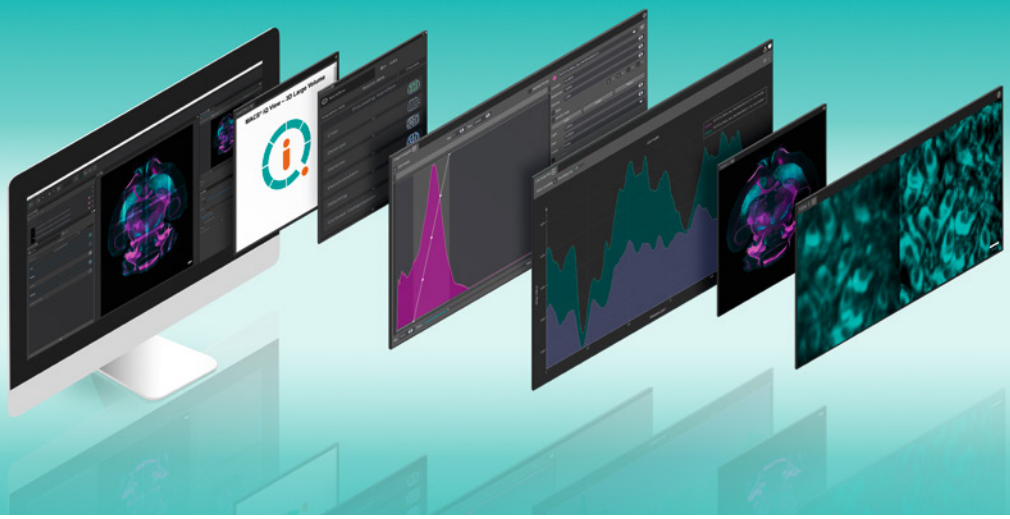
Miltenyi Biotec



MACS® iQ View – 3D Large Volume

Unlock the full potential of your light sheet microscopy data

Introduction

Light sheet microscopy is a 3D imaging technique that enables optical sectioning of large biological samples at high speed with a high signal-to-noise ratio, while also minimizing photobleaching and phototoxic effects. These features have made light sheet microscopy popular in various research fields, such as neuroscience, immuno-oncology, and cell biology.[1] Among the light sheet microscopes on the market, the UltraMicroscope Platform, i.e., UltraMicroscope Blaze™ and UltraMicroscope Choros™, stand out as they enable 3D imaging of exceptionally large biological samples, including entire mouse or human organs, or even whole mice, all at single-cell resolution and at unprecedented speed.[2]

While light sheet microscopy offers numerous advantages, researchers often encounter challenges following data acquisition, such as stitching together large mosaic datasets, striping artifacts, and low axial resolution. To address these challenges and to achieve the best possible image quality, we have developed MACS iQ View – 3D Large Volume (fig. 1), a software specifically designed for processing images acquired with the UltraMicroscope Systems.

MACS iQ View – 3D Large Volume provides a user-friendly interface and a simple design, making it easy for users to understand and interact with the software. It simplifies complex image processing, enabling users to enhance image quality with ease and gain meaningful insights. The streamlined workflow ensures efficient operation and an optimal user experience.

Key benefits

Seamless integration with UltraMicroscope Platform
MACS iQ View – 3D Large Volume seamlessly integrates with the UltraMicroscope Blaze and UltraMicroscope Choros, allowing users to directly access and process data acquired with these systems without the need to export or convert the data. This eliminates a time-consuming step in the workflow and enables researchers to quickly and easily begin processing their data on the same workstation that is used for data acquisition.

Intuitive and streamlined image processing workflow
The software includes a comprehensive suite of image processing modules that can be used to perform a variety of tasks, including 3D cropping, destriping, denoising, deconvolution, stitching, and contrast compression.

