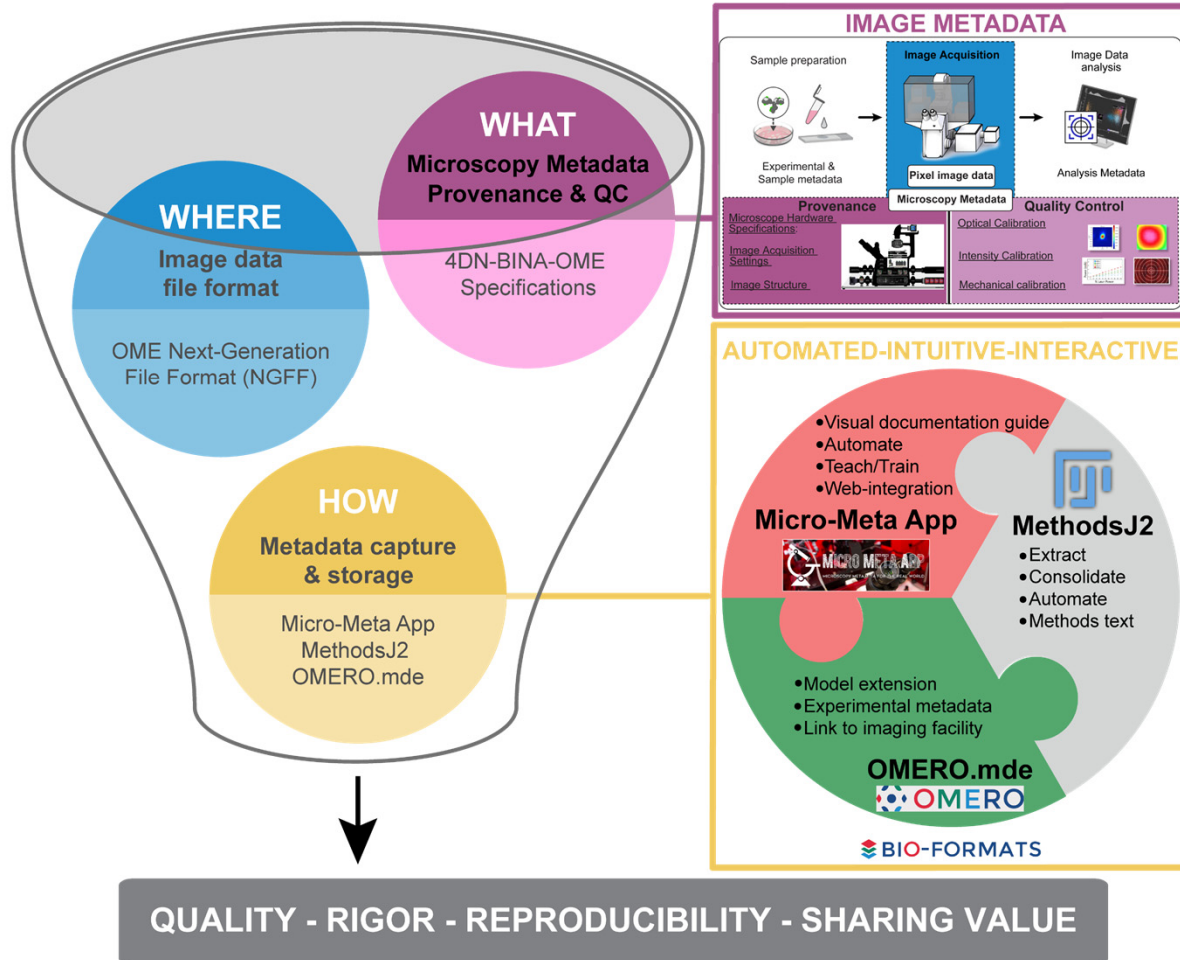


Supplementary information

MethodsJ2: a software tool to capture metadata and generate comprehensive microscopy methods text

In the format provided by the authors and unedited



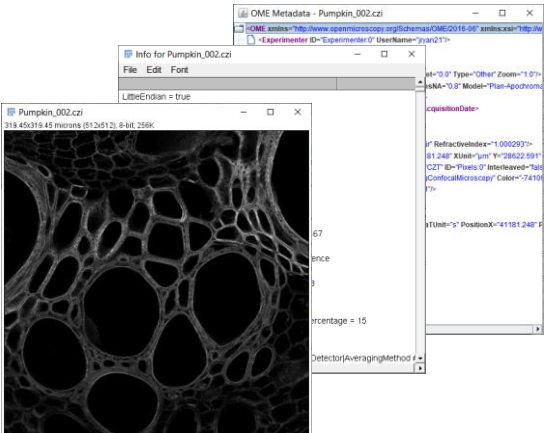
Supplemental Figure 1: Quality, rigor, reproducibility and sharing value for imaging experiments require the definition of community-driven Microscopy Metadata specifications and the adoption of easy-to-use metadata collection tools to facilitate the documentation and quality control tasks for experimental scientists. The establishment of FAIR (Wilkinson et al., 2016), community-driven Microscopy Image Data Standards implies parallel development on three interrelated fronts: (*WHERE*) Next-Generation File Formats (NGFF) *where* the ever-increasing scale and complexity of image data and metadata would be contained for exchange (Moore et al., 2021); *blue bubble*). (*WHAT*) Community-driven specifications for *what* ‘data provenance’ information (microscope hardware specifications, image acquisition settings and image structure metadata) and quality control metrics are essential for rigor, reproducibility, and reuse and should therefore be captured in Microscopy Metadata (*magenta bubble*). (*HOW*) Shared rules for *how* the (ideally) automated capture, representation and storage of Microscopy Metadata should be implemented in practice (*yellow bubble*). Micro-Meta App, MethodsJ2 and OMERO.mde are three highly interoperable tools and complementary that function to: 1) train users on the importance of documentation and quality control; 2) facilitate metadata extraction, collection, and storage; 3) automatically write Methods sections; and 4) facilitate the development of experimental metadata specifications in connection with local core facilities. To facilitate adoption by users with different use-styles and preferences, the three tools each work in a specialized environment: Micro-Meta App is used as stand-alone app or can be integrated in third-party image data portals. MethodsJ2 works as an ImageJ plugin. OMERO.mde works in the context of the OMERO image data repository. The different tools are based on different software platforms in order to appeal to the broadest community including microscope builders, imaging scientists working in core facilities and experimental scientists. The concept is to bring the tools to software platforms people are already using and lower the bar to enable broad uptake.

