CHAPTER 8 3D FOR LSM 510

CONTENTS

Page

8	3D for LSM 510	8-3
8.1	Overview and Explanations	
8.1.1	The Image Sequence	
8.1.2	The Image Properties	
8.1.3	Memory Usage	
8.2	User Interface	
8.2.1	Introduction	
8.2.2	Main Window	
8.2.3	Display Window	
8.3	Functions	
8.3.1	Functions in the File Menu	
8.3.2	Functions in the Edit Menu	
8.3.3	Functions in the Process Menu	
8.3.4	Functions in the View Menu	
8.3.5	Functions in the Measurement Menu	

3D FOR LSM 510 Contents

8 3D FOR LSM 510

8.1 Overview and Explanations

8.1.1 The Image Sequence

The "3D for LSM" handles image sequences generated by the Zeiss LSM software. This can be three-dimensional image data or a time sequence of two-dimensional images (slices). Each slice (as well as the sequence) can consist of up to eight channels. An image sequence consists of a series of individual (2D) images and has a name that designates the entire sequence. In general an image sequence is handled as a single object in the system. Individual channels or slices can be addressed.

The following terms and definitions apply for the "3D for LSM" software.

- An image sequence is a number of individual sequential images (usually called slices in the dialog boxes), the spacing between which is equal.
- Image sequences can contain up to 12 bit of image data (per channel).
- A sequence (slice) can consist of up to eight channels.
- The maximum size of an image sequence is limited by the provided memory of the operating system.







- A voxel is the smallest element of an image sequence (the equivalent of a pixel in a 2D image). All
 voxels in a given image sequence are the same size.
- The coordinate system originates in the left upper front corner of the image sequence. This point has the coordinates 0, 0, 0.
- All angles are positive for rotations to the right in the direction of the positive coordinate axis (righthanded coordinate system).
- A slice is an individual image in a sequence of images. The numbering of the slices starts with "1".

Image sequences can consist of several channels. Most functions and the **Display window** are providing buttons to select all or a subset of channels stored in the selected image sequence. The **Output** image sequence will only get those channels which are selected on the input side. The button selects all channels in the image sequence to be used clicking with the left mouse button on it.

Clicking with the left mouse button on any of the number buttons toggles the state of this single channel.

Clicking with the right mouse button on any of the number buttons selects this single channel exclusively. All other channels are deselected.

8.1.2 The Image Properties

Every image sequence has its own set of properties. They contain the scaling and the scaling units. The scaling and its units are required for 3D reconstruction and measurement. If a sequence of LSM-TIFF images is read in, the image properties are loaded automatically from the file header and allocated to the image properties of the new image sequence.

8.1.3 Memory Usage

All images shown in the **Gallery** are currently loaded in the system memory of the operating system. Some functions need additional temporarily used memory during their execution.

If the memory is running low delete some images from the **Gallery**. If the images are needed afterwards they must be saved to disk first. Normally all functions produce a new result (output) image sequence. In order to save some memory, other image sequences currently presented in the **Gallery** can be selected as result position. The output image is overwritten by entry execution of a function.

8.2 User Interface

8.2.1 Introduction

This section describes the following main components of the system:

- Main window Main window with the Menu, the Tool bar and Gallery. All general system functions are located here.
- **Gallery** Normally several images are required in order to accomplish a particular task. These images are displayed in reduced size to provide an overview and facilitate selection. This area is located just below the **Tool bar**.





Tool bar

This menu shows all image processing functions.

3D FOR LSM 510 User Interface

Display window This window is used to display image sequences.



Fig. 8-3 Display window

Dialog boxes All dialog boxes provide three buttons. Pressing the **OK** button executes the function with the defined parameters and closes the dialog window. Selecting the **Cancel** button does not execute the function, restores the parameters, and closes the dialog window. Pressing the **Apply** button executes the function with the defined parameters; the dialog window will stay opened.

8.2.2 Main Window

The Main window includes:

the **Menu**

<u>File Edit Process View Measure Windows Help</u>

the Tool bar



and the Gallery



File Menu

Ê	Open Image	Opens a file selector dialog to load an image sequence.
	Save Image As	Opens a file selector to save an image or image sequence.
	Save Display As	Saves the currently shown contents of the Display window as a single colour image.
5	Print	The printer parameters can be set with this tool. The standard Windows printer dialog is opened.
	Exit	Terminates the application.

Edit Menu

	Сору	Copies the contents of the Display window to the clipboard.
	Edit Channels	Allows to add or to remove channels to a single or multichannel image.
	Delete All Images	Deletes all images and image sequences from the memory.
Proces	s Menu	
+- *÷	Arithmetics	Adds or subtracts the grey values of two image sequences (Add , Subtract).
٩	Contrast	Enhances the contrast and brightness of an image sequence (Interactive, Automatic, Linearize).
	Smooth	Smoothes an image sequence.
•:•	Morphology	Performs morphological operations on image sequences (Erode , Dilate , Open , Close).
	Segment	Segmentates an image sequence to propose measurement (Interactive , Automatic).
8∕∥	Boolean	Combines two image sequences by Boolean operations (And , Or , Not , Xor , Mask).
× ×	Scrap	Selects or deletes objects of a defined size.
Ŝ	Fill Holes	Fills holes in objects.

LSM 510 LSM 510 META		3D FOR LSM 510 User Interface	Carl Zeiss
View	Menu		
	Set Channel Colour	The colour and the weight of the single channels can be defined	
	Properties	The properties of the image (e.g. scaling, use laser etc.) are displa	ayed.
9	Render	Calculates 3D reconstructions of an image sequence (Surface, Al	pha).
Measu	urement Menu		
A	Automatic Object	Measures geometrical and densitometrical features (General , Ol Features, Volume Features, Condition).	oject
Windo	ows Menu		
	Arrange All	Arranges the windows automatically.	
	Display	The current image is displayed in this window.	
Help I	Menu		
	Content	Opens the help for the software.	
	About 3D for LSM	Displays status and release message of the software.	

Tool Bar

This bar provides buttons with iconized images of nearly all functions. Clicking on one of the buttons will open a dialog window to define the function parameters. Selecting an entry from the menu alternatively can activate the same functions. Placing the cursor on a tool bar button will show a short description, if the window is activated.

8-10

Carl Zeiss

Gallery

The **Gallery** is used as an overview of the images available in memory and their contents. It is located just below the **Tool bar**. Each small image represents a sequence. The middle slice of each image sequence is shown. The status bar of each image shows the name. The name might be a number or a string.

Every image sequence has its own channel colour assignment (see **Display window**). When an image is copied the channel colour assignment is copied too. Drag and drop techniques can be applied to copy images or define the function parameters **Input** and **Output** using the **Gallery** thumbnails.

- Position the cursor on an image in the **Gallery**.
- Press the left mouse button.
- Hold the mouse button down and move the mouse to the destination position.
- At the destination release the left mouse button, the destination image will be overwritten.

To delete an image, drag it, move it to the wastebasket, and drop it.

8.2.3 Display Window

This window is used to display an image sequence, regardless of size or type. To show multiple channel sequences each channel could have its own base colour. The user can set these colours and the

weighting for each channel by pressing the corresponding button \square at the bottom of the window. To display a different image or image sequence, it can be dragged from the **Gallery** and dropped to the **Display window**.

The image can be displayed in full size (one pixel on the screen represents one pixel of the image) or in a zoomed size. To zoom the display view click and hold down the right mouse button on the window border and resize the window. The aspect ratio of the image will not be changed. Clicking on the button

resets the **Display window** to a full size view of the image (see above).

The title bar shows the currently displayed sequence name. The status bar displays the size of the current sequence and the selected slice on the left. On the right the cursor position within the window and the corresponding intensity (grey) value of each channel is shown.

The **Display window** can be closed without any effect to the image processing functions. If no **Display** window is opened select the entry **Display** in the **Window menu**.

The scroll bar at the lower right of the window enables to show the images in a sequence. The range reaches from one to the maximum slice provided by the current sequence.

LSM 510 LSM 510 META

To start the automatic animation of an image sequence start the Player tool by clicking on the button The colour selection for the channels can be activated by clicking on the button . A colour image can be displayed as a grey value image by clicking on the button .

Player

This function plays back the sequential images of an image sequence.

