

MethodsJ2

Joel Ryan, Thomas Pengo, Alex Rigano, Paula Montero Llopis,
Michelle S. Itano, Lisa Cameron, Guillermo Marqués,
Caterina Strambio-De-Castillia, Mark A. Sanders and Claire M. Brown

<https://github.com/ABIF-McGill/MethodsJ2>

Problem to address

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- Global need for standardization of image metadata

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 - Poor reproducibility
- Global need for standardization of image metadata

Standardization of image/microscope metadata

- Common language:

e.g. *“Light source”* vs *“Illumination”* vs *“incident light”*

Standardization of image/microscope metadata

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- Proper databasing

Standardization of image/microscope metadata

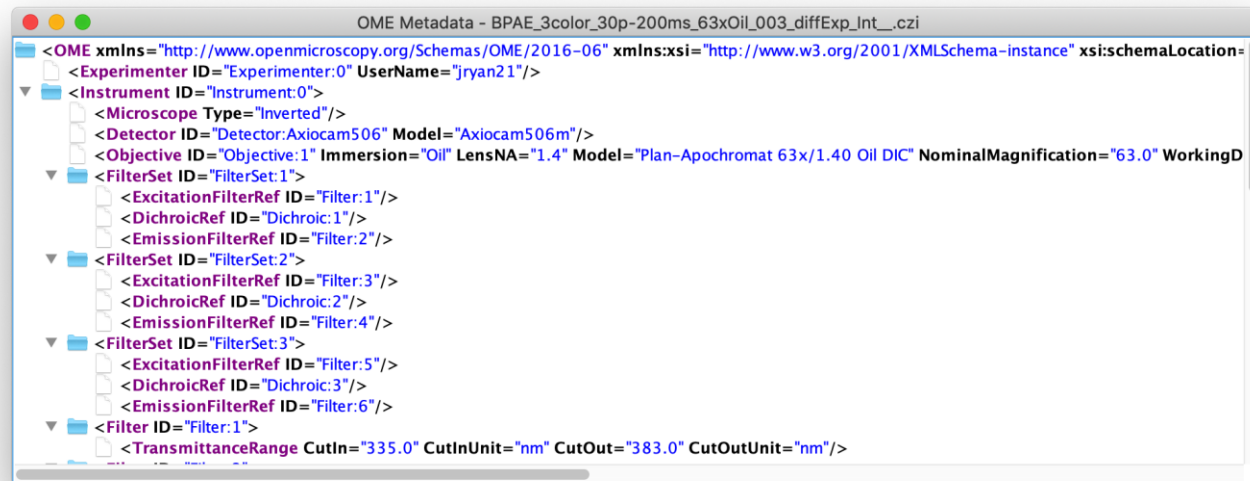
- Common language:

e.g. *“Light source” vs “Illumination” vs “incident light”*

- Proper databasing
- Help users/authors determine what is crucial for reporting

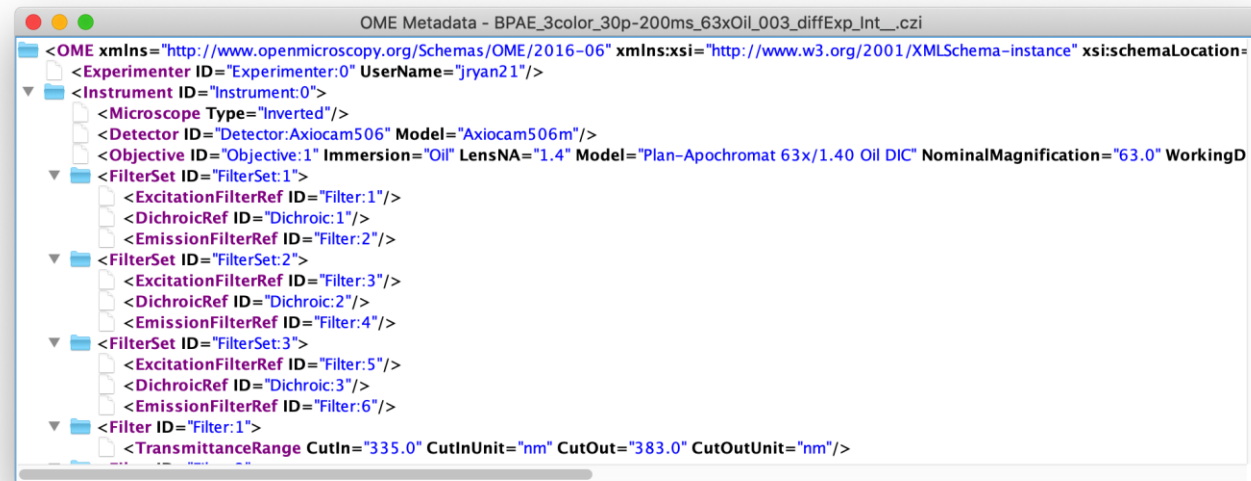
Standardization of image/microscope metadata

- Image metadata: Open Microscope Environment (OME)



Standardization of image/microscope metadata

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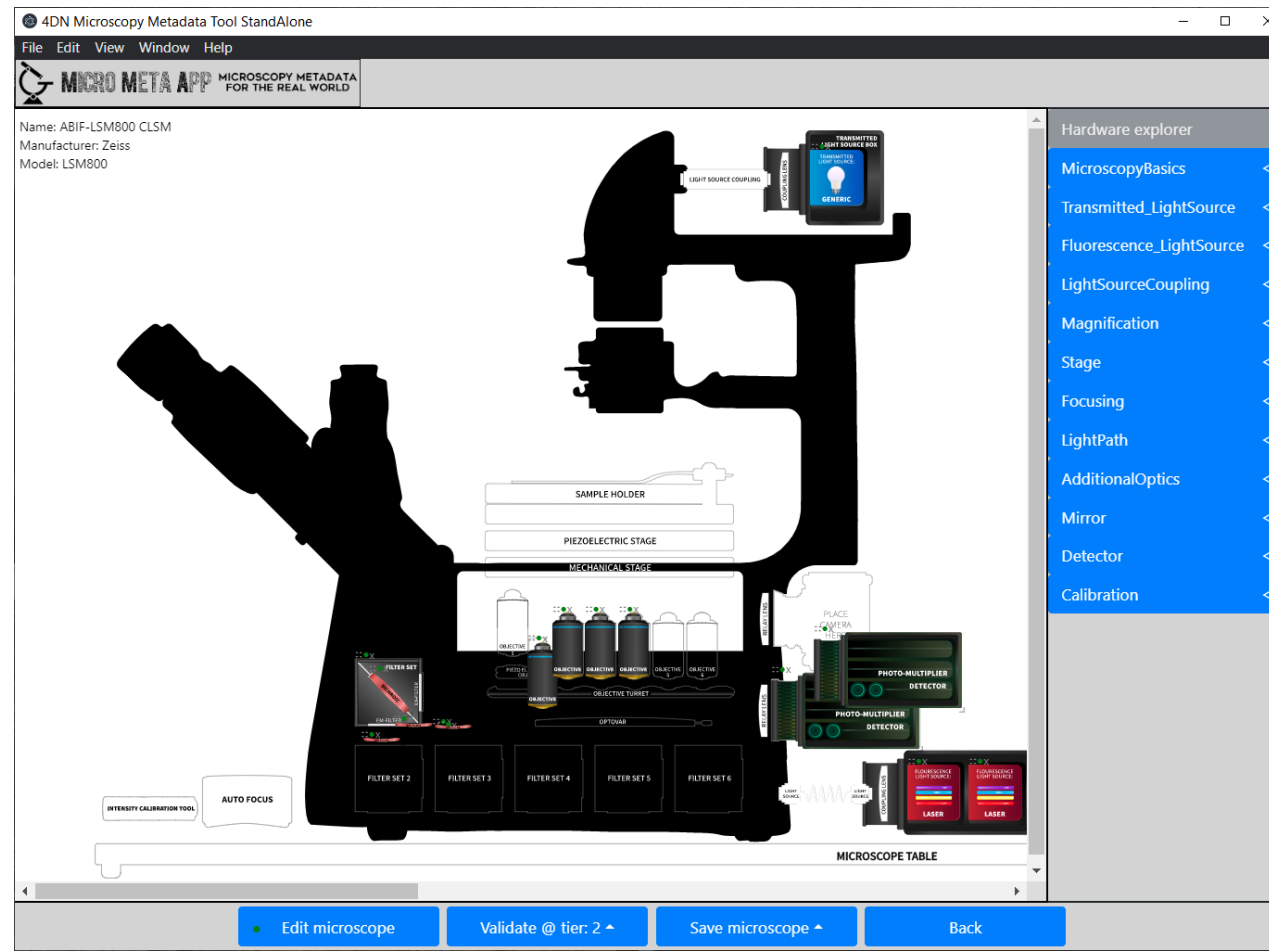


