

EER File Format

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ThermoFisher
SCIENTIFIC

Version history

Version	Date	Changes	Author(s)
1.0	30 Aug 2019	Initial version	P. Bootsma
2.0	02 Dec 2019	Added 7-bit compression	A. Dumitrescu
2.1	10 Dec 2019	Added Final image directory	M. Balasubramanian
2.2	17 Jan 2020	Added acquisition metadata	A. Dumitrescu, P. Bootsma
2.3	25 Feb 2021	Added orientation	D. van der Steen
2.4	1 April 2021	Update acquisition metadata	L. Oosterhof
2.5	4 April 2021	Update Fields	D. van der Steen
2.6	11 May 2021	Sensor pixel size units changed to meters	G. Singla
2.7	18 May 2021	Update acquisition metadata	G. Singla
2.8	20 May 2021	Added camera name	D. van der Steen
2.9	15 Sep 2021	Removing camera type from metadata	G. Singla
3.0	03 Mar 2023	Added compression details	D. van der Steen

References

No	Title	Author(s)
[TIFF]	TIFF Revision 6.0	Adobe Systems Inc.
[BIGTIFF]	The BigTIFF File Format	Aware Systems

Introduction

Some cameras can produce EER encoded images. These images are stored in a file format called the EER file format, identified by the extension “.eer”. EER files are based on BigTIFF, but define a number of extensions to this format to store all EER-related information in one file.

The EER file writer and the exact file format are still in development. This document describes the currently implemented file format. The following features are planned for future inclusion in EER files:

- Gain reference data
- Defect mask
- Frame metadata

Note that these are the current plans, and that plans can change.

BigTIFF Container Format

The EER file format is an extension of the BigTIFF file format defined by Aware Systems [BIGTIFF]. BigTIFF is an extension of the regular TIFF file format [TIFF] to support files of more than 4 gigabyte. EER files add a custom compression scheme for the EER data and custom tags to include the relevant metadata.

This document describes how EER data is stored within the BigTIFF container format. Interpretation of the data is outside of the scope of this document. The LibTIFF library [<http://simplesystems.org/libtiff>] can read BigTIFF files, and therefore also EER files.

TIFF is a flexible container format by nature, and readers should adhere to a set of requirements as specified in section 7 of the TIFF standard. The following requirements are the most important ones to ensure future extensibility of the file format:

- Entries with unknown tag number or field type must be ignored.
- Image file directories with an unexpected compression scheme must be skipped.

Extensions

This section describes the extensions added to the BigTIFF container format.

Compression schemes

The EER file format extends the default compression scheme of BigTIFF. This value is used in the compression field (tag 259) to indicate that an IFD contains EER compressed data (see Image File Directories).

The subpixel information is encoded in subpixel bits, the number of bits used may vary per compression scheme. The total number of subpixels can be derived based on the number of horizontal and vertical bits:

$$\text{number of subpixels} = 2^{(\text{bits}_{\text{horizontal}} + \text{bits}_{\text{vertical}})}$$

e.g., when using a subpixel depth of 2 for both horizontal and vertical the number of subpixels is:

$$\text{number of subpixels} = 2^{(2+2)} = 16$$

Fields

The EER file format adds several fields to BigTIFF. These fields can be present in IFDs as indicated later in this document.

Field	Tag	Type	Comment
Acquisition Metadata	65001	STRING	A non-zero-terminated string containing metadata concerning the entire acquisition.
Final image metadata	65006	STRING	A non-zero terminated string containing metadata concerning the final image
Frame metadata	65002	STRING	A non-zero terminated string containing metadata concerning a single frame

Image File Directories

An EER file contains one or more image file directories (IFDs). Every IFD contains either an EER frame or other image data.

EER Frames

EER compressed data is stored like a bilevel image in a regular TIFF. It is stored in one or more strips. If multiple strips are used, the entire frame can be reconstructed by concatenating the data in the separate strips. An EER frame contains required and optional fields. Unknown or unexpected fields must be ignored.

Required fields

Field	Tag	Type	Count	Comment
Image Width	256	SHORT or LONG	1	The number of columns in the frame
Image Length	257	SHORT or LONG	1	The number of rows in the frame
Compression	259	SHORT	1	The compression scheme used; the following values are possible: <ul style="list-style-type: none"> - 65000: <ul style="list-style-type: none"> o 8-bit EER compressed data o 2 bits horizontal subpixel information o 2 bits vertical subpixel information - 65001: <ul style="list-style-type: none"> o 7-bit EER compressed data o 2 bits horizontal subpixel information o 2 bits vertical subpixel information - 65002: EER compressed format is in the "Compression Details" (see optional fields)
Rows Per Strip	278	SHORT or LONG	1	The number of rows in each strip (except possibly the last strip, which can contain less rows)
Strip Offsets	273	SHORT, LONG or LONG8	1	For each strip, the byte offset of that strip
Strip Byte Counts	279	SHORT, LONG or LONG8	1	For each strip, the number of bytes in that strip
Orientation	274	SHORT	1	The orientation of the frame

Optional fields

Field	Tag	Type	Count	Comment
Run Length Encoding Bit Depth	65007	SHORT	1	Only available when 65002 in “Compression” tag is used. The number of bits for the RLE skip count.
Subpixel Horizontal Bit Depth	65008	SHORT	1	Only available when 65002 in “Compression” tag is used. The number of bits to provide horizontal subpixel information.
Subpixel Vertical Bit Depth	65009	SHORT	1	Only available when 65002 in “Compression” tag is used. The number of bits to provide vertical subpixel information.