1 , i	Player							×
1	, 10	, 20	30	, 40	, 50	60	7074	32 1 74
-				I		ÞI		
Increment 1 Wait Time 0			1 0	- -			▶ 17 ▶ 01	74 1000

Fig. 8-4

The image sequence is displayed in the **Display window**. The display process is working as a background task; other functions can be executed while the player is running. There are several ways to stop the player:

by closing the player window

by pushing the red Stop button of the player window (the window remains open)

by closing the image window.

The **Increment** parameter specifies whether each sequential image (1) should be displayed or whether some sequential images should be skipped during display. The value 2 skips one image for every sequential image displayed, in other words, it displays only every second image.

The parameter **Wait Time** states the delay in milliseconds between two successive sequential images. The maximum display speed depends mainly on the hardware. The sequential images are always displayed in their entirety, regardless of the set delay.

Control Element of the Player

The three arrow shaped controls on the scale show the start slice and the currently displayed sequential image. The values (positions) can be changed using the mouse. Press and hold the left mouse button and move the pointer to the desired position. The set values are shown in the numerical windows at right.

- Start slice
- Currently displayed sequential image
- 1 End slice

The buttons in the left group start and stop playback of an image sequence.

- Reverse playbackForward playback
- Play forward and then backward again (jojo)
- Stop playback
- Pause playback

The buttons in the middle group control the settings of the current sequential image.



Reset to start slice.

- Single step backward (1 sequential image each regardless of Increment).
- Single step forward (1 sequential image each regardless of Increment).



Set to end slice.

- **Increment** Image increment.
- **Wait Time** Displays delay between two images (in milliseconds).

Set Channel Colour

This function sets the colour and weight for the channels.

💘 Set Channe	el Colour			×
Image	8			
		Weight 0.	200%	Colour
Channel 1	100		• 0 200	
Channel 2	100		• 0 200	
Channel 3	100	•	• 0 200	
Channel 4	100	4	▶ 0 200	
Channel 5	100	4	▶ 0 200	
Channel 6	100	4	► 0 200	
Channel 7	100	4	▶ 0 200	
Channel 8	100	4	▶ 0 200	
				1
		<u>0</u> K	<u>C</u> ancel	Apply



Each image sequence can get its own colour definitions. All functions will inherit the colour definition from the **Input** sequence to the **Output** sequence. By default the colours are set to 100 % weighting and the pure base colours (red, green, blue) are defined.

The weight can be any value between 0 % and 200 %. The colour can be redefined by clicking on the coloured button on the right of the dialog. The standard Windows colour selection dialog is opened. The solution is done by clicking on one of the colours or by entering appropriate numbers in the corresponding edit boxes.

Pressing the **OK** button will close the colour selection dialog and update the **Display window** immediately.

Only those channels, which are available in the image sequence, can be defined.

Parameters:

Image	Image sequence to edit
Weight	Colour weighting for each channel
Colour	Base colour for each channel

3D FOR LSM 510 Functions

B 45-0008 e 10/02

Carl Zeiss

8.3 Functions

8.3.1 Functions in the File Menu

Open Image

This function reads a Zeiss LSM 510 (*.lsm), Zeiss LSM TIFF (*000.tif) or Carl Zeiss Vision (*0.img) image sequence from a disk or network drive.

Den Image Files C:\LSM510)\3DforLsm\Images\Mitosis\AA016.TIF	×
File:	Path: C:\LSM510\3DforLsm\Images\Mitosi	Preview
AA011.TIF AA012.TIF AA013.TIF AA013.TIF AA015.TIF AA015.TIF AA017.TIF AA018.TIF	C:\ LSM510 JDforLsm Images Mitosis	
Files of Type:	Drive:	512*512*15, 3 Ch
All TIF Images (*.tif)	□ c: ▼	4 >
9 New		
	Open Cancel	



The individual files of a Zeiss TIFF image sequence are read and saved as an image sequence in image memory. In addition, the image properties are read out of the TIFF files and allocated to the image sequence **Input**.

The directories of the current drive are listed in the **Directories** list box. Use the **Drives** list box to choose a different drive.

In case of choosing the TIFF-format in the **Files of Type** box, three number characters are always expected before the dot in the filename extension. The first number must be 000 at the end of the filename. From a complete sequence only this file is listed in the dialog, if "LSM TIF Images (*000.tif)" is selected in the **Files of Type** box. To view all TIFF files "All TIF Images (*.tif)" in the **Files of Type** box must be selected. This selection enables to start with a different file than with the very first (named *000.tif) at the end of the filenames three number digits.

Currently the Carl Zeiss Vision file format "KE Images (*0.img)" is supported. Two files per channel are saved.

LSM 510	3D FOR LSM 510	
LSM 510 META	Functions	Carl Zeiss

Carl Zeiss Vision image sequences must have a number digit at the end of the base filename. They are used to indicate the different channels in a multichannel sequence. The numbering starts with zero (0). If a sequence is saved in the Carl Zeiss Vision format the numbers are generated automatically. To load such an image sequence "KE Images (*0.img)" in the **Files of Type** box must be selected.

The window incorporates the usual file selection controls. The bottom half displays a selection of the image properties that are stored in the image sequence.

Parameters:

BaseName	Base name of the TIFF files (image sequence) to be loaded. Only the letters before
	the first number are stated.

Input Name of the resulting image in which the image sequence will be saved.

Save Image As

This function saves an image or image sequence to disk or network drive.

Save Image As	×
File	
C:\LSM5	510\3DforLsm\Images\SensoryCell*.lsm
File:	Path:
*.lsm	C:\LSM510\3DforLsm\Images\Sensc
	C:\ SM510 SJ forLsm Images
Files of Type:	Drive:
LSM5 Images (*.lsm)	C:
_ Input	
4. 5. ▼	
	Save As Close

Fig. 8-7

All the files in the current directory that have the selected image format are listed in the **File Name** list box.

The directories of the current drive are listed in the **Directories** list box. Use the **Drives** list box to choose a different drive.

Use the list box **Files of Type** to select the image format. Currently the LSM 510 image format (*.lsm) and the Carl Zeiss Vision file format "KE Images (*0.img)" is supported.

By choosing the Carl Zeiss Vision file format "KE Images (*0.img)", two files per channel are saved. On one hand the Carl Zeiss Vision type image sequence file, on the other hand the file with the image properties. One pair of files is written per channel. They are numbered automatically, starting with zero. A one number digit is added to the end of the filenames. The two files share the same filename but have different filename extensions (*.img and *.3d).

The content of the **Gallery** is shown in the **Input** section. The selection of the sequence to save is done by highlighting one of the provided names or by drag and drop from the **Gallery**.

Parameters:

Input Name of the image sequence to be saved

Filename Name of the file to be used on disk

Save Display As

This function saves the current **Display window** contents to a disk or network drive.

Save Display	As		? >	×
Save jn:	🕞 Images 💌 主	<u>r</u>	0-0- 0-0- 0-0-	
File <u>n</u> ame:	Display.bmp		<u>S</u> ave	
Save as <u>t</u> ype:	BMP Images (*.bmp)		Cancel	



Before the execution of this function any image or image sequence can be selected to be displayed. From a multichannel sequence any channel status (on or off) combination can be defined. The colours of the shown channels can be set with the function **Set Channel Colour**.

The current zoom factor of the **Display window** is not taken into account, the image is saved without any zoom.

The image is saved as a true colour image with 24-bit resolution. From the **Save as Type** list box one of the provided formats can be selected.

Parameters:

Print

This function prints the current **Display window** contents.

The standard Windows print dialog is opened.

Before the execution of this function any image or image sequence can be selected to be displayed. From a multichannel sequence any channel status (on or off) combination can be defined. The colours of the shown channels can be set with the function **Set Channel Colour**.

Parameters:

None

Exit

This function terminates the application completely.

All images and image sequences shown in the **Gallery** will be deleted from the memory. Save those images which might be used for any further processing.

Parameters:

8.3.2 Functions in the Edit Menu

Сору

This function copies the current **Display window** contents to the clipboard. No dialog is shown.

Before the execution of this function any image or image sequence can be selected to be displayed. From a multichannel sequence any channel status (on or off) combination can be defined. The colours of the shown channels can be set with the function **Set Channel Colour**.

The current zoom factor of the **Display window** is not taken into account; the image is copied without any zoom.

The image is copied as a true colour image with 24-bit resolution. Afterwards the contents can be pasted to any other Windows application.

Parameters:

3D FOR LSM 510 Functions

Carl Zeiss

Edit Channels

This function allows to add or to remove channels to a single or multichannel image.

