

Image-Pro[®] Plus Product Note

Extended Depth of Field

Introduction

Acquiring through-focus image stacks is becoming more and more commonplace in microscopy applications. The hardware required for such a technique is readily affordable, as are the computers and graphics cards required to display the resulting images. The benefits of looking simultaneously at all frames in an image stack as a composite are numerous, from elucidating previously unseen structures to determining relationships between existing ones. From standard brightfield to confocal techniques, composite images are very useful in making direct qualitative assessments about the structure and composition of image volumes.

Applications and Examples

The Extended Depth of Field function in Image-Pro Plus provides you with the ability to display a stack of images as a composite, building in effect a three dimensional image. The composite images reveal a great deal of information within the sample that may otherwise be obscured or unnoticed.

Extended Depth of Field requires an image volume comprised of at least two image planes. The planes may be of any single-plane image format supported by Image-Pro Plus v. 4.5, including those generated by many third-party software packages (Table 1).

<p>Image file formats supported by Image-Pro Plus:</p> <ul style="list-style-type: none"> • Molecular Dynamics (GEL) • Universal Imaging (STK) • BioRad (PIC) • Zeiss (LSM) • AutoQuant (DEB, AVZ, HDR) • Leica (LEI) • Gatan Digital Micrograph (DM3) • Fuji (INF) • Nikon C1 (ICS) • NIH (RAW)

Table 1. Third-Party File Formats Supported in Image Pro Plus v. 4.5

The following example shows a fetal mouse heart labeled with FITC. Thirty individual image planes were acquired through a confocal microscope and are

displayed in sequential fashion (Fig. 1) using the **Process|Sequence Gallery...** command (see Media Cybernetics Product Note- Sequence Gallery)

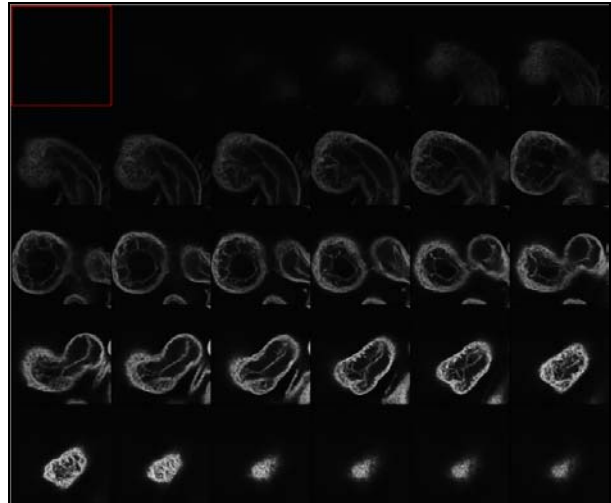


Fig 1. Sequence Gallery of Individual Planes. Sample represents an FITC-labeled fetal mouse heart acquired on a confocal microscope (courtesy Bob Zucker- USDA, Beltsville, MD).

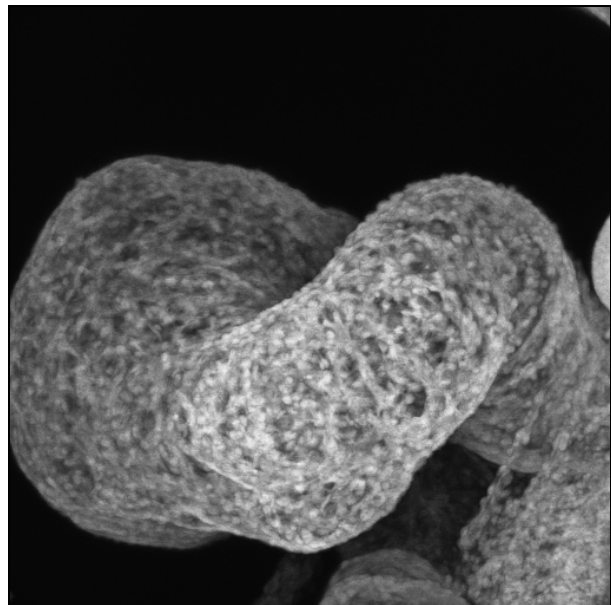


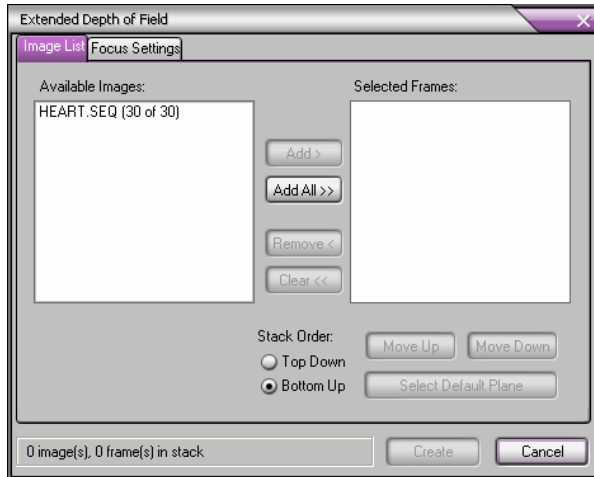
Fig 2. Maximum Intensity Projection of Individual Planes.