- Microscopy metadata: *4DN-BINA-OME Microscopy specifications*

----> Micro-Meta App

Micro-Meta App

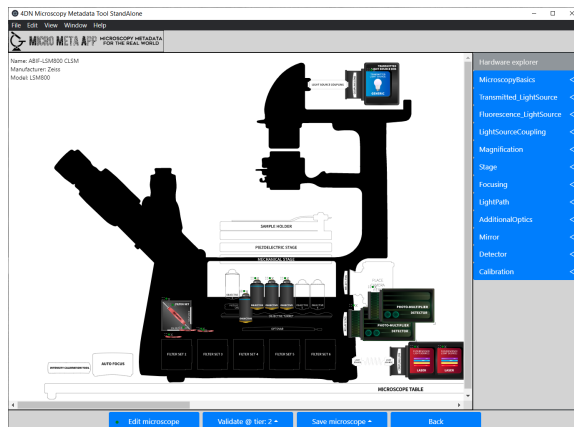
- GUI-based software to build a microscope hardware file using standardized metadata language



Rigano et al,
2021

Micro-Meta App

“Building” your microscope generates a standardized configuration file for that microscope



```
1 {
2   "Name": "ABIF Axiovert1",
3   "Schema_ID": "Instrument.json",
4   "ID": "93efae8d-1042-43aa-994e-2f5bc2a92fb1",
5   "Tier": 3,
6   "ValidationTier": 1,
7   "ModelVersion": "2.01.0",
8   "AppVersion": "1.2.2-b1",
9   "MicroscopeStand": {
10    "Name": "ABIF-Axiovert1",
11    "Schema_ID": "InvertedMicroscopeStand.json",
12    "ID": "370f843c-b4fb-4c75-a385-d4b6049eb3cb",
13    "Tier": 1,
14    "ModelVersion": "2.01.0",
15    "Extension": "Basic",
16    "Domain": "MicroscopeHardwareSpecifications",
17    "Category": "MicroscopeStand",
18    "Manufacturer": "Zeiss",
19    "Model": "Axio Observer Z1",
20    "CatalogNumber": "999",
21    "Type": "Compound",
22    "Origin": "Commercial-custom modified"
23  },
24  "components": [
25    {
26      "Name": "Zen",
27      "ID": "df2a3244-27c4-4a2b-b0b4-6969509f64a5",
28      "Tier": 1,
29      "Schema_ID": "AcquisitionSoftware.json",
30      "ModelVersion": "2.01.0"
31    }
32  ]
33 }
```

Micro-Meta App ---> MethodsJ2

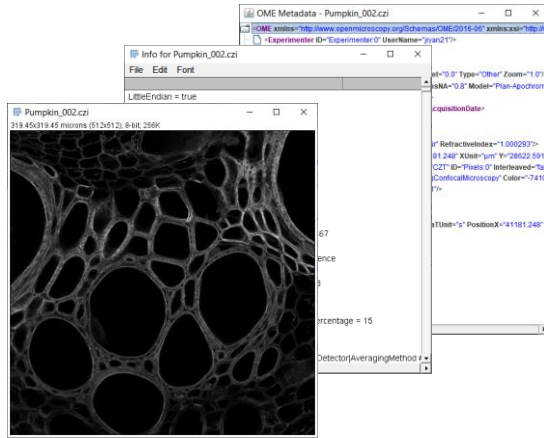
- Problem to address:

Authors of research papers tend to write incomplete methods sections for microscopy data/experiments

- Solution:

Build a Fiji script to help write methods sections

MethodsJ2 – Information input



Image, metadata,
OME metadata



MethodsJ2

Python script
running in Fiji

OME Metadata - Pumpkin_002.czi

OME xmls="http://www.openmicroscopy.org/Schemas/OME/2016-06/" xml:base="http://www.openmicroscopy.org/Schemas/OME/2016-06/" xml:lang="en" xml:space="preserve"><Experiment O="Experiment 0" User="Name" User="27">

Info for Pumpkin_002.czi

File Edit Font

LittleEndian = true

Pumpkin_002.czi

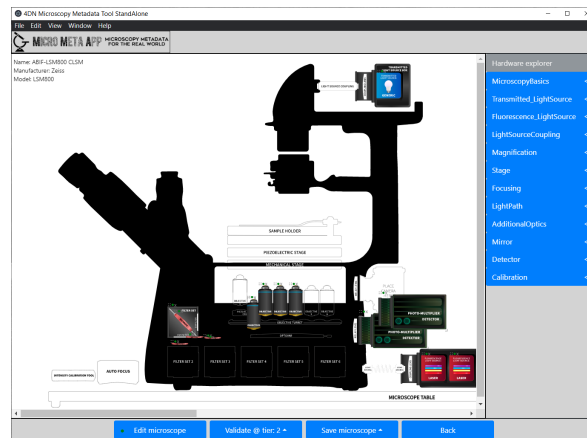
318 45x318 45 microns (612x512) 8-bit, 256K

67
ence
n
percentage = 15
DetectorAveragingMethod =

st="0.0" Type="Other" Zoom="1.0716283529663014" />
sName="0.0" Model="Plan-Apochrom
acquisitionDate">
/>
ReflectedIndex="1.0002937" />
61248" XSize="612" YSize="28622 59
CZT ID="Plots 0" Interleave="To
ConfocalMonoscopy" Color="741
7">
tTime="3" PositionX="41181 248"

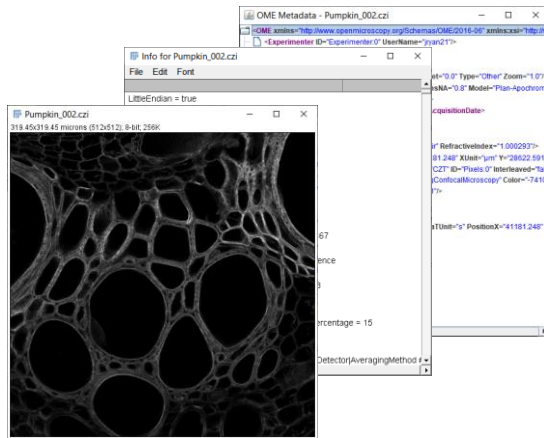
MethodsJ2

Contains objectives,
filters, detectors, etc..

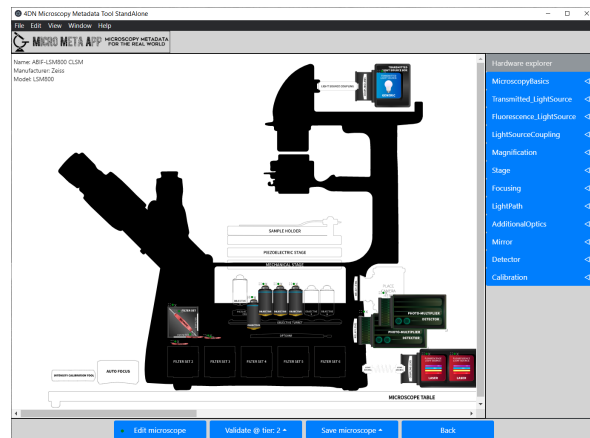


Micro-Meta App microscope hardware specifications file

MethodsJ2 – Information input



Image, metadata,
OME metadata



Micro-Meta App microscope
hardware specifications file

MethodsJ2

Python script
running in Fiji

User input,
Data validation

e.g.:
- validate detected
exposure time,
- choose objective from
those available on the
selected microscope

[illegible]