On the **Add Channel** tab sheet the channels of (different) **Input** sequences can be defined to add (combine) channels to an **Output** sequence.

Edit Channels		li l	×
Add Channel	elete Channel		
Input 1	1	All 1 2 3	
Input 2	3	All 1	
Output	2	New	
Output Size	 Input 1 Input 2 		
		OK Cancel Apply]



This operation is useful to add a segmented channel (or any other result of a function) to the original image sequence. The selected channels of **Input 1** and **Input 2** are copied to **Output**. The maximum number of channels in an image sequence is eight.

If the image sequences do not have the same extents **Output Size** defines which input is taken as a reference. This selection also defines the properties for scaling and units in the output image sequences.

Parameters:

Input 1	First input image sequence
Input 2	Second input image sequence
Output	Output image sequence
Output size	Defines source image sequence for size, scaling, and units

On the **Delete Channel** tab sheet channels of the **Input 1** image sequence can be selected to delete channels.

Edit Channels	×
Add Channel	elete Channel
Input 1	1 2 3
	, p ,
Output	2 New
	OK Cancel <u>Apply</u>



This operation might save time and memory for further processing if not all channels are needed. Only the selected channels of **Input 1** are copied to **Output**.

Parameters:

Input 1	Input image sequence
Output	Output image sequence

Delete All Images

This function deletes all images and image sequences from the memory (Gallery).

The function is used whenever a completely new image sequence should be processed. In order to drop the images item by item to the wastebasket all of them can be deleted by a single function.

If any image or image sequence is needed for further use save them first.

Parameters:

8.3.3 Functions in the Process Menu

Arithmetics - Add

This function adds two image sequences.

Arithmetics			X
Add Subtract			
Input 1	4	All 1	
Input 2	4	All 1	
Output	5	New	
Mode	 Wrap Clip Normalize 		
		OK Cancel A	.pply



The **Add** tab sheet of the **Arithmetics** dialog window must be selected.

If one or both input sequences are multichannel sequence, any number or combination can be selected. The number of selected channels for **Input 1** and **Input 2** must be the same. They will be combined from left to right.

LSM 510	3D FOR LSM 510	
LSM 510 META	Functions	Carl Zeiss

This function adds the two image sequences **Input 1** and **Input 2** voxel by voxel and generates the image sequence **Output**. Note that a resulting grey value may be greater than 255 (4095). The parameter **Mode** determines how a range overflow is handled:

- 1 Wrap No normalization the grey values are displayed modulo 256 (4096). If the result is greater than 255 (4095), the value 256 (4096) is subtracted from it.
- 2 Clip Grey values which exceed 255 (4095) are replaced with 255 (4095).
- 3 Normalize The resulting grey value range is scaled to the range 0...255 (0...4095).

Parameters:

Input 1	First input image sequence	
Input 2	Second input image sequence	
Output	Output image sequence	
Mode	1 - Wrap 2 - Clip 3 - Normalize	

Arithmetics - Subtract

This function subtracts two image sequences.

Arithmetics		×
Add Subtract		
Input 1	3	All 1 2 3
Input 2	4	All 1 2 3
Output	5	New
Mode	 Wrap Clip Normalize Shift/Clip 	
		OK Cancel Apply



The **Subtract** tab sheet of the **Arithmetics** dialog window must be selected.

If one or both input sequences are multichannel sequence, any number or combination can be selected. The number of selected channels for **Input 1** and **Input 2** must be the same. They will be combined from left to right.

This function subtracts the two image sequences **Input 1** and **Input 2** voxel by voxel and generates the image sequence **Output**. Note that a resulting grey value may be less than 0. The parameter **Mode** determines how a range overflow (negative values) is handled.

LSM 510 LSM 510 META	3D FOR LSM 510 Functions	Carl Zeiss
1 - Wrap	No normalization - the grey values are displayed modulo 2	

	is less than 0, the value 256 (4096) is added to it.
2 - Clip	Negative values are set to 0.
3 - Normalize	The resulting grey value range is scaled to the range 0255 (04095).
4 - Shift/Clip	128 (2048) is added to the difference, then negative values are set to 0. Values greater than 255 (4095) are set to 255 (4095).

Parameters:

Input 1	First input image sequence		
Input 2	Second input image sequence		
Output	Output image sequence		
Mode	1 - Wrap 2 - Clip		

- 3 Normalize 4 Shift/Clip

Contrast - Interactive

This function allows interactive changes of the contrast of an image sequence.

🗲 Contrast	×
Interactive Autom	natic Linearize
Input	1 All 1
Output	2 New 8 12
Channel	All 1
Clip Grey Values	
Input Histogram	L 36 C 145 H 255 O 255
Output Histogram	
	OK Cancel Apply



The Interactive tab sheet of the Contrast dialog window must be selected.

LSM 510	3D FOR LSM 510	
LSM 510 META	Functions	Carl Zeiss

A grey value range of the **Input** image sequence is scaled to another range in the **Output** image sequence. Both ranges can be edited interactively. This function is used to achieve a better view of an image sequence, or to scale a range of grey values to single value for a special coding in an image sequence. The function does not improve the result of the linear segmentation function **Segment**.

Input indicates the sequence to enhance. If it is a multichannel sequence, a single channel, all channels, or any number can be selected. The **Input** histogram shows the grey value distribution of the selected channels of the **Input** image sequence.

Output defines the name of the result sequence. It will get only those channels which are chosen by the **Input** parameter. The buttons labeled with 8 and 12 define the grey value (intensity) resolution in bit. Normally the result will get the same resolution as the **Input** sequence. A change will be needed if image sequences with different resolutions should be combined. Rising the grey value range to 12 bit will not enhance the display quality or measurement accuracy. The smooth and morphology functions will produce results with finer gradations.

If **Clip Grey Values** is selected, the output grey values are clipped to the **Low (L)** and **High (H)** values. If **Clip Grey Values** is not selected, output grey values beyond the **Low** and **High** value range are possible.

The **Output** histogram shows the resulting histogram. The horizontal axis represents the grey values from 0 to the maximum, which is either 255 or 4095, depending whether the input is 8 bit or 12 bit. The vertical axis represents the pixel count. The selected range is marked by the borderlines in the histogram. The blue line or **L** indicates the lower boundary, the red line or **H** the upper one, **C** indicates the center of the range.

There are three ways to change the range: clicking and dragging the borderlines with the mouse.

Entering a new value in the appropriate text boxes, clicking on the buttons or using the arrow keys from the keyboard. To alter the values within the histogram move the mouse pointer over one of the three coloured lines until the shape changes. Press and hold the left mouse button to move the line to a new position. To change the values with the arrow keys click once into the histogram. Using the left or right arrow key by its own will move the whole range. Pressing the **Shift** key additionally moves the lower boundary, the **Control** key the upper boundary.

The vertical scale of the histogram is set using the scroll bar. The units are percents of the maximum grey value distribution. This setting has no influence on the function.

Parameters:

Input	Input image sequence
Output	Output image sequence
Channel	Selection of the channel numbers for the Output image after contrast enhancement
Clip Grey Values	Clipping of grey values to the Low (L) and High (H) output grey values boundaries
Input L	Lower boundary of grey value range Input
Input C	Center of grey value range Input
Input H	Upper boundary of grey value range Input
Output L	Lower boundary of grey value range Output
Output C	Center of grey value range Output
Output H	Upper boundary of grey value range Output

Contrast - Automatic

This function scales the grey values of an image sequence to the maximum possible range.

Contrast Interactive Autom	natic Linearize
Input	1
Output	2 New 8 12
Channel	AII 1
Threshold	0 • 0 1000
Input Histogram	L 0 C 127 H 255 O 255
Output Histogram	L 0 127 H 255 0 255
	OK Cancel Apply



The Automatic tab sheet of the Contrast dialog window must be selected.

This function enhances the contrast of an image sequence by spreading the grey value distribution over the maximum possible range. This function is used to achieve a better view of an image.

The light and dark grey value ranges with a low share of pixels are excluded from the operation by the parameter **Threshold**. The **Threshold** units are in thousandths of the total number of voxels. Using a value of 10 means that the scale interval is set so that 5/1000 of the total number of voxels on the light side, and 5/1000 of the total number of voxels on the dark side of the grey value distribution are excluded.