MethodsJ2 step-by-step overview

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>

MethodsJ2 - Workflow



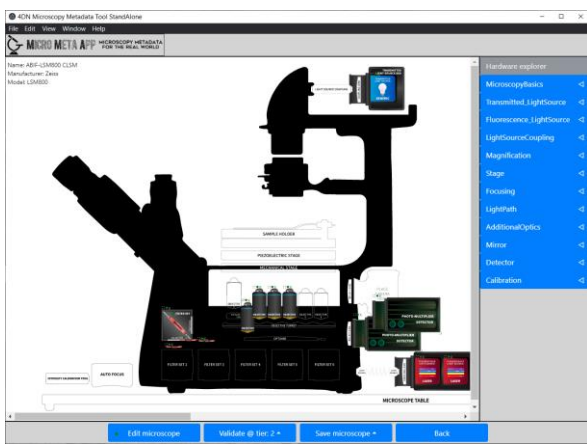
Image, metadata, OME metadata

MethodsJ2 structure file for dialog boxes and text generation

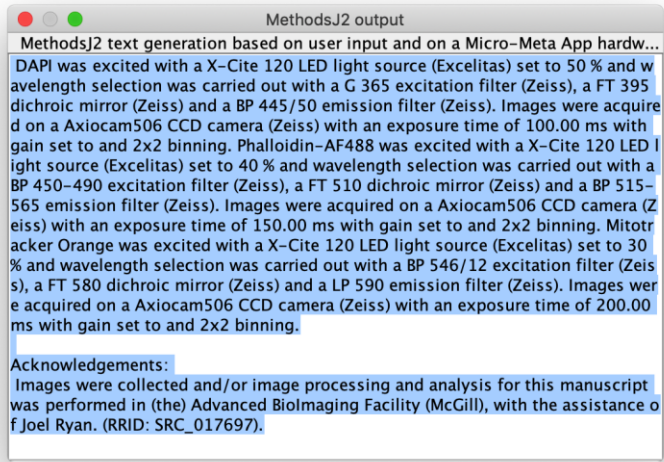
MethodsJ2
Python script running in Fiji

User input, guided by core facility staff

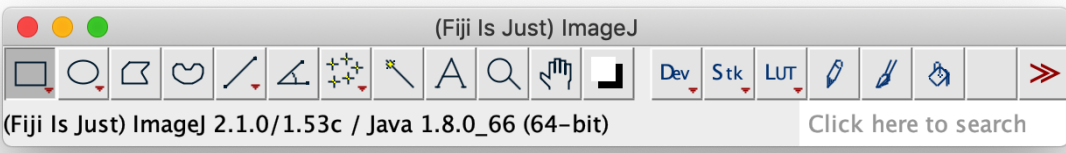
Generates materials and methods section for imaging experiments, based on community guidelines



