Minimum Information guidelines for fluorescence microscopy: increasing the value, quality, and fidelity of image data

Maximiliaan Huisman^{1*}, Mathias Hammer^{1*}, Alex Rigano², Farzin Farzam¹, Renu Gopinathan¹, Carlas Smith¹, David Grunwald^{1#}, and Caterina Strambio-De-Castillia^{2#}

¹RNA Therapeutics Institute and ²Program in Molecular Medicine UMass Medical School, Worcester MA 01605, USA.

*These two authors share first-authorship.

1 - ABSTRACT

High-resolution digital microscopy provides ever more powerful tools for probing the real-time dynamics of subcellular structures, and adequate record-keeping is necessary to evaluate results, share data, and allow experiments to be repeated. In addition to advances in microscopic techniques, post-acquisition procedures such as image-data processing and analysis (i.e., feature counting, distance measurements, intensity comparison and colocalization studies) are often required for the reproducible and quantitative interpretation of images. While these techniques increase the usefulness of microscopy data, the limits to which quantitative results may be interpreted are often poorly quantified and documented. Keeping notes on microscopy experiments and calibration procedures should be relatively unchallenging, as the microscope is a machine whose performance should be easy to assess. Nevertheless, to this date, no widely adopted 'data provenance' and quality control metadata guidelines to be recorded or published with imaging data exist. Metadata automatically recorded by microscopes from different companies vary widely and pose a substantial challenge for microscope users to create a good faith record of their work.

Similarly, the complexity and aim of experiments using microscopes vary, leading to different reporting and quality control requirements from the simple description of a sample to the need to document the complexities of sub-diffraction resolution imaging in living cells and beyond. To solve this problem, the 4DN Imaging Standards Working Group has put forth a tiered system of microscopy calibration and metadata standards for images obtained through fluorescence microscopy. The proposal is an extension of the OME data model and aims at increasing data fidelity, ease future analysis, and facilitate objective comparison of different datasets, experimental setups, and essays.

Keywords: Imaging, microscopy, fluorescence, metadata, calibration, standards, image-processing, super-resolution, instrumentation, data-formats

^{*}These two authors share senior-authorship.

2 - INTRODUCTION

The application of microscopy in biomedical research has come a long way since Antonie van Leeuwenhoek's discovery of unicellular organisms through his hand-crafted microscope. Countless innovations have propelled imaging techniques and have positioned fluorescence microscopy as a cornerstone of modern biology and as a method of choice for connecting "omics" datasets to their biological and clinical correlates. Still, regardless of how convincing imaging results look, they do not always convey meaningful information about the conditions in which they were acquired, processed and analyzed to achieve the presented results. Adequate record-keeping and quality control are therefore essential to ensure experimental rigor and data fidelity, to allow experiments to be reproducibly repeated and to promote the proper evaluation, interpretation, comparison and re-use of the results. Microscopy images must be accompanied by complete descriptions of experimental procedures, biological samples, microscope hardware specifications, image acquisition parameters, and metrics detailing instrument performance and calibration. Despite considerable effort (Goldberg et al., 2005; Linkert et al., 2010), however, universal data standards and reporting guidelines for the fair (Wilkinson et al., 2016) assessment and comparison of microscopy data have not been established. To understand this discrepancy and propose a way forward, we examine the benefits, pitfalls, and limitations of shared standards for fluorescence microscopy.

2.1 The rise of fluorescence microscopy

Long since the invention of microscopes, the capture and analysis of microscopy images had been severely limited by the ability to record and measure light. While the meticulous drawings of early microscopists revealed an unimagined realm ripe for discovery, they would nowadays be regarded as illustrations rather than scientific data. The introduction of photography and fluorescence drastically improved the objectivity of observations made through microscopy, but it was not until the invention of computers and digitized light detectors that another revolution would unfold in this field.

The invention of digital photodetectors changed fluorescence microscopy in three profound ways. First, it has allowed the increasingly accurate recording of progressively lower amounts of light, enabling the quantification of emitters that would be orders of magnitude too dim to be even distinguished by eye. Combined with major advances in genetic and biochemical labeling strategies, this increased sensitivity means that we can now view many sub-cellular events—often at the single-molecule (SM) level. Second, the advent of digital image formation and processing has enabled new imaging modalities, such as Laser Scanning Confocal Microscopy (LSCM), Structured Illumination Microscopy (SIM), and super-resolution (SR) imaging techniques that allow high-resolution imaging of live samples in three dimensions. Third, digital imaging has led to signal processing and computational methods that allow the extraction of quantitative information from images, including feature recognition and counting, motion detection, distance measurements, and intensity comparison. As a result of these advances in imaging, fluorescence microscopy has emerged as a key quantitation tool for biomedical research, as it allows the visualization and quantitative measurement of molecular interactions at the level of single cells—sometimes even single molecules—with high temporal resolution and specificity.

2.2 The metadata challenge in microscopy imaging: the great variability of data formats and metadata reporting practices

Notwithstanding the technological advances, the proliferation of imaging techniques has created a new challenge. Namely. the field of fluorescence microscopy lacks universally accepted standards for imaging data and guidelines for metadata—i.e., any and all information about an imaging experiment that allows its evaluation, interpretation, and comparison and that is generally recorded in the image data file header or in supplemental files. Much of the problem is an information or record-keeping one. All the procedures required to carry out each stage of a typical fluorescence microscopy experiment can add variability (Figure 1). Thus, to document possible sources of uncertainty, allow comparison and foster reproducibility, microscopy images need to be accompanied by complete descriptions of experimental procedures, biological samples, microscope hardware specifications, image acquisition parameters, and instrument performance and calibration metrics (i.e., quality control). Among all experimental steps described in Figure 1, image formation perhaps contributes the most quantifiable and manageable uncertainty, so long as the microscope and imaging system are properly maintained and operated. Standardized calibration and documentation are therefore essential for imaging data quality, accessibility, and utility. Keeping notes on microscopy experiments should be relatively straightforward, but microscopists trying to create a good-faith record of their work may encounter any of the following challenges:

- 1. **Biological questions being addressed by microscopy are significantly diverse and complex.** This has led to the development of multiple microscopy modalities, each of which can be implemented in manifold ways. Because each technique requires a different reporting and quality control approach, this situation poses a notable challenge when it comes to choosing the correct validation method for the experiment at hand.
- 2. The working conditions, theoretical performance, and capabilities of the microscope are often unknown and hard to assess by the average user.
- 3. The **relevant hardware or software parameters are difficult to retrieve** from available documentation and the user is not aware of how such variables might affect imaging results.
- 4. Lack of automation and of intuitive software tools make recordkeeping unduly burdensome, forcing experimental biologists to choose between scientific rigor and productivity.
- 5. Appropriate recordkeeping is hampered by a **lack of generally accepted microscopy file format standards and metadata documentation guidelines**. This in turn results in a huge variability in the information content (i.e., different parameters, different terms, different data structure) microscope manufacturers provide alongside images and in the need for data-files to be converted before they can be meaningfully compared. This often yields to a significant loss of metadata and in some cases, the image data itself may be inadvertently compromised in the conversion process.

2.3 The importance of standardization

While the need for rigorous standards to document microscope hardware, image acquisition settings and instrument performance to ensure scientific rigor and reproducibility has been widely recognized (Hoffmann et al., 2008; Hng and Dormann, 2013; Resch-Genger et al., 2005; Hoffmann et al., 2005; Stack et al., 2011), commonly utilized guidelines have not yet emerged. The Open Microscopy Environment (OME) community (Swedlow et al., 2003) has pioneered efforts to unify the bio-imaging field with the development of the OME data model, which includes the OME-TIFF file format and the OME-XML metadata model to capture imaging metadata(Goldberg et al., 2005). The BioFormats library produced by OME has substantially eased import and conversion of disparate file formats, significantly improving interoperability across different microscopes and acquisition platforms(Linkert et al., 2010). Articles that discuss best performance testing and instrument calibration practices (North 2006; Demmerle et al. 2017; Murray et al. 2007; Waters 2009; Lambert and Waters 2014; Petrak and Waters 2014; Jost and Waters 2019; Deagle et al. 2017; Mubaid et al. 2019; Theer et al. 2014; Ferreira et al. 2014; Hng and Dormann 2013) and data-submission requirements from journals (Hill, 2008) also offer some guidance. Nevertheless, taken together these criteria lack normative value, are often cryptic, incomplete, and out-of-date—especially with respect to state-of-the-art technologies. As a consequence, it remains quite challenging to figure out which parameters are relevant to the imaging experiment at hand and best practice guidelines are frequently ignored. The reason for this state of affairs is quite understandable: even when agreed upon in principle, the community may perceive standardization as overly complicated, expensive, cumbersome, or intrusive. Even if like-minded stakeholders are unified in their vision that a standard must be set, they must bridge differences in opinion to make it happen—difficult for any field, let alone one that has been developing for centuries in a mostly unregulated fashion. Despite these difficulties, much would be gained from establishing shared fluorescence microscopy standards. First,

despite these difficulties, much would be gained from establishing shared hidrescence microscopy standards. First, addressing these difficulties would allow to document each step of the imaging protocol, to minimize error, and to quantify residual uncertainty associated with each step of the procedure. This, in turn, would provide a wealth of valuable information -- typically referred to, respectively, as data provenance(Ram and Liu, 2008, 2012)(Ram and Liu, 2008, 2012) -- increasing the value of microscopy results. Namely, such details would make it possible to reliably evaluate scientific claims based on imaging data, facilitate comparisons within and between experiments, foster reproducibility, and maximize the likelihood that data can be analyzed by other scientists using current and future image processing and analysis methods. Furthermore, as publishing raw imaging data associated with scientific manuscripts becomes more common (i.e, making fluorescence microscopy a modern data-driven discipline akin to structural biology, genomics, and transcriptomics), and as public image repositories and resources come on line—e.g., Image Data Resource(Williams et al., 2017), Allen Cell Explorer(Allen Institute for Cell Science, 2019), Cell Image Library(Center for Research in Biological Systems, 2019), JCB DataViewer(Hill, 2008), Movincell(Observatoire Océanologique de Villefranche Sur Mer, 2015), and BioImage Archive(Ellenberg et al., 2018)—the need for shared documentation and quality control standards for imaging data will only continue to grow.

