Digital Slides and Third-Party Data Interchange

User Resources

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ImageServer is intended for use with the svs file format (the native format for digital slides created by scanning glass slides with the ScanScope scanner). Educators will use Aperio software to view and modify digital slides in Composite WebSlide (CWS) format.

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Contact Information

Headquarters:	Aperio Technologies, Inc.	European Office:	Aperio
	1360 Park Center Drive		3 The Sanctuary
	Vista, CA 92081		Eden Office Park
	United States		Ham Green
			Bristol BS20 0DD, UK

United States of America	
	Tel: 866–478–4111 (toll free)
	Fax: 760–539–1116
Customer Service	Tel: 866–478–4111 (toll free)
Technical Support	Tel: 866–478–3999 (toll free)
	Email: support@aperio.com
Europe	
	Tel: +44 (0) 1275 375123
	Fax: +44(0) 1275 373501
Customer Service	Tel: +44 (0) 1275 375123
Technical Support	Tel: +44 (0) 1275 375123
	Email: europesupport@aperio.com

Digital Slides and Third-Party Data Interchange

This document describes the file formats used for digital slides by Aperio, and discusses the different types of compression used. It then goes on to discuss data interchange with third-party software and systems, including desktop software, PACS systems, and LIMS systems.

Contents

The information in this document is completely non-proprietary and can be shared with anyone, including especially Aperio customers and partners.

Digital Slide Images

Aperio's ScanScope systems scan entire Pathology slides to create digital image files. By any standard these are *large* files, and their size drives a lot of the considerations in the way the data are stored and managed.

Digital Slide Image Sizes

Digital slides are typically captured with a 20x or a 40x objective lens. A 20x objective lens acquires image data with a resolution of .46 microns per pixel[1]. A 40x objective lens has twice the resolution, or .23 microns per pixel.

A typical slide has about 15mm x 15mm of tissue area—an area about the size of your thumbnail. Some slides may have larger tissue areas, such as 30mm x 20mm. For bright field scanners each pixel has 24 bits of information, 8 bits each of red, green, and blue color[2], so each pixel requires 3 bytes. The formula for a bright field image size is this:

(width / resolution) x (height / resolution) x 3 bytes

Therefore a 15mm x 15mm tissue area scanned at 20x results in an image with 30,000 x 30,000 pixels, or about 2.7GB of image information. A 30mm x 20mm tissue area scanned at 40x results in an image with 120,000 x 80,000 pixels, or about 28.8GB of image information. The following table shows some typical digital image sizes:

width	height	objective	dimensions	image size
15mm	15mm	20x	30,000 x 30,000	2.7GB
15mm	15mm	40x	60,000 x 60,000	10.8GB
30mm	20mm	20x	60,000 x 40,000	7.2GB
30mm	20mm	40x	120,000 x 80,000	28.8GB

Even the smallest example in this table, 2.7GB, is a very large amount of data.

For dark field fluorescent scanners digital slides are also captured with a 20x or a 40x objective lens. There is only one "color" channel, intensity of response, in a single fluorochrome probe scan resulting in a grayscale or monochrome image. However, each pixel has 10 bits per pixel of

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[1] One micron = 1 millionth of a meter.
[2] Since ScanScopes use a line-scan camera rather than a two-dimensional CCD, ScanScope digital images have full-color resolution, no Bayer masks or other types of interpolation are involved.

intensity which requires 2 bytes to store. The formula for image size of a typical fluorescent slide:

(width / resolution) x (height / resolution) x 2 bytes

which is 2/3rds that of single bright field scan at the same objective. It is typical to scan 3 probes on a fluorescent slide which then produces 3 related image files and in total twice as much storage as a single bright field slide.

Oil scanners capture their images with an 80x or a 100x objective lens. This results in pixel resolutions of 0.115 microns per pixel and 0.092 microns per pixel respectively. Like the bright field scanners with 20x and 40x objective lenses, each pixel has 24 bits of information, 8 bits each of red, green, and blue color[3], so each pixel requires 3 bytes. The formula for image size of a typical oil slide is identical to that of any brightfield slide but the image files are tremendously larger.

(width / resolution) x (height / resolution) x 3 bytes

The following table shows some typical digital image sizes for an oil scanner:

width	height	objective	dimensions	image size
15mm	15mm	80x	120,000 x 120,000	43.2GB
15mm	15mm	100x	160,000 x 160,000	76.8GB

Compression

Among the techniques used to manage image data is *compression*. Using various compression mechanisms, the amount of information in a digital image can be reduced to store it in a disk file.

Some compression techniques are *lossless*, which means no data are lost and the image will remain exactly as it was acquired. Other techniques are *lossy*, which means that some data are lost and the image will not remain exactly the same. Lossless compression techniques can typically only reduce the amount of data by a factor of 2 or 3. This is quite helpful, but image file sizes still remain very large. Lossy compression techniques can reduce the amount of data by a factor of 10 to 50, depending on the technique and the *quality*. High quality lossy compression techniques are preferred for digital slide data because of the large amounts of data.

Many Pathologists and other researchers are initially hesitant to use lossy compression; they want to store the image information exactly as it was acquired. Once they see the quality of compressed images they usually understand that lossy compression does not affect their ability to make a diagnosis or run analysis algorithms with the images. The degree of data loss with a

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[3] Since ScanScopes use a line-scan camera rather than a two-dimensional CCD, ScanScope digital images have full-color resolution, no Bayer masks or other types of interpolation are involved.

particular compression technique can be measured using a value called RMS (root mean square). The differences between each color value for each pixel in the original image and the compressed image are squared and added together. The square root of the sum is the RMS value[4].

LZW

LZW is the most commonly used type of *lossless* compression. (The acronym LZW stands for Lempel Ziv Welch, named after the three mathematicians who developed the technique.) LZW compression is rather compute-intensive, but has the useful property that *encoding* images with LZW takes much longer than *decoding* them. Typical compression factors with LZW compression range from 2 to 3.

JPEG and JPEG2000

JPEG and JPEG2000 are two commonly used types of *lossy* compression. (The acronym JPEG stands for Joint Photographic Experts Group, the name of the committee which developed the standards.) Despite their similar names and origins, JPEG and JPEG2000 are very different techniques. JPEG uses *arithmetic encoding* to compress images in 8 pixel x 8 pixel blocks. JPEG2000 uses *wavelet encoding* to compress images in much larger blocks of configurable size. In general JPEG2000 is a newer and better technique, yielding higher quality images with higher compression ratios than JPEG. The only disadvantage is that encoding images with JPEG2000 requires significantly more computer processing time.

