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# Converting microscopy image data and metadata with Microfile+

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## ABSTRACT

Enrich the metadata and FAIRness of microscopy image data by converting into OME-TIFF and/or JPEG2000 format with Microfile+.

## PROTOCOL CITATION

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## Conversion Setup

- 1 *MicroFile+ is a free tool compatible with Windows workstations. Download and install Microfile+ by going to <https://www.mbfbioscience.com/microfileplus>.*

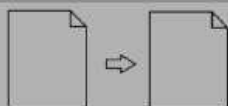
Once installed, launch the MicroFile+ application.

## MicroFile+ (RRID: SCR\_018724)

by MBF Bioscience

### 2 Select **Convert image(s)**.

Images can be converted from image to image, series to image, many channels to multichannel, and serie. The appropriate type of conversion will be image data dependent.



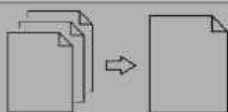
Convert image(s)



Combine 2D images into a 3D stack



Combine images into a multichannel image or stack



Combine images into a projection  
(min, max, or deep focus) image

### 3 In the Save Format section, select **Both** to save images as **JPEG2000** and **OME-TIFF**.

Investigators are encouraged to convert SPARC source image data as JPEG2000 and OME-TIFF formats. The JPX format is a compressed, web streamable version of the source microscopy image data. The OME-TIFF version complies with FAIR data standards by providing a version of the source image that can be viewed by anyone using

free tools such as the ImageJ viewer. Both image formats conserve the original metadata from the source image file and enable enrichment of additional metadata to the converted versions.

- 4 For **JPEG2000 Compression**, select **Lossy** and enter 40:1 compression.

**OME-TIFF data is always written using lossless compression (and therefore no selection is available for compression for this format).**

Because the images in each experiment could be acquired with different imaging devices, cameras, or objectives, the resolution of the images may be different. For each experiment, an appropriate compression level will need to be selected to ensure the compressed JPX images do not contain any artifacts. Artifacts caused by image compression will be most visible in regions of gradient transitions. The original image is likely to have crisp lines at these junctions where the compressed image versions will have a smaller range of color available to represent the gradients. Transitions will appear more pixelated and blurry. This can be severe for some images, making the compressed version uninformative and unrepresentative of the source image. The compression will need to be scaled back for images within that experiment.

- 5 In **Advanced** options, ensure **Enforce required metadata** is checked.

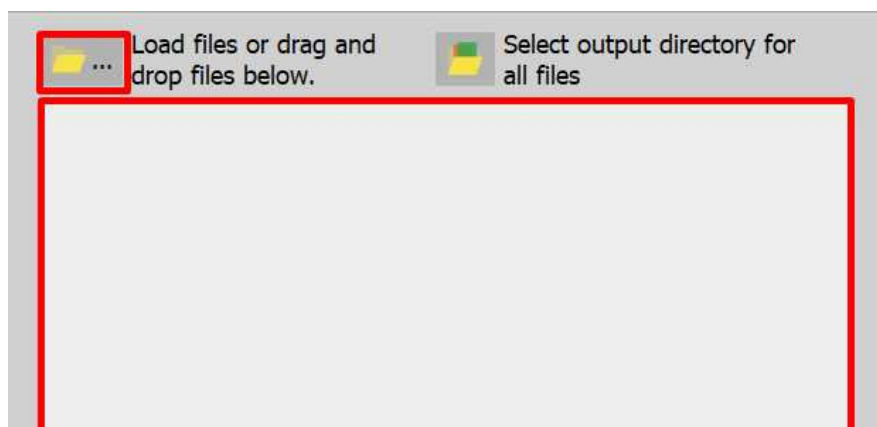
For SPARC conversions, it is necessary to ensure the required metadata values are provided:

