ADVANCED BIOIMAGING FACILITY

TOOLS FOR AUTOMATING MICROSCOPY METHODS REPORTING

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

MethodsJ2, Micro-Meta App, MicCheck

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

Methods sections for microscopy experiments tend be incomplete:

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Methods sections for microscopy experiments tend be incomplete:

Imaging methods are vastly underreported in biomedical research

Marqués, et al., *eLife* 2020

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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

Methods sections for microscopy experiments tend be incomplete:

"Images were acquired on a microscope."

Problems to address

Methods sections for microscopy experiments tend be incomplete:

"Images were acquired on a microscope (Zeiss)."

Problems to address

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

Methods

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 µm 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×/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 × 1 binning, a frame size of 1024 × 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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Mulholland, et al. 2020 *Nat Comms*

Complete methods sections are important

- Helps evaluate the work
- Enables reproducibility
- Enables data reusability…

Where to start?

WHERE TO START?

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?

Best practices and tools for reporting reproducible fluorescence microscopy methods

Examples of how different

hardware parameters affect

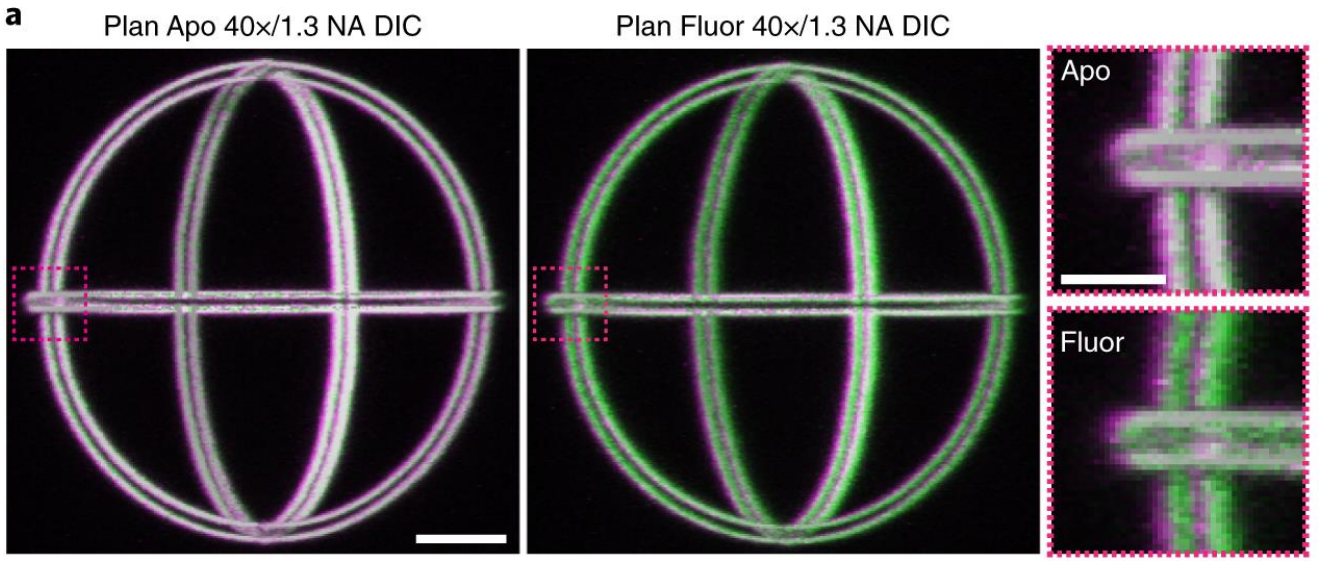
images!

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

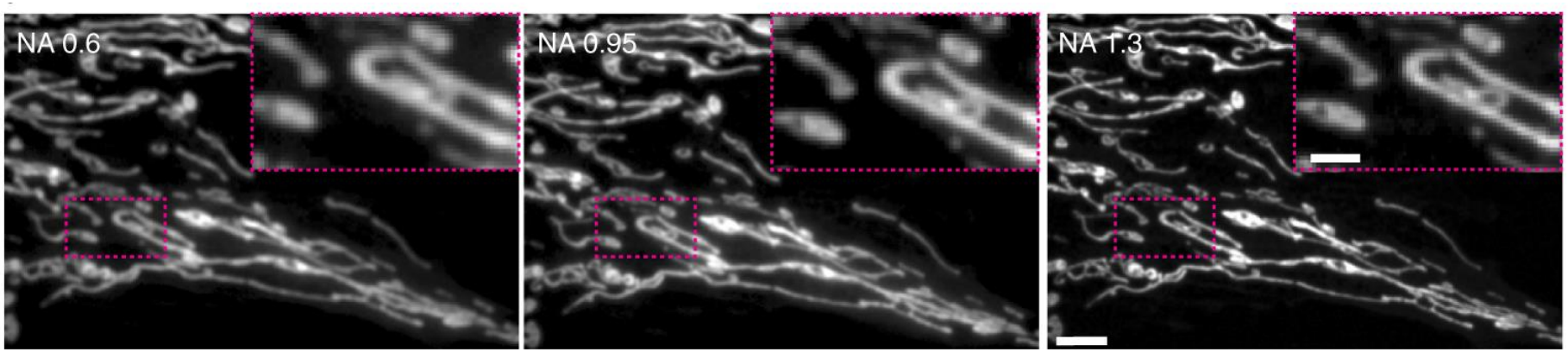
WHERE TO START?

Best practices and tools for reporting reproducible fluorescence microscopy methods