Both JPEG and JPEG2000 can compress images with different quality settings. The quality setting is usually given as a number on the scale from 0 to 100, where 0 is very bad quality and 100 is excellent quality, usually indistinguishable from the original. The following table shows some typical compression factors and RMS quality values for both types of compression at various quality levels:

compression type	quality	compression factor	RMS value
JPEG	30	21	3.4
JPEG	50	17	2.7
JPEG	70	13	2.2
JPEG2000	30	55	3.8
JPEG2000	50	42	3.2
JPEG2000	70	25	2.6

The actual compression factor which results when compressing a digital slide depends significantly on how much "glass" is in the scanned area; since glass has few features, it is highly

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[4] Because RMS is computed by squaring the differences and then taking a square root of the sum, large differences are given more weight than small differences, resulting in a good measure of the quality of the compression.

compressible. The figures in the table above are averages taken from four very different slides (see the Compression Measurements section below for details).

These RMS values are all quite low; even the largest represent an indistinguishable difference between the original image and the compressed image. In general, RMS values lower than 5 are difficult for human vision to detect. Because JPEG compresses images in 8 pixel x 8 pixel blocks, the edges of these blocks are sometimes visible as image artifacts, particularly when magnified. Therefore JPEG2000 images are generally higher quality than JPEG images for the same measured RMS value.

The following table combines the image size information with the compression type information to show average file sizes:

width	height	objective	image size	compression	quality	file size
15mm	15mm	20x	2.7GB	JPEG	30	129MB
15mm	15mm	20x	2.7GB	JPEG2000	50	66MB
30mm	20mm	40x	28.8GB	JPEG	30	1.4GB
30mm	**20mm**	**40x**	**28.8GB**	**JPEG2000**	**50**	**686MB**
15mm	15mm	80x	43.2GB	JPEG	30	2.1GB
15mm	15mm	80x	43.2GB	JPEG2000	50	1.0GB
15mm	15mm	100x	76.8GB	JPEG	30	3.7GB
15mm	15mm	100x	76.8GB	JPEG2000	50	1.9GB

Note the fourth entry in this table. Using JPEG2000 compression, a digital slide with 29GB of image data can be stored in a file of size 680MB! This is a huge difference. A 680MB file is very large, but still manageable, whereas a 29GB file is really too large to be practically useful. So the need and benefit for lossy compression with digital slides can be seen.

Single File Formats

When digital slides are scanned, the data are captured as a two-dimensional array of pixels, with three bytes of color information for each pixel. Various standard types of files may be used to store these data on magnetic media.

> Note that the *format* of the file is a different matter from the *compression* used on the data in the file. Frequently these two are related, but they are separate concepts.

This section gives an overview of the four most common standard file types used to store image data, including digital slides. A later section "The Aperio SVS File Format" on page 14 discusses the file format used by Aperio in more detail.

JFIF

JFIF is the most common file format for image data. (This acronym stands for JPEG File Interchange Format.) The JFIF file format is, as the acronym implies, used to store images which have been compressed with JPEG compression. For this reason JFIF files are often called "JPEG files." (This can be confusing, since when used this way "JPEG" is both a compression type *and* a file format.) **JFIF/JPEG files usually have the file extension .JPG**.

JFIF or JPEG files have a *stripped* organization. That means the image data in the file are stored in *strips* which extend across the entire image. The illustration below shows a stripped file organization:
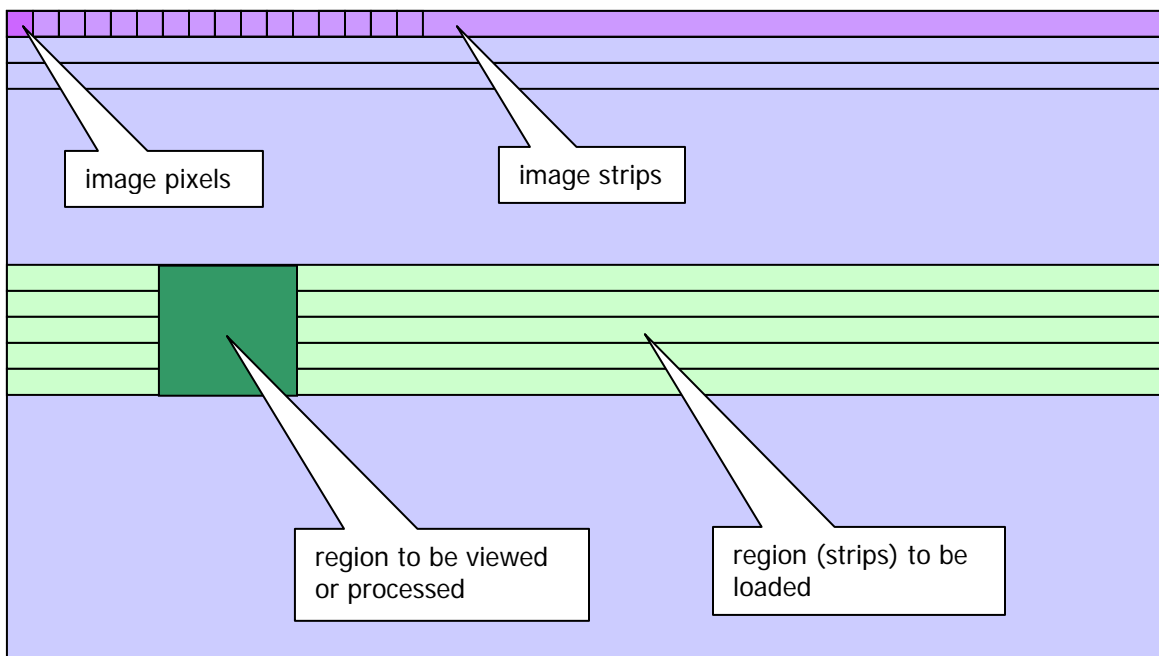

Image pixels are stored starting from the upper left corner (dark purple square), in strips all the way across the image (medium purple stripe). All the pixels in the image are stored as strips, like text running across a page.