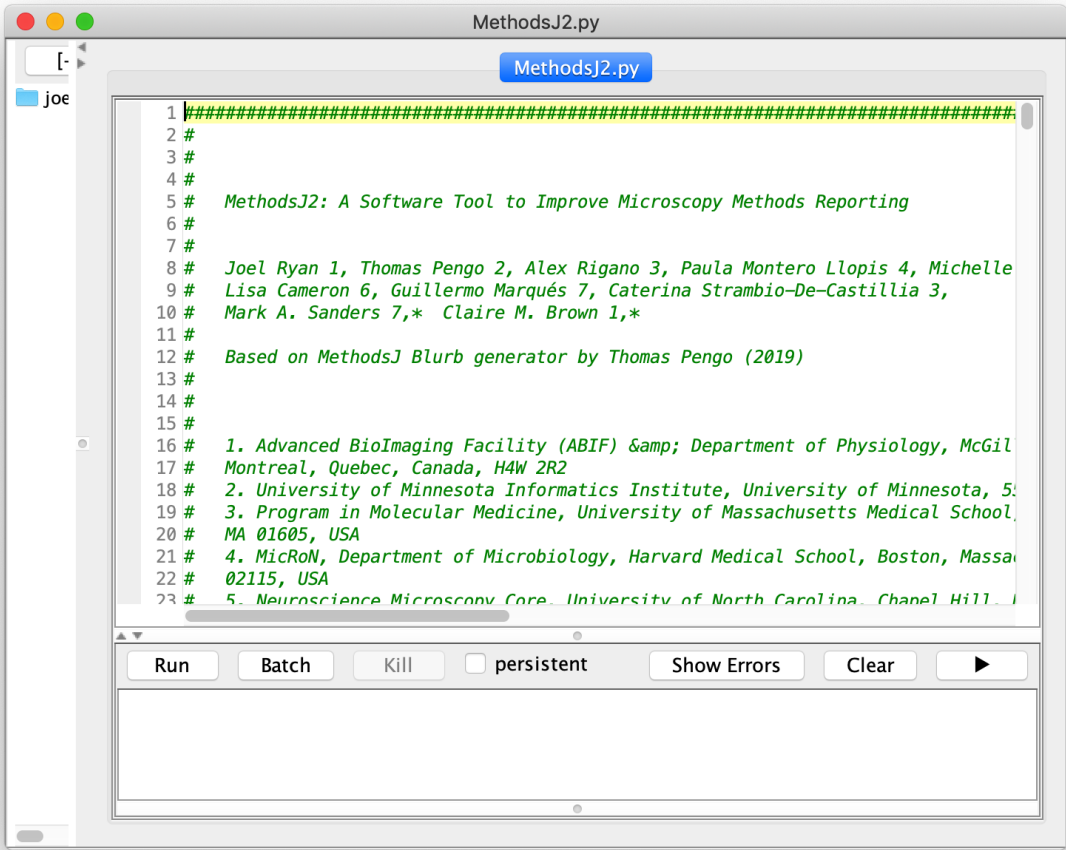
Micro-Meta App microscope hardware specifications file



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

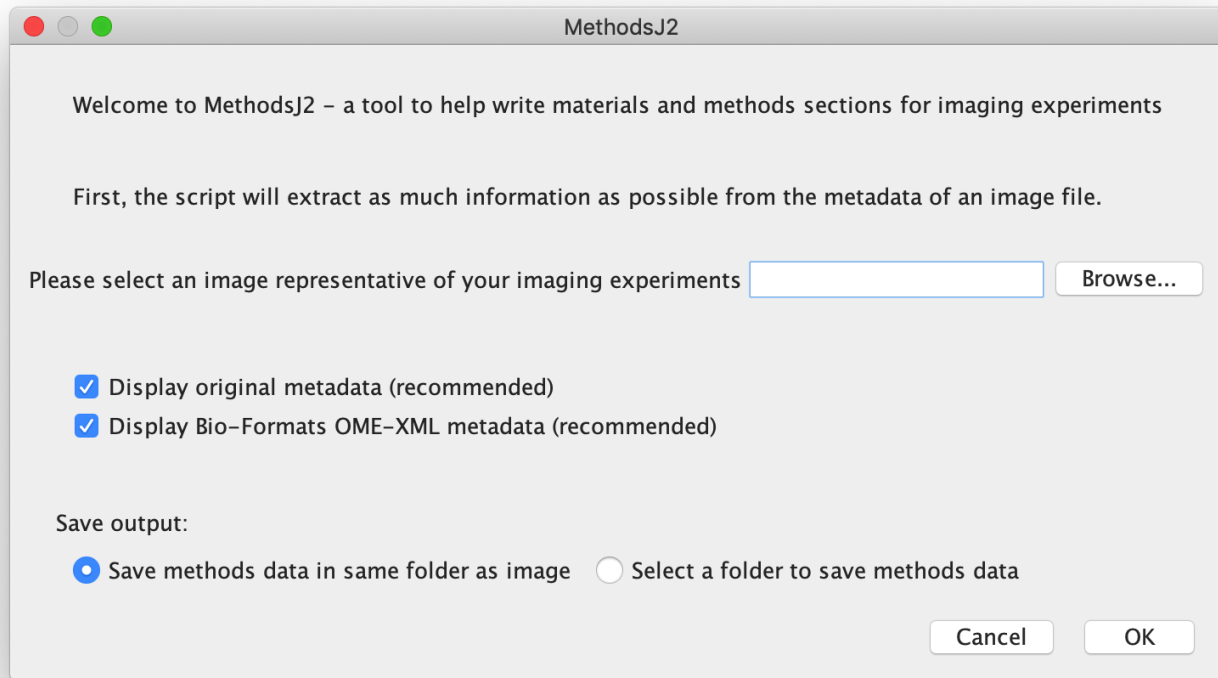


Check language: click Language and select Python

Once the script is loaded is ready, click Run

It may take a few seconds to start.

MethodsJ2 – select image



The screenshot shows a macOS-style window titled "MethodsJ2". The window contains the following text and controls:

- Header: "Welcome to MethodsJ2 – a tool to help write materials and methods sections for imaging experiments"
- Instruction: "First, the script will extract as much information as possible from the metadata of an image file."
- Text: "Please select an image representative of your imaging experiments" followed by a text input field and a "Browse..." button.
- Checkboxes:
 - Display original metadata (recommended)
 - Display Bio-Formats OME-XML metadata (recommended)
- Section: "Save output:" followed by two radio buttons:
 - Save methods data in same folder as image
 - Select a folder to save methods data
- Buttons: "Cancel" and "OK" at the bottom right.

