doi:10.1017/S1431927620017225
Microsc. Microanal. 26 (Suppl 2), 2020

Quantitative Image Format for Electron Microscopy

Grigore Moldovan and Michael Zabel

point electronic GmbH, Halle (Saale), Sachsen-Anhalt, Germany

Abstract

Experimental data, simulation, data analysis and visualisation require image file formats that are open source and able to contain and manage quantitative data. Quantification techniques bring the new challenge of managing image calibration parameters and formulas in an open and efficient format, compatible with routine microscopy workflows. A practical approach to quantitative image format is presented and discussed here, relying on open and extensible file formats - Tagged Image File (TIF) and Extensible Metadata Platform (XMP).

Introduction

Quantitative is a keyword in electron microscopy used to describe that image or spectrum intensities present physical values related to the experiment and the specimen under investigation. It's generally used alongside a technique name, such as EELS, EDX, BSE or EBIC, because quantitative acquisition or workflow require special hardware and software not normally found in routine microscopy. Quantification is perhaps most popular in microanalysis techniques, such as BSE, EDX and EELS, where it is primarily used to measure composition. Further applications are found in electrical analysis techniques, such as EBIC and RCI, where it is used to measure properties such as internal quantum efficiency. A growing field for quantification are transmission based techniques, such as HAADF, where it's used to measure not only composition, but also thickness.

In a wider context, quantification may be first seen as an advanced approach of using electron microscopes to make measurements. From this initial characterisation-base approach, quantification is now progressing to a model-base approach where the output is a quantitative model of the specimen. In this context, quantification is key in providing a direct link between theoretical simulations and experimental data, particularly obvious for techniques that rely on iterative computation between model and experiment. A further need for quantification arrises from automation, where physical parameters of instrument and specimen are required in order to determine automatic settings for operation.

Hardware and software

Somewhat similar to analog photography and photometry, quantification in electron microscopy used to present difficult challenges in acquisition, as the operator had to perform additional steps related to calibration of the film, such as recording reference data, digitisation and intensity calibration. Whilst automated image analysis may replace some of the manual steps, including digitisation and intensity calibration, a modern quantitative workflow uses calibrated electronics that no longer require additional calibration steps. This is referred as 'standard-less' and solves much of the complexities relating to the hardware. However, calibrated electronics must be added to the microscope, as normal electronics do not have this advanced degree of design and production.

Software must also be able to manage the calibrated hardware and signals. At a basic level this includes management of signal name, value and unit, such as 'Voltage', '0.250' and 'V'. This therefore includes a transformation from raw intensity in Analog to Digital Unit (ADU) to experimental value in physical units, which in practice means management of an offset and scale from calibration. Software must then be able to also abstract differences in operation, such as brightness and contrast, and therefore it must manage further settings related to amplification and the respective formulas they apply onto signals. A typical example is provided by 'Contrast', which is an amplification gain with a value and a unit, which is abstracted away from the measured signal by the use of a formula that describes the inputs and outputs of the amplifier. This in turn means that the software must manage a complete model of the calibrated hardware, including labels, values, formulas and units - i.e. the calibration metadata.

Image metadata

Handling of quantitative data requires not only exporting measurements, which may be a plain text file of measurements, but also file saving. Saving presents additional challenges compared with conventional exports, which relate to ease of use, performance and asset management. These are solved in electron microscopy by adoption of popular image file formats, primarily Tagged Image File (TIF), which is widely used and open source (1). Whilst standard open-source metadata formats, such as defined for TIFF, Image File Directory (IDF) or Exchangeable Image File (EXIF) provide partial support for custom information, such as 'MakerNote' in EXIF, they lack an open and extendable infrastructure to include complex items such as labels, units and formulas.

It is proposed here that a good metadata infrastructure is provided by the standard and open-source Extensible Metadata Platform (XMP) format (2). This may be embedded in TIF and JPEG image headers, and it is written in plain text, so easily inspected using a text editor. XMP allows for introduction of new namespaces to fully describe the calibration information, all in the Extensible Markup Language (XML) format that is easily read and parsed. XMP is also widely supported across development environments, from C# to Python, and therefore an ideal medium for supporting extended metadata.

An example is given in Figure 1, showing XMP metadata from a calibrated image file acquired using Digital Image Scanning System (DISS6) with a Multi-channel Signal (MICS) amplifier and a detector with embedded preampfification. As illustrated, namespaces are given for each device (Detector, MICS and DISS6), each with a section listing their settings when the image was acquired. A transformation namespace is then used to describe the ADU to physical value calibration, followed by a formula namespace describing the signal amplification. This metadata therefore contains all the information required to determine input physical values from the raw 16-bit pixel data.

https://doi.org/10.1017/S1431927620017225 Published online by Cambridge University Press

```xml
<x:xmpmeta xmlns:x="adobe:ns:meta/" x:xmptk="XMP Core 5.1.2">
  <rdf:RDF xmlns:rdf="http://www.w3.org/1999/02/22-rdf-syntax-ns#">

    <rdf:Description rdf:about=""
        ...
        xmlns:detectors="http://ns.pointelectronic.com/Detectors/1.0/">
      <detectors:Items>
        <rdf:Seq>
          <rdf:li rdf:parseType="Resource">
            ...
            <ddet:PreAmpGain1>1e-7</dseg:PreAmpGain>
          </rdf:li>
        </rdf:Seq>
      </detectors:Items>
    </rdf:Description>

    <rdf:Description rdf:about=""
        xmlns:mics="http://ns.pointelectronic.com/MICS/1.0/">
      <mics:Gain1>2</mics:Gain1>
      <mics:InputOffset1>-0.000716572031</mics:InputOffset1>
      <mics:OutputOffset1>-0.00316389565</mics:OutputOffset1>
      ...
    </rdf:Description>

    <rdf:Description rdf:about=""
        ...
        xmlns:d6tr="http://ns.pointelectronic.com/DISS6/1.0/types/Transformation#">
      <diss6:ChannelInfo rdf:parseType="Resource">
      </diss6:ChannelInfo>
      <diss6:ChannelId>S1</diss6:ChannelId>
      <diss6:Transformation rdf:parseType="Resource">
        <d6tr:Scale>1</d6tr:Scale>
        <d6tr:Offset>-0.5</d6tr:Offset>
        <d6tr:Unit>V</d6tr:Unit>
      </diss6:Transformation>
      ...
    </rdf:Description>

    <rdf:Description rdf:about=""
        xmlns:formulas="http://ns.pointelectronic.com/Formulas/1.0/"
        xmlns:fml="http://ns.pointelectronic.com/Formulas/1.0/types/Formula#">
      <formulas:Items>
        <rdf:Seq>
          <rdf:li rdf:parseType="Resource">
            <fml:Formula>(({diss6:S1} - {mics:OutputOffset1}) / {mics:Gain1} - {mics:InputOffset1}) / {ddet:PreAmpGain1}</fml:Formula>
            <fml:Name>Detector1 Formula</fml:Name>
            <fml:Unit>A</fml:Unit>
            <fml:Label>Detector1 Current</fml:Label>
          </rdf:li>
          ...
        </rdf:Seq>
      </formulas:Items>
  </rdf:RDF>
</x:xmpmeta>
```

Figure 1. Example of XMP metadata embedded in TIF image file, containing all information required for quantification.

References

(1) Tagged Image File (TIF) format**,** https://www.adobe.io/open/standards/TIFF.html
(2) Extensible Metadata Platform (XMP) format, https://www.adobe.io/open/standards/xmp.html

https://doi.org/10.1017/S1431927620017225 Published online by Cambridge University Press