This is a simple organization, but it has an important limitation for large images like digital slides: To view or process a subset of the image, a much larger subset of the image must be loaded. For example, in the illustration above the dark green rectangle indicates a region of the image to be viewed or processed. The light green region indicates the region of the image which must be loaded to access the dark green region. Each strip in the region of interest must be loaded, all the way across the image.

The maximum file size of a JFIF file is 4GB, although in actual practice files that large contain too much image information to be processed. Since the JPEG compression technology typically results in a compression factor of 15 or more, a 4GB file would contain 60GB of image data; far more than can be practically managed because of the stripped organization.

TIFF

TIFF is another common type of file for image data, especially for large images. (The acronym TIFF stands for Tagged Image File Format.) Unlike JFIF/JPEG files, TIFF files are not associated with any specific compression type or organization. TIFF files may contain data with no compression, with LZW lossless compression, or with JPEG or JPEG2000 lossy compression. **TIFF files usually have the file extension .TIF**.

Additionally, TIFF files may be stripped like JFIF/JPEG files, or they may have a *tiled* organization. This means image data are stored in square or rectangular tiles. The illustration below shows a tiled file organization:
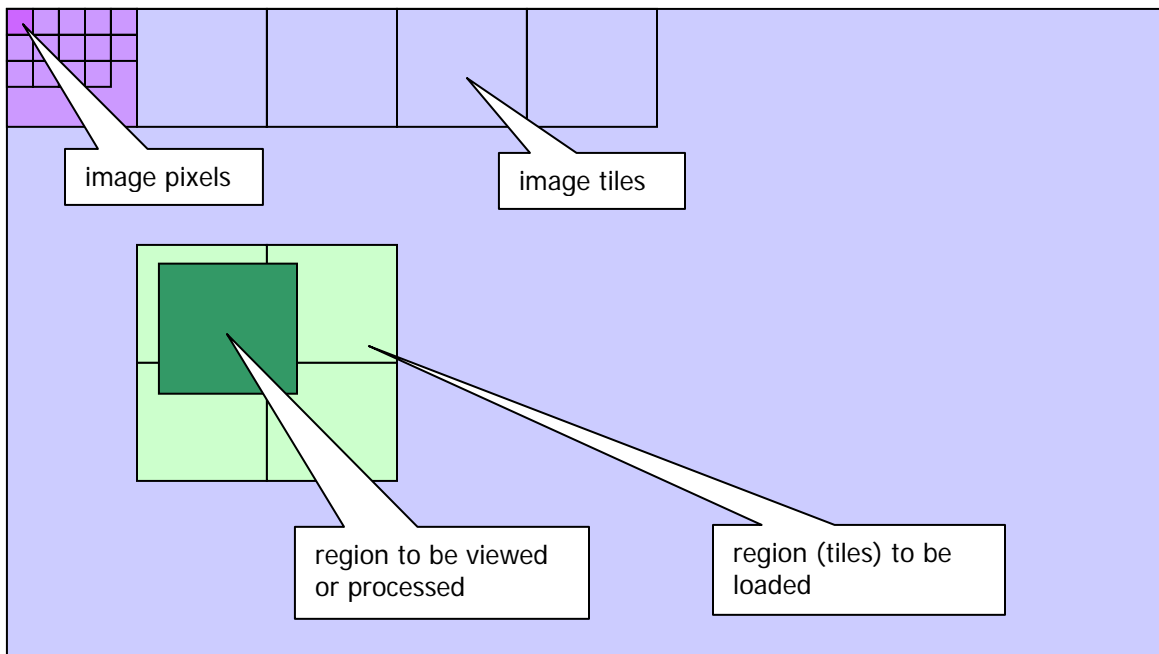

Image pixels are stored starting from the upper left corner (dark purple square), in tiles (medium purple rectangle). All the pixels in the image are stored as tiles, like the pages in a book.

This organization is more complicated than stripped files, but it has an important advantage for large images like digital slides: To view or process a subset of the image, only a small subset of the image must be loaded. For example, in the illustration above the dark green rectangle indicates a region of the image to be viewed or processed. The light green region indicates the tiles of the image which must be loaded to access the dark green region.

The maximum file size of a TIFF file is 4GB. Tiled TIFF files can actually store that much information in a way that can be practically managed; with LZW compression a 4GB TIFF file can hold about 8-12GB of image information, and with JPEG2000 compression a 4GB TIFF file can hold as much as 100GB of image information or more.

BigTIFF

As large as the TIFF file limit of 4 GB is, there are scanning situations where it can be exceeded even with compression. For example, when the tissue area is very large and the compression quality very high or when the scan objective lens is a very high power (small resolution).

The solution to the 4 GB boundary is the BigTIFF file format which extends the TIFF file format standard so much that there is no practical limit to the file size. BigTIFF files have a .TIFF file extension just like ordinary TIFF files and retain the same organizational structure discussed above.

On a practical note, when an application that is "BigTIFF aware" is writing to a TIFF file format and the 4GB limit is exceeded, the software smoothly transitions to the BigTIFF format. The result is a TIFF file using the BigTIFF format.

JP2

JP2 files contain JPEG2000 compressed data, just as JFIF files contain JPEG compressed data. The JP2 file format is structurally similar to JFIF, and these files are often called "JPEG2000 files." As you might expect **JP2 files usually have the file extension JP2**[5].

Like TIFF files, JP2 files support striped or tiled organization and can store multiple images in a single file. The maximum file size of a JP2 file is 4GB. Since the JPEG2000 compression technology typically results in a compression factor of 25 or more, a 4GB file could contain 100GB of image data.

GIF

GIF is one other type of file commonly used to store image data, in addition to JFIF/JPEG, TIFF/BigTIFF and JP2/JPEG2000. (This is an acronym for Graphics Interchange Format.) GIF files can only store image data with lossless LZW compression, and are limited to 16-bit image data. Additionally, GIF files always have a stripped organization. For these reasons GIF is never used to store digital slide data.