Objective lens

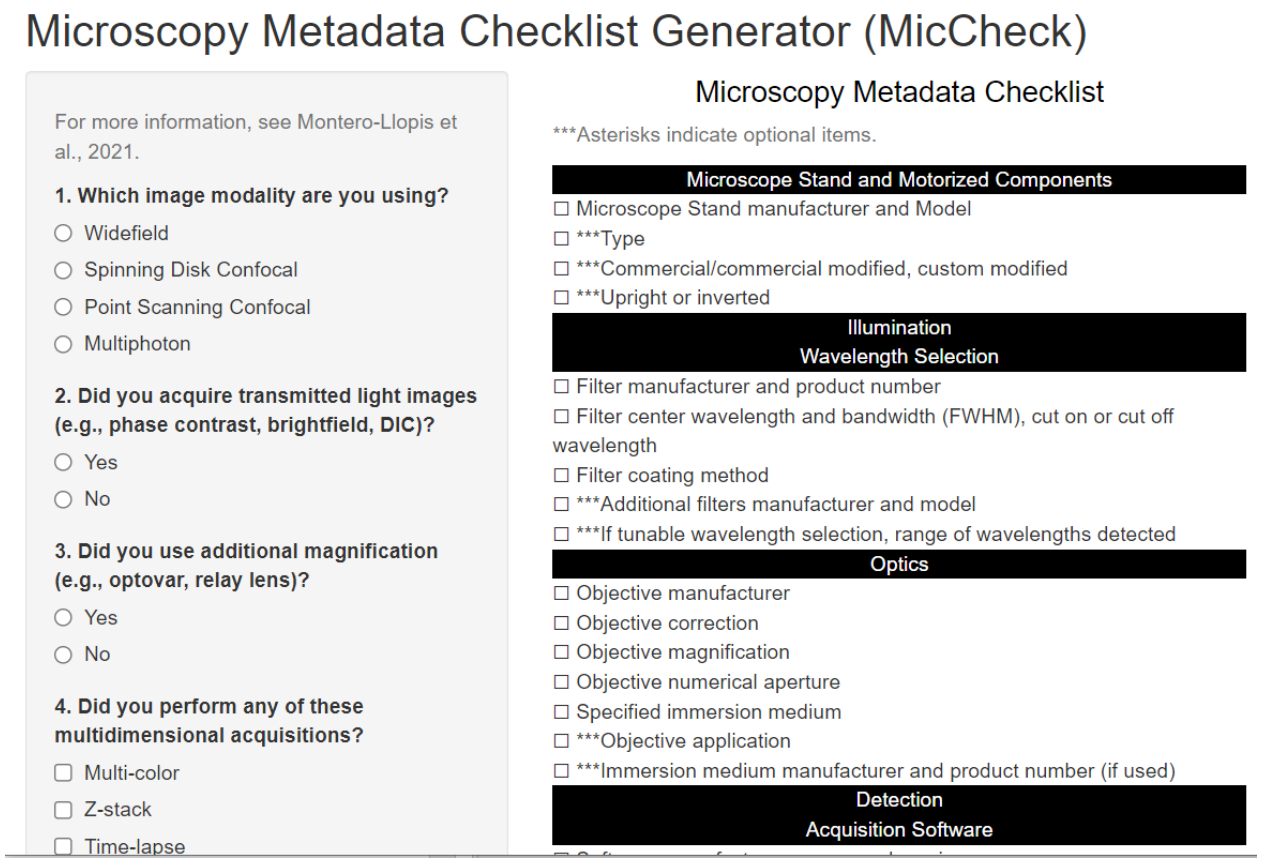
Numerical Aperture

(N.A.)



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

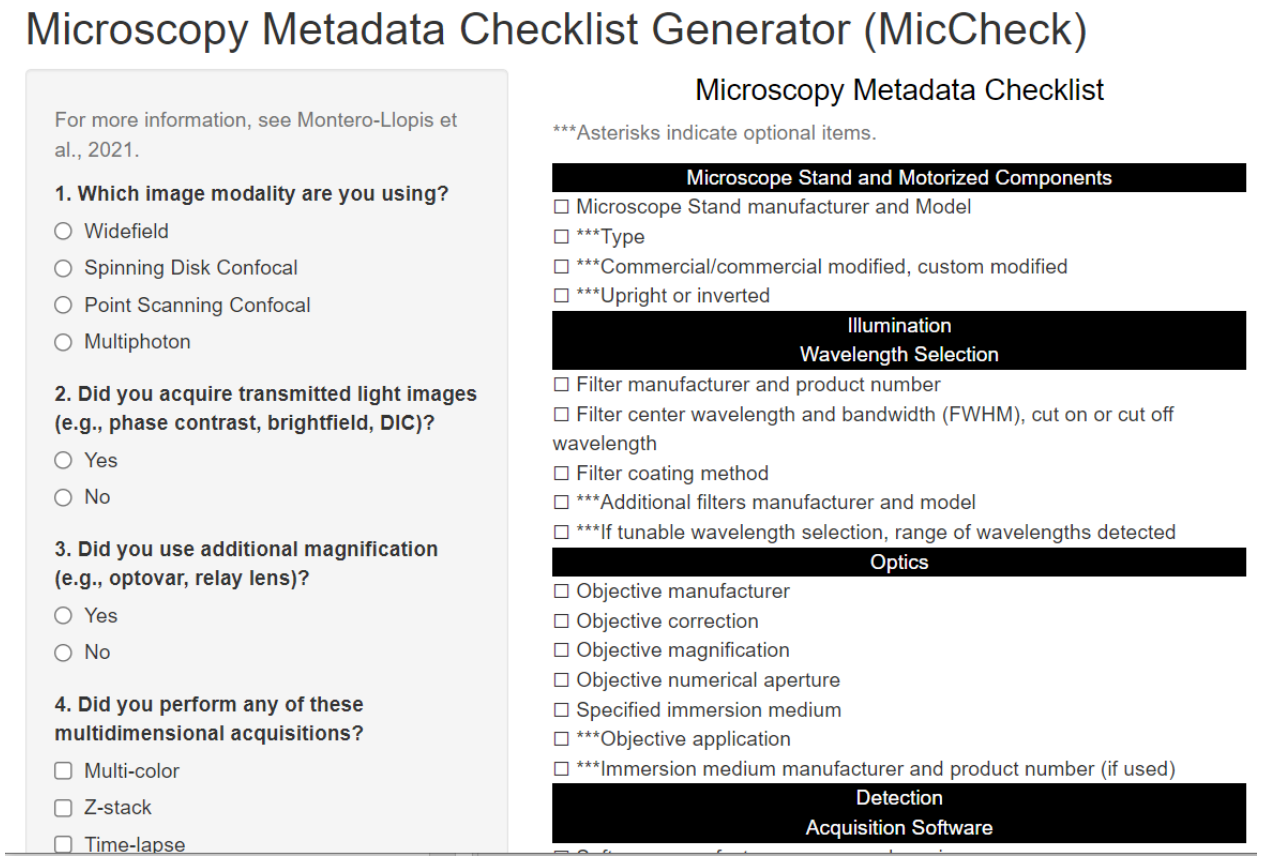
MicCheck



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

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

MicCheck



- 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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MethodsJ

Automating Methods Reporting...

Imaging methods are vastly underreported in biomedical research

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MethodsJ → **Methods text generation based on image metadata**

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

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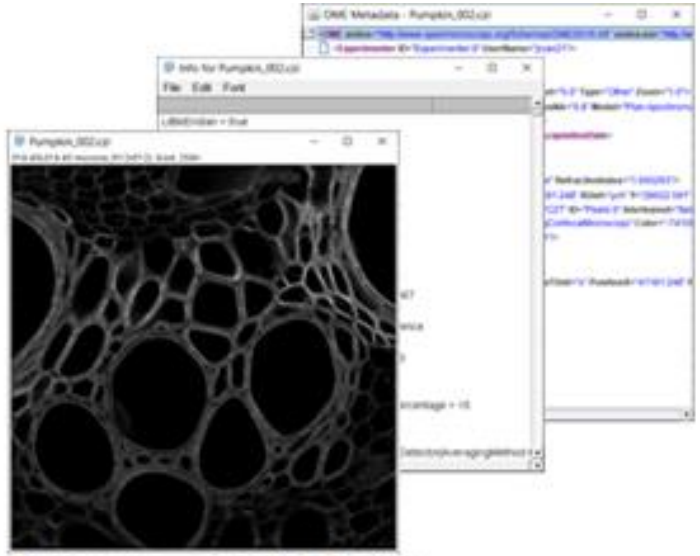
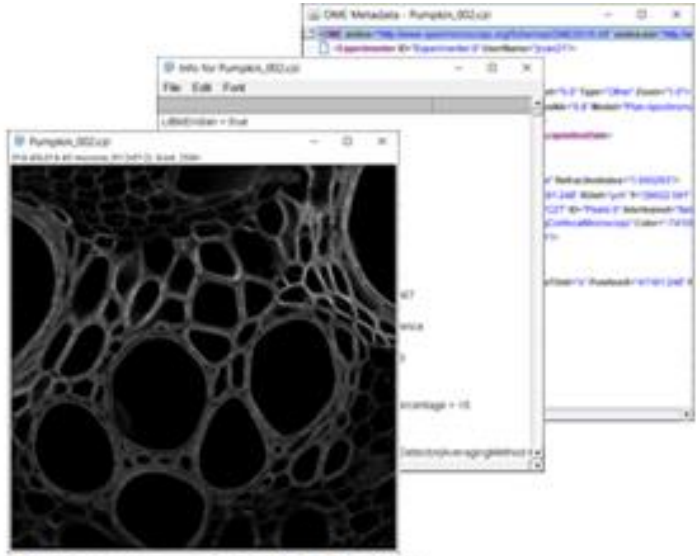
MethodsJ2 - sources of information



