Technical Note

Importing VisiView images in ImageJ



The VisiView acquisition software stores channels, time points and stage positions of multidimensional image sequences in separate files (either as single-plane MetaMorph .tif format or as stacks with the extension .stk). Importing them into ImageJ can be tedious. We recommend to let the VisiView software save image sequences automatically to disk. It will create file names according to a naming convention and create a file with the extension .nd for each experiment. This file contains meta-information which can be interpreted by an ImageJ plugin (Bio-Formats/LOCI Tools) and used to automatically re-assemble the entire experiment in a single ImageJ hyperstack.

Installation of Bio-Formats/LOCI Tools

Download the plugin from <u>http://loci.wisc.edu/software/bio-formats</u> and copy it into the plugins folder of ImageJ. In the Fiji distribution of ImageJ (<u>http://fiji.sc/Fiji</u>), the plugin is pre-installed and automatically updated. The following menu commands will be available:

🗊 (Fiji Is Just) ImageJ	l	
File Edit Image Process Analyze	Plugins Window Help Macros Shortcuts Shortcuts Utilities Shortcuts Shortcuts New Shortcuts Strate Compile and Run Install Strg+Umschalt+M	
	3D 3D Viewer Analyze Bio-Formats	Bio-Formats Importer
	Color Inspector 3D Examples Feature Extraction Image5D Integral Image Filters	Bio-Formats Exporter Bio-Formats Remote Importer Bio-Formats Windowless Importer Bio-Formats Macro Extensions
	LOCI Landmarks Mosaic Optic Flow Process Registration	 Stack Slicer Bio-Formats Plugins Configuration Bio-Formats Plugins Shortcut Window Update Bio-Formats Plugins Help

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Importing data

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Data can be imported either using the menu command 'Bio-Formats Importer' (select the .nd file as target), or simply by dragging the .nd file from a file manager (Windows Explorer) onto the main ImageJ window. A window with options will appear. Click 'OK'.

Stack viewing	Metadata viewing	Information	
View stack with: Hyperstack	Display metadata	Display OME-XML metadata -	-
Stack order: XYCZT	Display OME-XML metadata	standardized into the OME data	
Dataset organization	Memory management	regardless of file format, though some formats will populate more information than others.	
Open files individually	Specify range for each series		
 Swap dimensions Open all series Concatenate series when compatible 	Crop on import Split into separate windows	• The title of the dataset is listed under OME > Image > Name.	
Stitch tiles	C Split channels	 The time and date when the dataset was acquired 	
Color options	Split focal planes	is listed under OME >	
Color mode: Default	Split timepoints	The physical pixel sizes	
Autoscale		of each plane in microns	-

If one or more options in the sections 'Dataset organization' or 'Memory management' are checked, more popups will appear upon clicking 'OK', allowing to re-organize imports or loading subsets (in any dimension). If the files contain multiple stage postions, a list with all of them will be shown:

III Bio-Formats Series Options	
✓ Series 1: Stage1 "Pos478": 444 x 324; 18 planes (2C x 3Z x 3T)	\parallel
☐ Series 2: Stage2 "Pos479": 444 x 324; 18 planes (2C x 3Z x 3T)	$\langle \cdot \rangle$
☐ Series 3: Stage3 "Pos480": 444 x 324; 18 planes (2C x 3Z x 3T)	
OK Cancel	Select All Deselect All

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Windowless import

The import options dialog can be disabled via the command 'Bio-Formats Plugins Configuration'. In the 'Formats' tab of the window appearing, select 'Metamorph STK' and check the option 'Windowless'. From now on, files are imported with the options used last (*Note:* windowless import cannot be used if the experiments contains multiple stage positions. The stage positions list is also disabled. Only the first stage position will be imported).

Bio-Formats Plugins Configuration			
Formats Libraries Upgrade Log			
MIAS MINC MRI Medical Research Council Metamorph STK Metamorph TIFF Micro-Manager Minolta MRW Molecular Imaging	*	Extensions stk, nd, tif, t Enabled 📝 Windowless 📝	iff

Importing single .stk files

Individual .stk stacks can be imported by drag and drop onto the main ImageJ window. Drag and drop, however, always passes the file to ImageJ's default file reader, which does not correctly read the voxel dimensions of VisiView files. To force ImageJ to use the Bio-Formats plugin (which reads all metadata properly) and still use drag and drop, open the shortcut window of the plugin (menu command 'Bio-Formats Plugins Shortcut Window'). This window is drag and drop enabled and will open any dropped file with Bio-Formats. This option works with any of over 130 image formats currently supported by Bio-Formats.

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Bio-Formats Importer			
Bio-Formats Exporter			
Bio-Formats Remote Importer			
Bio-Formats Windowless Importer			
Bio-Formats Macro Extensions			
Stack Slicer			
Bio-Formats Plugins Configuration			
Update Bio-Formats Plugins			
Help			
About Bio-Formats Plugins			