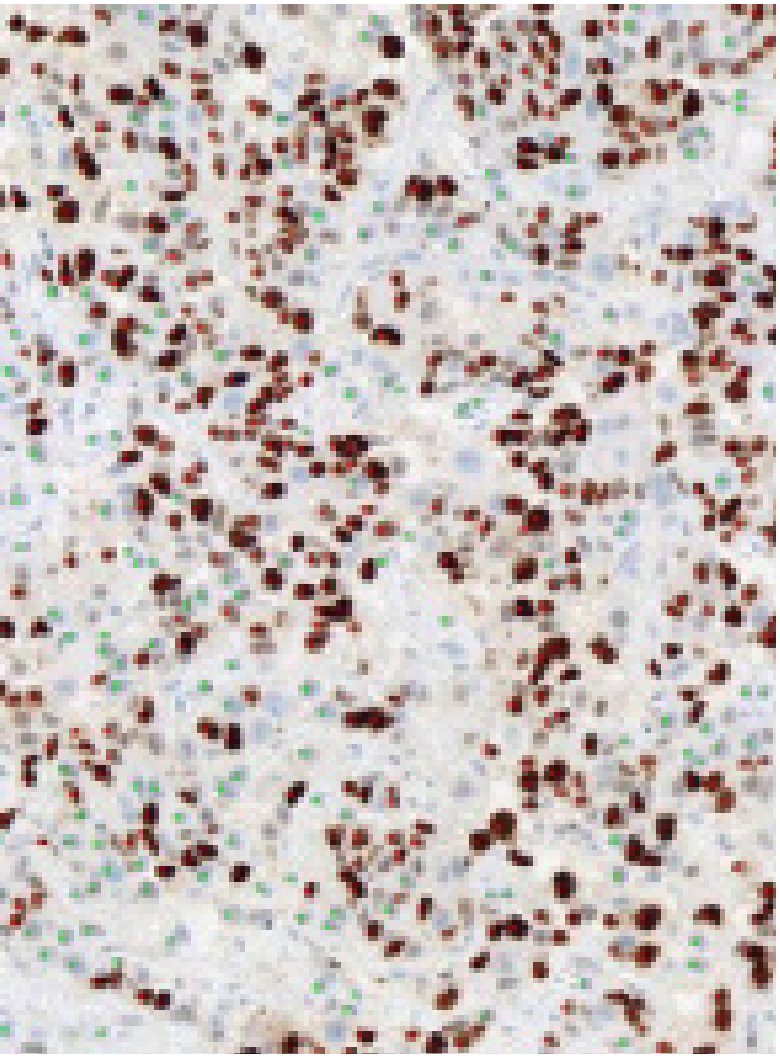
# Roche Digital Pathology

*BIF image file format for digital pathology*



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**Version 1.0**

# Glossary

| Acronym | Description |
|---|---|
| **AOI** (Area of Interest) | Area of interest, a rectangular region on the slide marked for scanning. AOIs have to be located outside of the label area of the slide. AOIs are proposed by the scanner software based on the perceived presence of tissue and also can be created, edited and deleted by the User. |
| **BIF** (BioImagene Image File) | Image file format used by Roche Tissue Diagnostics Digital Pathology scanner. Named after the original scanner manufacturing company. BIF-Files are fully compatible with the BigTIFF-variant of the TIFF v6.0 definition. |
| **BigTIFF** (Big Tagged Image File Format) | A variant of the TIFF-format, originally proposed in 2007 and implemented in LibTIFF v 4.0 (2011). It overcomes limitations to the size of an image stored in a TIFF-file by using 32bit pointers instead of 16bit pointers. While standard TIFF files cannot exceed 4GB in total file size, BigTIFF files can be up to 18 HexaBytes in size.[1] |
| **DP** | Digital Pathology |
| **ICC** (International Color Consortium) | A vendor-sponsored non-profit organization chartered with the development and promotion of vendor-neutral, cross-platform color management systems. |
| **IFD** (Image File Directory) | Logical unit defined by the TIFF-standard for storing a single image. Each TIFF-file contains at least one IFD, which encapsulates meta-data and pixel data for a single image. |
| **LOC** | Library of Congress |
| **LibTIFF** | A library implementation of the TIFF-standard originally implemented by Sam Leffler[2]. |
| **TIFF** | A file format for storing one or more images in a single file. While originally conceived for storing binary (BW) images only, it evolved into a flexible and adaptive file format still in widespread use almost 40 years after its conception. It stores meta-data describing the image geometry and other properties using keyword-value pairs. Pixel data can be stored uncompressed or using one of over 20 different compression methods.[3] The copyright for the file format is currently with Adobe Systems.[4] |
| **TIFF-Tag** | A marker (DWORD 32bit integer) of a known value, used by the TIFF-format to precede the corresponding data value. |
| **Tiffdump.exe** | A LibTIFF command line utility for extracting the contents of a TIFF-file in human readable form. |
| **RTD** | Roche Tissue Diagnostics |
| **VENTANA DP 200** | Roche Tissue Diagnostics Digital Pathology brightfield whole slide scanner. This document only addresses BIF-files generated by this scanner. |
| **VENTANA iScan Coreo** | Roche Tissue Diagnostics Digital Pathology brightfield whole slide scanner. BIF-files generated by this scanner are not addressed by this document. |
| **VENTANA iScan HT** | Roche Tissue Diagnostics Digital Pathology high-throughput brightfield whole slide scanner. BIF-files generated by this scanner are not addressed by this document. |
| **WSI** | Whole Slide Imaging |
| **XML** | Extended Markup Language |

[1] https://www.awaresystems.be/imaging/tiff/bigtiff.html
[2] https://gitlab.com/libtiff/libtiff
[3] http://www.simplesystems.org/libtiff/
[4] https://www.adobe.io/open/standards/TIFF.html

# BIF format

**With the acceleration of immunotherapies and the development of more complex assays, Roche is committed to an open environment for digital pathology. Roche encourages the development of reliable image analysis algorithms that can provide pathologists with tools to improve efficiency and precision for diagnosis. To that end, Roche is making the specifications of the Roche proprietary BIF file format publicly available.**

***Note:*** *To better understand this document, the reader should be familiar with the relevant TIFF and BigTIFF specifications and terminologies.*

The RTD BIF-image file format is fully compliant with the BigTIFF-variant of the TIFF-standard release 6.0. At 40x magnification, the image of a double-wide tissue slide can have up to 200,000 x 200,000 pixels. Considering the possibility of volumetric scans with multiple focus layers, the whole-slide image files can easily break the 4GByte size limit imposed by the use of 32-bit file pointers defined by the TIFF-standard, even when compressed. Using 64-bit file pointers, the BigTIFF-variant of the TIFF-standard overcomes the file-size barrier.