MethodsJ2

User input,
guided by core
facility staff

MethodsJ2 output

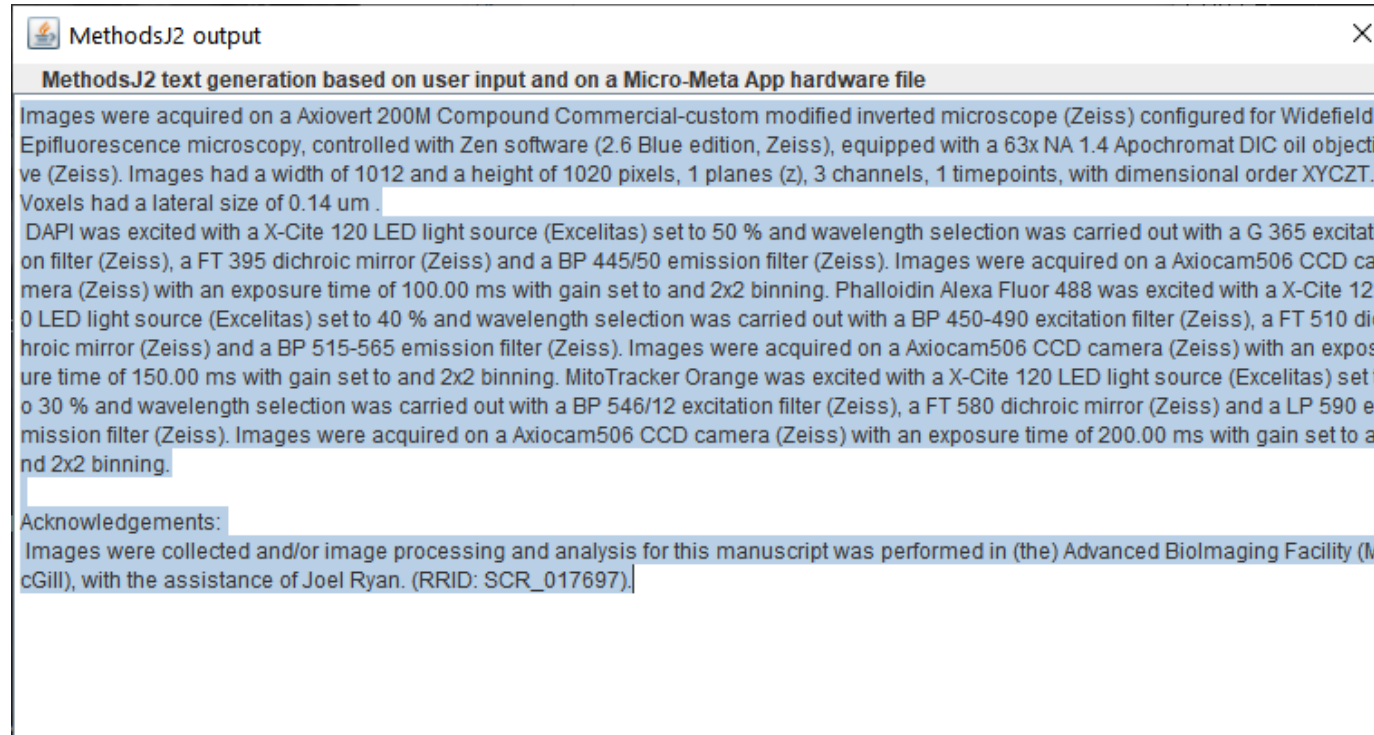
MethodsJ2 text generation based on user input and on a Micro-Meta App hardw...

DAPI was excited with a X-Cite 120 LED light source (Excelitas) set to 50 % and wavelength selection was carried out with a G 365 excitation filter (Zeiss), a FT 395 dichroic mirror (Zeiss) and a BP 445/50 emission filter (Zeiss). Images were acquired on a Axiocam506 CCD camera (Zeiss) with an exposure time of 100.00 ms with gain set to and 2x2 binning. Phalloidin-AF488 was excited with a X-Cite 120 LED light source (Excelitas) set to 40 % and wavelength selection was carried out with a BP 450-490 excitation filter (Zeiss), a FT 510 dichroic mirror (Zeiss) and a BP 515-565 emission filter (Zeiss). Images were acquired on a Axiocam506 CCD camera (Zeiss) with an exposure time of 150.00 ms with gain set to and 2x2 binning. Mitotracker Orange was excited with a X-Cite 120 LED light source (Excelitas) set to 30 % and wavelength selection was carried out with a BP 546/12 excitation filter (Zeiss), a FT 580 dichroic mirror (Zeiss) and a LP 590 emission filter (Zeiss). Images were acquired on a Axiocam506 CCD camera (Zeiss) with an exposure time of 200.00 ms with gain set to and 2x2 binning.

Acknowledgements:

Images were collected and/or image processing and analysis for this manuscript was performed in (the) Advanced Biolmaging Facility (McGill), with the assistance of Joel Ryan. (RRID: SRC_017697).

MethodsJ2 – text output



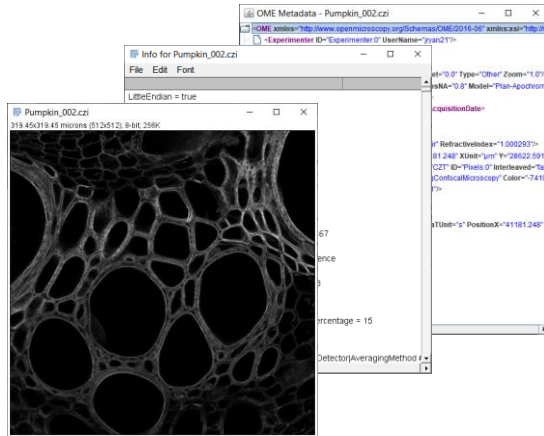
A draft of an experimental section text is displayed in a popup window and copied to the clipboard, to be pasted into a manuscript for revision.

MethodsJ2 – csv output

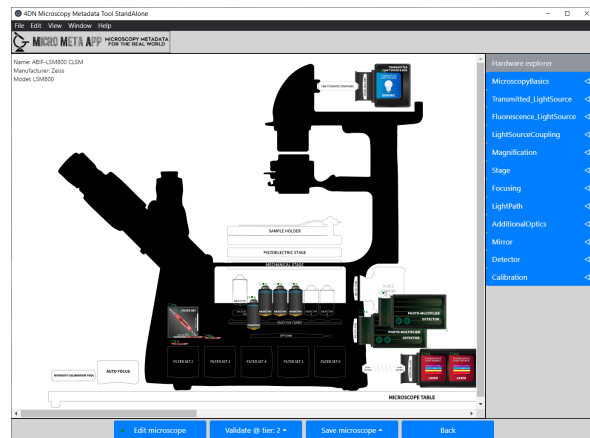
	A	B	C
1	Label	Image metadata value	User input value
2	Script		MethodsJ2 v1.2
3	Date		8/9/2021 11:40:58 AM
4	Image file:		C:\Users\joelr\Documents\GitHubRepositories\MethodsJ2\BPAE_3color_30p-200ms_63xO
5	MJ2 structure file:		https://raw.githubusercontent.com/ABIF-McGill/MethodsJ2/main/MJ2_structure_files/MJ2
6	Sample description:		Cultured BPAE cells
7	Sample preparation:		grown on No. 1.5 glass coverslips, fixed with 4% PFA and stained with DAPI, Phalloidin Alex
8	Mounting medium:		mounted in Cytoseal
9	Coverglass:		
10	Sample holder:		on glass slides
11	Image width in pixels (X):	1012	1012
12	Image height in pixels (Y):	1020	1020
13	Number of slices (Z):	1	1
14	Number of channels (C):	3	3
15	Number of frames (T):	1	1
16	Dimension order:	XYCZT	XYCZT
17	Pixel size XY (micron):	0.14	0.14
18	Voxel size Z (micron):	n/a	n/a
19	Time interval:	n/a	n/a
20	Micro-Meta App json file:		C:\Users\joelr\Documents\GitHubRepositories\MethodsJ2\abif_axiovert1.json
21	Microscope:	Zeiss wide field	Zeiss Axiovert 200M Compound (ABIF Axiovert1)
22	Please select the best descriptor for this system		Widefield Epifluorescence
23	Acquisition software:		Zen
24	Select objective:	63x NA 1.4	63X PLAN APOCHROMAT, NA=1.40, OIL, DIC
25	Channel Description (e.g. fluorophore, labeled protein or cell type):		DAPI
26	Light source:		X-Cite light source
27	Light source intensity:		30%
28	Select excitation filter:		G 365 - DAPI excitation filter
29	Select dichroic:		FT 395 - DAPI beamsplitter

A csv file is generated and saved containing the data collected from the image metadata as well as the data input by the user (either manually or selected from options sourced from the microscope.json file), and the methods text generated.

MethodsJ2 – how it works...



