

# TOOLS FOR AUTOMATING MICROSCOPY METHODS REPORTING

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VIRTUAL ILLUMINATE MEETING

MARCH 17 2022

JOEL RYAN – MCGILL ADVANCED BIOIMAGING FACILITY

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

Ryan et al., *Nat Methods* 2021

- ❖ 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>

## **Micro-Meta App: an interactive tool for collecting microscopy metadata based on community specifications**

Rigano, et al., *Nat Methods* 2021

## **Best practices and tools for reporting reproducible fluorescence microscopy methods**

Montero Llopis, et al., *Nat Methods* 2021

# PROBLEMS TO ADDRESS

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**Methods sections for microscopy experiments tend be incomplete:**

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**Methods sections for microscopy experiments tend to be incomplete:**

**Imaging methods are vastly underreported in biomedical research**

Marqués, et al., *eLife* 2020

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**Methods sections for microscopy experiments tend to be incomplete:**

**Imaging methods are vastly underreported in biomedical research**

Marqués, et al., *eLife* 2020

**> Less than 20% of 240 papers contained enough information to replicate microscopy experiments**

# PROBLEMS TO ADDRESS

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**Methods sections for microscopy experiments tend be incomplete:**

*“Images were acquired on  
a microscope.”*

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**Methods sections for microscopy experiments tend be incomplete:**

*“Images were acquired on  
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# PROBLEMS TO ADDRESS

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**Methods sections for microscopy experiments tend be incomplete:**

*“Images were acquired on  
a microscope (Zeiss).”*

- ❖ Difficulty in evaluating the work
- ❖ Poor reproducibility

# MICROSCOPY METHODS REPORTING

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## Methods

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### Immunofluorescence and Live-cell imaging

For immunofluorescence, stacks of optical sections were collected on a Nikon TiE microscope equipped with a Yokogawa CSU-W1 spinning-disk confocal unit (50  $\mu\text{m}$  pinhole size), an Andor Borealis illumination unit, Andor ALC600 laser beam combiner (405 nm/488 nm/561 nm/640 nm), Andor IXON 888 Ultra EMCCD camera, and a Nikon 100 $\times$ /1.45 NA oil immersion objective. The microscope was controlled by software from Nikon (NIS Elements, ver. 5.02.00). DAPI or fluorophores were excited with 405 nm, 488 nm, or 561 nm laser lines and bright-field images acquired using Nikon differential interference contrast optics. Confocal image z-stacks were recorded with a step size of 200 nm, 16-bit image depth, 1  $\times$  1 binning, a frame size of 1024  $\times$  1024 pixels, and a pixel size of 130 nm. Within each experiment, cells were imaged using the same settings on the microscope (camera exposure time, laser power, and gain) to compare signal intensities between cell lines. [...]

Mulholland, et al. 2020 *Nat Comms*

Complete methods sections are important

# MICROSCOPY METHODS REPORTING

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Complete methods sections are important

❖ Helps evaluate the work

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Complete methods sections are important

- ❖ Helps evaluate the work
- ❖ Enables reproducibility

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Complete methods sections are important

- ❖ Helps evaluate the work
- ❖ Enables reproducibility
- ❖ Enables data reusability...

# WHERE TO START?

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## **Best practices and tools for reporting reproducible fluorescence microscopy methods**

Paula Montero Llopis, Rebecca A. Senft, Tim J. Ross-Elliott, Ryan Stephansky, Daniel P.

Keeley, Preman Koshar, Guillermo Marqués, Ya-Sheng Gao, Benjamin R. Carlson, Thomas

Pengo, Mark A. Sanders, Lisa A. Cameron & Michelle S. Itano

*Nature Methods* 18, 2021

# WHERE TO START?

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## Best practices and tools for reporting reproducible fluorescence microscopy methods

Examples of how different  
hardware parameters affect  
images!

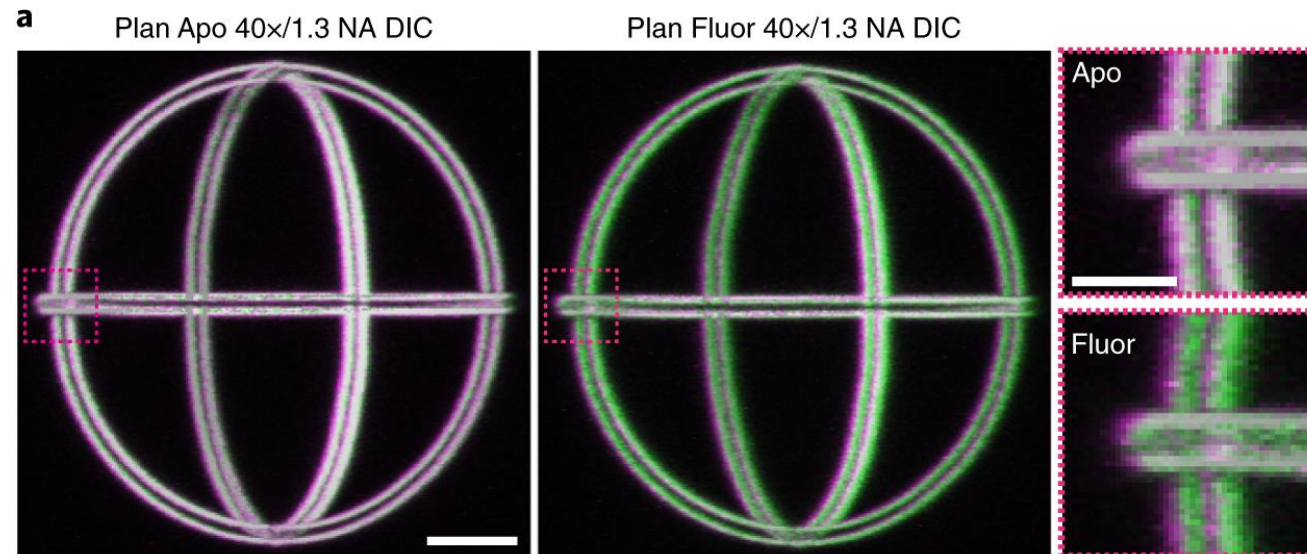
Montero Llopis, et al., *Nat Methods* 2021



# WHERE TO START?

## Best practices and tools for reporting reproducible fluorescence microscopy methods

Objective lens correction:



Montero Llopis, et al., *Nat Methods* 2021

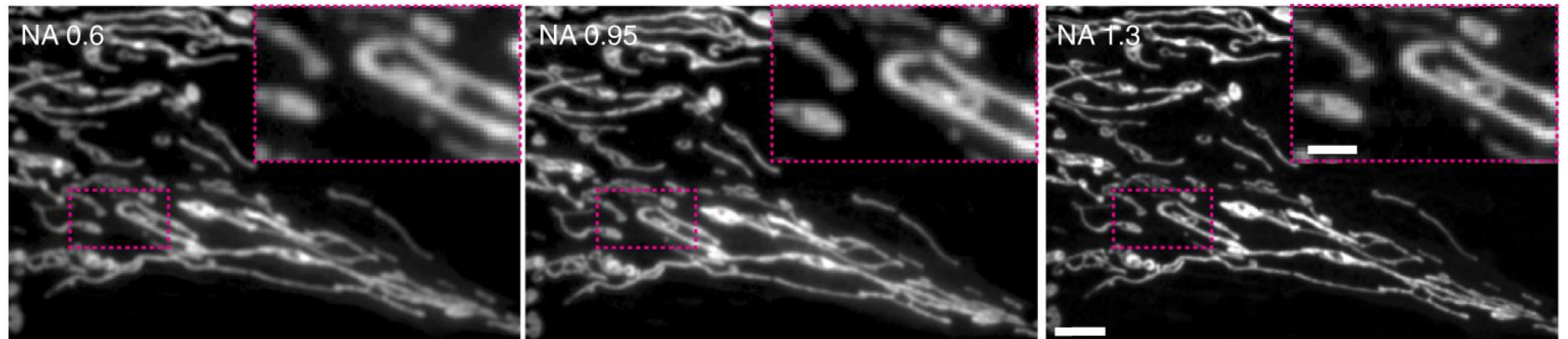
# WHERE TO START?

