

ZISRAW (CZI) File Format

Design specification

V 1.2.2

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1 General

1.1 Definitions and wording

Within this document we focus on the description of the CZI format for image storage. The data storage schema itself is more fundamental and is defined by ZISRAW, which means "Zeiss Integrated Software RAW" data format.

This format is intended to be a general purpose data format to be used in other parts of the software to store streamed data in a binrary file with some XML and binary metadata attached.

Currently, CZI is the only ZISRAW based implementation in the ZEN software.

1.2 Requirements

The definition of this file format was inspired by requirements for format simplicity (flat file), maximum data safety and performance but should also provide flexibility for future extensions.

1.3 Revision History

- V 1.01 added CZFOC Attachment schema (focus positions in micrometers)
- V 1.02 OME acknowledgements
- V 1.03 V Index
- V 1.04 added MVM attachment
- V 1.04a added Label and Prescan attachment
- V 1.05 general review
- V 1.06 ZEN black topography images (see ZEN black Topography images)
- V 1.07 fixed compression value for JPG/XR
- V 1.1 adapted to latest ZEN release version
- V 1.2 Topography Image Extensions

1.4 Acknowledgements

Applies to Metadata definitions

This format has been developed to be as close as possible to the OME specification, Copyright 2002-2011 OME (Open Microscopy Environment). The XSD has been defined to show a maximum compatibility with the OME tiff and XML data formats while maintaining the essential requirements to run Carl Zeiss ZEN software optimally. Carl Zeiss Microscopy GmbH acknowledges the copyright of OME and those parts of the czi format which are similar to the OME xml scheme are marked appropriately.

2 Implementation overview

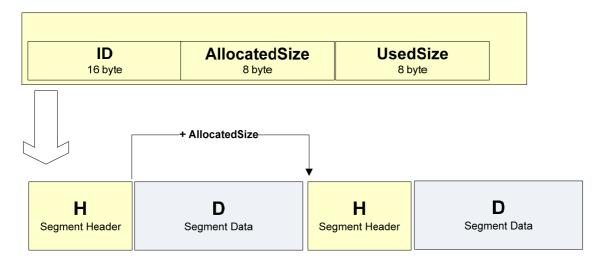
2.1 Segments and chaining

ZISRAW / CZI was designed to enable streaming of huge amount of data with a maximum of data safety.

The chosen architecture is a chain of "segments". Each segment is identified by a header with defined Identifier (SID). Segments are aligned on 32 byte boundaries. This improves the speed of the recovery process when rescanning the file in case of system crashes etc. A single search step in case of a missing segment header can move to the next multiple of 32 bytes instead of advancing byte by byte.

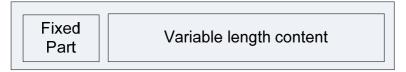
A SegmentHeader consists of

- A unique 16 byte ANSI character **ID**, each prefixed with "ZISRAW"
- An allocated size (**AllocatedSize**) determining the space reserved for the segment data. Using the value, the reader can advance to the next segment. Allocated Size is always a multiple of 32 bytes.
- A usage size (**UsedSize**) determining the number of bytes already used in this segment. If zero, it is assumed that UsedSize is equal to AllocatedSize.



2.2 Segment Data

A segment's data consists of a defined fixed part and a variable length part.



The reader of a segment will first read the fixed part to determine the content and the data size of the variable part.

The data structure of the fixed part is given by the segment ID (SID) of the segment header.

2.3 Main content data types

2.3.1 Pixels (ImageSubBlock)

An **ImageSubBlock** contains the image pixels and closely related metadata. Raw, uncompressed pixel blobs may be multi-dimensional, e.g. stacks of 2D images.

The dimensionality is given by a definition structure array using an entry for each of the contained dimension. This entry defines the logical and physical (stored) size in means of pixels. The sequence of the entries defines the storage sequence within the pixel Blob.

2.3.2 Common Metadata

Each file has one Metadata segment containing

- an XML string matching a defined schema
- a binary section with additional data in ZIP format.

2.3.3 Attachments (Embeddings)

Any kind of content, e.g. TimeStamp Blobs can be embedded in the storage using the **Attachment** segments. Attachments are identified using a **name** instead of the dimension information used by the **SubBlock** segment.

Reserved names are e.g.: "Preview" and "Thumbnail" which are JPG files to be used in shell extensions and image browsers.

2.4 SubBlock / Attachment - Directories

Readers can access the whole file using the segment's *AllocatedSize* to advance from segment to segment. This may be time consuming with many segments, so the most commonly used segment types (*SubBlock* and *Attachment*) have **Directory** information parts that are cached in a summary segments.

A **SubBlock** Segment contains a directory entry (currently: <u>DV-variable length</u> schema) with index /and size information as each *SubBlock* is identified by its bounds in a x-dimensional hyperspace and resolution (sub/supersampling)



An **Attachment** segment contains a directory entry (currently: Schema A1) with a name and a globally unique identifier (GUID).



Each DV/E or A1 entry contains the file part and the binary segment offset within the file.

The corresponding summary segments are **SubBlockDirectory** and **AttachmentDirectory**. They both contain a fixed part, followed by a list / array of DV or A1 entries. In most situations, this provides enough information about the related item, so full loading of the corresponding segment can be deferred until really needed.

3 Container

3.1 Byte order

Data is stored in "little-endian" (x64 compatible) format.

3.2 General

Data storage is based on "**Segments**" with defined schemas. Each of those segments contains data in Binary (Pixels, attachments) or XML format.

In any scenario, there is at least one master ZISRAW file containing the common directories and metadata.

The following storage scenarios are possible:

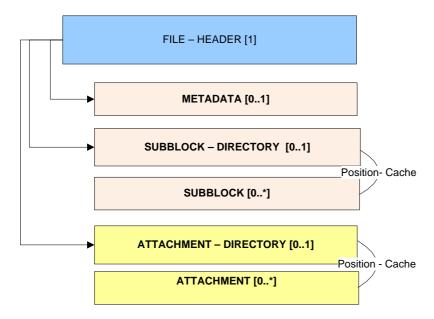
- A single file.
- Multi File (master file and file parts)
- [planned] CZI container file and pixel data in external files. Pixels may be saved as JPG / TIFF etc.

3.3 File structure

Each ZISRAW file consists of one or more of the following segment types:

- **FileHeader** one segment per file.
- Metadata zero or one segment per file.
- **SubBlock** one segment per SubBlock optionally containing (XML) metadata and attached binary data like a thumbnail representation.
- **SubBlockDirectory** directory of subblocks zero or one segment per file. If a SubBlock segment exists, the *SubBlockDirectory* is required.
- Attachment one segment per attachment (embedding) identified via name. Contains any kind of data.
- **AttachmentDirectory** directory of attachments zero or one segment per file (optional). The directory contains the names and file offsets of the attachment segments. If an Attachment segment exists, the **AttachmentDirectory** is required.

New segment types may be introduced as required without breaking compatibility.



3.4 Single File Container

All data is contained in the same physical file. All file references are offsets into this file.

3.5 Multi – File container

The multi – file scenario is used in the following contexts:

- Avoid exceeding a certain size limit for a single file (e.g. a DVD chunk size)
- Split a multi-dimensional data storage based on the contained dimension to get independent files (e.g. one file per Z-Stack) to be used either stand-alone or in the context with others.

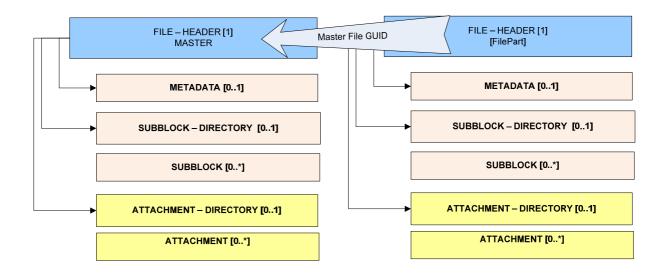
Each of the files of the multi-file storage is a complete valid image – maybe with its own thumbnail and preview.

If the user opens the **MasterFile** storage, the file parts are opened in addition and the contents of the various Directories are merged. The original file part number(s) is/are remembered.

When saving a multi-file based data storage, each file part directory is written separately using the **FilePart** information in the directory entry.

The following information is contained in the MasterFile only:

- The **Experiment** attachment segment
- The Metadata segment
- The **Preview** and **Thumbnail** attachments.



3.5.1 Multi-File naming schema

Using a strict naming schema we can avoid the need for embedding file names and enable renaming on file basis.

Master File: Name.czi
File Part 1 Name (1).czi
File Part 2 Name (2).czi
File Part 3 Name (3).czi

The names "File (x). czi " never appear in the stored data, only the part numbers (1 .. 3).

The naming pattern was chosen to be compatible with the *Windows Explorer* multi-file renaming implementation. The user may multi-select all files in the multiple-file set – renaming the master file will also rename the file parts. The space between the name and the file part (in brackets) is optional, but it is added when renaming the files in *Windows Explorer*.

3.6 Recommendations for multi-file usage

When using the multi-file schema, it is recommended to follow those conventions:

- Use a separate folder for each file set (even if it is possible to store multiple file sets in a single folder).
- Name the folder according to its master file, e.g. folder "Name" contains Name.czi, Name (1).czi, Name (2).czi etc.

4 Data Storage - Segment schemas

4.1 Segment header

SchemaID = SegmentHeader

| 2.5 | | | | | | |
|---------------|--------|--------|------|---|--|--|
| Item | Type | Offset | Size | Comments | | |
| Id | Byte[] | 0 | 16 | A sequence of up to 15 Ansi – characters 'A''Z', e.g. "ZISSUBBLOCK" | | |
| | | | | The special name "DELETED" marks a segment as deleted – readers should ignore or skip this segment. | | |
| AllocatedSize | Int64 | 16 | 8 | The total numer of bytes allocated for this segment. | | |
| UsedSize | Int64 | 24 | 8 | The currently used number of bytes. | | |

Segment headers are aligned on 32 byte boundaries. A reader knowing nothing about the content of a segmented file can read the 32 byte header, take the first 15 characters as display text and then use **AllocatedSize** to advance the file pointer to the next segment.

4.1.1 Currently defined segment IDs (SID)

| SID | Comments |
|-----------------|---|
| ZISRAWFILE | File Header segment, occurs only once per file. The segment is always located at position 0. |
| ZISRAWDIRECTORY | Directory segment containing a sequence of "DirectoryEntry" items. |
| ZISRAWSUBBLOCK | Contains an ImageSubBlock containing an XML part, optional pixel data and binary attachments described by the <i>AttachmentSchema</i> within the XML part. |
| ZISRAWMETADATA | Contains Metadata consisting of an XML part and binary attachments described by the AttachmentSchema within the XML part . |
| ZISRAWATTACH | Any kind of namend Attachment, some names are reserved for internal use. |
| ZISRAWATTDIR | Attachments directory. |
| DELETED | Indicates that the segment has been deleted (dropped) and should be skipped or ignored by readers. |

If a segment update would result in an overflow (**UsedSize** would exceed **AllocatedSize**), the segment is marked **DELETED** and a new segment is added to the end of the file.

Additional schemas may be defined if required.

4.2 File (Header) Segment

One segment per file.

4.2.1 Overview

SID = ZISRAWFILE

FileHeaderSegment 512 Byte (binary)

Minor/Major Version
PrimaryFileGuid
FileGuid
FilePart
DirectoryPosition
MetadataPosition
AttachmentDirectoryPosition

4.2.2 Segment content schema

SchemaID = FileHeader

| Item | Туре | Offset | Size | Comments |
|-----------------|------|--------|------|---|
| Major | Int | 0 | 4 | "1" |
| Minor | Int | 4 | 4 | "0" |
| Reserved1 | Int | 8 | 4 | - |
| Reserved2 | Int | 12 | 4 | - |
| PrimaryFileGuid | GUID | 16 | 16 | Unique Guid of Master file (FilePart 0) |
| FileGuid | GUID | 32 | 16 | Unique Per file |

| FilePart | Int32 | 48 | 4 | Part number in multi-file scenarios |
|---------------------------------|-------|----|---|--|
| DirectoryPosition | Int64 | 52 | 8 | File position of the SubBlockDirectory Segment |
| MetadataPosition | Int64 | 60 | 8 | File position of the Metadata Segment. |
| UpdatePending | Bool | 68 | 4 | 0xffff, 0 |
| | | | | This flag indicates a currently inconsistent situation (e.g. updating Index, Directory or Metadata segment). |
| | | | | Readers should either wait until this flag is reset (in case that a writer is still accessing the file), or try a recovery procedure by scanning all segments. |
| AttachmentDirectory Position | Int64 | 72 | 8 | File position of the AttachmentDirectory Segment. |

Single file: **PrimaryFileGuid** and the **FileGuid** are identical. The FilePart is 0.

Multi file: In the master file, the **PrimaryFileGuid** and the **FileGuid** are identical. In file Parts, the **PrimaryFileGuid** is the Guid of the master file and **FileParts** are > 0.

4.3 Metadata segment

One segment per file. This segment is used for global (once per image) metadata. The current Segment Position (seek offset) within the file is available from the file header (**MetadataPosition**)

4.3.1 Overview

SID = ZISRAWMETADATA

MetadataSegment (header part)

256 Byte (binary)

XmlSize AttachmentSize

Xml (1)

<lmageDocument>

/ImageDocument>

Attachment (0..1)

4.3.2 Segment content schema

SchemaId = *MetadataSegment*

| Item | Туре | Offset | Size | Comments |
|-----------------|-----------------|--------|-------|---|
| XmlSize | Int32 | 0 | 4 | Size of the XML data. |
| AttachmentSize | Int32 | 4 | 4 | Size of the the (binary) attachments. NOT USED CURRENTLY. |
| <spare></spare> | <spare></spare> | 8 | 256-8 | |

4.3.3 XML

XML data is stored as a string using UTF8 encoding.

4.4 SubBlock Segment

A SubBlock contains pixel data for a homogenous set / subset of the image. In light microscopy applications, most of the SubBlocks are two–dimensionsional where X and Y represent the camera frame. Confocal acquisition modes require other or more dimensions.

4.4.1 Overview

SID = ZISRAWSUBBLOCK

SubBlockSegment (header part)

MetadataSize DataSize AttachmentSize

Directory entry

SubBlock-Metadata (0..1)

<Metadata> <DataSchema/> <AttachmentSchema/> <../>
</Metadata>

Data (0..1)

Pixels

(Compressed / Uncompressed / RAW)

Attachment (0..1)

4.4.2 Segment content schema

SchemaId = SubBlockSegment

| Item | Туре | Offset | Size | Comments |
|----------------|------------------|---|-----------------------------------|--|
| MetadataSize | Int32 | 0 | 4 | Size of the metadata section. |
| AttachmentSize | Int32 | 4 | 4 | Size of the optional attachment section. |
| DataSize | Int64 | 8 | 8 | Size of the data section. |
| DirectoryEntry | DirectoryEntryDV | 16 | variable | Subset indices and size information, a 1:1 copy will be stored as part of the File's SubBlockDirectory Segment. The length of this information depends on the directory schema. |
| Fill | | n = variable + 16 | Max(256 – n, 0) | Fill segment up to Minimum of 256 bytes |
| | | | | |
| [Metadata] | String | off = Max(256, n) | <metadatasize></metadatasize> | Metadata |
| [Data] | <any></any> | off + <metadatasize></metadatasize> | <datasize>></datasize> | Data (Pixels) |
| [Attachments] | <any></any> | off + <metadatasize> +<datasize>></datasize></metadatasize> | <attachmentsize></attachmentsize> | Attachments (binary, text, etc, may be complete files, e.g. a ZIP file) |

Thus, the offset of the metadata section is 256 or the end of the directory entry if the size of the directory entry is greater than 240 (256-16)..

4.4.3 Metadata (XML)

XML data is stored as a string using UTF8 encoding.

4.4.4 Attachment

Optional attachments. Schema available in the AttachmentSchema node of the Metadata

4.4.5 Data

Main data (Pixels) either compressed or uncompressed as indicated by the "Compression" mode in the Directory part.

4.4.6 Directory Entry – Schema DV [Directory Variable length]

This schema uses a variable length entry to represent an variable number of dimensions. Each entry is represented by a **DimensionEntry** structure of one of *DimensionEntryDV*.