Composite File Formats

Occasionally it may be useful to store digital slide images as *composite* files, rather than as a single file. A composite file is a set of files which together store a single digital slide. Aperio software supports two kinds of composite files. The first is a simple format invented by Aperio

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[5] In the early days of Aperio we stored files with the file extension JP2, but these were TIFF files with JPEG2000 compression, *not* "true" JP2 files. We have since stopped creating files using the JP2 file extension but files with this extension are still supported by our software.

called ACI, or "Aperio Composite Image." The second is a standard format called CWS, or "Composite WebSlide."[6]

ACI Composite Files

ACI composite files consist of one or more standard single-file format images, and a text file with the file extension .ACI which describes how the images are *combined* to form a single large image. ACI files may be created by Aperio ScanScopes[7], and also manually for testing and other purposes. Aperio software such as ImageServer and ImageScope supports ACI files for input. ACI files are sometimes used to store *very large images*, since they are not limited by the size of any one individual file. For testing purposes, Aperio has created ACI-format digital slide images which are 500,000 x 500,000 pixels (750GB of image data)!

CWS Composite Files

CWS composite files are *directories*. A CWS image directory contains a large number (hundreds or thousands) of small JFIF files (.JPG), and one or two text .INI files which describe how the JFIF files are combined to form a single image. CWS files are used for two reasons: First, for compatibility purposes with other software which create or display files in this format, Second, CWS files can be accessed remotely *without any special server software*. A CWS directory may be copied to any webserver and accessed "in place" because each "block" of the image is stored as a separate file.

Panning and Zooming

When Pathologists view digital slide images they need to be able to *pan* and *zoom*.

Panning

Panning refers to moving around from side to side and up and down within the image. The viewing window of the computer displays a region of the slide's data, and panning causes the region within the window to change. The challenge of panning is nicely addressed by using a tiled file organization, as discussed in the previous section. The dark green rectangle in the "stripped" and "tiled" diagrams above could be a computer viewing window. As shown by the diagrams, using a tiled organized is significantly more efficient than a stripped organization, in that less data need to be loaded from the file.

As an example, consider a digital slide made from a 15mm x 15mm sample scanned with a 20x objective. As noted above, this image will have dimensions of 30,000 x 30,000 pixels. Assume the computer has a display window which is 1,600 x 1,200 pixels in size. To display one particular view of the slide from a stripped file, it is necessary to load 30,000 x 1,200 pixels, which comprise 108MB of data. To display the same view from a tiled file, say with a tile size of 240 pixels square, it is necessary to load 7 x 6 tiles of size

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[6] CWS is based on the standard established for "WebSlides" by Bacus Labs, Inc.
[7] Digital slides may be scanned by ScanScopes directly into ACI format by specifying DoWrite = True.

1,680 x 1,440 pixels, which comprise just 7MB of data. This difference becomes even more dramatic with larger tissue sizes, and with higher scanning resolution.

Zooming

Zooming refers to changing the display resolution of the image. At lower resolution more of the image is displayed, but with less detail. The challenge of zooming is partially addressed by using a tiled file organization, but not entirely. At lower resolutions progressively more data must be loaded from the file, since larger areas of the slide are displayed. In the limit when the view is "zoomed out" all the way, the entire image must be loaded! This is illustrated by the following diagram:
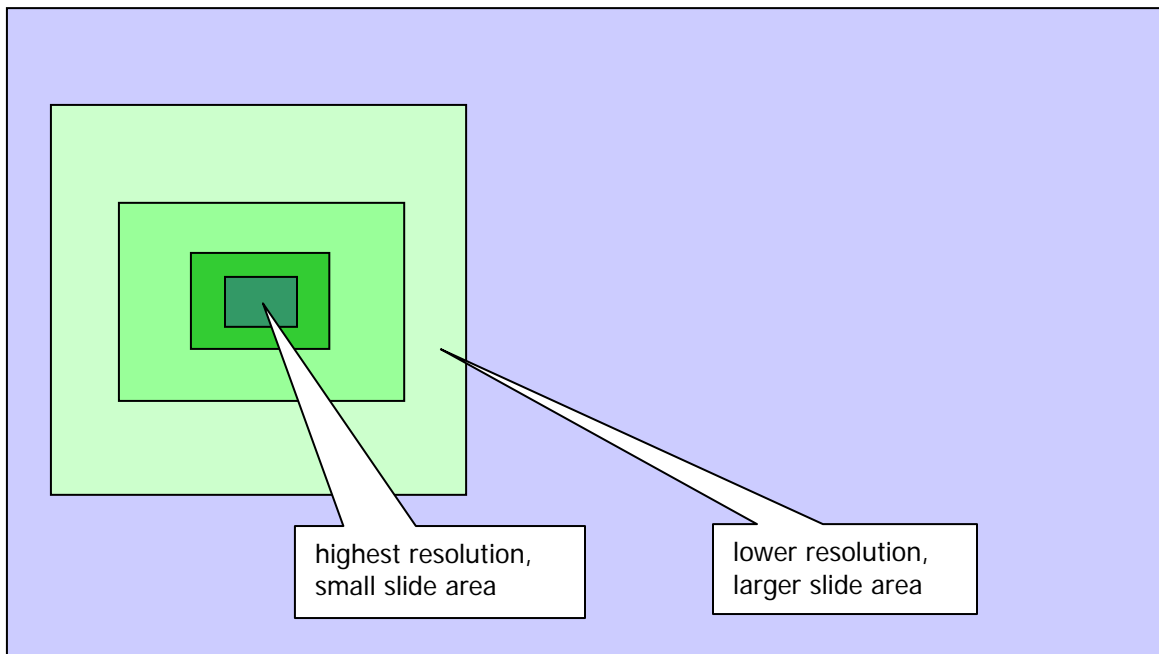
The darkest green rectangle is a view of the slide at the highest resolution, the resolution used when the slide was scanned. A relatively small area of the slide is needed to display this view. Progressively lighter green rectangles show views of the slide at progressively lower resolution, which require correspondingly larger areas of the slide to display this view. In the limit, the lowest resolution view would be the entire slide (light blue area), which would require all the slide data.