Input indicates the sequence to enhance. If it is a multichannel sequence, a single channel, all channels, or any number can be selected. The **Input** histogram shows the grey value distribution of the selected channels of the **Input** image sequence.

Output defines the name of the result sequence. It will get only those channels which are chosen by the **Input** parameter. The buttons labeled with 8 and 12 define the grey value (intensity) resolution in bit.

Normally the result will get the same resolution as the **Input** sequence. A change will be needed if image sequences with different resolutions should be combined. Rising the grey value range to 12 bit will not enhance the display quality or measurement accuracy. The smooth and morphology functions will produce results with finer gradations.

The **Output** histogram shows the resulting histogram. They are not editable. The horizontal axis represents the grey values from 0 to the maximum, which is either 255 or 4095, depending whether the input is 8 bit or 12 bit. The vertical axis represents the pixel count. The vertical scale of the histogram is set using the scroll bar. The units are percentages of the grey value distribution maximum. This setting has no influence on the function.

Parameters:

Carl 7eiss

Input	Input image sequence
Output	Output image sequence
Threshold	Exclusion value - 01000
Input L	Lower boundary of grey value range Input
Input C	Center of grey value range Input
Input H	Upper boundary of grey value range Input
Output L	Lower boundary of grey value range Output
Output C	Center of grey value range Output
Output H	Upper boundary of grey value range Output

3D FOR LSM 510

Contrast – Linearize

This function scales a range of grey values of an image sequence to equal area fractions in the histogram.

Input	
Output	2 New
Channel	All 1
Skip Black	
Input Histogram	L O C 127 H 255 O 255
Output Histogram	L 0 C 127 H 255 O 255



The **Linearize** tab sheet of the **Contrast** dialog window must be selected.

This function enhances the contrast by linearizing the histogram of the image sequence to equal area fractions in the histogram. The areas (voxel count multiplied by grey value range) of all grey values in the **Output** histogram are the same. This function is used to achieve a better view of an image sequence. When **Skip Black** is checked the grey value 0 will not be taken into account for linearization.

Input indicates the sequence to enhance. If it is a multichannel sequence, a single channel, all channels, or any number can be selected. The **Input** histogram shows the grey value distribution of the selected channels of the **Input** image sequence.

Output defines the range of the result sequence. It will get only these channels which are chosen by the **Input** parameter. The grey value (intensity) resolution will be the same as the one from **Input**.

3D FOR LSM 510 Functions

The **Output** histogram shows the resulting histogram. The horizontal axis represents the grey values from 0 to 255. The vertical axis represents the pixel count. The vertical scale of the histogram is set using the scroll bar. The units are percentages of the grey value distribution maximum. This setting has no influence to the function.

Parameters:

Image	Input image sequence	
Output	Output image sequence	
SkipBlack	0 - Grey value black is ignored 1 - Grey value black is taken into account	
Input L	Lower boundary of grey value range Input	
Input C	Center of grey value range Input	
Input H	Upper boundary of grey value range Input	
Output L	Lower boundary of grey value range Output	
Output C	Center of grey value range Output	
Output H	Upper boundary of grey value range Output	

Smooth (Gauss)

This function performs a Gauss filter.

📥 Gauss	X
Input	3 All 1 2 3
Output	4 New
Size	3 4 3 31
	OK Cancel <u>Apply</u>

Fig. 8-16

The noise in the image sequence is reduced, the edge shape is nearly unchanged, local maxima are leveled, the dynamic range is reduced.

Image sequences should be smoothed before they are reconstructed or segmented. For most sequences a **Size** value of 3 is sufficient enough. If **Input** is a multichannel sequence, any number and combination of channels can be selected. **Output** will only get the selected channels as results.

The grey value of every pixel is substituted by a weighted average of its surrounding neighbors. The neighbors are defined by a cube. The affected pixel is the central pixel of the filter cube. The weighted filter cube is approximated by a binomial distribution. The size of the filter cube is set using the **Size** scroll bar. Even numbers are set to the next odd value. The **Size** defines the strength of the smoothing.

Parameters:

Input	Input image sequence	
Output	Output image sequence	
Size	Filter size (331, only odd numbers)	

Morphology

The following four functions perform basic operations of mathematical morphology on image sequences.

Horphology		×
Erode Dilate	Open Close	
Input	1	AT 1
Output	2	New
Shape	•	
Count	1	111
Grey Morph	ology	OK Cancel <u>Apply</u>

Fig. 8-17

As generalization of the morphology of two-dimensional images to three dimensions the structural elements are small volumina.

Literature

Bomans, M.; Höhne, K.-H.; Tiede, U.; Riemer, M.: 3D-Segmentation of MR Images of the Head for 3-D Display IEEE Transactions on Medical Imaging 9, 1990, 177-183 Schiemann, T.; Bomans, M.; Tiede, U.; Höhne, K.-H.: Interactive 3D-Segmentation of Tomographic Image Volumes 14. DAGM-Symposium Mustererkennung, Springer-Verlag 1992, 73-80

LSM 510	3D FOR LSM 510	
LSM 510 META	Functions Car	

The input image sequence is analyzed voxel by voxel with a selected shape (Shape). The voxel to be analyzed is always the central voxel of the shape. The shape type determines which neighboring voxels are used to compute the resulting voxel.

The following structural elements are available for all morphological operations. They represent approximated spheres with an increasing radius.

Sequential image:

Volume view:

Cross shape



Sequential image:



Volume view:

Cross shape



3D FOR LSM 510 Functions

LSM 510 LSM 510 META

Cube cross shape: created through application of "cube" and "cross" one after the other.

Sequential image:

Carl 7eiss



Volume view:

For regions (voxels) that are at the edge of the image sequence, it assumed for erosion that there are white voxels with a grey value of 255 (4095) outside the edge. For dilation, it is assumed that there are black voxels with the grey value 0 outside the image sequence.

If the **Grey Morphology** tickbox is activated, erosion sets the grey value of the central voxel to the minimum of all neighboring voxels affected by the structural element; dilation sets the grey value of the central voxel to the maximum.

If the **Grey Morphology** tickbox is not activated, the neighboring voxels are only distinguished by grey value 0 and non-0. For erosion the central voxel is set to 0 if any of the neighbors is 0. It is set to 255 (4095) if any neighbor is not 0. For dilation the central voxel is set to 255 (4095) if any of the neighbors is not 0. It is set to 0 if all neighbors are 0.

Erosion reduces the size of bright regions, separates thin connections between them, and makes small regions disappear. Dilation, on the other hand, makes bright regions of the image grow in size, fills gaps, and smoothes small contour details.
B 45-0008 e	10/02	

LSM	510	
LSM	510	META

The result of erosion and dilation is called opening. On the one hand, this maintains to some extent the original size of the regions while not losing the smoothing effect of erosion on the image. This name stands for the operation of reducing convex bulges in the contour of the region. Thin connections between regions are eliminated, broken borders between regions are connected, and small regions disappear.

The opposite operation (first dilation, then erosion) is called closing. Concave bulges in the contours of regions are filled in; connections are formed between adjacent regions.

The following example illustrates the operations "Open" and "Close" in two dimensions:

Open = Erosion + Dilation



Close = Dilation + Erosion



Fig. 8-19

The "cube cross" shape was used for the operations shown.



Morphology - Erode

This function erodes structures in an image sequence.

Horphology 🖽		×
Erode Dilate	Open Close	
Input	1	All 1
Output	2	New
Shape	•	
Count	1	▶ 1 11
Grey Morpho	blogy	OK Cancel Apply

Fig. 8-20

In the **Morphology** dialog window, the tab sheet **Erode** must be selected.

Erosion makes bright regions smaller on a dark background. It also results in separation of thin connections between regions. Small regions disappear entirely.

If **Input** is a multichannel sequence any number and combination of channels can be selected. **Output** will only get the selected channels as results. The Input image sequence is eroded **Count** times with the shape **Shape**. The **Count** scroll bar determines the number of recursive operations.

The following shapes (numbered 1 to 3 from left to right) are available:



If **Grey Morphology** is selected the function will respect all grey value shades of the sequence **Input**. If **Grey Morphology** is not selected the function will distinguish between 0 and non-0 only. The result **Output** will be a binary sequence.

Parameters:

Input	Input image sequence
Output	Resulting image sequence
Shape	Shape used 1 - cross 2 - cube 3 - cube cross
Count	Number of recursive operations
Grey Morphology	0 - Distinguish between 0 and non 0 only 1 - All grey value shades are taken into account

Morphology - Dilate

This function dilates structures in an image sequence.