SchemaID = *DimensionEntryDV1(20 bytes)*

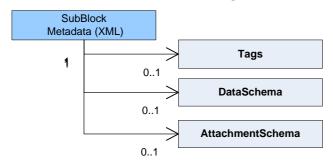
| Senemals Dimensionaling (20 dynes) | | | | | | |
|------------------------------------|---------|--------|------|--|--|--|
| Item | Туре | Offset | Size | Comments | | |
| Dimension | Byte[4] | 0 | 4 | Typically 1 Byte ANSI e.g. 'X', see <u>Dimensions / dimensions indices</u> | | |
| Start | Int32 | 4 | 4 | Start position / index. May be < 0. | | |
| Size | Int32 | 8 | 4 | Size in units of pixels (logical size). Must be > 0. | | |
| StartCoordinate | Float | 12 | 4 | Physical start coordinate (units e.g. micrometers or seconds) | | |
| StoredSize | Int32 | 16 | 4 | Stored size (if sub / supersampling, else 0) | | |

SchemaID = *DirectoryEntryDV* (32 bytes + EntryCount * 20)

| Item | Туре | Offset | Size | Comments |
|------------------|----------------------|--------|--------------------------------|--|
| SchemaType | Byte[2] | 0 | 2 | "DV" |
| PixelType | Int32 | 2 | 4 | The type of the image pixels, see <u>PixelTypes</u> . |
| FilePosition | Int64 | 6 | 8 | Seek offset of the referenced SubBlockSegment relative to the first byte of the file |
| FilePart | Int32 | 14 | 4 | Reserved. |
| Compression | Int32 | 18 | 4 | See Compression constants |
| PyramidType | Byte | 22 | 1 | [INTERNAL] Contains information for automatic image pyramids using SubBlocks of different resolution, current values are: None=0, SingleSubblock=1, MultiSubblock=2. |
| spare | Byte | 23 | 1 | Reserved |
| spare | Byte[4] | 24 | 4 | Reserved |
| DimensionCount | Int32 | 28 | 4 | Number of entries. Minimum is 1. |
| DimensionEntries | DimensionEnt ryDV | 32 | Dime nsion Count * 20 | Variable length array of dimensions. Each dimension can occur only once. |

4.5 SubBlock specific Metadata

The small Metadata section for each **ImageSubBlock** defined to have the following content:



4.5.1 Tags

Tags is a list of Name/Value pairs to provide any kind of additional information.

Sample:

```
<Tags>
    <FocusPosition>10.34</FocusPosition>
    <StageXPosition>103.4</StageXPosition>
    <StageYPosition>203.4</StageYPosition>
    <AcquisitionTime>2010-05-30T09:30:10.5</AcquisitionTime>
</Tags>
```

Physical positions

| Thysical positions | | | | |
|--------------------|----------|--|--|--|
| Tag | Туре | Info | | |
| FocusPosition | double | Focus position in micrometers. | | |
| AcquisitionTime | DateTime | Acquisition Time in Xml "RoundTrip" format, e.g. 2010-05-30T09:30:10.5 | | |
| StageXPosition | double | Stage axis X position in micrometers. | | |
| StageYPosition | double | Stage axis Y position in micrometers. | | |

4.5.2 DataSchema

The optional **DataSchema** node carries information about the structure of the data contained in the *Data* section. This can be the pixel format identifier, the pixel component details (e.g. BGR Triples, XYZ tokens) etc. and also data to decode the pixels if they are in RAW format.

Most elements within this schema are optional. for pixels encoded using a "Raw"-encoded format (AxioCam etc), RawDeoderParameters and RawDecoderContextId are mandatory.

Image files using Raw formats can be decoded into standard formats using specific conversion tools. The "Raw" encoding schemas are not part of this documentation.

| Name | Type | Info |
|----------------------|-------------|---|
| DataFormat | String | Information about the data format. Valid values are: |
| | | • Pixels (default) = n-dimensional pixel array— each pixel is defined by the PixelType identifier in the directory header. |
| Name | String | A user defined name. |
| MinValue | Double | Minimum value of a pixel. For Composite / RGB types, the minimum component value. |
| MaxValue | Double | Maximum value of a pixel. For Composite / RGB types, the maximum component value. |
| LowValue | Double | Recommended normalized Low value for display mapping with respect to the range of the date value. A value of 0 means: start mapping with data value 0. |
| HighValue | Double | Recommended normalized High value for display mapping with respect to the range of the date value. A value of 1.0 means: the maximum value for the given data type. |
| ValidBitsPerPixel | Int | The number of bits with meaningful value. Will be set to 12 for 12 Bit and to 14 for 14 Bit sensors. For multi-component types, the value is the sum of all components, e.g. 12 Bit RGB camera has a ValidBitsPerPixel of 36. |
| RawDecoderParameters | XML Node | Decoder parameters as Xml fragment. The decoder will de-serialize this information if required. |
| RawDecoderContextId | String | The Id (name) of an attachment containing additional binary data for the image decoder. |

4.5.3 AttachmentSchema

This optional AttachmentSchema node contains storage details of the data in the "Attachments" section.

<AttachmentSchema>

<DataFormat>MeasurementRegion/DataFormat>

</AttachmentSchema>

DataFormat for attachment schema

| Name | Type | Info |
|------------|--------|---|
| DataFormat | String | Information about the attachment data format. |
| | | |

4.5.4 Chunk-Container

A chunk-container is denoted by the DataFormat "CHUNKCONTAINER". The purpose of a chunk-container is to put several variable-size data-blocks into a single blob. Its layout is:

| Offset | Type | Purpose |
|------------|--------|---|
| 0 | Guid | Identifies the type of the payload |
| 16 | Int | Size of payload |
| 2020+size | Byte[] | The first chunk (payload) |
| 20+size | Guid | (optionally) identifes the type of the (next) payload |
| 20+size+16 | Int | Size of payload |
| 20+size+20 | Byte[] | The second chunk (payload) |

A chunk starts with a Guid which uniquely identifies what kind of data is to be found at the payload-section. The Guid is followed by an integer which gives the size of the payload that immediately follows. If there is a second chunk in the chunk-container, it will immediately follow after the payload (with its Guid, size and payload).

The size of the chunk-container is implictly given (because it is put into an attachment section), and the total size of the chunk-container is used to terminate the search for a new chunk. That is, the last chunk has been found if the offset pointed to by its size-field is greater or equal than the size of the whole attachment. The steps to enumerate all chunks are therefore (under the precondition, that its total size is known):

- 1. Read chunk-header
- 2. Determine offset of next chunk
- 3. If offset of next chunk \geq total size of attachment \rightarrow stop
- 4. Goto step 1

4.5.5 Mask

A mask is a bitonal bitmap (i.e. one bit per pixel) with the same size as the bitmap in a subblock. It is commonly used to give information about the validity of each pixel. A mask is commonly wrapped into a chunk-container, and put into a subblock-attachment.

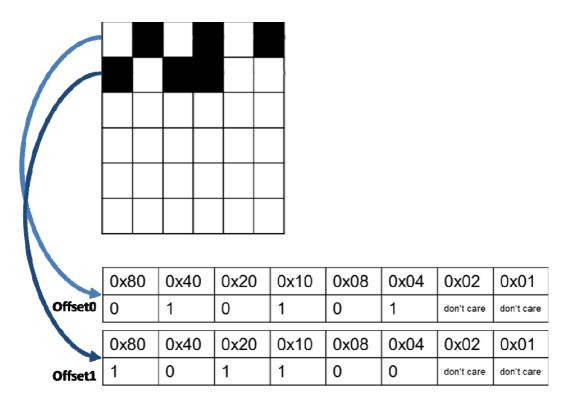
The memory layout is:

| Offset | Type | Name | Comment |
|--------|--------|--------------------------------|----------------------------------|
| 0 | Int | width | The width (specified in pixels) |
| 4 | Int | height | The heigth (specified in pixels) |
| 8 | Int | Type of representation | |
| 12 - ? | Byte[] | Specific to the representation | Depends on the previous field |

Type of representation informs how the mask is represented. Currently, the only defined type is "0" which means "represented as a uncompressed bitonal bitmap" – for this type, the memory-layout is as follows:

| Offset | Type | Name | Comment |
|---------------------------------------|--------|--|---|
| 0 | Int | width | The width (specified in pixels) |
| 4 | Int | height | The heigth (specified in pixels) |
| 8 | Int | Type of representation = 0 | The following is only valid for "type of representation = 0"! |
| 12 | Int | Stride | Length of a line specified in bytes |
| 1616+stride-1 | Byte[] | (first line of) mask data in bitmap representation | |
| (16+stride) (16+ stride*Height- 1) | Byte[] | (second to height) lines of mask | |

In the bitmap-representation, pixels are written in a "highest value to the left"-fashion. This means, that the pixel with x=0 is stored in in the highest order bit in byte[0] of the mask. The following diagram may clearify this:

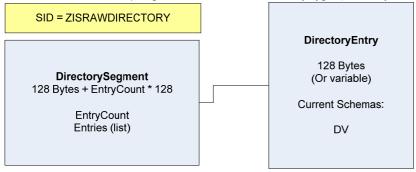


4.6 SubBlock - Directory

One segment per file.

4.6.1 Overview

The SubBlockDirectory segment contains entries of any type (currently the only supported schema is DV).



4.6.2 Segment content schema

SchemaID = *SubBlockDirectorySegment*

| Item | Туре | Offset | Size | Comments |
|------------|------------------|--------|----------|--|
| EntryCount | Int | 0 | 4 | The number of entries |
| Reserved | byte | 4 | 124 | |
| Entry[] | DirectoryEntryDV | 128 | variable | List of EntryCount items. |
| | | | | Each item is a copy of the DirectoryEntry in the referenced SubBlock segment. |

4.7 Attachment Segment

Multiple segments per file.

4.7.1 Overview

SID = ZISRAWATTACH

AttachmentSegment
256 Byte (binary)

DataSize
AttachmentEntry

Data
(E.g. embedded file)

4.7.2 Segment content schema [256 byte]

SchemaId = AttachmentSegment

| Item | Туре | Offset | Size | Comments |
|-----------------|-------------------|--------|-----------------------|--|
| DataSize | Int32 | 0 | 4 | Size of the data section. |
| <spare></spare> | Byte | 4 | 12 | reserved |
| AttachmentEntry | AttachmentEntryA1 | 16 | 128 | Core information, an 1:1 copy will be stored as part of the File's AttachmentDirectory Segment. |
| <spare></spare> | Byte | 144 | 112 | reserved |
| [Data] | <any></any> | 256 | <datasize></datasize> | Embedded file. |

4.7.3 Data

Binary or textual data as specified in the **AttachmentEntry** information.

4.7.4 AttachmentEntry - Schema A1 [128 bytes]

SchemaID = AttachmentEntryA1 (fixed length = 128 bytes)

| Item | Type | Offset | Size | Comments |
|-----------------|----------|--------|------|--|
| SchemaType | Byte[2] | 0 | 2 | "A1" |
| Reserved | Byte[10] | 2 | 10 | Reserved |
| FilePosition | Int64 | 12 | 8 | Seek offset relative to the first byte of the file |
| FilePart | Int | 20 | 4 | Reserved |
| ContentGuid | GUID | 24 | 16 | Unique Id to be used in strong, fully qualified references |
| ContentFileType | Byte[8] | 40 | 8 | Unique file type Identifier (see table below) |
| Name | Byte[80] | 48 | 80 | Null terminated (80-1) character UTF8 encoded string defining a name for this item. May be used in references instead of GUID. |

ContentFileType identfier strings (samples)

File types correspond to the file extension when the attachment is saved as a separate file.

| Туре | Comments | |
|-----------|--|--|
| ZIP | ZIP compressed stream | |
| ZISRAW | Embedded ZISRAW file | |
| CZTIMS | Time stamp list. | |
| CZEVL | Event List | |
| CZLUT | Lookuptable. | |
| CZPML | Pal molecule List. | |
| JPG, DOC, | Any registered MIME file type | |
| XLS, | , 18 211 1 2 2 1 1 1 1 1 1 1 1 1 1 1 1 1 | |

4.7.5 Reserved Attachment names

The following values for Name in AttachmentEntryA1 schema are reserved and have determined contents.

| Item | DataFormat | Content FileType | Comments |
|-----------------|------------|------------------------------------|--|
| Thumbnail | JPG file | JPG | Contains a thumbnail representation of the image. Typically, a thumbnail is downscaled to match a raster of 256 x 256 pixels, but other sizes are also possible. |
| | | | Thumbnails do not necessarily match the individual pixel SubBlocks, instead. They can represent be a symbolic representation to quickly identify the content. |
| Preview | Media file | JPG (or other media type) | The optional preview image / media file is used to implement a more detailed view of the image using some representative data (e.g. one of the center slices of a Z-Stack). Typical sizes will fit in a 1024 x 1024 raster. |
| Experiment | XML | CZEXP | If the image is the result of a (multidimensional) experiment, this attachment contains the original experiment definition. |
| | | | The Schema is described in the Metadata section as Experiment . |
| HardwareSetting | XML | CZHWS | Contains the initial hardware setting / configuration used to record this image. |
| | | | The Schema is described in the Metadata section as <u>HardwareSetting</u> . |
| TimeStamps | Binary | CZTIMS | Time stamps in seconds relative to the start time of the acquisition engine. |
| EventList | Binary | CZEVL | EventList stores the events reported during a timeseries. |
| LookupTables | Binary | CZLUT | Contains the properties of the lookup tables. |
| PalMoleculeList | Binary | CZPML | The PalMoleculeList provides access to the list of molecules and fiducials which have been detected by the PAL-M method for resolution enhancement. |
| | | | A PalMolecule contains the results for the PAL-M single molecule detection method for resolution enhancements of a single molecule. The list entries are generated form a series of TIRF images by subpixel localization of molecules with the highest intensity in the single images by fitting to the PSF of the microscope. |
| FocusPositions | Binary | CZFOC | Focus positions relative to the start position of the acquisition engine. |
| MVM | XML | CZMVM | Contains information for MultiviewMicroscopy. |
| Label | CZI file | CZI | Label image for slide scan. |
| Prescan | CZI file | CZI | Prescan image for slide scan. |
| SlidePreview | CZI file | CZI | Preview image for slide scan. |
| FiberMatrix | XML | CZFBMX | Contains the fiber matrix definition. |

| LsmMatTopographyReference | CZI file | CZI | The reference image which is attached to each z-stack when using an lsm. It's used to correct |
|---------------------------|----------|-----|---|
| | | | objective errors. |

Please note that the combination of 'Item' and 'Content FileType' is generally not arbitrary. For example, the item 'Thumbnail' must be of content filetype 'JPG'. The only exception is the item Preview, where JPG is expected but other media types are possible. The entry names are case sensitive.