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3.1 Different categories of metadata

Metadata is data that provides information about other data. In addition to basic metadata such as the name and date of a file, rich metadata consists of any and all additional information that allows data to be found, evaluated, cited and re-used by third party users and (ideally) by automatic means. In general, rich metadata adds value to the data by improving the degree to which its fidelity can be measured and its ability to meet quality criteria (i.e., FAIR principles) (Wilkinson et al., 2016), can be assessed. Many distinct categories of metadata exist. Basic metadata categories include descriptive metadata (i.e., file name, description, and author); structural metadata (i.e., metadata referring to the image structure); and administrative metadata (i.e., data owner, time of creation). Rich metadata categories are more variable. For the purpose of this discussion, we distinguish two main categories: 'data provenance' metadata (DPM) and quality control metadata (QCM). In general, the combination of all types of metadata makes data analysis and the development of analysis tools more cost-efficient and less time-consuming compared to using lower quality datasets.

3.1.1 Data Provenance Metadata

'Data provenance' also referred to as data "lineage" or data "pedigree", is an overloaded phrase with multiple definitions (Ram and Liu, 2008). In essence, 'data provenance' consists of an electronic version of the scientific record that is routinely maintained any time experiments are performed to document the steps that went into the creation of the results (i.e., experimental setup; sample preparation; instrument characteristics and settings; analysis algorithms and parameters). Hence, data provenance extends the basic scientific requirement of thorough experimental documentation to the domain of "big-data". Because the sheer amount of data makes manual annotation infeasible, data provenance increasingly relies on computational processes that are obscure to the experimenter (i.e., data formatting; data processing steps; algorithms source code). In this context, steps must be taken to ensure that all information pertaining to the origin of every relevant data product is logged, recorded, and maintained to foster the reproducibility and validity of the data, and to support the evaluation, selection, integration, re-use, and meta-analysis of data.

In order to formally define data provenance, Ram and Liu proposed an ontological model they called W7 (Figure 2), which might be used to track events that affect data during its lifetime (Ram and Liu, 2009). The W7 model defines provenance as the ensemble of metadata that facilitate scientific reproducibility, repeatability, validation, and interpretation by: (1) describing "what" happened to each data element (i.e. data manipulation events such as acquisition, processing, and analysis); and (2) providing answers to six interconnected "who", "how", "when", "where", "which", and "why" questions describing each data manipulation event. Following the W7 model, there are four aspects of data provenance that are generally considered:

- 1. Tracing the origin of each piece of raw, intermediate, and final data.
- 2. Recording information about scientific experiments and sample preparation.
- 3. Keeping track of data processing, analysis, and visualization.
- 4. Annotating data with 1, 2 & 3.

The types of DPM that need to be recorded in a typical fluorescence microscopy experiment (Figure 1) are listed in Table I.

3.1.2 Quality Control Metadata

A fluorescence microscopy image is "a digital representation of an optical image of the distribution of fluorophores that were introduced in the specimen" (Jost and Waters, 2019). As such, the process of image formation can be subdivided into multiple steps each of which is a potential source of error. Each image formation step must, therefore, be documented, and its impact on error propagation must be quantified to determine how well the resulting image accurately represents the biological phenomenon being studied. We will define quality control metadata (QCM) as all metadata that adds rigor and fidelity to the data. QCM typically comprises metrics related to the quantitation and calibration of instrument performance and to the estimation of error propagation (see Table II). Together, DPM and QCM maximize the repeatability, reproducibility, accuracy, and precision of results, establish whether the conclusions are warranted, and enable comparisons both within and between datasets.

Table I: Categories of data provenance metadata typically associated with microscopy experiments.

Category	Description	Example	Collection method	Standards
Experimental	This entails metadata that captures any and all information that would be routinely recorded on a lab notebook to document the experimental setup	Tissue culture conditions; Number of conditions; the number of biological replicates	Mostly manual data entry	Domain-specific. Example: MISFISHIE; CSMD; FBbi*
Sample preparation	This entails metadata that captures any and all information that would be routinely recorded on a lab notebook to document the sample preparation protocol	Fixation conditions; staining conditions; mounting conditions;	Mostly manual data entry	Domain-specific. Example: BRISQ; FBbi**
Image acquisition (microscopy)	This metadata describes how the image was acquired.	Microscope specification; Imaging settings	Should be easy to automatically capture, log and maintain during	OME [#]
Image data structure	This metadata captures the structure of the image data file	Number of focal planes; the number of channels; the number of time points; dimension order; pixel size	acquisition; varies significantly based on microscope manufacturer and image file format	
Data analysis	This metadata describes all steps of the data processing, analysis, and visualization workflow	Algorithm name; algorithm version; algorithm parameters	Should be easy to automatically capture, log and maintain during analysis; mostly not done	. Example: MIAPTE##
Data dissemination	This metadata describes the information that is required for publication and data dissemination	Submission title; submission identification; submission description	Easy to automatically capture	Example: SampleTab [^]

Legend: * (ICA Project, 2019; MISFISHIE Standard Working Group; FlyBase, 2019; Stoeckert et al., 2007; Smith et al., 2007; Omenn, 2014); ** (Equator Network, 2019; FlyBase, 2019); # (Goldberg et al., 2005; Allan et al., 2012); ## (Rigano and Strambio-De-Castillia, 2017, 2016); ^ (European Bioinformatics Institute (EBI), 2019)

3.2 How can metadata be represented?

Metadata can be easily represented as lists of key-value pairs, where the first term is a descriptive term for a specific attribute and the second term is the value of the attribute, including units for numerical values. Another important aspect to be considered in defining metadata guidelines is the need to develop a model for the data that provides an abstract representation of the real-world system to be described. Ideally, such a data model accounts for the components of the system, the attributes that need to be recorded for each component, and the relationship between components. One useful formalism for developing and describing an appropriate data model is represented by the Entity-Relationship (ER)

diagram (Chen, 1976). In addition, the Extensible Markup Language (XML) Schema Definition (XSD) language is the method sanctioned by the World Wide Web Consortium (W3C; https://www.w3.org/) to represent a model schema in a machine-readable manner.

3.3 Describing the origin of the data and how it was produced

3.3.2 Describing the instrument

When dealing with fluorescence microscopy, an intimate knowledge of the specific microscopic hardware used for the experiment may be just as important as a detailed documentation of the experimental context and of the sample under study. While the choice of instrumentation could be considered an experimental choice or variable like any other, factors like the availability of certain hardware, the compatibility of components, and the overall state of the equipment play an important role and often limit the options available during experimental design. Either way, the following categories of information about the microscope are important to consider as they clearly impact the results:

- Microscope hardware specifications As imaging systems become more complex, optical, electronic, and mechanical design increasingly affects imaging interpretation. Information about the detector is often the most relevant, as it offers clues about image quality and which quantitative analysis strategies should be used. Similarly, knowledge about the light source(s), its power, and the path taken by light on its way to the sample can help identify effects like bleed-through between color channels, high background fluorescence, and non-linear photo-physical effects. It is therefore imperative to detail the physical characteristics of the microscope and of all other components that were utilized during the image formation and acquisition process (e.g., microscope manufacturer and model number, objective nominal magnification and numerical aperture, filter excitation and emission wavelength, etc.), as well as the software and version used to drive image acquisition.
- Microscope configuration: Microscopes are often upgraded and can be configured in various ways. The choice of optical components (e.g., objective lenses, emission filters, and dichroic mirrors) can dramatically affect the acquired images, especially if they are not ideally suited for the experiment. Similarly, the invocation of options like scaling adapters, optical relays, or mechanical stabilizers (e.g., drift-correction and autofocus) can affect data interpretation by altering microscope performance.

3.3.1 Describing the imaging experiment

When designing and executing biomedical imaging experiments, experimenters make a lot of decisions, from the fluorescent tag that labels a molecule of interest to the exposure time of the camera. The value assigned to each parameter is relevant to most if not all fluorescence microscopy experiments and can vary greatly between studies. Acknowledging that there is no exhaustive list of potential metadata entries, the following are prime categories:

- **Administrative information:** As with any experimental procedure, it is invariably essential to keep a persistent record of when, where, and by whom were the images acquired.
- **Minimal sample information:** Some details about sample preparation do not need to be included as metadata for the image. However, key details required for interpreting the results and for assessing the suitability of the sample for the imaging technique include the staining or fluorescent labeling technique, the fluorophores used for visualization, and any special sample preparation, fixation, or mounting methods.
- Imaging technique: Each imaging technique comes with particularities, which are often relevant to the interpretation of the resulting image. Imaging modalities like SIM or stochastic SR techniques -- like *Photoactivated Localization Microscopy* (PALM), *Stochastic Optical Reconstruction Microscopy* (STORM) or *Super-resolution Optical Fluctuation Imaging* (SOFI) and their derivatives -- can produce artifacts. Power-intensive techniques -- like *Stimulated emission depletion* (STED) and *REversible Saturable Optical Fluorescence Transitions* (RESOLOFT) -- pose a high risk of biological impact on the sample through photo-toxicity. By keeping the pros and cons of the imaging method in mind, the results can be interpreted more confidently.
- Sample illumination: Fluorescent molecules can only be efficiently excited within a certain wavelength range. The brightness of the fluorescent signal is proportional to the excitation power, but so are the photo-bleaching rate and the potential of light-induced damage to the cell. Under these conditions, knowledge of the excitation wavelength and power are crucial for observing the intended photo-physical effects and for establishing with some confidence that the

observed phenomenon is not an artifact of either the staining or the excitation conditions.