Horphology		X
Erode Dilate	Open Close	
Input	1 AT 1	
Output	2 New	
Shape	⊕ ■ ■	
Count	1 1 11	
Grey Morph	nology OK Cancel	Apply



In the **Morphology** dialog window, the tab sheet **Dilate** must be selected.

Dilation makes bright regions larger on a dark background. It also results in the filling of gaps and smoothing of small contour details.

If **Input** is a multichannel sequence any number and combination of channels can be selected. **Output** will only get the selected channels as results.

The **Input** sequential image is dilated **Count** times with the shape **Shape**. The **Count** scroll bar determines the number of recursive operations.

The following shapes (numbered 1 to 3 from left to right) are available:



If **Grey Morphology** is selected the function will respect all grey value shades of the sequence **Input**. If **Grey Morphology** is not selected the function will distinguish between 0 and non-0 only. The result **Output** will be a binary sequence.

Input image sequence
Resulting image sequence
Shape used 1 - cross 2 - cube 3 - cube cross
Number of recursive operations
0 - Distinguish between 0 and non 0 only 1 - All grey value shades are taken into account

Morphology - Open

This function carries out an opening.

🖽 Morphology		×
Erode Dilate	Open Close	
Input	1	All 1
Output	2	New
Shape	+ =	
Count	1	111
Grey Morp	hology	OK Cancel Apply

Fig. 8-22

In the **Morphology** dialog window, the tab sheet **Open** must be selected.

This function carries out an erosion followed by a dilation. For the most part, the opening maintains the original size of the regions. Thin connections between regions and small regions themselves disappear. Convex bulges in the contours of the regions are reduced. The opening is applied to the grey value image sequence **Input Count** times with the shape **Shape**. If **Input** is a multichannel sequence any number and combination of channels can be selected. **Output** will only get the selected channels as results.

The **Count** scroll bar determines the number of recursive operations.

The following shapes (numbered 1 to 3 from left to right) are available:



If **Grey Morphology** is selected the function will respect all grey value shades of the sequence **Input**. If **Grey Morphology** is not selected the function will distinguish between 0 and non-0 only. The result **Output** will be a binary sequence.

Input	Input image sequence
Output	Resulting image sequence
Shape	Shape used 1 - cross 2 - cube 3 - cube cross
Count	Number of recursive operations
Grey Morphology	0 - Distinguish between 0 and non 0 only1 - All grey value shades are taken into account

Morphology - Close

This function carries out a closing.

🗰 Morphology		×
Erode Dilate	Open Close	
Input	1	AT 1
Output	2	New
Shape	+	
Count	1	▶ 1 11
Grey Morp	hology	OK Cancel Apply

Fig. 8-23

In the **Morphology** dialog window, the tab sheet **Close** must be selected.

This function carries out a dilation followed by an erosion. For the most part, the closing maintains the original size of the regions. Connections are formed between adjacent regions; gaps and bright concave bulges in the contours of regions are filled in. The closing is applied **Count** times to the grey value image sequence Input with the shape **Shape**. If **Input** is a multichannel sequence any number and combination of channels can be selected. **Output** will only get the selected channels as results.

The **Count** scroll bar determines the number of recursive operations.

The following shapes (numbered 1 to 3 from left to right) are available:



If **Grey Morphology** is selected the function will respect all grey value shades of the sequence **Input**. If **Grey Morphology** is not selected the function will distinguish between 0 and non-0 only. The result **Output** will be a binary sequence.

Input	Input image sequence
Output	Resulting image sequence
Shape	Shape used 1 - cross 2 - cube 3 - cube cross
Count	Number of recursive operations
Grey Morphology	0 - Distinguish between 0 and non 0 only1 - All grey value shades are taken into account

Segment - Interactive

This function carries out a grey value segmentation by means of thresholding.

Segment	2	۲
Interactive Autom	natic	
Input	1 1	l
Output	2 New	
Colour Binary	 Green O Blue / Red 	
Invert		I
Input Histogram		
	OK Cancel Apply	ļ

Fig. 8-24

The Interactive tab sheet of Segment dialog window must be selected.

Segmentation is especially used to generate binary regions. These are required for the measurement.

Two threshold values determine which grey value range of the **Input** image sequence is preserved and/or deleted in the **Output** image sequence. Only one channel of a multichannel sequence can be selected as **Input**. **Output** will always be a single channel sequence.

The vertical scaling of the histogram can be adjusted with the scroll bar at the right edge of the histogram. This setting has no influence on the function.

The thresholds **L**ow and **H**igh are determined either by moving the borderlines in the grey value histogram or by the scroll bars underneath. Furthermore, the values for **L**ow, **C**enter and **H**igh can be set through entry in the corresponding fields.

To move the lower (L) and upper (H) thresholds at the same time, move the vertical line in the grey value histogram or set the scroll bar (C).

The **Green** and **Blue/Red** option buttons of the parameter **Colour** determine whether the voxels within (**Green**) or outside (**Blue/Red**) of the grey value interval [**L**, **H**] are displayed with the corresponding colour.

3D FOR LSM 510 Functions

If **Green** is selected, the voxels within the selected interval are highlighted in green. The rest of the image retains its original grey values. The voxels with the grey values **L**ow and **L**ow+1 are displayed in blue. The voxels with the grey values **H**igh and **H**igh-1 are displayed in red.

If **Blue/Red** is selected, the voxels with grey values within the interval Low, **H**igh remain unchanged. Voxels with grey values less than Low are highlighted in blue; those with grey values higher than **H**igh are highlighted in red.

If the **Invert** option is selected, the grey values outside the defined interval will be segmented.

If the option **Binary** is selected, then all grey values in the range from **L**ow to **H**igh will be set to white (grey value 255) in the **Output** image sequence, while all others will be set to black (grey value 0). If the option is not selected, the grey values within the selected interval remain unchanged, while those outside the range will be set to black. The measurement function accepts both results without any difference in the results.

Parameters:

Carl 7eiss

Input	Input image sequence
Output	Resulting image sequence
Colour	Green - Selected interval is displayed in green
Blue/Red	Grey values below the selected interval are displayed in blue, grey values above in red
Binary	0 - Selected voxels retain the original grey value 1 - Selected voxels are set to grey value 255, the rest to grey value 0
Invert	0 - Grey values inside the selected interval are segmented 1 - Grey values outside the selected interval are segmented
L	Low grey value threshold
С	Center of threshold interval
н	High grey value threshold

Segment - Automatic

The function carries out an automatic grey value segmentation by means of thresholding.

Segment	×
Interactive Auto	matic
Input	1 1
Output	2 New
Colour	 ⊙ Green ⊙ Blue / Red
Invert	
Input Histogram	L 0 C 127 H 255 0 255
	OK Cancel Apply



The **Automatic** tab sheet of the **Segment** dialog window must be selected. Segmentation is especially used to generate binary regions. These are required for the measurement.

The function calculates the two strongest local minimums in the histogram of the **Input** image sequence. These values are used for the discrimination. Only one channel of a multichannel sequence can be selected as **Input**. **Output** will always be a single channel sequence. The vertical scaling of the histogram can be adjusted with the scroll bar at the right edge of the histogram. This setting has no influence on the function.

The **Green** and **Blue/Red** option buttons of the parameter **Colour** determine whether the voxels within (**Green**) or outside (**Blue/Red**) of the grey value interval [**L**, **H**] are displayed with the corresponding colour.

If **Green** is selected, the voxels within the selected interval are highlighted in green. The rest of the image retains its original grey values. The voxels with the grey values **L**ow and **L**ow+1 are displayed in blue. The voxels with the grey values **H**igh and **H**igh-1 are displayed in red.

If **Blue/Red** is selected, the voxels with grey values within the interval Low, **H**igh remain unchanged. Voxels with grey values less than Low are highlighted in blue; those with grey values higher than **H**igh are highlighted in red.

If the **Invert** option is selected, the grey values outside the defined interval will be segmented.

If the option **Binary** is selected, then all grey values in the range from **L**ow to **H**igh will be set to white (grey value 255 (4095)) in the **Output** image sequence, while all others will be set to black (grey value 0). If the option is not selected, the grey values within the selected interval remain unchanged, while those outside the range will be set to black.