The BIF-file format also utilizes some TIFF-extension properties as defined in Part II of the standard. For example, it implements a multi-page TIFF-file for storing multiple images in a single TIFF-file. Any TIFF-reader implementing the TIFF-baseline feature set (Part I of the standard) is able to load and display the first Image File Directory (IFD), which is the overview image of the slide.

Additional TIFF-extension features utilized in BIF-images are (a) storing some of the images organized in smaller tiles, and (b) JPEG-compression as defined in the Technical Note 2[5] using YCbCr encoded images.

The BIF-format also supports storage of volumetric slide scans with multiple image layers using the private TIFF-tag IMAGE_DEPTH (0x80E5) assigned to Silicon Graphics International.

While traditional tiled TIFF-images typically abut each other without any overlap, some BIF-image tiles overlap to better account for the stitching of the potentially large tissue images. Information about tile overlap is included as meta-data for the TIFF-tag XMP (0x02BC).

The TIFF-tag XMP describes the content of each IFD and how to interpret the meta-data necessary for reconstructing the whole slide image contained in BIF-files. This description of the BIF-file format can only be used for reconstructing images acquired with whole slide scanners belonging to the VENTANA DP family of scanners (e.g., VENTANA DP 200). Images created with older scanners, such as VENTANA iScan Coreo or VENTANA iScan HT, cannot be reconstructed correctly based on the information included in this document and should not be attempted.

***Note:*** *Feeding pixel data extracted from BIF-files created by iScan Coreo slide scanner or iScan HT slide scanner into segmentation algorithms will most likely result in incorrect object counts. Again, this document only covers the contents of BIF-files with the IFD0:iScan>>ScannerModel-attribute set to 'VENTANA DP 200'.*

***Note:*** *The scan image pixel data are stored in the device-dependent color space. To achieve accurate color reproduction comparable to viewing the tissue slide on a brightfield microscope with a D-65 illuminator, the raw pixel data need to be transformed into the color space of the display device using the ICC-profile (version 4) included in the ICC_PROFILE-tag (0x8773). Failure to apply the correct color transformation before viewing scanned images or engaging algorithmic analysis of pixel data will most likely yield incorrect results.*

***Note:*** *The VENTANA DP 200 slide scanner can also create regular BigTIFF-images (.TIFF file extension) with no overlap between any of the image tiles. The meta-data included in those image files differ to some extent from what is described in this document.*

[5] https://awaresystems.be/imaging/tiff/specification/TIFFTechNote2.txt

## Whole slide imaging process

VENTANA DP 600 slide scanner can image single-wide slides and VENTANA DP 200 slide scanner can image single-wide and double-wide slides at 20x and 40x magnification as defined by ISO:8037-2[6]. Single-wide slides measure 25mm x 75mm and double-wide slides measure 50mm x 75mm. The top 25mm are reserved for affixing a label. The lower 50mm are reserved for the tissue sample (see Figure 1 for details).

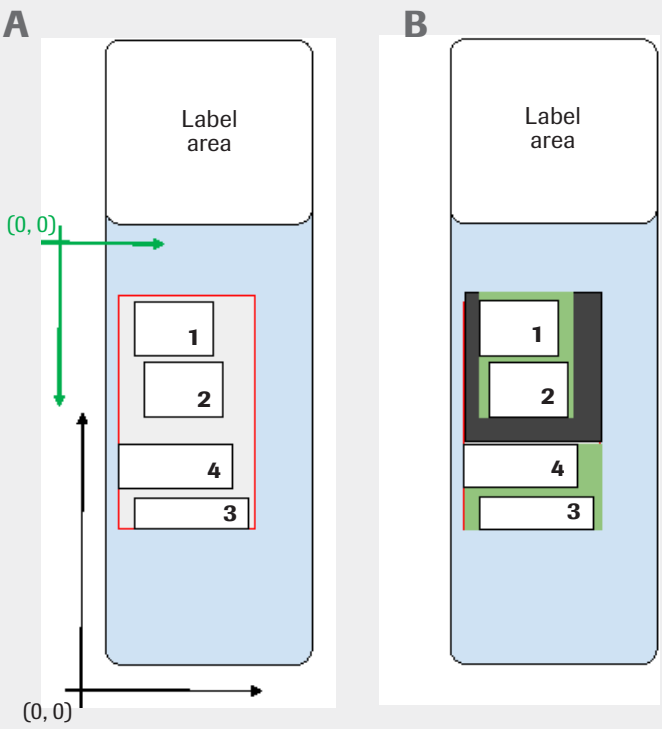
At 20x magnification, each image pixel represents 0.465µm x 0.465µm in tissue space. At 40x magnification, each pixel represents 0.25µm x 0.25µm. For single-wide slides the tissue area is 25mm x 50mm, and for double-wide slides the tissue area is 50mm x 50mm. If the entire tissue area of a slide is scanned, the 40x image for a single wide slide can have up to 100,000 x 200,000 pixels and double-wide slide can have up to 200,000 x 200,000 pixels.

While the user can select multiple rectangular, non-overlapping areas of interest (AOI) for scanning, those areas of the BIF-file represents a single rectangular area corresponding to the convex hull of all (Figure 1A). Depending on the geometry and location of the AOIs, some areas of the merged image may not have physically been imaged by the scanner (Figure 1B). Unscanned areas are represented by empty image tiles (zero pixels), which should be replaced by tiles filled with the white-value included in the metadata included in the BIF-file.

**Figure 1.** Looking at the slide with the label facing up, the origin of the physical slide coordinate system is located at the lower left corner, with both X and Y increasing in the right and up direction. The scanner coordinate system (shown in black) is in millimeters. The image origin (shown in green) is in the top left corner, with the pixel coordinates increasing down and right.