Using **Process|Extended Depth of Field...**, the overall structure of the mouse heart is revealed (Fig 2). The structure is not evident from the individual planes, but becomes quite obvious when compiling the images into their resulting composite views. In this particular

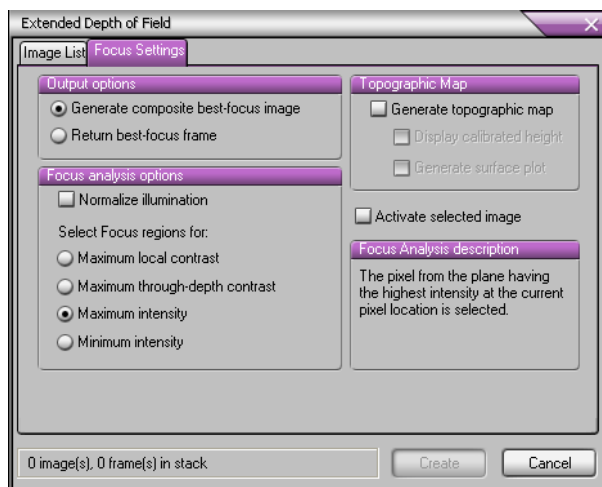
case, a maximum intensity projection of the individual planes has been created.

Implementation

Process|Extended Depth of Field...



The Extended Depth of Field- Image List Tab



The Extended Depth of Field- Focus Settings Tab

- Click ‘Select Default Plane’ when using either ‘Maximum Local Contrast’ or ‘Maximum Through Depth Contrast’. This allows you to choose an image plane to represent signal from image planes in the list not containing enough in-focus information.

- Click the Focus Settings tab. Here is where you can select the method by which you would like to display the composite image.
 - In the Output Options Group, click the ‘Generate composite best-focus image’ to create a composite image. To return the most in-focus frame, click ‘Return best-focus frame’
 - In the Focus analysis options group, choose the method by which the composite will be created:
 - Maximum Local Contrast- Pixels are analyzed in the area around the current pixel location. The pixel from the plane with the largest variance or local contrast is selected.
 - Maximum Through Depth Contrast- The mean value of the pixels from each plane at the current location is calculated. The pixel from the plane with the largest variance from the mean is selected.
 - Maximum Intensity- Good for fluorescent images. The pixel from the plane having the highest intensity at the current pixel location is selected.
 - Minimum Intensity- Good for brightfield images. The pixel from the plane having the lowest intensity at the current pixel location is selected.
 - Click the ‘Normalize illumination’ check box to normalize illumination in all the planes. This function helps to correct lamp flickering that may be present during image acquisition.

- Select **File|Open...** to load image file stack
- Select **Process|Extended Depth of Field...**
- Click the Image List file tab. Highlight the image of choice in the Available Images: list and click ‘Add’
 - Click the Top Up or Bottom Down button to change the order of images in the list.
 - Click ‘Move Up’ or ‘Move Down’ to reposition a frame in the sequence. Highlight the frame of interest to position the frame in the appropriate location.

In the Topographic Map group, click 'Generate topographic map' to create a topographic map of the selected image stack. It is important to note color images will be displayed in grayscale.

- Click the 'Display calibrated height' checkbox to translate the intensity values returned in the topographic map. These values represent the plane number from which the pixel intensity was taken. It is important to note these values will be different based upon the Focus analysis options that are used to create the composite.
- Click the 'Activate selected image' checkbox and click on the name of an image in the Selected Images list on the Image List tab to activate that image.

- Click 'Create' to generate the composite three-dimensional image or return the most in-focus frame.

Applications/Solutions Notes

Media Cybernetics Product Note- Sequence Gallery
Media Cybernetics Solution #1350- EDF Test Strips
Macro

Related Products

- Advanced Fluorescence Acquisition (AFA™) Microscope and Peripheral Automation Plug-In
- Scope-Pro™ Plug-In
- SharpStack™ Deconvolution Plug-In
- 3D Constructor™ Three Dimensional Display and Rendering Plug-In

How To Order

For more information on Image-Pro Plus, and to locate a Media Cybernetics' reseller in your area, visit our website at www.mediacy.com.

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