Parameters:

Input	Input image sequence
Output	Resulting image sequence
Colour	Green - Selected interval is displayed in green Blue/Red - Grey values below the selected interval are displayed in blue, grey values above in red
Binary	0 - Selected voxels retain the original grey value 1 - Selected voxels are set to grey value 255 (4095), the rest to grey value 0
Invert	0 - Grey values inside the selected interval are segmented 1 - Grey values outside the selected interval are segmented
L	Low grey value threshold
С	Center of threshold interval
н	High grey value threshold

3D FOR LSM 510

Carl Zeiss

from left to right.

Input 1	First input image sequence
Input 2	Second input image sequence
Output	Resulting image sequence

B 45-0008 e 10/02

This function carries out a bit-by-bit **And** calculation for the image sequences **Input 1** and **Input 2**.

All 1

All 1

ΟK

If one or both input sequences are multichannel sequences, any number or combination can be selected. The number of selected channels for **Input 1** and **Input 2** must be the same. They will be combined

New

Cancel

Boolean - And

Boolean

Input 1

Input 2

Output

Fig. 8-26

And Or Xor Not Mask

1

2

3

The And tab sheet of the Boolean dialog window must be selected.

This function is especially well suited for masking images.

LSM 510

×

Apply

8-49

B 45-0008 e 10/02

Carl Zeiss

Boolean - Or

This function carries out a bit-by-bit **Or** calculation for the images **Input 1** and **Input 2**.

Boolean			х
And Or Xor	Not Mask		_
Input 1	1	All 1	
Input 2	2	All 1	
Output	3	New	
		OK Cancel Apply	.)

Fig. 8-27

The **Or** tab sheet of the **Boolean** dialog window must be selected.

This function can be used to combine binary masks or regions.

If one or both input sequences are multichannel sequences, any number or combination can be selected. The number of selected channels for **Input 1** and **Input 2** must be the same. They will be combined from left to right.

Parameters:

Input 1First input image sequenceInput 2Second input image sequenceOutputResulting image sequence

Boolean - Xor

This function carries out a bit-by-bit **Xor** calculation for the images **Input 1** and **Input 2**.

≫ Boolean		×	<
And Or Xo	r Not Mask		1
Input 1	1	All 1	
Input 2	2	All 1	
Output	3	New	
		OK Cancel Apply]
			1



The Xor option button of the Function option group in the Boolean dialog window must be selected.

This function can be used to combine binary masks or regions.

If one or both input sequences are multichannel sequences, any number or combination can be selected. The number of selected channels for **Input 1** and **Input 2** must be the same. They will be combined from left to right.

Input 1	First input image sequence
Input 2	Second input image sequence
Output	Resulting image sequence

Boolean - Not

This function carries out a bit-by-bit negation of an image.

🏷 Boolean		2	<
And Or Xor	Not Mask		_
Input 1	1	All 1	
Output	2	New	
		OK Cancel Apply	

Fig. 8-29

The Not tab sheet of the Boolean dialog window must be selected.

If **Input** is a multichannel sequence any number or combination can be selected.

Parameters:

Input Input image sequence

Output Resulting image sequence

Boolean - Mask

This function masks a grey value image sequence.

🏹 Boolean		×
And Or Xo	Not Mask	
Input 1	1	All 1
Input 2	2	All 1
Output	3	New
		OK Cancel Apply

Fig. 8-30

The Mask tab sheet of the Boolean dialog window must be selected.

This function modifies the **Output** image sequence depending on the mask image sequence used.

If the grey value in **Input 2** is higher than 0, then the voxel values are copied from **Input 1** to the image sequence **Output**. If the grey value of the voxel is 0, then the voxel value of the **Output** image sequence is taken over.

If one or both input sequences are multichannel sequences, any number or combination can be selected. The number of selected channels for **Input 2** must be 1 or the same as for **Input 2**. They will be combined from left to right.

Input 1	First input image sequence
Input 2	Second input image sequence
Output	Resulting image sequence

Scrap

This function deletes or selects objects in a specified size range.

§⊈ Scrap	X
Input	1 All 1 2 3
Output	2 New
Minimum Volume	0.0 • 0 15362.0 micrometer ³
Maximum Volume	100.0 • 0 15362.0 micrometer ³
Select	
	OK Cancel <u>Apply</u>

Fig. 8-31

The operation deletes or selects objects on the basis of their total volume in voxels. Objects with a volume within the range **MinVolume** to **MaxVolume** are effected.

To delete objects outside the range, the parameter **Select** must be active. If the parameter is not activated objects outside the defined volume range are deleted.

Input	Input image sequence	
Output	Output image sequence	
MinVolume	Minimum object size	
MaxVolume	Maximum object size	
Select	0 - Select the objects outside the size range1 - Select the regions within the size range	

Fill Holes

This function fills holes in all objects.

🖏 Fill Holes	×
Input	1 All 1 2 3
Output	2 New
	OK Cancel <u>Apply</u>

Fig. 8-32

All holes in objects are filled by this operation. Holes are structures, which have a grey value of 0 and are surrounded completely by voxels with a grey value not equal to 0. It is assumed that regions outside the image are black. Holes, which touch the image border, are retained.

Input	Input image sequence
Output	Output image sequence

8.3.4 Functions in the View Menu

Render - Surface

This function displays an image sequence according to the **gradient shading method**.

🔮 Render	X
Surface Alpha	
Input	1 All 1 2 3
Output	2 New
Number of Views	1 1
Angle X	0 • -180 180 • Start
Angle Y	0 • -180 180 • End
Angle Z	0 • 180 180
Channel	All 1 2 3
Grey Low	55 • 0 255
Grey High	255 • 0 255
Aperture	3 • • 1 10
Reflection(%)	64 • 1 100
Auto Update	OK Cancel Apply

Fig. 8-33

The **Surface** tab sheet of the **Render** dialog window must be selected.

<u>Method</u>

The **Input** sequence defines the data to be reconstructed. If it is a multichannel sequence one or all channels can be selected for the reconstruction.

Output sets the name of the result image (sequence). If the sequence exists it is overwritten. Pressing the button **New** will generate a new name (number). The size of the sequential images in **Output** is determined by the size of the sequential images in **Input**.

Number of Views determines the number of reconstructions which should be computed. The radio buttons **Start** and **End** define which angle settings are currently shown. A definition for the angle **End** is only necessary if **Number of Views** is higher than 1. If this is true the result sequence will get views from the **Start** to the **End** angle definition. The other reconstructions are determined through the linearly interpolated intermediate angles. The direction of view is determined from the angles as follows:

The angle **Angle Z** determines the rotation of the direction of view on the Z-axis. The angle **Angle Y** determines the rotation of the direction of view on the Y-axis that has been rotated by the angle **Angle Z**. The angle **Angle X** determines the rotation of the direction of view on an X-axis that is rotated by **Angle Z** and **Angle Y**.

Channel defines if the following parameters are valid for **All** or just for one. Defining the thresholds for the channels independently is useful if the grey value boundaries of the objects differ too much in the different channels. The thresholds **Grey Low** and **Grey High** define the grey value range of the objects.

The parameter **Aperture** is a measure of the size of the highlights. Small values generate large highlights. Large values generate small highlights (similar to a spot).

3D FOR LSM 510 Functions

Use the parameter **Reflection** to control the ratio of diffuse and reflective brightness components, i.e., the overall basic brightness compared with the highlights. When the value of **Reflection** is low, the highlights predominate; when the values are high, the region appears to be uniformly illuminated and the highlights are not so pronounced. When **Auto Update** is selected, the reconstruction is updated automatically whenever a parameter is modified (except **Input, Output**, or **Number of Views**). **Show Cube** defines whether a wire frame cube is shown in the **Display window** or not.

<u>Application</u>

Carl 7eiss

This method can be applied, if the structures in the **Input** sequence can be segmented by grey value thresholding. Because the gradient is calculated for every pixel, the **Output** appears in very fine detail.

Noisy **Input** sequences must be smoothed (function **Smooth**) before rendering, otherwise the surface appears rough.

