Towards community-driven metadata standards for light microscopy: tiered specifications extending the OME model

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Supplementary Material

Supplementary OME Description: an informatics framework for biological image data

Since its launch in 2003[1–6] the OME initiative has played a leading role in the foundation and development of bio-image informatics[7], a new field of biological informatics which concentrates explicitly on building technology for the sharing and dissemination of reproducible and quality controlled biomedical image data. Specifically, the OME Data Model initially published in 2005[8–10] and its related software implementations[2,5,9] were developed to provide an informatics framework for the accurate capture, storage and management of rich metadata representations of all the information required for interpreting, comparing, reproducing, sharing and publishing biological microscopy data (Figure 2, *Microscopy Metadata*) independent of a proprietary file format. The OME Data Model is first and foremost implemented in the Bio-Formats library[9] which allows the interpretation of the more than 150 different proprietary file formats and their translation into the OME-TIFF and OME-NGFF[11] open file formats for biological imaging. This facilitates the transfer of image data between different commercial vendors and open-source software tools thus reducing the barriers between different analytical and data management tools. Finally, the OME Data Model is also implemented in OMERO, a relational database and application server to import, store, process, view, and export data[2].

One of the OME Data Model's main purposes 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 sessions (i.e., laser power, exposure times and detector gain). Since the physical setup of microscopes tends to be fixed, while imaging settings are typically adjusted for different samples and acquisition sessions, the OME Data Model subdivides Microscopy Metadata into two main sections:

1) The **<Instrument> core element** (Figure 2B) describes the imaging instrument and is used to store the relatively static description of a given microscope and its hardware components (e.g., objectives, illumination sources, filters, detectors).
2) The **<Image> core element** (Figure 2A) stores the specific instance of an acquired image and, in addition to describing the structure of the, possibly multi-dimensional, image, it documents the image acquisition settings utilized when that image was acquired. To this aim, the <Image> data element stores references to specific hardware components defined in the <Instrument> alongside any necessary configurations and parameter settings utilized for a given image dataset (e.g., excitation power, filter set and detector gain).

While this structure is very robust and allows to document core microscopy concepts such as <LightSource>, <Objective>, <Filter>, <Detector>, etc.,), the OME specifications have not kept pace with the wide range of technologies that are now routinely used in the life and biomedical sciences.

Supplementary 4DN-BINA-OME Tier-system description - Axis 1

1. Minimum Information required for full documentation → Tier 1

Tier 1 covers experiments that fall into two general sub-categories:

1. Qualitative evaluation: Experiments that require only qualitative assessment of image data for meaningful conclusions to be drawn. Examples include developmental and stem cell-biology experiments in which qualitative analysis of image data is used to support major findings as well as experiments that serve as minor supporting evidence in a project or manuscript (e.g., transfection controls, developmental stage and viability tests).
2. Simple image processing and analysis: Experiments that require simple processing and analyses to support conclusions. This category includes studies that require the identification, counting, intensity and morphometric analyses of features whose size is above the limit of resolution of the system. Examples include cell-counting, the measurement of reporter intensity, the localization of reporters in the nucleus vs. cytoplasm or the estimation of the size and shape of individual cells.

Hence, this descriptive tier does not require metadata describing advanced hardware features of the microscope or quantifying microscope performance. The complete description of metadata fields to be included in Tier 1 is available on GitHub[12–15].

2. Advanced image analysis, live-imaging and super-resolution -- Tier 2

Advances in microscope technology have been accompanied by an increased dependence on complex image analysis methods. Some imaging techniques require digital image processing and image analysis for the very construction of the images (e.g., assembling individual pixel intensities into the pixel array that constitute an image for CLSM, Structured Illumination Microscopy - SIM, and stochastic SR methods like Photo-Activated Localization Microscopy - PALM, and Stochastic Optical Reconstruction Microscopy - STORM). Others use model-driven data-processing to enhance the resolution of the data (e.g., deconvolution) and improve quantitative accuracy and reliable interpretability by combatting issues such as illumination shading, nonspecific background, limited signal, and optical aberrations. In addition, many imaging experiments require advanced image analysis for extracting quantitative information from the raw data. These techniques increase the usefulness of microscopy data, but they often require that the data meets certain criteria for the analysis to be useful and reliable, thus requiring more detailed data provenance and performance calibration information for correct interpretation. Specifically, Tier 2 experiments fall into two general, non-mutually exclusive, sub-categories:

1. Advanced quantification: Experiments that aim to draw conclusions about features that are near or below the limits of resolution, as well as experiments in which conclusions require post-acquisition reconstitution (i.e., deconvolution). Imaging techniques that use probability-based detection frameworks also fall into this category, since they often rely on advanced processing and quantitative analysis. Typical examples include SMLM (e.g., PALM and STORM), SM Fluorescence In Situ Hybridization (FISH) and distance/distribution measurements that aim to achieve SR precision. Because these sophisticated imaging techniques require the optical system to be performing at its theoretical best and often take into account the photophysical behaviors of the fluorophores and the detector, Optical and Intensity Calibration[16] are an essential requirement.

