High dynamic range (HDR) microscopy optimizes assay sensitivity and dynamic range in quantitative, high-plex spatial biology applications

J. Spencer Schwarz[1], Matthew H. Ingalls[1], Xenia Meshik[1], Adam Northcutt[1], O. Braubach[1]
Canopy Biosciences®- A Bruker Company, [1]St. Louis, Missouri, USA

AACR American Association for Cancer Research
3486

High dynamic range optimizes mIF data capture

The CellScape™ Precise Spatial Multiplexing platform is the only spatial biology system that ensures linear relationships between measured fluorescence intensities in a single field of view and over multiple fields of view across a tissue section. Conventional single-exposure imaging is incapable of maintaining linear relationships between all biologically relevant signals.

CellScape produces high dynamic range (HDR) images from multi-exposure captures. This ensures that all relative fluorescence intensities in a tissue section maintain linear relationships—ultimately this allows for true comparison of signal intensities which would otherwise fall outside the sensitivity of a standard microscopy camera.

Here, using the VistaPlex™ Spatial Immune Profiling Assay Kit, we dissect the process of generating an HDR image and explore its utility in spatial biology. We demonstrate two use-cases wherein HDR enables the delineation of discrete cellular phenotypes based on expression levels of a single biomarker like CD4 or Ki67. Our results establish the CellScape's HDR imaging as a method for truly differentiated quantitative spatial biology.

HDR capture extends the dynamic range of biomarker intensities in spatial biology experiments

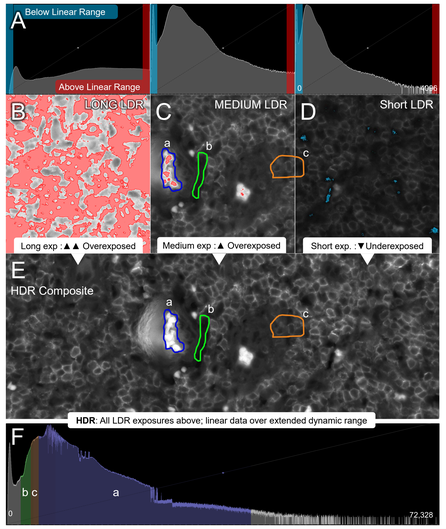


Figure 1: HDR Microscopy Enables Linear Fluorescence Comparisons with a Range that Far Exceeds the Capacity of Single Exposure Microscopy.
A scientific camera has a limited response range where intensity values maintain linear relationships—limiting the range of sensitivity for valid quantification (**A**). Under-saturated pixels in an image are not valid for measurement (cyan in **D**). Neither are over-saturated pixels (red in **B**). No single exposure can avoid over/under saturation completely (**C**). **HDR summation combines the linear intensity content from each LDR single exposure so direct comparisons can be made** for biomarker intensities exceeding the linear range of an LDR exposure (**E** and **F**).

Lettered ROIs (a-c) in **E** show portions of the image that require multiple exposures to measure appropriately. The same letters (a-c) in **F** display the linearized data from these regions within the composite HDR intensity histogram.

HDR extended dynamic range reveals the wide range of intensity measurements found among mIF datasets

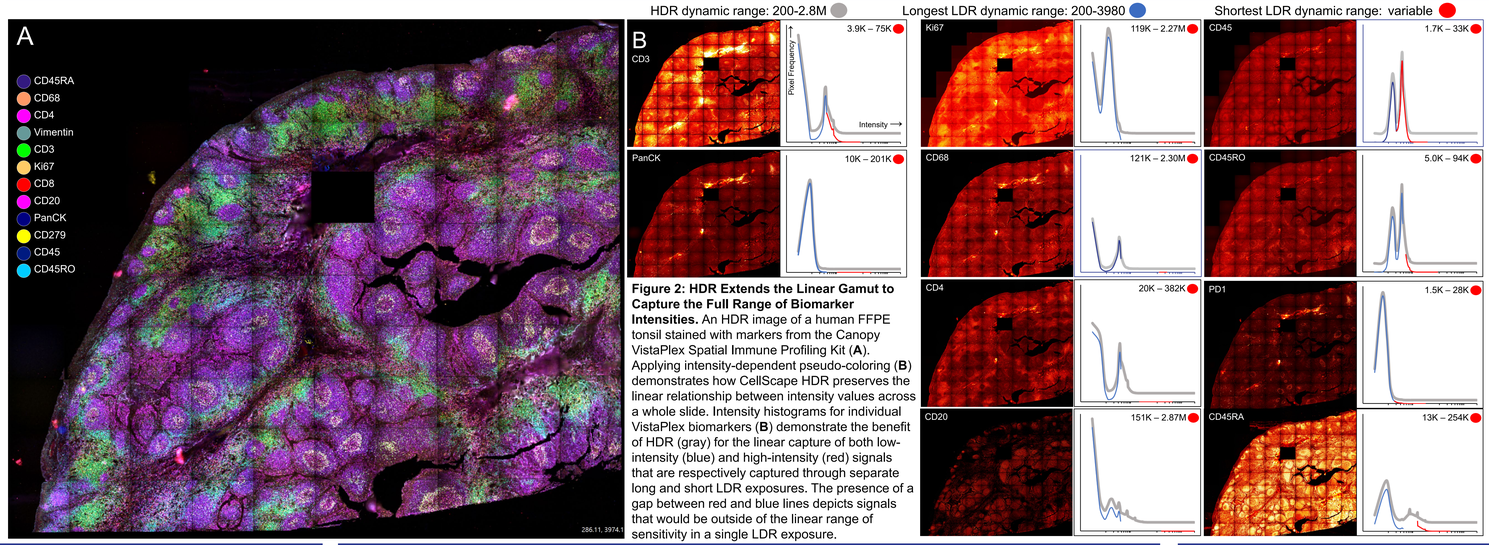
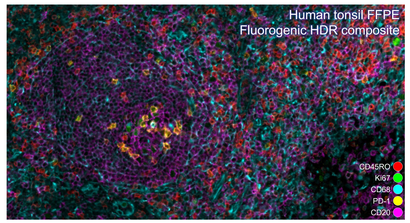