METHODSJ2 - SOURCES OF INFORMATION

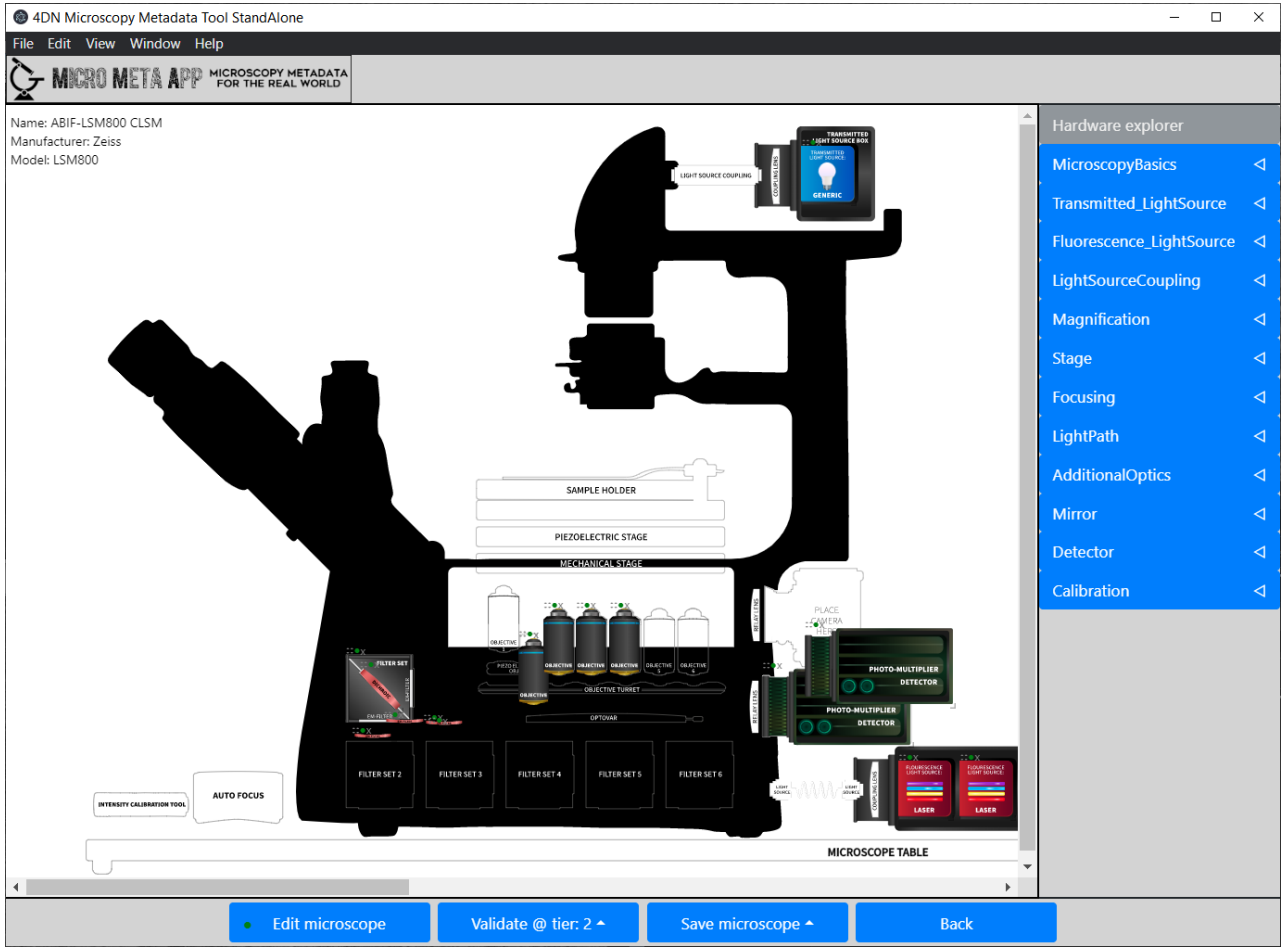


Image, metadata,
OME metadata

Micro-Meta App microscope
hardware specifications file

MICRO-META APP

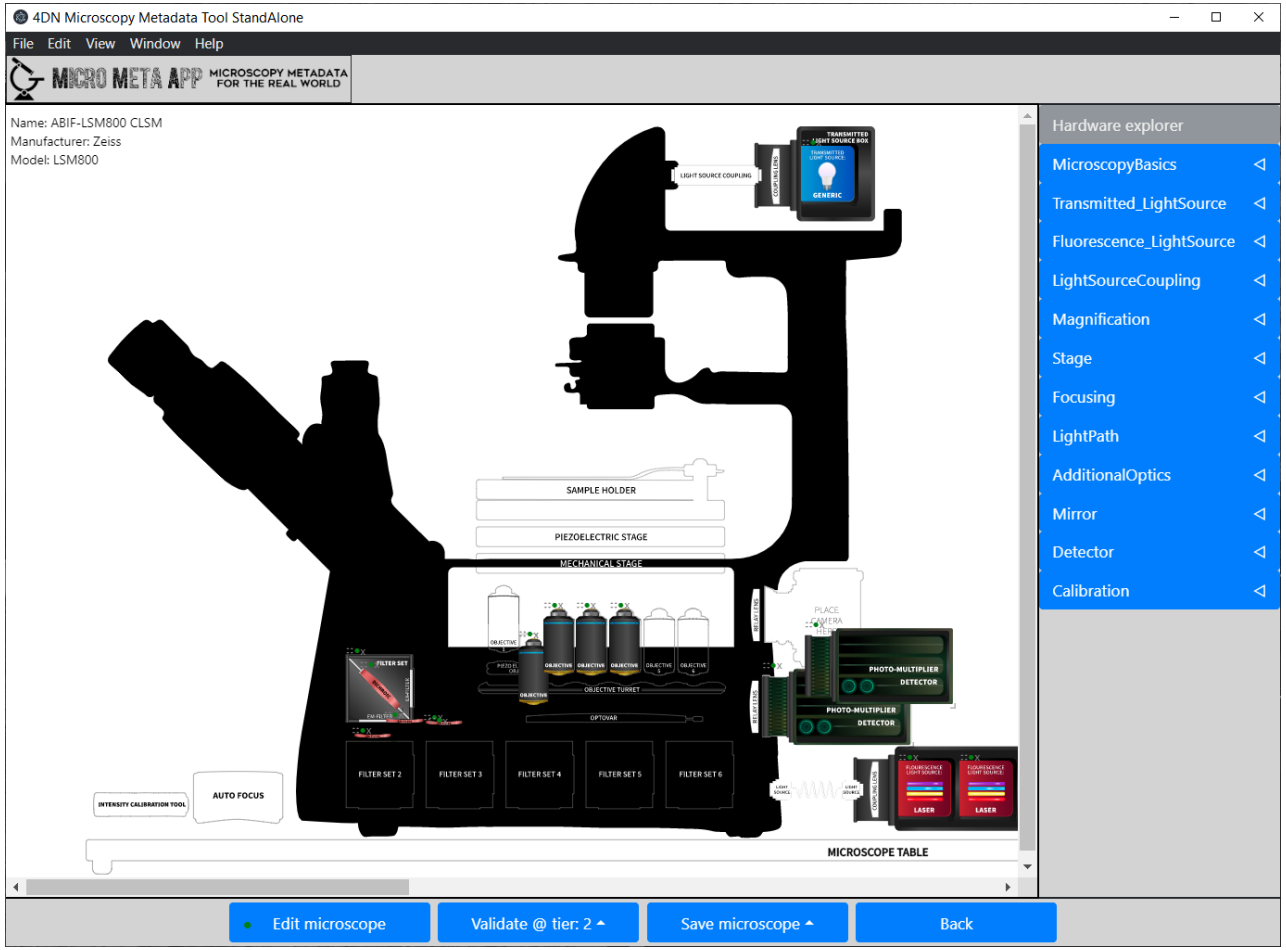
- "Build" your microscope *in silico*
- Uses standardized metadata language