2. Live cell imaging: Experiments that use light microscopy on live specimens that require complex multidimensional acquisition set-ups and/or are destined for advanced processing and analysis. These experiments require detailed information about the environmental conditions, phototoxicity data, and focal and stage stability measurements.

Typical examples include applications following the real-time dynamics of cellular events and real-time single-particle tracking (SPT) experiments. Because the stability of the system across time is often crucial for reproducibility, Mechanical Calibration [16] is also recommended for these experiments.

The capacity of microscope users to provide *bona fide* documentation of microscopy experiments is often limited by the degree to which they are made aware of all parts of the instrument by the manufacturer. Accordingly, Tier 2 represents the most demanding requirement for data obtained by microscope users using well-established microscope instrumentation, processing algorithms and analysis procedures that have been shown to be quantifiable across a range of conditions. The complete description of metadata fields to be included in Tier 2 is available on GitHub[12–15].

3. Manufacturing, technical development and full documentation → Tier 3

Tier 3 is intended to be used by manufacturers of microscope hardware components and for the developers of pioneering technologies. While it is inherently impossible to design a comprehensive future proof metadata standard for new technologies, all efforts should be made by instrument developers and manufacturers to provide any piece of information required, implicitly or explicitly, to interpret a given image formation or data processing method. As such, Tier 3 furnishes manufacturers and developers with clear community-sanctioned specifications about what provenance reporting and quality control information should be provided to microscope users to ensure scientific rigor, full reproducibility, and re-use value. The complete description of metadata fields included in Tier 3 is available on GitHub [12–15]. Additional method-specific information is expected to be required for most applications.

Supplementary 4DN-BINA-OME Extensions system - Axis 2

1. Basic 4DN-BINA-OME extension

The Basic 4DN-BINA extension of the OME Data Model was designed to better capture the technical complexity of transmitted light and wide-field fluorescence microscopy and is graphically presented in Figure 4, and Supplementary Figures 1A and 2 [12–14]. This extension puts forth several types of modifications:

1) Extension of already existing elements such as `<LightSource>`, `<Objective>`, `<Filter>`, and `<Detector>` by the introduction of additional attributes (Figure 4 and Supplementary Figure 1, blue and blue/red elements).
2) Mimicking the hierarchical structure of `<LightSource>`, introduction of several additional Abstract Parent Elements (APE) to describe hardware components, such as `<LightSourceCoupling>`, `<Filter>`, `<MirroringDevice>`, and `<Detector>`, that can be subdivided in specialized categories to streamline the structure of the model and avoid data duplication (i.e., the `<Detector>` category can be subdivided in `<Camera>` and `<PointDetector>`).
3) Establishment of the concept of individual `<WavelengthRange>` to facilitate the description of multi-pass excitation sources, filters, dichroic-mirrors and detectors.
4) Introduction of additional concepts to better capture the settings of individual hardware components that are employed during the acquisition of a specific image and are stored in the core OME `<Image>` element (i.e., `<MicroscopeStandSettings>`, `<CameraSettings>` and `<PointDetectorettings>`).
5) In order to facilitate the capture and integration of multi-spot/multi-scale imaging modalities, introduction of novel elements such as `<StageInsert>`, `<SamplePositioning-APE>`, `<FocusingDevice>` and `<SamplePositioningSetting>` to capture the complexity of microscope hardware components and settings commonly encountered in the field (Figure 4 and Supplementary Figure 1, grey elements), and which combine with the new `<PlaneTransformMatrix>` affine transform element to encode locations in real-world coordinates and allow the spatial integration of images collected at different positions with respect to the optical system.
6) In order to facilitate the capture, the light path complexities that are typically encountered in modern light microscopy modalities and their integration in multi-dimensional / multi-modal imaging:
   a) Expansion of the concept of `<LightPath>` and introduction of the new `<LightPathMap>` element to describe the order of optical components that might be placed between the excitation source and the detector other than the filter and dichroic, such as for example `<LightSourceCoupling>`, `<Prism>`, `<PolarizationOptics>`, `<Lens>`, and `<OpticalAperture>`.
   b) Introduction of the `<AdditionalDimensionMap>` and associated elements to flexibly describe dimensions beyond the five canonical X, Y, Z, T (time) , and C (color) dimensions, such as fluorescence lifetime, polarization angle, lambda, and different imaging techniques (e.g., immuno-fluorescence combined with SM FISH).