Input	Input image sequence		
Output	Resulting image sequence		
Number of Views	Number of reconstructions to be calculated		
Angle X	Angle of rotation on the X-axis, start position		
Angle Y	Angle of rotation on the Y-axis, start position		
Angle Z	Angle of rotation on the Z-axis, start position		
Channel	All - The following parameters are valid for all channels X - The following parameters are valid for the selected channel only		
Grey Low	Low grey value threshold of the region to be displayed		
Grey High	High grey value threshold of the region to be displayed		
Aperture	Measure of the extent of the highlights		
Reflection	Weight of the defuse brightness components in comparison to the highlights		
Auto Update	 0 - Function execution is performed on OK or Apply 1 - Function execution for the current angle is performed on any parameter change 		
Show Cube	0 - The wire frame cube is not shown 1 - The wire frame cube is shown in the Display window		

Render - Surface: Method Description

This method displays the surface of structures in the **Input** sequence shaded as if a light illuminated it. The position of the light is behind the view point with parallel rays in the direction of the sequence.

The input sequence is segmented into object and background by grey value thresholding: object voxels are within the grey value range **Grey Low** to **Grey High**.

Each **Output** pixel corresponds to a point at the surface at which the ray in view direction through the **Output** pixels hits the surface. All rays are parallel.

The surface normal required for shading in this gradient renderer is the grey value gradient in the **Input** volume at the surface voxel position. It is not the geometric surface normal. The grey value gradient is determined from the grey values in a 3x3x3 cube around the surface voxel by averaging e.g. the x-gradient in y- and z-direction [4].

There is no depth cueing (far objects would appear darker).

The illumination model is a Phong model [1] (surface normal is determined for each **Output** pixel) with diffuse reflection and specular reflection. Diffuse reflection means that the surface reflects light with equal intensity in all directions. The brightness of a given surface patch depends not on the view-direction, but only on the angle between light and surface normal. Specular reflection is observed on shiny surfaces as a highlight. The light is reflected as from a mirror. The maximum intensity is observed when the view direction is the one of the mirrored light direction.



Render - Alpha

This function displays an image sequence according to the **alpha rendering method**.

🐑 Render	6	<
Surface Alpha		
Input	1 All 1 2 3	
Output	2 New	l
Number of Views	1 1	
Angle X	0 • 180 180 • Start	
Angle Y	0 • 180 180 • End	
Angle Z	0 • -180 180	
Channel	All 1 2 3	
Threshold	50 • 0 255	
Ramp	130 • 0 255	
Max. Opacity	150 • 0 255	
🗖 Auto Update	OK Cancel Apply	
🔽 Show Cube		



The Alpha tab sheet of the Render dialog window must be selected.

One or more reconstructions of the input image sequence are computed according to the alpha rendering method. This type of reconstruction should be used if there is no possibility to segment the structures in the image sequence and also if the objective is to make deeply layered structures visible.

<u>Method</u>

The **Input** sequence defines the data to be reconstructed. If it is a multichannel sequence one or all channels can be selected for the reconstruction.

Output sets the name of the result image (sequence). If the sequence exists it is overwritten. Pressing the button **New** will generate a new name (number). The size of the sequential images in **Output** is determined by the size of the sequential images in **Input**.

Number of Views determines the number of reconstructions which should be computed. The radio buttons **Start** and **End** define which angle settings are currently shown. A definition for the angle **End** is only necessary if **Number of Views** is higher than 1. If this is true the result sequence will get views from the **Start** to the **End** angle definition. The other reconstructions are determined through the linearly interpolated intermediate angles.

The direction of view is determined from the angles as follows:

The angle **Angle Z** determines the rotation of the direction of view on the Z-axis. The angle **Angle Y** determines the rotation of the direction of view on the Y-axis that has been rotated by the angle **Angle Z**. The angle **Angle X** determines the rotation of the direction of view on an X-axis that is rotated by **Angle Z** and **Angle Y**.

Channel defines if the following parameters are valid for **All** or just for one. Defining the opacity for the channels independently is useful when the brightness and contrast of the channels differ too much. **Threshold** defines the range with no opacity. It is completely transparent. The range starts at grey value 0.

The length of slope is defined by **Ramp**. The maximum opacity value is set with the parameter **Max**. **Opacity**. This range ends at the maximum grey value. The **Opacity Table** shows the grey value histogram of Input with the opacity definition as a red line.

When **Auto Update** is selected, the reconstruction is updated automatically whenever a parameter is modified (except **Input, Output**, or **Number of Views**). **Show Cube** defines whether a wire frame cube is shown in the **Display window** or not.

Application

- 1. This method can be applied, if the structures in the Input sequence are unsharp so that objects are poorly defined by their grey value.
- 2. In this case, the Opacity Table is defined as a ramp. Low grey values have weight 0 to suppress the background voxels. The opacity rises with increasing grey values, depending on the parameter Ramp. The value of Max. Opacity defines the weight of the high grey values. High grey values above a threshold have weight 255 to show the "object" voxels unsuppressed. Of course a smooth step can be used.
- 3. The result is a display with inside structures shining through. A 3D impression can be obtained by rendering with several view directions.
- 4. In contrast to this, a voxel renderer like the gradient renderer would display only the surface of objects that are defined by grey value-thresholds. This surface would appear shaded as if illuminated by a light.
- 5. The method can also be applied to visualize pronounced structures within other enclosing structures, if the structures have different grey value ranges.
- 6. In this case, the Opacity Table is defined as a step. Low grey values (background) have weight 0. High grey values (inside structures) have maximum weight.

Input	Input image sequence		
Output	Resulting image sequence		
Number of Views	Number of reconstructions to be calculated		
Angle X	Angle of rotation on the X-axis, start position		
Angle Y	Angle of rotation on the Y-axis, start position		
Angle Z	Angle of rotation on the Z-axis, start position		
Channel	All - The following parameters are valid for all channels X - The following parameters are valid for the selected channel only		
Threshold	Grey value where the opacity starts rising		
Ramp	Length of the opacity slope		
Max. Opacity	Maximum opacity value		
Opacity Table	Maximum opacity value		
Auto Update	0 - Function execution is performed on OK or Apply 1 - Function execution is performed on any parameter change		
Show Cube	0 - The wire frame cube is not shown 1 - The wire frame cube is shown in the Display window		

Render - Alpha: Method Description

Each **Output** pixel is a weighted sum of the **Input** voxels along a ray in view direction through the **Input** sequence. Each **Input** voxel has an opacity value, dependent only on its grey value. The opacity values are defined by the parameters **Threshold**, **Ramp**, and **Max. Opacity**.

3D FOR LSM 510

Accumulation of pixels proceeds along the ray from back to front, i.e. from far pixels to near pixels. If a new pixel is added, it increases the result intensity by its grey value weighted by the opacity value, and attenuates the previously accumulated intensity according to the opacity value. Full intensity stops accumulation.

This calculation must be repeated for each pixel of the ray to generate one **Output** pixel. Then for each **Output** pixel to produce a 2D **Output** image for the selected view-angle. Then for each view-angle to produce an output sequence for **Number of Views** different view angles.

Render - References

LSM 510

[1] J.D. Foley, A.van Dam, S. K. Feiner, J.F.Hughes, Computer Graphics: Principles and Practice, Addison Wesley, Reading, MA, 1990.

[2] M. Levoy, Display of Surfaces from Volume Data, IEEE Computer Graphics & Applications, May 1988, 29-37.

[3] J. Ylä-Jääski, F.Klein, O. Kübler, Fast Direct Display of Volume Data for Medical Diagnosis, VGIP:Graphical Models and Image Processing 53,1991,7-18.

[4] K.H. Höhne, R. Bernstein, Shading 3D-Images from CT Using Gray-Level Gradients, IEEE Transactions on Medical Imaging, 5, 1986, 45-47.

[5] D.Gordon, R.A. Reynolds, Image Space Shading of 3-Dimensional Objects, CVGIP 29, 1985, 361-376.

3D FOR LSM 510 Functions

Carl Zeiss

8.3.5 Functions in the Measurement Menu

Z-1

Ζ

Z+1

Measurement Concept

Measurement is based on regions (objects) in three-dimensional space. Segmenting an image sequence generates these. The image segmentation process produces a mask image that defines the region.

A region is a group of voxels that touch at the surfaces or at the edges, but not at the corners (18 voxel neighborhood).

This is illustrated by the following example. The voxels marked black in sequential image Z-1, Z, Z+1 all belong to the same region as the grey central voxel in sequential image Z. The volume view shows the neighborhood interrelationships as a 3D projection.