## Best practices and tools for reporting reproducible fluorescence microscopy methods

Objective lens

Numerical Aperture

(N.A.)



Montero Llopis, et al., *Nat Methods* 2021

## Microscopy Metadata Checklist Generator (MicCheck)

For more information, see Montero-Llopis et al., 2021.

**1. Which image modality are you using?**

☐ Widefield

☐ Spinning Disk Confocal

☐ Point Scanning Confocal

☐ Multiphoton

**2. Did you acquire transmitted light images (e.g., phase contrast, brightfield, DIC)?**

☐ Yes

☐ No

**3. Did you use additional magnification (e.g., optovar, relay lens)?**

☐ Yes

☐ No

**4. Did you perform any of these multidimensional acquisitions?**

☐ Multi-color

☐ Z-stack

☐ Time-lapse

**Microscopy Metadata Checklist**

\*\*\*Asterisks indicate optional items.

**Microscope Stand and Motorized Components**

☐ Microscope Stand manufacturer and Model

☐ \*\*\*Type

☐ \*\*\*Commercial/commercial modified, custom modified

☐ \*\*\*Upright or inverted

**Illumination**

**Wavelength Selection**

☐ Filter manufacturer and product number

☐ Filter center wavelength and bandwidth (FWHM), cut on or cut off wavelength

☐ Filter coating method

☐ \*\*\*Additional filters manufacturer and model

☐ \*\*\*If tunable wavelength selection, range of wavelengths detected

**Optics**

☐ Objective manufacturer

☐ Objective correction

☐ Objective magnification

☐ Objective numerical aperture

☐ Specified immersion medium

☐ \*\*\*Objective application

☐ \*\*\*Immersion medium manufacturer and product number (if used)

**Detection**

**Acquisition Software**

<https://rebecca-senft.shinyapps.io/MicCheck/>

Montero Llopis, et al., *Nat Methods* 2021

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**Detection**

**Acquisition Software**

- Generates a checklist of recommended microscope data
- Helps users write methods section following

<https://rebecca-senft.shinyapps.io/MicCheck/>

Montero Llopis, et al., *Nat Methods* 2021

# AUTOMATING METHODS REPORTING...

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## **Imaging methods are vastly underreported in biomedical research**

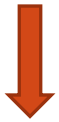
Marqués, et al., *eLife* 2020

# AUTOMATING METHODS REPORTING...

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**Imaging methods are vastly underreported in biomedical research**

Marqués, et al., *eLife* 2020



**MethodsJ**

# AUTOMATING METHODS REPORTING...

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**Imaging methods are vastly underreported in biomedical research**

Marqués, et al., *eLife* 2020





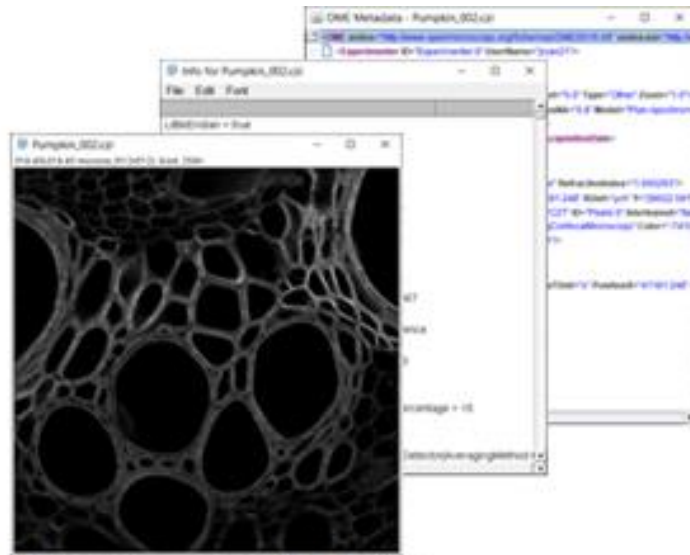
## MethodsJ2: a software tool to capture metadata and generate comprehensive microscopy methods text

Ryan et al., *Nat Methods* 2021

- ❖ 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
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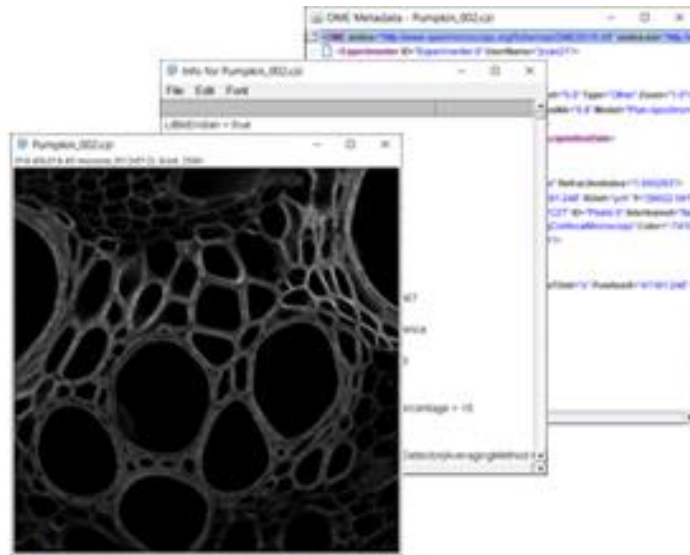
# METHODS J2 - SOURCES OF INFORMATION

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Image, metadata,  
OME metadata