4.7.6 TimeStamps content schema

SchemaId = *TimeStampSegment*

| Item | Type | Offset | Size | Comments |
|------------------|----------|--------|----------------------|---|
| Size | Int32 | 0 | 4 | Size of the whole block used for time stamps. |
| NumberTimeStamps | Int32 | 4 | 4 | Number of time stamps in the list. |
| TimeStamps | double[] | 8 | NumberTimeStamps * 8 | Time stamps in seconds relative to the start time of the acquisition engine. |

4.7.7 Focus Positions content schema

SchemaId = *FocusPositions*

| Item | Type | Offset | Size | Comments |
|-----------------|----------|--------|--------------------|---|
| Size | Int32 | 0 | 4 | Size of the whole block used for focus positions. |
| NumberPositions | Int32 | 4 | 4 | Number of positions in the list. |
| Positions | double[] | 8 | NumberPositons * 8 | Focus positions in micrometers relative to the Z start position of the acquisition engine. |

4.7.8 EventList content schema

SchemaId = EventListSegment

| Item | Туре | Offset | Size | Comments |
|--------------|------------------|--------|----------|--|
| Size | Int32 | 0 | 4 | Size of the whole block in bytes. |
| NumberEvents | Int32 | 4 | 4 | Number of recorded events in the list. |
| Events | EventListEntry[] | 8 | variable | |

4.7.9 EventListEntry content schema

 $SchemaId = \textit{EventListEntry} (fixed \ length = 16 \ bytes + DescriptionSize)$

| Item | Type | Offset | Size | Comments |
|-----------|--------|--------|------|--|
| Size | Int32 | 0 | 4 | Size of the entry in bytes. |
| Time | double | 4 | 8 | Time of the event in seconds relative to the start time of the LSM electronic module controller program. |
| EventType | Int32 | 12 | 4 | Can be one of the following values: |
| | | | | EV_TYPE_MARKER (= 0) - Experimental annotation |

| | 1 | | | |
|-----------------|--------|----|----------|---|
| | | | | EV_TYPE_TIMER_CHANGE (= 1) - The time interval has changed |
| | | | | EV_TYPE_BLEACH_START (= 2) - Start of a bleach operation |
| | | | | EV_TYPE_BLEACH_STOP (= 3) - End of a bleach operation |
| | | | | EV_TYPE_TRIGGER (=4) |
| | | | | - A trigger signal was detected on the user port of the electronic module. |
| DescriptionSize | Int32 | 16 | 4 | Size of the description character array. |
| Description | Byte[] | 20 | variable | Null terminated (80-1) character UTF8 encoded string defining a description for this event. |

4.7.10 LookupTables content schema

SchemaId = LookupTablesSegment

| Item | Туре | Offs et | Size | Comments |
|---------------------|--------------------|------------|----------|---|
| Size | Int32 | 0 | 4 | Size of the whole description block including this header in bytes. |
| NumberLookupT ables | Int32 | 4 | 4 | Number of lookup tables handled in the description block. |
| LookupTables | LookupTableEntry[] | 8 | variable | 12 bit to 12 bit LUT for the corresponding channels 1N. |

4.7.11 LookupTableEntry content schema

SchemaId = *LookupTableEntry*

| Item | Туре | Offset | Size | Comments |
|------------------|------------------|--------|----------|---|
| Size | Int32 | 0 | 4 | Size of the description block without the size in bytes. |
| Identifier | Byte[80] | 4 | 80 | Null terminated (80-1) character UTF8 encoded string defining a name for this lookup table. |
| NumberComponents | Int32 | 84 | 4 | Number of components handled in the description block. |
| Components | ComponentEntry[] | 88 | variable | Component part of the LookupTable. |

4.7.12 ComponentEntry content schema

SchemaId = ComponentEntry

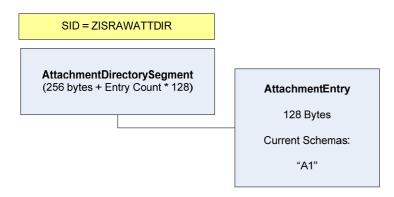
| Item | Type | Offset | Size | Comments |
|-------------------|---------|--------|---------------------|--|
| Size | Int32 | 0 | 4 | Size of the description block without the size in bytes. |
| ComponentType | Int32 | 4 | 4 | Component type: |
| | | | | CO_TYPE_RGB (= -1) - All (Rgb) |
| | | | | CO_TYPE_RED (= 0) |
| | | | | - Red |
| | | | | CO_TYPE_GREEN (= 1) |
| | | | | - Green |
| | | | | CO_TYPE_BLUE (= 2) |
| | | | | - Blue |
| | | | | |
| NumberIntensities | Int32 | 8 | 4 | Number of intensities. |
| Intensity | Int16[] | 12 | NumberIntensities*2 | Intensities for the component of the lookup table. |

4.8 AttachmentDirectory Segment

Zero or one segment per file.

The **AttachmentDirectory** segment is used to manage attached / embedded files which may, in turn, be valid ZISRAW files. Typical usage this segment is to hold commonly used parts like a **Thumbnail** or a **Preview**.

4.8.1 Overview



4.8.2 Segment content schema

SchemaID = AttachmentDirectorySegment

| Item | Туре | Offset | Size | Comments |
|------------|-------------------|--------|------------------|--------------------------|
| EntryCount | Int | 0 | 4 | The number of entries |
| Reserved | Byte | 4 | 252 | |
| Entry[] | AttachmentEntryA1 | 256 | EntryCount * 128 | List of EntryCount items |

5 Pixel storage

5.1 PixelTypes

| Id | Value | Bytes/Pixel | Info |
|--------------------|-------|-------------|---|
| Gray8 | 0 | 1 | 8 bit unsigned |
| Gray16 | 1 | 2 | 16 bit unsigned. |
| Gray32Float | 2 | 4 | 32 bit IEEE float |
| Bgr24 | 3 | 3 | 8 bit triples, representing the color channels Blue, Green and Red |
| Bgr48 | 4 | 6 | 16 bit triples, representing the color channels Blue, Green and Red |
| Bgr96Float | 8 | 12 | Triple of 4 byte IEEE float, representing the color channels Blue, Green and Red |
| Bgra32 | 9 | 4 | 8 bit triples followed by an alpha (transparency) channel |
| Gray64ComplexFloat | 10 | 8 | 2 x 4 byte IEEE float, representing real and imaginary part of a complex number |
| Bgr192ComplexFloat | 11 | 24 | A triple of 2 x 4 byte IEEE float, representing real and imaginary part of a complex number, for the color channels Blue, Green and Red |
| Gray32 | 12 | 4 | 32 Bit integer [planned] |

| Grav64 13 8 Double precision floating point [planned] | Grav64 | 13 | 8 | Double precision floating point [planned] |
|---|--------|----|---|---|
|---|--------|----|---|---|

5.2 Dimensions / dimensions indices

The *Bounds* definition of a **SubBlock** defines the index within a multi-dimensional hyperspace. All indices are integers but,, Using the **StartCoordinate** member of the directory, it is possible to associate a floating point value, e.g. to implement sub-pixel accurancy for image tiles (X/Y offset).

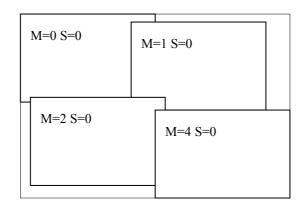
Dimensions in both metadata and binary information blocks are identified by a unique ANSI/ASCII character 'A', 'Z'. The following dimensions are currently defined, more will be added -

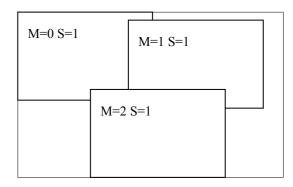
| Id | Info |
|----|--|
| X | Pixel index / offset in the X direction. Used for tiled images. |
| Y | Pixel index / offset in the Y direction. Used for tiled images. |
| С | Channel in a Multi-Channel data set |
| Z | Slice index (Z – direction). |
| Т | Time point in a sequentially acquired series of data. |
| R | Rotation – used in acquisition modes where the data is recorded from various angles. |
| S | Scene – for clustering items in X/Y direction (data belonging to contiguous regions of interests in a mosaic image). |
| I | Illumination - illumination direction index (e.g. from left=0, from right=1). |
| В | (Acquisition) Block index in segmented experiments. Note: This index has been dropped. Instead of the B index multiple single CZI images will be generated when saving segmented experiments. |
| M | Mosaic tile index – this index uniquely identifies all tiles in a specific plane |
| Н | Phase index – for specific acquisition methods. |
| V | View index (for multi – view images, e.g. SPIM) |

Note on M-index: For a mosaic image it is mandatory that all sub-blocks in a specific plane contain an M-index. The M-index enumerates all tiles in a specific plane, starting with 0. The M-index must be unique among all tiles in a specific plane. A plane in this context means: an X-Y-region in which all subblocks have the same C-, Z-, T-, R-, S-, I-, H- and V-coordinate. The M-index is associated with the order of the tiles when a tile-composite is considered. Higher M-Index means that the tile is to be placed on-top of tiles with a lower M-index.

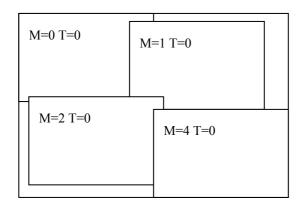
Some examples of correct usage of M-index are given below:

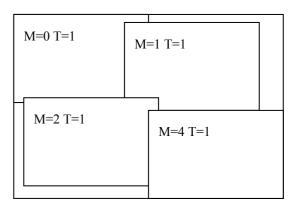
a) Document with mosaic and tiles



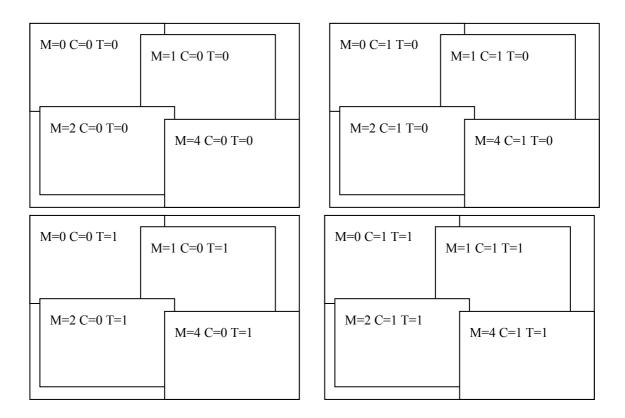


b) Document with mosaic and T-dimension





c) Document with mosaic and C-dimension and T-dimension



If the document does not contain mosaics (which means: there is never more than one tile in one plane) then the usage of M-index is optional.

5.3 Compression

Pixel data may be either compressed or uncompressed. Compression is determined by a *Compression* constant other than *Uncompressed*.

| Compresion | Value | Info | |
|--------------|-------|--|--|
| Uncompressed | 0 | Data contains uncompressed pixels | |
| | | Data is a stream of items with the specified PixelType and Size information. Each item is adressed by evaluating the "StoredSize" information in dimension order. | |
| | | The total data size can be calculated from BytePerPixel(PixeType) * multiplication of all stored sizes in all contained dimensions. | |
| LZW | 2 | Data contains pixels compressed with the Lemple-Ziff-Welch algorithm. | |
| | | This compression mode is currently not used in widefield acquisition. | |
| JpgFile | 1 | Data contains a single RGB or monochrome JPEG file. | |
| | | This compression mode can only be used with 2D SubBlocks. | |
| | | The summary information about the stored size represents the bitmap size in pixels. Anyway, the reader should check the size obtained from the JPEG decoder. | |
| JpegXrFile | 4 | Pixel Blob is a valid Jpeg-XR aka HDP-file (ISO/IEC 29199-2) using the WIC-WmpImageCodec. Valid PixelFormats are float (32 bit), 24 bit (3x 16 bit) color, 24 bit (3 x 8 bit) color, 8 bit and 16 bit greyscale, | |

| | | This compression mode can only be used with 2D SubBlocks. The summary information about the stored size represents the bitmap size in pixels. Anyway, the reader should check the size obtained from the JPEGXR decoder. | |
|--------|---------|---|--|
| Camera | 100-999 | Camera specific RAW data. | |
| System | 1000- | System specific RAW data. | |

6 Metadata (XML)

6.1 General

Metadata is always in XML format and is stored as UTF-8.

This chapter is intended to provide a course overview. Detailed schema documentation is available as a separate HTML or CHM based documentation.

6.2 Acknowledgements

This part of the specification is close to the OME specification, Copyright 2002-2011 OME (Open Microscopy Environment), see our acknowledgment in the header.

6.3 Versioning

Versioning in ZISRAW has two aspects which will be named Reorganization-Versioning and Sub-Versioning. Sub-Versioning means a 'friendly extension' of a given version while Reorganization-Versioning aims at a redesign of one or many given structures.

6.3.1 Sub-Versioning

To understand the idea of Sub-Versioning we have to make certain assumptions in advance. They refer to the way we treat XML and XSD. Sub-Versioning is characterized by the digits after the dot e.g. 1.00, 1.01, 1.02. This is stressed by the filename e.g. czi v1.xsd so the Sub-Versioning is 'internal'.

The following assumptions are fundamental

- XML data is always saved back in full range. This is especially true for XML data that cannot be interpreted completely so that parts that could not be understood persist.
- XSD is only enlarged as far as data and structure is concerned. This implies that changes to the XSD just
 mean new features have been added, like new elements, new attributes or even new structures. Therefore
 reorganization of already given structures, renaming of structures, elements or attributes or changing of
 semantics of elemens is not allowed in this context.
- Must-Entries are not used
- For the validation of the Sub-Version we refer to three different scenarios.

Scenario 1 – XML and XSD contain same data

- Validation is successful.

 The program can continue without errors
- Validation failed.

There exists a more or less severe error. The user has to be informed. The object model catches the error and gives detailed information. If possible, the program proceeds with the given limitations. In severe situations the opening of the image is stopped and the user has the possibility to reorganize the file to use it later on with a minimum of functionality.

Scenario 2 – XML is an older version than XSD

- Validation is successful.

 As the XSD has more capabilities than the XML needs, the XML can always be validated. This is even true when the XML and the Version of ZIS are older than the XSD itself. In this case it depends on the program version and its object model how much of the data can be used.
- Validation failed.
 See Scenario 1.

Scenario 3 – XSD is an older version than XML

• The Versions are recognized and the problem is known to the program

- The user is asked to download the actual XSD directly via internet. If this is not possible, e.g. a connection to the internet is not found, the url and the target directory are shown to the user and he is asked to manually supply the most recent XSD. After this procedure Scenario 1 or Scenario 2 is applicable.
 - In any case the user is able to go on working because we make the assumption that the data is valid and not corrupted. The software is simply supplied with more data than it can work with.
- In case the validation is turned on, the software must guarantee that the ZEN version can at least access its corresponding XSD. This is achieved by compiling the XSD directly into the versions of ZEN. The software looks for the latest version of the XSD, especially within the Sub-Version of a required Version.

6.3.2 Reorganization-Versioning

Reorganization-Versioning means rearrangement of already given structures, renaming of structures, elements or attributes, changes in definitions or any other severe, far reaching changes.

Reorganization-Versioning is characterized by the digits in front of the dot e.g. 2.00, 3.01, 4.02. Therefore each version has its own file and the xml tells the software which XSD to load.

As the XSD consists of several XSD-files like 'Experiment.xsd', 'HardwareSettings.xsd', 'Information.xsd', 'DisplaySettings.xsd' etc. it is possible to reorganize any specific part of the schema. This means that for instance a reorganized 'Experiment_2.00.xsd' may coexist with previous versions like 'HardwareSettings_1.09.xsd', 'Information 1.09.xsd'

Although an earlier version of the software will be able to validate the xml (see Scenario 3) it will automatically detect areas that cannot be handled. The degree of incompatibility depends on the degree of rearrangement or changes in the XSD compared to the object model holding the data. The software with the new XSD is automatically able to verify an old czi file, because the xml knows the version and the older versions are also supplied in an xsd-folder of the ZIS-software or Scenario 3 is applicable. For that reason internally the old way to access the data must be maintained to show and handle the information as expected.

Optional: When saving an image the user is asked, whether the old or the new format is to be applied.

6.4 General XML information

In ZISRAW files, XML metadata is entirely optional as all information about dimensions, pixel types etc. is contained in the binary SubBlock and / or SubBlockDirectory segments .

However, to provide useful information and enable advanced processing, some elements should be provided. In the following chapters, the column "req" (required) indicates one of

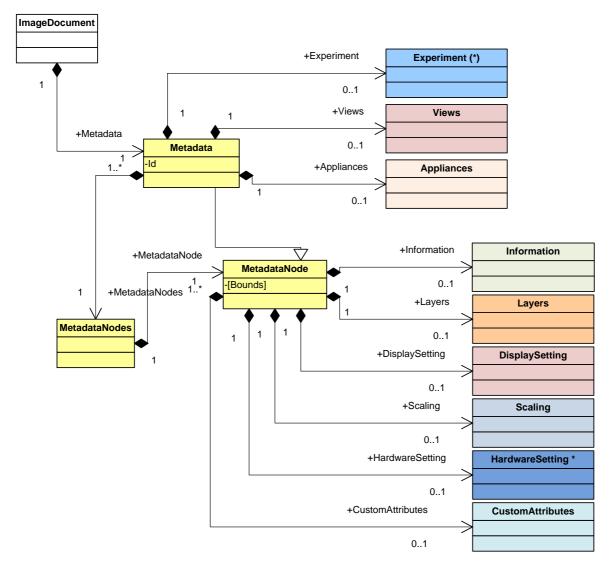
- Y mandatory, marked as required in the schema defintion
- B basic information, optional in schema definition but required to show GUI elements like rulers and display setting
- A requirement is application dependent, e.g. required for 3D deconvolution

Unless otherwise noted, the default value for all values is as follows

- xs:double : NaN
- **xs:string**: "" (an empty string) or the string constant shown in bold face if the string uses an enumerated restriction.
- xs:boolean : false

6.5 Storage structure

XML Storage is based on the *MetadataNode* schema containing the predefined elements **Information**, **Layers**, **DisplaySetting**, **Scaling** and **CustomAttributes**.



