

### **Carl Zeiss**

Microscope Division D-73446 Oberkochen

**Technical Information:** 

# LSM-TIFF

# The Tag Imaged File Format in the LSM

#### **SYNOPSIS**

The Carl Zeiss LSM creates images in the TIFF (Tag Image File Format) format. This format is also used to store LSM specific data with a privat tag.

#### WHAT IS TIFF?

Tiff is a file format that describes image data coming from scanners, frame grabbers and paint/photo retouch programs. The primary goal was to provide a rich environment within which the exchange of image data between application programs can be accomplished. This richness is required in order to take advantage of the varying capabilities of imaging devices. Though Tiff is a rich format, it can easily be used for simple applications as well.

However, most (and not only simple) Tiff applications do not support the full, rich Tiff standard and this is the reason for incompatibilities between different applications.

Tiff is capable of describing bi-level, grayscale, palette color and full color image data in several color spaces. It includes a number of compression schemes, is portable because it does not depend on particular operating systems or file systems and, allows privat tags to be defined that are specific to a particular application or organization.

#### FILE STRUCTURE

A Tiff file is a sequence of 8-bit bytes. Every Tiff file begins with an 8-byte Image File Header.

#### Image File Header

Bytes 0-1: byte order

0x4949

(=II) Intel byte order

0x4d4d

(=MM) Motorola byte order

Bytes 2-3: Tiff version

0x2a

(=42) this Tiff version has never changed

Bytes 4-7:

byte offset to the first Image File Directory.

## Image File Directory

An IFD (= image file directory) consists of a 2-byte count of the number of entries, followed by a sequence of 12-byte field entries, followed by a 4-byte offset to the next IFD or 0 after the last IFD. So it is possible to save more than one IFD in one file. This means: more than one image can be found in one Tiff file. However, most of the Tiff applications use only the first IFD. Each 12-byte IFD entry has the following format:

Bytes 0-1: Tag that identifies the field

Bytes 2-3: Type of the field Bytes 4-7: Length of the field

Bytes 8-11: Value Offset, file offset in bytes

The entries in an IFD are sorted in ascending order by Tag. The Value Offset is interpreted to contain the value instead of pointing to the value if the value fits into 4 bytes.

The usual field Types are:

1=BYTE 8-bit unsigned integer

8-bit containing 7-bit ASCII code, null terminated 2=ASCII

3=SHORT 16-bit unsigned integer 32-bit unsigned integer 4=LONG

5=RATIONAL Two LONGs, the first represents the numerator of a fraction, the second the denominator

There are more than 70 different Tags. Some of the most used are:

0x0100 ImageWidth 0x0101 **ImageLength** 0x0102 BitsPerSample 0x0103 Compression

0x0106 PhotometricInterpretation

So a valid 12-byte IFD entry look like:

0x0100 Tag=ImageWidth Type=LONG 0x0004 0x0000.0001 Length=1

0x0000.0200 Value=0x200=512

This entry specifies an image x-size of 512 pixels.

The image data can be organized in one strip, line by line, starting at the left upper corner, or in more strips. One strip contains one or more lines of the image, so that the size of the strip is about 8kB. The start addresses of the strips can be found by the Tag StripOffsets. So it is easy to find the image data: Read the header, determine the byte order, find the first IFD, search for the Tag StripOffsets, find the first strip of image data.

For detailed information, please refer to:

TIFF, Tag Image File Format, Revision 6.0, Draft 1, Feb 14, 1992

by Aldus Corporation

Techinfo: LSM-TIFF

# **HOW THE LSM USES TIFF?**

One goal for the Tiff format was to store the LSM images in a format that makes it possible to import the data into other applications. So you can use wordprocessors or DTP programs to generate reports with images, paint and retouch programs to modify and print images, and, not at last, image processing programs to process the image data and do some image analysis. There are also image format converters on the market that accept Tiff and create other formats.

#### THE LSM TIFF WRITER

We use the INTEL byte order only.

The LSM program stores images in in following Tiff classes:

Grayscale Images, Palette Color Images, RGB Full Color Images.

For all classes we store following tags:

NewSubfileType = 0, full resolution, no multipage, no mask

ImageWidth = image x size

ImageLength = image v size

Compression = 1, no compression

Make = "Carl Zeiss, Oberkochen, Germany"

Model = "Laser Scan Microscope"

Software = "ZIF 1.81 MAR-93", software version that generated this file

This means, that we always store an uncompressed full resolution image in the first IFD. With means of the Make, Model and Software Tags you can always specify the source of the specified image.

We use these tags for Grayscale Images:

BitsPerSample = 8

PhotometricInterpretation = 1, black is zero

StripOffsets = offset of the first strip

SamplesPerPixel = 1

StripByteCounts = byte count of one strip

You see, that we have only 8-bit images with black represented by the value 0. All the data is stored in one strip.