Image, metadata,
OME metadata



Micro-Meta App microscope
hardware specifications file

MethodsJ2

Python script
running in Fiji

- “asks the questions”
- Downloaded by users

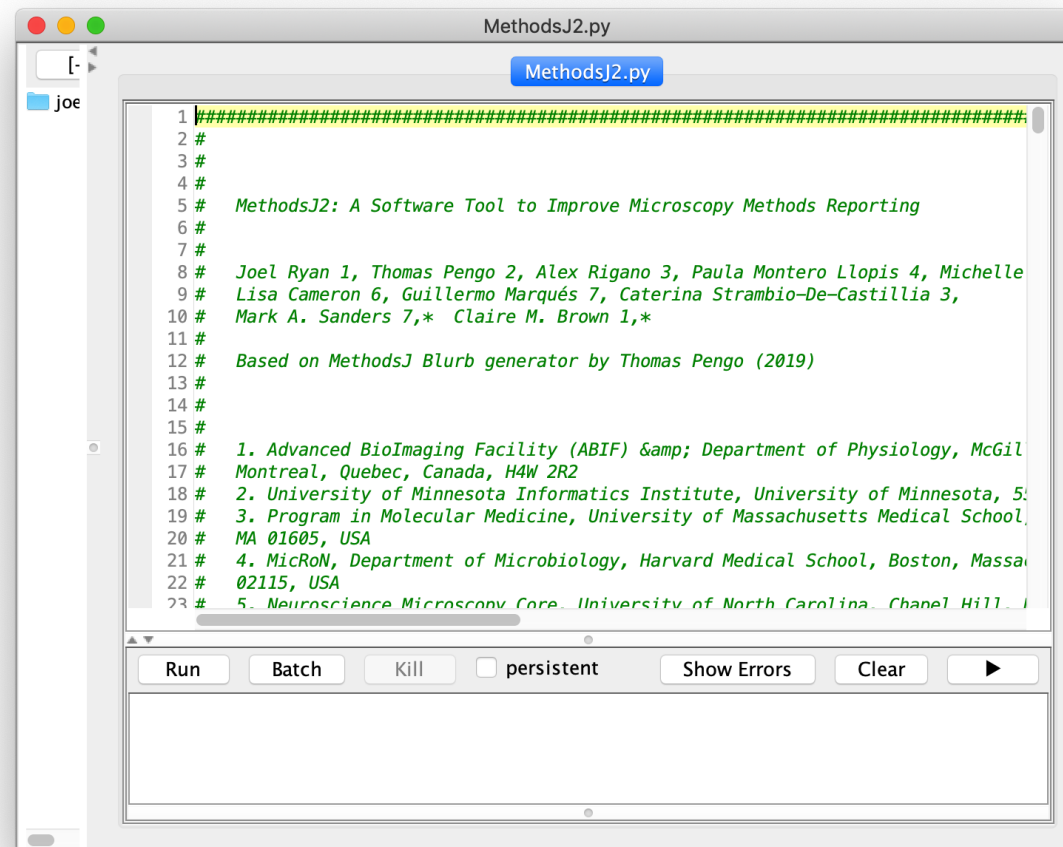
MJ2
structure file

- “defines the questions”
- Stored online

MethodsJ2 – structure file (online)

```
292     {
293         "Dialog_Box": "Channel Settings",
294         "category": "general",
295         "Dialog_Type": "addChoice",
296         "Setting": "Select excitation filter: ",
297         "Add_to_same_row": 0,
298         "CheckHardwareJSON": 1,
299         "Schema_ID": "ExcitationFilter.json",
300         "attributes": [
301             "Model",
302             "Manufacturer"
303         ],
304         "blurb": "and wavelength selection was carried out with a %s excitation filter (%s), "
305     },
```

MethodsJ2 - usage



- Download MethodsJ2 python script from Github
- Open in macro/script editor in Fiji.

AUTOMATED-INTUITIVE-INTERACTIVE

- Visual documentation guide
- Automate
- Teach/Train
- Web-integration

Micro-Meta App



MethodsJ2

- Extract
- Consolidate
- Automate
- Methods text

- Model extension
- Experimental metadata
- Link to imaging facility

OMERO.mde



MethodsJ2

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<https://github.com/ABIF-McGill/MethodsJ2>

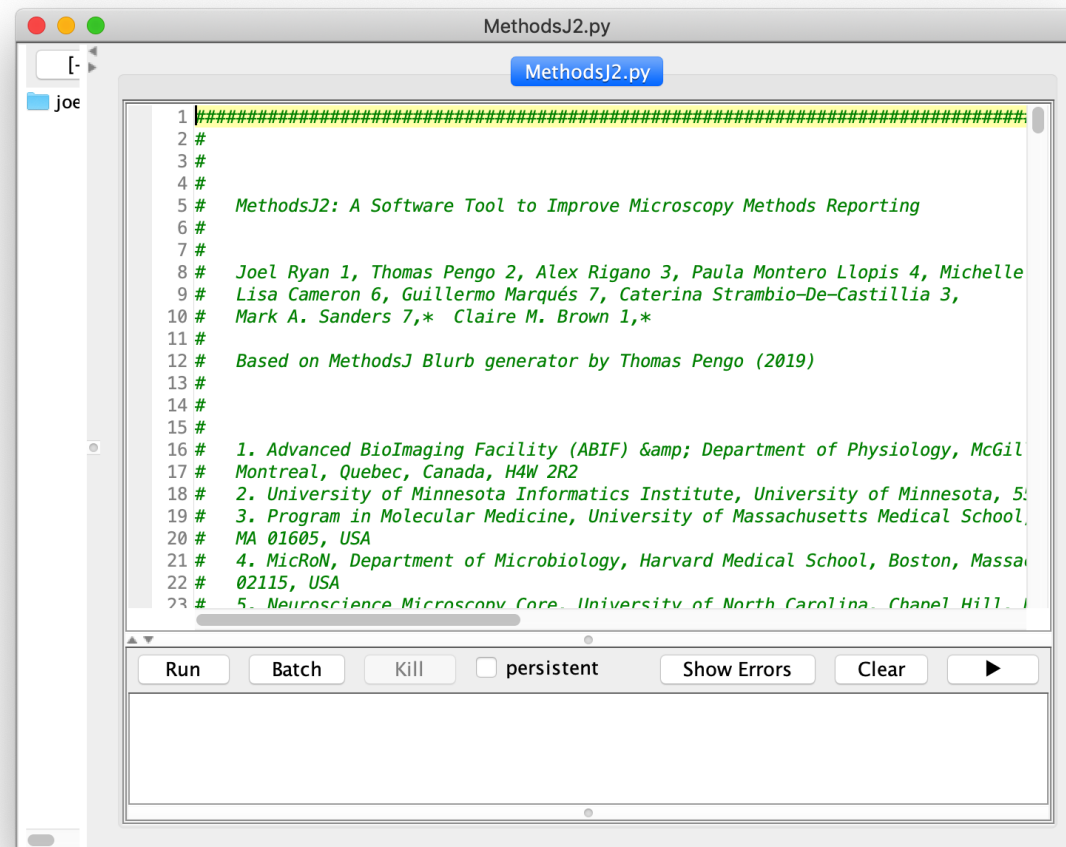
<https://www.biorxiv.org/content/10.1101/2021.06.23.449674v1>

MethodsJ2 - run



Drag and drop MethodsJ2.py on the main Fiji toolbar.

Alternatively, click File > New > Script, then in the Script Editor, Click File > Open, and select MethodsJ2.py