NOTE: Items marked with (*) may be stored in separate attachment segments, they are:

- **Experiment** the full experiment description at the time when the acquisition process was started. Normally this XML node is created once. The Experiment schema is currently undocumented.
- HardwareSetting the hardware setting / configuration at the time the experiment was started. This XML node represents the full hardware state including all the metadata used to reconstruct the GUI elements if required. State changes during the flow of the experiment are expressed via the nodes HardwareSetting node of the subset specific Metadata nodes. HardwareSettings are an advanced feature and are currently undocumented.

| Element | req | Type (XSD) | Sample | Description |
|-------------|-----|---------------------------|--------------------------------|---|
| Version | | xs:string | 1.0 | Version information for this XML content, expressed as <major>.<minor>.</minor></major> |
| | | | | Default Value is 1.0. |
| Experiment | | <unspecific></unspecific> | n/a in this context | Serialized Experiment. Separate schema is available as element Experiment in Experiment.xsd. |
| Views | | Views | <complex content=""></complex> | Reserved. Stores multiple predefined views for the data set. |
| Applicances | | Appliances | <complex content=""></complex> | Reserved. Stores data specific for named |

| | | | | appliances like measurement or advanced data analysis. |
|------------------|---|---------------------------|--|--|
| Information | В | Information | <pre><complex content=""></complex>, see <u>Information</u>.</pre> | Typical document properties like comments, keywords, author, etc. |
| Layers | | Layers | <pre><complex content=""></complex>, see <u>Layers</u>.</pre> | A collection of graphical (overlay) layers, either global or subset specific. |
| DisplaySetting | В | DisplaySetti ng | <pre><complex content=""></complex>,see DisplaySetting.</pre> | Default or subset specific multi-channel display setting (channels selection, colors, display mapping curves, gamma etc.) |
| Scaling | В | Scaling | <pre><complex content=""></complex>,see <u>Scaling</u></pre> | Scaling values for the various dimensions. Normally, only X,Y and Z have useful entries. |
| HardwareSetting | | <unspecific></unspecific> | <pre><complex content=""></complex></pre> | Detailed information about the recording hardware in original manufacturer format. Separate schema documentation is available as HardwareSetting.xsd. |
| CustomAttributes | | CustomAttri butes | <pre><complex content=""></complex>, see Custom attributes</pre> | Location for unlimited, but unchecked storage of named custom values (simple or complex types). |

6.6 Custom attributes

CustomAttributes is an opaque list of elements that should be preserved when loading and saving files. The reader of this element must store the XML of all child elements, e.g. in a dictionary, to be restored when saving.

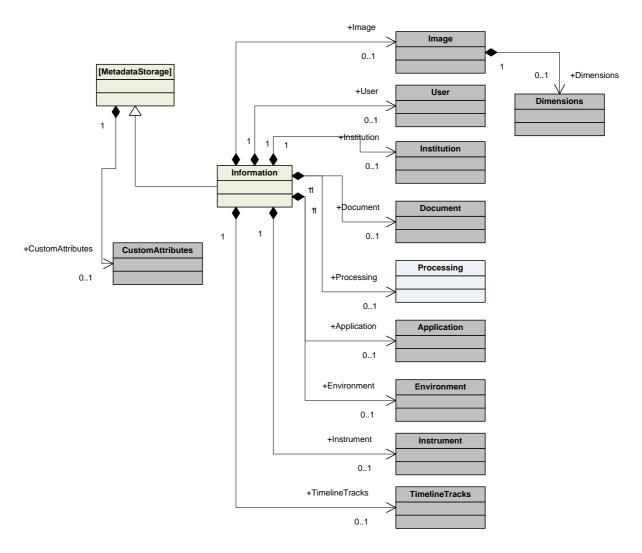
6.6.1.1 Sample: CustomAttributes

```
<CustomAttributes>
  <Test>The content<Test>
  <Test2/>
  <Test2/>
  <CustomAttributes>
```

6.7 Information

The **Information** element carries all the data used to implement the information views with the application and the various GUI elements to work with a multi-dimensional image.

All elements within the information storage are based on the *MetadataStorage* schema and provide a **CustomAtttributes** node for user-defined extensions without breaking the schema's compatibility.

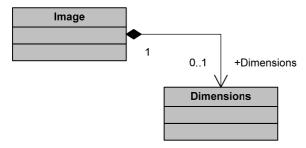


| Element | req | Type (XSD) | Sample | Description |
|-------------|-----|-------------|--|---|
| Image | В | Image | <pre><complex content=""></complex>, see Information/Image</pre> | Describes the image's main attributes like multidimensional bounds and dimension specific data. |
| User | | User | <complex content=""></complex> , see <u>Information/User</u> | User related information added provided by the application (Name etc.) |
| Institution | | Institution | <complex content=""></complex> ,see Information.Institution. | Information about the institution where the image was recorded (name, address, email etc). |
| Document | | Document | <pre><complex content=""></complex>, see Information/Document.</pre> | Typical document properties like comments, keywords, author, etc. |
| Processing | A | Processing | complex content, see <u>Information/Processing</u> . | Specific data for use within processing algorithms, e.g. the PSF information for deconvolution. |
| Application | | Application | <pre><complex content=""></complex>,see Information.Application.</pre> | Information about the creating application (program). |

| Environment | | Environment | <complex content=""></complex> | Information for experiment / recording environment (temperature, humidity, etc). |
|----------------|----|----------------|---|--|
| Instrument | BA | Instrument | <pre><complex content=""></complex>,see Information.Instrument</pre> | Detailed information about the recording hardware. |
| TimelineTracks | | TimelineTracks | <pre><complex content=""></complex>, see Information.TimelineTracks</pre> | Information about timeline tracks and events (e.g. bleaching, user markers, user settings, incubation, focus action, digital input etc.) |

6.7.1 Information / Image

Contains basic image information like dimensions and their sizes and optional details about the contained Dimensions.



The **Sizes** and pixel data related metadata information is provided in addition only to provide a convenient method to display summary information by reading the XML segment only.

Sizes define the overall dimensions (bounds) of the multi-dimensional data set. All values must be > 0, the default value for each dimension is 1.

When opening an image, its "real" bounds should be calculated from the contained **SubBlock** segments (as specified in the directory information).

Special for "req" column: If enclosed in brackets, e.g. (B) means that this element should be available if the image contains this dimension.

| Element | req | Type (XSD) | Sample | Description |
|---------|-----|---------------|--------|---|
| SizeX | В | xs:integer | 200 | Number of pixels in x direction (width). |
| SizeY | В | xs:integer | 200 | Number of pixels in y direction (height). |
| SizeC | (B) | xs:integer | 4 | Number of channels. |
| SizeZ | (B) | xs:integer | 20 | Number of Z slices. |
| SizeT | (B) | xs:integer | 100 | Number of timepoints. |
| SizeH | (B) | xs:integer | 1 | Number of phases. |
| SizeR | (B) | xs:integer | 1 | Number of rotation angles (indices). |
| SizeS | (B) | xs:integer | 1 | Number of scenes. |
| SizeI | (B) | xs:integer | 1 | Number of illumination direction indices. |

| SizeM | (B) | xs:integer | 1 | Number of mosaic tiles (regular mosaics only). |
|----------------------|-----|---|---|--|
| SizeB | (B) | xs:integer | 1 | Number of acquisition / recording / blocks Each block may have specific dimensions expressed via Information.Image in a subset specfic Metadata node. |
| SizeV | (V) | xs:integer | 1 | Number of views in a multi-view image. Each view has associated metadata describing ist X/Y/Z/Angle position. |
| PixelType | В | PixelType (xs:string restriction) | Gray8 | preferred pixel type (individual types are defined in binary image data and / or channel XML information) one of Gray8, Gray16, Bgr24, Bgra32, Gray32Float, Bgr48, Bgr96Float, Gray64ComplexFloat, Gray32Float, Bgr192ComplexFloat. |
| ComponentBitC ount | В | xs:integer | 12 | ComponentBitCount for the entire image is for informative purpose only. In <i>Dimensions/Channels/Channel</i> each Channel has its own <i>ComponentBitCount</i> element. |
| | | | | If not specified or 0, the component bit count is derived from the pixel type. Normally, this value is used with 16 bit images only to indicate the maximum possible valid bits of the sensor, e.g. 12 defines a 12 bit camera. |
| OriginalScanD ata | | xs:boolean | true | Set to true if the image is the output of a scanning process and has not been modified up to now. |
| Dimensions | В | Dimensions | <pre><complex content=""></complex>, see Information.I mage.Dimens ions</pre> | More detailed information about each contained dimensions. |

6.7.1.1 Sample: Image

6.7.2 Information / Image / Dimensions

Contains information about contained dimensions.

| Element | req | Type (XSD) | Sample | Description |
|--------------|-----|--|--|--|
| Channels | | DimensionChan nel | complex content, see Information / Image / Dimensions / Channels / Channel | A collection of Channel information |
| Tracks | | <anonymous complex="" type=""></anonymous> | complex content. See Information / Image / Dimensions / Tracks | Contains information about tracks. |
| LambdaStacks | | <anonymous></anonymous> | | |
| Т | | <anonymous></anonymous> | complex content | Associates a timestamp to each t-coordinate. |
| Z | | <anonymous></anonymous> | complex content | Associates a spatial point for each Z Index. |

6.7.3 Information / Image / Dimensions / Channels / Channel

XSD complex type **DimensionChannel**.

There must be at least one element per channel in the Image, even for a single-plane image.

And information about how each of them was acquired is stored in the various optional *Ref elements.

The IlluminationType element is a string enumeration which may be set to 'Transmitted', 'Epifluorescence', 'Oblique', or 'NonLinear'.

Contains user and site information.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|-----------|--|
| Id | Y | xs:string | Channel:0 | Unique ID within a list of siblings. Recommended format is Channel: <nr>.</nr> |
| Name | | xs:string | DAPI | Default, user supplied, name of the channel. |

| Element | req | Type (XSD) | Sample | Description |
|-------------------|-----|--------------------------|-------------|--|
| PixelType | В | restriction of xs:string | Gray8 | Information about the pixel type of this channel (should match the binary data in the SubBlock segment) |
| | | | | One of Gray8, Gray16, Bgr24, Bgra32, Gray32Float, Bgr48, Bgr96Float, Gray64ComplexFloat, Gray32Float, Bgr192ComplexFloat. |
| ComponentBitCount | В | xs:string | 12 | ComponentBitCount for the channel If not specified or 0, the component bit count is derived from the pixel type. Normally, this value is used with 16 bit images only to indicate the maximum possible valid bits of the sensor, e.g. 12 defines a 12 bit camera. |
| AcquisitionMode | | restriction of | Brightfield | AcquisitionMode describes the type of |

| | | xs:string | | microscopy performed for each channel. |
|----------------------------|---|----------------------------|-----------------|---|
| | | As.sumg | | One of: |
| | | | | Brightfield, |
| | | | | LaserScanningConfocalMicroscopy, SpinningDiskConfocal, SlitScanConfocal, MultiPhotonMicroscopy, StructuredIllumination, |
| | | | | SingleMoleculeImaging, TotalInternalReflection, FluorescenceLifetime, SpectralImaging, FluorescenceCorrelationSpectroscopy, NearFieldScanningOpticalMicroscopy, SecondHarmonicGenerationImaging, PALM, STORM, STED, TIRF, FSM, LCM, SPIM, |
| IlluminationType | A | restriction of xs:string | Transmitte d | Other The method of illumination used to capture the channel |
| | | xs.sumg | u | One of |
| | | | | Transmitted, Epifluorescence(*1), Oblique, NonLinear, Other |
| ContrastMethod | A | restriction of xs:string | Brightfield | ContrastMethod describes the technique used to achieve contrast for each channel. |
| | | | | One of |
| | | | | Brightfield, Phase, DIC, HoffmannModulation, ObliqueIllumination, PolarizedLight, Darkfield, Fluorescence, MultiPhotonFluorescence, Other |
| IlluminationWavelengt h | A | SpectrumChar acteristic | 340 | Illumination Wavelength as either a single Peak or a list of Ranges. This characterizes the light used for illuminating the specimen. More specific, it is about that the light that actually hits the specimen, not the light as it leaves the light source. |
| DetectionWavelength | A | SpectrumChar acteristic | 320 | Detection Wavelength as either a single Peak or a list of Ranges. This characterizes the part of the spectrum for which the detector used for this channel is actually sensitive for. It gives in any case the "net result"-it does not matter what technique is used to limit the detection range. |
| ExcitationWavelength | A | xs:double | 400 | Wavelength of excitation for this channel in nanometers. This characterizes the fluorochome - the fluochrome one was interested in for this channel. |
| | | | | It has just an informative meaning - it is not meant to characterize the fluochrome in depth, that is what the DyeId is meant for. [units:nanometers]. |
| EmissionWavelength | A | xs:double | 400 | Wavelength of emission for this particular channel, in nanometres[nm]. |
| DyeId | A | xs:double | 400 | The dye id which is unique within the original |

| | | | | dye database. |
|-------------------|---|---------------------------------------|----------|--|
| DyeDatabaseId | | xs:string | | The id (Guid) of the original database this dye is taken from. |
| PinholeSize | A | xs:double | 1.2 | The optional PinholeSize element allows specifying adjustable pin hole diameters for confocal microscopes. |
| | | | | The Pinhole is track specific i.e. channels of the same track need to have the same value here. [units:micrometers]. |
| PinholeSizeAiry | A | xs:double | | The size of the pinhole in units of the airy disc. Since this value cannot (easily) be derived from the above <i>PinholeSize</i> (in micrometers), the rule is that we store this value separately. |
| | | | | The Pinhole is track specific i.e. channels of the same track need to have the same value here. [units:micrometers]. |
| PinholeGeometry | A | restriction of xs:string | Circular | The geometry of the pinhole, either circular or rectangular. |
| | | | | One of Circular, Rectangular, Other |
| Fluor | | xs:string | | The Fluor element is used for fluorescence images. This is the name of the fluorophore used to produce this channel [plain text string]. This element is just for informative purposes. The fluorochrome is far better identified by the Dyeld. However - if you do not have a Dyeld at hand, you may use this field in order to give at least an informative string. |
| NDFilter | | xs:float | | The <i>NDfilter</i> element is used to specify the combined effect of any neutral density filters used. [units:optical density expressed as a PercentFraction] |
| PockelCellSetting | | xs:integer | | The <i>PockelCellSetting</i> used for this channel. is the amount the polarization of the beam is rotated by. [units:none] |
| Color | В | restriction of xs:string | #5566ff | The original color of the channel (the color that was defined by the dye or the user before the acquisition). |
| ExposureTime | В | restriction of xs:string | 4000 | Exposure Time used to acquire this channel for informative purposes. |
| | | pattern value="\s*((\d +) (\d+- | | The value may be given as a single number or a range. |
| | | \d+))\s*" | | Examples: "4000" "89944" "887-1100" "100-1000" |
| | | | | This is element gives just an informative value for the exposure-time used to acquire this channel. It must not be understood to have the meaning of "exposuretime is constant for all pictures in this channel". If the exposure time is not constant, then a range may be given |

| | | | here. If it does not apply at all (e.g. because no CCD-camera or similar was used as the detector), leave it out. [units:nanoseconds]. |
|----------------------|-------------------------------------|---|--|
| SectionThickness | xs:double | 12.3 | For SIM this gives the thickness of the section. [unit: micrometers]. |
| DetectorSettings | ChannelDecte ctorSettings | <pre><complex content=""></complex>, see ChannelDe ctectorSetti ngs</pre> | Supplements or overrides detector parameters for acquisition of this particular channel. |
| LightSourcesSettings | ChannelLight SourcesSetting s | <pre><complex content=""></complex>, see ChannelLi ghtSources Setting</pre> | Supplements or overrides light sources parameters for acquisition of this particular channel. |
| LightPath | ChannelLight Path | <pre><complex content=""></complex></pre> | For the moment, the LightPath is track specific i.e. channels of the same track need to have the same value here. |
| FilterSet | FilterSetRef | <pre><complex content=""></complex></pre> | Refers to an instance of InstrumentFilterSet in Information/Instrument/FilterSets. |
| LaserScanInfo | ChannelLaser ScanInfo | <pre><complex content=""></complex></pre> | Here we find information how the laser operated when scanning the field. |
| Reflector | xs:string | | |
| CondenserContrast | xs:string | | |
| NACondenser | xs:double | 0.45 | |
| Ratio | Ratio | | The ratio between two active channels. |

^{(1) *&}quot;Epifluorescence" in this context just describes the fact that the objective is used to bring the fluorescence inducing light ("the illumination") to the specimen (in contrast to transmitted illumination). This does not necessarily require that we do a fluorescence acquisition, maybe "lightthroughobjective" would be less confusing...