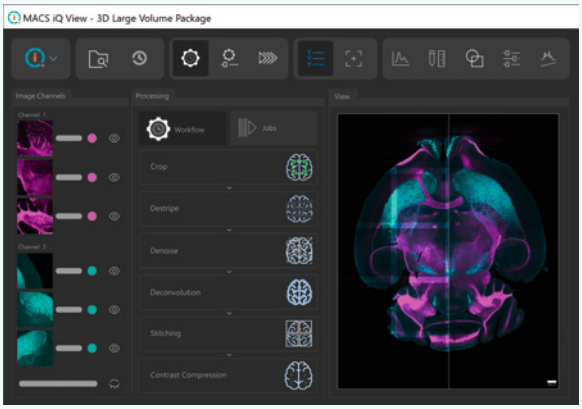


Figure 1: MACS iQ View – 3D Large Volume graphical user interface.

The software seamlessly incorporates these processing modules into an intuitive workflow and streamlines the entire process. The modules are organized in a hierarchical order, making it easy to set up workflows intelligently. Specific modules offer predefined options (subtle, standard, and progressive) for users to fine-tune processing parameters without feeling overwhelmed by an excessive number of choices, striking a balance between flexibility and simplicity (fig. 2).

Automation from image acquisition to processing
Users can connect MACS iQ View – 3D Large Volume to the acquisition software of the UltraMicroscope Platform, ImSpector. This connection allows users to assign and schedule individual processing steps, or even custom workflows, directly to a measurement while setting up an imaging experiment in ImSpector. Once the imaging acquisition is complete, processing starts automatically, eliminating the need to start it manually. This automation saves time and makes light sheet 3D imaging easy.

Batch processing
The software supports batch processing, allowing users to queue and initiate various workflows across multiple datasets. This feature proves especially valuable when dealing with extensive datasets and imaging multiple samples using the UltraMicroscope Blaze or Choros, particularly in the LightSpeed Mode, ensuring a smooth processing workflow.

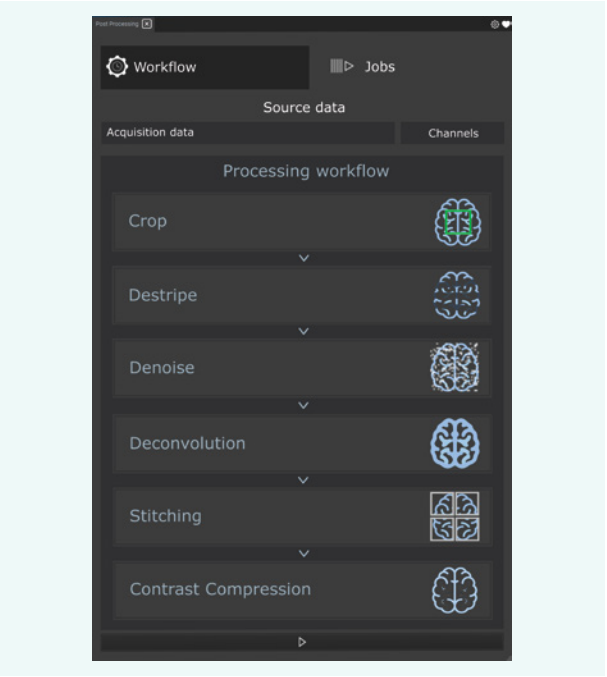


Figure 2: Processing workflow of MACS iQ View – 3D Large Volume. The image processing workflow facilitates the straightforward assignment of processing modules in a proposed step-by-step sequence, enabling automatic execution of each step.
This streamlined approach ensures ease of operation and eliminates the need for manual intervention at every stage.