## Palette Color Images have following tags:

BitsPerSample = 8

PhotometricInterpretation = 3, Palette Color

StripOffsets = offset of the first strip

SamplesPerPixel = 1

StripByteCounts = byte count of one strip

ColorMap = offset of the lookup table

The only difference is the lookup table that is stored as 768 16-bit values in a redgreen-blue sequence.

#### RGB Full Color Images are specified by following tags:

BitsPerSample = 8,8,8

PhotometricInterpretation = 2, RGB

StripOffsets = offset of chunky data or offset of the 3 planes

SamplesPerPixel = 3

StripByteCounts = byte count of chunky data or 3 byte counts of 3 planes

PlanarConfiguration = 1 (chunky format, RGB pixel sequence) or PlanarConfiguration = 2 (planar format, R G and B in seperate planes)

There is nearly no difference in speed between storing in chunky or in planar format. Especially for data export to other applications, chunky format is more accepted (see also the test list).

We don't use following tags:

RowsPerStrip, default is 2\*\*32-1, that is, the entire image is one strip.

ResolutionUnit

**XResolution** 

**YResolution** 

RowsPerStrip = 0xffff.ffff has been found to be a problem for the PhotoStyler Tiff reader, so we removed it and use the default value. The Resolution tags are normally required for all classes of Tiff images, but don't make sense for LSM images and have been left out. There is no problem for other Tiff readers with this missing tags.

#### Tests

This full resolution image (first IFD) has been accepted by a lot of applications:

- Microsoft WinWord 2.0 (no RGB planar format)
- MicroSoft PowerPoint 3.0 (no RGB planar format)
- Micrografx Designer 3.1
- Aldus PhotoStyler 1.1
- PaintShop Pro 1.0 (no RGB planar format)
- Carl Zeiss AxioDoc
- Shareware pv.exe (no RGB planar format)

## **Privat tags**

Two new tags may be found in our Tiff files. One for the LSM settings and the other for an additional comment. A change was necessary, because ALDUS (the TIFF administrator) assigned us privat tags that will never be changed again.

34412=0x866c (former 0x9bc4) is made to store LSM specific information

34413=0x866d (former 0x9bc5) is made for an additional comment

The format of the LSM-information:

| Offset | Туре | Description | n                              |
|--------|------|-------------|--------------------------------|
| 0000h  | WORD | Code 494Ch  |                                |
| 0002h  | WORD | Version (00 | 002h)                          |
| 0004h  | WORD | Image type  |                                |
|        |      | Bit 0/1     | Number of 8 bit planes         |
|        |      | Bit 2/3     | Number of channels per pixel   |
|        |      | Bit 4       | 0: Original data,              |
|        |      |             | 1: Calculated data (animation) |

```
Bit 5
                             1: Time series
                     Bit 6
                             Reserved (0 or 1)
                     Bit 7
                             1: Image is part of sequence
                     Bit 8
                             1: y-direction is Time
                     Bit 9
                             1: x-direction is Time
 0006h WORD
                   Reserved
 0008h WORD
                   x size of image
 000ah WORD
                   y size of image
 000ch WORD
                   x position of ROI
 000eh WORD
                   x position of ROI
 0010h WORD
                   x size of image display mask
 0012h WORD
                   y size of image display mask
 0014h WORD
                   Reserved
 0016h WORD
                   Reserved
 0018h WORD
                   Image position number in a sequence
 001ah BYTE
                   Reserved
 001bh BYTE
                   Number of valid channel parameters (1..3)
 001ch BYTE
                   Number of lasers (1..4)
 001dh 3 bytes
                   Reserved
 0020h float
                   x size of a pixel (µm or s)
 0024h float
                   y size of a pixel (µm or s)
> 0028h float
                   z distance in a sequence (µm or s)
√002ch float
                   Sequence value (µm / s)
 0030h 8 x WORD
                   List of laser lines (nm)
                    ______
 0040h
        64 bytes
                   | Channel parameters 1
                    _____
 0080h
        64 bytes
                   Channel parameters 2 (if available)
                    ______
 00c0h
        64 bytes
                   | Channel parameters 3 (if available)
                    ______
 0100h 16 x char
                   User text 1
 0110h 16 x char
                   User text 2
 0120h 16 x char
                   Date and time text
 0130h 16 x char
                   Beam splitter text (channel 1, should be valid
for all)
                   The time in seconds since midnight (00:00:00),
 0140h
       time_t
                   January 1, 1970, Universal Coordinated Time
 0144h WORD
                   Fraction of a second in milliseconds
                   Timezone difference in minutes
 0146h short
 0148h short
                   Daylight saving flag
 014ah float
                   Real scan time for one image (s)
```