6.7.3.1 ChannelDectectorSettings complex type

| Element | req | Type (XSD) | Sample | Description |
|-------------|-----|--------------------------|---|--|
| Detector | | DetectorRef | <pre><complex content=""></complex></pre> | Refers to an instance of <i>InstrumentDetector</i> in Information/Instrument/Detectors. |
| Binning | | restriction of xs:string | 1x1 | Represents the number of pixels that are combined to form larger pixels. One of 1x1, 2x2, 4x4, 8x8, Other |
| Gain | | xs:double | 1.45 | The Gain of the detector. [units:none] |
| DigitalGain | | xs:double | 0.78 | The digital Gain of the detector. [units:none] |
| Offset | | xs:double | 0.6 | The Gain Offset of the detector. [units:none] |
| EMGain | | xs:double | 0.2 | The EM Gain |
| Voltage | | xs:double | 6.78 | The Voltage of the detector. volts[V] |
| ReadOutRate | | xs:double | 10.5 | The speed at which the detector can count pixels. |
| | | | | Units of <i>ReadOutRate</i> is MHz. This is the bytes per |

| | | | second that can be read from the detector (like a baud rate). megahertz[MHz] |
|---|------------|------|---|
| UseBrightness ContrastCorre ction | xs:boolean | true | The brightness and contrast correction for stacks and z-scans was active during acquisition and is defined by sets of variable values for AOTF power, PMT gain and detector amplifier gain and offset for the positions of the focus drive. Default: <i>false</i> . |

6.7.3.2 ChannelLightSourcesSettings complex type

The ChannelLightSourcesSettings type is a sequence of *LightSourceSettings* elements.

| Element | req | Type (XSD) | Sample | Description |
|-------------------------|-----|-----------------------------|--|--|
| LightSourceSe ttings | | ChannelLightSo urceSettings | <pre><complex content=""></complex>, see</pre> | Describes a light source by light source reference, attenuation etc. |
| | | | ChannelLightSour | |
| | | | ceSettings | |

6.7.3.3 ChannelLightSourceSettings complex type

Channel acquisition specific overrides for the Light Source.

| Element | req | Type (XSD) | Sample | Description |
|-------------|-----|----------------|---|---|
| LightSource | | LightSourceRef | <pre><complex content=""></complex></pre> | Refers to an instance of <i>InstrumentLightSource</i> in Information/Instrument/LightSources. |
| Wavelength | | xs:double | 420 | The Wavelength of the light source. nanometres[nm]. |
| Attenuation | | xs:double | 0.45 | The Attenuation of the light source [units:none] A fraction, as a value from 0.0 to 1.0. A value of 0.0 means "no attenuation", and 1.0 means "all light was blocked". So, this is 1 - light_intensity_after_attenuation / light intensity before attentuation. |
| Intensity | | xs:double | 0.78 | The intensity of the light source. The intensity might be set in percent or in Volt; the unit is part of the string value. |

6.7.4 Information / Image / Dimensions / Tracks

| Element | req | Type (XSD) | Sample | Description |
|----------------|-----|---|--|---|
| MultiplexType | | restriction of xs:string | Frame | Specifies when a switch to the next track is done. Possible values are: |
| | | | | • Frame - after a frame, |
| | | | | • Line - after a line. |
| | | | | The value is the same for all tracks in a recording. |
| MultiplexOrder | | <ananymous complex type></ananymous | <pre><complex content=""></complex></pre> | The switch order of tracks. This type consists of a sequence of Track references (via Id) |
| Track | | DimensionTr ack | <pre><complex content=""></complex>, see Information /</pre> | Individual track information |

| | | Image / | |
|--|--|----------------|--|
| | | Dimensions / | |
| | | Tracks / Track | |

6.7.5 Information / Image / Dimensions / Tracks / Track

XSD complex type **DimensionTrack**.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|------------------|--|
| Id | Y | xs:string | Track:1 | Unique ID within a list of siblings. Recommended format is Track: <nr>.</nr> |
| Name | | xs:string | Bleaching, Phase | The name of the track as specified by the user. |

| Element | req | Type (XSD) | Sample | Description |
|-------------|-----|------------|--------------------------------|--|
| ChannelRefs | Y | ChannelRef | <complex content=""></complex> | Sequence of references to the contained channels (Information / Image / Dimensions/Channels) via ID. |

6.7.6 Information / User

Contains user and site information.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|--------|---|
| Id | Y | xs:string | User:1 | Unique ID within a list of siblings. Recommended format is User: <nr>.</nr> |

| Element | req | Type (XSD) | Sample | Description |
|-------------|-----|------------|----------------------|--|
| DisplayName | | xs:string | John Doe | Name to be displayed in user interface elements. |
| FirstName | | xs:string | John | First name, sometimes called christian name or given name or forename. |
| MiddleName | | xs:string | Mc. | Any other names. |
| LastName | | xs:string | Doe | A person's last name sometimes called surname or family name. |
| Email | | xs:string | john@doe.com | A person's email address |
| Institution | | xs:string | Carl Zeiss Munich | A person's institution. |
| UserName | | xs:string | | This is the username of the experimenter (in a 'logon' or 'database' sense). |
| Phone | | xs:string | +49 989 7898 | Phone number in stanard notation. |
| Fax | | xs:string | +1 8989 8998 | Fax number in standard notation |
| Address | | xs:string | Main Street 2 | Street and number. |
| City | | xs:string | Munich | User's city. |
| Country | | xs:string | Germany | User's country. |
| State | | xs:string | Bavaria | State (required for US address specification) |

6.7.6.1 Sample: User

6.7.7 Information / Document

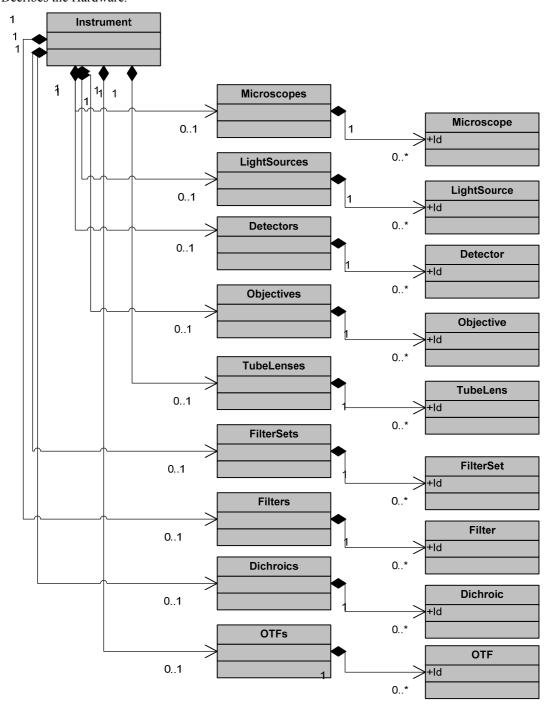
Contains general "document" information.

| Element | req | Type (XSD) | Sample | Description |
|--------------|-----|-------------|-------------------------|--|
| Name | | xs:string | FluoCells | Specific name given to this image. |
| Author | | xs:string | John Doe | The document's author (typically the experimenter or image generator). |
| UserName | | xs:string | jdoe | User name in unique format, e.g. system account name. |
| SubType | | xs:string | Image | Document sub-type to further specifiy the individual generation process and usage of this image. Samples are "PSF", "SIM". The default is an empty string or "Image". |
| Title | | xs:string | My first experiment | A reasonable title given to the image. |
| CreationDate | | xs:dateTime | 2001-01- 28T00:00:05 | Date and time when the document was created. |
| Description | | xs:string | Cells in a | Advanced (may be lengthy) description of what was acquired and in which context. |
| Thumbnail | | xs:string | cells.jpg | A thumbnail to be displayed as the image's icon / symbol (application / organization specific location). |
| Comment | | xs:string | Annotated by XY | Additional comments. |
| Rating | | xs:integer | 1 | A rating value from 03. |
| Keywords | | xs:string | cells john | A list of keywords for full text searches etc |

6.7.7.1 Sample: Document

6.7.8 Information / Instrument

Decribes the Hardware.



| Element | req | Type (XSD) | Sample | Description |
|--------------|-----|---------------------------------------|---|---|
| Microscopes | | Microscopes, InstrumentMicroscope | see Information/Instrume nt/Microscopes/Micr oscope | Defines the microscopes used. Typically there is only a single element of this type. |
| LightSources | | LightSources InstrumentLightSource | see <u>Information/Instrume</u> nt/LightSources/Light | A collection of available light sources. |

| | | <u>Source</u> | |
|------------|-----------------------------------|--|--|
| Detectors | Detectors InstrumentDetector | see <u>Information/Instrume</u> <u>nt/Detectors/Detector</u> | A collection of available light detectors. |
| Objectives | Objectives InstrumentObjective | see <u>Information/Instrume</u> nt/Objectives/Objecti <u>ve</u> | A collection of available objectives. |
| TubeLenses | TubeLense InstrumentTubeLens | see <u>Information /</u> <u>Instrument /</u> <u>TubeLenses /</u> <u>TubeLens</u> | A collection of available tube lenses. |
| FilterSets | | | A collection of available filter sets. |
| Filters | | | A collection of available filters. |
| Dichroics | | | A collection of available dichroics. |
| OTFs | | | A collection of available OTFs. |

6.7.8.1 Information / Instrument / Microscopes / Microscope

XSD Complex type: **InstrumentMicroscope**.

Describes the microscope (stand).

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|--------------|---|
| Id | Y | xs:string | Microscope:1 | Unique ID within a list of siblings. Recommended format is Microscope: <nr>.</nr> |

| Element | req | Type (XSD) | Sample | Description |
|--------------|-----|--------------|---|---|
| Manufacturer | | Manufacturer | <pre><complex content=""></complex></pre> | Information about the manufacturer of this hardware. |
| System | | xs:string | LSM700 | A list of components where each part is divided by a colon. The purpose is to name the whole system.For a confocal system the schema is "[LsmName][, RtScannerName][, CameraName][, StandName]. |
| Туре | | xs:string | Upright | The type of the microscope. One of <i>Upright, Inverted, Dissection, Electrophysiology or Other</i> . |

6.7.8.2 Information / Instrument / LightSources / LightSource

 $XSD\ Complex\ type: \textbf{InstrumentLightSource}.$

The lightsource for the instrument. An instrument may have several light sources.

The type of lightsource is specified by one of the child-elements which are 'Laser', 'Filament', 'Arc' or 'LightEmittingDiode'.

Each of the light source types has its own *Type* attribute to further differentiate the light source (eg, Nd-YAG for Laser or Hg for Arc).

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|---------------|---|
| Id | Y | xs:string | LightSource:1 | Unique ID within a list of siblings. |
| | | | | Recommended format is LightSource: <nr>.</nr> |

| | | | A LightSource ID must be specified for each light source, and the individual light sources can be referred to by their LightSource IDs (e.g. from Channel) |
|------|-----------|---------|--|
| Name | xs:string | AttoArc | The name of this hardware component. |

| Element | req | Type (XSD) | Sample | Description |
|-----------------|-----|-------------------------|---|--|
| Manufacturer | | Manufacturer | <pre><complex content=""></complex></pre> | Information about the manufacturer of this hardware. |
| Power | | xs:float | 23.5 | The light-source power, units milliwatts[mW] |
| LightSourceType | | choice of complex types | <laser><td>The type of the light source. One of complex types <i>Laser</i>, <i>Filament</i>, <i>Arc</i>, <i>LightEmittingDiode</i></td></laser> | The type of the light source. One of complex types <i>Laser</i> , <i>Filament</i> , <i>Arc</i> , <i>LightEmittingDiode</i> |

6.7.8.3 Information / Instrument / Detectors / Detector

XSD Complex type: InstrumentDetector.

The detector used to capture the image. The Detector ID can be used as a reference within the *Channel* element in the *Image* element.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|------------|---|
| Id | Y | xs:string | Detector:1 | Unique ID within a list of siblings. Recommended format is Detector: <nr>.</nr> |
| Name | | xs:string | | The name of this hardware component. |

| Element | req | Type (XSD) | Sample | Description |
|----------------------|-----|--------------------------|---|---|
| Manufacturer | | Manufacturer | <pre><complex content=""></complex></pre> | Information about the manufacturer of this hardware. |
| Gain | | xs:float | 1.2 | The Detector Gain for this detector, as a float. [units:none] |
| Voltage | | xs:float | 5.6 | The Voltage of the detector (e.g. PMT voltage) as a float. volts[V] |
| Offset | | xs:float | 0.1 | The Detector Offset. [units:none] |
| Zoom | | xs:float | 1.78 | The Zoom or "Confocal Zoom" or "Scan Zoom" for a detector. [units:none] |
| AmplificationGa in | | xs:float | 0.5 | Gain applied to the detector signal. This is the electronic gain (as apposed to the inherent gain) that is set for the detector. [units:none] |
| AmplificationOf fset | | xs:float | 0.1 | Offset applied to the detector signal. [units:none] |
| Туре | | restriction of xs:string | | Type of the detector, one of CCD, IntensifiedCCD, AnalogVideo, PMT, Photodiode, Spectroscopy, LifetimeImaging. CorrelationSpectroscopy, FTIR, EMCCD, APD, CMOS, EBCCD, Other |
| Adapter | | DetectorAdapter | <pre><complex content=""></complex></pre> | If the detector type is supposed to have an adapter, this is the respective adapter data. |

| | | | E.g. a CCD can have a camera adapter with a magnification, a PMT or a Photodiode doesn't have an adapter. |
|--------------|----------|-----|--|
| | | | DetectorAdapter contains the elements Manufacturer, Magnification and CustomAttributes. |
| GammaDefault | xs:float | 1.1 | The default gamma value. This value will be set by the Reset DisplaySetting function. [units:none] default is 1.0. |

6.7.8.4 Information / Instrument / Objectives / Objective

XSD Complex type: InstrumentObjective

A description of the microscope's objective lens.

Required elements include the lens numerical aperture, and the magnification, both of which a floating point (real) numbers.

The values are those that are fixed for a particular objective: either because it has been manufactured to this specification or the value has been measured on this particular objective.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|-------------|--|
| Id | Y | xs:string | Objective:1 | Unique ID within a list of siblings. Recommended format is Objective: <nr>.</nr> |
| Name | | xs:string | | The name of this hardware component. |

| Element | req | Type (XSD) | Sample | Description |
|---------------------------|-----|--------------------------|---|--|
| Manufacturer | | Manufacture r | <pre><complex content=""></complex></pre> | Information about the manufacturer of this hardware. |
| Correction | | restriction of xs:string | PlanApo | This is the type of correction coating applied to this lens. |
| | | | | One of |
| | | | | UV, PlanApo, PlanFluor, SuperFluor, VioletCorrected, Achro, Achromat, Fluor, Fl, Fluar, Neofluar, Fluotar, Apo, PlanNeofluar, Other |
| Immersion | | restriction of xs:string | Oil | This is the type of immersion medium the lens is designed to work with. |
| | | | | It is not the same as 'Medium' in Information/Image/ObjectiveSettings (a single type) as here Immersion can have compound values like 'Multi'. |
| | | | | One of |
| | | | | Oil, Water, WaterDipping, Air, Multi, Glycerol, Other |
| ImmersionRefractiv eIndex | | xs:float | 1.234 | The refractive index of the immersion. [units:none] |
| | | | | If this field is empty, the refractive index is assumed to be the default refractive index of the specified immersion. |

| LensNA | AB | xs:float | 0.7 | The numerical aperture of the lens expressed as a floating point (real) number. |
|--------------------------|----|--------------------------|----------|--|
| | | | | Expected range 0.02 - 1.5 [units:none] |
| | | | | The depth of focus can be retrieved via the formula: depthOfField = 0.55 / (LensNA * LensNA). |
| NominalMagnificati on | AB | xs:float | 40 | The magnification of the lens as specified by the manufacturer - i.e. '40' is a 40x lens. [units:none]. |
| CalibratedMagnific ation | | xs:float | 39.978 | The magnification of the lens as measured by a calibration process - i.e. '39.987' for a 40x lens. [units:none]. |
| WorkingDistance | AB | xs:float | 105.534 | The working distance of the lens expressed as a floating point (real) number. Units are microns[um]. |
| Iris | | xs:boolean | false | Records whether or not the objective was fitted with an Iris. [flag] |
| PupilGeometry | AB | restriction of xs:string | Circular | Records what type of phase-rings ("Phasenringe") the objective has. |
| | | | | One of |
| | | | | Circular, Annular, PhaseRing1, PhaseRing2, PhaseRing3, Other |

6.7.8.5 Sample: InstrumentObjective

6.7.8.6 Information / Instrument / TubeLenses / TubeLens

XSD Complex type: InstrumentTubeLense

A description of the tube lens.

