

## High Definition Imaging Software: Enhanced Visualization and Overlay of Molecular and Optical Images

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This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

To highlight the new tools for manipulation of ion and optical image overlays in Waters High Definition Imaging (HDI) Software version 1.4. This includes tools for alignment and verlay blending modes.

### Benefits

Overlay mass spectrometry images onto histology or other optical and molecular images for multi-modal imaging data comparison.

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### Introduction

Mass spectrometry imaging (MSI) is already widely adopted in the field of analytical science and is now

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receiving significant interest for clinical research applications. As MSI becomes further established within this field, the software used for acquisition and subsequent manipulation of datasets must evolve. To meet the needs of these users, Waters has developed a next generation software solution – High Definition Imaging (HDI), v1.4. The software solution incorporates:

- An intuitive user interface for enhanced presentation of imaging mass spectrometry data as well as images obtained from other imaging modalities, i.e. H&E stained optical images.
- Functionality for the overlay of both molecular and optical/histological images.
- A data management pane designed for efficient organization and handling of image layers.

The ability to overlay mass spectrometry images onto other imaging data such as optical/histological images enables clinical research scientists to easily compare their MSI data with morphological information. This could also include optical images from an immunohistochemistry experiment or other imaging modalities where a conventional file format is applied. The combination of these approaches could lead to the discovery of valuable biochemical information. Similarly, multiple ion images can be overlaid to investigate the relationships between co-localized molecules in various morphological and histological features.

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## Results and Discussion

### Overlay of mass spectrometry ion images onto histology data

Optical images can be uploaded into HDI v1.4 alongside MSI datasets. This functionality enables the comparison of histological features and molecular distributions observed in ion images, with the two datasets aligned and overlaid.

HDI v1.4 features a new toolbar (Figure 1) for accurate alignment of optical and MSI data, providing the user with full control of the images being overlaid – either simultaneously or individually. This includes the rotation, resizing, and movement of all images with the addition of stretch and compression functions for optical images. Figure 1 also highlights another new feature in the software, whereby the co-registered pixel coordinates are displayed as the cursor moves across the image display. This allows the user to pinpoint the exact position of

features of interest and therefore further mine continuum raw data (i.e MS spectra) *in-situ*.