**A** The user can define multiple non-overlapping AOIs (white rectangles labeled 1-4). The absolute position of the user-defined AOIs is stored in the meta-data of the BIF-file.

**B** All individual AOIs are combined into a single rectangular BIF-image. Depending on the relative position of the different AOIs, the scanner may combine individual AOIs into a smaller number of larger scan-AOIs (green rectangles). Unscanned tiles have a length of zero (see text for details).




[6] https://www.iso.org/obp/ui/#iso:std:iso:8037:-2:en

The origin of the physical coordinate system is located in the lower left corner of the slide (facing the tissue coverslip with the label up). However, the origin of the scanned image is in the top left corner (pixel 0, 0).

In order to speed up access to the potentially large image, the pixel data of the high-resolution scan are stored as individual square image tiles (see Section 'IFD 2: High Resolution Image' for details). While various limitations affect the size of the image tiles, they are typically in the order of 1024x1024 pixels each.

The high-resolution image is decomposed into the smaller image tiles in a serpentine fashion (Figure 2).

If the convex hull of all AOIs combined in the BIF-image is not a multiple of the tile size, the image will be padded with empty white pixels to the top and right.

For details about the overlap between certain tiles, see Section 'IFD 2: High-Resolution Scan Image.'

The scanners also can be configured to scan multiple planes above and below the nominal focus plane (Figure 3). While the private IMAGE_DEPTH-tag (0x80BE) is used to store all image planes in a single file, the image corresponding to the nominal focus plane is always stored first in the respective IFD. This allows TIFF Part II compatible readers (that ignore the IMAGE_DEPTH tag) to load the in-focus image plane.

BIF-files discussed in this document always refer to RGB brightfield images, with 8 bits per color channel.

**Figure 2.** To facilitate fast access to the potentially large image, the scan image is split into smaller square image tiles. The tile numbering system starts in the lower left corner and continues up towards the label in a serpentine fashion.

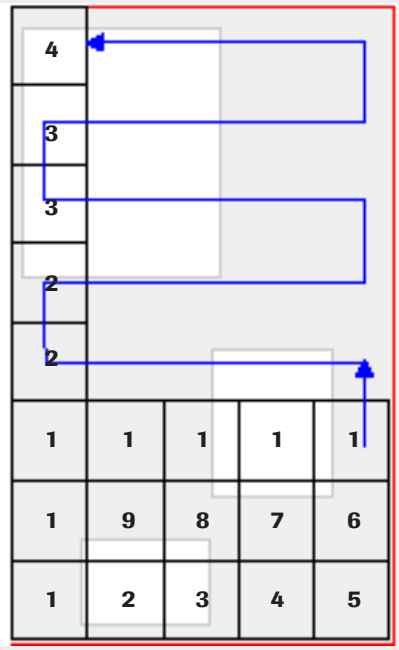
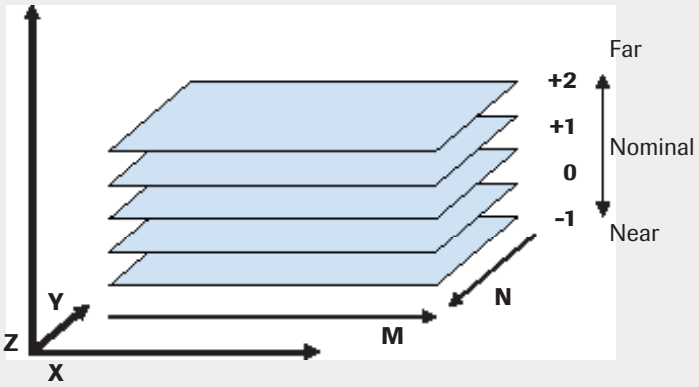



**Figure 3.** Volumetric scans including multiple image layers are stored in the BIF-file using the private tag IMAGE_DEPTH (0x80E5). The first M x N entries in the TILE_OFFSETS-tag (0x0144) correspond to the nominal focus layer 0, followed by M x N entries for each of the near focus layers, followed by M x N entries for each of the image tiles making up the far focus layers. Image readers that do not support the private IMAGE_DEPTH-tag still can read the first M x N image tiles, which correspond to the nominal focus plane.



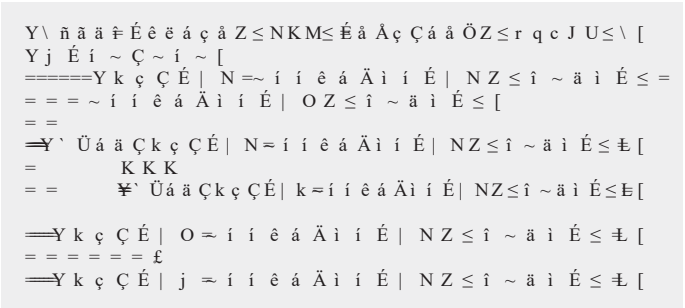
## File structure

Each BIF-file contains at least three image directories, plus several lower resolution image directories:

**IFD 0:** Overview image
**IFD 1:** Tissue probability image
**IFD 2:** High-resolution scan image
**IFD 3 and higher:** Dyadic resolution pyramid images

The images in IFD 3 and higher are added to the BIF-file to facilitate fast image display. These images contain lower resolution versions of the scan image, each image being downsampled by a factor of two from the prior layer.

The total number of images included in the resolution pyramid depends on the total size of the scanned slide area. The down-sampling process stops when the entire image fits into a single image tile.



Each IFD includes its own set of TIFF-tags (see Appendix A for a complete list).

The payload for the XMP-tag included in several IFDs represents a valid UTF-8 encoded XML data blob.

The content of the <MetaData>–child-nodes varies between the different TIFF-pages and is described in the following sections. The relationship between parent and child nodes is indicated with increasing a '>' preceding the name of the child node, adding another '>' character for each node level. The attributes, their contents, data types, and constant values are broken out for each xml-node in a separate table.

***Note:*** *Some XML-nodes and attributes are not relevant for the extraction of the stored images. These elements are included for completeness and labeled 'NA' without further explanation of their contents.*

### IFD 0: Overview image

The overview image includes the entire slide, including both the label and the tissue area. The image is stored as a JPEG-compressed, striped image. Unlike the high-resolution scan image, the overview image is an sRGB-color image and no additional color management is necessary. Refer to 'Appendix A: TIFF-Tags by IFD' for information about which tags can be used to elucidate information about the overview image.