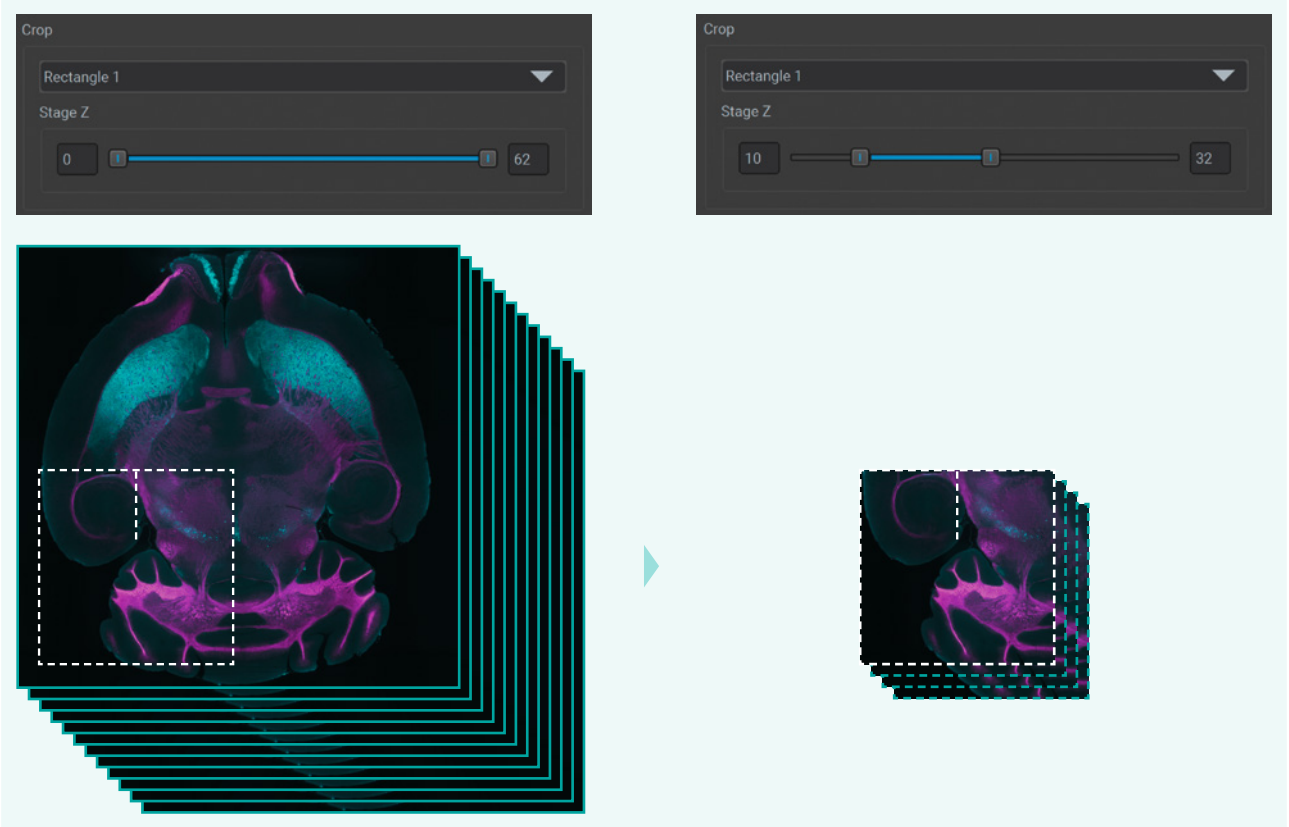


Figure 3: Crop. The ROI can be defined by drawing a rectangle (left). In the next step, there is the possibility to define the range of the z-planes to be cropped (right).

Side-by-side visualization
Upon completion of the image processing, users can view the original and processed data side-by-side to assess the results.

Various output format options
The output data is presented in the widely compatible OME-TIFF format, ensuring seamless integration with a variety of imaging analysis softwares. Image snapshots can be exported in standard formats such as TIFF, PNG, and JPEG. In addition to these options, users have the possibility to convert their data directly into a format optimized for 3D visualization.

Image processing modules

Crop
The first module in the workflow is Crop. It allows for cropping the data along the x and y-axes by defining a region of interest (ROI), as well as along the z-axis (fig. 3). To address the substantial data volumes generated by light sheet microscopy (e.g. 10–100s of GB), this module empowers researchers to downsize data, enabling efficient parameter testing and workflow optimization.

Destripe
One of the challenges in light sheet microscopy is the presence of striping artifacts caused by light scattering or absorption by small structures (e.g., impurities like dust particles, air bubbles, or pigmentation foci) along the illumination path. We have developed Destripe to effectively eliminate these artifacts while preserving the genuine sample features. With the "Automatic destriping decision" feature enabled, the software analyzes the image and applies destriping only when needed, using the automatically calculated mean stripe width. The algorithmic strategy identifies the unwanted stripe-like structures. It then accurately adjusts the local brightness variations of the gray value structures in these stripes. Figure 4 demonstrates an example where Destripe was effectively applied.