- Image acquisition settings: Microscope settings such as the magnification, or the Numerical Aperture (NA) of the objective, are essential information for almost any piece of microscopy data, as they give a sense of the scale and resolution of the image. More complex experiments (e.g., three-dimensional imaging, time-lapse acquisition, low-signal conditions) will require additional parameters (e.g., optical sectioning distance, frame rate, and exposure time) be reported. In general, more sophisticated systems also require more parameters to be set and reported. For example, if using a highly sensitive Electron-Multiplying Charge-Coupled Device (EMCCD) camera, the EM-gain and sensor temperature must be recorded in addition to the exposure time and pixel size.
- Image-structure information The data structure of a digital image is often multidimensional and quite complex. The basic digital image structure consists of a plane composed of a two-dimensional matrix of pixels (i.e., X and Y dimensions). In addition, most modern microscopy images include frames obtained under different illumination conditions (i.e., color-channels; C dimension). Finally, the acquisition of images including multiple focal planes (i.e., Z dimension) and time-frames (i.e., T dimension) is quite commonplace. Given this variability, information indicating how the digital structure of the images should be interpreted (e.g., pixel size, number of dimensions utilized, which dimensions constitute different focal planes, color-channels, etc.) is crucial.

3.4 Measuring instrument performance, estimating error and reporting quality-control metrics

In a perfect world, every measurement obtained using a given microscope would reflect its stated capabilities, would minimize the amount of bias and imprecision attributable to the instrument, and would be readily interpretable. In reality, microscopes are complex assemblies that require frequent tuning and calibration, careful handling, and well-trained users that are informed on the limits to which they should interpret the data obtained. Even well-maintained microscopes may have multiple users performing different kinds of experiments that may require settings to be altered or filters and optics to be exchanged, which can cause accidental dealignment or soiling. To make matters worse, subtle—even imperceptible—performance defects can dramatically affect imaging results and therefore the interpretation of an experiment. Whereas obvious artifacts may readily raise alarm, subtle defects will only be detected by careful measurement. This is especially true for data that requires quantitative analysis and/or pushes the limit of the specified technical capabilities of the microscope. The closer an experiment gets to the microscopes full capabilities, the greater the repercussions of sub-optimal calibration can become—and the harder it becomes to assess from the images alone whether the required resolution, stability, registration, et cetera, are indeed achieved. To facilitate the repeatability, reproducibility, and comparison of experimental results arising from imaging experiments—even long after images are acquired—image datasets must be annotated with detailed, quantitative accounts of the microscope performance as measured at the time of acquisition.

3.4.1 Measuring instrument performance and calibration

Given that otherwise identical microscopes can perform quite differently, standardized performance evaluation should be conducted a priori to prevent expensive and time-consuming do-overs. At a minimum, such knowledge should be obtained a posteriori as it can help determine the extent to which the data may be interpreted. Either way, the categories of metrics about the microscope performance listed in Table II and discussed below are important to consider as they clearly impact the results:

• Quality control metrics Various metrics (North, 2006; Demmerle et al., 2017; Murray et al., 2007; Waters, 2009; Lambert and Waters, 2014; Petrak and Waters, 2014; Jost and Waters, 2019; Deagle et al., 2017; Mubaid et al., 2019; Theer et al., 2014; Ferreira et al., 2014) can be used to measure microscope performance depending on the type of experiment and the available tools. Together, these measurements increase the depth and reliability of a variety of assessments, analyses, and comparisons performed on fluorescence microscopy images. Such calibration metrics can be generally subdivided into five categories: (1) optical; (2) field; (3) detector; (4) excitation; and (5) mechanical (Table II). Metrics in the first four categories evaluate the great majority of fluorescence data. Mechanical calibration metrics become most useful in experiments that involve time-lapse imaging or the tiling of multiple Fields Of View (FOV).

Category	Metric	Description	
Optical	lateral resolution	Spatial resolving power achieved in x and y	
Optical	axial resolution	Spatial resolving power achieved in z	
Optical	planarity	Deformations of the focal plane across the Field of View (FOV), due to misalignment/malfunction	
Optical	asymmetry	Deviation from axial symmetry of the PSF as observed across the FOV	
Optical	lateral chromatic shift	Offset between color channels (and variance) in x and y	
Optical	axial chromatic shift	Offset between color channels (and variance) in z	
Detector	detector dark value	The average intensity in the absence of incoming light	
Detector	detection limit	Minimum detectable signal	
Detector	linear range	Intensity range of linear response	
Detector	photometric gain	The conversion factor between arbitrary units (a.u.) of intensity and photons	
Detector	noise character	Noise type and variance	
Excitation	excitation power	Irradiance at the sample plane	
Excitation	excitation wavelength	The center wavelength of the irradiance	
Excitation	excitation variance	Irradiance variation over the FOV	
Field	field flatness	Response variations over the FOV, due to dissimilarities in detector sensitivity and distortions in the optical path.	
Mechanical	lateral drift	Change in (x, y) position over time	
Mechanical	focal drift	Change in z position over time	
Mechanical	position repeatability	Precision in returning to a previously visited position (useful for tiling).	
Mechanical	settling time	The time it takes to stabilize a target position (useful for fast three-dimensional acquisitions)	

3.4.2 How to obtain standardized metrics for instrument calibration

Intuitive, repeatable, and inexpensive routines are essential to standardize the calibration of fluorescence microscopes. Different calibration metrics (Table II) require different measurement procedures, and have unique advantages and disadvantages (Theer et al., 2014; Ferreira et al., 2014; Hoffmann et al., 2008; Resch-Genger et al., 2005; Nieuwenhuizen et al., 2013; Hoffmann et al., 2005). Moreover, most calibration methods require costly equipment (e.g., reference slides, power meters, calibrated light-sources) and entail levels of skill and time commitment that cannot be reasonably expected from most microscope users or even facility managers. To bridge this gap, the 4DN-OME metadata model proposes a standard for QCM entries, accompanied by simple and inexpensive calibration procedures to obtain them. Integral to this effort, a custom-made tool called Meta-Max was developed to standardize detector and excitation calibration—particularly useful when dealing with image data that has a low Signal-to-Noise Ratio (SNR) and is subject to statistics-based image analysis. These methods and tools will be described in a series of parallel manuscripts [REF].

3.5 Ideal properties of microscopy metadata standard guidelines

The absence of a universally accepted data standard precludes the full realization of microscopy's potential to become a reliable source of unbiased and reproducible quantitative data. We propose that a realistic and attainable data standard should strive towards ideal criteria (listed below), which comply with the emerging Minimum Information (Taylor et al., 2008) and Findable Accessible Interoperable and Reusable (FAIR; (Wilkinson et al., 2016) principles for data stewardship:

- 1. **Metadata and data should be easy to comprehend and find by experimental scientist** The terms utilized by the standard needs to be self-explanatory, domain-relevant, broadly applicable, generally understood by the community, and easy for end-users to interpret.
- 2. **Metadata and data should be easy for machines to access and interpret** Machine-readable metadata are essential to maximize automation thus minimizing the dataset annotation overhead for experimental scientists, minimize redundancy, and facilitate the datasets through searchable resources. For this purpose, datasets should be unequivocally identifiable and metadata should contain explicit links to the data they describe.
- 3. **Formal data model** Metadata should be structured in a formal data model describing the real-life components that need to be documented, the relationship between components and the attributes that need to be recorded to sufficiently describe each component.
- **4. Reusability** To optimize the reuse of data, metadata and data should be well-described so that they can be replicated, compared and combined with data obtained by others. The standard should clearly stipulate a <u>rich plurality of relevant metadata parameters</u> to describe the data, specify acceptable units of measure, and guarantee easy access and navigation.
- 5. Interoperability Data and metadata should be easy to integrate with other data and should be readily accessible with available applications or workflows for analysis, storage, and processing. For this purpose, the standard should include strong links that connect data and metadata to other relevant data and metadata.
- **6.** Tracing the origin of data and all intermediate steps The standard should stipulate that all necessary information to trace the 'provenance' (i.e., origin, source) of data (i.e., such as what microscope and what imaging settings used to acquire the image, and which software was used to analyze the image data) should be documented in detail.
- 7. Maximal usability and minimal annotation burden
 - **a. Minimal information** The standard should require <u>only</u> those parameters that can be reasonably expected to influence the outcome of the imaging experiment (i.e., imaging settings as well as information about the hardware and its performance);
 - **b.** Adaptable system of guidelines Recognizing that different experiments often have different annotation requirements, the standard should follow an adaptable system of norms that scales with experimental and technological complexity and that clearly specifies which data provenance, and quality control metadata should be provided in each case thus minimizing the documentation burden on the experimenter.
- **8. Standardized instrument calibration and error estimation** The standard should provide clear norms about recommended quality control procedures and about the frequency with which each should be performed to ensure experimental rigor and data fidelity.

We acknowledge that a perfect standard may never exist. We are nonetheless convinced that a clear system with sensible compromises would go a long way toward getting the most out of fluorescence microscopy data and would help the field mature in ways that benefit the entire life sciences community. In the next section, we propose a set of imaging guidelines that have emerged from work conducted by the Imaging Standard Working Group (IWG) of the National Institutes of Health (NIH) Common Fund's 4D Nucleome (4DN) Consortium (Dekker et al., 2017).

