Results of community surveys organized by the members of the BioImaging North America – Quality Control and Data Management Working Group to understand the microscopy reporting and reproducibility needs of the bioimaging community

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Introduction

In January 2022, members of the BioImaging North America (BINA) Quality Control and Data Management Working Group, held a Community Conversation to introduce a series of articles that had been featured in the FOCUS on Microscopy Reporting and Reproducibility published in the December 2021 issue of Nature Methods.

During this event, the authors of the papers featured on the FOCUS issue were invited to present their work and interact with members of the BINA community.

A series of community surveys were conducted during this Community Conversation to better understand the audience, their current reporting, and reproducibility practices and their interest in tools and resources to help them better take advantage of these practices.

While the results of these polls are limited by the small sample size, this document is published in the hope that these results could be useful to the community to guide the future development of Research Data Management metadata specifications and software tools.

Summary

During the Community Conversation attendants were asked to participate to six polls under the condition of anonymity and with the proviso that polls results could be shared in aggregate manner.

The six polls were entitled as follows

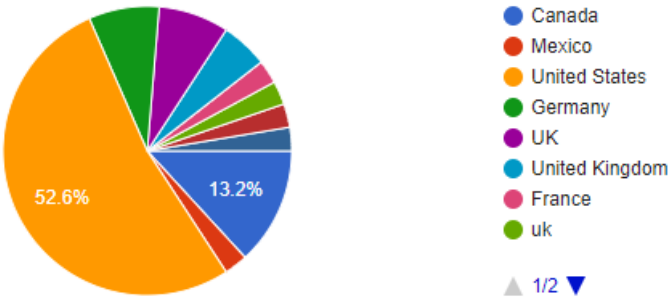
1. **Poll 1**- Learning about the audience attending the Community Conversation
2. **Poll 2** - Interest in reporting, reproducibility, and quality control for imaging experiments - collected before the presentation of the articles featured on the Nature Methods December 2021 FOCUS issue.
3. **Poll 3** - Interest in reporting, reproducibility, and quality control for imaging experiments -collected after the presentation of the articles featured on the Nature Methods December 2021 FOCUS issue.
4. **Poll 4**- Current microscope instrumentation calibration and quality control practices.

Survey results

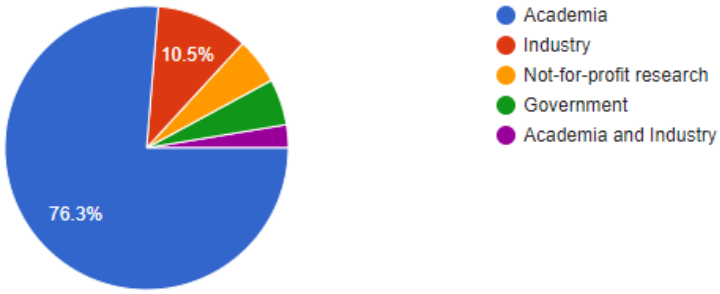
The results with some ‘take home points’ are summarized below.

Poll 1 - *Learning about the audience attending the Community Conversation*

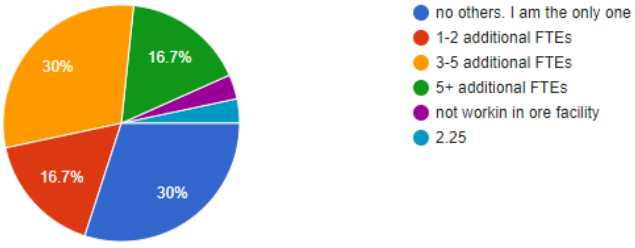
(38 respondents)



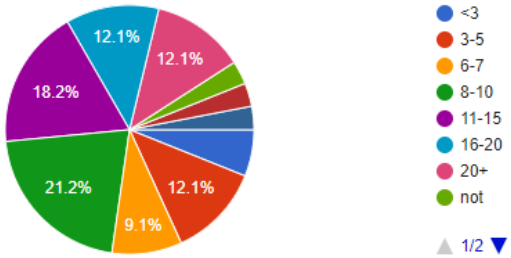
The audience was largely from the US (53%) with representation from the UK (~16%), Canada (13%) and Germany (8%) then followed by Mexico, France, Japan and Australia at ~3% (1 person each).