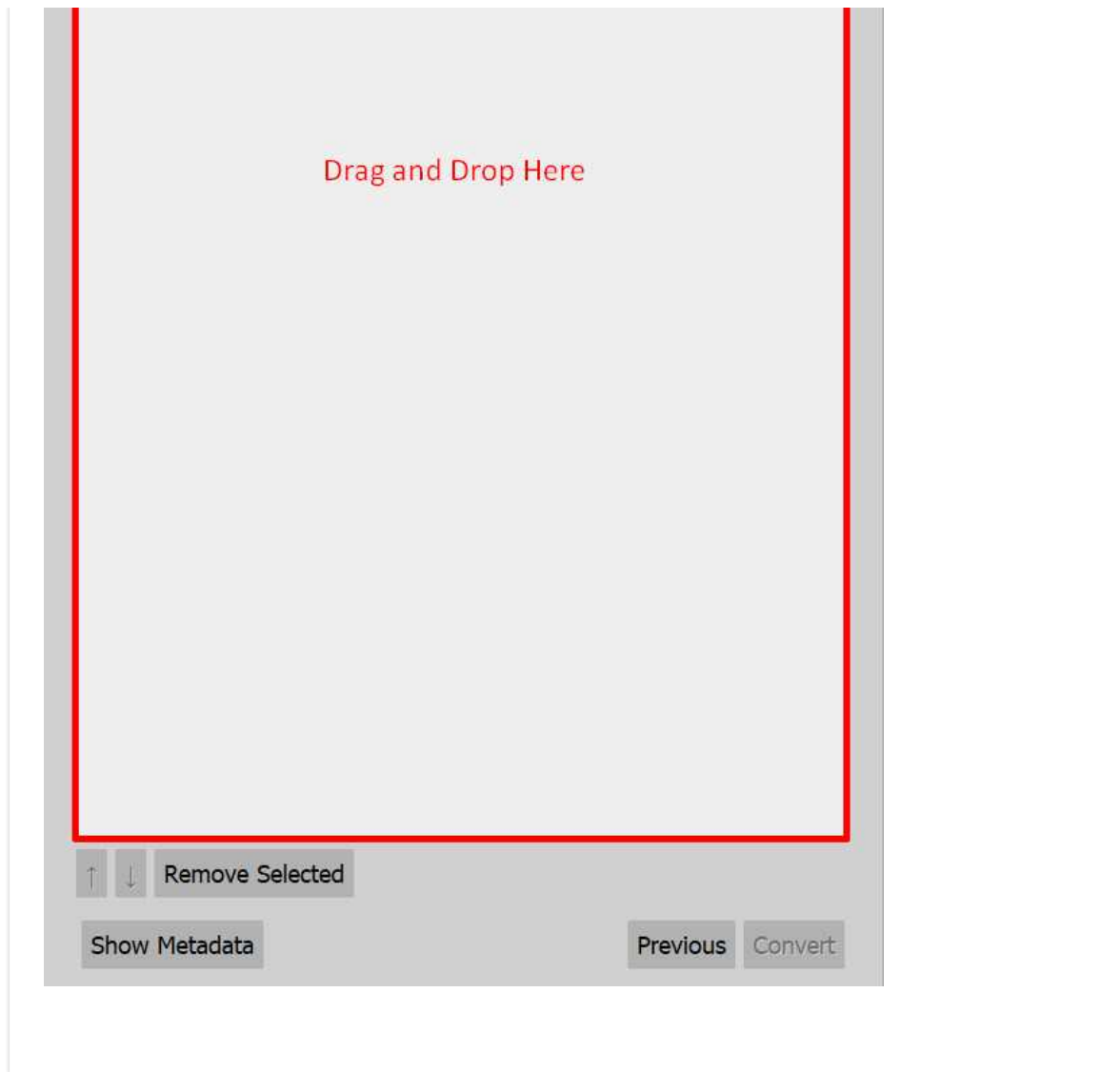
1. Image Name
2. Channel Target Label
3. Pixel size in micrometers in X, Y, and Z
4. Compression

- 6 Click **Next**.

#### Enrich Metadata

- 7 Load the original image file(s) into the converter by either a) clicking on the **Load files or drag and drop files below** button in the upper left corner and browsing to the file or b) dragging and dropping the file(s) into the image selection panel. The image file and conversion result information will populate the table for each file loaded into the converter.





- 8 Select an image from the file list and click **Show Metadata** to open the metadata generation window.
- 9 Metadata conserved from the original file will populate automatically. To add metadata that is missing or change metadata, either a) input the information manually or b) create an Experiment preset that can be selected and applied (see Step 10.1 for more information).