4 - THE OME-4DN MICROSCOPY METADATA GUIDELINES PROPOSAL

4.1 A tier-based system of guidelines for fluorescence microscopy metadata collection

While a one-size-fits-all solution for microscopy metadata requirements might be unreasonable, it is worth noting that microscopy documentation appears to follow a cumulative pattern, whereby increasingly elaborate imaging experiments require additional metadata on top of those required for more basic experiments. On this account, a graded system for metadata requirements would not only be appropriate, but it also would minimize the burden of collecting metadata for each experiment while maximizing the opportunities for reproducibility, evaluation, analysis, and comparison.

Table III: Tiers for fluorescence microscopy metadata collection proposed by the Imaging Standards Working Group of the 4D Nucleome initiative. Each tier accommodates an increasingly complex group of experiments and therefore requires progressively more metadata

Category	Nr.	Name	Description	Example
Descriptive	1	Documentation	Reporting qualitative effects without quantification	Transfection control, viability assay
	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, the study of cellular sub-compartments
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
	4	Live Cells Imaging	Tracking of intracellular dynamics	Cell tracking, single-particle tracking, dynamic expression level quantification
	5	Technical Development	Full reproducibility of microscopic setup and image acquisition	Development of novel unproven technology or of new gold-standard; full reproducibility of microscopy set-up and image acquisition settings
Legend:				

We, therefore, propose a flexible system in which different imaging communities (i.e., consortia, individual research institutions, individual fields of knowledge) would a priori define sets of criteria whereby microscope hardware and imaging experiments are classified on the basis of their complexity, imaging modality, and analytical requirements. Along these lines, the tiered system of guidelines put forth by the IWG of the 4DN consortium (Table III) and delineated below is not intended to meet the needs of all imaging communities. Rather, this set of tiers should be considered as an example of how different imaging experiment types could be placed on a complexity scale to facilitate the collection of the most appropriate minimum set of metadata required for reproducibility and comparison of each category. We therefore expect that the tier system and accompanying metadata model proposed here will invariably be customized by other communities to meet their individual needs. Specifically, the definitions of individual tiers might need to be adapted as well as individual metadata fields might need to be modified.

4.2 The 4DN tiered microscopy guidelines proposal

A robust, maximally useful, and efficient metadata standard would be tailored around the different reporting requirements of experiments of increasing complexity. We, therefore, propose a five-tier system (Table III) in which imaging experiments are classified on the basis of three sets of criteria:

- 1. Do meaningful results require quantitative image analysis?
- 2. Are the samples fixed or alive?
- 3. Are the microscope and the acquisition modality being used readily available or does it require custom development?

Consistent with minimum information principles, the system should not be considered exhaustive, rather its purpose is to represent a minimal set of metadata required for each tier—especially for complex experiments. Moreover, the scope of the guidelines is circumscribed exclusively to the information relevant for the interpretation of the imaging experiment itself. That is, while the proposed standard encompasses information about the sample and its preparation that directly impacts the imaging conditions (e.g., cell type, labeling method, embedding medium), it is not intended to replace the description of experimental protocols or other more appropriate experimental ontologies.

On the basis of these criteria we propose two Descriptive (Tiers 1 and 2) and three Analytical (Tiers 3-5) tiers.

Table IV: Suggested minimum (i.e., Tier 1) experimental description requirements (Tier 1) for any fluorescence microscopy experiment that meets this data-standard.

Element	Attribute	Description	
Experiment	ID	A unique identifier for this experiment.	
	Name	A user assigned name for this Experiment	
	Labelling Method	The method used for the molecular component under study in this experiment	
	Experimenter	The scientist that performed this experiment.	
	Description	A description of the imaging experiment.	
Sample	ID	A unique identifier for this sample.	
	Name	A user assigned name for this sample	
	Description	A description of the sample that was imaged in this case.	
Legend:			

4.2.1 Descriptive metadata tiers

Experiments that fall into descriptive tiers (Tiers 1 and 2) produce images that can be interpreted with confidence in a visual manner. This criterion excludes experiments in which images are acquired under signal-limited conditions (i.e., SM detection, single-particle tracking - SPT), experiments that require the detection of diffraction-limited features (i.e., SR methods), or experiments in which conclusions require post-acquisition reconstitution (i.e., deconvolution). For an experiment to be considered descriptive, meaningful conclusions need to be justified on the basis of the qualitative evaluation or the simple processing and quantitative analysis (e.g., segmentation) of data. Thus, descriptive tiers do not require metadata that describe advanced hardware features of the microscope or that quantify microscope performance. Whereas, Tier 1 metadata describe qualitative image data, Tier 2 metadata is suitable for basic quantification of image features (e.g., counting objects, and intensity, shape or size estimations).

Table V: Suggested minimum (i.e., Tier 1) microscope specifications requirements for any fluorescence microscopy experiment that meets this data-standard.

Element	Attribute	Description	Example values
Microscope	ID	A unique identifier for this component.	
	Name	A user-defined name for this component.	
	Manufacturer	The manufacturer of this component.	Nikon, Leica, etc.
	Туре	The type of this microscope.	Upright, Inverted, Dissection, Stereomicroscope, Electrophysiology, Custom
Light source	Id	A unique identifier for this component.	
	Name	A user-defined name for this component.	488 Laser
	Manufacturer	The manufacturer of this component.	
	Type	The type of LightSource used in this case.	Arc, Filament, Laser, Light Emitting Diode, Generic
Optovar	ID	A unique identifier for this component.	
	Name	A user-defined name for this component.	Nikon optovar
	Manufacturer	The manufacturer of this component.	Nikon, Leica, etc.
	Magnification	The magnification of the optovar lens as specified by the manufacturer.	1.60
Objective	ID	A unique identifier for this component.	
	Name	A user-defined name for this component.	Nikon 100x
	Manufacturer	The manufacturer of this component.	Nikon, Leica, Other
	Nominal Magnification	The magnification of the lens as specified by the manufacturer - i.e. '60' is a 60X lens	100
	LensNA	The numerical aperture of the lens expressed as a floating-point (real) number. Expected range 0.02 - 1.5.	1.40

4.2.1.1 Tier 1: Documentation

Tier 1 experiments only require the qualitative documentation of raw images for meaningful conclusions to be drawn and do not require any further processing or analysis. Tier 1 images are therefore often part of control experiments or serve as minor supporting evidence in a project or manuscript. Typical examples of Tier 1 experiments are transfection controls or viability tests. Tier 1 constitutes the minimum information required for fluorescence microscopy-based experiments to meet the proposed standard. Any data that might be shared or published should, at the very least, contain the metadata stipulated in this tier (Figure 3 and Tables IV, V, and VI). Metadata appropriate for this tier includes parameters that are

generally reported in journals. As such, Tier 1 metadata alone could save considerable effort in looking for bits of information scattered across different data-files, hardware setups, and lab notebooks, or searching the various sections of publications for information that may or may not be included. Example metadata values that would be used to describe a Tier 1 experiment are presented in Table V.

4.2.1.2 Tier 2: Simple quantification

Applications that require simple processing and analyses to support conclusions fall into this tier. Thus, Tier 2 includes studies that require the identification, counting, intensity and morphometric analyses of features whose signal is above the limit of detection and whose size is above the limit of resolution of the system. Examples include cell-counting, the measurement of reporter intensity, the estimation of the size and shape of individual cells. Supplemental Figure 1 describes metadata fields that must be included in addition to Tier 1 metadata in order to meet Tier 2 criteria.

4.2.2 Analytical metadata tiers

Advances in technology have brought tremendous analysis capabilities to the field of imaging. While some imaging techniques require digital image-processing for the very construction of the images (e.g., LSCM, SIM and stochastic SR methods like PALM and STORM), other methods produce images that need some kind of processing or quantification to be reliably interpreted (e.g., SPT or SM fluorescence in situ hybridization (smFISH) experiments). Smart image-processing and -analysis strategies can extract a wealth of data that cannot be readily or unequivocally discerned in the image. On top of that, model-driven data-processing can enhance the resolution of the data—e.g., through deconvolution, exhaustive photo reconstitution (EPR (Carrington et al., 1995; Carrington and Fogarty, 1987) or Super-resolution Optical Fluctuation Imaging (SOFI)—and help combat issues like unspecific background, limited signal, and optical aberrations. These techniques generally increase the usefulness of microscopy data, but require additional information. Moreover, the data may need to meet certain conditions for the analysis to be reliable; to assess the limits to which the results can be interpreted, it is highly desirable—if not essential—to have metadata that proves that the analysis was justified.

As some analyses are more demanding than others, we submit three Analytical tiers (Tier 3-5) for fluorescence microscopy data. Tier 3 and 4 involve the use of well-established microscope instrumentations and analysis and processing procedures that have been shown to work and have been quantified in a range of conditions. Tier 5 is reserved for the development of pioneering technologies whose reproducibility requires exhaustive details.

Admittedly, it is difficult to draw a hard distinction between Tiers 3 and 4. Nevertheless, Tier 3 generally involves SR images, diffraction-limited, or signal-starved imaging in fixed cells, and Tier 4 encompasses live-cell time-lapse experiments. In both tiers, the optical system is assumed to be performing at its theoretical best; regular characterization of the performance of the optical system is therefore highly recommended. Compliance with Tiers 3 and 4 will help ensure that data is reliable, comparable, and reproducible and maximize the possibility that data can be reanalyzed using future methods, which would allow new information and insights to be obtained from existing image data.

4.2.2.1 Tier 3: Advanced quantification

Tier 3 covers all fluorescence microscopy experiments that utilize fixed samples and that aim to draw conclusions about features that are near or below the detection and the diffraction limits. As such, these applications often rely on advanced processing and quantitative analysis.

Typical examples include SR methods, smFISH experiments, high SNR Single-Molecule Location Microscopy (SMLM) and distance/distribution measurements that aim to achieve precision near or below the diffraction-limited resolution of the optical system. As sophisticated image-processing techniques often take into account the photophysical behaviors of the fluorophores and of the detector, optical and intensity calibration are an essential requirement for Tier 3 experiments. Metadata entries shown in Supplemental Figure 2 are required, as they allow quantitative analysis and help ensure that the results are repeatable and comparable between datasets.