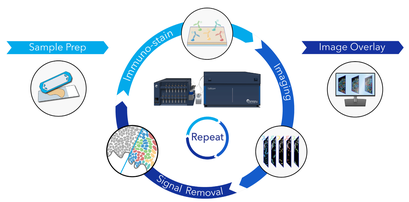
Figure 2: HDR Extends the Linear Gamut to Capture the Full Range of Biomarker Intensities. An HDR image of a human FFPE tonsil stained with markers from the Canopy VistaPlex Spatial Immune Profiling Kit (**A**). Applying intensity-dependent pseudo-coloring (**B**) demonstrates how CellScape HDR preserves the linear relationship between intensity values across a whole slide. Intensity histograms for individual VistaPlex biomarkers (**B**) demonstrate the benefit of HDR (gray) for the linear capture of both low-intensity (blue) and high-intensity (red) signals that are respectively captured through separate long and short LDR exposures. The presence of a gap between red and blue lines depicts signals that would be outside of the linear range of sensitivity in a single LDR exposure.

Data generation with VistaPlex Spatial Immune Profiling Assay Kit



Data were collected using CellScape and the VistaPlex Spatial Immune Profiling Assay Kit. VistaPlex kits are ready-to-use assay panels and enable researchers to obtain robust mIF data on the CellScape platform. The Spatial Immune Profiling Kit enables spatial phenotyping of key immune populations and epithelial cells in human formaldehyde-fixed, paraffin-embedded (FFPE) tissues.

VistaPlex Spatial Immune Profiling Biomarkers		
CD3	CD68	Ki-67
CD4	CD45	PD-L1
CD8	CD45RA	Pan-CK
CD20	CD45RO	Granzyme B
FoxP3	PD-1	DNA



HDR single marker delineation of human Helper T-Cells from myeloid mononuclear cells

