

Using ImageJ with file formats created on our microscopes

There are excellent manuals and tutorials available on the [NIH ImageJ website](#). In addition to the official ones, the [ImageJ Documentation wiki](#) and [Fiji Cookbook](#) are good.

Components needed

- ImageJ ([download from ImageJ website](#))
- Bio-Format Plugins for ImageJ ([download from OME website](#))
- an alternative is to just get Fiji which is just ImageJ packaged with some commonly used plugins, including Bio-Format ([download from Fiji website](#))

bit-depth

- the system outputs 12-bit data and images can be acquired with 8, 12 or 16-bit resolution

image resolution	grey scale level	lif file type
8-bit	256	8-bit, uint8
12-bit*	4096	16-bit, uint16
16-bit (scaled from 12-bit)	65536	16-bit, uint16

*16-bit files with 12-bit resolution will appear dark unless the intensity is scaled
e.g., set Minimum displayed value to 0 and Maximum displayed value to 4095 for full scale display
1. Do Image > Adjust > Brightness/Contrast...
2. click on Set and enter the desirable displayed values or click on Auto to use the min and max intensity values stored in the image

- some software cannot deal with 16-bit files, you can convert the image to 8-bit by doing Image > Type > 8-bit

lif

1. launch ImageJ/Fiji
2. drag and drop the lif file onto ImageJ's status bar to open the file
3. after a few moments of thinking, loci_tools' Bio-Formats Import Options dialog box will open
 - for Stack Viewing, set View stack with: Hyperstack
 - under Color options, check Autoscale if you want to use the min and max intensity values stored in the image
4. click OK and wait for loci_tools' Bio-Formats Series Options dialog box which allows selection of specific contents
5. select the desirable images and click OK

multidimensional files

- sequential multichannel file opens correctly using View stack with: Hyperstack
- sometimes simultaneous multichannel files may open with channels interleaved, and here is a workaround
 1. need to use View stack with: Standard ImageJ and Stack order: Default (xyzct)
 2. convert the opened stack to hyperstack, do Image > Hyperstack > Stack to hyperstack..., use the default xyzct order and fill in the appropriate c, z, t value

other simple tasks

- if you want to break down the multidimension series e.g., XYZCT
 1. do it during file open with the Split into separate windows options
Split channels, focal planes, timepoints
 2. after the image series is opened as a hyperstack, you can do Plugins > LOCI > Stack Slicer and check the appropriate options
- If you feel like breaking down a lif into individual TIFF, you can try this ImageJ macro

```
macro "Break up a lif into individual TIFF" {
// open the file manager to select a lif file to break it into TIFFs
// in this case, only the metadata specific to a series will be written

path = File.openDialog("Select a File");

run("Bio-Formats Macro Extensions");
Ext.setID(path);
Ext.getCurrentFile(file);
Ext.getSeriesCount(seriesCount);

for (s=1; s<=seriesCount; s++) {
// Bio-Formats Importer uses an argument that can be built by concatenate a set of strings
run("Bio-Formats Importer", "open=&path autoscale color_mode=Default view=Hyperstack stack_order=XYZCT serie
out_path = getDirectory("image") + getTitle() + ".tif";
saveAs("tiff", out_path);
close();
}
}
```

- ...

(more to come)