4.2.2.2 Tier 4: Live cell imaging

Tier 4 represents the most demanding requirement for data obtained using established methods. Similar to Tier 3, data that belong to this category is expected to be diffraction-limited and destined for advanced processing and analyses. Besides, Tier 4 experiments also require detailed information about the environmental conditions in which the sample is kept during imaging, phototoxicity, and focal stability. Typical examples include applications that are designed to follow the

Table VI: Suggested minimum (i.e., Tier 1) image acquisition settings requirements for any fluorescence microscopy experiment that meets this data-standard.

Element	Attribute	Description	
Image	ID	A unique identifier for this Image.	
	Name	A user assigned name for this Image.	
	Acquisition Date	The acquisition date of the Image.	
	SizeX	X size of the pixel data array.	
	SizeY	Y size of the pixel data array.	
	SizeZ	Number of Z-planes.	
	SizeC	Number of Channels used in this Image.	
	Physical Size X	The physical width of a pixel.	
	Physical Size Y	Physical height of a pixel.	
	Physical Size Z	The physical distance between optical planes.	
	Microscope Settings	This field refers to the microscope used to acquire this image and holds the setting that was applied to the microscope in this case.	
	Plane	Each Image has to have at least one Plane and one Channel. The Plane object holds the microscope stage and image timing data for a given channel/z-section/timepoint.	
	Channel	Each Image has to be at least one Plane and one Channel. The Channel element stores information about how each image channel was acquired.	
Microscope Settings	Total Effective Magnification	This is the total effective magnification used in this case. This number can be obtained by multiplying Optovar/Magnification times the Objective/Magnification.	
Plane	ID	A unique identifier for this Plane.	
	TheZ	The Z-section to which this plane corresponds. This is numbered from 0.	
	TheT	The time point to which this plane corresponds. This is numbered from 0.	
	TheC	The channel to which this plane corresponds. This is numbered from 0.	
Channel	ID	A unique identifier for this Channel.	
	Name	A name for the channel that is suitable for presentation to the user.	
	Acquisition Mode	This element describes the type of microscopy performed for each channel.	
	Contrast Method	This element describes the technique used to achieve contrast for each channel.	
	Color	Color used to render this channel.	

real-time dynamics of cellular events and single-particle tracking experiments. Imaging techniques that use probability-based detection frameworks to function in signal-starved conditions also fall in this category. Mechanical calibration is highly recommended for this tier. The metadata keys in Supplemental Figure 3 specify some of the information to be included; but method-specific metadata are also expected to be required for some applications.

4.2.2.3 Tier 5: Technical development

It is inherently impossible to design a comprehensive metadata standard for future technologies. It seems reasonable, however, to hold technologies that aim to break the boundaries of resolution and detection to the highest possible metadata standard. Thus, any piece of information that is required, implicitly or explicitly, by the proposed image formation or data processing method should be included as metadata alongside the images. As such, Tier 5 provides developers with the option of fully documenting the proposed technique, thus ensuring full reproducibility and validation. While Supplemental Figures 4 and 5 specify some of the metadata fields that need to be reported for Tier 5, additional method-specific information is expected to be required for most applications.

4.3 The 4DN proposal for an update of the OME data model

4.3.1 OME: an informatics framework for biological-image data

Deriving information from images is completely dependent on contextual information, such as sample preparation, fluorescent labeling and image acquisition details, knowledge of the optical transfer function, spectral properties and noise characteristics of the microscope as well as knowledge of the precise characteristics of the algorithms used to extract quantitative information from images. The OME (Swedlow et al., 2003) data model (Goldberg et al., 2005)—and related software implementations (Allan et al., 2012; Linkert et al., 2010)—was developed in response to this challenge to provide an informatics framework for the accurate storage, processing and analysis of biological image data obtained from a wide range of microscopic technologies and capabilities. This new kind of bioinformatics, termed bio-image informatics, specifically concentrates on providing rich metadata representations of the microscope instrument, its configuration, and the parameters used during image acquisition, while providing minimal information about sample preparation, and experimental protocols. To address the huge variability between image datasets while at the same time avoiding undue burdens that would be imposed by a single universal model, the OME data model supports extensibility and the capacity of adapting to many different types of data.

The OME data model is implemented in OMERO, a relational database and application server to import, store, process, view and export data (Allan et al., 2012). The data model is also implemented in an XML file format, facilitating transfer between OME databases and exchange with other software, including those provided by commercial vendors (Linkert et al., 2010). Thus, OME serves as a neutral broker among a multitude of otherwise incompatible software tools.

4.3.1.1 The OME data model overview

OME is designed with four hierarchical levels: Project, Dataset, Image, and Feature. Each image is defined as being part of a dataset and project, and when necessary, a given plate and screen. The stored data is also related to the experimenter that collected the data and his or her group.

One of the main aims of the OME model is to describe the different hardware components of the microscope, define the light path of each channel and document the settings used for individual image acquisition session (i.e., laser power, exposure times and detector gain). Since the physical setup of microscopes tends to be fixed, while some of the parameters change over time, and the imaging settings are adjusted for different samples, the OME model subdivides microscopy metadata in two main sections:

- 1) The **Instrument>** core element is used to store the relatively static description of a given microscope and of its hardware components (i.e. one or more light sources, objectives, filters, detectors). Because microscopes do not typically change from image to image, these descriptions can be utilized for every image that was collected using the same instrument.
- 2) The **Image** core element is used to document the image acquisition settings utilized for a specific imaging session. To this aim, the metadata fields and sub-elements of the Image data element store references to specific hardware components defined in the Instrument alongside any necessary configurations and parameter settings utilized for a given image dataset (i.e., excitation power, filter set and detector gain).

4.3.1.2 OME: extensibility

Despite offering innumerable advantages, standardization can limit severely the type of data that can be stored and massively increase the administrative burden associated with imaging experiments. As it is not possible to know a priori the complexity and diversity inherent to experimental details and imaging modalities that might be developed, the OME data model was designed from the onset to meet strict extensibility requirements.

More specifically, a key design feature of the OME data model are mechanisms that allow to describe custom model elements that can be used to extend the core of the data model. As the main goal for OME is to respond to the needs of the microscopy community, the core of OME defines a common ontology for fluorescence microscopy and provide shared means of describing the microscope instrument hardware, its configuration as well as the imaging parameters.

Building upon this foundation, the core of the model can be extended by defining additional types of information (i.e., specialized or vendor-specific metadata; analysis-related metadata; quality control metadata) and its relationship with core model elements. As these custom model elements (Rigano and Strambio-De-Castillia, 2017, 2016; Masuzzo, 2017) become more commonly used they can then be incorporated into the core on the basis of community announcements and related vetting processes to meet expanding community needs.

4.3.2 The 4DN-OME proposal: a major extension of the core OME data model

OME represents the ideal starting point for the 4DN-OME extension presented here. The proposed 4DN extension of the OME xml model (Figures 4 and 5) has the following key features:, 1) a tiered-system of reporting guidelines that scales required metadata content with experimental complexity. 2) A metadata model designed to better capture the technical complexity of high-resolution single-molecule localization and single-particle tracking experiments. 3) The introduction of standards for fluorescence microscope calibration and quantitative instrument performance assessment.

In addition to introducing the concept of graded documentation requirements based on a tiered-system of guidelines (Table III), the 4DN-OME proposal extends the existing the OME-core classes <Instrument> (Figure 4) and <Image> (Figure 5) to reflect the technological advances and the quality control requirements associated with single-molecule, super-resolution microscopy. To this aim, the proposal put forth several types of modifications. First, additional classes and attributes were introduced to capture the complexity of microscope hardware components commonly encountered in the field, their configuration, their settings and their calibration requirements (i.e., <Additional Optics>; <CalibrationTool>; <OpticalCalibration>, <IntensityCalibration> etc.) as described in Table II. Second, mimicking the hierarchical structure of <LightSource>, several additional abstract concepts were proposed to describe the hardware components that commonly require specialization (i.e., <LightSourceCoupling>, <Filter>, <Mirror>, and <Detector>). This streamlined the structure of the model and reduced data duplication. Third, the concept of individual 'WavelengthRange' class was established to facilitate the description of multi-pass excitation sources, filters and dichroic-mirrors. Furthermore, the structure of <LightPath> was expanded to describe optical components that might be placed between the source and the detector (i.e., <LightSourceCoupling>, <Prism>, <PolarizionOptics>, <Mirror>) to accommodate a variety of different technical modalities. Finally, while the core OME < Image> class remains central to the 4DN-OME extension as the main storage element for acquisition settings, additional concepts were introduced to better capture the settings of individual components (i.e., <MicroscopeSettings>, <CameraSettings> <PMTSettings>).

5 - CONCLUSION

Because the information content of image data is not machine-readable, microscopy images need to be accompanied by thorough documentation of the microscope hard-ware and imaging settings to ensure a correct interpretation of the results. A significant challenge with the reproducibility of microscopy results and with their integration with other data types, such as chromatin folding maps generated by the 4DN consortium, lies in the lack of shared super-resolution microscopy reporting guidelines and of instrument perfor-mance and calibration standards. Despite a growing consensus that a shared standard for fluorescence microscopy is desirable, previous efforts to develop shared microscopy data models and application programming interfaces (Swedlow et al., 2003; Linkert et al., 2010; Goldberg et al., 2005) have not yet succeeded in the establishment of a universal set of norms.

The proposed 4DN extension of the OME model is put forth to help address this challenge. In addition to aligning the OME metadata model to current technological developments, the standard advanced by the 4DN IWG focuses on the

maximization of usability via the introduction of a tiered system of documentation requirements, and on the development of quality-control standards for fluorescence imaging.

