyTOF system for analysis. The Hyperion CyTOF system (formerly branded as Helios®), is a mass cytometer that employs state-of-the-art Time-of-Flight Inductively Coupled Plasma mass spectrometry (ICP-MS) technology in combination with elemental tags that have higher molecular weights than those elements that are naturally abundant in biological systems. In this way, the Hyperion CyTOF has the exquisite ability to simultaneously resolve >40 elemental probes at high acquisition rates without the need for compensation associated with fluorometric techniques, thereby maximizing the data obtained from a single sample in a single experiment.

The Hyperion Imaging platform incorporates a laser to sequentially ablate protein bound metal-tagged antibodies from individual (<1 μm) regions of fixed tissue sections or cell smears which are then individually atomized and ionized. The atomic ions are separated by mass and the high mass ions derived from the probes (but not low mass endogenous cellular ions) are counted. The presence of the metal tag indicates the presence of the associated target protein, and the intensity of the signal is directly proportional to the number of antibodies bound in each ablation spot. The elemental composition of each micron is thus separately determined, with the data from its (x,y) position being noted and rasterized in a digital image which can be analyzed using a variety of software packages with widely used single cell segmentation and other bioinformatics algorithms.

In summary, the Hyperion Imaging platform enables the semi-quantitative analysis of >40 parameters in tissue sections and cell smears without the need for spectral compensation or subtraction of autofluorescent background. This unique spatial and parametric definition of the cells *in situ* enables understanding of protein behavior and interactions to drive biological breakthroughs and define clinical biomarkers.

<u>The above attributes, and others highlighted below, are unique to the Hyperion Imaging platform sold by Fluidigm Corporation.</u>

Additional unique attributes:

- <1 μm ablation spot size of solid tissue, or cells on slides
- Up to 52 elemental tags for analysis without re-staining (135 channels available)
- Rastering speed of 200 pixels/second
- 0.3% sensitivity for ^{159}Tb
- Detection limit of 400 antibody copies per μm^2
- Dynamic range of 4 orders of magnitude
- Automated calibration
- 7.2TB RAID mirrored data storage

These attributes are required for high parameter IMC. **Hyperion Imaging** is the only platform that meets those mandatory specifications. **Fluidigm Corporation** is the sole manufacturer of this platform.