2. Advanced and Confocal 4DN-BINA-OME Extension

This extension is designed to better capture experiments requiring the use of confocal microscopy and tunable optics (Figure 4, green elements). As depicted graphically in Figure 4, and Supplementary Figures 1B and 3 [12–14], this extension consists primarily in the introduction of novel concepts required to capture hardware components and image acquisition settings that are needed for confocal microscopy and other advanced acquisition modalities that require tunable excitation and emission light selection such as for example, `<ConfocalScanner>`, `<AcoustoOpticalBeamSplitter>`, `<LiquidCrystalTunableFilter>`, and `<PinHole>`.

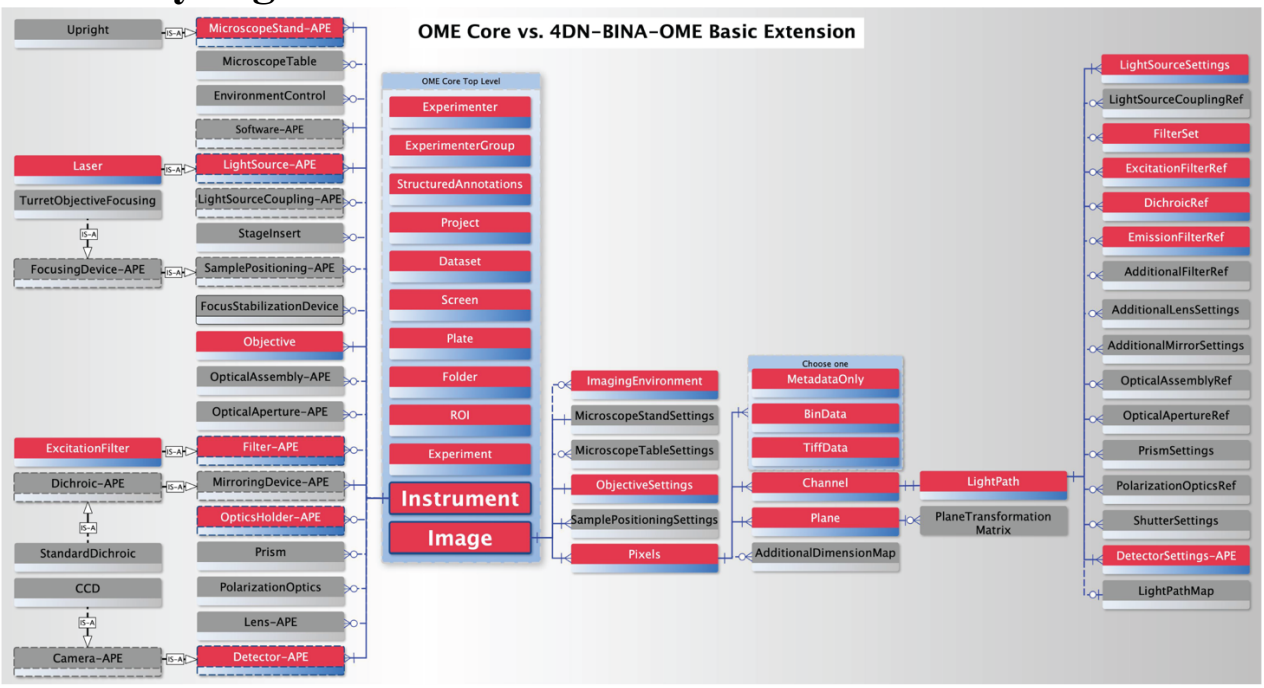
3. Calibration and Performance 4DN-BINA-OME Extension