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

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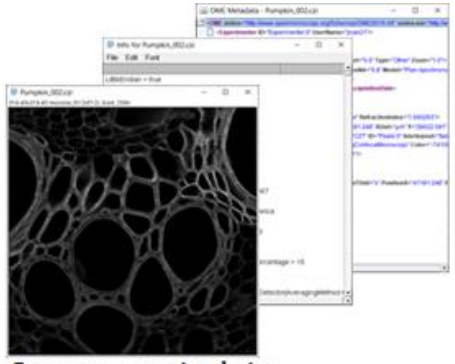
METHODSJ2

METHODSJ2



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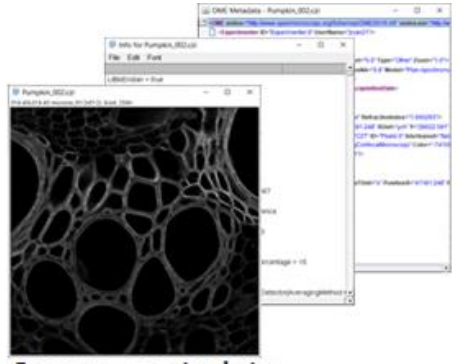

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Micro-Meta App microscope
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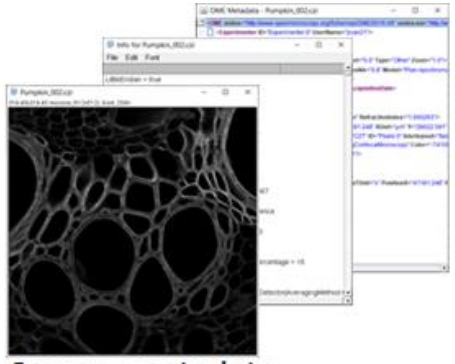

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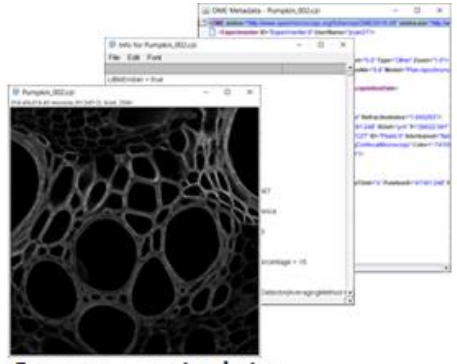
MethodsJ2

Python script
running in Fiji

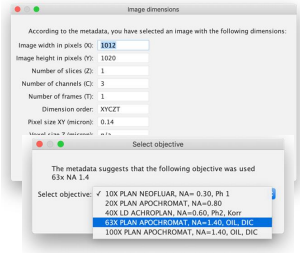
METHODSJ2



Image, metadata, OME metadata

Micro-Meta App microscope hardware specifications file

User input, guided by core facility staff



MethodsJ2

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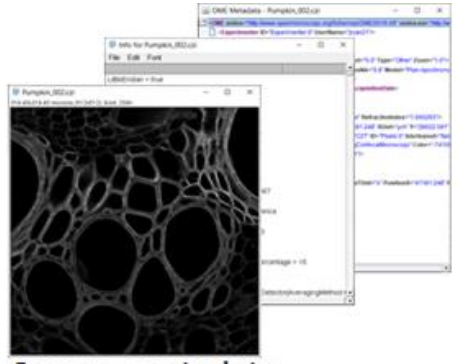

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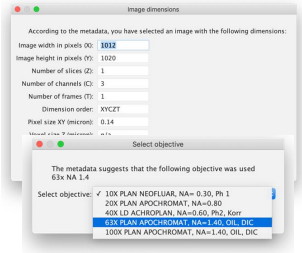
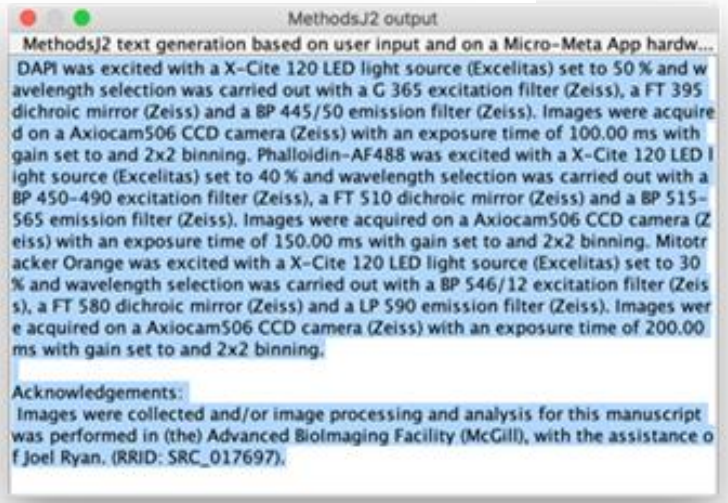