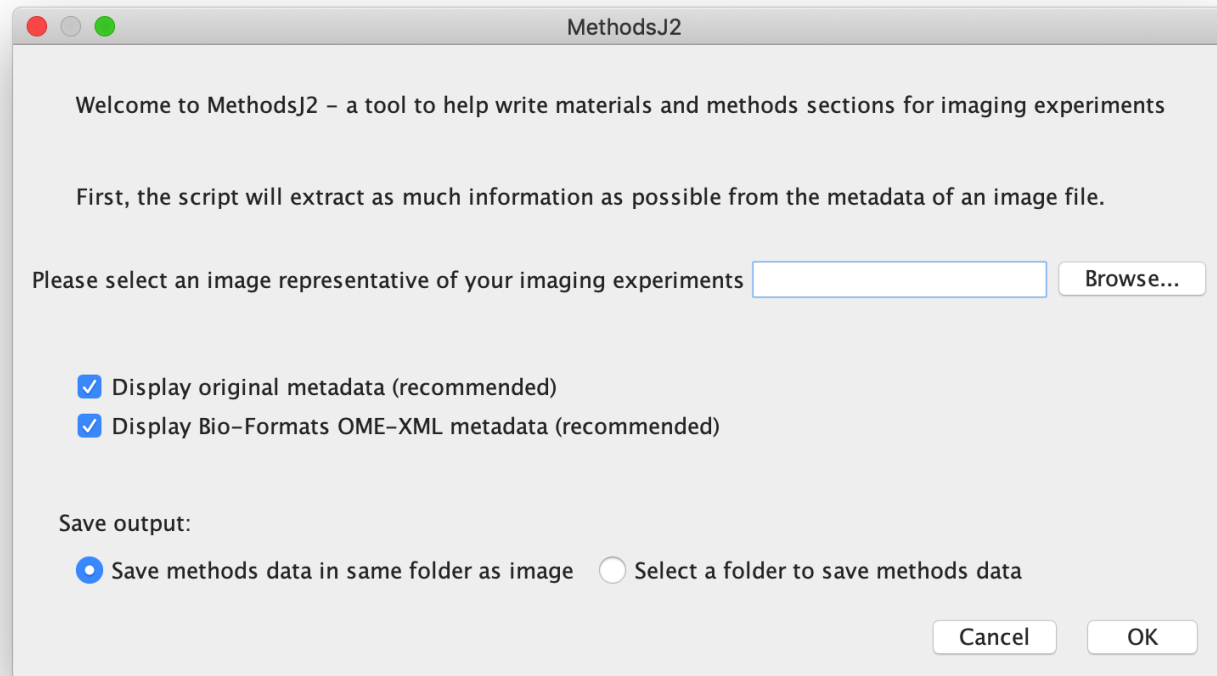


Check language: click Language and select Python

Once the script is loaded is ready, click Run

It may take a few seconds to start.

MethodsJ2 – select image



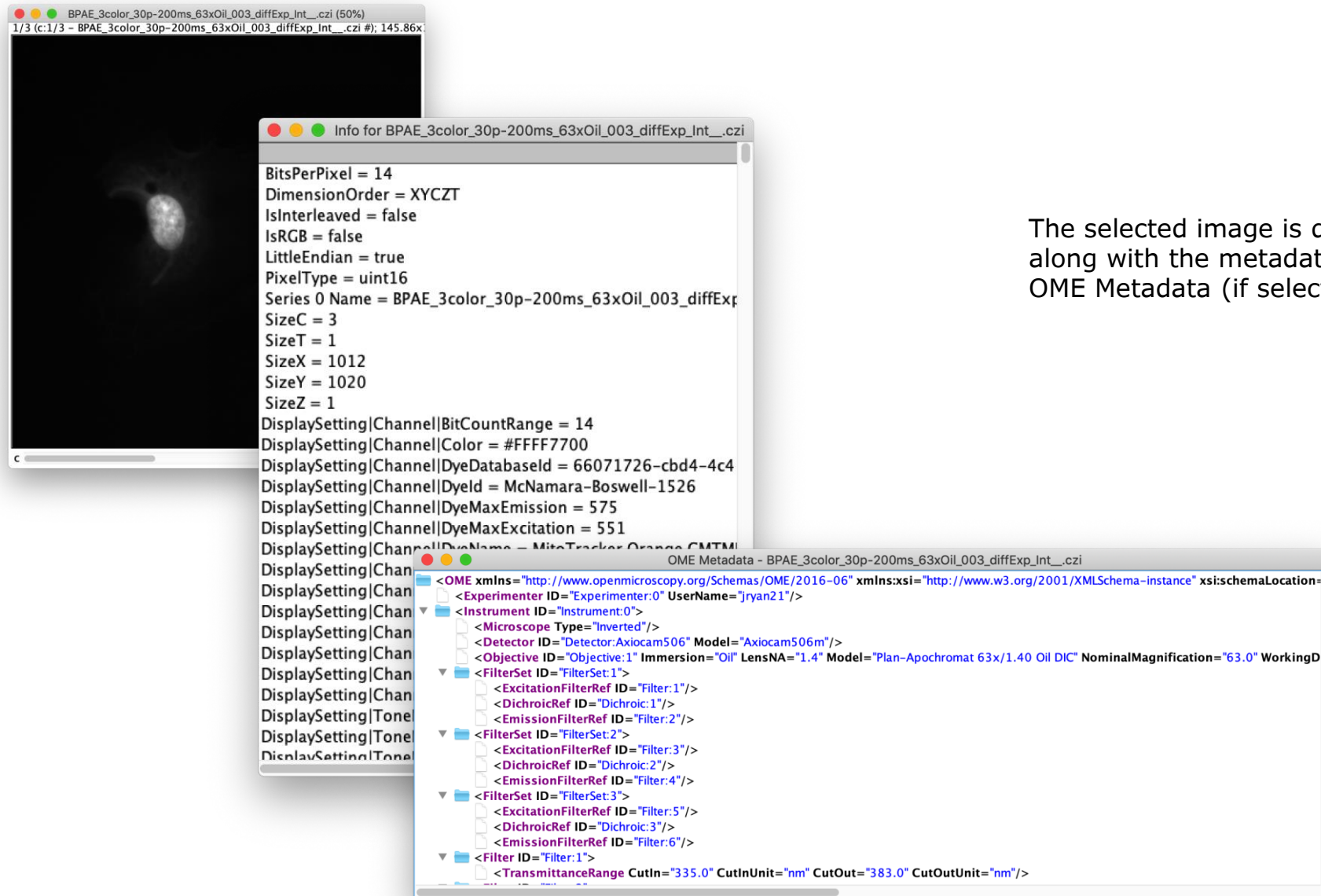
Select an image to load and to source metadata.

Click on Browse and navigate to the image, or drag and drop in image file into the text input field

Optional: display metadata windows (useful for filling out dialog boxes later)

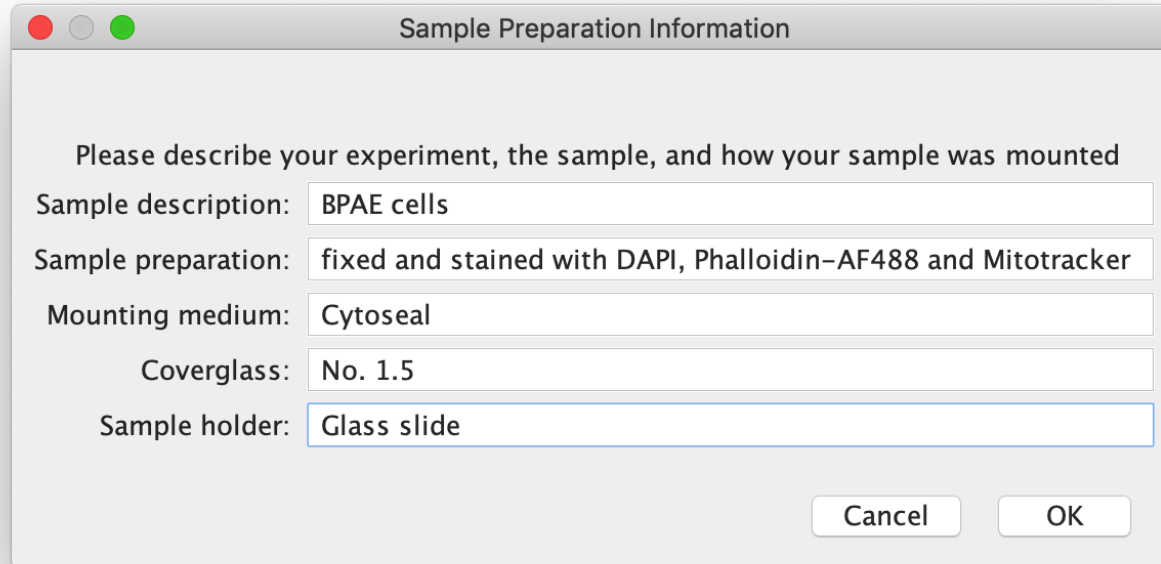
Select where to save the csv file output of the script.

MethodsJ2 – select image



The selected image is displayed,
along with the metadata and
OME Metadata (if selected)

MethodsJ2 – Sample Preparation



A screenshot of a macOS-style dialog box titled "Sample Preparation Information". The dialog has a light gray background and a title bar with red, yellow, and green window control buttons. Inside the dialog, there is a prompt: "Please describe your experiment, the sample, and how your sample was mounted". Below this prompt are five text input fields, each with a label to its left. The labels and their corresponding values are: "Sample description:" with "BPAE cells", "Sample preparation:" with "fixed and stained with DAPI, Phalloidin-AF488 and Mitotracker", "Mounting medium:" with "Cytoseal", "Coverglass:" with "No. 1.5", and "Sample holder:" with "Glass slide". At the bottom right of the dialog are two buttons: "Cancel" and "OK".