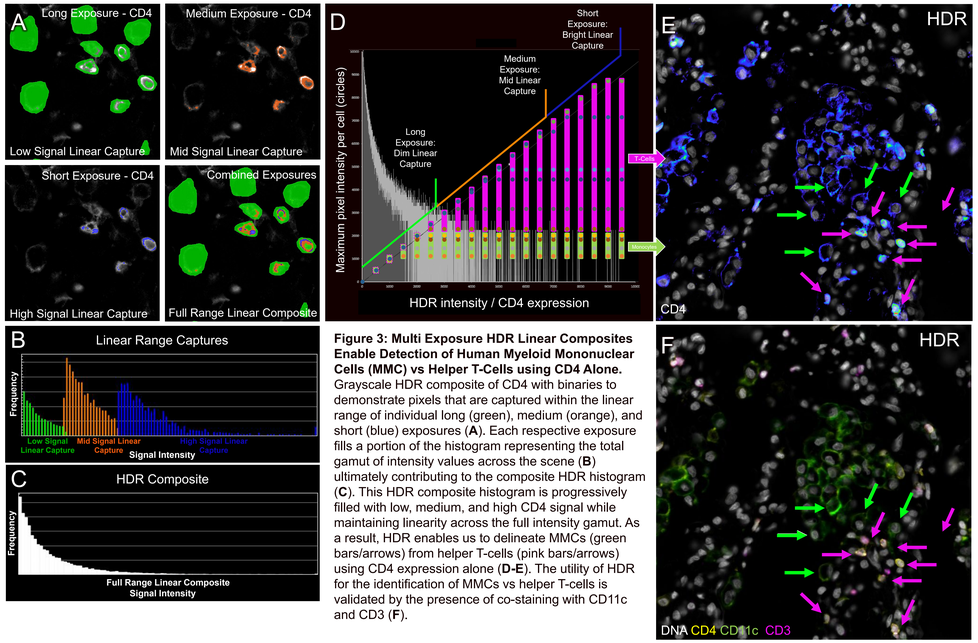


Figure 3: Multi Exposure HDR Linear Composites Enable Detection of Human Myeloid Mononuclear Cells (MMC) vs Helper T-Cells using CD4 Alone. Grayscale HDR composite of CD4 with binaries to demonstrate pixels that are captured within the linear range of individual long (green), medium (orange), and short (blue) exposures (**A**). Each respective exposure fills a portion of the histogram representing the total gamut of intensity values across the scene (**B**) ultimately contributing to the composite HDR histogram (**C**). This HDR composite histogram is progressively filled with low, medium, and high CD4 signal while maintaining linearity across the full intensity gamut. As a result, HDR enables us to delineate MMCs (green bars/arrows) from helper T-cells (pink bars/arrows) using CD4 expression alone (**D-E**). The utility of HDR for the identification of MMCs vs helper T-cells is validated by the presence of co-staining with CD11c and CD3 (**F**).

HDR microscopy powers precise spatial biology

HDR Imaging enables greater precision when annotating proliferating cells via Ki67 expression

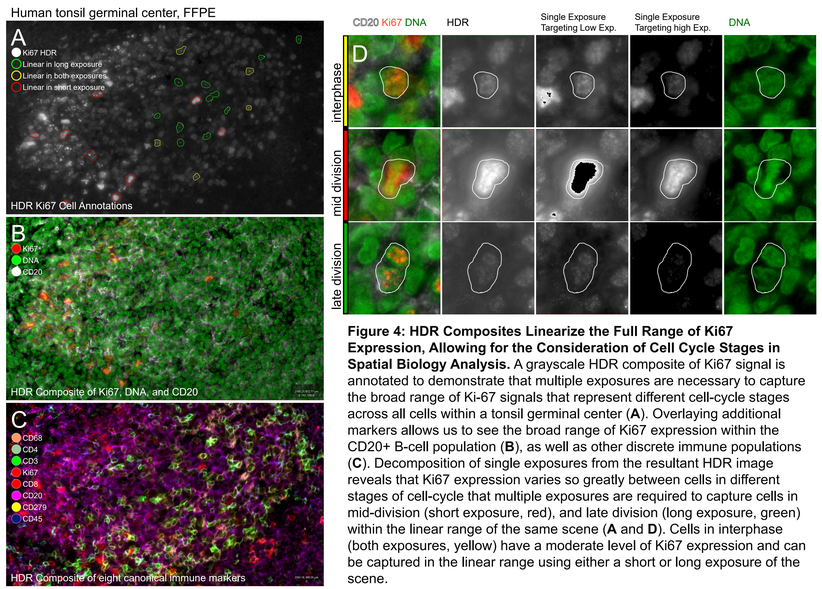


Figure 4: HDR Composites Linearize the Full Range of Ki67 Expression, Allowing for the Consideration of Cell Cycle Stages in Spatial Biology Analysis. A grayscale HDR composite of Ki67 signal is annotated to demonstrate that multiple exposures are necessary to capture the broad range of Ki-67 signals that represent different cell-cycle stages across all cells within a tonsil germinal center (**A**). Overlaying additional markers allows us to see the broad range of Ki67 expression within the CD20+ B-cell population (**B**), as well as other discrete immune populations (**C**). Decomposition of single exposures from the resultant HDR image reveals that Ki67 expression varies so greatly between cells in different stages of cell-cycle that multiple exposures are required to capture cells in mid-division (short exposure, red), and late division (long exposure, green) within the linear range of the same scene (**A** and **D**). Cells in interphase (both exposures, yellow) have a moderate level of Ki67 expression and can be captured in the linear range using either a short or long exposure of the scene.

Conclusions

- CellScape HDR maintains linear relationships between all fluorescence intensity values in a scene, a significant advantage over conventional single-exposure microscopy.
- With CellScape HDR, all detectable signals from multi-exposure captures are incorporated into the resultant composite image—ensuring inter and intra-assay precision as no biologically relevant signal is missed.
- CellScape HDR presents researchers with the opportunity to push the boundaries of multiplex immunofluorescence and make novel discoveries by performing **truly quantifiable spatial biology**.

Contact

J. Spencer Schwarz
John.Schwarz@Bruker.com

For more information, contact
hello.canopy@Bruker.com
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