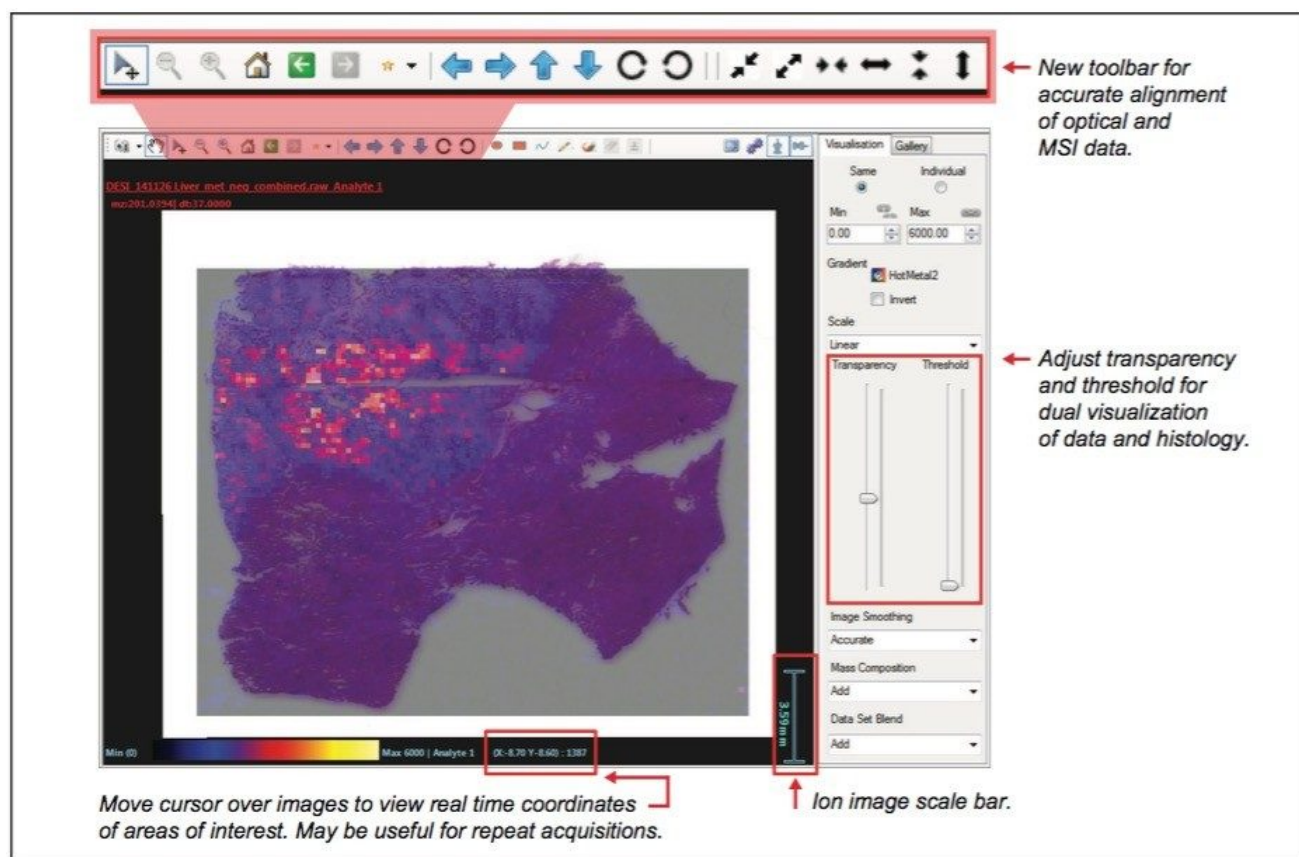


Figure 1. Ion image (MSI data) overlaid onto an optical image of a consecutive H&E stained section. Attention is drawn to new features including live pixel co ordinates, transparency sliders, and a live scale bar.

Clear viewing of the optical image underneath the overlaid mass spectrometry images is readily achieved. The transparency and intensity threshold of overlaid ion images can be individually altered as demonstrated in Figure 2A and 2B. Additionally, a scale bar has been added to the image display window, enabling the display of scientific data for export and publication in scientific and peer-reviewed journals.