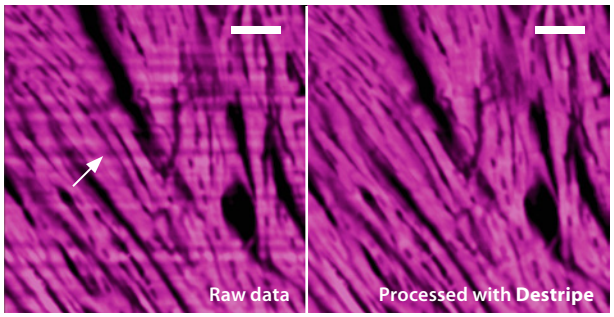


Figure 4: Destripe. An xy-plane of data from mouse cardiac muscle cells (magenta, autofluoresence) before (left) and after (right) processing with Destripe. The arrow indicates the stripe artifacts in the image. The scale bar is 50 µm. To demonstrate the power of the Destripe function, stripe-like artifacts were enhanced by using only one light sheet.

Denoise
Denoise reduces image noise and maintains or even enhances the spatial resolution of the image through a stochastically motivated iterative computation process. Therefore, it improves the visualization of fine structures and weak signals, facilitating image interpretation. Rather than employing a traditional image filtering algorithm, it takes into account the noise characteristics of both the fluorescence signal and the camera. Based on this, a statistical mathematical-physical approach is used to calculate the best estimate of the ideal noise-free image. This approach is supported by a tailored regularization scheme that guarantees convergence to the best estimate.[3] Figure 5 demonstrates an example where Denoise was effectively applied.

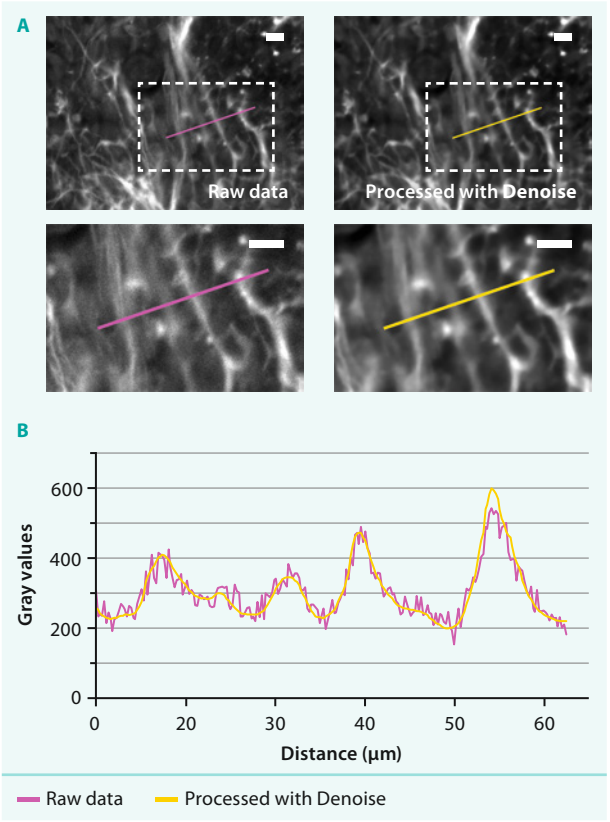


Figure 5: Denoise. An xy-plane of a small region of a human cerebral organoid with β Tubulin 3 staining (β-Tubulin 3 Antibody, anti-human/mouse/rat, Vio® G570, REAfinity®) before (left) and after (right) processing with Denoise (progressive setting). (A) The images in the lower panel provide enlarged views of the framed areas shown in the upper panel. The scale bar is 10 µm. The line graph (B) shows the gray values along the line (line profile) in the images before (magenta) and after (yellow) Denoise.