Required elements include the magnification.

A tube lens might be a tube lens, an optovar lens or a Bertrand lens.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|------------|---|
| Id | Y | xs:string | TubeLens:1 | Unique ID within a list of siblings. Recommended format is TubeLens: <nr>.</nr> |
| Name | | xs:string | | The name of this hardware component. |

| Element | req | Type (XSD) | Sample | Description |
|---------------|-----|---------------|---|--|
| Manufacturer | | Manufacture r | <pre><complex content=""></complex></pre> | Information about the manufacturer of this hardware. |
| Magnification | Y | xs:float | 1.4 | The magnification of the lens as specified by |

| | | | the manufacturer [units:none]. |
|------|----------------------------|---------|--|
| Туре | (restriction of) xs:string | Optovar | The type of the lens in the revolver element (might be a tube lens, an optovar lens or a Bertrand lens). |
| | | | One of (no restriction up to now!) |
| | | | Optovar, TubeLens, BetrandLens |

6.7.8.7 Sample: InstrumentTubeLens

6.7.9 Information / TimelineTracks

The timeline tracks contain event information about bleaching, user markers, user settings, incubation, focus action etc.

The TimelineTracks type is a sequence of *TimelineTrack* elements.

6.7.9.1 Information / TimelineTracks / TimelineTrack

| Element | req | Type (XSD) | Sample | Description |
|------------------|-----|---|-----------------|--|
| Id | Y | xs:string | Track:1 | Unique ID within a list of siblings. Recommended format is Track: <nr>.</nr> |
| Name | | xs:string | Bleaching Track | Name of this timeline track. |
| TimelineElements | | <pre><complex content=""></complex></pre> | | Sequence of TimelineElement elements. |

6.7.9.2 Information / TimelineTracks / TimelineTrack / TimelineElements / TimelineElement

| Element | req | Type (XSD) | Sample | Description |
|------------------|-----|--|-----------------|--|
| Time | | xs:dateTime | Track:1 | Unique ID within a list of siblings. Recommended format is Track: <nr>.</nr> |
| Start | | xs:string | Bleaching Track | Name of this timeline track. |
| Duration | | xs:double | | Sequence of TimelineElement elements. |
| Bounds | | <pre><complex content=""></complex>, see SubsetBoun ds</pre> | | Bounds associated with this event. |
| Trigger | | restriction of xs:string | | Information about how this event was triggered. Possible values: AtFixedTime, AtFixedBounds, UserInteraction, TriggeredByAction. |
| EventInformation | | xs:choice of <complex content=""></complex> | | |

6.7.9.3 TimelineElement / EventInformation

The EventInformation can be set by one of the following complex types:

- BleachingEvent

- UserMarkerEvent
- UserSettingEvent
- IncubationRecordingEvent
- FocusActionEvent
- ExecutionInformationEvent
- DigitalInputEvent

More detailed schema documentation is available as a separate HTML or CHM based documentation.

6.7.9.4 Sample: TimelineTracks

```
<TimelineTracks>
  <TimelineTrack Id="Track:1" Name="Bleaching Track">
    <TimelineElements>
      <TimelineElement Id="1">
        <Time>2012-01-26T11:02:26.8920202Z</Time>
        <Duration>0.30</Duration>
        <EventInformation>
          <Bleaching>
            <Type>BleachWithLaser</Type>
            <Laserlines>
              <Laserline>
                <Intensity>84.07960199005</Intensity>
                <Wavelength>405</Wavelength>
              </Laserline>
              <Laserline>
                <Intensity>36.81592039801</Intensity>
                <Wavelength>488</Wavelength>
              </Laserline>
            </Laserlines>
          </Bleaching>
        </EventInformation>
      </TimelineElement>
    </TimelineElements>
  </TimelineTrack>
  <TimelineTrack Id="Track:2" Name="UserMarkers Track">
    <TimelineElements>
      <TimelineElement Id="1">
        <Time>2012-01-26T11:02:26.8920202Z</Time>
        <Duration>0.00</Duration>
        <EventInformation>
          <UserMarker>
            <Type>PressedButton</Type>
            <Comment>Cola getrunken ...
          </UserMarker>
        </EventInformation>
      </TimelineElement>
      <TimelineElement Id="2">
        <Time>2012-01-26T12:02:26.8920202Z</Time>
        <Duration>0.00</Duration>
        <EventInformation>
          <UserMarker>
            <Type>PressedButton</Type>
            <Comment>Cola getrunken...
          </UserMarker>
        </EventInformation>
      </TimelineElement>
    </TimelineElements>
  </TimelineTrack>
  <TimelineTrack Id="Track:3" Name="UserSettings Track">
   <TimelineElements>
      <TimelineElement Id="1">
        <Time>2012-01-26T11:02:26.8920202Z</Time>
```

```
<Duration>0.00</Duration>
      <EventInformation>
        <UserSetting>
          <Type>Setting</Type>
          <Comment></Comment>
        </UserSetting>
      </EventInformation>
    </TimelineElement>
  </TimelineElements>
</TimelineTrack>
<TimelineTrack Id="Track:4" Name="IncubationRecording Track">
  <TimelineElements>
    <TimelineElement Id="1">
      <Time>2012-01-26T11:02:26.8920202Z</Time>
      <Duration>0.00</Duration>
      <EventInformation>
        <IncubationRecording>
          <Components>
            <MTBIncubationTemperatureChannel1 Name="Channel 1">
              <TargetValue>30</TargetValue>
              <Value>23.9</Value>
            </MTBIncubationTemperatureChannel1>
            <MTBIncubationO2Channel Name="O2 Channel">
              <Value>23.9</Value>
            </MTBIncubationO2Channel>
          </Components>
        </IncubationRecording>
      </EventInformation>
    </TimelineElement>
  </TimelineElements>
</TimelineTrack>
<TimelineTrack Id="Track:5" Name="FocusActions Track">
  <TimelineElements>
    <TimelineElement Id="1">
      <Time>2012-01-26T11:02:26.8920202Z</Time>
      <Duration>5.00</Duration>
      <EventInformation>
        <FocusAction>
          <a href="#">Action>SoftwareAutofocusRun</action></action>
          <Result>Success</Result>
          <StartPosition>28.0</StartPosition>
          <ResultPosition>33.4/ResultPosition>
        </FocusAction>
      </EventInformation>
    </TimelineElement>
    <TimelineElement Id="2">
      <Time>2012-01-26T11:06:26.8920202Z</Time>
      <Duration>10.00
      <EventInformation>
        <FocusAction>
          <action>SoftwareAutofocusRun</action>
          <Result>Failure
          <StartPosition>33.4</StartPosition>
          <Hint>Hit the search range boundary before finding a maximum./Hint>
        </FocusAction>
      </EventInformation>
    </TimelineElement>
    <TimelineElement Id="3">
      <Time>2012-01-26T11:08:26.8920202Z</Time>
      <Duration>0.00</Duration>
      <EventInformation>
        <FocusAction>
          <action>DefiniteFocusStabilize</action>
          <Result>Success</Result>
          <StartPosition>35.3</StartPosition>
          <ResultPosition>36.4</ResultPosition>
```

6.7.10 Information / Application

Main application specific information. Expandable via CustomAttributes.

| Element | req | Type (XSD) | Sample | Description |
|---------|-----|------------|----------------|---|
| Name | Y | xs:string | AimApplication | Name of the application (executable) which created the image application. |
| Version | | xs:float | 1.1 | Version of the application which created the image |

6.7.11 Information / Processing

This node contains specific data for special processing data requirements, e.g.

- DFT
- DCV
- Colocalization
- Stitching

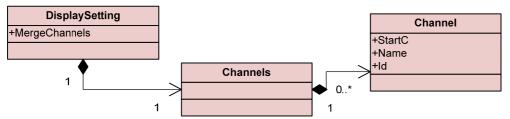
The persisted information may be obtained by the processing function upon first usage. It uses other metadata like Information/Instrument to get the values, provides modification features in the GUI and stores the results for later retrieval.

6.7.11.1 Sample: Processing

```
<Processing>
      <BlockShift>true/BlockShift>
      <ImageType>Basic</ImageType>
      <ProcessingDimension>2</processingDimension>
      <RefPixelType>3</RefPixelType>
      <ThirdDimension>4</ThirdDimension>
      <WindowMode>4</WindowMode>
    </DFT>
    <PSF>
      <AnticipatedPolarHeight>10</AnticipatedPolarHeight>
      <AxialResolution>3.5</AxialResolution>
      <Defocus>10</Defocus>
      <DesignCoverglassThickness>1e-3/DesignCoverglassThickness>
      <AnticipatedPolarWidth>3</AnticipatedPolarWidth>
      <Dimension>3</Dimension>
      <UsedImmersionIndex>3</UsedImmersionIndex>
      <Type>3</Type>
      <Source>3</Source>
      <WorkingDistance>3.4</WorkingDistance>
      <ZStackDirection>3</ZStackDirection>
    </PSF>
```

6.8 DisplaySetting

Contains generic multi-channel display adjustments (Low/High/Gamma) and advanced information like channel color mapping via palettes (lookup – tables).



The main use of DisplaySetting is to parametrize the GUI elements used in image visualization.

| Element | req | Type (XSD) | Sample | Description |
|--------------------|-----|---------------------|---|--|
| MergeChannels | | xs:boolean | true | Indicates whether display should be in multichannel (merged) mode. |
| ShowRangeIndicator | | xs:boolean | true | If true, shows display the image in "range idicator" mode to visualize under- and overexposure |
| Channels | Y | Channels Channel | <pre><complex content=""></complex></pre> | One entry for each contained image channel |

6.8.1 DisplaySetting / Channels / Channel

Defines the standard / current display settings for a single channel.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|-----------|--|
| Id | | xs:string | Channel:0 | Unique channel ID must match the identifiers in Information / Dimension / Channels to uniquely address a channel. Id not specified, the correspondance is via collection index. |
| Name | | xs:string | | The (user-defined) name of this channel. |

| Element | req | Type (XSD) | Sample | Description |
|-----------------------|-----|--------------------------|--------|--|
| Low | | xs:double | 0.1 | The normalized low (=black) value of the mapping range. Default value is 0.0. |
| High | | xs:double | 0.5 | The normalized high (=white) value of the mapping range. Default value is 1.0. |
| Gamma | | xs:double | 1.0 | The gamma value to be applied to the mapping range. Default value is 1.0. |
| IsAutoApplyEnabled | | xs:boolean | false | Indicates whether the command specified in <i>AutoApplyMode</i> is applied each time a new image subset is selected (e.g. when the player is running). Default valus is false. |
| AutoApplyMode | | restriction of xs:string | MinMax | Mode to be applied when the <i>AutoApplyMode</i> is set to true. One of <i>MinMax</i> , <i>BestFit</i> . |
| LowerBestFitThreshold | | xs:double | 0.1 | The lower threshold for the <i>BestFit</i> operation. The value is useful e.g. to skip large nearly |

| | | | black areas with some noise. Default is 0.1. |
|-----------------------|--|---|---|
| UpperBestFitThreshold | xs:double | 0.1 | The upper threshold for the <i>BestFit</i> operation. The value is useful e.g. to skip large white or nearly white areas. Default is 0.1. |
| Mode | restriction of xs:string | Spline | The mode: can be <i>Spline</i> , <i>Ramp or None</i> . Default is <i>None</i> . |
| Points | xs:string | 0.1, 0.7 | If <i>Mode</i> is Spline, these are the Spline Points. |
| | | 0.3, 0.6 | If mode is <i>Ramp</i> , these are the Points for the Ramp mode. |
| | | | If mode is <i>None</i> , there is supposed to be no Points node, i.e. Points are ignored. |
| Description | xs:string | | User defined description of the channel. |
| DyeName | xs:string | | The original Dye name taken from the acquisition information when the image was acquired. |
| ShortName | xs:string | | The information displayed in small GUI elements like channel buttons. |
| | | | If not set, <i>ShortName</i> is derived from the <i>Name</i> or the <i>DyeName</i> . |
| Color | (restriction of) | #FF0077 | The color in which to display the channel if <i>ColorMode</i> = Color. |
| | xs:string | | The string must be in #RRGGBB or #AARRGGBB format where R,G or B are the Red, Green and Blue components in hexadecimal notation. |
| ColorMode | restriction of xs:string | Color | The color mode in which to display the channel. |
| | | | One of Indeterminate, None, Color, Palette, Dye, Custom. |
| | | | If set to <i>Color</i> , uses the <i>Color</i> value, if set to <i>Palette</i> uses the <i>PaletteName</i> name to select a predefined system palette. <i>None</i> means: use original channel color. |
| | | | Currently, the other values are not used. |
| OriginalColor | (restriction of) xs:string | #FF0077 | The original color of the channel, i.e. the color the channel had on the most recent save event. |
| IsSelected | xs:boolean | true | If the DisplaySetting is in <i>MergeChannels</i> mode, the channel is only displayed if <i>IsSelected</i> is true. |
| PaletteName | xs:string | dawn | If <i>ColorMode</i> = <i>Palette</i> , the channel is displayed with the lookup-table (LUT) defined by the <i>PaletteName</i> . |
| ChannelWeight | xs:float | 1.0 | The channel weight (ratio among all selected channels). |
| ChannelUnit | <anonymous< td=""><td><pre><complex content=""></complex></pre></td><td>The <i>ChannelUnit</i> contains scaling factor, offset and unit for each image channel. The data are currently used by images with ion concentration data. The display value</td></anonymous<> | <pre><complex content=""></complex></pre> | The <i>ChannelUnit</i> contains scaling factor, offset and unit for each image channel. The data are currently used by images with ion concentration data. The display value |

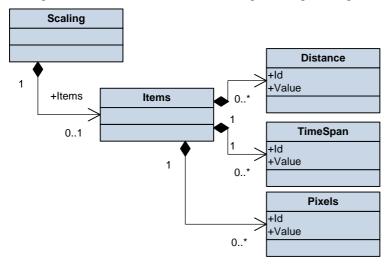
| | | is calculated by * PixelIntensity / M | DisplayValue = Factor axPixelIntensity + Offset. |
|--|--|---------------------------------------|--|
| | | | ensity" is the maximum ity for the data type (4095 |

6.8.1.1 Sample: DisplaySetting

```
<DisplaySetting>
 <Channels>
    <Channel StartC="0" Name="FITC" Id="Channel:0">
      <Color>#00FF00</Color>
      <Gamma>0.3</Gamma>
     <IsAutoApplyEnabled>true</IsAutoApplyEnabled>
     <Low>0.0</Low>
      <ColorMode>Color</ColorMode>
     <LowerBestFitThreshold>0.1/LowerBestFitThreshold>
      <PaletteName>dawn</PaletteName>
      <Weight>1.0</Weight>
    </Channel>
    <Channel StartC="1" Name="DAPI" Id="Channel:1">
      <Color>#FF00FF</Color>
      <Gamma>0.3</Gamma>
     <DyeName>Dye1</DyeName>
     <IsAutoApplyEnabled>true</IsAutoApplyEnabled>
      <ColorMode>Color</ColorMode>
      <IsSelected>true</IsSelected>
      <Weight>1.0</Weight>
    </Channel>
    <Channel StartC="2" Name="Rhodamin" Id="Channel:2">
      <Color>#FF0000</Color>
      <Gamma>0.3</Gamma>
      <DyeName>Dye1</DyeName>
     <IsAutoApplyEnabled>true</IsAutoApplyEnabled>
      <Low>0.0</Low>
      <IsSelected>true</IsSelected>
      <High>0.7</High>
     <PaletteName>dawn</PaletteName>
      <Mode>Spline</Mode>
      <Points>0.1,0.09 0.24,0.54 0.63,0.40 0.79,0.90</Points>
      <Weight>1.0</Weight>
    </Channel>
   </Channels>
 </DisplaySetting>
```

6.9 Scaling

Scaling is a collection of **UnitItems** – each representing an image dimension via Key (string).



In addition, scaling carries some information about parameters required to implement the "automatic scaling" feature (select a scaling from a predefined value based on the current hardware setting).