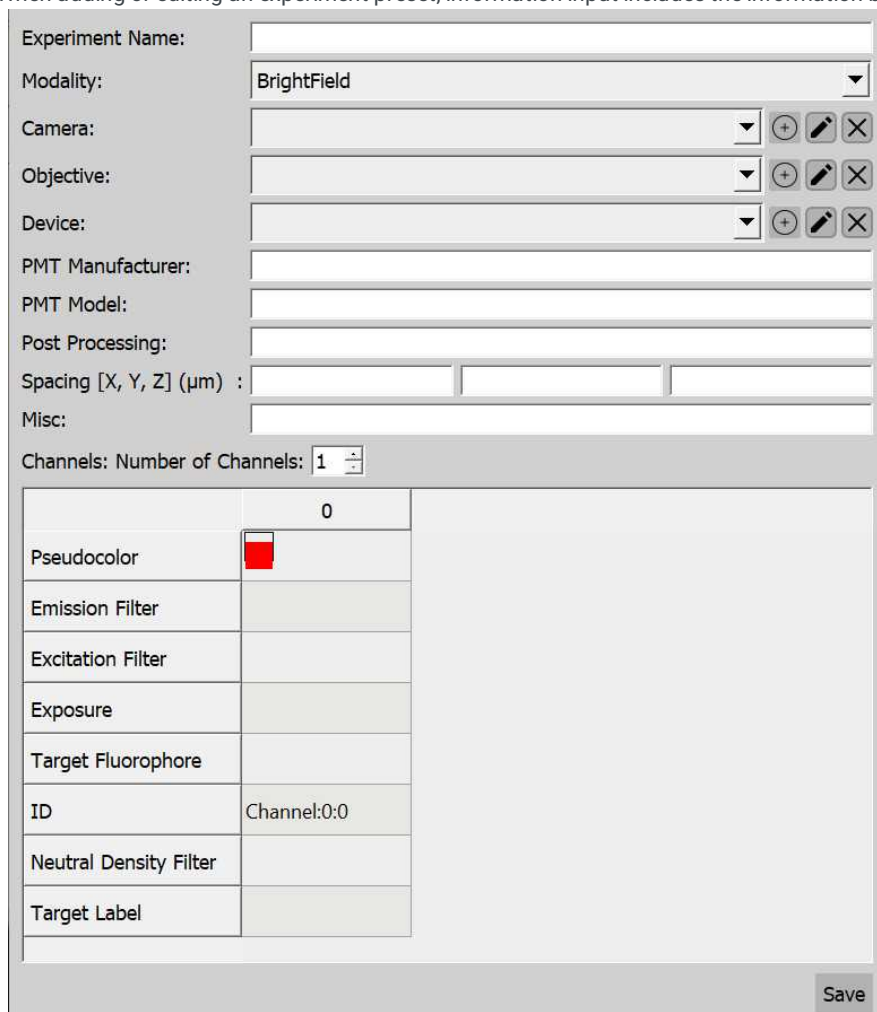
Metadata is collected and/or added for fields pertaining to the channel(s), device/camera, image, information, objective, and photomultiplier tube (PMT). See definitions for each image metadata element in the [Image Metadata Glossary](#).

Enable the **Show all editable fields** checkbox to view all fields that may have metadata written to them.

☒ **Show all editable fields**

- 10 Experiment presets can be created for images with consistent microscope hardware and channel metadata. If this metadata is not included in the source data, it can be added to a set of images with this batch process instead of subjecting users to adding it by hand to hundreds of images.

10.1 When adding or editing an experiment preset, information input includes the information below:



The screenshot shows a form for creating or editing an experiment preset. The fields are as follows:

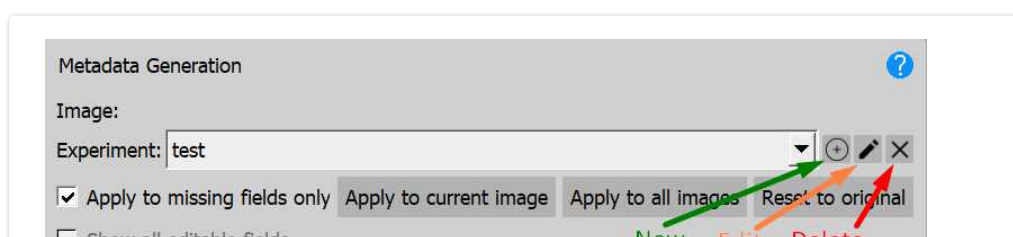
- Experiment Name:
- Modality:
- Camera:  (+) (pencil) (X)
- Objective:  (+) (pencil) (X)
- Device:  (+) (pencil) (X)
- PMT Manufacturer:
- PMT Model:
- Post Processing:
- Spacing [X, Y, Z] (μm) :
- Misc:
- Channels: Number of Channels:

	0
Pseudocolor	<input type="checkbox"/>
Emission Filter	<input type="text"/>
Excitation Filter	<input type="text"/>
Exposure	<input type="text"/>
Target Fluorophore	<input type="text"/>
ID	Channel:0:0
Neutral Density Filter	<input type="text"/>
Target Label	<input type="text"/>

Save

This information is aligned with the metadata fields described in the Image Metadata Glossary.