metadata

- the metadata from the header of the lif file relevant to the image opened can be displayed by Image > Show Info...
- the same information can be displayed if you check "Metadata viewing, Display metadata in results window" option in the Bio-Format Import Options dialog box
- here is an example of the metadata in a xyz series, most entries are self explanatory, additional comments or commonly used parameters are in []; also, there are repeated entries and entries not applicable for our installation e.g., bUseCARS, bUseMP, and bUseSTED

```
[all these up to the long dash line below are metadata recognized by Bio-Format]
DimensionOrder = XYZCT [slice order]
IsInterleaved = false
IsRGB = false
LittleEndian = true
PixelType = uint8
SizeC = 1
SizeT = 1
SizeX = 4096
SizeY = 16
SizeZ = 240
Application 1 = LAS AF
Application 2 = LAS AF
Application 3 = LAS AF
Application 4 = LAS AF
Application 5 = LAS AF
ChannelDescription|BitInc 1 = 0
ChannelDescription|BytesInc 1 = 0
ChannelDescription|ChannelTag 1 = 0
ChannelDescription|DataType 1 = 0
ChannelDescription|IsLUTInverted 1 = 0
ChannelDescription|LUTName 1 = Red [ ]
ChannelDescription|Max 1 = 2.550000e+002 [max intensity]
ChannelDescription|Min 1 = 0.000000e+000 [min intensity]
ChannelDescription|NameOfMeasuredQuantity 1 =
ChannelDescription|Resolution 1 = 8 [bit depth]
ChannelDescription|Unit 1 =
ChannelScalingInfo|Automatic 1 = 0
ChannelScalingInfo|BackgroundLutName 1 =
ChannelScalingInfo|BlackValue 1 = 0
ChannelScalingInfo|GammaValue 1 = 1
ChannelScalingInfo|WhiteValue 1 = 1
DimensionDescription|BitInc 1 = 0
DimensionDescription|BitInc 2 = 0
DimensionDescription|BitInc 3 = 0
DimensionDescription|BytesInc 1 = 1
DimensionDescription|BytesInc 2 = 4096
DimensionDescription|BytesInc 3 = 65536
DimensionDescription|DimID 1 = 1
DimensionDescription|DimID 2 = 2
DimensionDescription|DimID 3 = 3
DimensionDescription|Length 1 = 2.460327e-005 [physical length x, m]
DimensionDescription|Length 2 = 9.012186e-008 [physical length y, m]
DimensionDescription|Length 3 = 1.002885e-005 [physical length z, m]
DimensionDescription|NumberOfElements 1 = 4096 [logical size x, pixel]
DimensionDescription|NumberOfElements 2 = 16 [logical size y, pixel]
DimensionDescription|NumberOfElements 3 = 240 [logical size z, pixel]
DimensionDescription|Origin 1 = 1.376765e-020 [physical origin x, m]
DimensionDescription|Origin 2 = 1.847741e-008 [physical origin y, m]
DimensionDescription|Origin 3 = -1.500012e-005 [physical origin z, m]
DimensionDescription|Unit 1 = m
DimensionDescription|Unit 2 = m
DimensionDescription|Unit 3 = m
HardwareSetting|FilterSettingRecord|AOTF (405) 1 = 0
HardwareSetting|FilterSettingRecord|AOTF (405) 2 = 0
HardwareSetting|FilterSettingRecord|AOTF (458) 1 = 0
HardwareSetting|FilterSettingRecord|AOTF (458) 2 = 0
HardwareSetting|FilterSettingRecord|AOTF (476) 1 = 0
HardwareSetting|FilterSettingRecord|AOTF (476) 2 = 0
HardwareSetting|FilterSettingRecord|AOTF (488) 1 = 1.00103766099005 [gain ]
HardwareSetting|FilterSettingRecord|AOTF (488) 2 = 0 [offset]
HardwareSetting|FilterSettingRecord|AOTF (514) 1 = 0
HardwareSetting|FilterSettingRecord|AOTF (514) 2 = 0
HardwareSetting|FilterSettingRecord|AOTF (561) 1 = 0
HardwareSetting|FilterSettingRecord|AOTF (561) 2 = 0
HardwareSetting|FilterSettingRecord|AOTF (633) 1 = 0
HardwareSetting|FilterSettingRecord|AOTF (633) 2 = 0
```