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| 014eh   | 2 bytes    | Reserved                                      |
|---------|------------|---|
| 0150h   | 16 x char  | Emission filter channel 1 text                |
| 0160h   | 16 x char  | Emission filter channel 2 text (if available) |
| 0170h   | 16 x char  | Emission filter channel 3 text (if available) |
| 0180h   | 32 x char  | Reserved for lens decription text             |
|         |            |   |
| Channel | Parameters | (64 bytes)                                    |
|         |            |   |
| Offset  | Type       | Description                                   |
| 0000h   | BYTE       | Source  |
|         |            | 1 Conv Refl                                   |
|         |            | 2 Conv Trans                                  |
|         |            | 3 Conv Overl                                  |
|         |            | 4 Conv Fluor                                  |
|         |            | 5 LSM Refl1                                   |
|         |            | 6 LSM Refl2                                   |
|         |            | 7 LSM Refl3                                   |
|         |            | 8 LSM Trans                                   |
|         |            | 9 OBIC  |
|         |            | 10 Extern                                     |
| 0001h   | BYTE       | Pinhole                                       |
| 0002h   | BYTE       | Emission filter                               |
| 0003h   | BYTE       | Flags   |
|         |            | Bit 0 TV                                      |
|         |            | Bit 1 Confocal                                |
|         |            | Bit 2 Reserved                                |
|         |            | Bit 3 Ratio                                   |
| 0004h   | BYTE       | Attenuation filter 1                          |
| 0005h   | BYTE       | Attenuation filter 2                          |
| 0006h   | BYTE       | Attenuation filter 3                          |
| 0007h   | BYTE       | Laser (each bit represents one laser line)    |
| 0008h   | BYTE       | Scanning time                                 |
| 0009h   | BYTE       | Bandwidth                                     |
| 000ah   | BYTE       | Beam splitter                                 |
| 000bh   | BYTE       | Lens  |
| 000ch   | BYTE       | Scan function                                 |
| 000dh   | BYTE       | Averaging mode (reserved)                     |
| 000eh   | WORD       | Number of averaging                           |
| 0010h   | WORD       | Contrast                                      |
| 0012h   | WORD       | Brightness                                    |
|         |            |   |