The specifications for the capture of metrics required for light microscope calibration and quality control captured in this extension were developed in collaboration with QUAREP-LiMi (quarep.org; Figure 4, and Supplementary Figures 1C and 4) [13–15,17,18] and are described in detail in an accompanying manuscript [16]. A diverse set of metrics [19–42] can be used to measure microscope performance and control image quality depending on the type of experiment being performed and the questions being asked. Together, these measurements increase the depth and reliability of a variety of assessments, analyses, and comparisons performed on light microscopy images. Such calibration metrics can be subdivided into four categories: 1) optical; 2) intensity/excitation; 3) intensity/detector, and 4) mechanical (see also Table III in: Huisman et al., 2021) [16]. Metrics in the first three categories evaluate the great majority of image data. Mechanical calibration metrics become most useful in experiments that involve time-lapse imaging or the tiling of multiple Fields-Of-View (FOV). In order to capture these metrics categories, the Calibration and Performance extension introduces the following new elements:

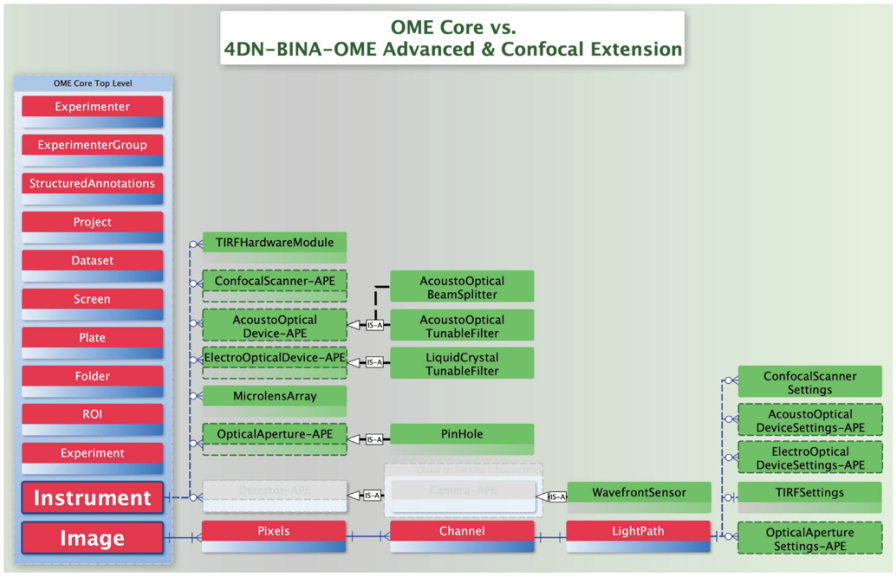
1) `<IntensityCalibrationTool>` and `<LightSensor>` represent hardware tools (e.g., power meter) used for performing specific intensity calibration procedures and represented as sub-elements of `<Instrument>`.
2) `<OpticalCalibration>`, `<ExcitationCalibration>`, `<DetectorCalibration>` and `<MechanicalCalibration>` store information that describes each respective calibration procedure, which might be performed to document individual image datasets and the resulting metrics. `<OpticalCalibration>`, and `<DetectorCalibration>` relate to the `<Image>` element. In turn, `<ExcitationCalibration>` and `<MechanicalCalibration>` are associated with the `<Channel>` and the `<Plane>` elements respectively.
3) `<ColoredBeadsSlide>` (Figure 4 and Supplementary Figure 1C), and `<CalibrationStandardSlide>`, `<FluorescenceReferenceSlide>` and `<DNAOrigami>` (not shown in Figure 4), which belong to the `<OpticalStandard>` category and store information that describe reference standards used for `<OpticalCalibration>` and other procedures including `<ChromaticRegistrationEvaluation>` and `<FieldUniformityEvaluation>`.