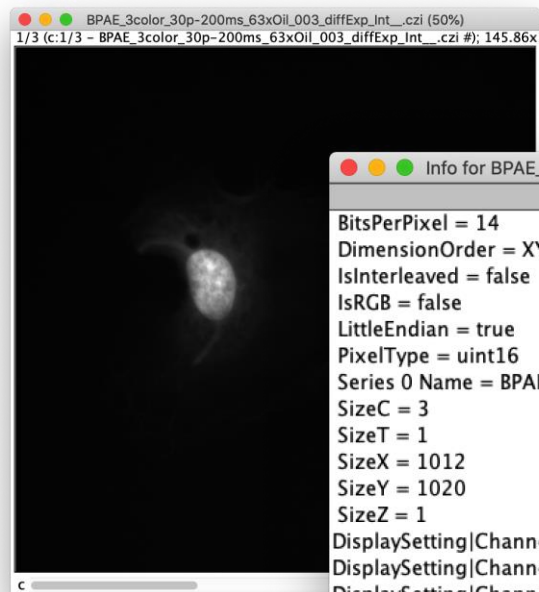
Select an image to load and to source metadata.

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

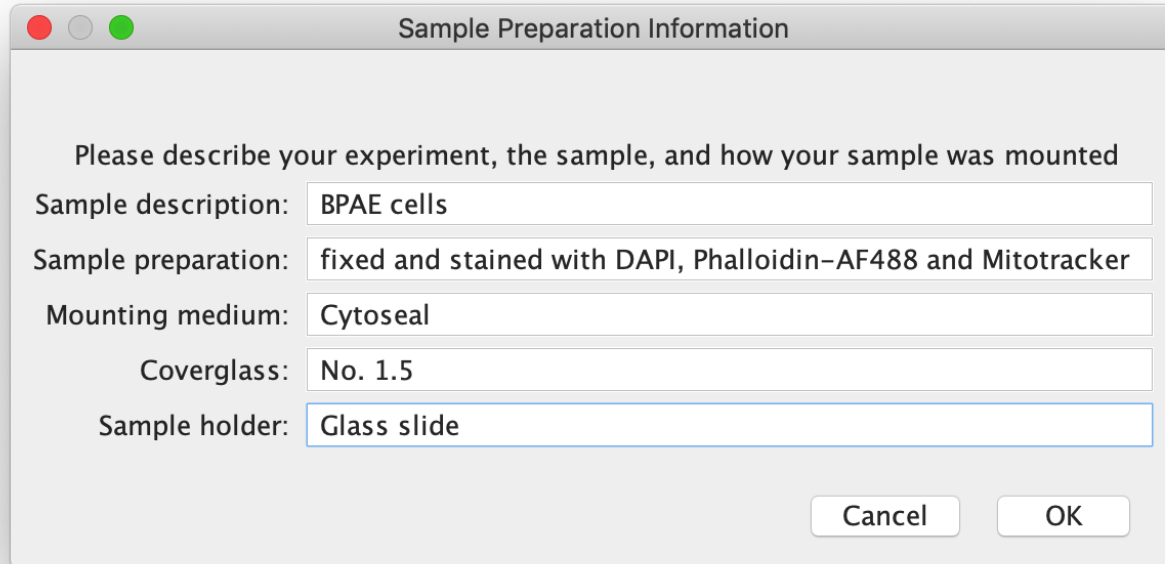


```
Info for BPAE_3color_30p-200ms_63xOil_003_diffExp_Int_...czi
BitsPerPixel = 14
DimensionOrder = XYZCT
IsInterleaved = false
IsRGB = false
LittleEndian = true
PixelType = uint16
Series 0 Name = BPAE_3color_30p-200ms_63xOil_003_diffExp
SizeC = 3
SizeT = 1
SizeX = 1012
SizeY = 1020
SizeZ = 1
DisplaySetting|Channel|BitCountRange = 14
DisplaySetting|Channel|Color = #FFFF7700
DisplaySetting|Channel|DyeDatabaseId = 66071726-cbd4-4c4
DisplaySetting|Channel|DyeId = McNamara-Boswell-1526
DisplaySetting|Channel|DyeMaxEmission = 575
DisplaySetting|Channel|DyeMaxExcitation = 551
DisplaySetting|Channel|DyeName = MitoTracker Orange CMTM
```

```
OME Metadata - BPAE_3color_30p-200ms_63xOil_003_diffExp_Int_...czi
<OME xmlns="http://www.openmicroscopy.org/Schemas/OME/2016-06" xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance" xsi:schemaLocation="
  <Experimenter ID="Experimenter:0" UserName="jryan21"/>
  <Instrument ID="Instrument:0">
    <Microscope Type="Inverted"/>
    <Detector ID="Detector:Axiocam506" Model="Axiocam506m"/>
    <Objective ID="Objective:1" Immersion="Oil" LensNA="1.4" Model="Plan-Apochromat 63x/1.40 Oil DIC" NominalMagnification="63.0" WorkingD
  <FilterSet ID="FilterSet:1">
    <ExcitationFilterRef ID="Filter:1"/>
    <DichroicRef ID="Dichroic:1"/>
    <EmissionFilterRef ID="Filter:2"/>
  <FilterSet ID="FilterSet:2">
    <ExcitationFilterRef ID="Filter:3"/>
    <DichroicRef ID="Dichroic:2"/>
    <EmissionFilterRef ID="Filter:4"/>
  <FilterSet ID="FilterSet:3">
    <ExcitationFilterRef ID="Filter:5"/>
    <DichroicRef ID="Dichroic:3"/>
    <EmissionFilterRef ID="Filter:6"/>
  <Filter ID="Filter:1">
    <TransmittanceRange CutIn="335.0" CutInUnit="nm" CutOut="383.0" CutOutUnit="nm"/>
```