Sample Preparation Information

Please describe your experiment, the sample, and how your sample was mounted

Sample description: BPAE cells

Sample preparation: fixed and stained with DAPI, Phalloidin-AF488 and Mitotracker

Mounting medium: Cytoseal

Coverglass: No. 1.5

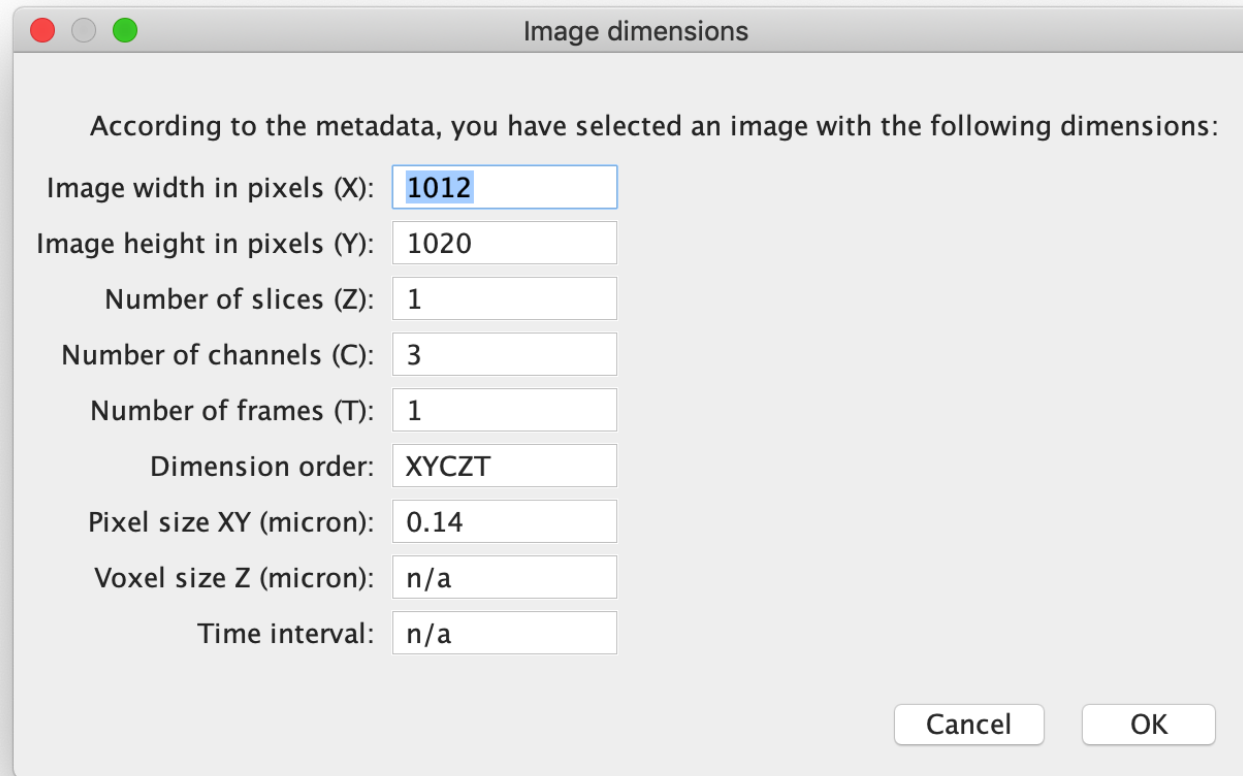
Sample holder: Glass slide

Cancel OK

Please provide information about the sample, and how it was prepared for imaging

Given the variety of samples and preparations, no text is generated for sample description. It is more of a reminder for users to provide complete sample information.

MethodsJ2 – Image dimensions

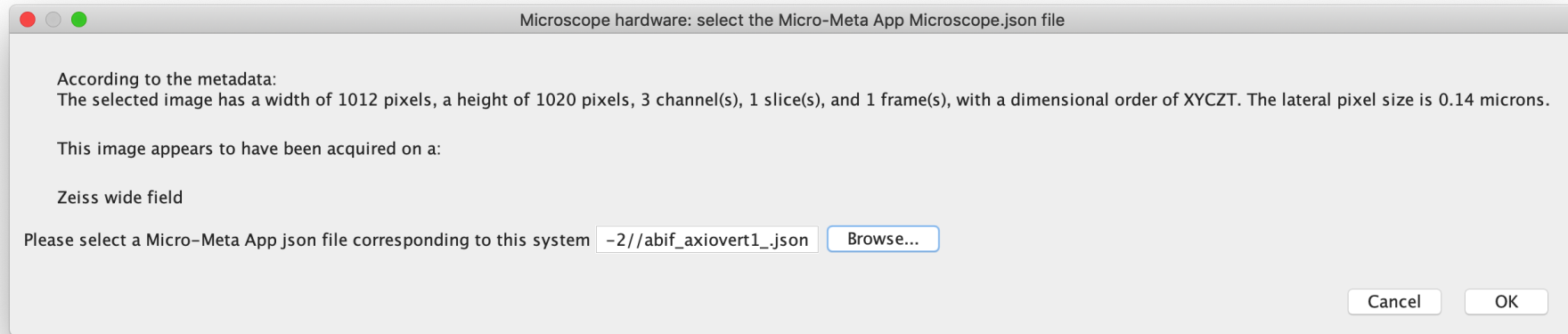


A macOS-style dialog box titled "Image dimensions" with standard red, yellow, and green window control buttons in the top-left corner. The dialog contains a text label "According to the metadata, you have selected an image with the following dimensions:" followed by a list of image properties, each with a corresponding text input field. The "Image width in pixels (X)" field is highlighted with a blue border and contains the value "1012". The other fields contain "1020", "1", "3", "1", "XYCZT", "0.14", "n/a", and "n/a" respectively. At the bottom right, there are "Cancel" and "OK" buttons.

Property	Value
Image width in pixels (X):	1012
Image height in pixels (Y):	1020
Number of slices (Z):	1
Number of channels (C):	3
Number of frames (T):	1
Dimension order:	XYCZT
Pixel size XY (micron):	0.14
Voxel size Z (micron):	n/a
Time interval:	n/a

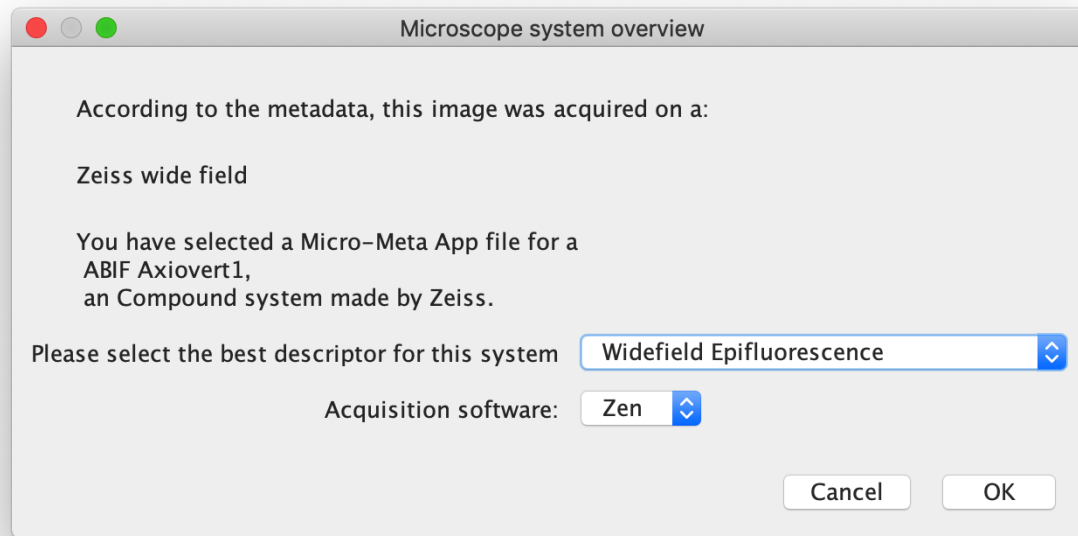
Please verify image dimensions.
Values are sourced from the
image metadata