A solution for efficient zooming is to create *multiple* images of the slide, at different levels of resolution. These images can be thought of as forming a pyramid, as shown in the following diagram:
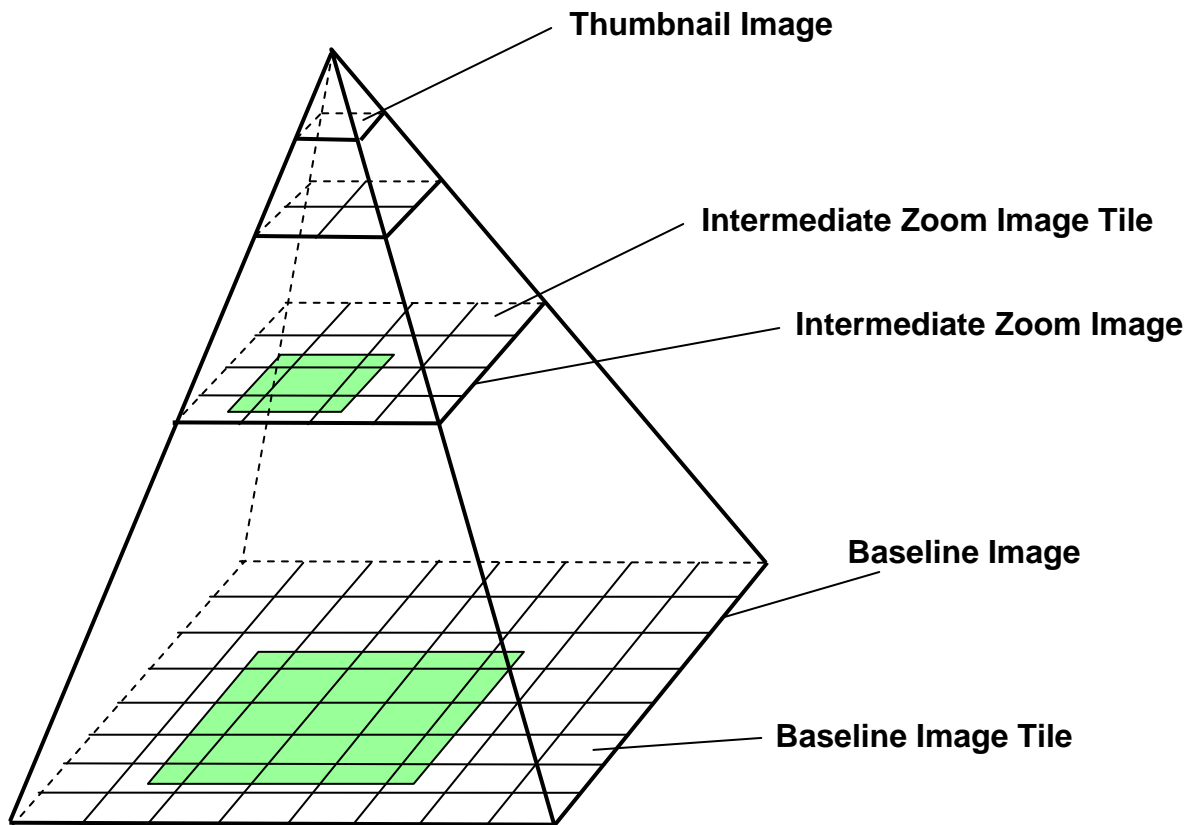
The full resolution image created when the slide was scanned in the "baseline" image. A very low resolution "thumbnail" is created which is the tip of the pyramid. In between, one or more "intermediate zoom" images are created. These are typically at integral multiples of the base resolution. For example, the first intermediate image might be $1/4^{th}$ the resolution of the base image, the next might be $1/16^{th}$, then next $1/64^{th}$, and so on.

The advantage of the pyramid for zooming can be seen in the diagram above. If the region illustrated by the green rectangle is required for viewing, it requires far more data to be read from the baseline image than from the intermediate zoom image. In the limit, if the entire image is to be displayed at the lowest resolution, only the thumbnail needs to be read!

> As an example, consider a digital slide made from a 15mm x 15mm sample scanned with a 20x objective. As noted, this image will have dimensions of 30,000 x 30,000 pixels. Assume the computer has a display window which is 1,600 x 1,200 pixels in size. In the example we noted that to display a view at full resolution required 7MB of data. However, to display a view at $1/5^{th}$ resolution (zoomed out to 20%) would require 25 times as much data, or 175MB! If an intermediate resolution image was available at $1/4^{th}$ resolution, it could be used to render the desired view, in which case only $5/4^{th}$s as much

data would be required, or 9MB. The difference becomes more dramatic with larger slides and viewing at lower resolutions.

To summarize, in order to provide efficient panning and zooming it is necessary to 1) have a tiled organization, and 2) have multiple images at different resolutions.

The TIFF file format not only supports different types of compression, it also supports tiled organization and multiple images within one file. TIFF is an open standard which has been available since the early 1980s, and is supported on a wide variety of computing platforms by a wide variety of software[8]. This makes TIFF an obvious choice for storing digital slide data.

Like TIFF, JP2 it also supports tiled organization and multiple images within one file. JP2 is an open standard which has been available since 2000 and is supported on a wide variety of computing platforms by a wide variety of software[9]. Like TIFF, JP2 is also a great choice for storing digital slide data.

The Aperio SVS File Format

Digital slide images created by Aperio's ScanScope slide scanners can be stored in the JP2 or the *SVS* file format. (SVS is an acronym for ScanScope Virtual Slide.) **SVS files are TIFF/BigTIFF files**. They are fully compatible with the TIFF standard, with no proprietary extensions. SVS files make use of the following aspects of the TIFF standard:

- They contain multiple images at different resolution levels. The first image in the file is always the baseline image (full resolution).
- They use a *tiled* image organization.
- The images may be compressed with LZW (lossless) compression, or with JPEG or JPEG2000 (lossy) compression (JPEG2000 compression is preferred).

The first image in an SVS file is always the baseline image (full resolution). This image is always tiled, usually with a tile size of 240 x 240 pixels. The second image is always a thumbnail, typically with dimensions of about 1024 x 768 pixels. Unlike the other slide images, the thumbnail image is always stripped. Following the thumbnail there may be one or more intermediate "pyramid" images. These are always compressed with the same type of compression as the baseline image, and have a tiled organization with the same tile size.