MethodsJ2

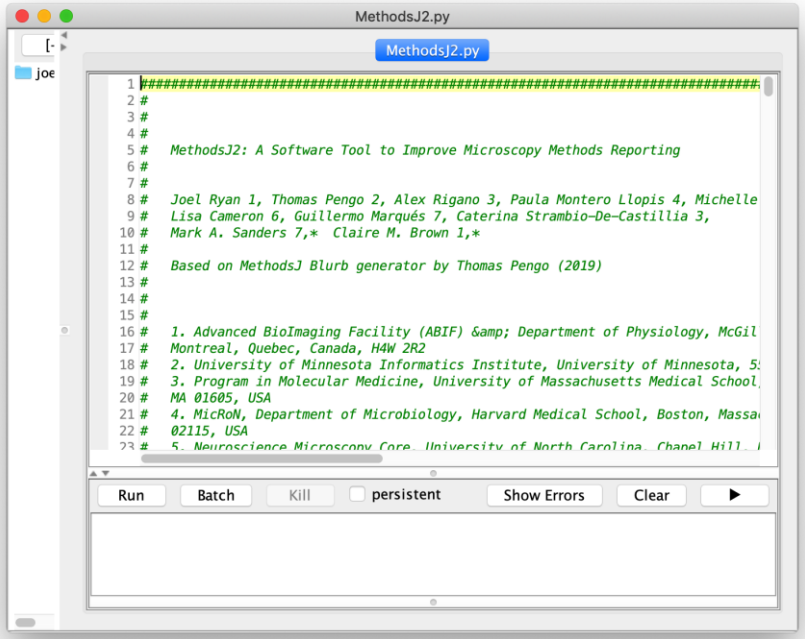
Python script running in Fiji

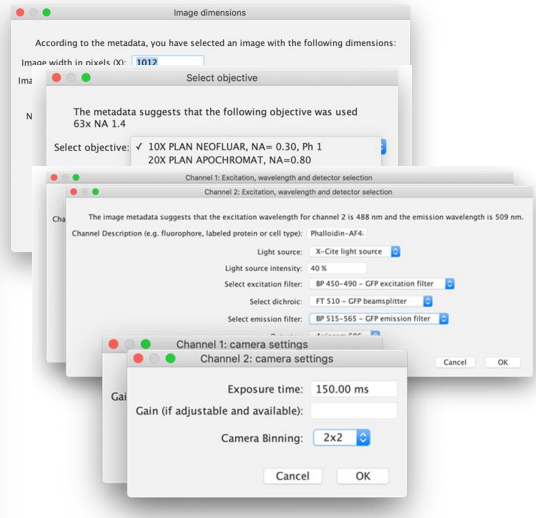
Generates materials and methods section for imaging experiments, based on community guidelines



METHODSJ2 - DEMO TODAY!

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





MethodsJ2 – required files

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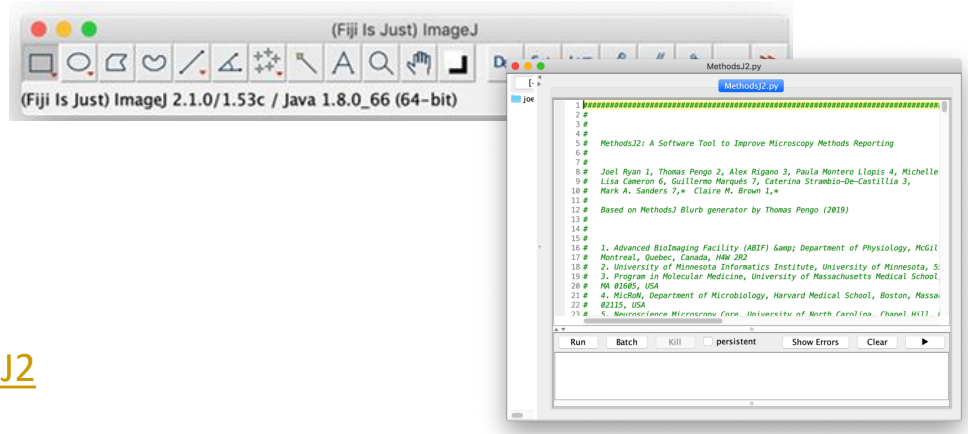

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- Fiji
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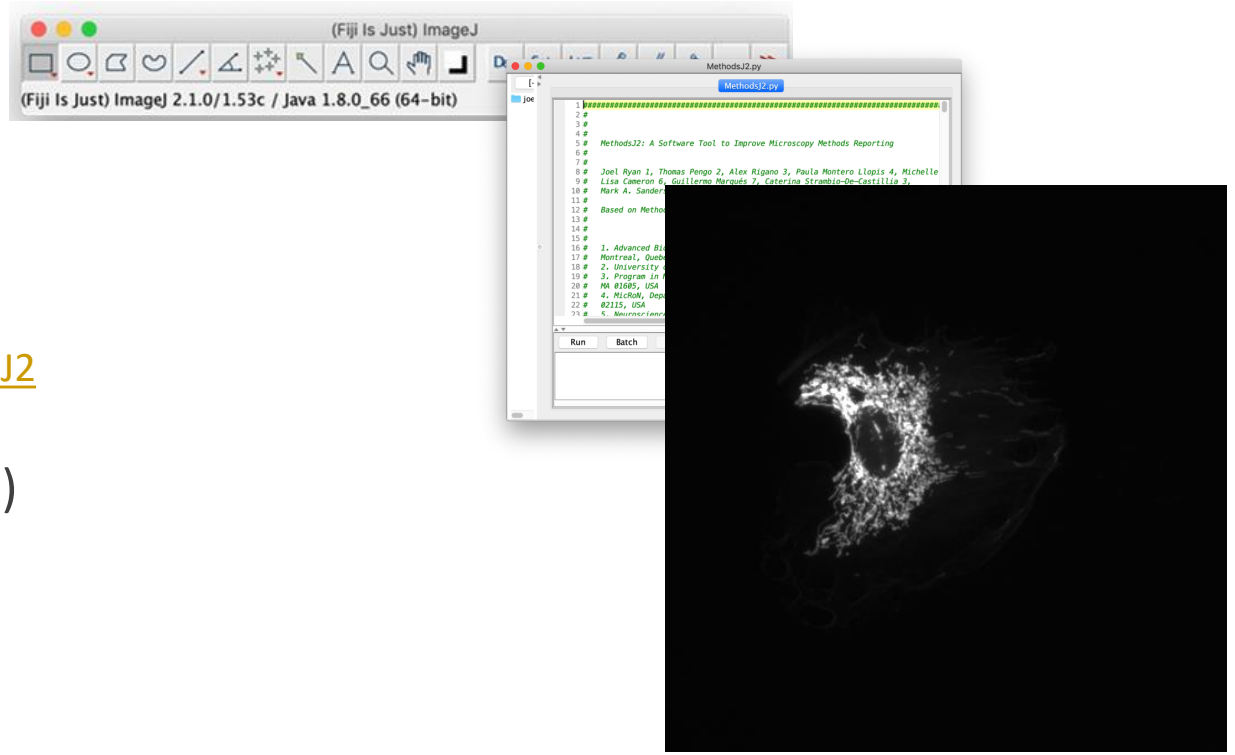