MethodsJ2 – select Microscope.json file



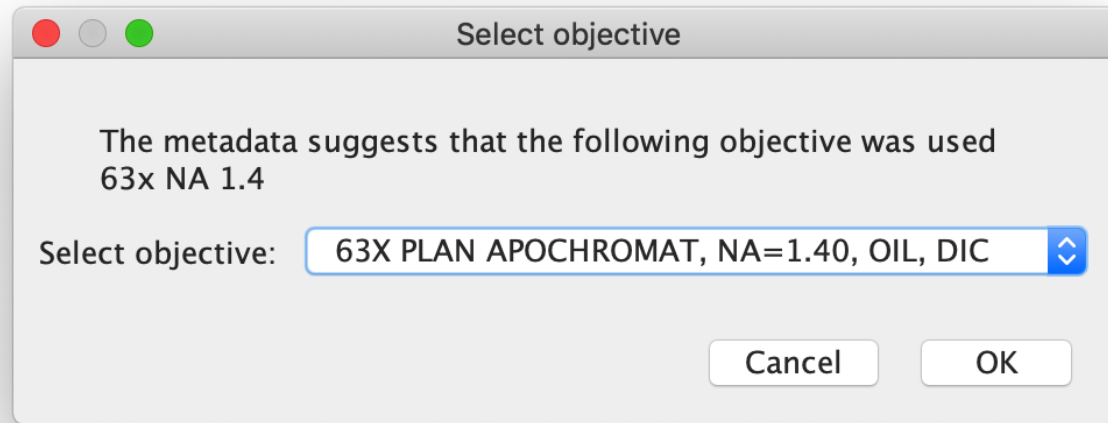
Choose Micro-Meta App hardware specifications file for the microscope used to acquire the selected image

MethodsJ2 – choose descriptor and software



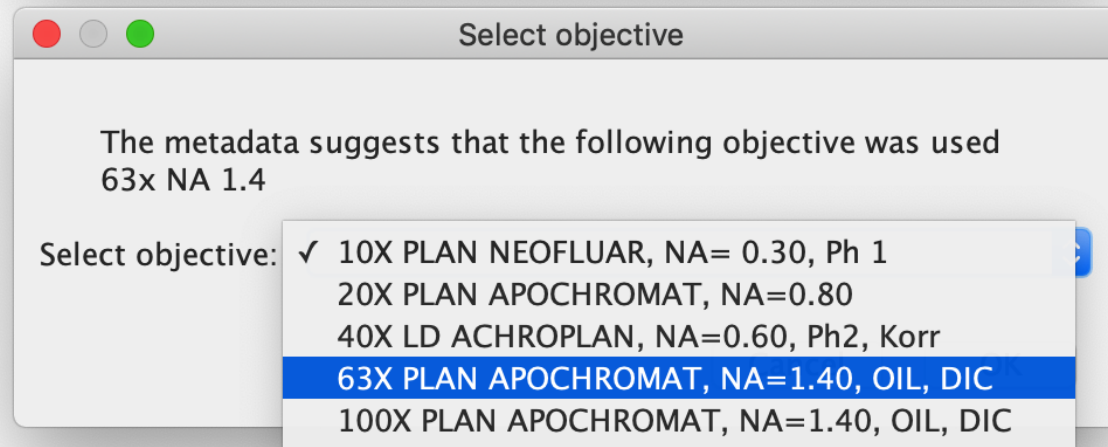
Please select the best descriptor for the selected microscope, as well as the acquisition software.

MethodsJ2 – select objective



Select the objective used for this experiment.

A suggestion is made based on the metadata, and the list of objectives to choose from is sourced from the microscope configurations file.



The drop-down menu is populated from objectives available in the Micro-Meta app hardware specifications file.

MethodsJ2 – Channel acquisition settings

Channel 1: Excitation, wavelength and detector selection

The image metadata suggests that the excitation wavelength for channel 1 is 353 nm and the emission wavelength is 465 nm.

Channel Description (e.g. fluorophore, labeled protein or cell type):

Light source:

Light source intensity:

Select excitation filter:

Select dichroic:

Select emission filter:

Detector:

Channel 1: camera settings

Exposure time:

Gain (if adjustable and available):

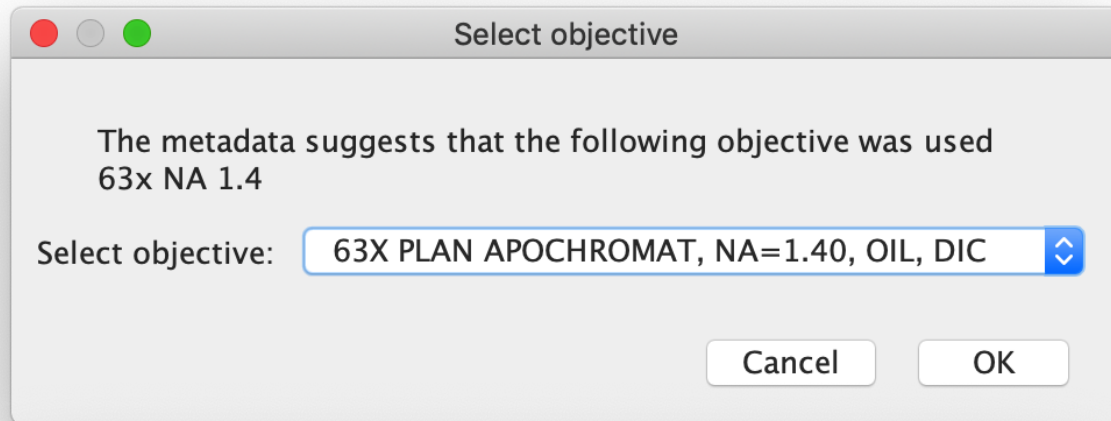
Camera Binning:

Please fill in information for the first channel

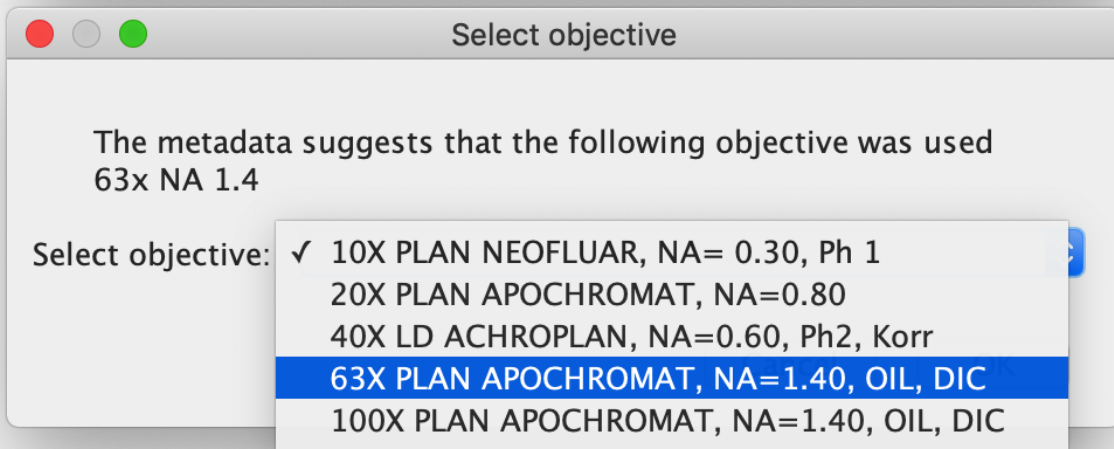
Options in drop-down menus are sourced from the microscope configuration file.

Detector settings are based on whether a camera or point detector is selected

MethodsJ2 – select objective



'Schema_ID': "Objective.json", make dropdown menu with 'Name'



```
return "Magnification",  
      "LensNA",  
      "Correction",  
      "ContrastModulation",  
      "DIC",  
      "ImmersionType",  
      "CorrectionCollar",  
      "Manufacturer"
```

MethodsJ2 – Channel settings

Channel 1: Excitation, wavelength and detector selection

The image metadata suggests that the excitation wavelength for channel 1 is 353 nm and the emission wavelength is 465 nm.