Deconvolution

A light sheet is not infinitely thin; it possesses a certain thickness that varies along the direction of propagation. Additionally, the point spread function (PSF) of the objective lens automatically convolves an image. Consequently, the acquired measurement data inherently contain a degree of unavoidable blurring.[5] Deconvolution enhances resolution in all three spatial directions, with a particular focus on improving it along the z-axis. The algorithm employs a stochastic iterative computation process by considering the spatial distribution of light sheet intensity and the PSF specific to the user's UltraMicroscope objective lens. It repeatedly refines a three-dimensional distribution of fluorescence source locations until it converges to the best estimate.[4,6]

Currently, the processing of images taken at 4× magnification with 1.6× and 2.5× zoom or 12× magnification with 1×, 1.6×, and 2.5× zoom is supported. To achieve the best results after Deconvolution, a special acquisition mode has been integrated into the acquisition software of the UltraMicroscope. When capturing images at a 12× magnification and 2.5× zoom, there is a 70% increase in XY resolution.

Deconvolution improves the scientific value of the processed image, allowing for more precise spatial data, such as diameter and volume measurements. It enhances image resolution, revealing details that were previously hidden.

Figure 6 shows a compelling example that demonstrates the effective application of Deconvolution within an image processing workflow.

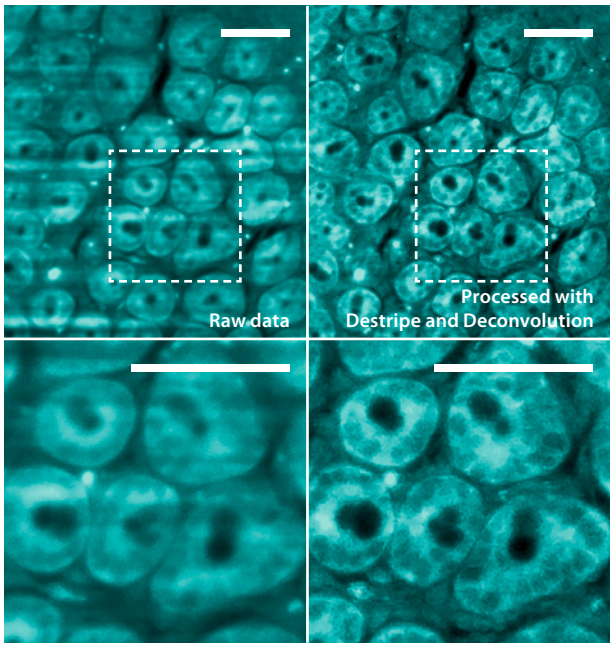


Figure 6: Destripe and Deconvolution. An xy-plane of data from mouse small instestine (autofluorescence) before (left) and after (right) processing with Destripe to remove striping artifacts and Deconvolution (standard setting) to enhance resolution. The images below provide enlarged views of the framed areas. The scale bar is 50 µm.

Stitching

The UltraMicroscope Platform allows users to capture large and intricate cleared biological samples, such as whole organs or an entire mouse, which often exceed the objective's field of view. When imaging these samples or using higher magnifications, it becomes necessary to acquire 3D mosaic images with a certain degree of overlap. Stitching is the process that combines these 3D tiles, which can be as large as terabytes of light sheet data, into a single, seamless, and high-resolution 3D stack. By simply clicking a button in MACS iQ View – 3D Large Volume, the 3D mosaic stacks are seamlessly stitched together without the need for any manual intervention. Figure 7 provides an example illustrating the utilization of Stitching.