## METHODS2 - SOURCES OF INFORMATION



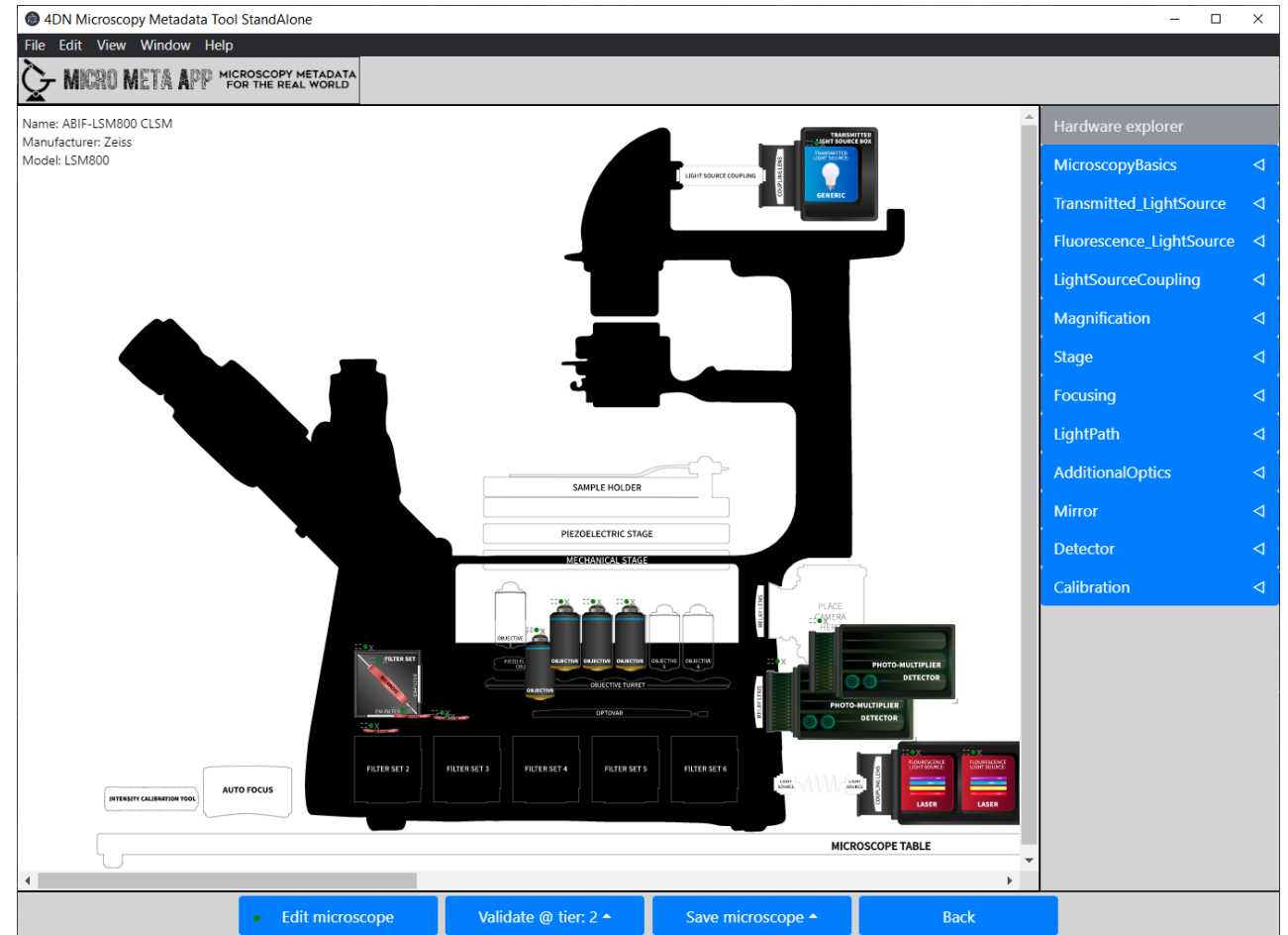
Image, metadata,  
OME metadata



## Micro-Meta App microscope hardware specifications file

# MICRO-META APP

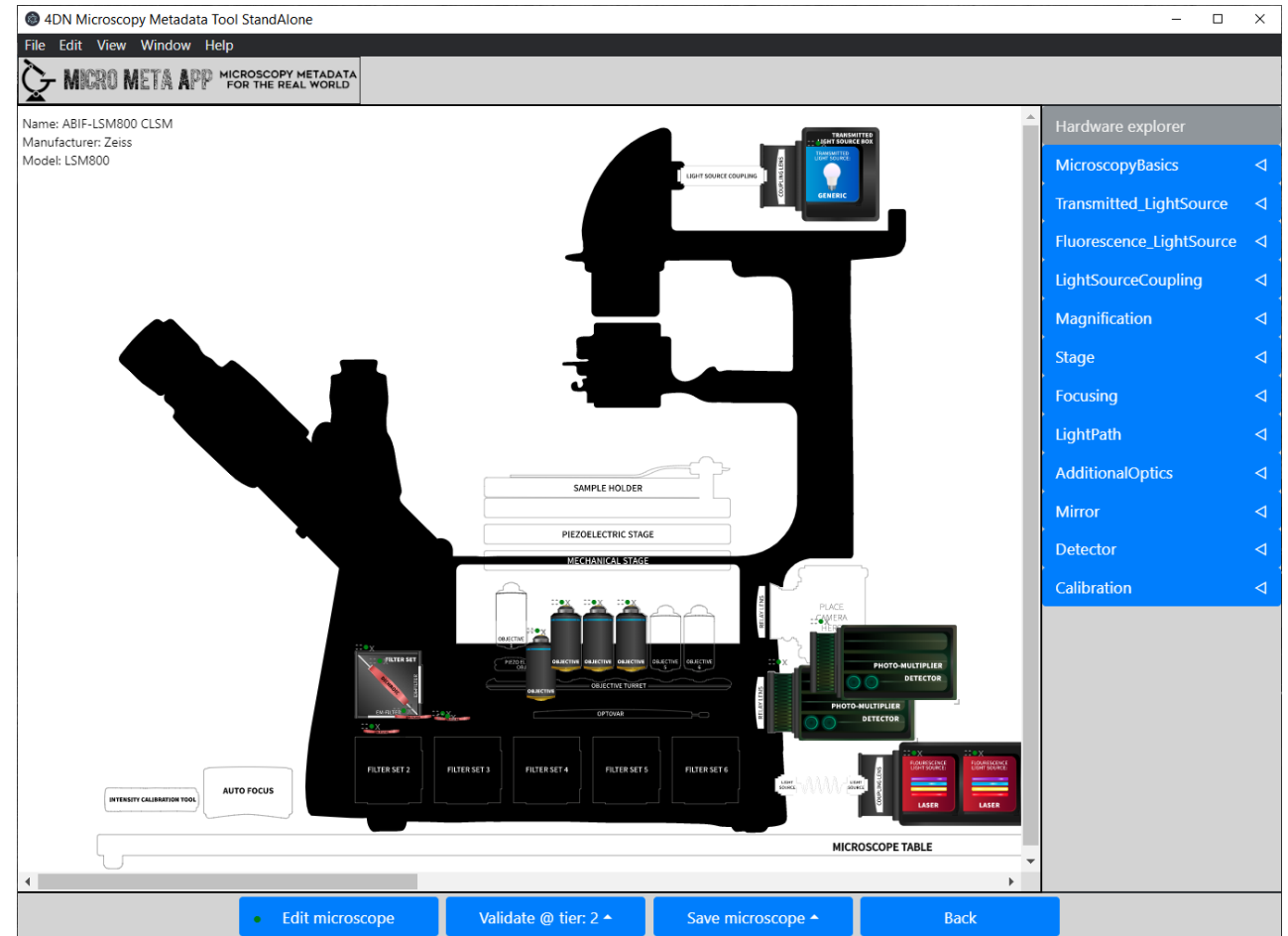
- “Build” your microscope *in silico*
- Uses standardized metadata language