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

MethodsJ2 – Sample Preparation



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

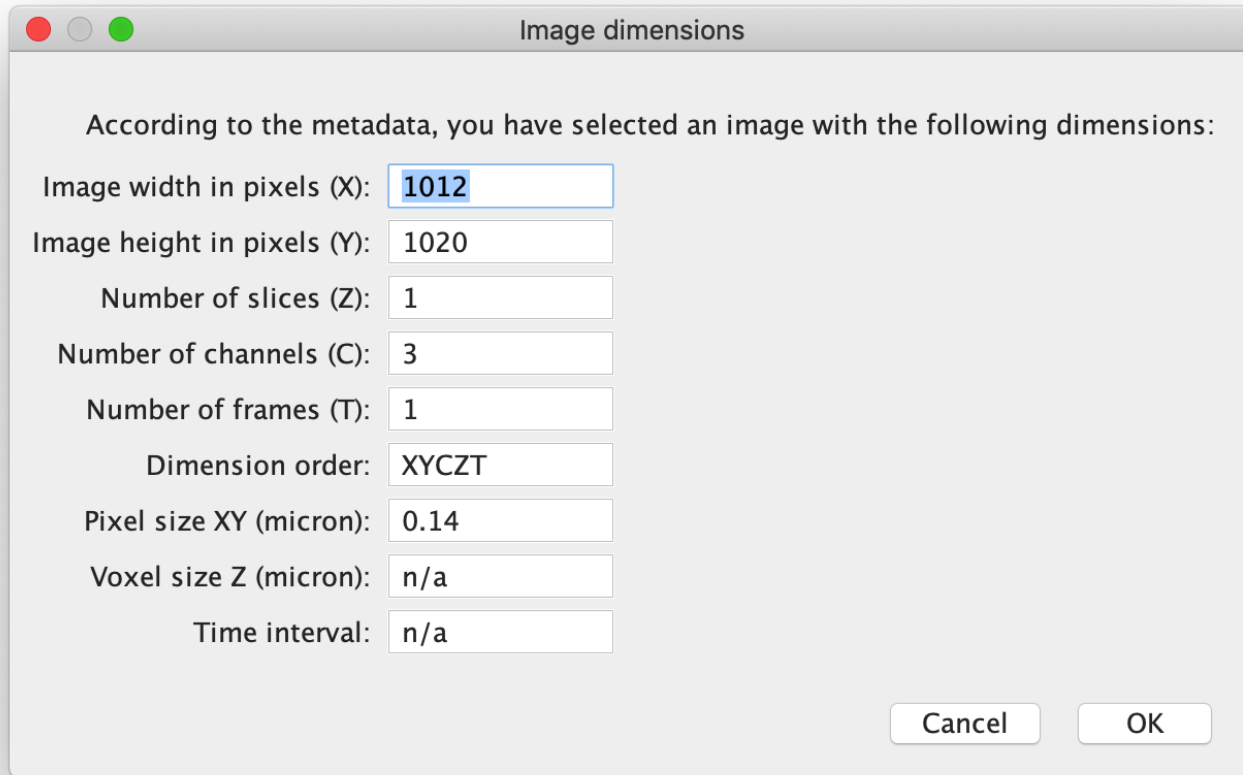


Image dimensions

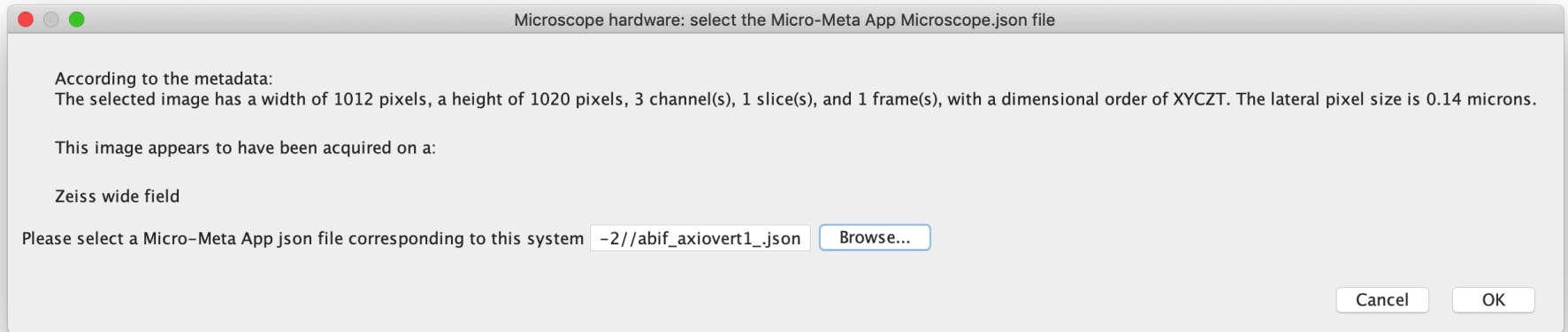
According to the metadata, you have selected an image with the following dimensions:

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

Cancel OK

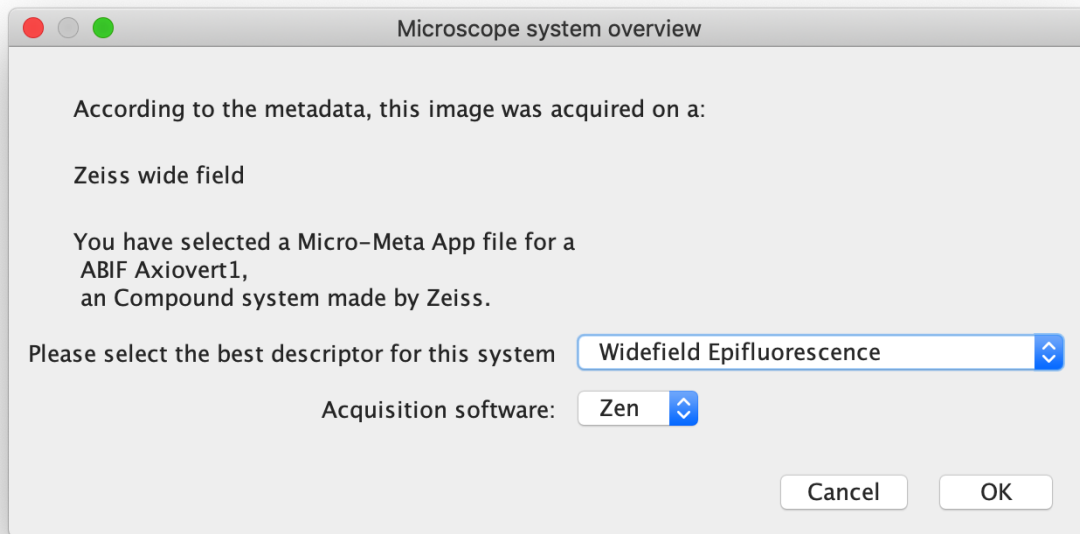
Please verify image dimensions.
Values are sourced from the
image metadata

MethodsJ2 – select Microscope.json file



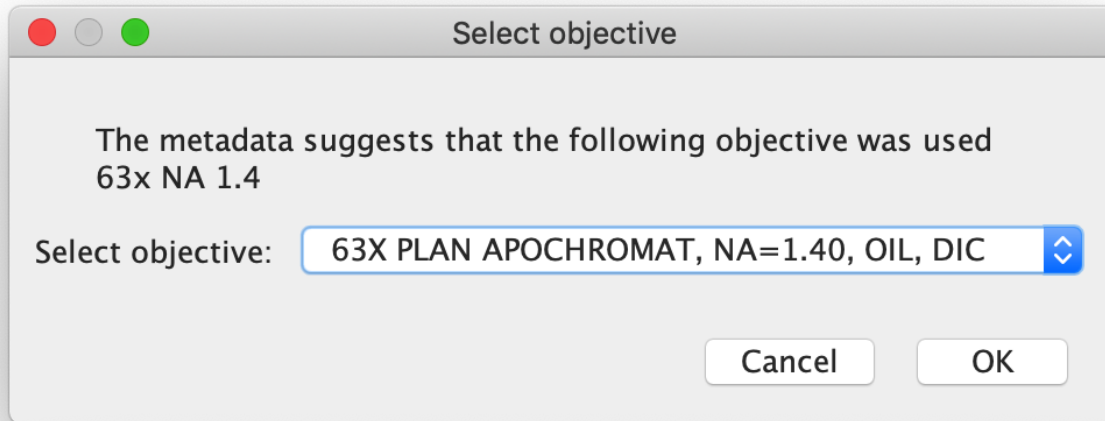
Choose Micro-Meta App hardware specifications file for the microscope used to acquire the selected image

MethodsJ2 – choose descriptor and software



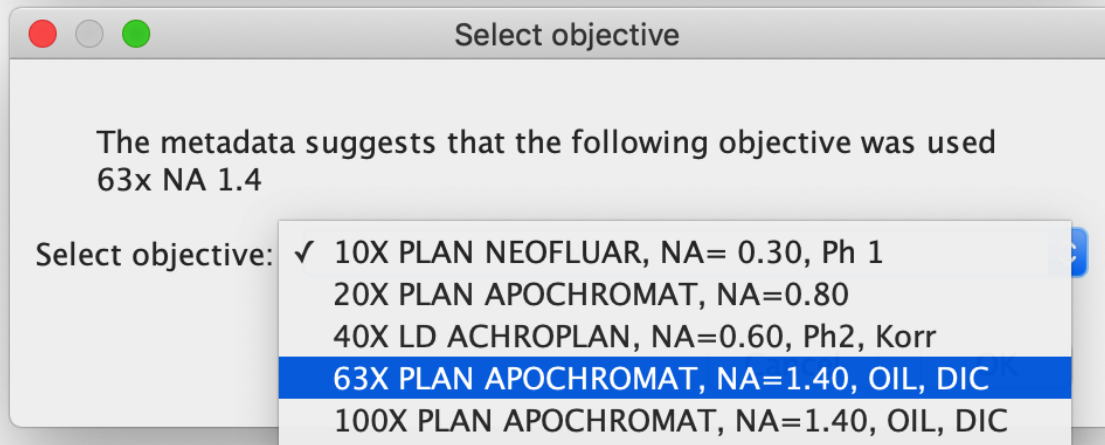
Please select the best descriptor for the selected microscope, as well as the acquisition software.