Optionally at the end of an SVS file there may be a slide label image, which is a low resolution picture taken of the slide's label, and/or a macro camera image, which is a low resolution picture taken of the entire slide. The label and macro images are always stripped. If present the label image is compressed with LZW compression, and the macro image with JPEG compression. By

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[8] More information about the TIFF file format is available at the website http://www.libtiff.org/

[9] More information about the JP2 file format is available at the website http://www.jpeg.org/jpeg2000/

storing the label and macro images in the same file as the high resolution slide data there is no chance of an accidental mix-up between the slide contents and the slide label information.

The intermediate resolution images in an SVS file are typically $1/4^{th}$ resolution of the previous image. The following table shows the pyramid from a typical large SVS file, using the example of a tissue area of 30mm x 20mm scanned with a 40x objective lens:

image	dimensions	image size	resolution	% total size
baseline	120,000 x 80,000	29GB	1:1	
1st level	30,000 x 20,000	1.8GB	4:1 – 25%	6%
2nd level	7,500 x 5,000	112MB	16:1 – 6%	0.4%
3rd level	1,875 x 1,250	7MB	64:1 – 1%	-
thumbnail	1,024 x 680	2MB	117:1	-

Note that most of the size of the file comes from the baseline image. Adding the pyramid only adds about 7% to the overall size of the file, but it has a dramatic effect on the performance of zooming, as discussed above.

Aperio SVS files usually contain image data compressed with a lossy technique like JPEG or JPEG2000. The compression factors offered by these technologies mean that the 4GB file size limit of TIFF does not cause problems. With LZW lossless compression, the compression factor is usually only about 2 or 3, which means the 4GB file size limit does come into play. **Many digital slides are too large to store in a single file when compressed with a lossless compression technique**. For customers which require lossless compression, they must either 1) use small scan areas, 2) store data at a reduced resolution, or 3) store image data in multiple files.

The Aperio JP2 File Format

Digital slide images created by Aperio's ScanScope slide scanners can be stored in the JP2 file format. Aperio implemented all of the same features in the JP2 file as it implemented in SVS file: pyramid image levels, thumbnails, a slide label image, and a macro camera image. Unlike SVS image files, all images in the JP2 image file are compressed with JPEG2000 compression. JP2 files are accessible by any other JP2-compliant third party program for viewing and editing.

The Aperio ACI File Format

ACI (Aperio Composite Images) consist of one or more standard single-file format image files, and a text file with the file extension .ACI which describes how the images are *combined* to form a single large image. ACI files may be created by Aperio ScanScopes[10], and also manually for testing and other purposes. Aperio software such as ImageServer, ImageScope, and Digital Slide Studio support ACI files for input.

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[10] Digital slides may be scanned by ScanScopes directly into ACI format by specifying DoWrite = True.

The .ACI files are text files which may contain lines with one of four formats:

```
# <comment...>
<layer> <Z-offset>
<image_path> <Y-offset> <X-offset> (<quality parameters...>)
<attribute> = <value>
```

Comment lines may be present in an ACI file, prefixed with a pound sign (#), and are ignored.

Layer lines are optional, and are used to describe three-dimensional slide images, which are organized into *layers*. Each layer line specifies the Z-offset for an image layer. All the following image lines define image files for that layer, until the next layer line is encountered. Layers must be described in ascending order of Z-offset. Any image lines before the first layer line are treated as belonging to the first layer with a Z-offset of zero.

Image lines specify an <image_path>, a relative path to an image file to be included in the composite image. The <Y-offset> specifies the pixel offset to this image within the virtual image in the Y direction (up and down) relative to the upper left corner of the virtual image, and the <X-offset> specifies the pixel offset in the X direction (across). In some cases one or more quality parameters may also be present; they are typically ignored. In the most common case all the files comprising the composite image are located in the same directory, and the <image_path>s are all simply the filenames. It is possible to combine any existing files into a larger single image using ACI files.

Attribute lines are optional, and specify various image attributes of the image. The image description is formed by concatenating all image attributes together, in the form <attribute>=<value>, delimited with vertical bar characters "|".

When images are composited together, if they are overlapped the image to the right or to the bottom is assumed to be "on top." That is, the virtual image is formed by layering images from the upper left to the lower right.

The Aperio CWS File Format

CWS (Composite WebSlide) images are *directories*[11]. A CWS directory contains a large number of small JFIF files (.JPG), and one or two text .INI files which describe how the JFIF files are combined to form a single image. **CWS images may be created directly from ScanScopes during scanning by specifying a CompressionType of 10**. Other Aperio software such as Digital Slide Studio can also create slides in CWS format. The **icomp** command-line tool may be scripted to convert large numbers of slides from SVS (single-file) format to CWS (composite directory) format if desired.

All Aperio software can read digital slides in CWS format, including ImageServer, ImageScope, Digital Slide Studio, and the Aperio Algorithm Framework. Since CWS image files are

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[11] The CWS composite format is a standard "WebSlide" format developed by Bacus Labs, Inc.

directories, images can be selected either by the directory itself or by selecting either .INI file inside the directory. Selecting an INI file is easier in common file open dialogs.

The main reason to use CWS format digital slides is that CWS directories may be accessed remotely via any webserver *without any special server software*. For example, Microsoft IIS (Internet Information Server, a standard component of Microsoft Windows) may be used for this purpose. Any CWS image directory can be copied to a webserver and then accessed remotely using tools like ImageScope or WebViewer, and analyzed using Aperio Algorithm Framework.

Aperio's ImageScope viewer supports accessing CWS format digital slides when hosted on a standard webserver. Using the File | Access Remote Server dialog box, specify the machine name and port number of the webserver instead of an ImageServer machine. ImageScope automatically determines that the machine is a standard webserver, and enables access to CWS format images on that machine.

A CWS image is organized as one or more levels at different resolutions. Each level consists of a collection of JFIF files which "tile" the image at this resolution. The level is described by a .INI file which gives the relative coordinates of the files within the level. The .INI files have the following names:

ScanSlide.INI - Lowest resolution level, typically magnification of 1.5x. If the slide has only one level, only this .INI file may be present.

FinalScan.INI - Higher resolution level(s), typically magnification of 20x or 40x. There may be intermediate levels between the highest resolution and the lowest resolution, in which case they are described in this file also.