Microscopy is not the only field in which recent technological advances have resulted in increasingly richer datasets. Recent examples are genomic DNA and transcriptomics RNA sequencing, which are in fact much younger than fluorescence microscopy. While in its early days, protocols varied substantially (the original images from the sequencer were kept with the determined sequence), it took only about a decade to establish metadata requirements. One factor that helped establish such metadata criteria was the NIH Encyclopedia of DNA Elements (ENCODE) consortium (de Souza, 2012; ENCODE Project Consortium, 2012). While the genomics field was certainly less technologically diverse at the time of ENCODE than the bio-imaging field ever was, it was the development of Standard Operating Procedures (SOPs) and shared benchmarks (i.e., gold-standards) within this closed group that was pivotal for the establishment of agreeable standards for practical day-to-day use.

In the interest of scientific progress and data fairness, data and metadata standards should not be dictated by individual laboratories or microscope manufacturers. Rather, they should emerge organically from discussions involving all community stakeholders who can benefit from standardization. This manuscript is published with the explicit intention of stimulating thoughts, critiques and further contributions from imaging scientists, instrument manufacturers, software developers, journals and funding agencies. Specifically, to ensure that the proposed metadata model can be evaluated and eventually adopted, we are collecting feedback by the following members of the imaging community: 1) funding agencies; 2) measurements and standards agencies such as the US National Institute of Standards and Technology (NIST), the UK National Physical Laboratory (NPL) and the German Bundesanstalt für Materialforschung und -prüfung (BAM); 3) microscopy community organization such as Global Bioimaging, EU Bioimaging, German Bioimaging and BINA; 4) vendors; 5) journals; 6) core facility directors. Furthermore, we are working in close collaboration with the OME community to establish general criteria and procedures to incorporate major model updates into the core of the model.

We hope that by targeting issues that have received less attention in the past, this proposal might serve as a further step towards building open microscopy standards, provided it is further improved on the basis of feedback from all the stakeholders in the imaging community.

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7 - REFERENCES

- Allan, C., J.-M. Burel, J. Moore, C. Blackburn, M. Linkert, S. Loynton, D. MacDonald, W.J. Moore, C. Neves, A. Patterson, M. Porter, A. Tarkowska, B. Loranger, J. Avondo, I. Lagerstedt, L. Lianas, S. Leo, K. Hands, R.T. Hay, A. Patwardhan, C. Best, G.J. Kleywegt, G. Zanetti, and J.R. Swedlow. 2012. OMERO: flexible, model-driven data management for experimental biology. Nat. Methods. 9:245–253.
- Allen Institute for Cell Science, 2019. © 2016 Allen Institute for Cell Science, Project Overview, alleninstitute.org.
- Carrington, W.A., and K.E. Fogarty. 1987. 3-D molecular distribution in living cells by deconvolution of optical section using light microscopy. In 13th Annual Northeast Bioengineering Conference. K.R. Foster, editor. 108–111.
- Carrington, W.A., R.M. Lynch, E.D.W. Moore, G. Isenberg, K.E. Fogarty, and F.S. Fay. 1995. Superresolution three-dimensional images of fluorescence in cells with minimal light exposure. Science. 268:1483–1487.
- Center for Research in Biological Systems. 2019. The CRBS Cell Image Library. www.cellimagelibrary.org.
- Chen, P.P.-S. 1976. The entity-relationship model—toward a unified view of data. ACM Transactions on Database Systems (TODS). 1:9–36. doi:10.1145/320434.320440.
- Deagle, R.C., T.-L.E. Wee, and C.M. Brown. 2017. Reproducibility in light microscopy: Maintenance, standards and SOPs. Int. J. Biochem. Cell Biol. 89:120–124. doi:10.1016/j.biocel.2017.06.008.
- Dekker, J., A.S. Belmont, M. Guttman, V.O. Leshyk, J.T. Lis, S. Lomvardas, L.A. Mirny, C.C. O'Shea, P.J. Park, B. Ren, J.C.R. Politz, J. Shendure, S. Zhong, and 4D Nucleome Network. 2017. The 4D nucleome project. Nature. 549:219–226. doi:10.1038/nature23884.
- Demmerle, J., C. Innocent, A.J. North, G. Ball, M. Müller, E. Miron, A. Matsuda, I.M. Dobbie, Y. Markaki, and L. Schermelleh. 2017. Strategic and practical guidelines for successful structured illumination microscopy. Nat. Protoc. 12:988–1010. doi:10.1038/nprot.2017.019.
- Ellenberg, J., J.R. Swedlow, M. Barlow, C.E. Cook, U. Sarkans, A. Patwardhan, A. Brazma, and E. Birney. 2018. A call for public archives for biological image data. Nat. Methods. 15:849–854. doi:10.1038/s41592-018-0195-8.
- ENCODE Project Consortium. 2012. An integrated encyclopedia of DNA elements in the human genome. Nature. 489:57–74. doi:10.1038/nature11247.
- Equator Network. 2019. BRISQ: Biospecimen Reporting for Improved Study Quality. FAIRsharing.org.
- European Bioinformatics Institute (EBI). 2019. Sample Tab: Sample Tabular Format. FAIRsharing.org.
- Ferreira, T.A., A.V. Blackman, J. Oyrer, S. Jayabal, A.J. Chung, A.J. Watt, P.J. Sjöström, and D.J. van Meyel. 2014. Neuronal morphometry directly from bitmap images. Nat. Methods. 11:982–984. doi:10.1038/nmeth.3125.
- FlyBase. 2019. FBbi: Biological Imaging methods Ontology. FAIRsharing.org.
- Goldberg, I.G., C. Allan, J.-M. Burel, D. Creager, A. Falconi, H. Hochheiser, J. Johnston, J. Mellen, P.K. Sorger, and J.R. Swedlow. 2005. The Open Microscopy Environment (OME) Data Model and XML file: open tools for informatics and quantitative analysis in biological imaging. Genome Biol. 6:R47.
- Hill, E. 2008. Announcing the JCB DataViewer, a browser-based application for viewing original image files. J. Cell Biol. 183:969–970. doi:10.1083/jcb.200811132.
- Hng, K.I., and D. Dormann. 2013. ConfocalCheck--a software tool for the automated monitoring of confocal microscope performance. PLoS One. 8:e79879. doi:10.1371/journal.pone.0079879.
- Hoffmann, K., U. Resch-Genger, and R. Nitschke. 2005. Simple tool for the standardization of confocal spectral imaging systems.
- Hoffmann, K., U. Resch-Genger, and R. Nitschke. 2008. Comparability of fluorescence microscopy data and need for instrument characterization of spectral scanning microscopes. In Standardization and Quality Assurance in Fluorescence Measurements II. Springer. 89–116.

- ICA Project. 2019. CSMD: Core Scientific MetaData model. FAIRsharing.org.
- Jost, A.P.-T., and J.C. Waters. 2019. Designing a rigorous microscopy experiment: Validating methods and avoiding bias. J. Cell Biol. 218:1452–1466. doi:10.1083/jcb.201812109.
- Lambert, T.J., and J.C. Waters. 2014. Assessing camera performance for quantitative microscopy. Methods Cell Biol. 123:35–53. doi:10.1016/B978-0-12-420138-5.00003-3.
- Linkert, M., C.T. Rueden, C. Allan, J.-M. Burel, W. Moore, A. Patterson, B. Loranger, J. Moore, C. Neves, D. MacDonald, A. Tarkowska, C. Sticco, E. Hill, M. Rossner, K.W. Eliceiri, and J.R. Swedlow. 2010. Metadata matters: access to image data in the real world. J. Cell Biol. 189:777–782.
- Masuzzo, P. 2017. MIACME 0.1. Cell Migration Standardization Organization.
- MGED: an ontology for microarray experiments in support of MAGE v.1. 2007.
- MISFISHIE Standard Working Group. MISFISHIE: Minimum Information Specification For In Situ Hybridization and Immunohistochemistry Experiments. FAIRsharing.org. Jan. 8, 2019 pp.
- Mubaid, F., D. Kaufman, T.-L. Wee, D.-S. Nguyen-Huu, D. Young, M. Anghelopoulou, and C.M. Brown. 2019. Fluorescence microscope light source stability. Histochem. Cell Biol. 151:357–366. doi:10.1007/s00418-019-01776-6.
- Murray, J.M., P.L. Appleton, J.R. Swedlow, and J.C. Waters. 2007. Evaluating performance in three-dimensional fluorescence microscopy. J. Microsc. 228:390–405. doi:10.1111/j.1365-2818.2007.01861.x.
- Nieuwenhuizen, R.P.J., K.A. Lidke, M. Bates, D.L. Puig, D. Grunwald, S. Stallinga, and B. Rieger. 2013. Measuring image resolution in optical nanoscopy. Nat. Methods. 10:557–562.
- North, A.J. 2006. Seeing is believing? A beginners' guide to practical pitfalls in image acquisition. J. Cell Biol. 172:9–18. doi:10.1083/jcb.200507103.
- Observatoire Océanologique de Villefranche Sur Mer. 2015. MULTI-DIMENSIONAL MARINE ORGANISMS DATAVIEWER. Movincell.
- Omenn, G.S. 2014. The strategy, organization, and progress of the HUPO Human Proteome Project. J. Proteomics. 100:3–7.
- Petrak, L.J., and J.C. Waters. 2014. A practical guide to microscope care and maintenance. Methods Cell Biol. 123:55–76. doi:10.1016/B978-0-12-420138-5.00004-5.
- Ram, S., and J. Liu. 2008. A Semiotics Framework for Analyzing Data Provenance Research. Journal of Computing Science and Engineering. 2:221–248. doi:10.5626/JCSE.2008.2.3.221.
- Ram, S., and J. Liu. 2009. A new perspective on semantics of data provenance. In Proceedings of the First International Conference on Semantic Web in Provenance Management-Volume 526. CEUR-WS. org. 35–40.
- Ram, S., and J. Liu. 2012. A Semantic Foundation for Provenance Management. J. Data Semant. 1:11–17. doi:10.1007/s13740-012-0002-0.
- Resch-Genger, U., K. Hoffmann, W. Nietfeld, A. Engel, J. Neukammer, R. Nitschke, B. Ebert, and R. Macdonald. 2005. How to improve quality assurance in fluorometry: fluorescence-inherent sources of error and suited fluorescence standards. J. Fluoresc. 15:337–362. doi:10.1007/s10895-005-2630-3.
- Rigano, A., and C. Strambio-De-Castillia. 2016. Minimum Information About Particle Tracking Experiments. Biosharing.org.
- Rigano, A., and C. Strambio-De-Castillia. 2017. Proposal for minimum information guidelines to report and reproduce results of particle tracking and motion analysis. bioRxiv. 155036.
- Smith, B., M. Ashburner, C. Rosse, J. Bard, W. Bug, W. Ceusters, L.J. Goldberg, K. Eilbeck, A. Ireland, C.J. Mungall, N. Leontis, P. Rocca-Serra, A. Ruttenberg, S.-A. Sansone, R.H. Scheuermann, N. Shah, P.L. Whetzel, and S. Lewis. 2007. The OBO Foundry: coordinated evolution of ontologies to support biomedical data integration. Nat. Biotechnol. 25:1251–1255.
- de Souza, N. 2012. The ENCODE project. Nat. Methods. 9:1046.