Rigano et al, 2021

# MICRO-META APP

- “Build” your microscope *in silico*
- Uses standardized metadata language
- Machine-readable hardware configuration file



Rigano et al, 2021

# MICRO-META APP

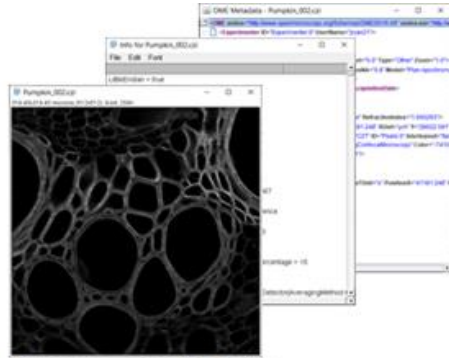
- “Build” your microscope *in silico*
- Uses standardized metadata language
- Machine-readable hardware configuration file

```
1 {
2   "Name": "ABIF Axiovert1",
3   "Schema_ID": "Instrument.json",
4   "ID": "93efaebd-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 }
```



# METHODS J2

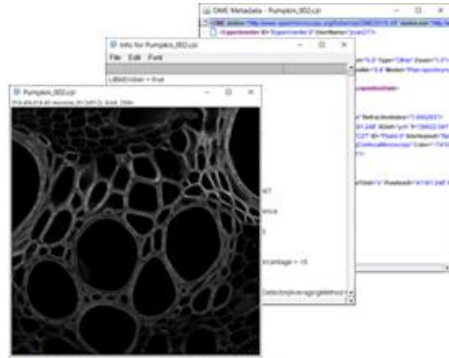
---



Image, metadata,  
OME metadata



## METHODS J2

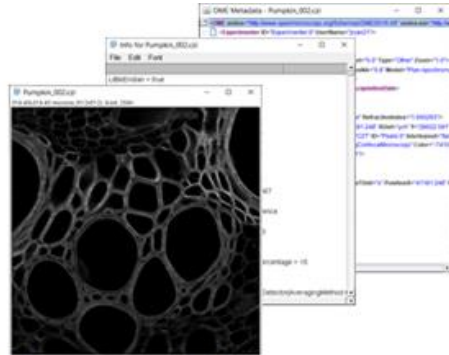


Image, metadata,  
OME metadata



Micro-Meta App microscope  
hardware specifications file

# METHODS J2



Image, metadata,  
OME metadata

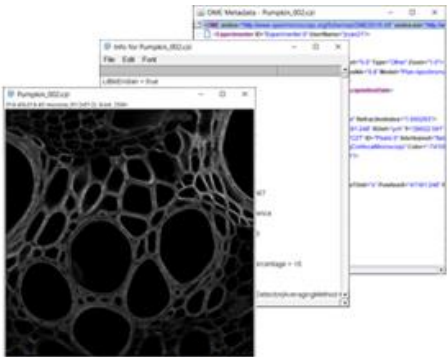


Micro-Meta App microscope  
hardware specifications file

MethodsJ2

Python script  
running in Fiji

# METHODS J2

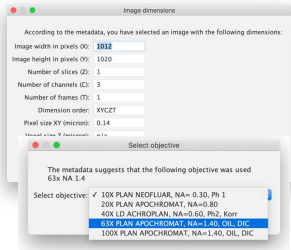


Image, metadata, OME metadata



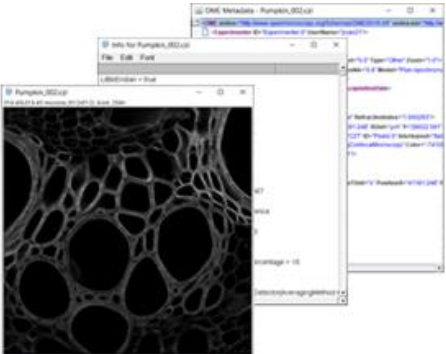
Micro-Meta App microscope hardware specifications file

User input,  
guided by core  
facility staff



MethodsJ2  
Python script  
running in Fiji

# METHODS J2



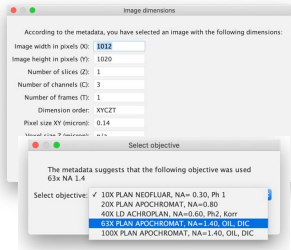
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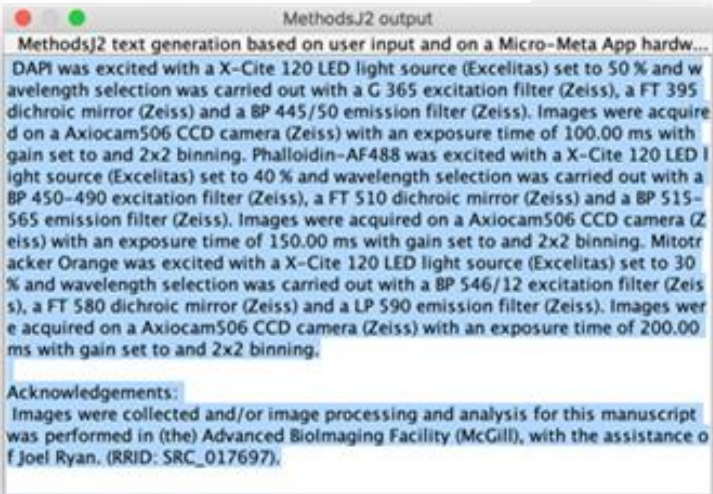


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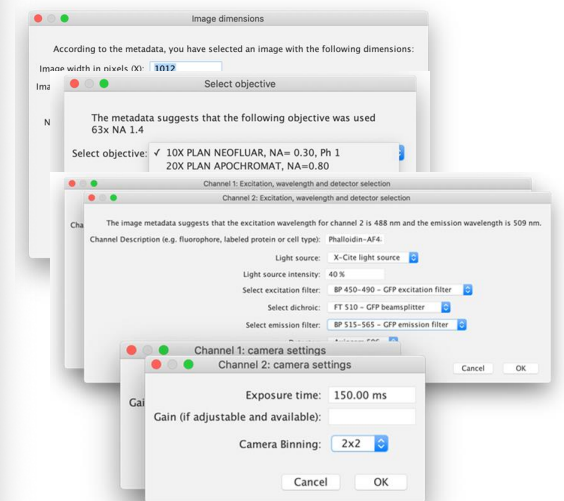
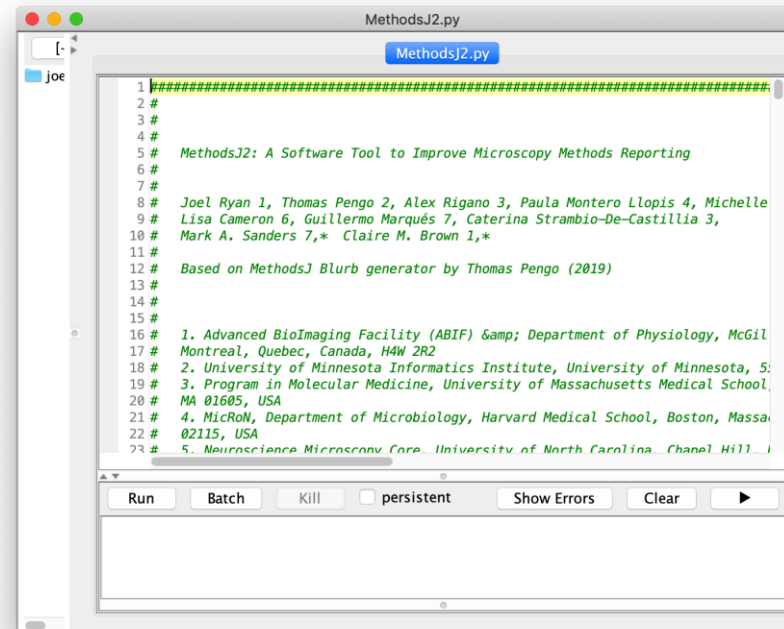