The entire format of the .INI files is beyond the scope of this document, but coordinates of images within the files are given in "stage units." A stage unit is about $1/8^{th}$ of a micron. The stage unit coordinate system has (0,0) in the center of a "stage," and the images are positioned relative to this; stage units ascend to the *left* and *up*. Coordinates of individual images are given based on the *center* of each image. A typical image block size is 752 x 480 pixels, although other dimensions are possible and supported. CWS images may have multiple Z-layers for the highest resolution level.

Data Interchange with Third-Party Systems

This section discusses data interchange with third-party software and systems, including desktop image processing and analysis software, PACS systems[12], and LIMS[13] systems.

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[12] PACS is an acronym for Picture Archive and Communication System, and is commonly used to describe systems used in clinical labs and hospitals for managing Radiology images.

[13] LIMS is an acronym for Laboratory Information Management System, and is commonly used to describe systems used in clinical labs and hospitals for managing Pathology workflow.

Interchange with Thirty Party Desktop Software

Frequently, customers and partners want to be able to open Aperio SVS files or JP2 files directly with third-party desktop software. And they are disappointed to find that they can't! There are two reasons, 1) image size, and 2) compression type.

As discussed above, digital slide images are *big files*. They are typically too big to be loaded entirely into the memory of a personal computer. Most third-party software processes images by loading them entirely into memory. So at a fundamental level, they cannot process Aperio digital slides, even with all considerations of file format and compression set aside. The only solution is to create smaller files by a combination of cropping and reducing image resolution.

The second issue is compression type. Although Aperio digital slides are *standard TIFF files or JP2 files*, as discussed above, TIFF files and JP2 files can have a variety of organizations and compression types. Aperio SVS files and JP2 files contain multiple images, use a tiled organization, and are compressed with JPEG or JPEG2000 lossy compression. A few third-party applications support all of these characteristics of TIFF files, but most do not. Frequently when a third-party application "supports TIFF files," it only supports the most basic type of TIFF file, with one image, a stripped organization, and no compression.

> Note: It is because Aperio SVS files are frequently incompatible with third-party applications that we use the .SVS file extension. If we used a .TIF extension it would be accurate but misleading, because most applications which can process TIFF files cannot process Aperio SVS files, for the reasons given above.

Aperio provides two solutions for this need. As with the file size issue, the solution is to extract a standard file from the Aperio digital slide file.

Aperio's ImageScope viewer has a facility to interface directly with third-party programs. Using this feature, users can seamlessly export an entire digital slide or a selected rectangular subset into a temporary JPEG or TIFF file, and then launch a specified third-party program passing the temporary file as a parameter. This facility automatically scales the exported image to a resolution supported by the third-party program.

For more flexibility, Aperio provides a utility program named Digital Slide Studio which enables a standard JPEG or [simple] TIFF file to be extracted from an Aperio digital slide. DSStudio provides facilities for cropping images and/or reducing their resolution, solving the file size problems, and it can output an extracted image as a standard JPEG or [simple] TIFF file. DSStudio also enables image colors to be adjusted and filters to be applied.

Compatibility with PACS Systems

Customers and partners often ask about Aperio's compatibility with PACS systems. A PACS system is a client/server computer system which stores, retrieves, archives, displays, and analyzes medical images, usually Radiology images such as X-rays and CT-scans. Other types of images may be managed by PACS systems as well; each supported discipline is often referred as a "modality" of the system.

PACS systems manage images which are DICOM-compliant. DICOM is an acronym for Digital Imaging and Communications in Medicine, and refers to a *file header standard*. DICOM headers can "wrap" many commonly used image file types, including JPEG and TIFF. DICOM files are limited in size to 2GB. In addition, most DICOM systems cannot manage TIFF files or JP2 files with a tiled organization, and/or TIFF files with JPEG or JPEG2000 compression. For these reasons **it is generally not possible to import Aperio SVS files directly into PACS systems**.

PACS systems use a store-and-forward idiom. All image data are stored on a central server. When a client wishes to view or process an image, the *entire* image is transmitted from the server to the client, and loaded into the client's memory. This means large images are slow to load, slow to view, and slow to process. As a practical matter, images larger than about 500MB are impossible to process.

Most PACS systems can import TIFF images (onto which they add the DICOM header), but many do not support the more sophisticated aspects of the TIFF standard or the JP2 standard, such as multiple image, tiled organization, and lossy compression (JPEG or JPEG2000). For these reasons PACS systems *cannot* generally important SVS or JP2 files directly, because 1) they are too large, and 2) they use incompatible organization and compression. Aperio provides two solutions for this need.

As the first solution, customers can store digital slide images in *two* parallel ways; first, as a full-resolution SVS file, and second, as a lower-resolution TIFF file which can be imported directly into a PACS system. The lower resolution file is managed as part of the customer's ordinary workflow, where it can be viewed, analyzed, etc. The PACS system client software then provides a link to the ImageScope viewer which a user can follow to display the digital slide at full resolution (using the SVS or JP2 file, served via Aperio's ImageServer). This solution has the advantages that the customer's existing workflow can be used without change, and it requires little to no change to the PACS system software. The disadvantage is that the customer must manage the SVS files as large image data *outside* their PACS system.

The second solution involves breaking SVS or JP2 files into multiple smaller pieces which can be imported directly into the PACS system and wrapped with DICOM headers. A lower resolution version of the SVS or JP2 file (corresponding to the 1st layer of the pyramid) can be used within the PACS system as part of the customer's ordinary workflow. The PACS system client software then provides a link to the ImageScope viewer which a user can follow to display the digital slide at full resolution. This requires the PACS system client to export the pieces of the SVS or JP2 file, and then ImageScope opens the collection of parts as a single digital slide image. This solution has the advantages that the customer's existing workflow can be used without change, and it provides that **all** image data are stored within the PACS system. The disadvantage is that the PACS system must provide the capability to export the SVS file pieces for viewing.

Compatibility with LIMS Systems

In many clinical applications, the customer will have a LIMS system which they wish to interface to the Aperio ScanScope system. (In this discussion LIMS is a placeholder for any

external system which manages slide *metadata*; sometimes called "workflow" systems or "database" systems.) These systems manage hospital/patient/case information, study/experiment/case information, etc.; these data originate outside the ScanScope system, and are typically maintained outside the ScanScope system. The need for customers and partners in this area is to interface into the ScanScope data.