The majority of participants (76%) were from Academia, with smaller representation from Industry (11%), non-profit research (5%), Government (5%) or a combination of Academic and Industry (1 participant ~3%)



Core Facility Managers/Directors & staff accounted collectively for 66% of the audience with some Staff Scientist (18%), Professor (13%) and Junior Faculty (5%) representation. Of those working in a Core Facility most were in facilities with either no other FTEs or cores with less than 5 FTEs (~80%) and a wide range of numbers of instruments→



Poll 2 - *Interest in reporting, reproducibility, and quality control for imaging experiments - collected before the presentation of the articles featured on the Nature Methods December 2021 FOCUS issue*

(32 total respondents)

The majority of respondents (30) currently record their experimental image acquisition data in "names of directories and files" (~57%), Electronic notebooks (~53%) or traditional paper notebooks (~37%) some (~3%) use Facility Online Management (FOM) software, BioFormats or commercial acquisition software or unspecified Metadata app.
~59% rely on the information provided by the manufacturer in the image file for assigning/attaching microscopy metadata (instrument hardware, acquisition settings and calibration) to their image files, while ~41% Record in names of directories and files.

When asked to identify how important respondents believed the following information/metadata is to ensuring reproducibility the following results were shared;

	Important	Somewhat	Not so important
1. Microscope manufacturer name and model	26	5	1
2. Date/Time of capture	18	10	3
3. Imaging environment (temp/ CO_2 etc.)	28	4	0
4. Illumination/excitation light power	27	5	0
5. Magnification and NA	32	0	0
6. Additional objective characteristics (e.g. corrections, working distance)	23	9	0
7. Excitation/ emission wavelength ranges	32	0	0
8. Description of light path	13	19	0
9. Camera/scanhead manufacturer & model	27	4	1
10. Exposure/ pixel dwell time	27	5	0
11. Pixel/ voxel size	32	0	0

When asked if there is/are additional information/metadata not included in the list above the following were shared;

- Digital processing / correction methods.
- biological conditions
- experimental condition
- Dye name and concentration
- Triggering out not for live imaging
- Bandwidth for fluo emission
- Measured resolution of your microscope, field flatness, maybe any of these of would help etc.
- type of sample holder (incl. coverslip thickness)
- Effective intensity

When asked of the same list of information/metadata which they report on in their research respondents indicated as follows

	Always	Sometimes	Rarely
1. Microscope manufacturer name and model	30	1	1
2. Date/Time of capture	17	4	11

3. Imaging environment (temp/ CO_2 etc.)	20	7	5
4. Illumination/excitation light power	18	8	6
5. Magnification and NA	30	2	0
6. Additional objective characteristics (e.g. corrections, working distance)	17	14	1
7. Excitation/ emission wavelength ranges	29	3	0
8. Description of light path	10	13	9
9. Camera/scanhead manufacturer & model	30	1	1
10. Exposure/ pixel dwell time	21	10	1
11. Pixel/ voxel size	28	4	0

When asked to identify the primary reason for reporting only "Sometimes" or "Rarely", on the items above respondents indicated the following reasons

	Not enough Information time	Don't know how	not available
1. Microscope manufacturer name and model	1	0	3
2. Date/Time of capture	7	2	2
3. Imaging environment (temp/ CO_2 etc.)	0	3	4
4. Illumination/excitation light power	1	1	6
5. Magnification and NA	0	0	1
6. Additional objective characteristics (e.g. corrections, working distance)	1	1	2
7. Excitation/ emission wavelength ranges	1	0	1
8. Description of light path	5	3	2
9. Camera/scanhead manufacturer & model	0	0	2
10. Exposure/ pixel dwell time	4	0	1
11. Pixel/ voxel size	1	0	1

The following were shared when asked to provide further comments about difficulties in capture and reporting metadata;

- People simply won't do it
- Difficult to get microscope specs from Vendors on equipment that came with instrument
- number of channels acquired, number of planes in z and/or t…
- Lack of user interest
- This information is captured, but not always reported in publications.
- as core staff, I encourage reporting all of these and try to educate people on why
- i think for the things reported "sometimes", that is often because I didn't (at the time of reporting) consider it critical to the results. E.g. temperature-wouldn't necessarily report room temp experiments.
- Filter set model numbers aren't available from microscope specs and differ from filter manufacturers

Related to the same list of information/metadata, respondents were asked to indicate how frequently they find this information/metadata reported in Materials and Methods sections of publications, enabling them to reproduce the finding?