Channel Description (e.g. fluorophore, labeled protein or cell type): (human)

Light source: (human)

Light source intensity: (human)

Select excitation filter: (human)

Select dichroic: (human)

Select emission filter: (human)

Detector: (human)

'Schema_ID': "Fluorescence_LightSource_LightEmittingDiode.json",
or "Fluorescence_LightSource_Laser.json"

'Schema_ID': "ExcitationFilter.json"

'Schema_ID': "StandardDichroic.json"

'Schema_ID': "EmissionFilter.json"

'Schema_ID': "CCD.json", "IntensifiedCamera.json" or "CMOS.json"

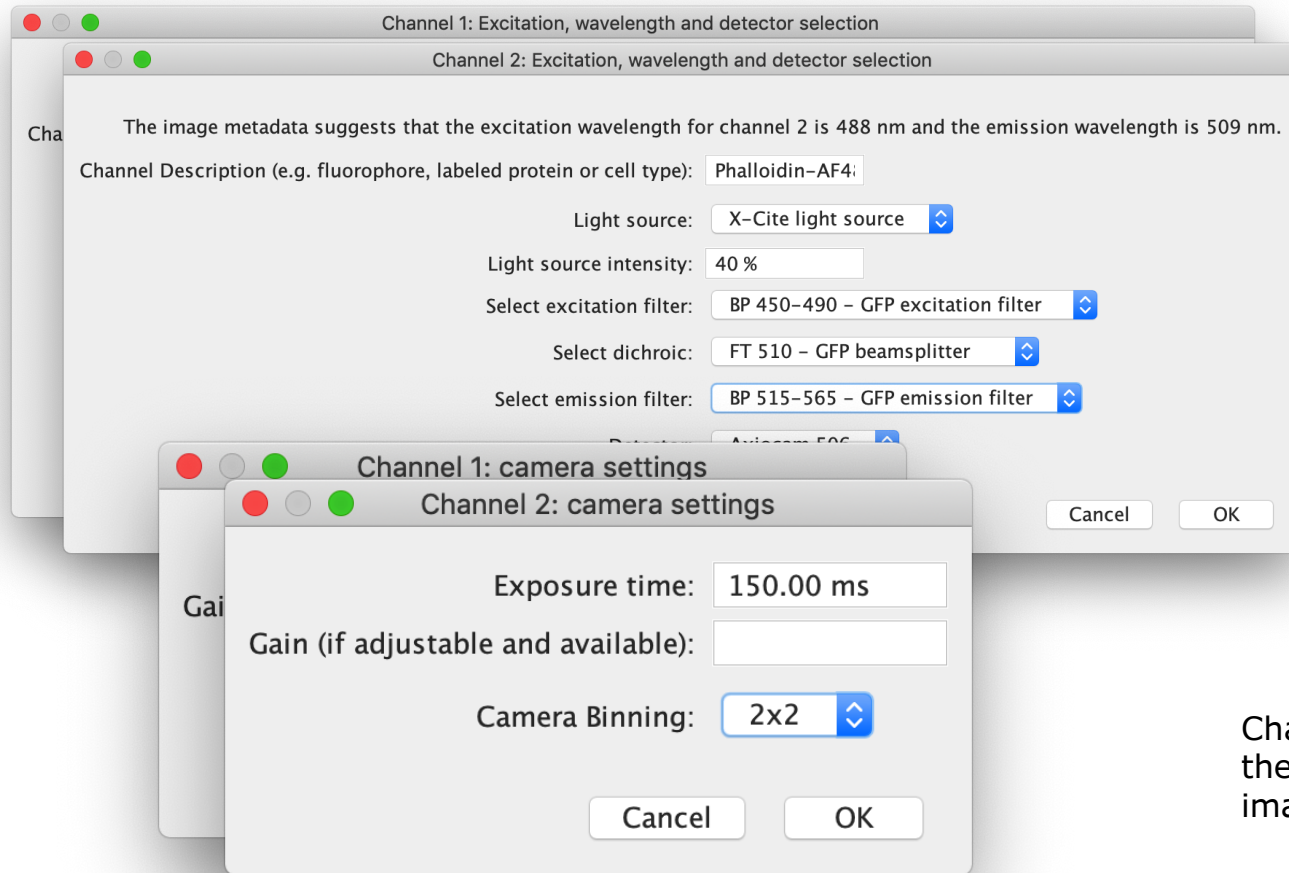
Channel 1: camera settings

Exposure time: (human)

Gain (if adjustable and available): (human)

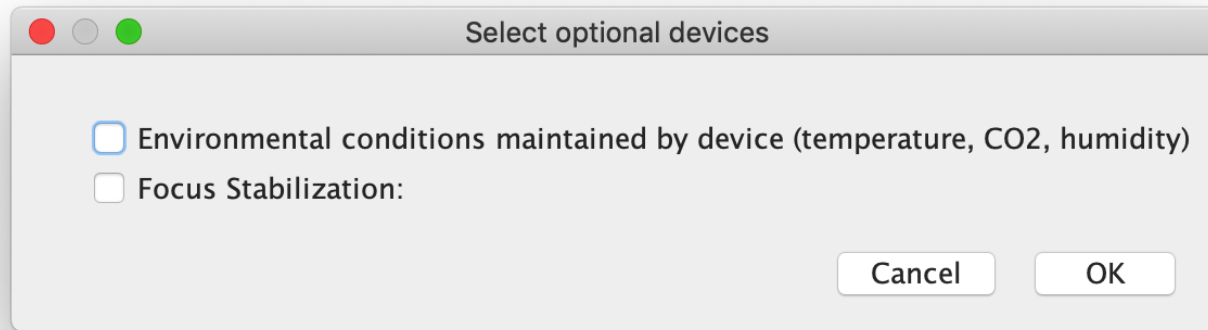
Camera Binning: (human, Dropdown choices: ["1x1", "2x2", "4x4"])

MethodsJ2 – Channel acquisition settings loop



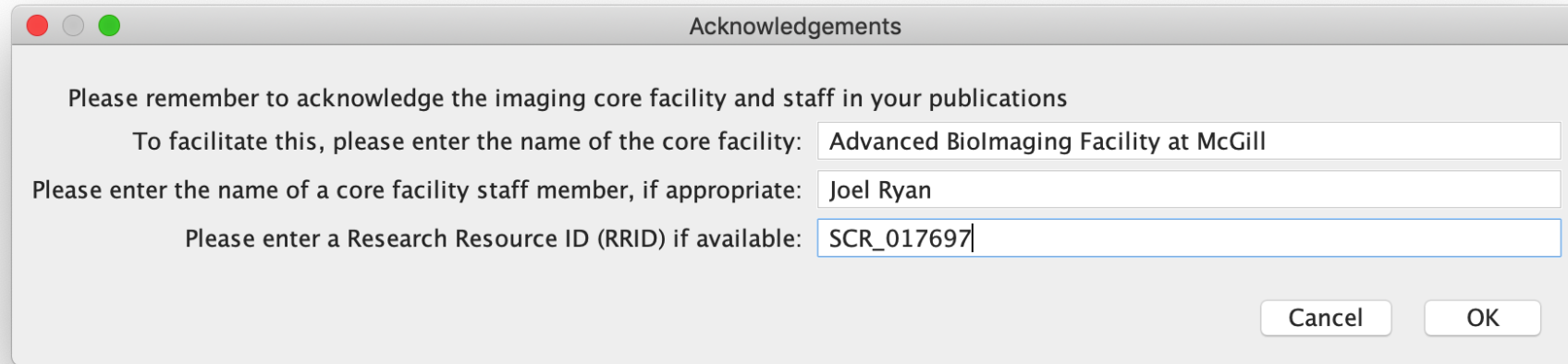
Channel menus will loop through the channels in the selected image.

MethodsJ2 – select optional devices



Choose whether optional devices from the microscope hardware specifications file were used for the selected image.

MethodsJ2 – Sample text for acknowledgement



Acknowledgements

Please remember to acknowledge the imaging core facility and staff in your publications

To facilitate this, please enter the name of the core facility: Advanced Biolmaging Facility at McGill

Please enter the name of a core facility staff member, if appropriate: Joel Ryan

Please enter a Research Resource ID (RRID) if available: SCR_017697

Cancel OK

Please enter the name of the core facility or laboratory which manages the microscope used for the acquisition of the selected image, as well as any imaging scientist who was helpful in the imaging experiment, and if available a Research Resource ID

MethodsJ2 – extensibility

```
282     {
283         "Dialog_Box": "Channel Settings",
284         "category": "general",
285         "Setting": "Light source intensity: ",
286         "Add_to_same_row": 0,
287         "CheckHardwareJSON": 0,
288         "Dialog_Type": "addStringField",
289         "blurb": "set to %s"
290     },
291     {
292         "Dialog_Box": "Channel Settings",
293         "category": "general",
294         "Dialog_Type": "addChoice",
295         "Setting": "Select excitation filter: ",
296         "Add_to_same_row": 0,
297         "CheckHardwareJSON": 1,
298         "Schema_ID": "ExcitationFilter.json",
299         "attributes": [
300             "Model",
301             "Manufacturer"
302         ],
303         "blurb": "and wavelength selection was carried out with a %s excitation filter (%s), "
304     },
305 }
```

Dialog boxes, drop-down menus, text generation can be added or modified by core facility staff, by modifying a MJ2 structure file and storing it locally or online (e.g. on Github)