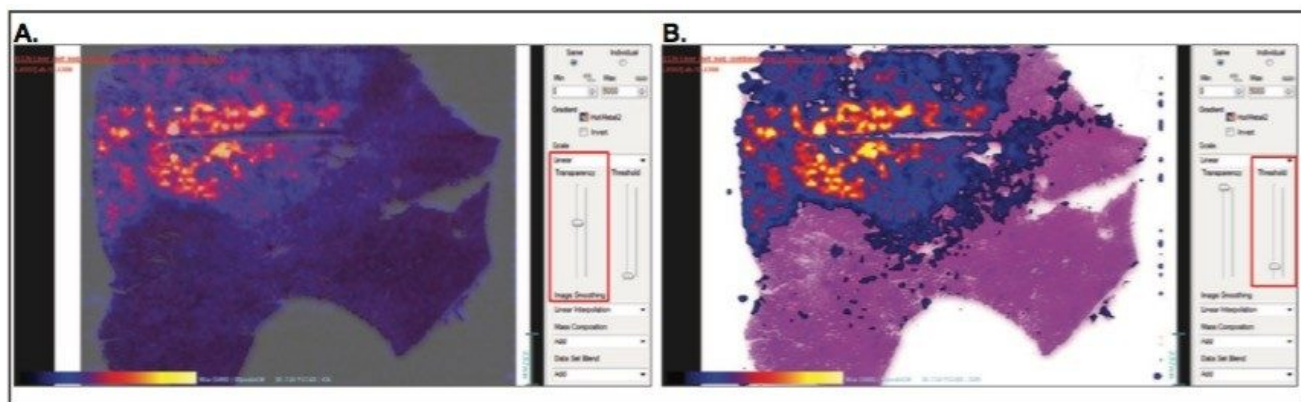


Figure 2. Effect of the transparency (A) and intensity threshold cut-off (B) on the ion images to aid the overlay and co-registration of the optical image with the mass spectrometry data.

## Enhanced manipulation of MS generated ion image overlays

Within HDI, layers can be selected individually, or a Red- Green-Blue (RGB) overlay can be generated through the selection of three ions in the mass list (Figure 3). Gradients for each layer can be set on either the same or individual scales, enabling full visualization of differential or co-localized distributions of the ions selected.



Figure 3. Generation of an RGB overlay allowing for manipulation of gradient scales combined, or on an individual basis.

It is also possible to overlay composite ion images via the selection of multiple masses and clicking the icon for a single layer, whereby a single color composite layer will be added. This could be highly valuable for overlaying different groups of peptides, known to correspond to particular proteins – essentially generating an overlay of peptide mass fingerprints.

Once an ion image overlay has been created, a variety of different blend modes can be implemented, providing optimum and appropriate visualization for a given experimental objective. This is a useful tool in applications where molecular interactions are of interest – for example, observing the co-localization of different  $m/z$  species, or for identifying the prominent ions detected in discrete structures or features of the analyzed samples. Blend modes available include: "Add," "Min," "Max," "Multiply," "None," and "Intensity Correlation" – four of which are displayed as applied to an RGB overlay in Figure 4.