	Often	Sometimes	Rarely
1. Microscope manufacturer name and model	24	6	0
2. Date/Time of capture	1	1	27
3. Imaging environment (temp/ CO_2 etc.)	6	19	4
4. Illumination/excitation light power	1	11	18
5. Magnification and NA	14	12	3

6. Additional objective characteristics (e.g. corrections, working distance)	3	9	17
7. Excitation/ emission wavelength ranges	5	19	5
8. Description of light path	2	5	22
9. Camera/scanhead manufacturer & model	9	14	6
10. Exposure/ pixel dwell time	1	11	17
11. Pixel/ voxel size	9	10	11

The following were shared when asked to provide current challenges related to quality, reporting and reproducibility (asked to select up to 4 from the list but the question wasn't "multiple choice format" so respondents could only select one or pick 'other' and then provide details.

1. Lack of time ~10%
2. Lack of tools ~26%
3. Absence of key metadata in imaging files ~10%
4. Lack of knowledge of what is important/necessary ~0
5. Multiple reporting metadata methods leading to inconsistent information ~13%
6. No agreed standard to work towards ~23%
7. Other
    a. People don't care and will not do it
    b. Lack of time and lack of tools and multiple reporting metadata methods
    c. Lack of tools/absence of key metadata/lack of knowledge/no agreed standards
    d. All the above
    e. Lack of user interest
    f. Lack of tools, lack of budget to buy tools, lack of knowledge on what is important/lack of standardized protocols.

Poll 3 *- Interest in reporting, reproducibility, and quality control for imaging experiments -collected after the presentation of the articles featured on the Nature Methods December 2021 FOCUS issue.*

(28 respondents)

After having heard the presentation, respondents were asked to indicate their level of agreement with the following statements:

	Agree	Disagree	Need more information
1. I plan to learn more about recording & reporting metadata & their impact on reproducibility	26	1	0
2. I plan to give recording & reporting metadata a higher priority	24	2	1
3. I plan to encourage my users to give higher priority to recording & reporting metadata	26	0	1
4. I plan to get more involved in advocating for recording & reporting metadata & their impact on reproducibility	24	0	2

When asked after the presentation if they were interested in using the tools shared, respondents answered as follows

	already use it	plan to use it	Unsure/need more information	do not plan to use it
1. MicroMetaApp	9	8	8	2
2. Methods J2	3	18	5	2
3. MicCheck	2	15	10	1
4. MDEmic (OMERO.mde)	0	9	14	4

When asked if respondents would like a dedicated workshop on the following tools, respondents answered as follows

- All 74% (17)
- MicroMeta App 13% (3)
- MethodsJ2 22% (5)
- MicCheck 17% (4)
- MDEmic (OMERO.mde) 4% (1)

When asked, what would be the most useful format for learning how to use these tools, respondents answered as follows

1. Virtual group workshop with ‘hands on examples’ 69% (18)
2. In person workshop with demo and case study 19% (5)
3. Video tutorial/On-line resource I could access 24/7. 92% (24)
4. All of the above 4% (1)

5. Any or all of above but video may be more useful for sending out to colleagues — 4% (1)

When asked if respondents were aware of available data management tools (OMERO) and initiatives to build public repositories (DR, BioImage Archive, SSDB) for archiving image data before the presentation responses were as follows;

Yes - 89% No - 11%

When asked for their interested in learning more about the following image data management and repository initiatives, responses were as follows;

	Yes	No	Need more Information
○ OMERO	21	2	3
○ IDR	14	3	6
○ BioImage Archive	16	3	6
○ SSDB	11	3	9

When asked about their level of interested in participating in QUAREP LiMi, responses were as follows;

- I am already a member — 75% (21)
- I plan to become a member — 11% (3)
- Unsure/need more information — 14% (4)
- I do not plan to become a member — 0%

Poll 4 - *Current microscope instrumentation calibration and quality control practices*

(21 respondents)

When asked what tools respondents use or are aware of for microscope calibration and performance evaluation related to light source and resolution (i.e. PSF) the following was indicated;

	Aware of	Use/Used	Unaware of
1. Power meter	4	16	1
2. Bead slide (‘homemade”)	6	14	1
3. Tetra Speck bead slide (ThermoFisher)	4	16	1
4. PSFcheck	6	10	5
5. Argolight slide	9	8	4
6. Other samples/tools	3	8	3

"Other samples/tools", used by respondents are listed below:

- reticles, solutions, other beads, filament preps, Plexiglas
- Chroma sides, GE's chromatic correction slide
- Zeiss calibration slide, homogeneous dye solution, dye slide
- Currently testing MetaMax, chroma slide modification,....
- plant tissue samples
- mirror slide
- Molecular probes sample slides for a known sample, Chroma plastic slides,
- MetaMax