Table 1 lists the information included in the 4 meta-data tags.

***Table 1.*** *(a) Meta-data tag contents, (b) XMP xml-contents. Note: entries labeled 'NA', may have a value associated with them, but the actual value is not relevant for decoding the image.*

| TIFF-Tag | Content |
|---|---|
| **IMAGE_DESCRIPTION** | Constant string: 'Label_Image' |
| **SOFTWARE** | String describing the name and version of the software generating the BIF-file, e.g.: 'ScanOutputManager 1.0.0.15101' |
| **DATE_TIME** | Scan date and time formatted as 'yyyy:mm:dd HH:MM:SS' e.g.: '2018:08:22 16:54:15' |
| **XMP** | Meta-data (see Table 1b for details)[7] |

| XML-Node | Content |
|---|---|
| **MetaData** | |
| **>iScan** | ***Note:*** *this node has a multiplicity of 1.* |

| Attributes | Type | Value description |
|---|---|---|
| **Mode** | string (const.) | Must be: "brightfield" |
| **Magnification** | integer | Scan magnification; must be "20" or "40." |
| **ScanRes** | float | "0.465" (Mag 20x) or "0.25" (Mag 40x). |
| **UnitNumber** | unsigned int | Scanner serial number, 2,000,000 or larger. |
| **ScannerModel** | string (const.) | Scanner model name; must be "VENTANA DP 200." Warning! Stop processing the BIF-file if the string does not match model name. |
| **Z-layers** | unsigned int | Number of image planes; must be an odd number. |
| **Z-spacing** | float | Image plane separation in [µm]. |
| **UserName** | string | Windows username, e.g., "Operator." |
| **BuildVersion** | version string | Creating software version number (major.minor[.build[.revision]] ), e.g., 1.0.0.1551 |
| **BuildDate** | DateTime | Creating software build date and time (m/d/yyyy H:M:S PM), e.g. 1/17/2018 0:7:11 PM. |
| **SlideAnnotation** | string | NA |
| **ShowLabel** | boolean | NA |

[7] Note: the payload

*Continued from previous page*

| | | |
|---|---|---|
| **LabelBoundary** | integer | NA |
| **Barcode1D** | string | Detected 1D barcode (if present). |
| **Barcode2D** | string | Detected 2D barcode (if present). |
| **FocusMode** | integer | NA |
| **FocusQuality** | integer | NA |
| **ScanMode** | integer | NA |
| **ScanWhitePoint** | unsigned byte | White point pixel value. |
| **Anonymization** | boolean | NA |

**>>AOI0** ***Note:*** *the attribute values use the Physical Coordinate System with the origin being the lower left corner of the slide (opposite to the label end of the slide). To draw an AOI correctly on top of the overview image, the Top and Bottom values need to be subtracted from the image height.*

| Attributes | Type | Value description |
|---|---|---|
| **Left** | unsigned int | Left boundary of AOI [pixel], Left is always less than Right. |
| **Top** | unsigned int | Top boundary of AOI [pixel], Top is always larger than Bottom. |
| **Right** | unsigned int | Right boundary of AOI [pixel], Right is always larger than Left. |
| **Bottom** | unsigned int | Bottom boundary of AOI [pixel], Bottom is always less than Top. |

| | |
|---|---|
| **>>AOI1** | ... |
| **...** | ... |
| **>> AOI[N-1]** | As for AOI0, N is the number of AOIs defined for the slide. |
| **>ProcessingParameters** | Note: this node has a multiplicity of 1. |
| **>>Registration** | NA |
| **>>Color** | NA |

### IFD 1: Tissue probability image

The tissue probability image is an 8-bit grayscale image with the same dimensions as the overview image. The probability image is always LZW-compressed. White pixels represent tissue; black pixels indicate no tissue was detected. The tissue-detection algorithm uses this probability map to propose the AOIs to scan.

Table 2 lists the information included in the 4 meta-data tags.

***Table 2.*** *(a) Meta-data tag contents, (b) XMP xml-contents*

| TIFF-Tag | Content |
|---|---|
| **IMAGE_DESCRIPTION** | Constant string: 'Probability_Image' |
| **SOFTWARE** | String describing the name and version of the software generating the BIF-file, e.g.: 'ScanOutputManager 1.0.0.15101' |
| **DATE_TIME** | Scan date and time formatted as 'yyyy:mm:dd HH:MM:SS', e.g.: '2018:08:22 16:54:15' |
| **XMP** | Meta-data (see Table 2b for details) |

| XML-Node | Content |
|---|---|
| **MetaData** | |
| **>PreScanData** | ***Note:*** *this node has a multiplicity of 1.* |

| Attributes | Type | Value description |
|---|---|---|
| **SlideIdentifier** | string | Internal slide name. |
| **SizeImage** | integer | Image dimension in the form of X x Y, where X is the width and Y is the height of the probability image in pixels, e.g., "1251x3685." |
| **PixelsPerUnit** | float | Conversion factor for translating pixels to physical units. |
| **Unit** | string (const) | Physical units resulting from applying the pixel-to-real world conversion factor. Currently the only legal value is "micron". |
| **PixelMode** | boolean | Flag indicating if SizeImage is in [pixels] or physical units. When set to '1,' the origin of the image is at the left-top and Y increases going down. In physical mode, the origin of the image is at the left-bottom and Y increases going up. Currently the only legal value is 1 (true). |

| | |
|---|---|
| **>>AOI0** | ***Note:*** *this node has a multiplicity of N, where N is the number of AOIs defined for this slide.* |

| Attributes | Type | Value description |
|---|---|---|
| **Index** | unsigned int | Zero-based index of the AOI. |
| **Identifier** | string | Unique identifier for AOI, e.g., "AOI0". |
| **Valid** | boolean | Flag indicating if the AOI was scanned successfully. 0=No, 1=Yes |
| **SizeImage** | unsigned int | Image size [pixels] of the probability image in the form X x Y. This property is identical to the SizeImage attribute of the PrescanData node, e.g. "1251x3685"[8] |

[8] Redundant, the value of this attribute is already defined in the SizeImage-attribute of the parent node PrescanData attribute SizeImage.