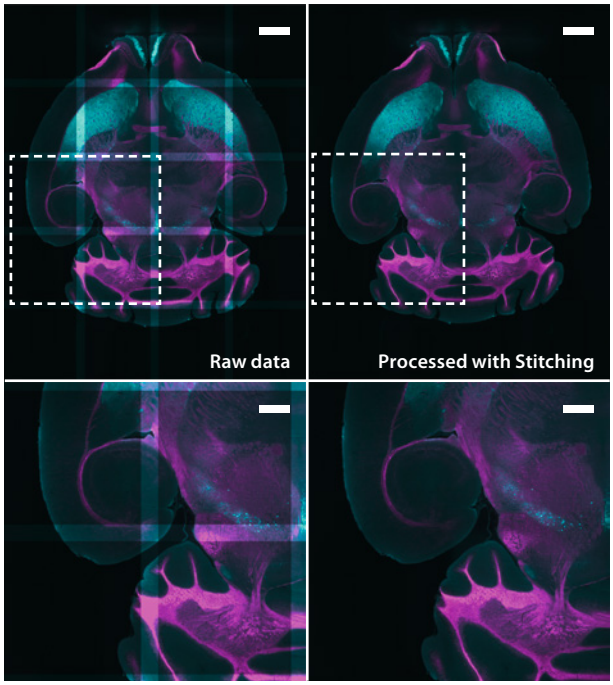


Figure 7: Stitching. An xy-plane of unstitched (left) and stitched (right) data from a mouse brain labeled with an antibody against myelin basic protein (magenta, MBP Antibody, anti-human/mouse, Vio R667, REAfinity) and tyrosine hydroxylase (cyan, Tyrosine Hydroxylase Antibody, anti-human/mouse/rat, Vio G570, REAfinity), which label the myelin basic protein and dopaminergic neurons, respectively. The scale bar is 1000 µm. The images below provide enlarged views of the framed areas. The scale bar is 500 µm.

Contrast Compression

In fluorescence microscopy, gray values typically span a wide dynamic range. This poses a challenge when attempting to display both bright and dim structures within the same channel. Contrast Compression simultaneously unveils the finest and brightest fluorophore deposits without the issues linked to traditional grayscale manipulation, which can frequently lead to underexposed or oversaturated image details (fig. 8). The contrast compression method used here employs a wavelet-based multiscale approach to transform the initial image into a specific contrast domain. On different scales within the contrast domain, the images are compressed and then recombined into a grayscale image that preserves overall structure and topology.[7]

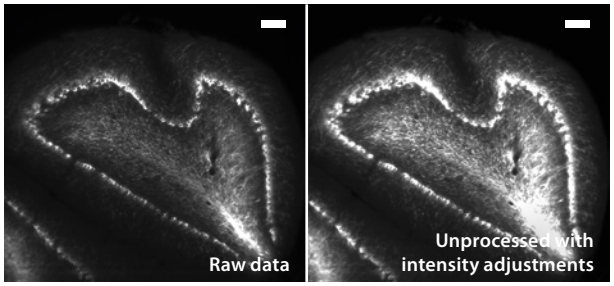


Figure 8: Intensity adjustments. An xy-plane from an adult mouse cerebellum labeled with an antibody against neurofilament protein (Neurofilament Antibody, anti-human/mouse, Vio R667, REAfinity). Unprocessed image (left), unprocessed with only intensity adjustments (right). As shown, using only grayscale manipulation it is impossible to display all the details of the data, and this often results in some structures becoming overexposed or underexposed. The scale bar is 100 µm.

In addition to its subtle, standard, and progressive processing strengths, the contrast compression algorithm has two modes: Fluorescence Mode and Structure Mode. In Fluorescence Mode, the resulting grayscale image still retains the character of a fluorescence microscopy image despite compression. This means that the varying distribution densities of fluorophores continue to influence the contrast-compressed image.

In Structure Mode, on the other hand, the original brightness differences are more strongly equalized. The resulting grayscale image emphasizes the faithful reproduction of the inherent image structure, while the reproduction of the original intensity takes a backseat. Figure 9 demonstrates the implementation of the structure mode of Contrast Compression. It is recommended to perform Denoise before contrast compression to prevent the amplification of background noise.

