Supplemental Table I: Extended view of the Tiers for the reporting of Light Microscopy Metadata as proposed by the Imaging Standards Working Group of the 4D Nucleome initiative and by the Quality Control and Data Management Working Group of Bioimaging North America.

Category	Tier Nr.	Name	Description	Example Experiment Type	Example Labelling	Experimental/ Sample	Microscope hardware specifications	Image acquisition settings	Optical calibration	Intensity calibration	Mechanical calibration
Descriptive	1	Minimum Information/ Qualitative or Basic Quantification/ Material & Methods	Reporting qualitative effects, or effects that require simple quantification including the identification of non-refractive limited objects followed by basic feature extraction and statistical analysis	Developmental and stem-biology experiments in which qualitative analysis of image data is used to support major findings, transfection control, viability assay, counting of cells and nuclei, expression level measurements, localization of markers in cellular sub-compartments	Histochemistry, Immuno-Histochemistry, Fluorescent In Situ Hybridization (FISH), Immuno Fluorescence (IF), Fluorescent Protein (FP) labelling	experimenter name; experiment description and date; sample description; mounting medium; temperature and CO2 conditions;	microscope manufacturer, model and type; light source manufacturer, wavelenght and type; objective manufacturer, magnification, NA and correction; filter/dichroic transmittance range; detector manufacturer and type	acquisition date; immersion liquid name and refractive index; illumination type and intensity; fluorophore; exposure time; pixel dwell time; channel name, color, contrast method and acquisition mode; image dimension order and number; physical pixel size x, y, and z	not required; recommended quarterly	not required; recommended annually	
Analytical	2	Advanced Quantification	Reporting quantitative effects that require advanced quantification including the localization of single molecules and tracking of intracellular dynamics	Diffraction-limited spot localization, measurement of distances, co-localization studies, detection of low-signal features, advanced processing, cell tracking and single-particle tracking, dynamic expression level quantification	All of the above + Single Molecule (SM) FISH, CasFISH, SM Proximity Ligation Assay (PLA), dCas9-based labelling, OligoPaint	O2 pressure, and humidity conditions; refractive index of the mounting medium; thickness of the coverglass	detailed environmental control device, microscope table, light source, light source coupling, transmittance light path, magnification, sample positioning, focusing, autofocus, filter, dichroic, additional optics and detector specification (e.g., lightsource spectral properties; objective correction properties; focusing device ZReproducibility, ZSettlingTime, ZResolution, etc.)	illumination attenuation; objective temperature and iris aperture; immersion liquid measured refractive index; sample positioning settings; detector integration; ligthpath configuration	required monthly	highly-recommended monthly to quarterly	
	3	Manufacturing/ Technical Development/ Full Documentation	Full documentation of microscopic setup, image acquisition and quality control	Microscopy hardware manufacturing; development of novel and yet to be validated technology in both commercial and academic settings; full reproducibility of microscopy set-up and image acquisition settings	All of the above	all the metadata specified by the data model - including any novel technology-specific metrics			required for every acquisition	required monthly to quarterly	

Legend: Each tier accommodates increasingly complex images, experiments, instrumentation, and analytical needs and therefore requires progressively more metadata. This tiered system is not intended to meet the needs of all imaging communities. Rather it is proposed as a framework that might need to be adapted and modified depending on the needs of individual data collection consortia, disciplines, or institutions.