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| | | |
|---|---|---|
| **Rectangle** | formatted string | Bounding rectangle of the AOI in the form X, Y + WxH, e.g., "262,1823+729x617" defines an AOI with the upper left corner at (262, 1823) if PixelMode=1 (see below). The AOI is 729 pixels wide and 617 pixels tall. |
| **PixelsPerUnit** | float | Conversion factor for translating pixels to physical units. |
| **Unit** | string (const) | Physical units resulting from applying the pixel-to-real world conversion factor. Currently the only legal value is “micron.” |
| **PixelMode** | boolean | Flag indicating if SizeImage is in [pixels] or physical units. When set to ‘1,’ the origin of the image is at the left-top and Y increases going down. In physical mode, the origin of the image is at the left-bottom and Y increases going up. Currently the only legal value is 1 (true). |

**>>>AnchorPointSet** ***Note:*** *this node has a multiplicity of 1.*

| Attributes | Type | Value description |
|---|---|---|
| **SizeAOI** | formatted string | Size of the AOI in the form of W x H. The units of measurement can be in pixels or physical units as defined by the ‘Unit’ attribute, e.g. "729x617" describes an AOI 729 pixels wide and 617 pixels tall if the value of the ‘Unit’ attribute is ‘pixels.’ |
| **FrameStep** | formatted string | Size of a single image tile in the form of X x Y in pixels or physical units. The units of measurement can be in pixels or physical units as defined by the ‘Unit’ attribute, e.g., “1024x1024” defines a square tile with 1K rows and columns if the ‘Unit’ attribute is ‘pixels.’[9] |
| **FrameCountAOI** | formatted string | Number of columns and rows of image tiles in the AOI in the form of C x R. e.g., “61x51” defines an AOI 61 image tiles wide and 51 images tiles tall. |
| **OnCenter** | boolean | NA |
| **FocusTime** | float | NA |
| **PixelsPerUnit** | float | Conversion factor for translating pixels to physical units. |
| **Unit** | string (const) | Physical units resulting from applying the pixel-to-real world conversion factor. Currently the only legal value is “micron.” |
| **PixelMode** | boolean | Flag indicating if SizeImage is in [pixels] or physical units. When set to ‘1,’ the origin of the image is at the left-top and Y increases going down. In physical mode, the origin of the image is at the left-bottom and Y increases going up. Currently the only legal value is 1 (true). |

**>>>>AnchorPoint** ***Note:*** *this node has a multiplicity of 1.*

| Attributes | Type | Value description |
|---|---|---|
| **Point** | formatted string | Anchor point coordinates relative to the top left corner of the AOI in the form of X, Y. The units of measurement can be in pixels or physical units as defined by the ‘Unit’ attribute. E.g., “24, 12” defines the anchor point position at 24 pixels from the left edge and 12 pixels from the top of the AOI if the ‘Unit’ attribute is ‘pixel.’ |
| **FrameCoord** | formatted string | Size of a single image tile in the form of X x Y in pixels or physical units, e.g., “1024x1024” defines a square tile with 1K rows and columns. |
| **Density** | float | NA |

[9] Redundant, the value of this attribute is already defined in the Rectangle-attribute in the parent AOI-node.

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| | | |
|---|---|---|
| **OnCenter** | boolean | NA |
| **OnHull** | boolean | NA |
| **Locked** | boolean | NA |
| **BackupFocus** | float | Conversion factor for translating pixels to physical units. |
| **Probability** | float | NA |
| **BackupFocus** | boolean | Flag indicating if SizeImage is in [pixels] or physical units. When set to '1,' the origin of the image is at the left-top and Y increases going down. In physical mode, the origin of the image is at the left-bottom and Y increases going up. Currently the only legal value is 1 (true). |
| **Time** | boolean | Flag indicating if SizeImage is in [pixels] or physical units. Currently the only legal value is 1 (true). |
| **PixelsPerUnit** | float | Conversion factor for translating pixels to physical units. |
| **Unit** | string (const) | Physical units resulting from applying the pixel-to-real world conversion factor. Currently the only legal value is "micron." |
| **PixelMode** | boolean | Flag indicating if SizeImage is in [pixels] or physical units. When set to '1,' the origin of the image is at the left-top and Y increases going down. In physical mode, the origin of the image is at the left-bottom and Y increases going up. Currently the only legal value is 1 (true). |

...

**>AOIParameters**

| Attributes | Type | Value description |
|---|---|---|
| **SlideIdentifier** | string | Internal slide name. |
| **XEnd** | unsigned int | Right edge of the slide (glass) detected in the overview image in [pixels]. |
| **YStart** | unsigned int | First pixel row of the image that is part of the tissue area, i.e., the first row of pixels outside the label area of the overview image. |
| **CornerGrayValue** | unsigned int | NA |
| **FolderPath** | string | NA |
| **AOIApproach** | integer | NA |
| **AOIRectExtendDim** | integer | NA |
| **MergeDistance** | float | NA |
| **PixelsPerUnit** | float | Conversion factor for translating pixels to physical units. |
| **Unit** | string (const) | Physical units resulting from applying the pixel-to-real world conversion factor. Currently the only legal value is "micron." |

### IFD 2: High resolution scan

The third page in the BIF-file (IFD 2) contains the high-resolution scan image. The image is stored as a tiled JPEG-compressed image (see Technote 2 reference).

IFD 2 also contains the TIFF-tag ICC_PROFILE (0x8773). Since the pixel data are stored in the device-dependent color space, the included ICC-profile (ICC v4.0) needs to be combined with the target display profile and the stored raw pixel values transformed to the display device color space to achieve the intended image quality. While the ICC-profile is only included in IFD-2, it also needs to be applied to all pixel data of the resolution pyramid layers of IFD3 and up. The included ICC-profile does not need to be applied to the overview image, since it is stored in sRGB color space.

Table 3 lists the information included in the 4 meta-data tags.