Supplementary Figures

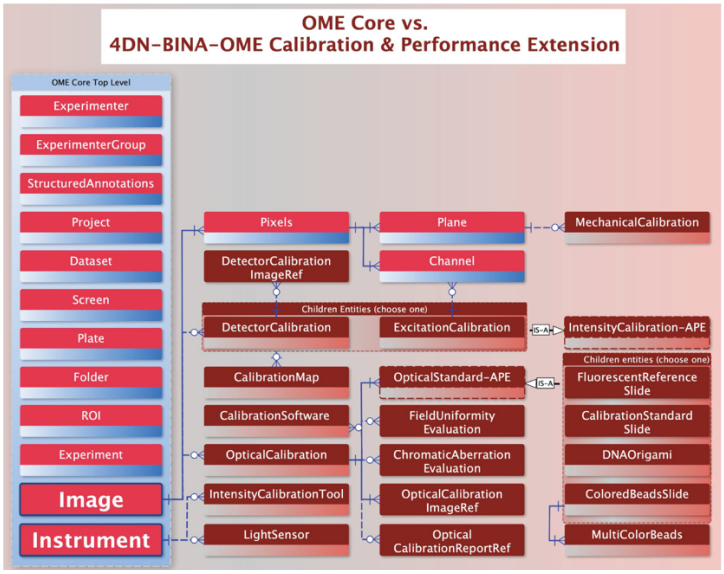
A



B



C

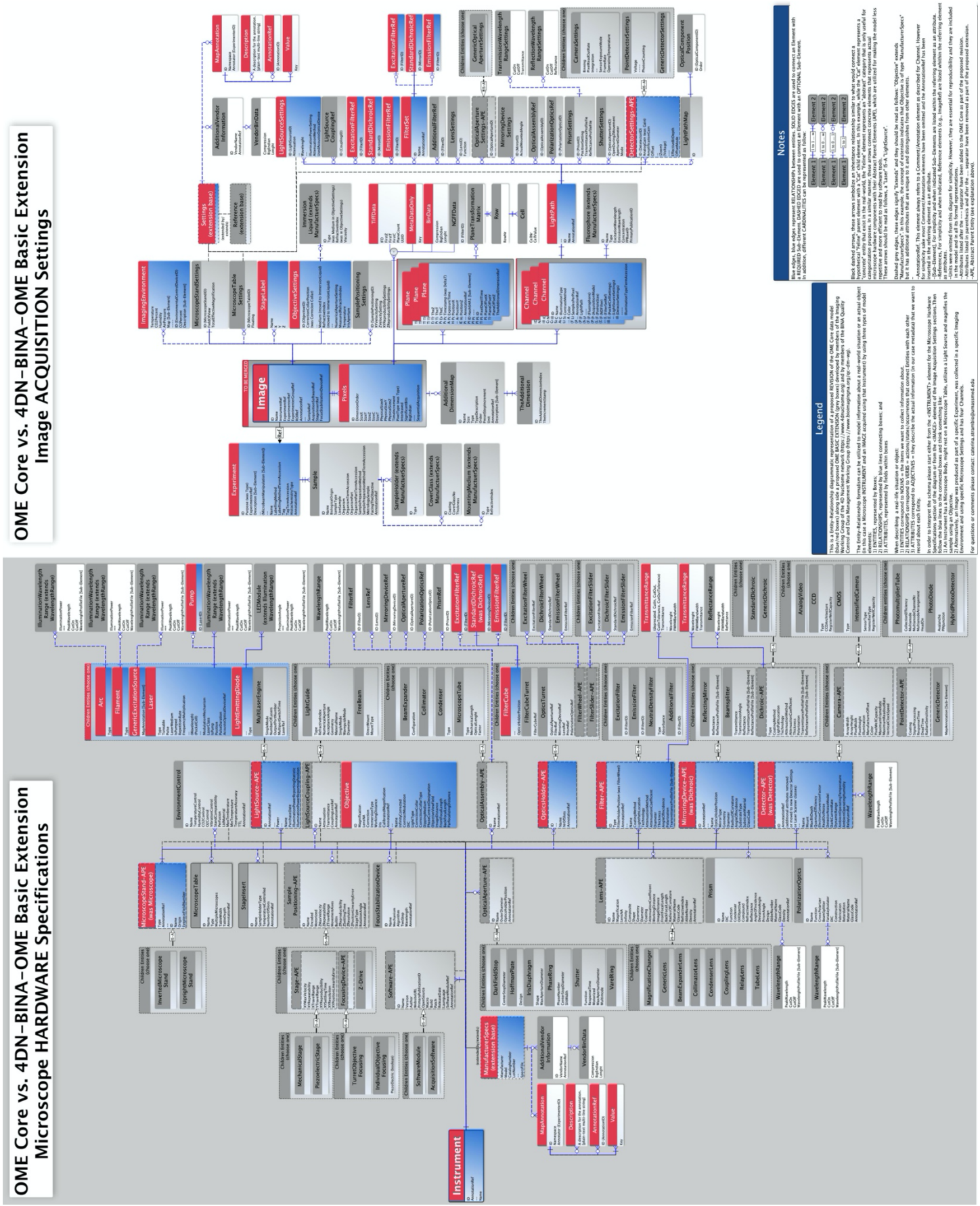


Supplementary Figure 1: Comparison between the Core of the OME Data Model and the 4DN-BINA-OME Extensions. Simplified Entity-Relationship (ER) diagrammatic representation of the extensions of the **<Instrument>** and **<Image>** elements of the OME Data Model that is put forth by 4DN and BINA as compared with the Core of the OME Data Model as originally proposed (*Red and Blue boxes*)[8]. The structure of the diagram is as described for Figure 6. In addition, for readability purposes, ER diagrams are organized hierarchically from "top-level" to "bottom-level" elements, on the basis of three criteria: 1) "top-level" elements that belong to the Core of the OME Data Model (i.e., <Project>, <Experiment>, <Instrument> and <Image>)[8] are enclosed within a light-blue box in the center of the diagram labelled ***OME Core Top Level***. 