MethodsJ2  
Python script  
running in Fiji

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



# METHODSJ2 - DEMO TODAY!



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

# METHODS J2 – REQUIRED FILES

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Fiji

 [fiji.sc/](https://fiji.sc/)



# METHODSJ2 – REQUIRED FILES



Fiji



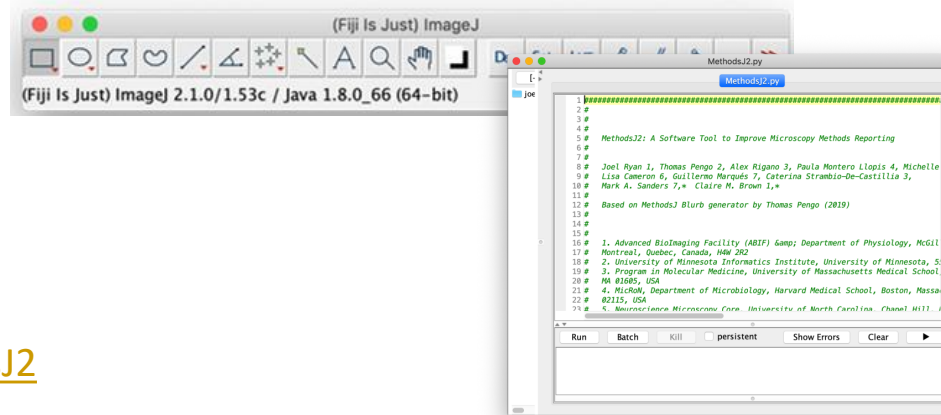
[fiji.sc/](https://fiji.sc/)



MethodsJ2 python script

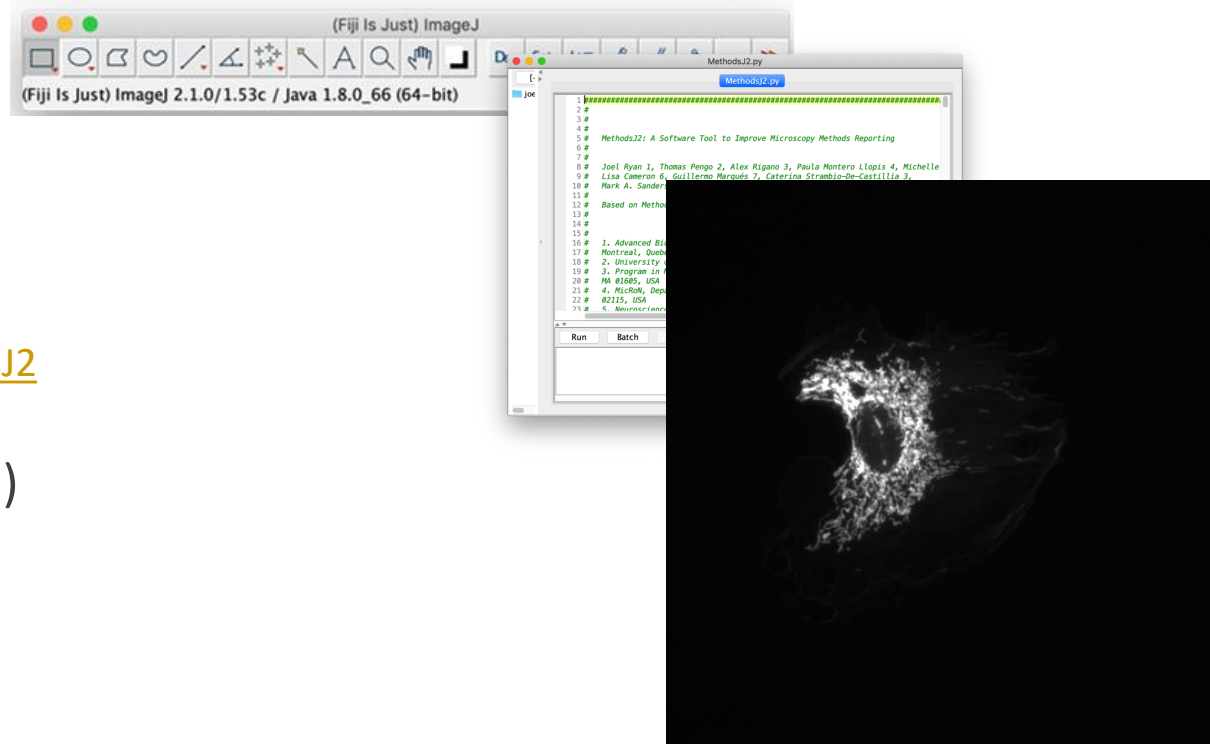


[github.com/ABIF-McGill/MethodsJ2](https://github.com/ABIF-McGill/MethodsJ2)