***Table 3.*** *(a) Meta-data tag contents, (b) XMP xml-contents*

| TIFF-Tag | Content |
|---|---|
| **IMAGE_DESCRIPTION** | Image description with 3 tokens, e.g., "level=0 mag=40 quality=95." The tokens are separated by a single SPACE character (ASCII 0x20). An EQUAL character (ASCII 0x3D) separates the key from the value within each token. |

| Key | Type | Value |
|---|---|---|
| **level** | unsigned int | Zero-based index into the resolution pyramid. |
| **mag** | float | Magnification of the image.<br>***Note:*** *this value accurately describes the resolution of the current pyramid layer. (Do not compute the magnification using other data).* |
| **quality** | integer | JPEG compression quality factor in the range (70, 100), with 100 representing the least compression (highest image quality) and 70 representing the highest compression (least image quality). |

| | |
|---|---|
| **SOFTWARE** | String describing the name and version of the software generating the BIF-file, e.g.: 'ScanOutputManager 1.0.0.15101' |
| **DATE_TIME** | Scan date and time formatted as 'yyyy:mm:dd HH:MM:SS', e.g.: '2018:08:22 16:54:15' |
| **XMP** | Meta-data (see Table 2b for details) |

| XML-Node | Content |
|---|---|
| **EncodeInfo** | |

| Attributes | Type | Value description |
|---|---|---|
| **Ver** | integer | Version of the EncodeInfo-node. Must be 2 or higher.<br>***Warning!*** *Stop processing the file if the attribute value is less than 2. This document does not provide sufficient information to correctly place the image tiles for these BIF-files.* |

**>SlideInfo**

| Attributes | Type | Value description |
|---|---|---|
| **Rack** | integer | NA |
| **Slot** | integer | NA |
| **BaseName** | string | Internal file name (ignore). |

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**>>SlideStitchInfo**

| Attributes | Type | Value description |
|---|---|---|
| **Left** | integer | NA |
| **Top** | integer | NA |
| **Right** | integer | NA |
| **Bottom** | integer | NA |

**>>>ImageInfo** ***Note:*** *this node has a multiplicity of N, where N is the number of AOIs scanned.*

| Attributes | Type | Value description |
|---|---|---|
| **AOIScanned** | boolean | Flag indicating if the AOI was scanned (1) or an error occurred during the scan (0). |
| **AOIIndex** | unsigned int | Zero-based AOI-index. |
| **NumRows** | unsigned int | Number of rows of image tiles in this AOI. |
| **NumCols** | unsigned int | Number of columns of image tiles in this AOI. |
| **Width** | unsigned int | Width of each image tile in pixels. |
| **Height** | unsigned int | Height of each image tile in pixels. |
| **Pos-X** | float | X-Position of the leftmost image tiles in stage coordinates [pixels]. |
| **Pos-Y** | float | Y-coordinate of the lowest tile in the AOI in stage coordinates [pixels]. |

**>>>>TileJointInfo** ***Note:*** *this node has a multiplicity of R(C-1) + C(R-1), where R is the number rows and C is the number of columns of image tiles in this AOI.*

| Attributes | Type | Value description |
|---|---|---|
| **FlagJoined** | boolean | Flag indicating if this tile joint is valid. This value should always have a value of 1 (true).<br>***Note****: stop processing the file if the value is different.* |
| **Confidence** | unsigned int | This value should always be 100.<br>***Note:*** *stop processing the file if the value is different.* |
| **Direction** | String (choice) | For tiles joined horizontally, this attribute is 'LEFT' or 'RIGHT. For tiles joined vertically, this attribute is 'UP' or 'DOWN'.<br>***Note:*** *stop processing the file if the value is different.* |
| **Tile1** | unsigned int | Tile index (physical coordinate system) of the first tile making up the joint. |
| **Tile2** | unsigned int | Tile index (physical coordinate system) of the second tile making up the joint. |
| **OverlapX** | integer | Number of overlapping pixels for a horizontally joined pair of tiles. (Direction is 'LEFT' or 'RIGHT') |
| **OverlapY** | integer | Number of overlapping pixels for a vertically joined pair of tiles. (Direction is 'UP' or 'DOWN')<br>***Note:*** *stop processing if OverlapY is not zero.* |

*Continued from previous page*

**>>>FrameInfo** — ***Note:*** *this node has a multiplicity of N, where N is the number of AOIs scanned.*

| Attributes | Type | Value description |
|---|---|---|
| **AOIScanned** | boolean | Flag indicating if the AOI was scanned (1) or an error occurred during the scan (0). |
| **AOIIndex** | unsigned int | Zero-based AOI-index. |

**>>>>Frame** — ***Note:*** *this node has a multiplicity of RxCxZ, where R is the number of image tile rows in this AOI, C is the number of image tile columns in this AOI, and Z is the number of image planes.*
***Note:*** *the order of the Frame-nodes is important, since it reflects the order in which the image tiles are stored in the TIFF-tag TILE_OFFSETS (0x144). That is, the first Frame-node refers to the tile pointed to by the first TILE_OFFSETS-element.*

| Attributes | Type | Value description |
|---|---|---|
| **XY** | string (formatted) | Zero-based row and column of the image tile in the form 'C,R' where C is the column index and R is the row index. Tile '0, 0' is the top-left corner of the AOI and C increases to the right and R increases away from the label area. For example, '12,34' describes the tile at the 13th column and the 35th row of the AOI. |
| **Z** | unsigned int | Zero-based image plane index. |
| **Focus** | integer | NA |

**>AoiOrigin** — ***Note:*** *the multiplicity of this node is equal to the number of AOIs.*
***Note:*** *AOI origins are always multiples of the tile size.*
***Note:*** *OriginX and OriginY are given in terms of pixel coordinates.*
***Note:*** *if there is only one AOI, OriginX and OriginY are both zero.*

**>>AOI0**

| Attributes | Type | Value description |
|---|---|---|
| **OriginX** | integer | Left coordinate of the AOI in the high-resolution image. |
| **OriginY** | integer | Top coordinate of the AOI in the high-resolution image. |

**>>AOI1**

| Attributes | Type | Value description |
|---|---|---|
| **OriginX** | integer | Left coordinate of the AOI in the high-resolution image. |
| **OriginY** | integer | Top coordinate of the AOI in the high-resolution image. |

**...** — ...

**AOI<N>**

| Attributes | Type | Value description |
|---|---|---|
| **OriginX** | integer | Left coordinate of the AOI in the high-resolution image. |
| **OriginY** | integer | Top coordinate of the AOI in the high-resolution image. |

### Image stitching process

Two different coordinate systems are necessary for assembling the whole slide from the image tiles included in IFD 2.