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```
0014h long
                  x motor (in motor steps)
0018h long
                  y motor (in motor steps)
001ch long
                   z motor (in motor steps)
0020h WORD
                   Zoom factor * 1000
0022h short
                   Angle of rotation (0.1 degree)
0024h WORD
                   Obic address 1
0026h WORD
                   Obic address 2
0028h short
                   Scan offset x
002ah short
                   Scan offset y
002ch BYTE
                   Attenuation filter 4
002dh 3 bytes
                   Reserved
0030h float
                   Objective magnification
0034h float
                   Objective apperture
0038h float
                   Reserved
003ch float
                   Reserved
```

The graphic overlay of the image can optionally be stored in the second IFD. But this data can only be read by the LSM program. There is no application known that accepts more than one IFD per file.

A subsample is prepared to be stored in a third IFD. This has the Tag NewSubfileType = 1 (reduced resolution).

The first version released was ZIF 1.30

Modifications/bug fixes of version ZIF 1.70:

The Tag StripByteCounts has been added, so we avoid some warning messages from other Tiff readers.

The Tag BitsPerSample was modified for RGB images to 8,8,8. More Tiff reader can now read our RGB format.

The Tag RowsPerStrip=0xffff.ffff made some problems with Tiff readers, so we removed it. The default is 0xffff.ffff.

Modifications/bug fixes of version ZIF 1.74:

Photometric Interpretation is set to 3 for 8 bit data with lookup table. It was 1 before and some Tiff readers don't like it and set wrong color tables.

Modifications/bug fixes of version ZIF 1.76:

The color lookup table has been improved for the AxioDoc software.

Modifications/bug fixes of version ZIF 1.81:

RGB images can be stored/loaded in the chunky format.

Most read/write operations have been tuned in speed.

The privat fields are now variable in length.

#### THE LSM TIFF READER

The Tiff reader of the LSM has the primary task to efficiently read the LSM Tiff files with all the options like overlay, low resolution image, privat info, privat comment. It is NOT designed to import Tiff data from other applications. Especially, the tiff reader

- do not read MOTOROLA format
- do only accept BitPerSample = 8
- do not read compressed data
- do not read data in more than 1 strip

To import Tiff images from other application, there is a Tiff import function for simple formats. This import function is automatically called in the LSM program if the Tiff reader wasn't successful.

#### THE LSM TIFF IMPORT

The Tiff import function accepts Motorola and Intel format. It reads uncompressed grayscale and palette color images that are 8 bits deep as well as 24 bit full color RGB images in the planar or chunky format. The image data may be in more than one strip. This function solves most of the import requirements.

 Date of first version (#1)
 Jan 12, 1993

 Version
 #3, Jun 17, 1994

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# Lsm - Tiff

This is a short description of Tiff (= Tag Image File Format) and how the LSM program uses it.

#### What is Tiff?

Tiff is a file format that describes image data coming from scanners, frame grabbers and paint/photo retouch programs. The primary goal was to provide a rich environment within which the exchange of image data between application programs can be accomplished. This richness is required in order to take advantage of the varying capabilities of imaging devices. Though Tiff is a rich format, it can easily be used for simple applications as well.

However, most (and not only simple) Tiff applications do not support the full, rich Tiff standard and this is the reason for incompatibilities between different applications.

Tiff is capable of describing bi-level, grayscale, palette color and full color image data in several color spaces. It includes a number of compression schemes, is portable because it does not depend on particular operating systems or file systems and, allows privat tags to be defined that are specific to a particular application or organization.

#### File Structure

A Tiff file is a sequence of 8-bit bytes. Every Tiff file begins with an 8-byte Image File Header.

Bytes 0-1: byte order

0x4949 (=II) Intel byte order 0x4d4d (=MM) Motorola byte order

Bytes 2-3: Tiff version

0x2a (=42) this Tiff version has never changed

Bytes 4-7: byte offset to the first Image File Directory.

An IFD (= image file directory) consists of a 2-byte count of the number of entries, followed by a sequence of 12-byte field entries, followed by a 4-byte offset to the next IFD or 0 after the last IFD. So it is possible to save more than one IFD in one file. This means: more than one image can be found in one Tiff file. However, most of the Tiff applications use only the first IFD. Each 12-byte IFD entry has the following format:

Bytes 0-1: Tag that identifies the field

Bytes 2-3: **Type** of the field Bytes 4-7: **Length** of the field

Bytes 8-11: Value Offset, file offset in bytes

The entries in an IFD are sorted in ascending order by Tag. The Value Offset is interpreted to contain the value instead of pointing to the value if the value fits into 4 bytes.

The usual field Types are:

1=BYTE 8-bit unsigned integer

8-bit containing 7-bit ASCII code, null terminated 2=ASCII

3 = SHORT16-bit unsigned integer 4 = LONG32-bit unsigned integer

5=RATIONAL Two LONGs, the first represents the numerator of a fraction,

the second the denominator

There are more than 70 different Tags. Some of the most used are:

0x0100ImageWidth  $0 \times 0101$ ImageLength  $0 \times 0102$ BitsPerSample  $0 \times 0103$ Compression

 $0 \times 0106$ PhotometricInterpretation

So a valid 12-byte IFD entry look like:

0x0100 $0 \times 0004$ 

 $0 \times 0000.0001$ 

0x0000.0200

Tag=ImageWidth

Type=LONG

Length=1

Value=0x200=512

This entry specifies an image x-size of 512 pixels.

The image data can be organized in one strip, line by line, starting at the left upper corner, or in more strips. One strip contains one or more lines of the image, so that the size of the strip is about 8kB. The start addresses of the strips can be found by the Tag StripOffsets. So it is easy to find the image data: Read the header, determine the byte order, find the first IFD, search for the Tag StripOffsets, find the first strip of image data.