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):

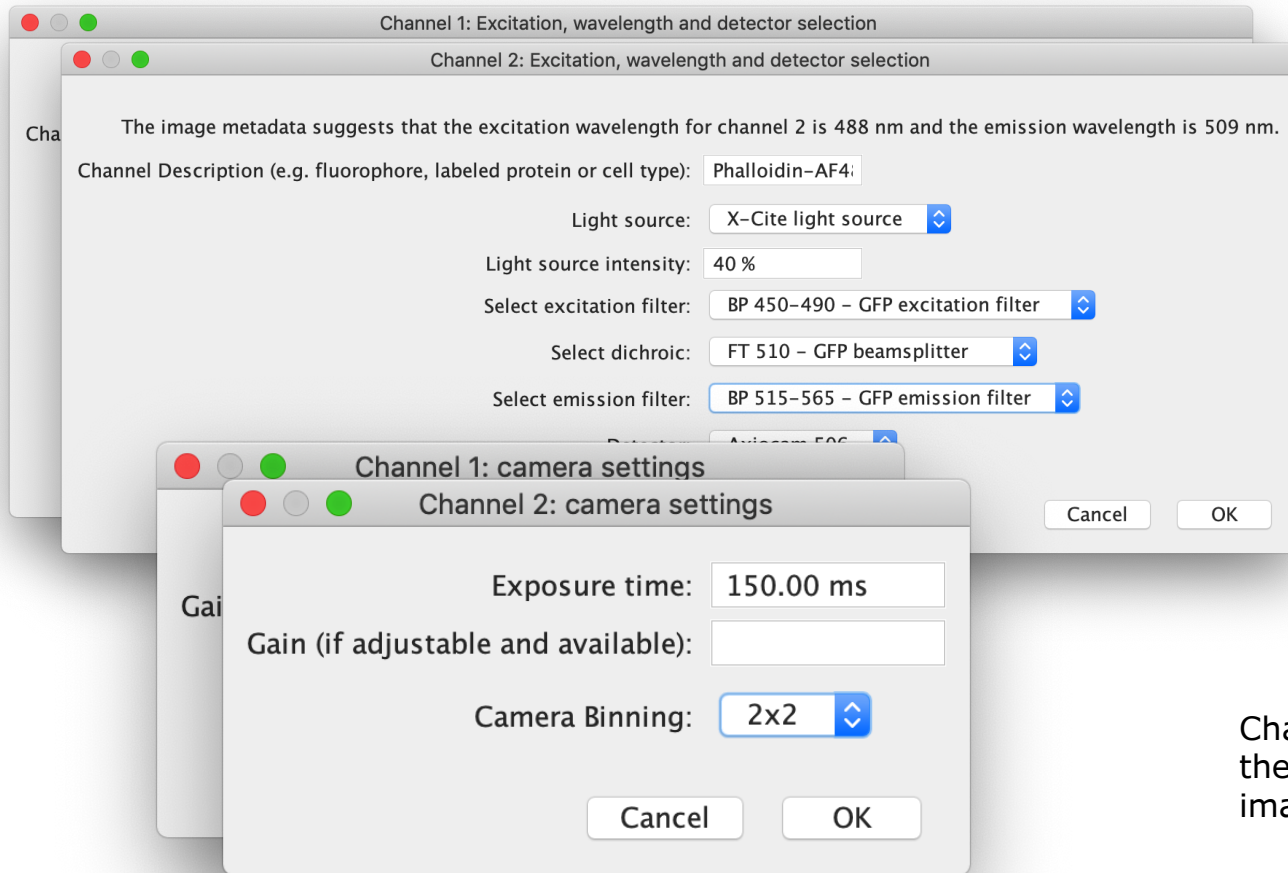
Camera Binning:

Please fill in information for the first channel

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

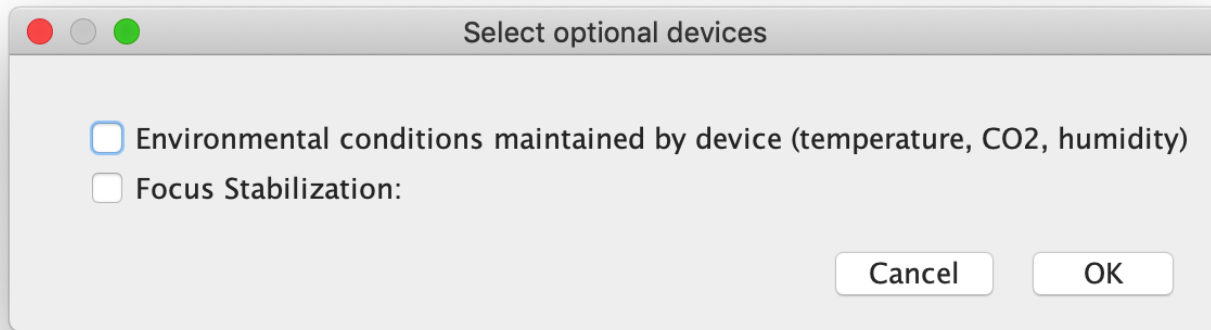
Detector settings are based on whether a camera or point detector is selected