# METHODS J2 – REQUIRED FILES

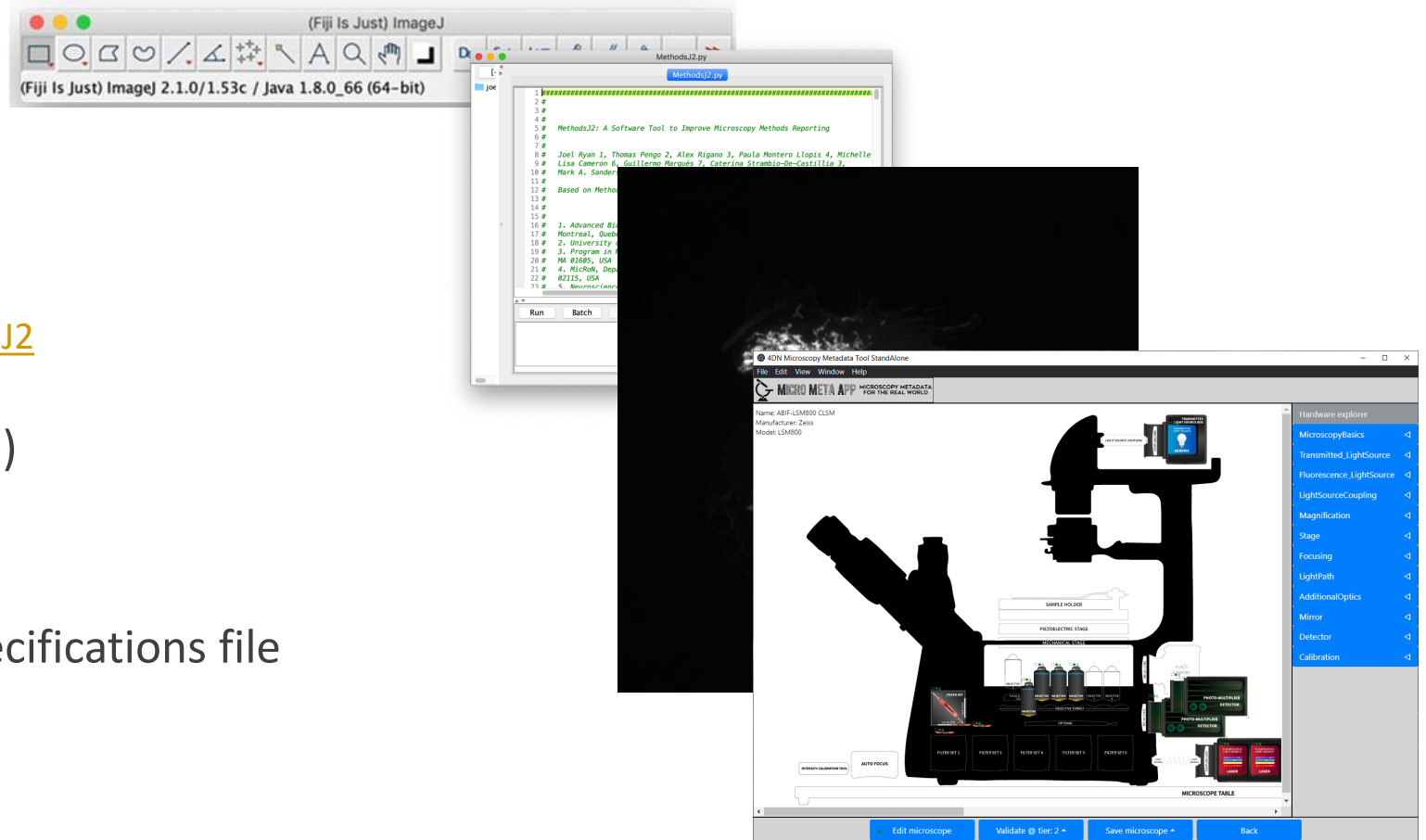
- ❖ Fiji
  - ❖ [fiji.sc/](https://fiji.sc/)
- ❖ MethodsJ2 python script
  - ❖ [github.com/ABIF-McGill/MethodsJ2](https://github.com/ABIF-McGill/MethodsJ2)
- ❖ Raw image file (with metadata)
  - ❖ Demo on MethodsJ2 github





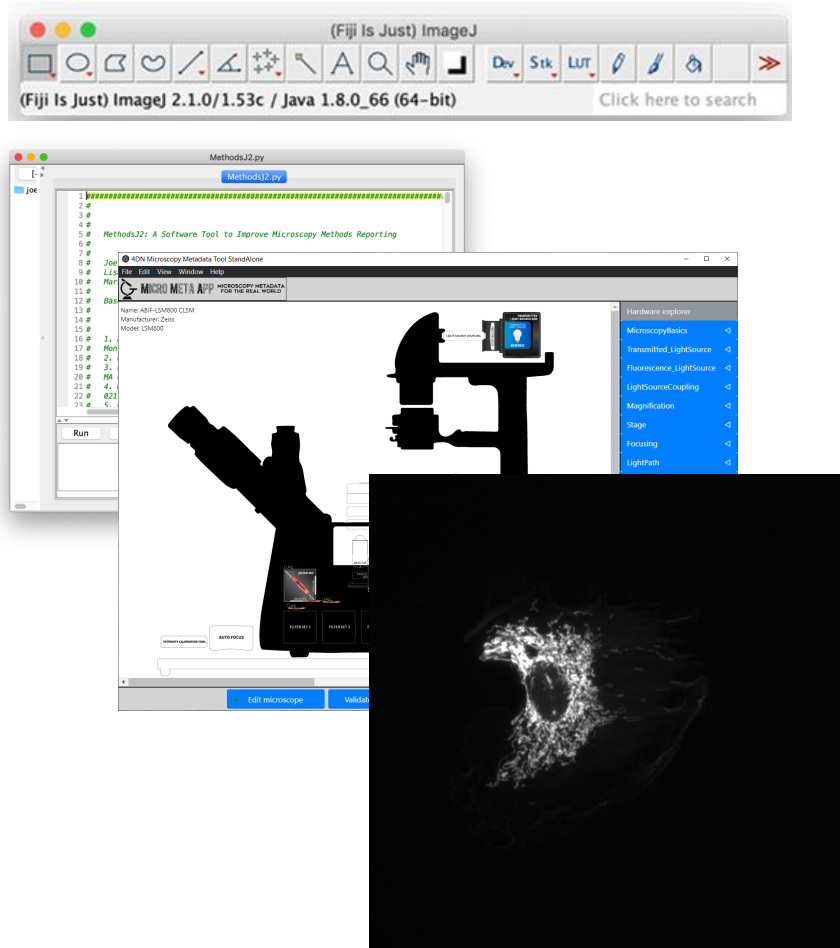
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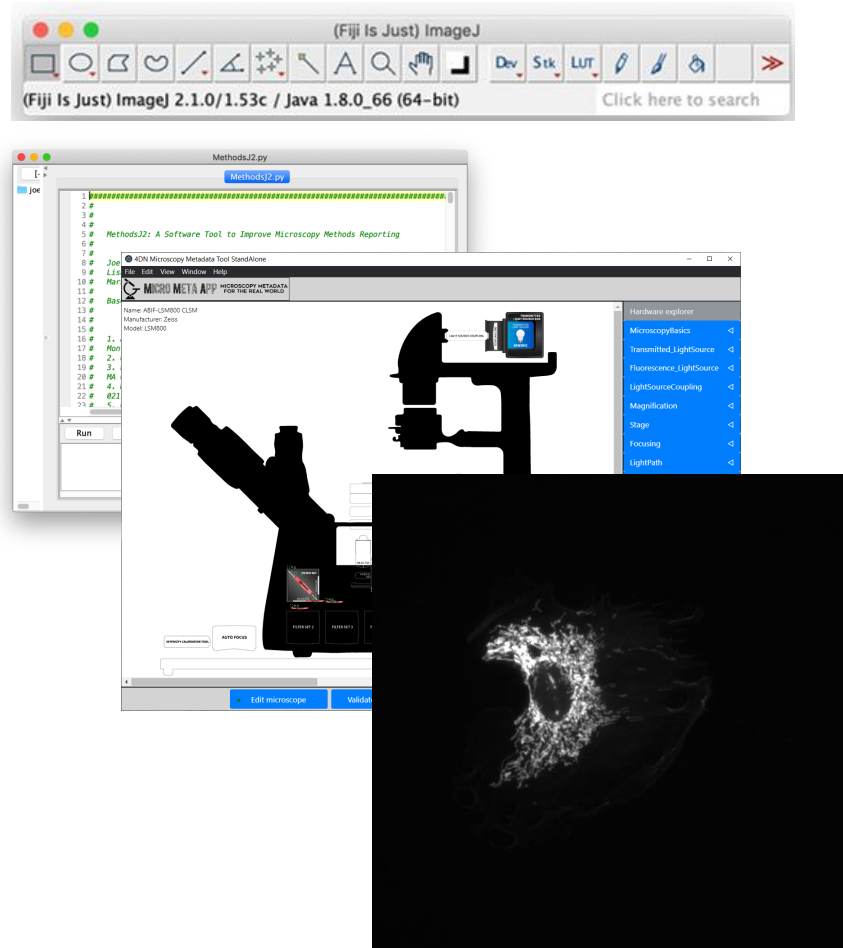
# METHODSJ2 – WORKFLOW