For detailed information, please refer to:

Tag Image File Format, Specification, Revision 5.0, Final, 8/8/88 by Aldus Corporation and Microsoft Corporation and:

Tag Image File Format, Revision 6.0, Draft 1, February 14, 1992 by Aldus Corporation

#### How the LSM uses Tiff?

One goal for the Tiff format was to store the LSM images in a format that makes it possible to import the data into other applications. So you can use wordprocessors or DTP programs to generate reports with images, paint and retouch programs to modify and print images, and, not at last, image processing programs to process the image data and do some image analysis. There are also image format converters on the market that accept Tiff and create other formats.

#### The LSM Tiff Writer

We use the INTEL byte order only.

The LSM program stores images in in following Tiff classes: Grayscale Images, Palette Color Images, RGB Full Color Images.

For all classes we store following tags: NewSubfileType = 0, full resolution, no multipage, no mask ImageWidth = image x size
ImageLength = image y size
Compression = 1, no compression
Make = "Carl Zeiss, Oberkochen, Germany"
Model = "Laser Scan Microscope"
Software = "ZIF 1.81 MAR-93", software version that generated this file

This means, that we always store an uncompressed full resolution image in the first IFD. With means of the Make, Model and Software Tags you can always specify the source of the specified image.

We use these tags for **Grayscale Images**:
BitsPerSample = 8
PhotometricInterpretation = 1, black is zero
StripOffsets = offset of the first strip
SamplesPerPixel = 1
StripByteCounts = byte count of one strip

You see, that we have only 8-bit images with black represented by the value 0. All the data is stored in one strip.

#### Palette Color Images have following tags:

BitsPerSample = 8
PhotometricInterpretation = 3, Palette Color
StripOffsets = offset of the first strip
SamplesPerPixel = 1
StripByteCounts = byte count of one strip
ColorMap = offset of the lookup table

The only difference is the lookup table that is stored as 768 16-bit values in a red-green-blue sequence.

#### RGB Full Color Images are specified by following tags:

BitsPerSample = 8,8,8
PhotometricInterpretation = 2, RGB
StripOffsets = offset of chunky data or offset of the 3 planes
SamplesPerPixel = 3
StripByteCounts = byte count of chunky data or 3 byte counts of 3 planes
PlanarConfiguration = 1 (chunky format, RGB pixel sequence) or
PlanarConfiguration = 2 (planar format, R G and B in seperate planes)

There is nearly no difference in speed between storing in chunky or in planar format. Especially for data export to other applications, chunky format is more accepted (see also the test list).

We don't use following tags: RowsPerStrip, default is 2\*\*32-1, that is, the entire image is one strip. ResolutionUnit XResolution YResolution

RowsPerStrip = 0xffff.ffff has been found to be a problem for the PhotoStyler Tiff reader, so we removed it and use the default value. The Resolution tags are normally required for all classes of Tiff images, but don't make sense for LSM images and have been left out. There is no problem for other Tiff readers with this missing tags.

This full resolution image (first IFD) has been accepted by a lot of applications:

- \* Microsoft WinWord 2.0 (no RGB planar format)
- \* MicroSoft PowerPoint 3.0 (no RGB planar format)
- \* Micrografx Designer 3.1
- \* Aldus PhotoStyler 1.1
- \* PaintShop Pro 1.0 (no RGB planar format)
- \* Carl Zeiss AxioDoc

\* Shareware pv.exe (no RGB planar format)

#### Privat tags:

Two new tags may be found in our Tiff files. One for the LSM settings and the other for an additional comment. A change was necessary, because ALDUS (the TIFF administrator) assigned us privat tags that will never be changed again.

34412=0x866c (former 0x9bc4) is made to store LSM specific information 34413=0x866d (former 0x9bc5) is made for an additional comment The format of the LSM-information:

| Offset         | Туре                 | Description   |
|----------------|----------------------|---|
| 0000h          | WORD                 | Code 494Ch  |
| 0002h          | WORD                 | Version (0100h)   |
| 0004h          | WORD                 | Image type  |
|                |                      | Bit 0/1 Number of planes  |
|                |                      | Bit 2/3 Number of channels  |
|                |                      | Bit 4 0: Original data,1: Calculated data (anim.)                             |
|                |                      | Bit 5 1: Time series  |
|                |                      | Bit 6 Reserved (0 or 1)   |
|                |                      | Bit 7 1: Image is part of sequence  |
|                |                      | Bit 8 1: y-direction is Time Bit 9 1: x-direction is Time                     |
| 00065          | MODD                 |   |
| 0006h<br>0008h | WORD<br>WORD         | Reserved  |
| 000ah          | WORD                 | x size of image y size of image   |
| 000an          | WORD                 | x position of ROI   |
| 000eh          | WORD                 | x position of ROI   |
| 0010h          | WORD                 | x size of image display mask  |
| 0012h          | WORD                 | y size of image display mask  |
| 0014h          | WORD                 | Reserved  |
| 0016h          | WORD                 | Reserved  |
| 0018h          | WORD                 | Image position number in a sequence   |
| 001ah          | BYTE                 | Reserved  |
| 001bh          | BYTE                 | Reserved  |
|                | BYTE<br>3 bytes      | Number of laser lines Reserved  |
| 0020h          | float                |   |
| 0020h          | float                | x size of a pixel (µm or s)   |
|                |                      | y size of a pixel (μm or s)   |
| 0028h          | float                | z distance in a sequence (µm or s)  |
|                | float<br>8 x WORD    | Sequence value (µm / s) List of laser lines (nm)                              |
| 003011         | 6 X WORD             | List of laser lines (mm)  |