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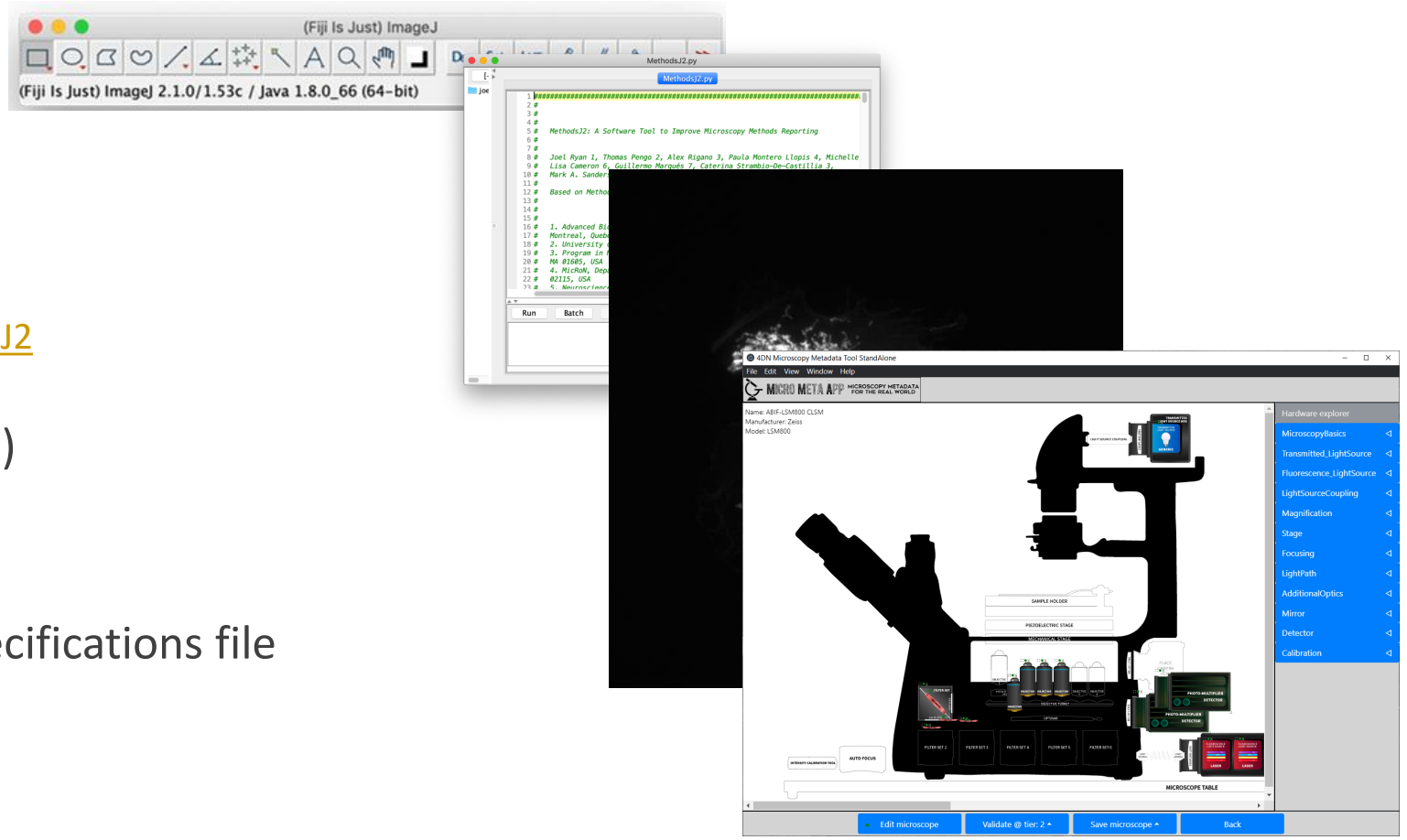
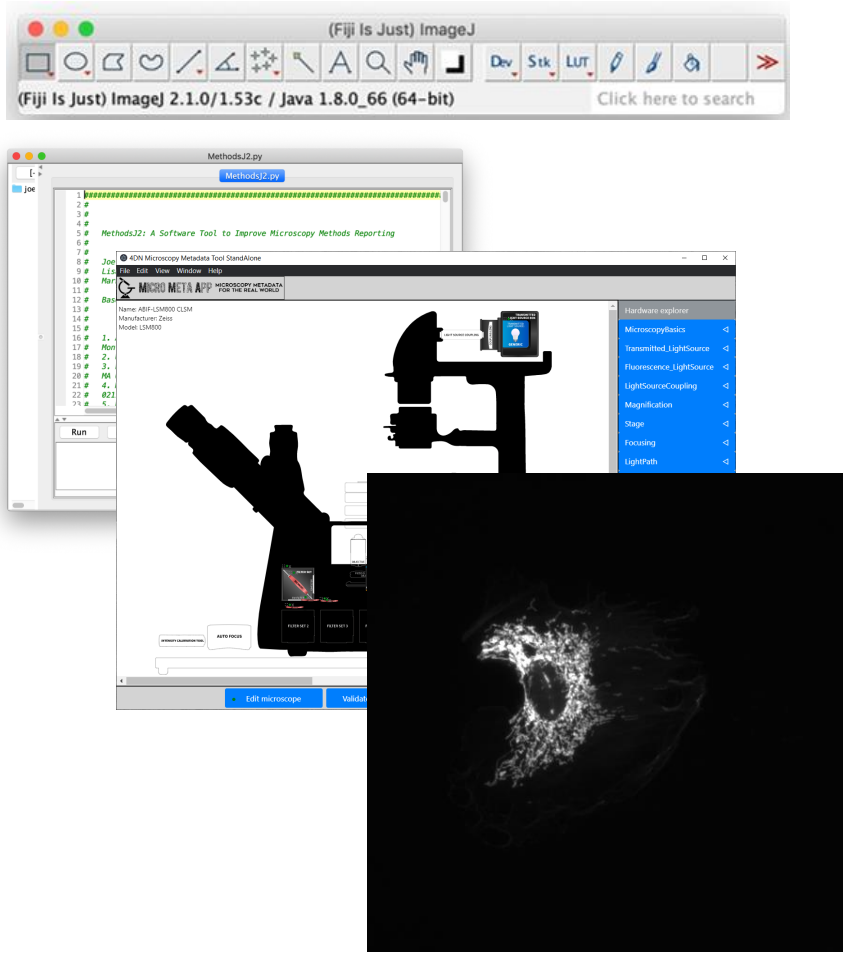