The optional **AutoScaling** node enables usage of this scaling for automatic scaling by providing elements that can be compared to the actual hardware state.

The value of the scaling items specify the units/index, or units/pixel in case of X and Y index, e.g.

```
<Distance Id="X">
  <Value>1.21e-6</Value>
  </Distance>
```

means that the image has a scaling of 1.21 micrometers (10⁻⁶ m) per pixel.

| Element | req | Type (XSD) | Sample | Description |
|-------------|-----|--|---|--|
| AutoScaling | | AutoScalingSettings | <pre><complex content=""></complex></pre> | Additional data to use this scaling for an Automatic scaling feature. Reserved for internal use. |
| Items | Y | choice of complex types <u>DistanceUnitItem</u> , <u>TimeSpanUnitItem</u> <u>PixelUnitItem</u> | <complex content=""></complex> | One item for each dimension, Accepted element names for the relted complex types are: Distance, TimeSpan or Pixels |

6.9.1.1 DistanceUnitItem complex type

Specifies a distance in meters.

| Attribute | req | Type (XSD) | Sample | Description |
|-----------|-----|------------|--------|---|
| Id | Y | xs:string | X | Identifier of the related dimension. One of <i>X,Y,Z,T</i> but may also specify other dimensions if units can be expressed in one of the supported UnitItems. |

| Element | req | Type (XSD) | Sample | Description |
|---------|-----|------------|--------|----------------------|
| Value | Y | xs:double | 1e-4 | Value in meters [m]. |

| DefaultUnitFormat | xs:string | um | Optional preselection of a display unit in GUI elements |
|-------------------|------------|-------|--|
| | | | One of <i>m</i> , cm, mm, u, μ, um, μm, nm, pm, i, inch, mil. |
| IsReciprocal | xs:boolean | false | If true, shows values as reciprocal units, e.g. 1 m shows as 1 m-1. |
| Origin | xs:double | 0.0 | Specifies an alternate origin (default is 0.0), used e.g. for rulers. |
| Direction | xs:integer | 0 | Optionally defines the scaling direction as follows: 1 positive, -1 negative, 0 (default) undefined. |

6.9.1.2 TimeSpanUnitItem complex type

Specifies a time span in seconds.

Also supports: Id, IsReciprocal, Origin and Direction – see $\underline{\text{DistanceUnitItem}}$.

| Element | req | Type (XSD) | Sample | Description |
|-------------------|-----|------------|--------|---|
| Value | | xs:double | 1e-4 | Value in seconds [s]. |
| DefaultUnitFormat | | xs:string | um | Optional preselection of a display unit in GUI elements |
| | | | | One of |
| | | | | s , ms , us , μs , ns , ps . |

6.9.1.3 PixelUnitItem complex type

Specifies pixel (or "no") scaling. For use in a Scaling context, the *Value* is normally 1.0.

Also supports: Id, IsReciprocal, Origin and Direction – see DistanceUnitItem

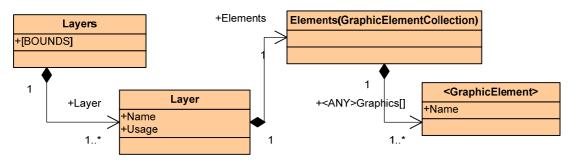
| Element | req | Type (XSD) | Sample | Description |
|-------------------|-----|------------|--------|---|
| Value | | xs:double | 1e-4 | Value in pixels [px]. |
| DefaultUnitFormat | | xs:string | um | Optional preselection of a display unit in GUI elements |
| | | | | One of |
| | | | | px , mpx. |

6.9.1.4 Sample: Scaling

```
<Scaling>
 <AutoScaling>
   <CameraFramePixelDistance>1</CameraFramePixelDistance>
   <CameraFrameBinning>1</CameraFrameBinning>
   <CameraAdapterMagnification>1</CameraAdapterMagnification>
   <Optovar>1</Optovar>
   <ReflectorMagnification>1</ReflectorMagnification>
   <Objective>Plan-Neofluar 1.25x/0.04</Objective>
 </AutoScaling>
 <Items>
   <Distance Id="X">
      <DefaultUnitFormat>um/DefaultUnitFormat>
     <Origin>10.1</Origin>
      <Value>1.21e-6</Value>
   </Distance>
   <Distance Id="Y">
```

6.10 Layers

A collection of (graphical) layers for overlays.

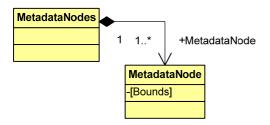


6.10.1.1 Sample: Layers

```
<Layers>
  <Layer Name="Layer1">
    <Usage>Annotation</Usage>
    <IsProtected>false</IsProtected>
    <LayerFlags>1</LayerFlags>
    <Elements>
      <Line Id="7">
        <Geometry>
          <X1>32.067510548523217</X1>
          <Y1>167.93248945147678</Y1>
          <X2>167.93248945147678</X2>
          <Y2>232.06751054852322</Y2>
        </Geometry>
      </Line>
      <Rectangle Id="10">
        <Geometry>
          <Left>215</Left>
          <Top>149</Top>
          <Width>170</Width>
          <Height>100</Height>
        </Geometry>
      </Rectangle>
      <Bezier Id="56">
        <Geometry>
          <Points>572.417721518987,302.95358649789 691.405063291139,304.64135021097
696.46835443038,432.911392405063 555.540084388186,483.544303797468</Points>
        </Geometry>
      </Bezier>
    </Elements>
  </Layer>
```

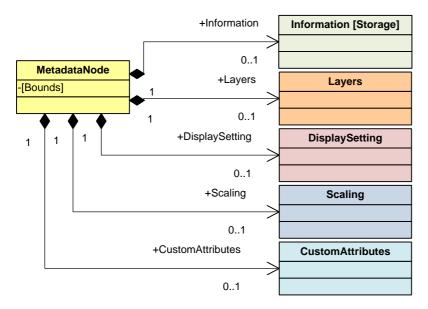
6.11 MetadataNodes

A collection of nodes, each containing metadata for an image subset.



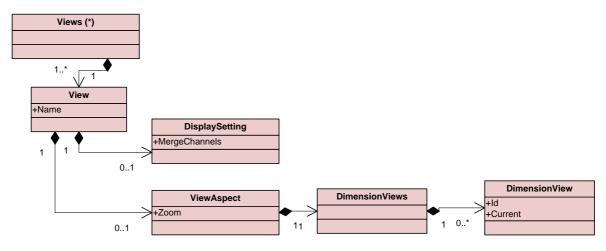
6.11.1 MetadataNode

Contains Metadata for a specific Image subset. The elements in this node are the same as the global information nodes in the **Metadata** element. The main usage is to define additional data for a given subset or information data overriding the default values.



6.12 Views

The **Views** tree contains a collection of various named views defining a specific aspect of the image (e.g. T2, Z5) to set the displayed position within a multi-dimensional image and optional display settings.



6.12.1.1 Sample: View

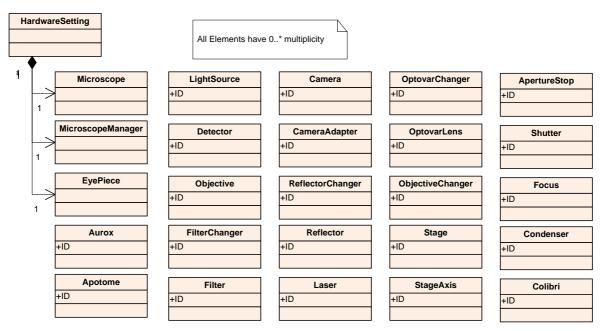
```
<View Id="Default" Name="Default">
   <ViewAspect>
     <DimensionViews>
       <DimensionView Id="Z">
         <Current>10</Current>
       </DimensionView>
       <DimensionView Id="T">
         <Current>5</Current>
       </DimensionView>
     </DimensionViews>
   </ViewAspect>
   <DisplaySetting>
     <Channels>
       <Channel Id="Channel:0">
         <Color>#220077</Color>
         <High>0.7</High>
       </Channel>
       <Channel Id="Channel:1">
         <Color>#ff0077</Color>
         <PaletteName>dawn</PaletteName>
         <Weight>1.0</Weight>
       </Channel>
       <Channel Id="Channel:2">
         <Gamma>0.3</Gamma>
       </Channel>
       <Channel Name="TexasRed" Id="Channel:4">
         <Color>#CC00FF</Color>
       </Channel>
     </Channels>
   </DisplaySetting>
 </View>
```

7 Additional Metadata

7.1 HardwareSetting

This schema is **currently not released for public use** as details are still under construction. Contains the (microscopy) hardware configuration related to acquisition of an image subset.

This element is currently not specified in detail.



HardwareSetting is a flat list of devices with their parameters. It represents the changes in the state of the components related to the general **HardwareConfiguration** element.

7.1.1 HardwareSetting (in root node)

HardwareSetting (in root node) represents the current hardware configuration at the time an acquisition process was started and the initial states of the various devices.

A summary of all hardware relevant information is available in the **Information.Instrument** element. Using **HardwareSetting** is an advanced topic. A full **HardwareSetting** includes all the metadata required to reconstruct the user interface, e.g. the *LightPath* visualization.

Because **HardwareSetting** contains huge amount of XML and is stored only once –during a possibly complex acquisition, it may not be included in the global Metadata segment which is updated various times. Instead, it may use an attachment segment named "**HardwareSetting**".

7.1.1.1 Sample: HardwareSetting (configuration)

```
<ReflectedLightPath>
    </ReflectedLightPath>
    <TransmittedLightPath>
    </TransmittedLightPath>
  </Microscope>
  <ApertureStop Id="MTBRLApertureStop" Name="Motorisierte Aperturblende Auflicht">
    <Motorization>Motorized/Motorization>
  </ApertureStop>
  <LightSource Id="MTBTLHalogenLamp">
    <LightPathLocation>TransmittedLight</LightPathLocation>
    <Model>AttoArc</Model>
    <SerialNumber>2345</SerialNumber>
    <Motorization>Motorized/Motorization>
  </LightSource>
  <Shutter Id="MTBRLShutter" Name="Aquila.RL_UniblitzShutter_mot">
    <NumberOfPositions>2</NumberOfPositions>
    <Motorization>Motorized/Motorization>
  </Shutter>
  <Camera Id="MTBCamera_MTBSideportChanger_Left" Name="AxioCam1">
    <Type>CCD</Type>
    <ColorMode>Color</ColorMode>
    <BlueReferenceHigh>1.34/BlueReferenceHigh>
    <AnalogGainEnabled>true</AnalogGainEnabled>
    <BlackReferenceEnabled>true/BlackReferenceEnabled>
  </Camera>
  <CameraAdapter Id="MTBCameraAdapter_MTBBaseportChanger_Frontport"</pre>
<NumberOfPositions>2</NumberOfPositions>
    <Magnification>1</Magnification>
    <CameraRef Id=" MTBCamera_ MTBBaseportChanger_Frontport"/>
  </CameraAdapter>
  <EyePiece Id="MTBEyePiece" Name="10x SF23" IsAvailable="true">
    <Magnification>10</Magnification>
    <TotalFieldOfView>23</TotalFieldOfView>
  </EvePiece>
  <Focus Id="MTBFocus" Name="Motorisierter Fokus">
    <Motorization>Motorized/Motorization>
    <Position>0</Position>
    <MinPosition>-14000/MinPosition>
    <MaxPosition>14000</MaxPosition>
    <HasLoadWork>true/HasLoadWork>
  </Focus>
</HardwareSetting>
```

7.1.2 HardwareSetting (delta in Root or in MetadataNodes)

Defines the deltas to the initial state during acquisition of specific image subsets.

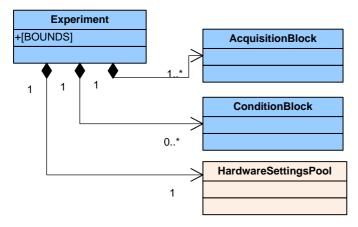
7.1.2.1 Sample: HardwareSetting (delta)

```
</Focus>
  <ObjectiveChanger Id="MTBObjectiveChanger">
    <Position>3</Position>
  </ObjectiveChanger>
  <OptovarChanger Id="MTBOptovarChanger">
    <Position>3</Position>
  </OptovarChanger>
  <ReflectedLightExtFilterChanger Id="MTBRLExtFilterChanger">
    <Position>8</Position>
  </ReflectedLightExtFilterChanger>
  <ReflectorChanger Id="MTBReflectorChanger">
    <Position>6</Position>
  </ReflectorChanger>
  <StageAxis Id="MTBStageAxisX">
    <Position>123.56</Position>
  </StageAxis>
  <StageAxis Id="MTBStageAxisY">
    <Position>123.56</Position>
  </StageAxis>
</HardwareSetting>
```

7.2 Experiment

An experiment contains a global pool of **HardwareSettings** and a series of various blocks:

- An **AcquisitionBlock** describes a multi-dimensional acquisition (details see following chapters).
- A **ConditionBlock** contains triggers and programmable conditions.



Discussion: Detailed schema information is subject to ongoing discussions, the current implementation uses the Light microscopy definitions only. Additional data for confocal acquisition have to be merged. Finalizing of those tasks is in the 2013 timeframe.

8 Topography Image Extensions - Metadata specification

8.1 Introduction

Motivation of this document is to describe

- organization of the topology data to be kept in an CZI file
- introduce and define the concepts of "heightmap" and "topography data item"

Basic idears to intoroduce a topography-concept in the CZI fileformat:

There is necessity to ...

- store a physical property ("height") for each pixel of an image. The solution is the ability to express that a specific channel contains a height-map.
- express the notion of "not measured" or "invalid measurement" for each pixel.
- group a set of entities (=heightmap and images that is).

The term "topo data" is understood as "height per pixel" – so that we can express an elevation on a plane. It is not understood in a broader definition (like e. g. describing the boundary of a 3-D object).

Our goal was to integrate the topography concept into the CZI-format as non-intrusive as possible, so that existing code (which is not aware of these concepts) is not broken. So, these additions to the CZI-format should have a minimum impact on existing implementations.

Sidenote:

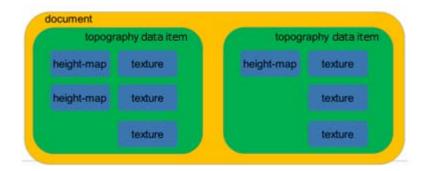
As a matter of fact, in various releases of the ZEN.Blue or ZEN.Black software different ways were implemented in order to store heightmaps and associated metadata, e.g. see chapter 'ZEN black Topography images'. All of these implementations are considered legacy, and for these CZI-documents appropriate code-paths must be considered and implemented if they are to be recognized correctly. Dealing with such legacy formats is not an automatic process that can be taken for granted, it will be handled on demand.

Going forward, only the information specified in this chapter is to be used.

8.2 Concepts

8.2.1 Topography Data Item

A *Topography Data Item* is a set of images, and is composed of heightmaps and textures. Heightmaps and textures are channels (on the level of the CZI-document). Conceptually, the organization is



It is important to recognize that the metadata describing the Topography Data Item is only complementary information; it does not invalidate or overrule other metadata in the CZI.

8.2.2 Heightmap

Heightmaps are organized as planar bitmaps, i.e. there is one number per pixel per heightmap. A Topography Data Item may very well contain more than one heightmap.

8.2.3 Textures

Textures are photographic images, and pixels at the same x-y-position (among different textures or heightmaps) refer to the same point in space (and the same specimen).

8.3 Metadata structure for topography

In the following the metadata is specified which is to be used to describe a topography document. This structure is mandatory in order to recognize the document as containing topographic information.

<Appliances>

8.3.1 TopographyData

The element TopographyDataItem describes one Topography Data Item. It is placed inside of an "Appliance"-node. The attribute Id of the node must contain the string "Topography:1" (exactly this string, e. g. no other numbers are allowed). This node may contain multiple TopographyDataItem-nodes; so in order to have an multiplicity >1 for the TopographyDataItem do not create an Appliance-Node with an Id different to "Topography:1" like e. g. "Topography:2", instead have multiple TopographyDataItem nodes inside the Items-node). The data format is as depicted here:

```
<Appliance Id="Topography:1">
    <Data>
      <Items>
        <TopographyDataItem Id="tdi1">
           <Textures>
             <Texture StartC="0"/>
             <Texture StartC="1"/>
                                                A Topography Data Item is
           </Textures>
                                                defined here by listing the
           <HeightMaps>
                                                textures and the heightmaps
             <HeightMap StartC="2"/>
           </HeightMaps>
        </TopographyDataItem>
        <TopographyDataItem Id="tdi2">
                                                      There can be any number of
           <Textures>
                                                      Topography Data Items given
             <Texture StartC="1"/>
                                                      here
             <Texture StartC="4"/>
           </Textures>
           <HeightMaps>
             <HeightMap StartC="5"/>
           </HeightMaps>
        </TopographyDataItem>
      </Items>
    </Data>
  </Appliance>
</Appliances>
```

Notes:

- Among all nodes of type TopographyDataItem the attribute Id must be unique.
- For the nodes of type Texture and HeightMap an attribute-group of type SubsetBounds is syntactically allowed. However, only the attribute StartC is to be honored, all other attributes (among the attribute-group SubsetBounds) are to be ignored.
- If the attribute StartC is not present, then StartC=0 is to be assumed.