| 0040h          | 64 bytes             | Channel parameters 1  |
| 0080h          | 64 bytes             | Channel parameters 2 (if available)   |
|                | 11 11 11 11 11       |   |
| 00c0h          | 64 bytes             | Channel parameters 3 (if available)   |
| 0100h          | 16 x char            | User text 1   |
| 0100h          | 16 x char            | User text 2   |
| 0120h          | 16 x char            | Date and time text  |
|                | 16 x char            | Beam splitter text  |
| 0140h          | time_t               | The time in seconds since midnight (00:00:00),                                |
|                |                      | January 1, 1970, Universal Coordinated Time                                   |
| 0144h          | WORD                 | Fraction of a second in milliseconds  |
| 0146h          | short                | Timezone difference in minutes  |
| 0148h          | short                | Daylight saving flag  |
| 014ah          | float                | Real scan time for one image (s)  |
| 014eh<br>0150h | 2 bytes<br>16 x char | Reserved Emission filter channel 1 text                                       |
| 0150h          | 16 x char            | Emission filter channel 1 text  Emission filter channel 2 text (if available) |
| 0170h          | 16 x char            | Emission filter channel 3 text (if available)                                 |
| 0180h          | 32 x char            | Reserved for lens decription text   |
|                |                      | <del>-</del>  |

# Channel Parameters (64 bytes)

| Offset         | Туре         | Description  |
|----------------|--------------|--|
| 0000h          | ВУТЕ         | Source 1 Conv Refl 2 Conv Trans 3 Conv Overl 4 Conv Fluor 5 LSM Refl1 6 LSM Refl2 7 LSM Refl3 8 LSM Trans 9 OBIC |
| 0001h          | BYTE         | 10 Extern Pinhole  |
| 0002h          | BYTE         | Emission filter  |
| 0003h          | BYTE         | Flags  |
|                |              | Bit 0 TV   |
|                |              | Bit 1 Confocal Bit 2 Reserved  |
|                |              | Bit 2 Reserved Bit 3 Ratio   |
| 0004h          | BYTE         | Attenuation filter 1   |
| 0005h          | BYTE         | Attenuation filter 2   |
| 0006h          | BYTE         | Attenuation filter 3   |
| 0007h          | BYTE         | Laser (each bit represents one laser line)   |
| 0008h          | BYTE         | Scanning time  |
| 0009h          | BYTE         | Bandwidth  |
| 000ah          | BYTE         | Beam splitter  |
| 000bh          | BYTE         | Lens   |
| 000ch          | BYTE         | Scan function  |
| 000dh          | BYTE         | Averaging mode (reserved)  |
| 000eh          | WORD         | Number of averaging  |
| 0010h          | WORD         | Contrast   |
| 0012h          | WORD         | Brightness   |
| 0014h          | long         | x motor (in motor steps)   |
| 0018h<br>001ch | long         | y motor (in motor steps)   |
| 001Ch          | long<br>WORD | z motor (in motor steps) Zoom factor * 1000  |
| 0020h          | short        | Angle of rotation (0.1 degree)   |
| 0024h          | WORD         | Obic address 1   |
| 0024h          | WORD         | Obic address 1   |
| 0028h          | short        | Scan offset x  |
| 002ah          | short        | Scan offset y  |
| 002ch          | BYTE         | Attenuation filter 4   |
| 002dh          | 3 bytes      | Reserved   |
| 0030h          | float        | Objective magnification  |
| 0034h          | float        | Objective apperture  |
| 0038h          | float        | Reserved   |
| 003ch          | float        | Reserved   |
|                |              |  |

The graphic overlay of the image can optionally be stored in the second IFD. But this data can only be read by the LSM program. There is no application known that accepts more than one IFD per file.

A subsample is prepared to be stored in a third IFD. This has the Tag NewSubfileType = 1 (reduced resolution).

The first version released was ZIF 1.30

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The Tag StripByteCounts has been added, so we avoid some warning messages from other Tiff readers.

The Tag BitsPerSample was modified for RGB images to 8,8,8. More Tiff reader can now read our RGB format.

The Tag RowsPerStrip=0xffff.ffff made some problems with Tiff readers, so we removed it. The default is 0xffff.ffff.

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Photometric Interpretation is set to 3 for 8 bit data with lookup table. It was 1 before and some Tiff readers don't like it and set wrong color tables.

Modifications/bug fixes of version ZIF 1.76:

The color lookup table has been improved for the AxioDoc software.

Modifications/bug fixes of version ZIF 1.81:

RGB images can be stored/loaded in the chunky format.

Most read/write operations have been tuned in speed.

The privat fields are now variable in length.

#### Samples:

See the Appendix for 4 sample headers, written by our Tiff writer.

cz\_gray.tif is a 512\*512 grayscale image

cz\_color.tif is the same as cz\_gray, with neon colors cz\_rgb1.tif is a 512\*480 RGB image in the planar format cz\_rgb2.tif is a 512\*480 RGB image in the chunky format

### The LSM Tiff Reader

The Tiff reader of the LSM has the primary task to efficiently read the LSM Tiff files with all the options like overlay, low resolution image, privat info, privat comment. It is NOT designed to import Tiff data from other applications. Especially, the tiff reader

- \* do not read MOTOROLA format
- \* do only accept BitPerSample = 8
- \* do not read compressed data
- \* do not read data in more than 1 strip

To import Tiff images from other application, there is a Tiff import function for simple formats. This import function is automatically called in the LSM program if the Tiff reader wasn't successful.

# The LSM Tiff Import

The Tiff import function accepts Motorola and Intel format. It reads uncompressed grayscale and palette color images that are 8 bits deep as well as 24 bit full color RGB images in the planar or chunky format. The image data may be in more than one strip. This function solves most of the import requirements.

# **Appendix**

file - cz\_gray.tif

Offset Name

Value

Header:

00000000 Byte Order

4949

