

Supplemental Table I

Category	Tier Nr.	Name	Description	Example Experiment Type	Example Labelling	Experimental/ Sample	Microscope hardware specifications	Image acquisition settings	Quality Control
Descriptive	1	Minimum Information/ 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	Transfection control, viability assay, counting of cells and nuclei, expression level measurements, localization of markers in cellular sub-compartments	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, wavelength 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 or recommended quarterly to annually depending on the type of procedure
	2	Advanced Quantification and/or Live Cell Imaging	Identification and localization of refraction-limited particles, super-resolution microscopy, tracking of intracellular dynamics	Diffraction-limited spot localization, measurement of distances, co-localization studies, signal-starved 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; lighthpath configuration	Highly recommended or required monthly to quarterly depending on the type of procedure
Analytical	3	Manufacturing/ Technical Development/ Full Documentation	Full documentation of microscopic setup, image acquisition and quality control	Microscopy hardware manufacturing; development of novel unproven 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 guidelines! - including any novel and technology-specific metrics			Required for every acquisition, monthly or quarterly depending on the type of procedure