METHODSJ2 – WORKFLOW

Fiji, input files



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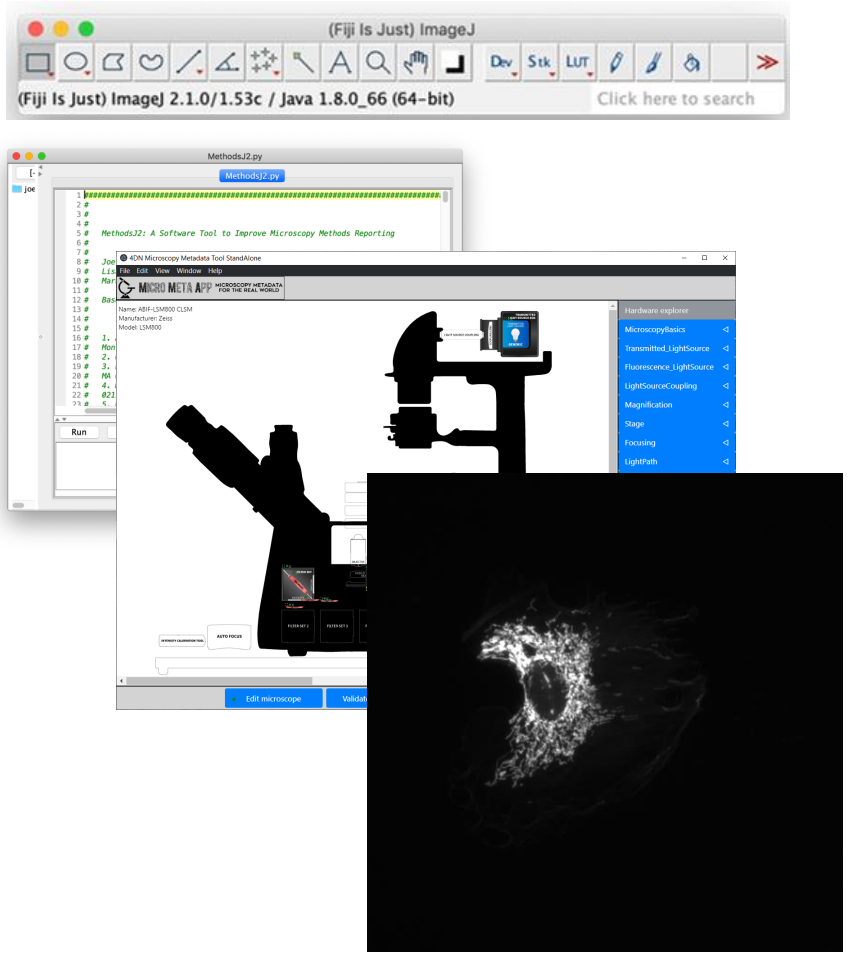
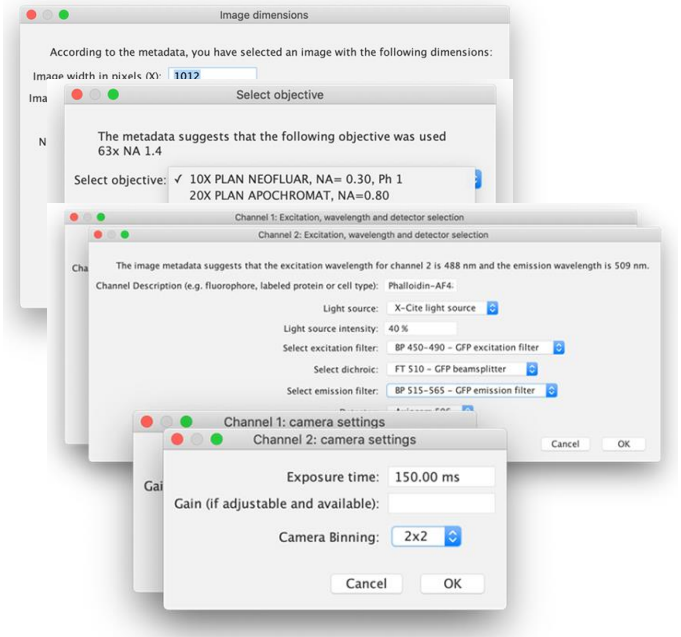
Fiji, input files

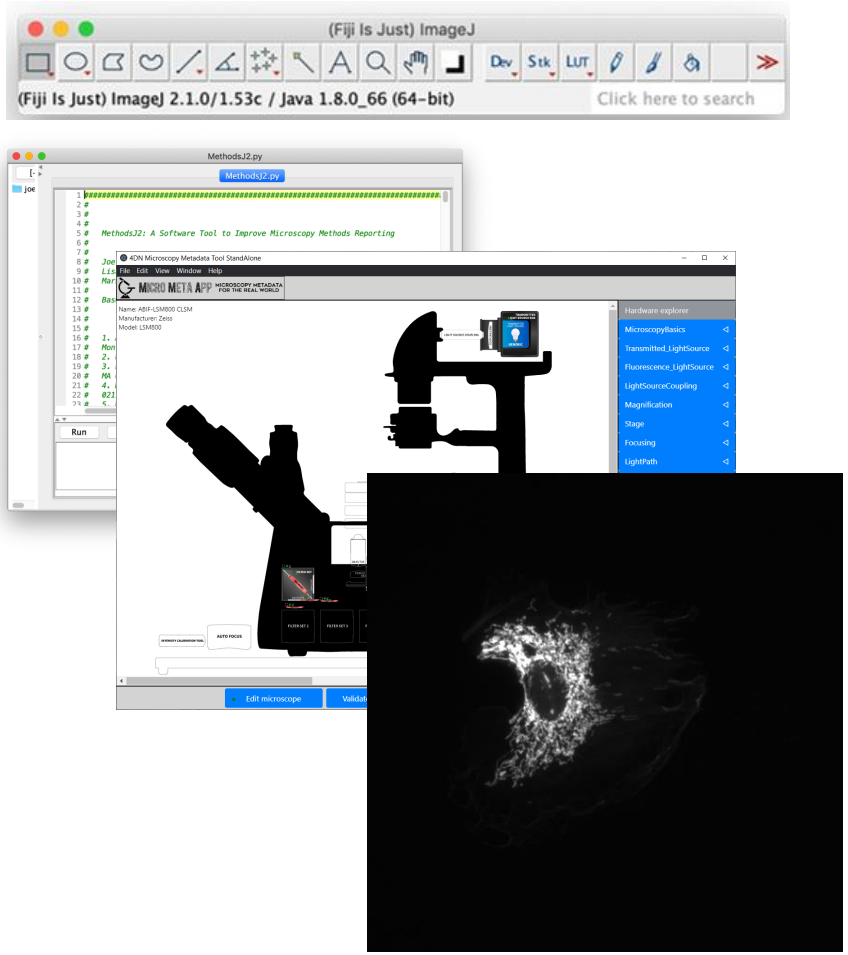


User input



MethodsJ2 – workflow

Fiji, input files



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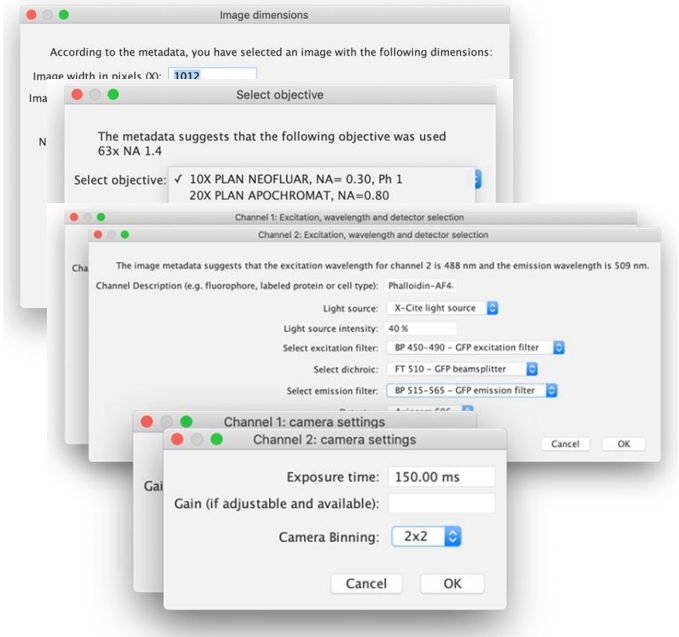
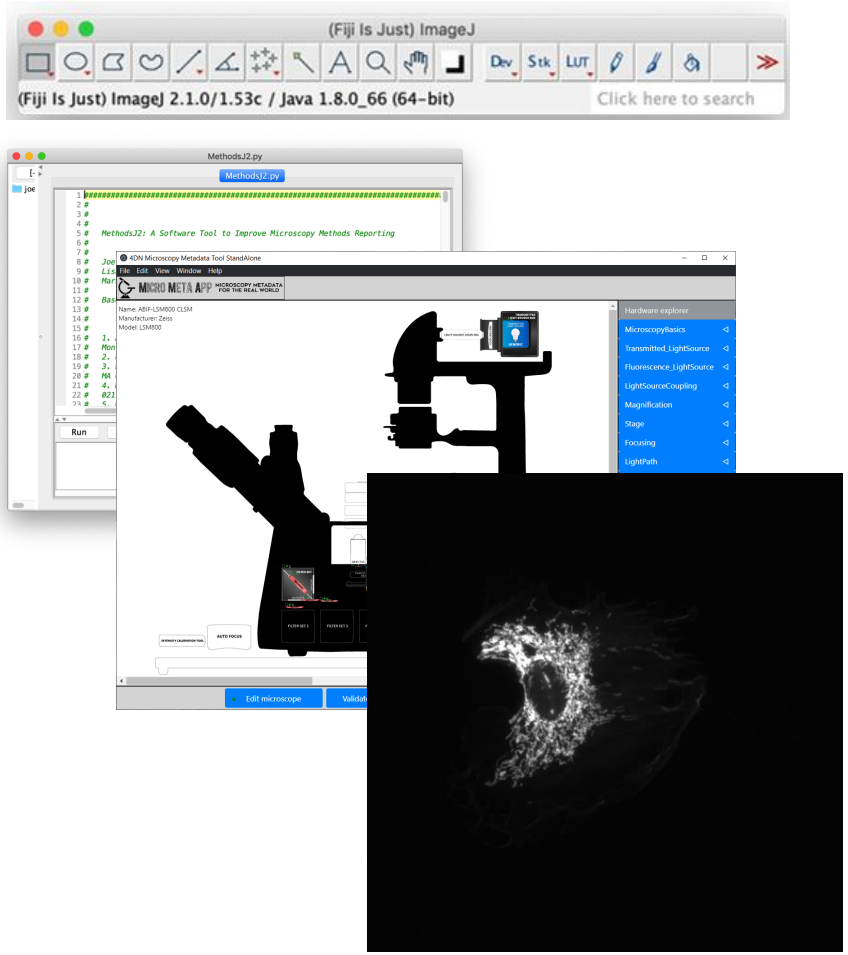