A. Physical Coordinate System: This coordinate system is also referred to as the Stage Coordinate System. Its origin is the lower left corner of the slide (facing the coverslip with the label pointed up) and positions are given using [pixel] units. Within the context of the physical coordinate system, image tiles are ordered in a serpentine path, starting with tile 1 in the lower left corner, proceeding to the right, then up and to the left, followed by up and to the right (Figure 2).

B. Image Coordinate System: The origin of the image coordinate system is in the top left corner of the image (facing the coverslip with the label pointing up). The units are [pixels] and the coordinate values increase to the right and down (away from the label). Within the context of the image coordinate system, image tiles are typically indexed row by row starting from the left (Figure 1).

The overlap between all tile pairs is included in the XMP-data for IFD2. For each AOI, the node EncodeInfo/SlideStitchInfo contains one ImageInfo-node (Table 3b). The overlap information for every possible horizontal and vertical pair of image tiles is encoded in a <Frame>-node. Using the convention for the physical coordinate system (serpentine path), the attributes ‘Tile1’ and ‘Tile2’ list the indices for the image tiles. Tile2 always is placed on top of Tile1, replacing the pixels in the overlap area. No blending operation should be performed in the overlapping tile region.

Figure 4 illustrates the placement for two pairs of tiles with horizontal overlap.

Yqáä Égçáåífå Ñçe=ä~Ögçáå ÉÇZ≤N≤`=çå ÑáÇÉå ÅÉZ≤NMM≤ =
aáêÉÅíáçåZ≤ibcq≤
qfáäÉNZ≤P≤qfáäÉOZ≤Q≤⊨îÉêä~éuZ≤OQ≤⊨îÉêä~évZ≤M≤L[
Yqáä Égçáåífå Ñçe=ä~Ögçáå ÉÇZ≤N≤`=çå ÑáÇÉå ÅÉZ≤NMM≤ =
aáêÉÅíáçåZ≤ibcq≤=
qfáäÉNZ≤NM≤qfáäÉOZ≤V≤⊨îÉêä~éuZ≤OQ≤⊨îÉêä~évZ≤M≤L[

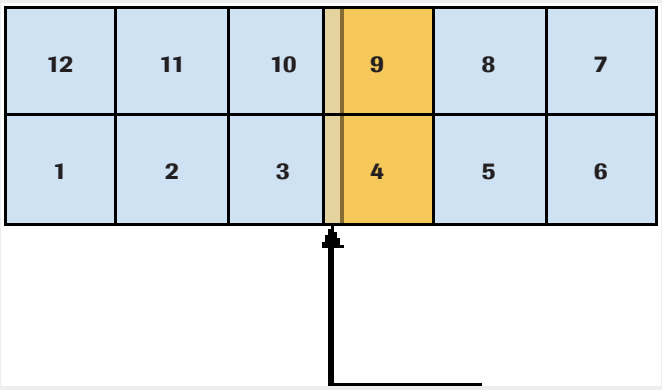
Similar rules apply for the overlap between vertical tile pairs, such as tiles 5 and 8 in Figure 4. However, BIF-files created with VENTANA DP 200 scanner do not contain vertical tile overlap and hence, vertical tile overlap does not need to be taken into account when assembling the image contained in IFD2.

The tile-pair overlap information is provided only once, even if the BIF-file contains a volumetric scan with multiple image planes. The different z-planes are optimally aligned in the XY-plane and all tiles of all image planes always have the same overlap. For example, for a BIF-file with 3 z-planes, if tiles 3 and 4 overlap by 24 pixels in z-plane 0, tiles 3 and 4 in image plane 1 and 2 also overlap by 24 pixels. The order in which the image tiles are stored in IFD 2 is described in the EncodeInfo/ SlideStitchInfo/ImageInfo-child nodes <Frame> for each AOI (see Table 3b).

The XY-attribute describes the placement of a tile within the AOI using the image coordinate system convention where the origin is located in the top left corner. For example, the image tile pointed to by the first element of the TILE_OFFSETS vector (IFD 2) has to be placed at the tile position indicated by the first <Frame>-node, and the tile pointed to by the second element of TILE_OFFSETS has to be placed at the X,Y-location indicated by the second <Frame> node.

While there is a <Frame>-node for each tile in each z-plane, only the tile positions for the first plane need to be read out (the first RxC <FRAME>-nodes). The sequence of the image tiles is always identical to that of the first image plane and the <Frame>-nodes with Z>=1 can be ignored.

**Figure 4.** The TileJointInfo-nodes encode the overlap information between pairs of neighboring tiles. The tiles are indexed using the serpentine path defined by the physical coordinate system convention with the lower left tile having index 1. The pixels of ‘Tile2’ replace the pixels of ‘Tile1.’ No mixed blending should be performed.




**Tile2 replaces Tile1 pixels in the overlap region (24 pixel)**

### AOI Positions within BIF-image and unscanned slide areas

Although multiple AOIs across the tissue slide area can be defined, the BIF image merges the individual AOIs into a single rectangular image. Figure 5 provides an example of a BIF-image including two separate AOIs. The position of the top left corner of each AOI is stored in the <AoiOrigin> node included in IFD 2 XML-data. The OriginX and OriginY attributes for the AOI<N> nodes encode the AOI origins in terms of pixel coordinates, which always are multiples of the tile size. The exact location of each AOI within the TILE_OFFSETS-vector of the IFD2 directory can be identified using the AoiOrigin child node attribute 'OriginX' and 'OriginY' and the 'NumRows' and 'NumCols' attributes of the <SlideInfo><AoiInfo>-nodes highlighted in gray in the Table below.

```
Y p ä á Ç É f å Ñ ç [
¥^ ç á f å Ñ ç ¤ f j ^ d b p f wb Z ≤ NOUM ≤ ¥ f j ^ d b p f wb Z ≤ NOUM ≤ =

==========k ì ã o ç ï ë Z ≤ N P ≤ =k ì ã ` ç ä ë Z ≤ Q ≤
mç ë J u Z ≤ OT NMO ≤ mç ë J v Z ≤ ROS VN ≤ ¤ á ê m~ í ÜZ ≤ ≤ L [
¥^ ç á f å Ñ ç ¤ f j ^ d b p f wb Z ≤ NOUM ≤ ¥ f j ^ d b p f wb Z ≤ NOUM ≤ =
==========k ì ã o ç ï ë Z ≤ N N ≤ =k ì ã ` ç ä ë Z ≤ O N ≤
mç ë J u Z ≤ NNUUS ≤ mç ë J v Z ≤ P QT T N ≤ ¤ á ê m~ í ÜZ ≤ ≤ L [
Y L p ä á Ç É f å Ñ ç [

= Y ^ ç á l ê á Öá å [
=¥ ^ l f M ∓ ê á Öá å u Z ≤ NRP S M ≤ ∓ ê á Öá å v Z ≤ M ≤ L [
=¥ ^ l f N ∓ ê á Öá å u Z ≤ M ≤ ∓ ê á Öá å v Z ≤ O MQ U M ≤ L [
= Y L ^ ç á l ê á Öá å [
```