## Fiji, input files

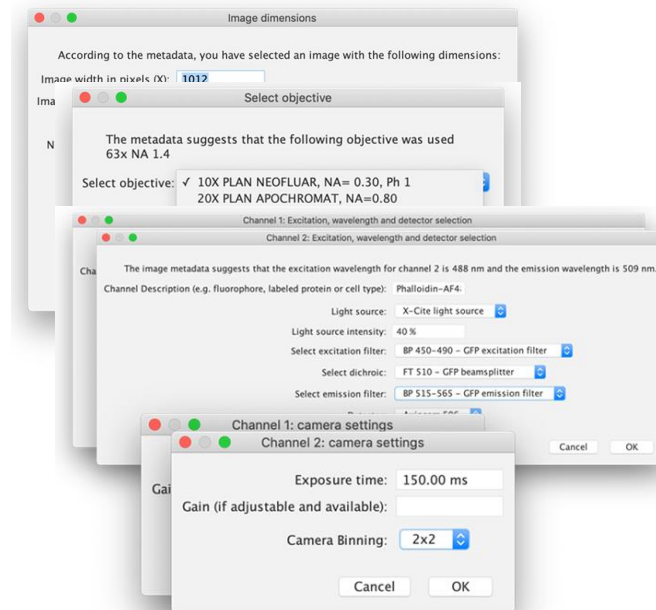


# METHODSJ2 – WORKFLOW

## Fiji, input files

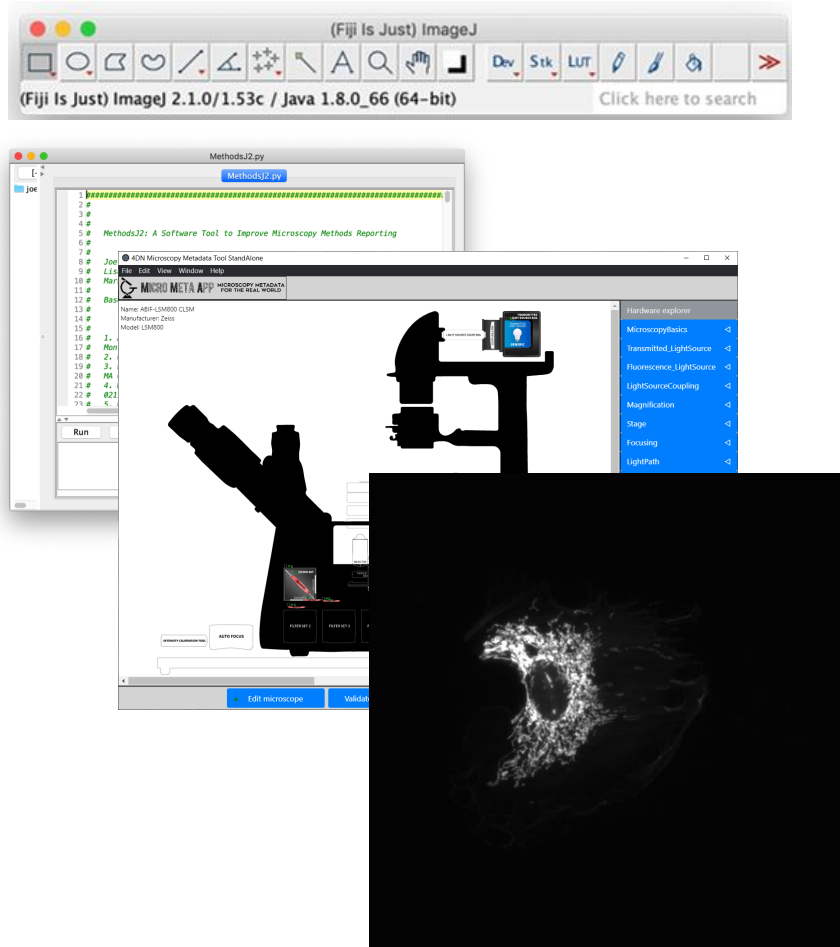


## User input

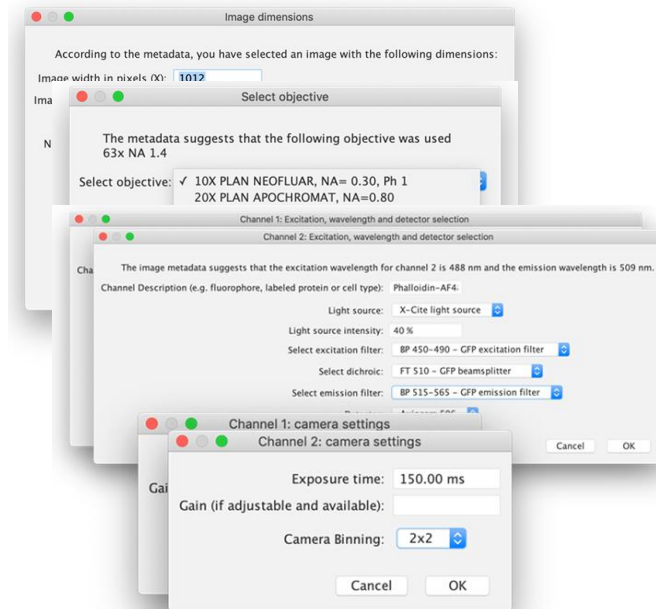


# METHODS J2 – WORKFLOW

## Fiji, input files



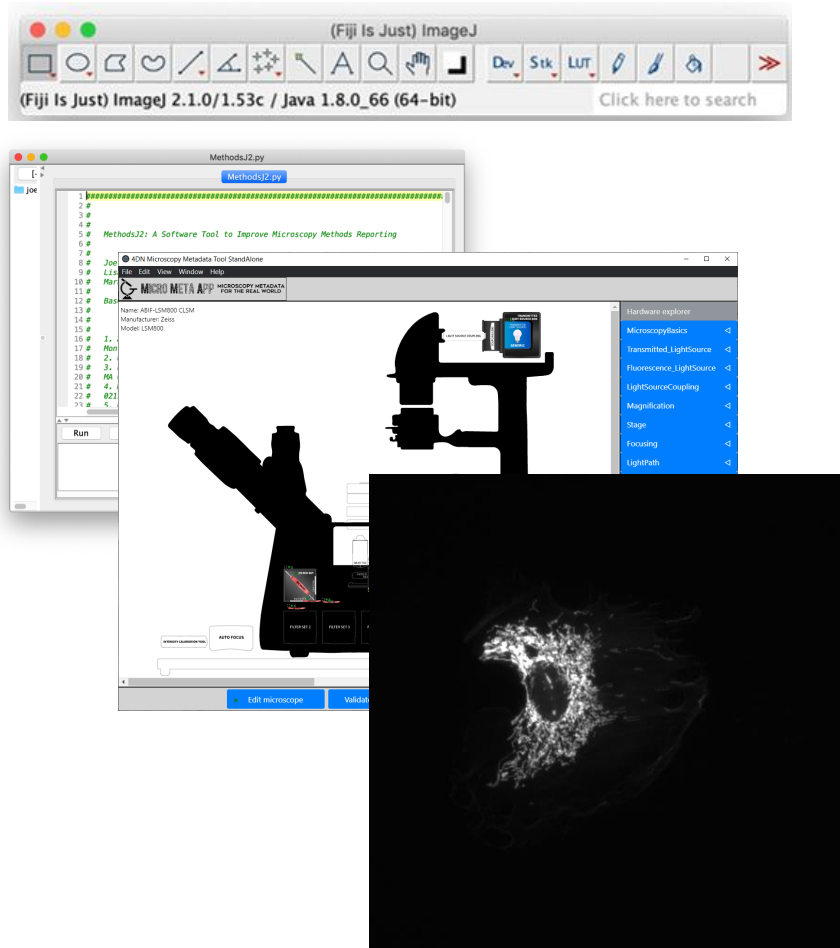
## User input



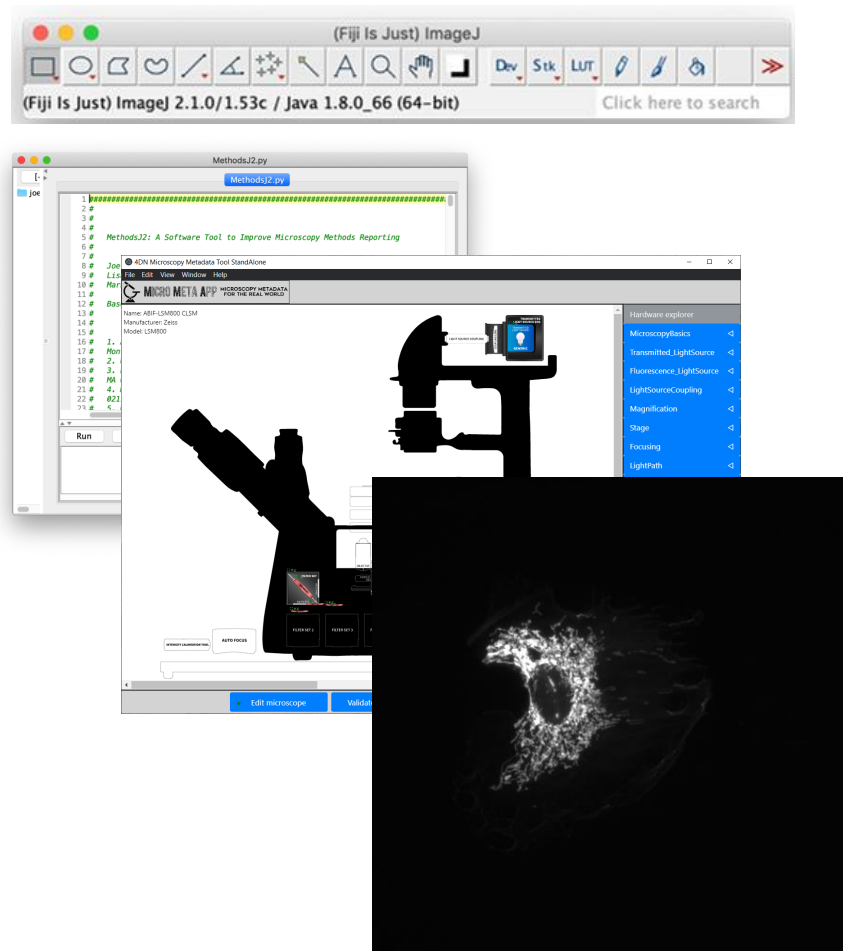
## Text output

*“Images were acquired on a AxioObserver Z1 upright inverted microscope equipped for widefield fluorescence using a 63x NA 1.4 Apochromat DIC oil immersion objective (Zeiss) [...]”*

# METHODSJ2 – WORK IN PROGRESS



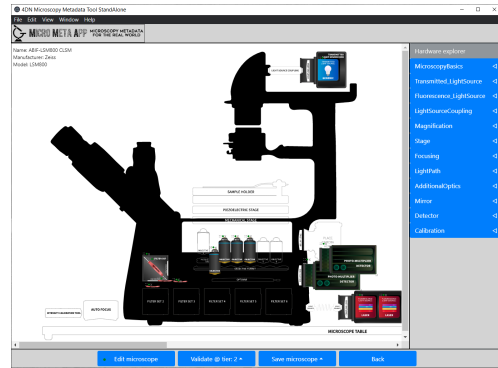
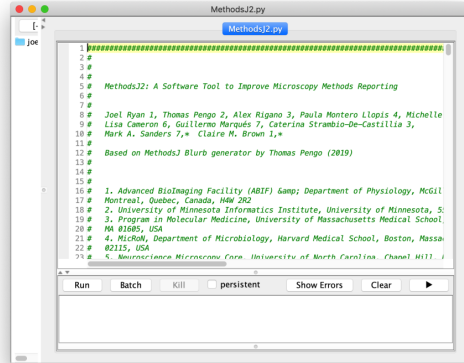
# METHODSJ2 – WORK IN PROGRESS



In the works...