HardwareSetting|FilterSettingRecord|DMI6000 Stage Pos x 1 = 0.03751998567257
HardwareSetting|FilterSettingRecord|DMI6000 Stage Pos y 1 = 0.00860454637572
HardwareSetting|FilterSettingRecord|DMI6000 Stage Pos z 1 = 0
HardwareSetting|FilterSettingRecord|DMI6000 Stage XOrigin 1 = 0
HardwareSetting|FilterSettingRecord|DMI6000 Stage YOrigin 1 = 0
HardwareSetting|FilterSettingRecord|DMI6000 Stage ZOrigin 1 = 0
HardwareSetting|FilterSettingRecord|Dummy Name (Obj.) 1 = Dummy5
HardwareSetting|FilterSettingRecord|Excitation Beam Splitter FW 1 = RT 30/70 []
HardwareSetting|FilterSettingRecord|Galvo Slider 1 = Galvo X Normal
HardwareSetting|FilterSettingRecord|Hardware Type No. 1 = 7
HardwareSetting|FilterSettingRecord|Laser output power 1 = 19.9115044247788 [Ar output power 20%]
HardwareSetting|FilterSettingRecord|Laser wavelength 1 = 405
HardwareSetting|FilterSettingRecord|Laser wavelength 2 = 458 [refers to Ar laser, not really using the 458
HardwareSetting|FilterSettingRecord|Laser wavelength 3 = 561
HardwareSetting|FilterSettingRecord|Laser wavelength 4 = 633
HardwareSetting|FilterSettingRecord|Magnification-Changer 1 = SCANx
HardwareSetting|FilterSettingRecord|Numerical aperture (Obj.) 1 = 1.4
HardwareSetting|FilterSettingRecord|Objective 1 = HCX PL APO CS 63.0x1.40 OIL UV []
HardwareSetting|FilterSettingRecord|Order number (Obj.) 1 = 11506188
HardwareSetting|FilterSettingRecord|PMT 1 1 = Inactive
HardwareSetting|FilterSettingRecord|PMT 2 (HV) 1 = 375.562676432441
HardwareSetting|FilterSettingRecord|PMT 2 (HV_Unit) 1 = V
HardwareSetting|FilterSettingRecord|PMT 2 (Offs.) 1 = 0
HardwareSetting|FilterSettingRecord|PMT 2 (Preamp) 1 = Standard
HardwareSetting|FilterSettingRecord|PMT 2 1 = Active []
HardwareSetting|FilterSettingRecord|PMT 3 1 = Inactive
HardwareSetting|FilterSettingRecord|PMT 4 1 = Inactive
HardwareSetting|FilterSettingRecord|PMT Trans 1 = Inactive
HardwareSetting|FilterSettingRecord|Phase 1 = -36.5224689097429
HardwareSetting|FilterSettingRecord|Polarization FW 1 = Empty 1
HardwareSetting|FilterSettingRecord|Position 1 = 3
HardwareSetting|FilterSettingRecord|Power State 1 = Off
HardwareSetting|FilterSettingRecord|Power State 2 = On []
HardwareSetting|FilterSettingRecord|Power State 3 = Off
HardwareSetting|FilterSettingRecord|Power State 4 = Off
HardwareSetting|FilterSettingRecord|RLD_Settings 1 = -1
HardwareSetting|FilterSettingRecord|Refraction index 1 = 1.518
HardwareSetting|FilterSettingRecord|Reson. Galvo Pan 1 = Galvo X Pan Center
HardwareSetting|FilterSettingRecord|Rotation Direction 1 = 1
HardwareSetting|FilterSettingRecord|SMD-Phase 1 = 0
HardwareSetting|FilterSettingRecord|SP Mirror Channel 1 (left) 1 = 406.25
HardwareSetting|FilterSettingRecord|SP Mirror Channel 1 (right) 1 = 458.75
HardwareSetting|FilterSettingRecord|SP Mirror Channel 1 (stain) 1 =
HardwareSetting|FilterSettingRecord|SP Mirror Channel 2 (left) 1 = 470 []
HardwareSetting|FilterSettingRecord|SP Mirror Channel 2 (right) 1 = 500 []
HardwareSetting|FilterSettingRecord|SP Mirror Channel 2 (stain) 1 =
HardwareSetting|FilterSettingRecord|SP Mirror Channel 3 (left) 1 = 616.25
HardwareSetting|FilterSettingRecord|SP Mirror Channel 3 (right) 1 = 668.75
HardwareSetting|FilterSettingRecord|SP Mirror Channel 3 (stain) 1 =
HardwareSetting|FilterSettingRecord|SP Mirror Channel 4 (left) 1 = 721.25
HardwareSetting|FilterSettingRecord|SP Mirror Channel 4 (right) 1 = 773.75
HardwareSetting|FilterSettingRecord|SP Mirror Channel 4 (stain) 1 =
HardwareSetting|FilterSettingRecord|Scan Field Rotation 1 = -1.39513951395139