- Stack, R.F., C.J. Bayles, A.-M. Girard, K. Martin, C. Opansky, K. Schulz, and R.W. Cole. 2011. Quality assurance testing for modern optical imaging systems. Microsc. Microanal. 17:598–606. doi:10.1017/S1431927611000237.
- Swedlow, J.R., I.G. Goldberg, E. Brauner, and P.K. Sorger. 2003. Informatics and Quantitative Analysis in Biological Imaging. Science. 300:100–102.
- Taylor, C.F., D. Field, S.-A. Sansone, J. Aerts, R. Apweiler, M. Ashburner, C.A. Ball, P.-A. Binz, M. Bogue, T. Booth, A. Brazma, R.R. Brinkman, A.M. Clark, E.W. Deutsch, O. Fiehn, J. Fostel, P. Ghazal, F. Gibson, T. Gray, G. Grimes, J.M. Hancock, N.W. Hardy, H. Hermjakob, R.K. Julian, M. Kane, C. Kettner, C. Kinsinger, E. Kolker, M. Kuiper, N. Le Novère, J. Leebens-Mack, S.E. Lewis, P. Lord, A.-M. Mallon, N. Marthandan, H. Masuya, R. McNally, A. Mehrle, N. Morrison, S. Orchard, J. Quackenbush, J.M. Reecy, D.G. Robertson, P. Rocca-Serra, H. Rodriguez, H. Rosenfelder, J. Santoyo-Lopez, R.H. Scheuermann, D. Schober, B. Smith, J. Snape, C.J. Stoeckert, K. Tipton, P. Sterk, A. Untergasser, J. Vandesompele, and S. Wiemann. 2008. Promoting coherent minimum reporting guidelines for biological and biomedical investigations: the MIBBI project. Nat. Biotechnol. 26:889–896.
- Theer, P., C. Mongis, and M. Knop. 2014. PSFj: know your fluorescence microscope. Nat. Methods. 11:981–982. doi:10.1038/nmeth.3102.
- Waters, J.C. 2009. Accuracy and precision in quantitative fluorescence microscopy. J. Cell Biol. 185:1135–1148. doi:10.1083/jcb.200903097.
- Wilkinson, M.D., M. Dumontier, I.J.J. Aalbersberg, G. Appleton, M. Axton, A. Baak, N. Blomberg, J.-W. Boiten, L.B. da Silva Santos, P.E. Bourne, J. Bouwman, A.J. Brookes, T. Clark, M. Crosas, I. Dillo, O. Dumon, S. Edmunds, C.T. Evelo, R. Finkers, A. Gonzalez-Beltran, A.J.G. Gray, P. Groth, C. Goble, J.S. Grethe, J. Heringa, P.A.C. 't Hoen, R. Hooft, T. Kuhn, R. Kok, J. Kok, S.J. Lusher, M.E. Martone, A. Mons, A.L. Packer, B. Persson, P. Rocca-Serra, M. Roos, R. van Schaik, S.-A. Sansone, E. Schultes, T. Sengstag, T. Slater, G. Strawn, M.A. Swertz, M. Thompson, J. van der Lei, E. van Mulligen, J. Velterop, A. Waagmeester, P. Wittenburg, K. Wolstencroft, J. Zhao, and B. Mons. 2016. The FAIR Guiding Principles for scientific data management and stewardship. Sci Data. 3:160018. doi:10.1038/sdata.2016.18.
- Williams, E., J. Moore, S.W. Li, G. Rustici, A. Tarkowska, A. Chessel, S. Leo, B. Antal, R.K. Ferguson, U. Sarkans, A. Brazma, R.E. Carazo Salas, and J.R. Swedlow. 2017. Image Data Resource: a bioimage data integration and publication platform. Nat. Methods. 14:775. doi:10.1038/nmeth.4326.

8 - FIGURES

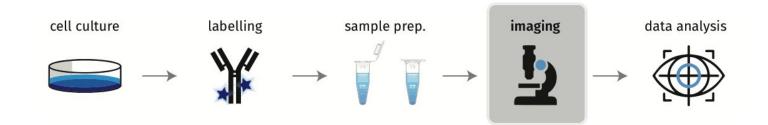


Figure 1: The position of image acquisition in the context of a typical bio-imaging experiment. Every phase of a typical bio-imaging experiment involves a host of different procedures and techniques at the center of which is 'imaging'. When it is properly utilized, carefully maintained, and operated following consistent experimental conditions, sample preparation, and labeling, a microscope is expected to display finite, and repeatable accuracy and precision, which is can be measured and is known to depend on the Signal-to-Noise-Ratio (SNR) conditions observed during image acquisition. It follows that while every experimental step contributes variability and uncertainty to the results, the relative impact of imaging can be minimized through instrument calibration and proper record-keeping. The resulting information referred to, respectively, as data provenance (Figure 2 and Table I) and quality control metadata (Table II), is essential for the evaluation, interpretation, comparison, and reproducibility of experimental results.

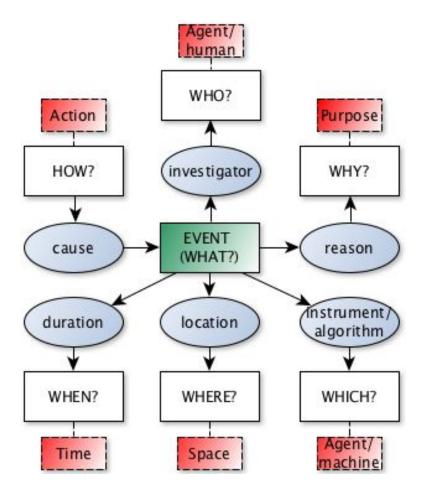


Figure 2: W7 model for data provenance. Conceptual graph describing the W7 model. White solid boxes represent concepts. Blue circles represent relationships between concepts. Colored dashed boxes represent the significance of each concept for documenting data provenance

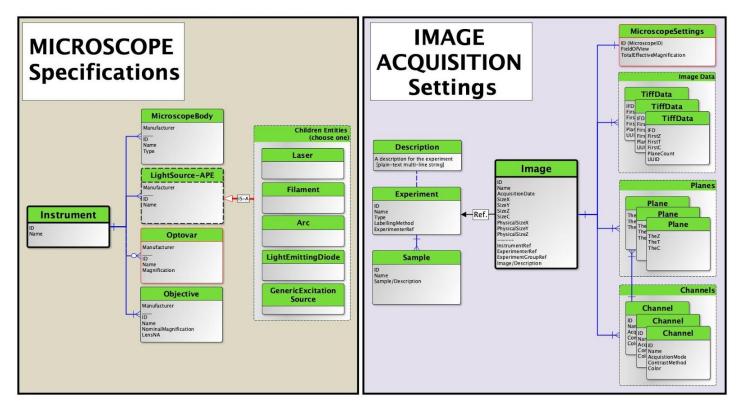


Figure 3: Entity-Relationship diagram representing the suggested Minimum Information for Fluorescence Microscopy proposal metadata model for Tier 1 experiments. Note that metadata referring to the sample is not meant to replace the sample preparation protocol. This metadata entries included describing parameters that are relevant to the interpretation of imaging results and can be used as a reference to retrieve further experimental and sample preparation metadata stored elsewhere.

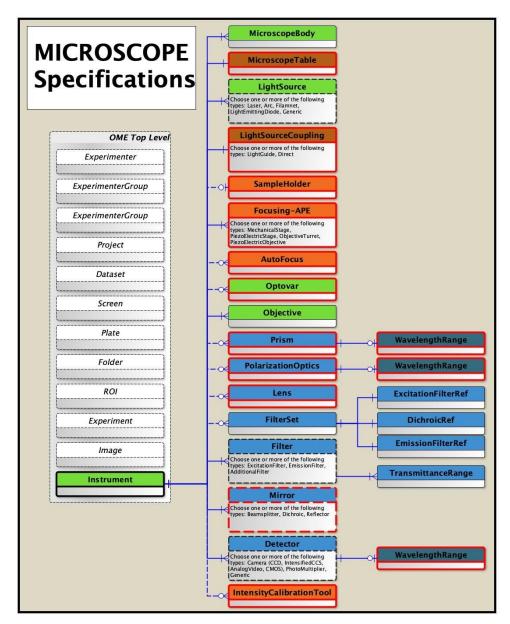


Figure 4: 4DN-OME data-model comparison: Microscope Specifications. Schematic representation of the extension of the Instrument element of the OME data model as proposed by the 4D Nucleome Imaging Standards Working Group. Red-lined boxes represent elements that were introduced de novo a result of the proposed extension. Dashed-lined boxes represent abstract parent elements that in the model are represented by concrete children elements (e.g., LightSource --> Laser, LED, Arc, Filament, etc.). Color codes represent the proposed Tier level: Green, Tier 1; Blue, Tier 2; Orange, Tier 3; Maroon, Tier 4; Dark blue, Tier 5.