The latter two statements are made for legacy reasons; for newly created document it is mandatory that the attribute "StartC" is present and that it is the only attribute.

8.3.2 Channel-Metadata

A channel containing a heightmap should be marked in the channel's metadata as ChannelType=Heightmap – like in this example:

```
<Image>
    <Dimensions>
      <Channels>
        <Channel Id="Ch0" Name="Ch0">
        </Channel>
        <Channel Id="Ch1" Name="Ch1">
        </Channel>
        <Channel Id="Ch2" Name="Ch2">
          <ChannelType>Heightmap</ChannelType>
        </Channel>
      </Channels>
    </Dimensions>
  </Image>
                                                                      This channel is a heightmap,
</Information>
                                                                      therefore it is strongly
                                                                      recommended to set the
<Appliances>
                                                                      ChannelType accordingly
  <Appliance Id="Topography:1">
    <Data>
      <Ttems>
        <TopographyDataItem Id="tdi1">
          <Textures>
            <Texture StartC="0"/>
            <Texture StartC="1"/>
          </Textures>
          <HeightMaps>
            <HeightMap StartC="2"/>
          </HeightMaps>
        </TopographyDataItem>
      </Items>
```

The only reason for stating that it "should be set" is for legacy reasons. For newly created documents, this condition must be met.

8.3.3 DocumentType

The field Information/Document/SubType is not required to be set to a specific value. It is also not recommended to check it for a specific value (as far as this specification is concerned). It is recommended not to honor this field in any respect.

8.4 Heightmap data format

8.4.1 Pixeltype

If a channel is declared as a heightmap, then it must have the pixeltype Float32. This applies to all subblocks for this channel (and all pyramid-subblocks). Other pixeltypes are not allowed.

8.4.2 Unit

The unit of length is micro-meter = 10^{-6} m. Each float pixel in a heightmap directly gives a unit of length in μ m.

8.4.3 Non-measured pixels

A float value of NaN (Not a Number) is used to mark a pixel as "not measured" or "invalid measurement". Software is expected to treat this value appropriately.

Currently, other special float values (like e.g. infinity) are not used.

8.5 Detecting the presence of a Topography Data Item

In order to detect the presence of a Topography Data Item, the following operations are recommended:

- 1. Enumerate all sub-nodes of type "Appliance" of node "Appliances", and search for a node (of type "Appliance") with the attribute Id = "Topography:1".
- 2. If such a node is found, navigate to the subnodes "Data/Items".
- 3. If .../Data/Items exists, then it must contain a list of nodes with type "TopographyDataItem". The content of this node gives the description of a Topography Data Item.
- 4. All channels listed as heightmaps (in all nodes of type TopographyDataItem at the specified location) are to be considered containing height-information (and are subject to the restrictions associated with it).

This condition is necessary (for a channel to be identified as a heightmap), a sufficient condition is also the channeltype (in the channel's metadata).

However, please note:

- For legacy reasons, the ChannelType may not be found in every document.
- There is no requirement that if a ChannelType=Heightmap is found, that this heightmap is a member of a Topography Data Item (or that there are any Topography Data Items at all).

It is recommended to treat the information from the node Appliance Id='Topography:1' as authorative.

9 Additional Material

9.1 ZEN black Topography images

There are two important entries in the CZI metadata XML that need to be analysed to import the topography images correctly.

The two entries are (with full path):

1) Image Subtype:

ImageDocument\Metadata\Information\Document\SubType

2) Channel Unit:

ImageDocument\Metadata\DisplaySetting\Channels\Channel\ChannelUnit

A complete XML example for the topography height map is given here:

```
<ImageDocument>
  <Metadata>
    <Version>1.0</Version>
    <Information>
      <Document>
        <SubType>Topography</SubType>
      </Document>
    </Information>
    <DisplaySetting>
      <Channels>
        <Channel Id="D83F925549DFF0A977EE888F8C95022E" Name="Ch1">
          <ChannelUnit>
            <FactorI>4.218e-005</FactorI>
            <OffsetI>0</OffsetI>
            <UnitI>Meter</UnitI>
          </ChannelUnit>
        </Channel>
      </Channels>
    </DisplaySetting>
  </ImageDocument>
</Metadata>
```

The following images can be saved with a CZI file:

1) Topography Height Map Image:

The topography height map image contains the height of the computed surface normalized to [0..1] range. The values are saved as float32 (4 Byte) data. The image contains height information for all pixels. No values will be set to zero due to intensity thresholds in the topography height map creation. To mask out pixels a separate image (Topography Mask Image, see point 3) is used. A Topography Height Map Image will always be accompanied by a Topography Maximum Intensity Image.

Note: There might be CZI and LSM images that contain only a Topography Height Map Image due to a bug in ZEN black topography viewer image export.

Sample XML:

2) Topography Maximum Intensity Image:

The topography maximum intensity image contains the intensity information of the surface pixel that has been computed during topography height map image creation.

Sample XML:

3) Topography Mask Image:

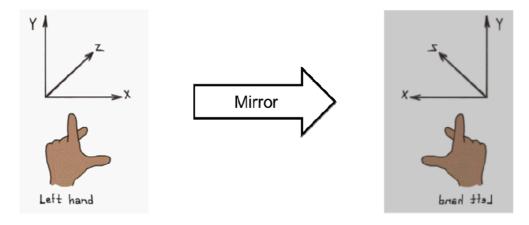
Used to mask out image pixel that have intensity values below or higher than a certain threshold during the topography height map image creation. This image is not mandatory to use and might not be saved in exported Topography Images.

```
Sample XML:
```

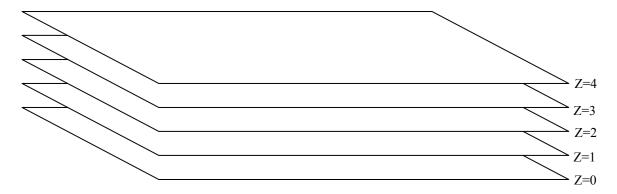
```
<SubType>TopoGraphyMask - Please note: use big "G" in
"TopoGraphyMask"
```

9.2 Spatial orientation (of Z-stacks)

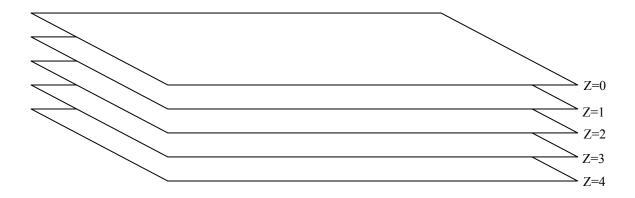
The following definitions are motivated by the necessity to define the chirality of voxel-data which is stored as z-slices (aka z-stack). If the specimen in a z-stack is reconstructed by layering the z-slices, the spatial orientation of the z-axis makes the difference between a left-handed and right-handed coordinate system – and in result a mirrored specimen.



It is therefore important (in a spatial reconstruction like e. g. in 3D-viewer) whether the z-slices are arranged this way



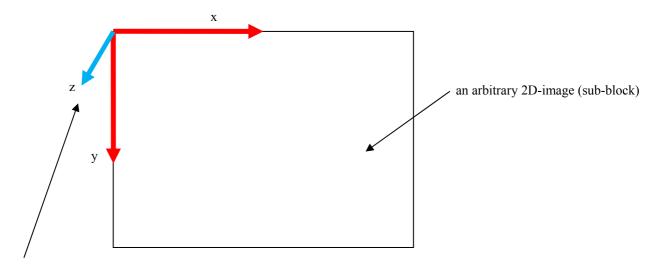
or that way:



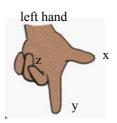
We therefore make the definition:

If the z-slices are stacked spatially according to their Z-index, the coordinate-system is defined in the following way:

The X- and Y-direction is defined by the images in the sub-blocks. The Z-coordinate is then taken to be perpendicular to them in either a left-handed or right-handed orientation.



The z-axis is pointing towards the reader \rightarrow left-handed coordinate system.



The chirality "left-handed" or "right-handed" is given in a new metadata-field "../Dimensions/Z/XYZHandedness". It can have the following values:

| value | description |
|-------------|---|
| LeftHanded | The coordinate-system (in which z-slices are to be spatially arranged according to their Z-indices) is left-handed (as shown in above picture). |
| RightHanded | The coordinate-system (in which z-slices are to be spatially arranged according to their Z-indices) is right-handed |
| undefined | The orientation is unknown - which means that the creator of the document states that the orientation is not known/not defined. |

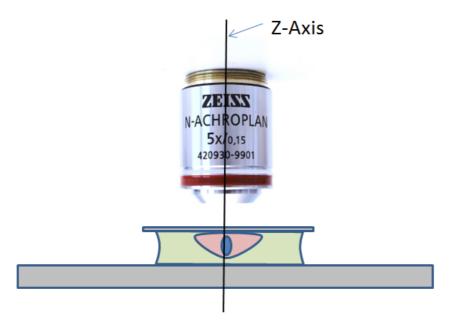


"undefined" is also the default value to be assumed if the field "Handedness" is not present. Therefore, legacy documents have an undefined chirality.

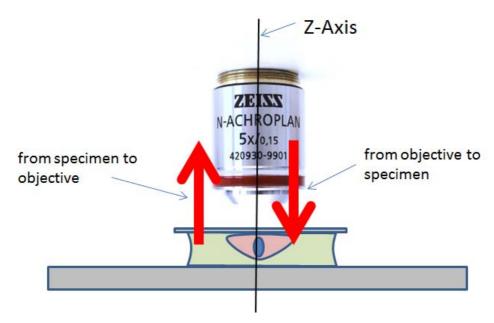
Note that this definition currently does not apply to the spatial arrangement according to the "associated Z-distances" (which are stated in Dimension/Z/Positions). The necessity for a strict definition has not yet arisen, and there has not been a consensus on this topic. It is nevertheless recommended at this point that the order of the z-slices is not changed when arranging them according to the "associated Z-distances".

9.3 Z-Axis Direction (experimental)

We define the Z-axis to be colinear with the optical axis, i. e. a line through the objective and the specimen. For a conventional microscope we have this:

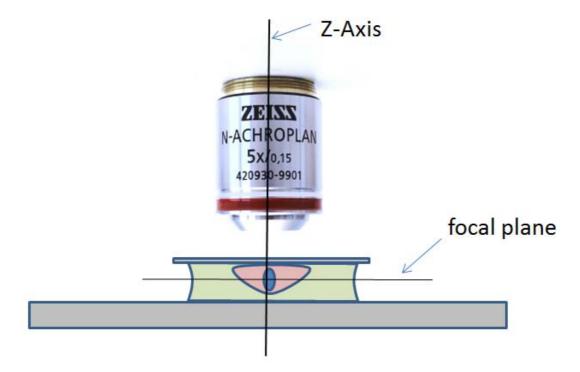


The direction of the Z-axis may either be from objective/viewer to specimen or from specimen to objective/viewer as shown here:



The Z-axis defined this way is used for the distances which are given in ../Dimensions/Z/Positions ("Z-labels") and for the focus-position found in the subblock-metadata (../Tags/FocusPosition). Conceptually, these positions specify

the Z-position of the focal plane in a coordinate-system where the specimen is at rest.



The origin of the z-Axis is not defined here – but the positions given in ../Dimensions/Z/Positions and in the subblock-metadata ((../Tags/FocusPosition) must refer to the same origin.

We define a field **ZAxisDirection** (located at ../Dimensions/Z) which gives the information about the direction of the z-axis (as defined above):

ZAxisDirection

We define the z-axis to be collinear with the optical axis. On this axis the z-coordinates of the focal plane are measured, and their distances are found in the Z-labels (defined unter Positions) and in the FocusPosition-field found in the subblock-metadata. The direction of the axis may either be "from specimen to objective" or "from objective to specimen". In this coordinate system the specimen is at a fixed position. Note that the origin of the coordinate system is _not_ defined here.

Possible values are: FromSpecimenToObjective, FromObjectiveToSpecimen and undefined. Undefined is the default (and to be assumed if this element is not present).

Some Q and A:

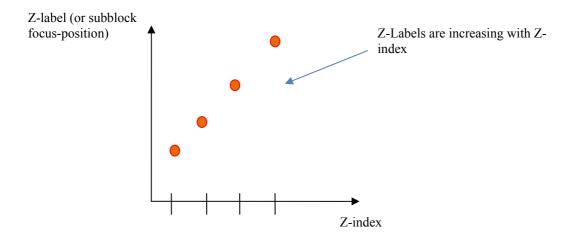
What is the definition of the Z-labels in case of a mosaic or a t-stack?

In case of a mosaic (or a t-stack or a muli-channel) the actual focus-positions may be different (per e. g. M-, T- or C-index). Since the Z-labels only assign **one** position to **one** Z-index, they cannot capture the modality accurately in this case. We expect that the Z-labels give a representative and meaningful position – without further defining what this means. Only the subblock-focus-positions capture all data.

How do I find out whether "lower Z-index" means "specimen closer to objective/viewer" or the opposite?

Check whether ZAxisDirection is "FromObjectiveToSpecimen" or "FromSpecimenToObjective". Next, you need to check the Z-Labels and determine whether the condition "from Z-Index Z1 > Z-Index Z2 follows that Z-Label(Z1) >

Z-Label(Z2)" holds (for all Zs) – or the opposite "from Z-Index Z1 < Z-Index Z2 follows that Z-Label(Z1) > Z-Label(Z2)". In other words – whether the function Z-Index \rightarrow Z-Label is strictly monotonic increasing or decreasing.



The first piece of information (ZAxisDirection) tells whether

| ZAxisDirection | Z-Label A > Z-Label B means that | |
|-------------------------|---|--|
| FromObjectiveToSpecimen | plane A is further away from objective than plane B | |
| FromSpecimenToObjective | plane A is closer to the objective than plane B | |

Taking this together with the monotony of the function Z-index → Z-Label gives us

| ZAxisDirection | function Z-index →Z-Label is monotonic | Z-Label A > Z-Label B means that |
|-------------------------|---|---|
| FromObjectiveToSpecimen | increasing | plane A is further away from objective than plane B |
| FromSpecimenToObjective | increasing | plane A is closer to the objective than plane B |
| FromObjectiveToSpecimen | decreasing | plane A is closer to the objective than plane B |
| FromSpecimenToObjective | decreasing | plane A is further away from objective than plane B |

How do I check whether the function Z-index →Z-Label is increasing or decreasing?

This is complicated by the fact that the relation Z-index vs. Z-Label may be given in three variants: either as an increment, as a list of numbers in XML or by a reference to a binary attachments (cf. ../Dimensions/Z/Positions). In the latter two cases, strictly speaking, one has to walk through all Z-indices and check each Z-Label individually. It is not sufficient to check just two labels, there is no strict requirement that the list is even monotonic. Furthermore, the above remark needs to be taken into consideration about the fact that Z-labels are just representative numbers. So, depending on the task at hand, it may also be necessary to check the subblock focus-positions individually.

What do I do if the ../Dimensions/Z/Positions – information is not present/not valid (or the subblock focus-positions)?

In this case the ZAxisDirection does not provide any usable information.

10 Terms and Abbreviations

| Id | Reference |
|-----------------------------|---|
| МТВ | Microscope Tool Box |
| GUI | Graphical User Interface |
| HDR | High Dynamic Range |
| CZI | Carl Zeiss Image |
| bitmap | The term bitmap is used in the sense "rectangular array of entities", where each entity is called a pixel and of a certain type (pixeltype), and all pixels of a bitmap have the same pixeltype |
| Z-labels | The distance associated with the Z-index (found in/Dimensions/Z/Positions) |
| subblock-focus- position | The focus-position found in the subblock-metadata (/Tags/FocusPosition) |