HardwareSetting|FilterSettingRecord|Scan Speed 1 = 400 []
HardwareSetting|FilterSettingRecord|System Number 1 = 5100001224
HardwareSetting|FilterSettingRecord|TLD_Settings 1 = 100
HardwareSetting|FilterSettingRecord|Target Slider 1 = Target Park
HardwareSetting|FilterSettingRecord|UV Lens FW 1 = Lens 63x/1.4 Oil
HardwareSetting|FilterSettingRecord|X Scan Actuator (Gain) 1 = 9.99996185317286
HardwareSetting|FilterSettingRecord|X Scan Actuator (Offs.) 1 = 8.67361737988404E-19
HardwareSetting|FilterSettingRecord|X Scan Actuator (POS) 1 = 0
HardwareSetting|FilterSettingRecord|X Scan Actuator 1 = Active
HardwareSetting|FilterSettingRecord|Y Scan Actuator (Gain) 1 = 9.99996185317286
HardwareSetting|FilterSettingRecord|Y Scan Actuator (Offs.) 1 = 1.16407704723806E-06
HardwareSetting|FilterSettingRecord|Y Scan Actuator (POS) 1 = 0
HardwareSetting|FilterSettingRecord|Y Scan Actuator 1 = Active
HardwareSetting|FilterSettingRecord|Y-Phase 1 = 0
HardwareSetting|FilterSettingRecord|Z Scan Actuator (POS) 1 = 0
HardwareSetting|FilterSettingRecord|Z Scan Actuator (POS) 2 = -4.97127053381973E-06
HardwareSetting|FilterSettingRecord|Z Scan Actuator 1 = Inactive
HardwareSetting|Name 1 = default
HardwareSetting|ScannerSettingRecord|SystemType 1 = TCS SP5
HardwareSetting|ScannerSettingRecord|bAFUseFixSliceNumber 1 = 0
HardwareSetting|ScannerSettingRecord|bEnableRoiScan 1 = 0
HardwareSetting|ScannerSettingRecord|bIs3DLimitedRoiScanEnable 1 = 0
HardwareSetting|ScannerSettingRecord|bIsSequential 1 = 0
HardwareSetting|ScannerSettingRecord|bStepSizeConstant 1 = 1
HardwareSetting|ScannerSettingRecord|bStepSizerActivated 1 = 0
HardwareSetting|ScannerSettingRecord|bUseCARSLight 1 = 0

```

HardwareSetting|ScannerSettingRecord|bUseChaserUVShutter 1 = 0
HardwareSetting|ScannerSettingRecord|bUseChaserVisibleShutter 1 = 0
HardwareSetting|ScannerSettingRecord|bUseFSOPOLight 1 = 0
HardwareSetting|ScannerSettingRecord|bUseMP2Shutter 1 = 0
HardwareSetting|ScannerSettingRecord|bUseMPS shutter 1 = 0
HardwareSetting|ScannerSettingRecord|bUsePulsed635VisibleLight 1 = 0
HardwareSetting|ScannerSettingRecord|bUsePumpLight 1 = 0
HardwareSetting|ScannerSettingRecord|bUseSTED1Light 1 = 0
HardwareSetting|ScannerSettingRecord|bUseSTED2Light 1 = 0
HardwareSetting|ScannerSettingRecord|bUseSTED3Light 1 = 0
HardwareSetting|ScannerSettingRecord|bUseSTED4Light 1 = 0
HardwareSetting|ScannerSettingRecord|bUseStokesLight 1 = 0
HardwareSetting|ScannerSettingRecord|bUseSuperContVisibleShutter 1 = 0
HardwareSetting|ScannerSettingRecord|bUseUV405Shutter 1 = 0
HardwareSetting|ScannerSettingRecord|bUseUVShutter 1 = 0
HardwareSetting|ScannerSettingRecord|bUseVisibleShutter 1 = 1
HardwareSetting|ScannerSettingRecord|bValidBegin 1 = 1
HardwareSetting|ScannerSettingRecord|bValidEnd 1 = 1
HardwareSetting|ScannerSettingRecord|csLutName0 1 = Green
HardwareSetting|ScannerSettingRecord|csLutName1 1 = Red
HardwareSetting|ScannerSettingRecord|csLutName2 1 = Blue
HardwareSetting|ScannerSettingRecord|csLutName3 1 = Gray
HardwareSetting|ScannerSettingRecord|csLutName4 1 = Gray
HardwareSetting|ScannerSettingRecord|csScanMode 1 = xyz [current scan mode]
HardwareSetting|ScannerSettingRecord|dblAFFocusRange 1 = 0.00005
HardwareSetting|ScannerSettingRecord|dblFocusStabilizerPSDOffset 1 = -2
HardwareSetting|ScannerSettingRecord|dblPinhole 1 = 4.77129376133485E-05 [pinhole, m]
HardwareSetting|ScannerSettingRecord|dblPinholeAiry 1 = 0.499475929206169 [pinhole, airy]
HardwareSetting|ScannerSettingRecord|dblSizeX 1 = 2.46032684568375E-05