The easiest way for customers to interface their LIMS/other external systems to a ScanScope system is via Aperio's ImageServer. This program provides an extensive open interface to all digital slide image data as well as the image metadata managed within the ScanScope system. These metadata include annotations, algorithm results, and scan data (for example, focus quality measurements), etc. The interface is provided via standard HTTP messaging, and the documentation is available publicly on the Aperio website (http://www.aperio.com/documents/) as *Aperio ImageServer Programmer's Reference*.

Another way for customers to interface their LIMS/other external systems to a ScanScope system is via Aperio's DataServer. This program provides an extensive open interface to all meta data managed within the ScanScope system as well as the image file location. The interface is provided via standard HTTP messaging, and the documentation is available publicly on the Aperio website (http://www.aperio.com/documents/) as *Aperio DataServer Programmer's Reference*.

In addition, some customers access the Aperio SQLServer database directly. The schema and other data information for the database are available to selected customers who require this capability. This method of interface is somewhat more difficult and error-prone than using the ImageServer interface so its use is discouraged unless specifically required.

For both types of interface (ImageServer and direct database access) the "key" for accessing slide data is the *bar code value* decoded from the slide. This value need not be anything in particular except that it must be unique; the Aperio system merely decodes and stores it.

In addition to interfacing to ImageServer and/or the ScanScope database for access to slide image data and metadata, some customers and/or LIMS vendors may wish to use the programming APIs provided by Aperio for deeper integration, as detailed in the next section. For example, LIMS client software may incorporate **Viewport** for image display.

Finally, and perhaps the most cost effective solution, a customer can contract with Aperio's Professional Services group which specializes in LIS/LIMS interfacing as well as custom solutions.

Aperio Programming APIs

Philosophically, Aperio intends that its data file formats and software interface be "open," with well-documented interfaces to enable second and third parties to interface and extend the Aperio ScanScope system. This section lists the interfaces which are available and indicates where detailed information can be found. (API is an acronym for Application Programming Interface, a standard software development mechanism.)

ImageServer

Aperio ImageServer is the main external interface to the ScanScope system. In most cases, second and third parties will find all the capabilities they require in this interface. Aperio ImageServer provides an extensive open API for all digital slide image data as well as the image metadata managed within the ScanScope system. These metadata include annotations, algorithm results, scan data (for example, focus quality measurements), etc. Under customer control, the ImageServer configuration can be set up to enable updates as well as inquiries, including authoring annotations, running algorithms, and maintaining digital slide metadata. The API is provided as a standard HTTP message interface, and the documentation for it is available publicly on our website (http://www.aperio.com/documents/) as *Aperio ImageServer Programmer's Reference*.

WebViewer

Aperio WebViewer is a tool which enables a gallery of digital slide images to be presented and each image viewed in any web browser with WebScope using no applets or plug-ins required. The WebViewer uses an interface to ImageServer to obtain slide image data.

WebViewer is designed to be incorporated easily into second- and third-party websites. This enables customers and partners to develop websites with their own formatting and content, but using WebViewer to display digital slide information. Documentation for the Aperio WebViewer is available on the Aperio website (http://www.aperio.com/documents/) as *Aperio WebViewer Programmer's Reference*.

WebScope

WebScope is a web-based, platform-independent digital slide viewer. A big advantage of using WebScope is that nothing needs to be installed on the client workstation and it can be used from any workstation (Macintosh, Linux, etc.) that uses an Internet browser that supports the Adobe Flash Player.

WebScope now offers a rich set of features that enhance your digital slide viewing. Many of these features will be familiar to ImageScope users, and WebScope continues to be enhanced to become more compatible with ImageScope. (ImageScope is Aperio's full-featured digital slide

viewer that is a Windows client application.) WebScope features include navigating the digital slide creating and viewing slide annotations, and analyzing the digital slides.

Viewport Control

Aperio provides an ActiveX control[14] called **Viewport** which can be easily incorporated into third-party applications to *display* digital slide data. Aperio's ImageScope and TMALab applications use **Viewport**, as does the Bioimagene application used for image analysis at USLABS. The **Viewport** API is a standard control interface, and the documentation for it is available publicly on the Aperio website (http://www.aperio.com/documents/) as *Aperio Viewport ActiveX (.OCX) Programmer's Reference*.

In addition to viewing capabilities, **Viewport** also enables more sophisticated capabilities such as data snapshots for third-party image data interfaces, annotation authoring and display, and running algorithms remotely (a "remote" interface to ImageServer).

TIFFComp Control

Aperio provides an ActiveX control called **TIFFComp** which can be easily incorporated into third-party applications to *create* digital slides in Aperio SVS format. The digital slide output processing in the ScanScope Controller software uses this module, as does Aperio's Digital Slide Studio (*nee* ImageCompressor). The **TIFFComp** API is a standard control interface, and the documentation for it is available on the Aperio website (http://www.aperio.com/documents/) as *Aperio TIFFComp ActiveX (.OCX) Programmer's Reference*.

TIFFComp is the core of the capabilities provided by Digital Slide Studio, including the ability to adjust image colors, crop images and scale images to any desired resolution, apply convolution filters, and provides detailed control over the format, organization, and compression of the output digital slide.

Algorithm Framework

The Aperio Algorithm Framework provides an environment for running analysis algorithms, which can be provided by Aperio, or developed by second or third parties. The framework is built into the ImageScope viewer enabling algorithms to be run "locally" (directly on a client machine). The framework can also be installed on one or more server machines enabling algorithms to be run in a batch mode "remotely." A key advantage of the Algorithm Framework is that the same algorithms can be written once and run both ways.

The framework includes a software development kit (SDK) which has development modules, sample algorithms, and documentation. The SDK documentation is available on the Aperio website (http://www.aperio.com/documents) as *Aperio Algorithm Framework (AAF) Programmer's Reference*.

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[14] ActiveX controls are a Microsoft technology for developing "plug-in" software modules

Aperio Image Client

In rare instances a customer or partner may require access directly to digital slide files, rather than using the interfaces described above. In general we discourage this because we would prefer customers and partners use a standard interface to the files; it gives us more freedom to innovate with the file format as we develop new features. For those customers who do require direct access, Aperio provides a C++ subroutine called Aperio Image Client (AIC) which encapsulates access to digital slide files. The AIC is an underlying component of Aperio's ImageServer, Viewport, TIFFComp, Algorithm Framework, etc.

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