2) Elements are grouped in those that relate to <Instrument>, which are positioned on the left (e.g., <Objective>), vs. those connected to <Image>, which are positioned on the right (e.g., <ImagingEnvironment>). 3) Finally, elements are organized in a manner that roughly follows the microscopy light path (i.e., <MicroscopeStand> is on top, <Detector> is at the bottom and in between there are <LightSource> followed by <Objective>, and <Filter>). For simplicity's sake only a subset of all available model elements are indicated in this summary diagram.

A) *Grey boxes* represent elements that were introduced, *de novo* as part of the ***4DN-BINA-OME Basic extension***.

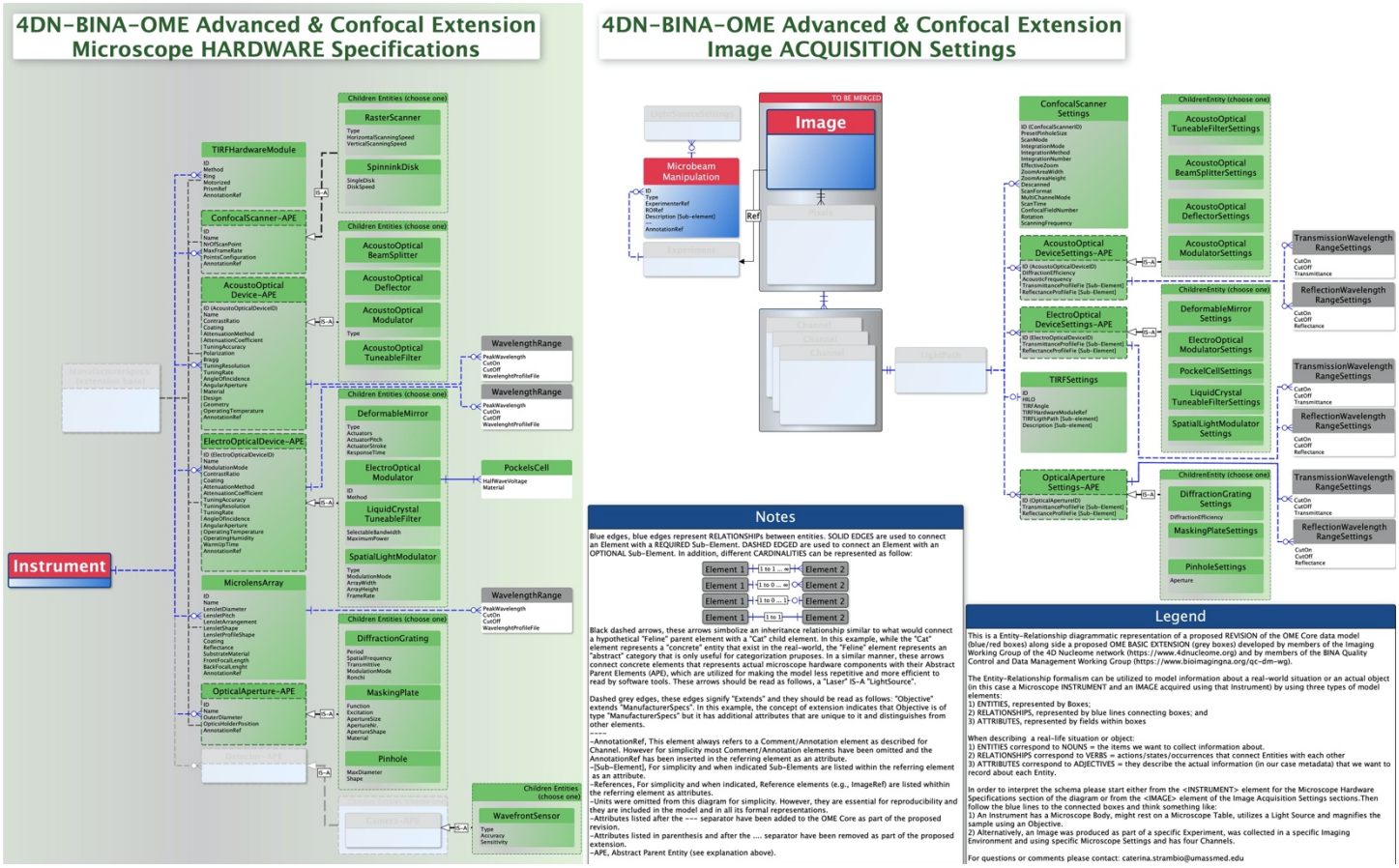
B) *Green boxes* represent elements that were introduced, *de novo* as part of the ***4DN-BINA-OME Advanced and Confocal extension***.