```
00000002 Version
00000004 1st IFD Pointer
                                                             002A
                                                             80000008
IFD:
00000008 Entry Count
                                                             000E

        0000000A
        NewSubfileType
        00FE
        0004
        00000001
        00000000

        00000016
        ImageWidth
        0100
        0004
        00000001
        0000020

        00000022
        ImageLength
        0101
        0004
        00000001
        0000020

        0000002E
        BitsPerSample
        0102
        0003
        00000001
        0008

        0000003A
        Compression
        0103
        0003
        00000001
        0001

        00000052
        PhotometricInterpretation
        0106
        0003
        00000001
        0001

0000000A NewSubfileType
                                                            010F 0002 00000020 000000B6
0110 0002 00000016 000000D6
0111 0004 00000001 00000122
00000052 Make
0000005E Model
0000006A StripOffsets
00000076 SamplesPerPixel
00000082 StripByteCounts
0000008E SoftWare
0000009A ?
                                                            0115 0004 00000001 0001
0117 0004 00000001 00040000
0131 0002 00000010 000000EC
866C 0001 00000017 000000FC
866D 0002 0000000F 00000113
000000A6 ?
000000B2 Next IFD Pointer
                                                             00000000
Fields pointed to by the tags:
000000B6 Make
                                                             "Carl Zeiss, Oberkochen, Germany"
000000D6 Model
                                                             "Laser Scan Microscope"
000000EC SoftWare
                                                             "ZIF 1.81 MAR-93"
000000FC ?
                                                             70 72 69 76 61 74 20 4C 53
                                                             4D 20 69 6E 66 6F 72 6D 61
                                                             74 69 6F 6E 00
00000113 ?
                                                             "privat comment"
file - cz_color.tif
Offset Name
                                                             Value
Header:
00000000 Byte Order
                                                             4949
00000002 Version
00000004 1st IFD Pointer
                                                             002A
                                                             80000000
IFD:
00000008 Entry Count
                                                             000F
0000000A NewSubfileType

        00FE
        0004
        00000001
        0000000

        0100
        0004
        00000001
        00000200

        0101
        0004
        00000001
        00000200

        0102
        0003
        00000001
        0008

00000016 ImageWidth
00000022 ImageLength
0000002E BitsPerSample
                                                           0103 0003 00000001 0001
0000003A Compression
00000046 PhotometricInterpretation 0106 0003 00000001 0003
00000052 Make
                                                            010F 0002 00000020 000000C2
0000005E Model
                                                            0110 0002 00000016 000000E2
0000006A StripOffsets
                                                          0111 0004 00000001 0000073C
00000076 SamplesPerPixel
                                                          0115 0003 00000001 0001
00000082 StripByteCounts
0000008E SoftWare
                                                            0117 0004 00000001 00040000
0131 0002 00000010 000000F8
0000009A ColorMap
                                                            0140 0003
866C 0001
                                                                                00000300 00000108
00000017 00000708
000000A6
                2
                                                             866D 0002 0000001D 0000071F
000000B2
                ?
000000BE Next IFD Pointer
                                                             00000000
Fields pointed to by the tags:
000000C2 Make
                                                             "Carl Zeiss, Oberkochen, Germany"
000000E2 Model
                                                             "Laser Scan Microscope"
000000F8 SoftWare
                                                             "ZIF 1.81 MAR-93"
00000108 ColorMap
                                                             0000 0000 0000 0000 0000 0000
                                                             0000 0000 0000 0000 0000 0000
```