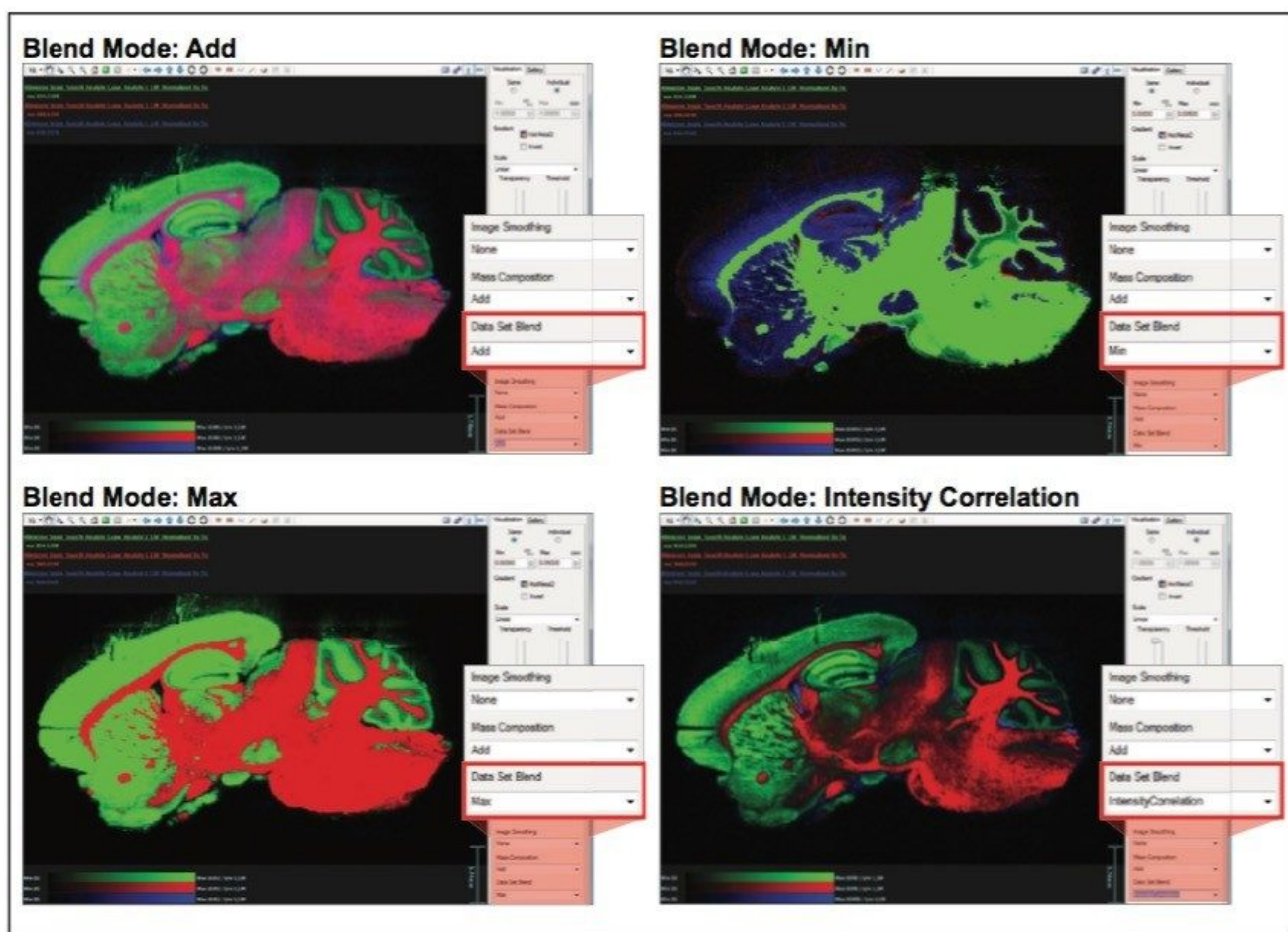


Figure 4. Blend modes as applied to an RGB overlay.

## Modes

- Add: When using the “Add” blending mode, the software will blend the overlay colors together where multiple  $m/z$  species are present in a single pixel. This blending mode is useful to assess co-localization of molecules in an RGB overlay. Pixels that have an  $m/z$  species displayed in blue and also a  $m/z$  species displayed in red will appear purple in color.
- Min: This blend mode displays the color of the  $m/z$  species of the lowest intensity in a given pixel.
- Max: This blend mode displays the color of the  $m/z$  species of the highest intensity in a given pixel.
- None: If no blending is applied, the layers will be stacked one on top of another in correlation with their position in the data management pane.

- Intensity Correlation: The  $m/z$  images are stacked. Pixels with lower signal intensity are more transparent, whereas those with increased signal intensity will appear more opaque. This means that where the top  $m/z$  layer has low signal, the layers underneath with higher signal will become more visible.

The ability to manage multiple ion image layers is now possible with the introduction of a designated overlay feature tab. This vastly improved visualization also makes use of a much wider palette of solid colors (Figure 5). Separate gradients for each of the color overlays are displayed in the image pane for added integrity and true comparison purposes. In every case, a data name and  $m/z$  stamp is displayed in the color of the corresponding layer – at the top left of the image pane – for added clarity.