- 10.2 To create a metadata preset for a set of images select the experiment + button . To edit the currently selected preset, select the **pencil** button and to delete the currently selected preset, select the **X** button. New experiment presets should be created for each set of images with differing microscope hardware, channel settings, etc.

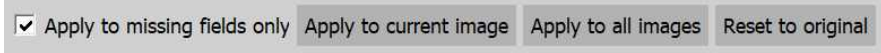


The screenshot shows the 'Metadata Generation' dialog box. It contains the following elements:

- Image:
- Experiment:  (+) (pencil) (X)
- ☒ Apply to missing fields only
- ☐ Show all editable fields
- Buttons: Apply to current image, Apply to all images, Reset to original
- Footer: New Edit Delete



- 10.3 Enable the **Apply to missing fields only** checkbox to apply an experiment preset only to metadata fields that are missing. If the **Apply to missing fields only** checkbox is disabled, applying an experiment preset will overwrite any conserved image metadata. Select the **Apply to current image** button to apply an experiment preset to the current image conversion. Select the **Apply to all images** button to apply an experiment preset to all image conversions. To reset the metadata inputs back to what was populated from the original file, click the **Reset to Original** button.



## 10.4


A screenshot of example metadata fields in Microfile+ for the example image are below:



Key	Value
Channel	
0	
0	
Modality	WideField
Pseudocolor	
CondenserNA	0.550000
ContrastMethod	Fluorescence
Emission Filter	465.000000
Excitation Filter	353.000000
Exposure	0.133333
Target Fluorophore	DAPI
ID	Channel:0
IlluminationType	Epifluorescence
Neutral Density Filter	0.8
Target Label	DAPI
1	
Modality	WideField
Pseudocolor	
CondenserNA	0.550000
ContrastMethod	Fluorescence
Emission Filter	618.000000
Excitation Filter	280.000000
Exposure	0.133333
Target Fluorophore	Alexa Fluor 594
ID	Channel:1
IlluminationType	Epifluorescence
Neutral Density Filter	0.4
Target Label	Alexa Fluor 594
Detector	
ID	Detector:0:0
Model	H7422
Name	Hamamatsu
Type	PMT
Device	
ID	Device:0:0
Model	LSM 800
Name	Zeiss
Type	Inverted
DeviceGroup	
Filter	
Image	
0	
BitsUsedPerPixel	12
Name	CZI Example.czi
OriginX	0.000000
OriginY	0.000000

Origin	0.000000
OriginZ	0.000000
SizeC	2
SizeX	1388
SizeY	1040
SizeZ	0
Info	
DateCreated	2020-11-17T11:04:09.482068
Misc	apprid=SCR_018724;;insrid=SCR_004314
Name	Experiment 1.1
NumDeviceGroups	1
NumImages	1
PostProcessing	Post Processing Measures
Software	MBF Bioscience, Microfile+ 2021.1.1
SourceFiles	CZI Example.czi
SpacingX	0.691888
SpacingY	0.691888
SpacingZ	1.0
UUID	urn:uuid:d496d5ba-d15f-46b4-a7dc-d74703090155
Objective	
ID	Objective:0:0
Immersion	Air
LensNA	0.800000
Magnification	20.000000
Make	Zeiss
RefractiveIndex	1
Model	Plan Aplanachromat
PMT	
Manufacturer	Hamamatsu
Model	R550

## Convert Images

- 11 Use the **save** button in the conversion results column to change the save location of the selected converted image from the default location. Use the **Select output directory for all files** button in the upper right corner to change the save location for all converted images from the default location.

... Load files or drag and drop files below.  Select output directory for all files

File	Conversion Result
... Example.lsm	...s/Image to Image testing/LSM Example.jp2  ✓
CZI Example.czi	...s/Image to Image testing/CZI Example.jp2  ✓

- 12 Click **Convert** to initiate the conversion process.