XIMAGESIZE and YIMAGESIZE provide the tile dimensions (1280 pixel for either dimension). Dividing the OriginX value for the first AOI indicates the first tile of AOI1 being located in row 0, column 12. The NUMRows and NUMCols attributes define the first AOI as 4 image tiles wide and 13 tiles tall. The second AOI starts at the first tile for row 16 and is 21 tiles wide and 11 image tiles tall.

The TILEOFFSETS and TILEBYTECOUNTS vectors for AOI1 are zero for the first 12 tiles, indicating that these tiles have not been scanned. Any algorithm analyzing image data must be aware of the fact that some areas included in the BIF-image have not been scanned and should not be used for calculations like PSNR or DR. Unscanned tiles should be displayed using the 'ScanWhitePoint' attribute value (<SlideInfo><iScan>-node) as RGB-pixel value.

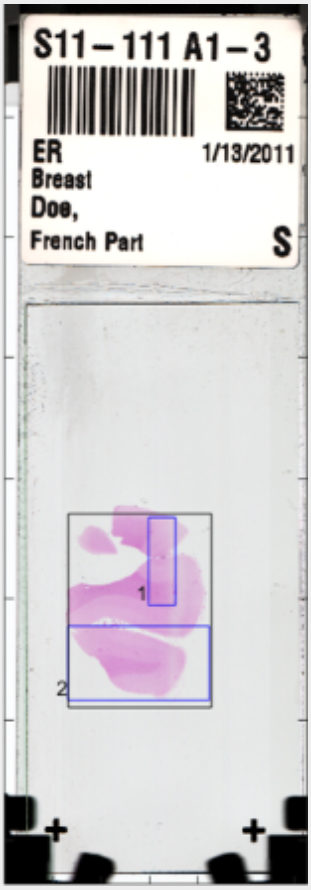
```
q f i b l c c p b q p ¤ MMMMMMMMMMMMU P P R QR U V U T S R MT T S =
P Q T N M Q R N = K K K = z

q f i b _ v q b ` l r k q p ¤ MMMMMMMMMMMMV O S P V V ∓ Q S N N N =
U Q M S V M = K K K = z
```

### IFD 3 and Higher

The contents of the meta-data tags IMAGE_DESCRIPTION, SOFTWARE, and DATE_TIME are identical to IFD 2. When parsing the BIF-file content, the IMAGE_DESCRIPTION-tag should be used to extract magnification and JPEG quality factor for each resolution layer. The XMP-tag is not present in these IFDs.

As for IFD 2, the downsampled images are stored in tiled TIFF-format using JPEG-compression. There is no overlap between lower-resolution image tiles, i.e., all tiles abut each other.

**Figure 5.** Multiple AOIs (blue rectangles) are merged into a single rectangular BIF-image (black outline). The BIF-image approximates the convex hull of all AOIs. Areas of the BIF-image not scanned as part of any of the AOIs are represented in the BIF-file using empty JPEG-tiles (zero length). The upper left corner of each AOI is encoded in the Attributes of the AoiOrigin child nodes <AOI1> through <AOIN>. AoiOrigin always falls online boundaries.



# Appendix A: Tiff-Tags by IFD

| Tag code (decimal) | Tag name | IFD 0: Label image | IFD 1: Tissue probability image | IFD 2: Scan image | IFD 3 and higher |
|---|---|---|---|---|---|
| **256** | ImageWidth | yes | yes | yes | yes |
| **257** | ImageLength | yes | yes | yes | yes |
| **258** | BitsPerSample | yes | yes | yes | yes |
| **259** | Compression | yes | yes | yes | yes |
| **262** | PhotometricInterpretation | yes | yes | yes | yes |
| **270** | ImageDescription | yes | yes | yes | yes |
| **273** | StripOffsets | yes | yes | **no** | **no** |
| **277** | SamplesPerPixel | yes | yes | yes | yes |
| **278** | RowsPerStrip | yes | yes | **no** | **no** |
| **279** | StripByteCounts | yes | yes | **no** | **no** |
| **284** | PlanarConfig | yes | yes | yes | yes |
| **305** | Software | yes | yes | yes | yes |
| **306** | DateTime | yes | yes | yes | yes |
| **322** | TileWidth | **no** | **no** | yes | yes |
| **323** | TileLength | **no** | **no** | yes | yes |
| **324** | TileOffsets | **no** | **no** | yes | yes |
| **325** | TileByteCounts | **no** | **no** | yes | yes |
| **347** | JPEGTables | **no** | **no** | yes (optional)[10] | yes (optional)[10] |
| **530** | YCbCrSubsampling | **no** | **no** | yes | yes |
| **532** | ReferenceBlackWhite | **no** | **no** | yes | yes |
| **700** | XMP | yes | yes | yes | **no** |
| **32997** | ImageDepth | **no** | **no** | yes (volumetric scan only) | Extended Markup Language. (volumetric scan only) |
| **34675** | ICCProfileOffset | **no** | **no** | yes | **no** |

[10] JPEGTables (347) was introduced by Technote 2 (new JPEG compression type 7) to extract JPEG identical coefficients from all image tiles. Removing the JPEG-coefficients from the compressed pixel data and storing only a single copy reduces the file size when the pixel data are organized in tile-format (typical BIF-file: 2–5%). Some versions of BIF-files covered by this document implement this TIFF-tag; some older versions store the JPEG-coefficients within each compressed image tile.

**For inquiries regarding VENTANA slide scanners, please contact your local Roche representative.**

**roche.com**
**diagnostics.roche.com**



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