C) *Maroon boxes* represent elements that were introduced, *de novo* as part of the proposed ***4DN-BINA-OME Calibration and Performance extension***.

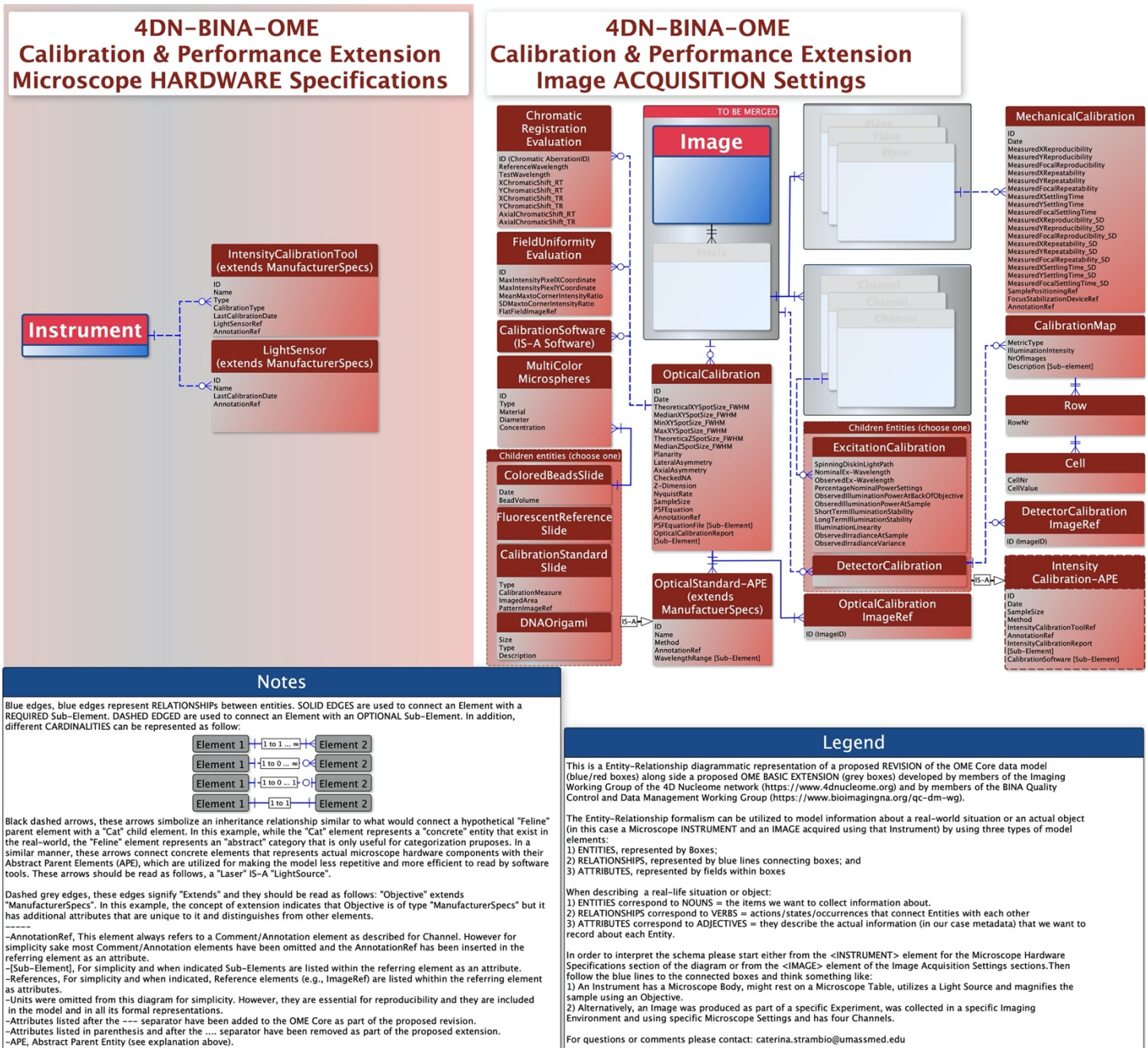


Supplementary Figure 2 | Entity-Relationship diagram representing the CORE/BASIC 4DN-BINA extension of the OME Data Model. This is an Entity-Relationship (ER) diagrammatic representation of the ***Basic Extension*** (*Grey boxes*) of the ***Core*** of the OME Data Model (*Red and Blue boxes*)[8], proposed by 4DN and BINA. The general structure and position

of elements in the diagram is as described for Figure 6 and Supplementary Figure 1. In summary, the ER diagram can be utilized to model information about a real-world situation (in this case a microscope Instrument and an Image acquired using that Instrument) by using three types of model elements: 1) Entities, represented by Boxes (i.e., nouns that represent real-life objects or situations); 2) Relationships, represented by lines connecting boxes (i.e., verbs that represent actions/states/occurrences that connect Entities with each other); and 3) Attributes, represented by fields within boxes (i.e., adjectives that describe the metadata that need to be collected about each Entity). *Blue lines* symbolize "HAS-A" relationships and should be read as: "An Instrument has one or more Objectives". *Black-dashed arrows* symbolize "IS-A" inheritance relationship that connect concrete "children" elements that represents actual microscope hardware or acquisition settings components (e.g., `<Camera>` or `<CameraSettings>`), with their a generalized "parent" abstract concept (e.g., `<Detector-APE>` or `<DetectorSettings-APE>`). These relationships are useful for making the model less repetitive and more efficient employed by software tools and should be read as: "A Laser is a type of Light Source". *Grey dashed lines* signify "Extends" and they should be read as follows: "Objective" extends "ManufacturerSpecs". To interpret the schema please start from either the ***<Instrument>*** or **<Image>** elements of the Microscope Hardware Specifications and Image Acquisition Settings sections of the diagram, respectively. Then follow the blue lines to the connected boxes and think something like, an Instrument has a Microscope Stand, might