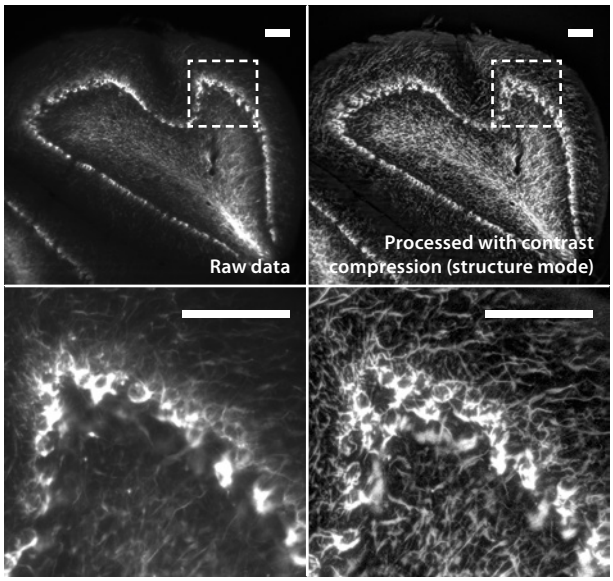


Figure 9: Contrast Compression. An xy-plane of data from an adult mouse cerebellum labeled with an antibody against neurofilament protein (Neurofilament Antibody, anti-human/mouse, Vio R667, REAfinity). Unprocessed image (upper left) and after processing with Contrast Compression (structure mode, standard setting, upper right). The images in the lower panel provide enlarged views of the framed areas. The scale bar is 100 µm.

Conclusion

To obtain high-quality 3D images in light sheet microscopy, the use of advanced image processing algorithms is essential. MACS iQ View – 3D Large Volume offers a comprehensive and user-friendly solution for processing images captured with the UltraMicroscope Blaze or UltraMicroscope Choros.

A unique feature of the software is the integration of various processing algorithms (Crop, Destripe, Denoise, Deconvolution, Stitching, and Contrast Compression) into a unified workflow. This allows users to process their images in a straightforward and efficient manner. Additionally, the side-by-side viewing of the unprocessed and processed data enables users to experiment with different settings and combinations of algorithms to find the best fit for their data.

The Deconvolution algorithm enhances the ability to discern fine structures and can increase xy resolution by up to 70% when using 12× and 2.5× magnifications. One significant difference in this software's Deconvolution compared to other solutions is that it is based on measurements of light sheet intensity and the PSF of the user's specific UltraMicroscope, rather than theoretical values. The improved contrast and resolution achieved through deconvolution may facilitate subsequent image analysis, such as segmentation.

The stitching algorithm simplifies the process of creating a single image from multiple images with just one click, eliminating the need for additional steps. This means that data captured with the UltraMicroscope can be directly opened in MACS iQ View – 3D Large Volume, eliminating the requirement to convert files to another format or manually align tiles for the stitching algorithm.

Moreover, the batch processing feature empowers users to initiate and queue customized workflows on multiple datasets, resulting in time savings and increased productivity. The output data is in OME-TIFF format, which is widely compatible with most imaging analysis software, allowing users to seamlessly continue their data analysis.

In summary, MACS iQ View – 3D Large Volume equips researchers to fully harness the potential of their light sheet microscopy data. It streamlines and accelerates the visualization and processing of high-dimensional data, providing researchers with improved image quality and resolution, to scale-up scientific investigations and image analysis.

References

1. Olarte, O.E. *et al.* (2018) Light-sheet microscopy: a tutorial. Advances in Optics and Photonics 10: 111-179.
2. Mai, H., *et al.* (2022) Scalable tissue labeling and clearing of intact human organs. Nature Protocols 17(10): 2188-2215.
3. De Witte, Y. *et al.* (2009) Bronnikov-aided correction for x-ray computed tomography. JOSA A 26: 890-894.
4. Yu, D.F. and Fessler, J.A. (2002) Edge-preserving tomographic reconstruction with nonlocal regularization. IEEE transactions on medical imaging 21: 159-173.
5. Yan, M. (2011) General convergent expectation maximization (EM)-type algorithms for image reconstruction with background emission and poisson noise. Inverse Probl 7: 1007-1029.
6. Fessler, J.A., and Erdogan, H. (1998) A paraboloidal surrogates algorithm for convergent penalized-likelihood emission image reconstruction. 1998 IEEE Nuclear Science Symposium Conference Record. 1998 IEEE Nuclear Science Symposium and Medical Imaging Conference (Cat. No. 98CH36255) 2: 1132-1135.
7. Mantiuk, R., Myszkowski, K., Seidel, H.P. (2006) A perceptual framework for contrast processing of high dynamic range images. ACM Transactions on Applied Perception (TAP) 3: 286-308.

LEARN MORE

Visit our website to learn more about the MACS iQ View – 3D Large Volume software

▸ **miltenyibiotec.com/ MACS-iQ-View-3D-LV**

Notes

Miltenyi Biotec

Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**

Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.

130-133-514.02