When asked whether they own or have access to the following tools for system checks and performance evaluation related to light source and PSF, respondents answered as follows;

	Own	Have access to	Neither
1. Power meter	11	5	5
2. Bead slide (‘homemade”)	11	4	5
3. Tetra Speck bead slide (ThermoFisher)	12	4	5
4. PSFcheck	6	4	10
5. Argolight slide	8	2	11
6. Other samples/tools	8	0	5

When asked, on average, how often respondents check for performance of microscopes in your facility, the following answers were given;

	N/a	Daily	Monthly	Annually	Never	Unsure
1. Wide field Microscopes	3	1	8	6	0	1
2. Confocal Microscopes	0	1	11	6	0	1
3. Multi-photon Microscopes	7	0	8	2	0	2
4. Super Resolution Systems	8	0	8	2	0	1
5. Light Sheet Systems	7	1	7	1	2	1
6. Atomic Force Microscopes	15	0	0	0	0	1
7. Micro CT Scanner	14	1	2	0	0	1
8. Optical Coherent Tomography	15	0	0	0	0	1

When asked how often their facility performs the following maintenance and calibration procedures, the following was shared;

1. Visual inspection and cleaning of microscope:
N/A 0, On Demand 7, Daily 1, 2-3 days 2, 2 weeks (14 days) 7, Once a month 2, >month 1, Never 0, Unsure 0

2. Regular intense objective cleaning
N/A 0, On Demand 6, Daily 0, 2-3 days 0, 2 weeks (14 days) 4, Once a month 6, >month 4, Never 0, Unsure 0

3. Inspection of the objective under stereomicroscope or high mag
N/A 0, On Demand 8, Daily 1, 2-3 days 0, 2 weeks (14 days) 2, Once a month 3, >month 6, Never 0, Unsure 0

4. Laser (LED, arc lamp) power measurement
N/A 0, On Demand 5, Daily 0, 2-3 days 1, 2 weeks (14 days) 0, Once a month 2, >month 9, Never 3, Unsure 0

5. Power over time measurement
N/A 0, On Demand 3, Daily 0, 2-3 days 0, 2 weeks (14 days) 1, Once a month 5, >month 5, Never 6, Unsure 0

5. Scanner calibration (linearity)
N/A 1, On Demand 5, Daily 0, 2-3 days 0, 2 weeks (14 days) 0, Once a month 3, >month 7, Never 4, Unsure 0

7. Scan-field rotation precision
N/A 1, On Demand 4, Daily 1, 2-3 days 0, 2 weeks (14 days) 0, Once a month 2, >month 7, Never 5, Unsure 0

8. PSF measurements
N/A 0, On Demand 8, Daily 0, 2-3 days 0, 2 weeks (14 days) 0, Once a month 4, >month 7, Never 1, Unsure 0

9. Chromatic aberration & registration
N/A 0, On Demand 6, Daily 1, 2-3 days 0, 2 weeks (14 days) 0, Once a month 4, >month 6, Never 3, Unsure 0

When asked, how important are each of the factors are in influencing/impacting respondents ability to perform system maintenance and calibration procedures, the following was shared;

	Very important	Somewhat	Not important
1. I do not have the time to conduct it	12	5	3
2. I do not have the equipment to conduct it	7	3	9
3. I do not have the necessary knowledge/protocols	5	7	6
4. I do not think my customers value it enough to do it	5	9	5
5. There is no incentive to do this	4	6	9

When asked what other challenges (not listed above) affect respondents' ability to perform system maintenance and calibration procedures, the following was shared;

- Having a second person/staff to double-check those calibrations are correct
- It costs a lot of money. Staff time and instrument blocked off. No one wants to pay for this.
- downstream analysis tools
- Experience
- Done at preventive maintenance as part of service contract

Which of the following software tools for PSF measurement evaluation do you know and/or use?

a. MetroloJ (Cordelières & Matthews) 43% (6)
b. MIPs for PSFs (Gelman & Rietdorf) 7% (1)
c. PSFj (Knob Lab) 29% (4)

d. PSF calculator (Zeiss) 21% (3)
e. Huygens PSF Distiller (SVI) 43% (6)
f. PyCalibrate (PSFcheck) 14% (2)
g. ImageJ 7% (1)
h. PSF WIzard in ZEN Blue 7% (1)
i. Day book from argoligth, PyCalibrate 7% (1)