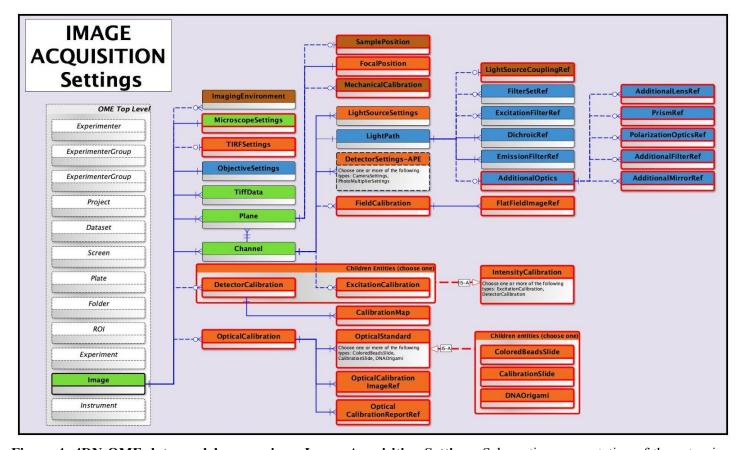
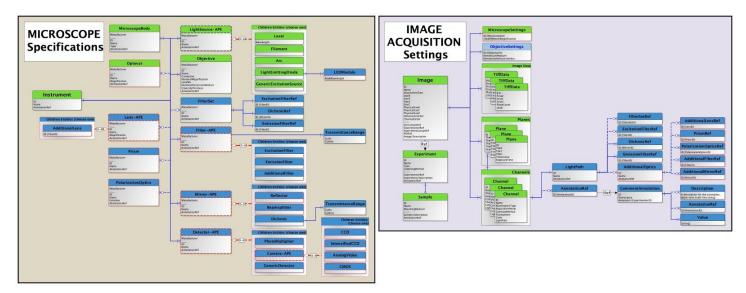
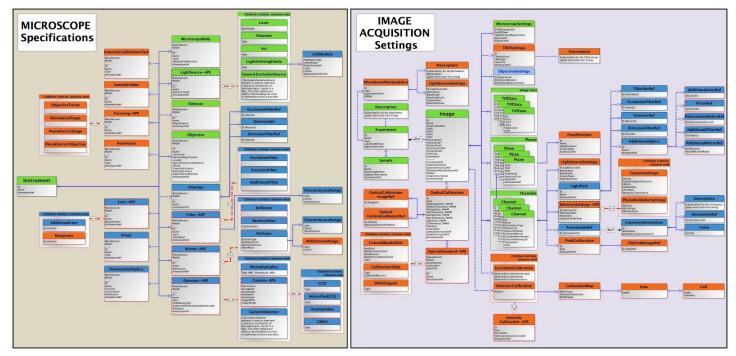


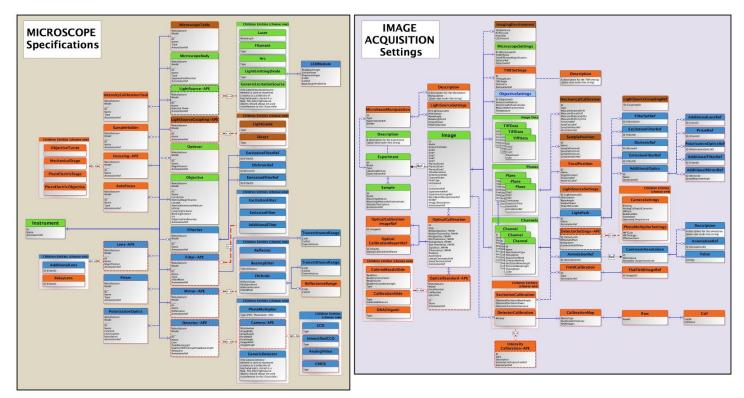
Figure 4: 4DN-OME data-model comparison: Image Acquisition Settings. Schematic representation of the extension of the Image element of the OME data model as proposed by the 4D Nucleome Imaging Standards Working Group. Red-lined boxes represent elements that were introduced de novo a result of the proposed extension. Dashed-lined boxes represent abstract parent elements that in the model are represented by concrete children elements. Color codes represent the proposed Tier level: Green, Tier 1; Blue, Tier 2; Orange, Tier 3; Maroon, Tier 4; Dark blue, Tier 5.



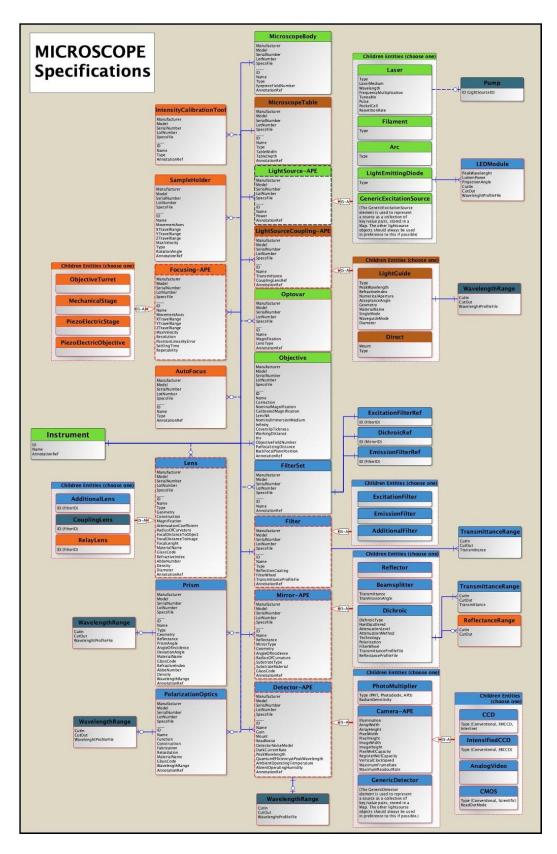
Supplemental Figure 1: Entity-Relationship diagram representing the suggested metadata model for Tier 2 experiments. This list is not exhaustive; depending on the type of analysis that is envisioned, more parameters might need to be added.



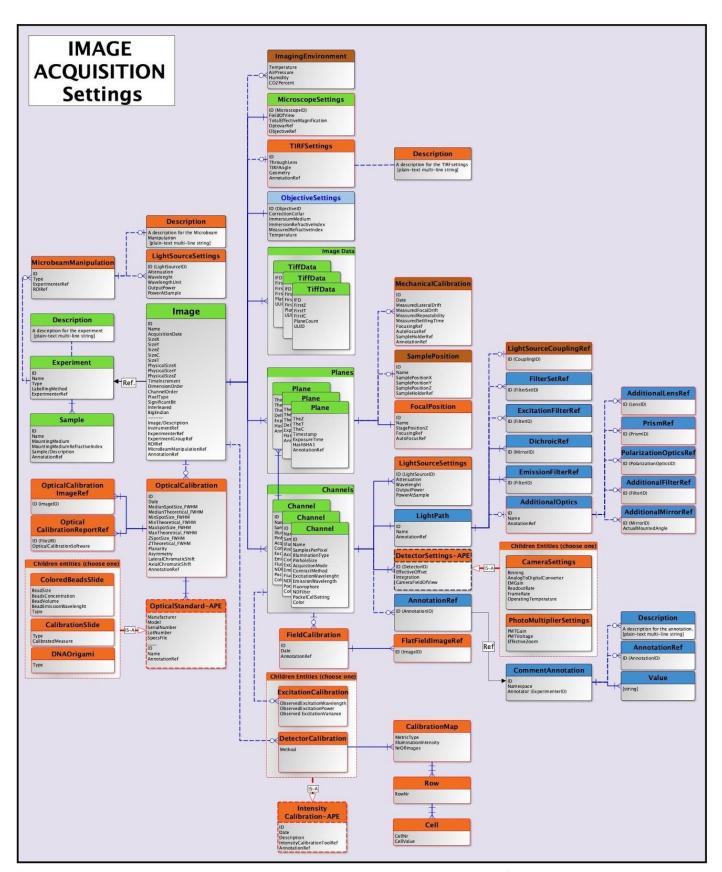
Supplemental Figure 2: Entity-Relationship diagram representing the suggested metadata model for Tier 3 experiments. Details on calibration procedures and metadata are presented in separate manuscripts [REF]. This list is not exhaustive; depending on the type of analysis that is envisioned, more parameters might need to be added.



Supplemental Figure 3: Entity-Relationship diagram representing the suggested metadata model for Tier 4 experiments. Details on calibration procedures and metadata are presented in separate manuscripts [REF]. This list is not exhaustive; depending on the type of analysis that is envisioned, more parameters might need to be added.



Supplemental Figure 4: Entity-Relationship diagram representing the suggested metadata model for the Instrument element at Tier 5 level. Metadata attributes presented here are not meant to represent an exhaustive list; depending on the type of instrumentation and analysis that is envisioned, more parameters might need to be added.



Supplemental Figure 5: diagram representing the suggested metadata model for the Image element at Tier 5 level. Metadata attributes presented here are not meant to represent an exhaustive list; depending on the type of instrumentation and analysis that is envisioned, more parameters might need to be added.