```
0000 0000 0000 0000 0000 0000
                                         0000 ...
                                        70 72 69 76 61 74 20 4C 53
4D 20 69 6E 66 6F 72 6D 61
74 69 6F 6E 00
00000708 ?
0000071F ?
                                         "cz_gray.tif with neon colors"
_____
file - cz_rgb1.tif
Offset Name
                                         Value
Header:
00000000 Byte Order
                                        4949
00000002 Version
00000004 1st IFD Pointer
                                         002A
                                        00000008
IFD:
00000008 Entry Count
                                        000F
0000000A NewSubfileType
                                        00FE 0004 00000001 00000000
00000016 ImageWidth
00000022 ImageLength
                                       0100 0004 00000001 00000200
                                       0101 0004 00000001 000001E0
00000022 Imageners
0000002E BitsPerSample
                                       0102 0003 00000003 000000C2
0000003A Compression
                                       0103 0003 00000001 0001
00000046 PhotometricInterpretation 0106 0003 00000001 0002
00000052 Make
                                       010F 0002 00000020 000000C8
                                   0110 0002 00000016 00000008
0111 0004 00000003 000000FE
0115 0003 00000001 0003
0117 0004 00000003 0000010A
011C 0003 00000001 0002
0131 0002 00000010 00000116
0000005E Model
0000006A StripOffsets
00000076 SamplesPerPixel
00000082 StripByteCounts
0000008E PlanarConfiguration
0000009A SoftWare
                                        866C 0001 00000017 00000126
000000A6 ?
                                        866D 0002 00000026 0000013D
000000B2 ?
000000BE Next IFD Pointer
                                        00000000
Fields pointed to by the tags:
                                        0008 0008 0008
000000C2 BitsPerSample
000000C8 Make
                                        "Carl Zeiss, Oberkochen, Germany"
000000E8 Model
000000FE StripOffsets
0000010A StripByteCounts
00000116 SoftWare
                                        "Laser Scan Microscope"
                                      00000163 0003C163 00078163 0003C000 0003C000
                                        "ZIF 1.81 MAR-93"
00000126 ?
                                        70 72 69 76 61 74 20 6C 73
6D 20 69 6E 66 6F 72 6D 61
74 69 6F 6E 00
0000013D ?
                                         "Clara Schumann on the 100 DM
banknote"
file - cz_rgb2.tif
Offset
         Name
                                        Value
Header:
00000000 Byte Order
                                         4949
00000002 Version
                                         002A
00000004 1st IFD Pointer
                                        00000008
IFD:
00000008 Entry Count
                                        000F
0000000A NewSubfileType
                                        00FE 0004 00000001 00000000
                                        0100 0004 00000001 00000200
00000016 ImageWidth
00000022 ImageLength
                                        0101 0004 00000001 000001E0
```

| 0000000   | Dit - D G 1               | 0100                    | 0000  |               |              |       |
|-----------|---------------------------|-------------------------|-------|---------------|--------------|-------|
| 0000002E  | BitsPerSample             | 0102                    | 0003  | 00000003      | 000000C2     |       |
| AE000000  | Compression               | 0103                    | 0003  | 00000001      | 0001         |       |
| 00000046  | PhotometricInterpretation | 0106                    | 0003  | 00000001      | 0002         |       |
| 00000052  | Make                      | 010F                    | 0002  | 00000020      | 000000C8     |       |
| 0000005E  | Model                     | 0110                    | 0002  | 00000016      | 000000E8     |       |
| 0000006A  | StripOffsets              | 0111                    | 0004  | 00000001      | 0000014A     |       |
| 00000076  | SamplesPerPixel           | 0115                    | 0003  | 00000001      | 0003         |       |
| 00000082  | StripByteCounts           | 0117                    | 0004  | 00000001      | 000B4000     |       |
| 0000008E  | PlanarConfiguration       | 011C                    | 0003  | 00000001      | 0001         |       |
| 0000009A  | SoftWare                  | 0131                    | 0002  | 00000010      | 000000FE     |       |
| 000000A6  | ?                         | 866C                    | 0001  | 00000017      | 0000010E     |       |
| 000000B2  | ?                         | 866D                    | 0002  | 00000025      | 00000125     |       |
| 000000BE  | Next IFD Pointer          | 0000000                 |       |               |              |       |
|           |                           |                         |       |               |              |       |
| Fields po | inted to by the tags:     |                         |       |               |              |       |
| 000000C2  | BitsPerSample             | 8000                    | 8000  | 8000          |              |       |
| 000000C8  | Make                      | "Carl                   | Zeiss | , Oberkoch    | en, Germany' | ı     |
| 000000E8  | Model                     | "Laser Scan Microscope" |       |               |              |       |
| 000000FE  | SoftWare                  | "ZIF 1.81 MAR-93"       |       |               |              |       |
| 0000010E  | ?                         |                         | 2 69  |               | 4 20 4C 5    | 53    |
|           |                           | 4D 2                    | _     | 6E 66 6       |              | 51    |
|           |                           | 74 6                    |       | 6E 00         |              | -     |
| 00000125  | ?                         |                         | Schum |               | 100 DM bank  | mote" |
|           | •                         | cara                    | Schan | aiii oii ciic | 100 DH bain  |       |

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