Text output

"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



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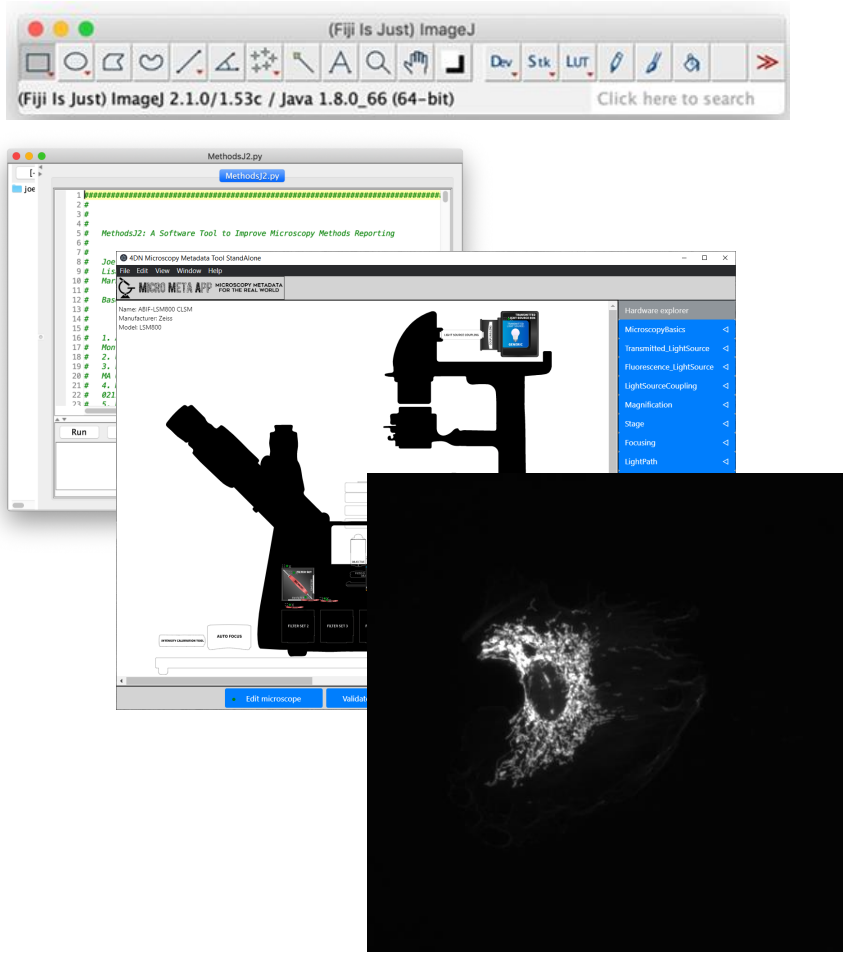
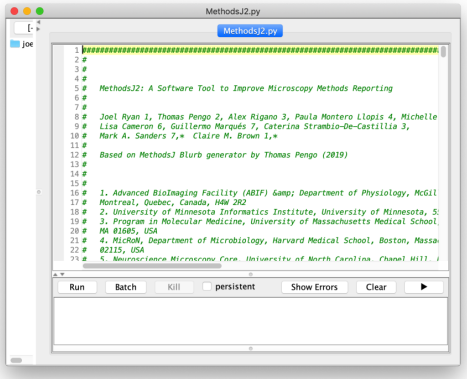
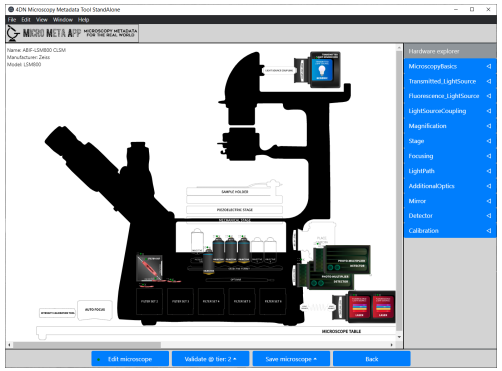


In the works…

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

METHODSJ2 – ACKNOWLEDGEMENTS





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

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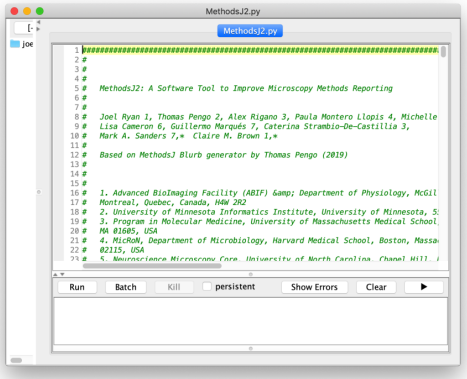

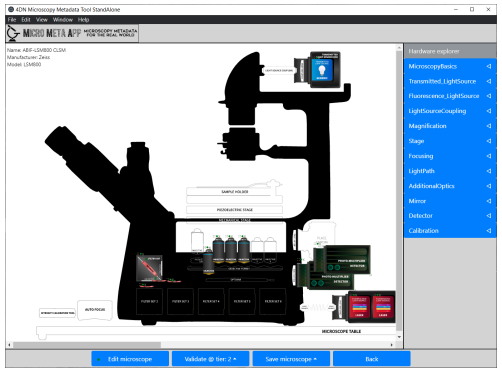

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