Camera compression details

The compression version used per camera may differ, the following versions are used:

Camera name	Compression Version	Supported until
Falcon 4	65001; 7-bit/subpixel bits 2 vertical and horizontal	2025
Falcon 4i	65001; 7-bit/subpixel bits 2 vertical and horizontal	2025
Falcon C	65002; 7-bit/subpixel 1 bit vertical and horizontal	

Optional fields

There are no optional fields specific to EER.

Final Image

An IFD containing the final pipeline image is included as part of the EER file if a final image was recorded with the acquisition. When included, the final image is always in the first IFD of the EER file. The presence of a final image in the first IFD is identified by the compression tag, which is set to no compression in case of a final image, as opposed to EER frames. Currently, the EER file format only supports EER frames and one optional final image frame.

Required fields

Field	Tag	Type	Count	Comment
Image Width	256	SHORT or LONG	1	The number of columns in the frame
Image Length	257	SHORT or LONG	1	The number of rows in the frame
Compression	259	SHORT	1	The compression scheme used; COMPRESSION_NONE (1) for final image data
Bits per sample	258	SHORT	1	The number of bits per channel. For final image data, it is 16 bits per sample (2 bytes)
Rows Per Strip	278	SHORT or LONG	1	The number of rows in each strip (except possibly the last strip, which can contain less rows)

Strip Offsets	273	SHORT, LONG or LONG8	1	For each strip, the byte offset of that strip
Strip Byte Counts	279	SHORT, LONG or LONG8	1	For each strip, the number of bytes in that strip
Orientation	274	SHORT	1	The orientation of the frame. For final image data, it is always TOPLEFT

Metadata

Metadata is stored in tags for the acquisition, frames and the final image. These tags contain an XML string structured as follows:

```
<metadata>
  <item name="[name]" unit="[unit]">[value]</item>
  <item name="[name2]">[value2]</item>
  ...
</metadata>
```

The unit attribute may be omitted for values that do not have a unit.

Acquisition metadata

In the first IFD metadata is written that applies to the entire acquisition. The metadata is written as a tag with ID 65001.

Name	Unit	Comments
acquisitionID		unique acquisition identifier (or label) used to identify the data set for access and offloading
cameraName		Camera name used for the acquisition
coincidenceCompensatedDose	e/pixel	The coincidence compensated dose per pixel for the entire acquisition
coincidenceCompensatedDoserate	e/pixel/s	The coincidence compensated dose rate per pixel for the entire acquisition
commercialName		The commercial name of the camera
eerGainReference		Relative location of the EER gain reference that was valid at the time of the EER acquisition
exposureTime	s	The duration of the acquisition
meanDoseRate	e/pixel/s	Average amount of electrons per pixel per second during the acquisition
numberOfFrames		Total number of frames in the acquisition
sensorImageHeight	pixels	The height of the sensor
sensorImageWidth	pixels	The width of the sensor
sensorPixelSize.height	m	The height of a pixel
sensorPixelSize.width	m	The width of a pixel
serialNumber		Serial number of the sensor. Used to identify the camera or relate set to EER gain references
timestamp	ISO 8601	Time synchronized with the TEM PC including time zone information, in millisecond accuracy

totalDose	e/pixel	Average amount of electrons per pixel in the entire acquisition
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Final image metadata

The first IFD will contain final image metadata when the final image is recorded. The metadata is written as a tag with ID 65006 and contains:

Name	Unit	Comments
roi.top		Top coordinate of the region of interest for the image in sensor coordinates, where 0 is the topmost pixel.
roi.left		Left coordinate of the region of interest for the image in sensor coordinates, where 0 is the leftmost pixel.
roi.bottom		Bottom coordinate of the region of interest for the image in sensor coordinates. The value is exclusive, so for a 4096 x 4096 image the top coordinate will be 0 and the bottom coordinate will be 4096.
roi.right		Right coordinate of the region of interest for the image in sensor coordinates. The value is exclusive, so for a 4096 x 4096 image the left coordinate will be 0 and the right coordinate will be 4096.
binning		The amount of pixels that have been binned together.
darkCorrection		A yes/no indication on whether the image is dark corrected
gainCorrection		A yes/no indication on whether the image is gain corrected.
numberOfFrames		The number of frames in the final image
timestamp	ISO 8601	Time synchronized with the TEM PC including time zone information, in millisecond accuracy
pixelValueToCameraCounts		The conversion from a pixel value to a camera count
countsToElectrons		The conversion from camera counts to electrons. The following formula gives the dose from 1 pixel: <i>pixelValue</i> x <i>pixelValueToCameraCounts</i> x <i>countsToElectrons</i>
exposureTime	s	The exposure time for the final image
meanPixelValue		The average pixel value of the final image
checksum		An indication on whether the checksum was valid.

driftCorrectionInformation.driftCorrected		A yes/no indication on whether drift correction was enabled.
driftCorrectionInformation.confidence		A confidence indication for the drift vectors ranging from 0 to 1.
driftCorrectionInformation.clipping		A yes/no indication on whether either vector was clipped.
driftCorrectionInformation.vectorXCoordinate		The drift in X direction
driftCorrectionInformation.vectorYCoordinate		The drift in Y direction

Frame metadata

All IFD's, except for the final image, will contain frame metadata. The metadata is written as a tag with ID 65002 and contains:

Name	Unit	Comments
frameID		The sequence number of the frame in the total acquisition
timestamp		The time at which the frame was acquired, expressed as a ISO8601 representation with timezone indication.
orientation		The TIFF orientation value
decompressionAlgorithmVersion		The algorithm that was used to process the EER data.
pixelFormat		The pixel format
rleCodeLength		The run-length-encoding code length
nrOfSubPixelPerDirection		The number of sub pixels per pixel in width and height
dose	e/pixel	The average dose per pixel in the frame