HardwareSetting|ScannerSettingRecord|dblSizeY 1 = 9.01218624792582E-08
HardwareSetting|ScannerSettingRecord|dblSizeZ 1 = 1.00288486755835E-05
HardwareSetting|ScannerSettingRecord|dblStepSize 1 = 4.196170993968E-08
HardwareSetting|ScannerSettingRecord|dblVoxelX 1 = 6.00812416528388E-09 [voxel-width, m]
HardwareSetting|ScannerSettingRecord|dblVoxelY 1 = 6.00812416528388E-09 [voxel-height, m]
HardwareSetting|ScannerSettingRecord|dblVoxelZ 1 = 4.196170993968E-08 [voxel-depth, m]
HardwareSetting|ScannerSettingRecord|dblZoom 1 = 9.99996185317286 [ ]
HardwareSetting|ScannerSettingRecord|dwChannelMask 1 = 4
HardwareSetting|ScannerSettingRecord|dwLogiChMask 1 = 2
HardwareSetting|ScannerSettingRecord|eAFAnalyseType 1 = 1
HardwareSetting|ScannerSettingRecord|eAFSubsystem 1 = 0
HardwareSetting|ScannerSettingRecord|eAFWorkflowTimelapse 1 = 1
HardwareSetting|ScannerSettingRecord|eAFWorkflowXY 1 = 1
HardwareSetting|ScannerSettingRecord|eAFZUseMode 1 = 1
HardwareSetting|ScannerSettingRecord|eDataSource 1 = 0
HardwareSetting|ScannerSettingRecord|eDirectional 1 = 1
HardwareSetting|ScannerSettingRecord|eDirectionalY 1 = 1
HardwareSetting|ScannerSettingRecord|eSequentialMode 1 = 0 [ ]
HardwareSetting|ScannerSettingRecord|eZUseMode 1 = 1
HardwareSetting|ScannerSettingRecord|nAFFixSliceNumber 1 = 0
HardwareSetting|ScannerSettingRecord|nAFPrecision 1 = 2
HardwareSetting|ScannerSettingRecord|nAFWorkflowTimelapseIterator 1 = 1
HardwareSetting|ScannerSettingRecord|nAFWorkflowXYIterator 1 = 1
HardwareSetting|ScannerSettingRecord|nAccumulation 1 = 1
HardwareSetting|ScannerSettingRecord|nAverageFrame 1 = 1 [ ]
HardwareSetting|ScannerSettingRecord|nAverageLine 1 = 3 [ ]
HardwareSetting|ScannerSettingRecord|nBegin 1 = 492830
HardwareSetting|ScannerSettingRecord|nBit 1 = 8
HardwareSetting|ScannerSettingRecord|nChannels 1 = 1
HardwareSetting|ScannerSettingRecord|nEnd 1 = 513862
HardwareSetting|ScannerSettingRecord|nFormatInDimension 1 = 4096
HardwareSetting|ScannerSettingRecord|nFormatOutDimension 1 = 16
HardwareSetting|ScannerSettingRecord|nLineAccumulation 1 = 1
HardwareSetting|ScannerSettingRecord|nLines 1 = 9.99996185302825
HardwareSetting|ScannerSettingRecord|nPages 1 = 9.99996185302735
HardwareSetting|ScannerSettingRecord|nSections 1 = 240
IsPreviewImage 1 = 0
Location = E:\pang\work\SP5\leica test files\z-res 02.lif [ ]
Memory|MemoryBlockID 1 = MemBlock_79
Memory|Size 1 = 15728640
Name 1 = PreviewMarker
Name 2 = HardwareSettingList
Name 3 = ChannelAttachment
Name 4 = ViewerScaling
Name 5 = ImageXMLDocument
Name 6 = Image028 [this entry belongs to the next series, don't know why it should be here]
Quantity|Unit 1 = m
Quantity|Unit 2 = m
Quantity|Unit 3 = V
Quantity|Unit 4 = V

```

```
Quantity|Value 1 = 0.00000047
Quantity|Value 2 = 0.0000005
Quantity|Value 3 = 375.562676432441
Quantity|Value 4 = 0
TimeStamp|HighInteger 1 = 30107055 [each slice, ordinal sort order]
TimeStamp|HighInteger 10 = 30107055
... [entries deleted for brevity]
TimeStamp|LowInteger 98 = 3385276720
TimeStamp|LowInteger 99 = 3389326720
```

```
-----

[these are metadata returned by ImageJ]
Title: z-res 02.lif - Series026
Width: 24.60 microns (4096)
Height: 0.09 microns (16)
Depth: 10.07 microns (240)
X Resolution: 166.482 pixels per micron
Y Resolution: 177.537 pixels per micron
ID: -14
Coordinate origin: 0,0,0
Bits per pixel: 8 (color LUT)
Display range: 0-255
Image: 1/240 (z:1/240 - Series026)
No Threshold
Magnification: 0.25
Uncalibrated
Path: E:\pang\work\SP5\leica test files\z-res 02.lif
No Selection
```