MethodsJ2 – Channel acquisition settings loop



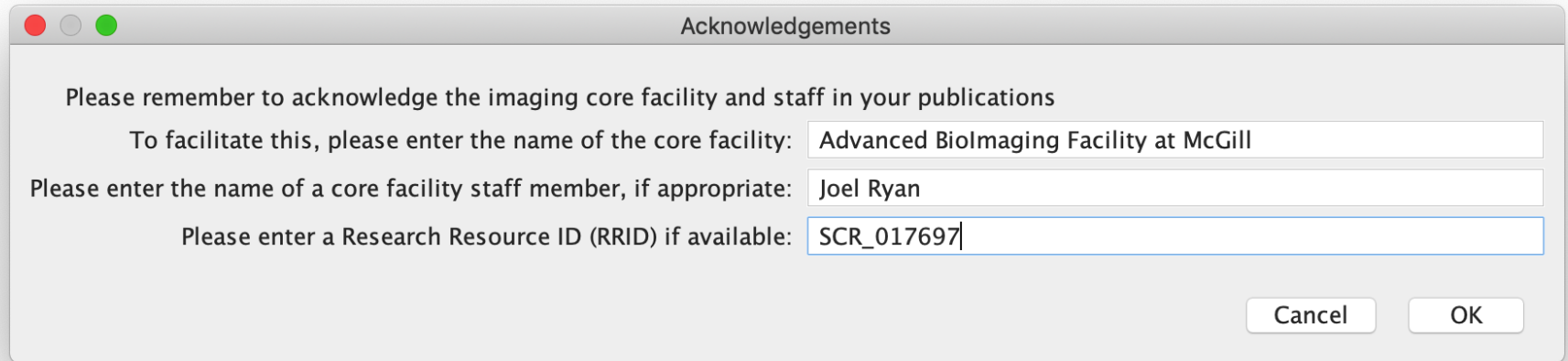
Channel menus will loop through the channels in the selected image.

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



A screenshot of a macOS-style dialog box titled "Acknowledgements". The dialog box has a title bar with three colored window control buttons (red, yellow, green) on the left. The main content area contains the following text and input fields:

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

To facilitate this, please enter the name of the core facility:

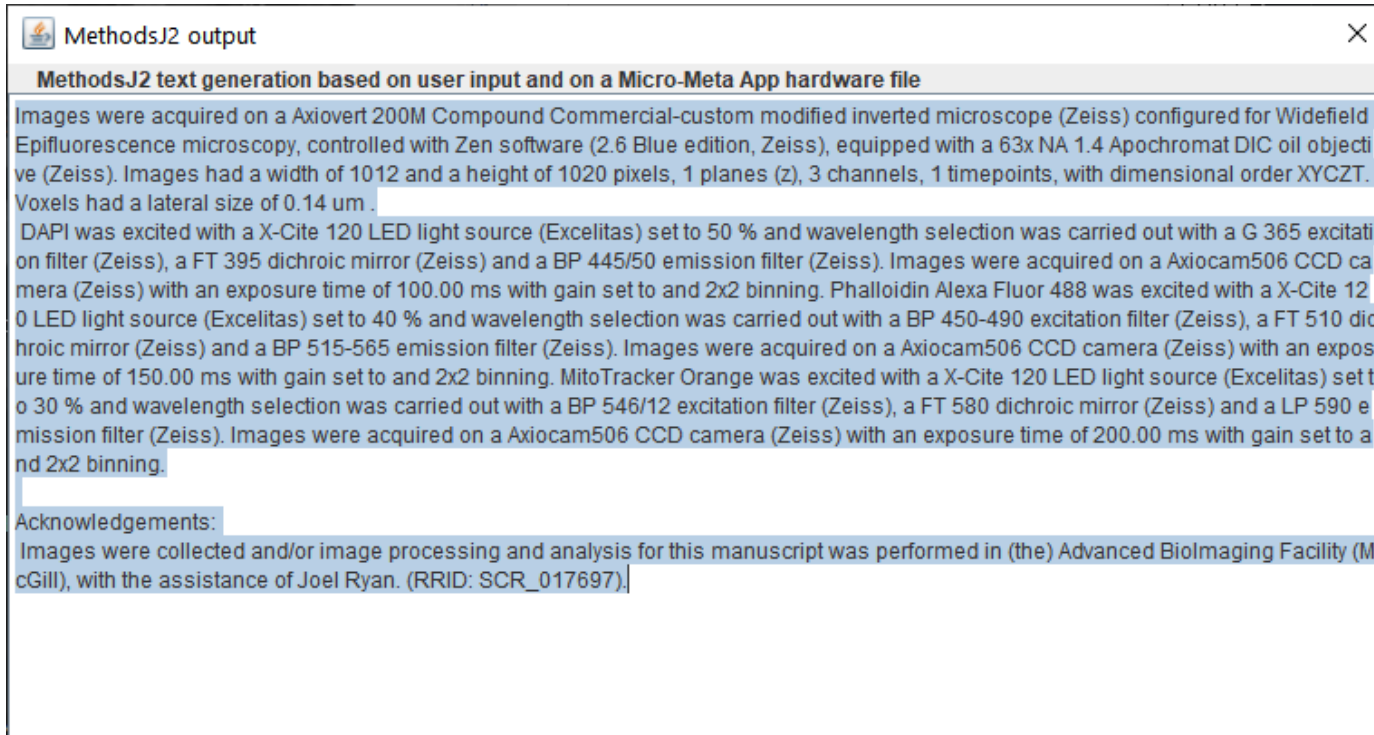
Please enter the name of a core facility staff member, if appropriate:

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

At the bottom right of the dialog box, there are two buttons: "Cancel" and "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 – 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 – 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 {
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 },
```

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)