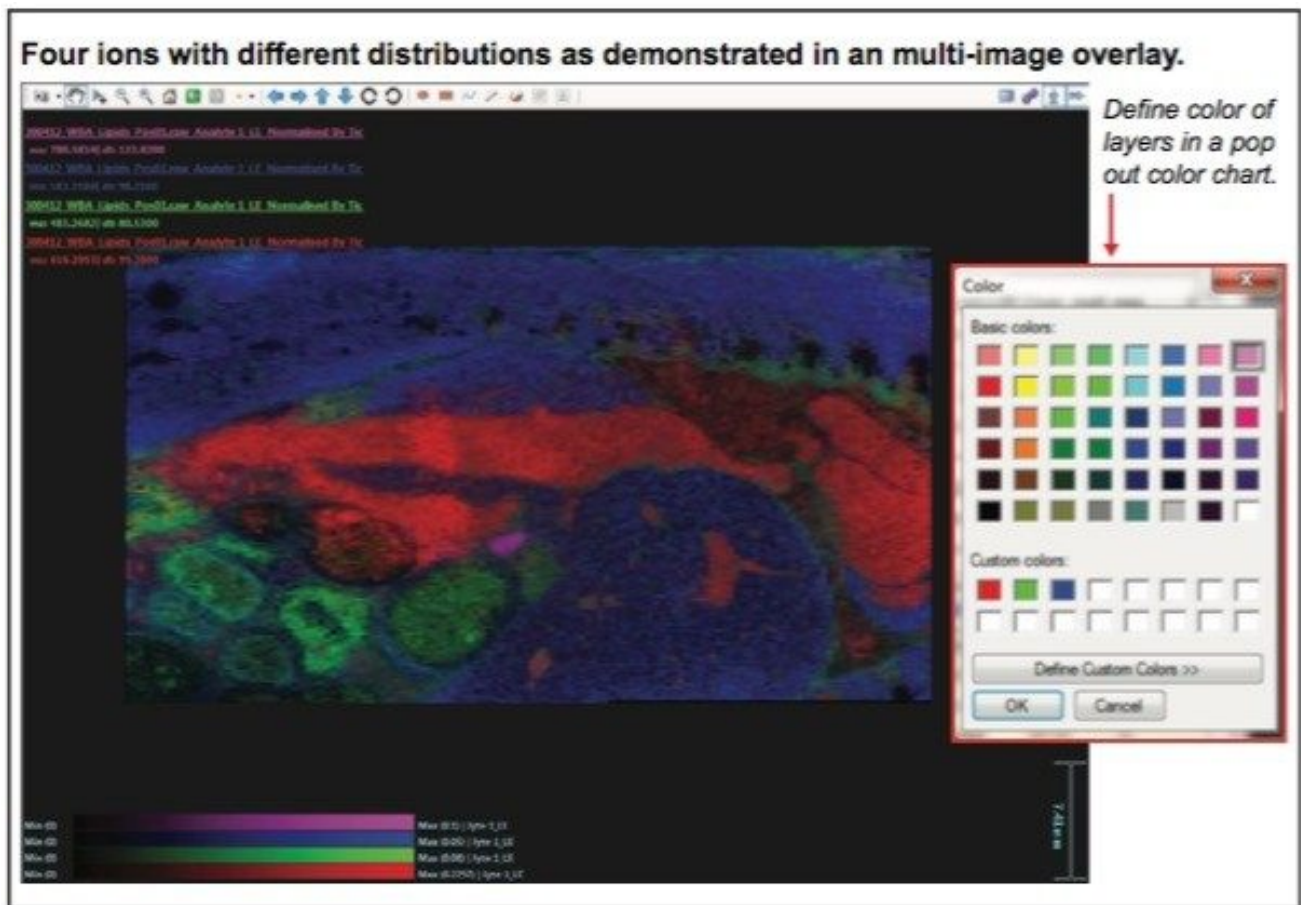


Figure 5. Multiple images overlaid to show differing distributions of four ions, with layer colors selected from an extensive palette on a selected region of rat whole body section.

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## Conclusion

The latest HDI Software enables simultaneous analysis of multiple (MALDI and DESI) imaging datasets and their corresponding optical images, delivering:

- Tools for improved alignment of optical/ histological images with the overlay of ion images.
- Ability to overlay in excess of three ion images using the new color chart.
- Improved and extended options for ion image overlays in a designated tab for easy manipulation.
- Blend modes for optimal manipulation and visualization of ion image overlays.

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## Featured Products

[High Definition Imaging \(HDI\) Software <https://www.waters.com/134833914>](https://www.waters.com/134833914)

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