

## **Bio-Rad File Conversion/Import**

### File Format.

The native file format for the Bio-Rad confocal microscope is a raw file possessing a 76 byte header followed by sequence of 8-bits/pixel images and ending with a block of image attributes. Bio-Rad native files are denoted by a “.PIC” filename extension. They may contain a single image from a single mixer channel, up to 5 images representing 5 channels of the same image plane, or multiple images from a single channel, such as a time-lapse series or a z-series. Time-lapse and z-series images collected from multiple channels will be saved as a separate image stack for each channel. Stacks from multiple channels will be denoted by the last 2 characters of their common filename having been set to “01”, “02”, “03”, “04” or “05”.

While specific import directions for Fiji are given below, the basic principles are applicable to all graphics software.

### Importing Bio-Rad Files using Fiji.

1. Select ‘Open’ from the File Menu;
2. Select the .PIC file or first stack of a multi-channel z-series, ending in “01”;
3. ‘OK’;

Image>Properties will display the pixel size, however the Voxel depth may display as a negative value. Image>Show Info... will display .pic format metadata.

Multi-channel images must be opened 1 channel at a time and their metadata may be slightly scrambled. This does not make sense, since the ‘Open’ command used Bio-Formats.

### Importing Bio-Rad Files using the Bio-Formats plugin.

4. Select ‘Bio-Formats’ from the Plugin Menu;
5. Click on the ‘Bio-Formats Importer’;
6. Select the .PIC file or first stack of a multi-channel z-series;
7. ‘OK’;

Multi-channel stacks will open correctly, Image>Show Info... and Image>Properties will correctly display .pic format metadata. The usual Bio-Formats options may be applied.

### **Fiji/ImageJ**

#### Importing Bio-Rad Files using the File Import Command.

1. Select ‘Import...’ from the File Menu;
2. Click on the ‘Custom’ radio button;
3. Click on the ‘Set’ button;
4. Enter Width and Height of the image (pixels);
5. Enter ‘76’ for offset;
6. Enter the number of slices;
7. ‘OK’;
8. ‘Open’;
9. Select any additional channels and repeat the process;
10. Merge into a color stack by the ‘Image/Color/Merge Channels’ command.

If the images open with a noticeable striped appearance, then you have incorrectly entered the image dimensions. An incorrect offset will shift the image to the right. If you are unsure how many slices are present, simply enter a large value. ImageJ will open until it runs out of images. Most images are 512x512, 1024x768 or 1024x1024.