Metadata and Performance Tracking for Fluorescent Microscopes I - Metadata

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Microscopy images need to be accompanied by a description of the sample, its preparation and experimental layout as well as technical parameters under which images were taken. The term “metadata” is used to refer to such accompanying information, but the exact meaning of “metadata” frequently varies with context. A major challenge with metadata for technical parameters is the large variability of what is recorded by different microscopes. Metadata can be as simple as the pixel size or as complex as the results of an entire internal instrument calibration routine – and everything in between.

To enable full quantitative analysis – to extract the maximal information content of images – and to make images from different microscopes comparable, we propose 1) an OME based, extended metadata model to capture complete hardware and settings used for image acquisition, 2) an extension of metadata to contain optical calibration- and performance documentation and 3) a tier system for metadata requirements that scales the amount of metadata to be reported with the complexity of the imaging data.

Here we present the proposed metadata model and tier system [1] and a tool [2] to collect metadata in a tier dependent manner through a GUI that enables non-experts to be thorough and complete in their metadata documentation of hardware and setting used. This work is accompanied by a second contribution on optical calibration and a third contribution on performance calibration.

Category	ID	Name	Description	Example	Example Labelling	Optical calibration	Intensity calibration	Mechanical calibration
Descriptive	1	Documentation	Reporting qualitative effects without quantification	Transfection control, viability assay	FISH, Immuno Fluorescence	recommended annually (not required)	not recommended	not recommended
	2	Simple Quantification	Identification of non-refractive limited objects followed by basic feature extraction and statistical analysis	Counting of cells and nuclei, expression level measurements, study of cellular sub-compartments	FISH, Immuno Fluorescence	recommended quarterly (not required)	not recommended	not recommended
Analytical	3	Advanced Quantification	Identification and localization of refraction-limited particles, super-resolution microscopy	Diffraction-limited spot localization, measurement of distances, co-localization studies, signal-starved features, advanced processing	SM FISH, CasFISH, Proximity Ligation Assay (PLA), SM-FP	required quarterly	recommended	recommended
	4	Life Cells Imaging	Tracking of intracellular dynamics	Cell tracking, single particle tracking, dynamic expression level quantification	dCas9-based labelling, fluoresent protein labelling, SM-FP	required monthly	highly-recommended	highly-recommended
	5	Pioneer	Full reproducibility of microscopic set up and image acquisition	Development of novel unproved technology or of new gold-standard; full reproducibility ofmicroscopy set up and image acquisition settings	all of the above	required for every acquisition		

[1] https://github.com/WU-BIMAC/MicroscopyMetadata4DNGuidelines
[2] https://github.com/WU-BIMAC/4DNMicroscopyMetadataTool