rest on a Microscope Table, utilizes a Laser, which is a type of Light Source, and magnifies the sample using an Objective. Alternatively, an Image was produced as part of a specific Experiment, was collected in a specific Imaging Environment using specific Microscope Settings and has four Channels. Units were omitted from this diagram for simplicity. However, they are essential for reproducibility, and they are included in the model and in all its formal representations. Attributes listed after the --- separator have been added to the OME Core as part of the proposed revision. Attributes listed in parenthesis and after the .... separator have been removed as part of the proposed extension. Abbreviations: APE, Abstract Parent Entity (see explanation above). A larger version of this image is available for download at: https://doi.org/10.5281/zenodo.4711229



Supplementary Figure 3: Entity-Relationship diagram representing the ADVANCED & CONFOCAL 4DN-BINA extension of the OME Data Model. This is an Entity-Relationship diagrammatic representation of the ***Advanced & Confocal Extension*** (*Green boxes*) of the ***Core*** of the OME Data Model (*Red and Blue boxes*)[8], proposed by 4DN and BINA. The general structure and position of elements in the diagram is as described for Figure 6 and Supplementary Figures 1 and 2. A larger version of this image is available for download at: https://doi.org/10.5281/zenodo.4711229



Supplementary Figure 4: Entity-Relationship diagram representing the CALIBRATION & PERFORMANCE 4DN-BINA extension of the OME Data Model. This is an Entity-Relationship diagrammatic representation of the ***Calibration & Performance Extension*** (*Maroon boxes*) of the ***Core*** of the OME Data Model (*Red and Blue boxes*)[8], proposed by 4DN and BINA. The general structure and position of elements in the diagram is as described for Figure 6 and Supplementary Figures 1 and 2. A larger version of this image is available for download at: https://doi.org/10.5281/zenodo.4711229.

Supplementary Tables

Supplementary Table I: Extended view of the Tiers for fluorescence microscopy metadata collection 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

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.

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