- ❖ Developing functionality for broader range of microscopes and hardware
  - ❖ Looking for input and help! :)
- ❖ Plugin for *napari*
- ❖ Long-term: integration into OMERO

# METHODSJ2 – ACKNOWLEDGEMENTS



BioImaging  
North America

## Guidance

Prof. Claire Brown

Prof. Caterina Strambio de Castillia  
(UMass)

## Development

Thomas Pengo (UMinn)

Alessandro Rigano (UMass)

Nicole Xue

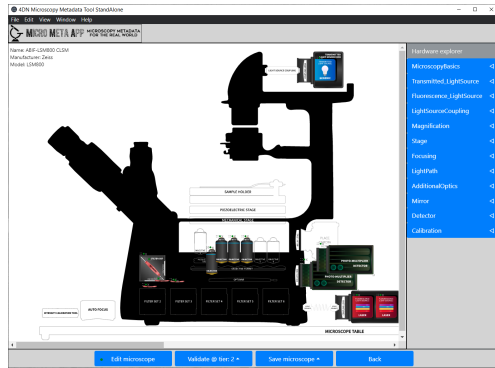
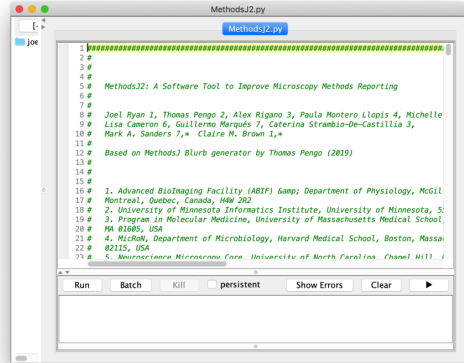
## Workgroups:

MethodsJ2 team

Micro-Meta App teams

Advanced BioImaging Facility

# METHODSJ2 – ACKNOWLEDGEMENTS



BioImaging  
North America

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