Sequential image:

Volume view:





LSM 510 META	Functions	Carl Zeiss
Measurement Process		
The measurement process consists of regions, and feature calculation.	^t three steps: region definition,	checking of the validity of the
Region definition:	- Automatically from the mask	c image
Region validation check depends on:	- Minimum volume - Measurement condition	
Feature calculation depends on	- Shape of the region	

3D FOR LSM 510

- Densitometric value distribution of the region
- Feature parameters



Fig. 8-36

LSM 510

All regions found are checked according to certain conditions. The voxel volume of each region must be equal to or greater than **MinVolume**. The measurement condition must be fulfilled. Only those regions that meet all the conditions are valid for the measurement. The region can be measured or labeled. Measurement is a process that produces data. Labeling is a process that generates an image volume.

Automatic Object Measurement – Object Features

A measurement feature describes a region characterized by a number (e.g. volume, area or a densitometrical statistic). The features can be selected on the **Object Features** and **Volume Features** tab sheets.

🚵 Automatic Object Measurement		×
General Object Features Volume Fe	eatures Condition	
Available Features: Volume Volume filled Ellipsoid Ellipsoid filled Surface area Surface area Surface area Surface area filled Sphere diameter	Selected Features:	
Sphere form factor Number of holes Mean densitometric Standard deviation densitometric Minimum densitometric Maximum densitometric	»	
Select All	Remove All	
	OK Cancel	Apply

Fig. 8-37

The scalings and units are taken automatically from the assigned sequence.

The measurement features can be selected individually for each measurement. The object features generate a result value for every single object.

LSM 510	3D FOR LSM 510	
LSM 510 META	Functions	Carl Zeiss

The dialog shows two lists. One shows the **Available Features** as groups (on the left). The other one shows the **Selected Features**. Double-clicking on items of the left list will add the **Selected Features** to the right list. Double-clicking on an item of the right list will remove this item from the list. **Selected Features** can also be transferred by clicking on the button in the middle (<< / >>) of the dialog.

The combo box above the right list represents predefined feature lists. Selecting one of the entries will fill the right list with these features; previously selected features will be overwritten.

The button **Select All** will copy all features to the list of selected features.

The button **Remove All** will clear the list of selected features.

Clicking on the **Apply** button will execute the measurement process and switch to the **General** tab sheet of the dialog.

Parameters:

Available Features	List of available object features
Selected Features	List of selected object features
Select All	Select all available object features for measurement
Remove All	Remove all object features from the selected features list

The following sections describe all measurement features which are defined in the system.

Object Features (geometric)

If **Object Features** are selected, one set of measurement data is calculated for each object.

Group Name	Name	Description
Volume	Volume	Volume of the object.
Volume Filled	VolumeF	Volume of the filled object.
Ellipsoid	EllipseA	Length of the main axis of the ellipsoid with the same geometrical moment of inertia as the object.
	EllipseB	Length of the middle axis of the ellipsoid with the same geometrical moment of inertia as the object.
	EllipseC	Length of the minor axis of the ellipsoid with the same geometrical moment of inertia as the object.
Ellipsoid filled	EllipseAF	Length of the main axis of the ellipse with the same geometric moment of inertia as the filled object.
	EllipseBF	Length of the middle axis of the ellipse with the same geometric moment of inertia as the filled object.
	EllipseCF	Length of the minor axis of the ellipse with the same geometric moment of inertia as the filled object.
Surface Area	SurfArea	Surface area of the object.
Surface Area Filled	SurfAreaF	Surface area of the filled object.
Sphere Diameter	Dsphere	Diameter of the sphere with the same volume. $\sqrt{6*VOLUMEF/\pi}$
Sphere Form Factor	Fsphere	Form factor of the object. $6 \cdot \sqrt{\pi} \cdot \frac{\text{VOLUMEF}}{\sqrt{\text{SUFFAFEAF}^3}}$
Number of Holes	Nparts	Number of holes within an object.

Object Features (densitometric)

Group Name	Name	Description
Mean Densitometric	MeanD	Densitometric mean value of an object.
Standard Deviation Densitometric	StdD	Standard deviation of the densitometric values of an object.
Minimum Densitometric	MinD	Minimum grey value of an object.
Maximum Densitometric	MaxD	Maximum grey value of an object.

Automatic Object Measurement - Volume Features

A measurement feature describes a region characterized by a number (e.g. volume, area, or a densitometrical statistic). The features can be selected on the **Object Features** and **Volume Features** tab sheets.

🔝 Automatic Object Measurement	×
Automatic Object Measurement General Object Features Volume Available Features: Count Volume Volume percentage Surface area Mean densitometric Standard deviation densitometric Minimum densitometric	Features Condition Selected Features: VolCount
Minimum densitometric Maximum densitometric	Remove All
	OK Cancel Apply



The measurement features can be selected individually for each measurement. The object features generate a result value for every single object.

The dialog shows two lists. One shows the **Available Features** as groups (on the left). The other one shows the **Selected Features**. Double-clicking on items of the left list will add the **Selected Features** to the right list. Double-clicking on an item of the right list will remove this item from the list. **Selected Features** can also be transferred by clicking on the button in the middle (<< / >>) of the dialog.

The combo box above the right list represents predefined feature lists. Selecting one of the entries will fill the right list with these features; previously selected features will be overwritten.

The button **Select All** will copy all features to the list of selected features.

The button Remove All will clear the list of selected features.

Clicking on the **Apply** button will execute the measurement process and switch to the **General** tab sheet of the dialog.

LSM	510	
LSM	510	META

3D FOR LSM 510 Functions

Available Features	List of available object features	
Selected Features	List of selected object features	
Select All	Select all available object features for measurement	
Remove All	Remove all object features from the selected features list	

Volume Features (geometric)

The volume-related measurement generates one measured value per image sequence. The following table contains the predefined volume characteristics.

Group Name	Name	Description
Count	VolCount	Number of regions measured.
Volume	VolVolume	Total volume of all regions.
Volume Percentage	VolVolumeP	Total volume of all regions, in relation to the volume of the image sequence.

Volume Features (densitometric)

Group Name	Name	Description
Surface Area	VolSurfArea	Total surface area of all regions.
Mean Densitometric	VolMeanD	Mean grey value of all regions.
Standard Deviatio Densitometric	nVolStdD	Grey value standard deviation of all regions.
Minimum Densitometric	VolMinD	Minimum grey value in the image sequence.
Maximum Densitometric	VolMaxD	Maximum grey value in the image sequence.
LSM 510	3D FOR LSM 510	
--------------	----------------	--
LSM 510 META	Functions	

Automatic Object Measurement - Condition

The measurement conditions are used to limit the objects to be evaluated (e.g. only objects with defined minimum value). All objects are tested against the defined conditions. If the conditions are fulfilled the feature values are written to the data table.

🔝 Automatic Objec	t Measureme	nt		×
General Object Fe	atures Volum	e Features	Condition	
Feature:	Operator:	Number:	>>	List of conditions:
Volume VolumeF ElipseA ElipseC ElipseAF ElipseBF ElipseBF ElipseCF SurfArea SurfAreaF DSphere FSphere NParts MeanD StdD		00 0 1 2 3 4 5 5 6 7 7 8 9		
Minimum Volume	8	\	/oxel	Remove All
				OK Cancel Apply

Fig. 8-39

To define the following parameter select the **Condition** tab sheet of the **Automatic Object Measurement** dialog window.

Carl 7eiss

The list on the very left at the dialog shows all the measurement **Features**. The second list provides the comparison **Operators** and the next **Numbers** to define a value. This gives the possibility to compose an expression to test a feature value against a constant value. The fields above the lists will show the composed (selected) string. Clicking on the desired list entry does the selection. The button with the ">>", characters adds this string to the **List of Conditions**. All lines of the **List of conditions** are combined with the AND expression automatically. To remove a condition line double-click on it.

The parameter **Minimum Volume** defines the minimum voxel volume for the measurement. This is an easy way to eliminate very small regions caused by noisy sequences and segmentation process.

The button **Remove All** will clear the list of defined conditions.

Clicking on the **Apply** button will execute the measurement process and switch to the **General** tab sheet of the dialog.

Parameters:

Feature	List of available object features
Operator	List of available condition operators
Number	List of numbers to compose the value
List of conditions	Defined condition list
Remove All	Remove all entries from the List of conditions
Minimum Volume	Minimum object volume in voxel

Automatic Object Measurement - General

This function carries out an automatic measurement and labeling.

🔔 Auto	omatic (eral) Obj) bject ect Fea	Measur tures V	ement 'olume Features	Condition				>	
Mas Den	k Image Is Image]	2							
_ Ou	utput —									
C	Object		Datab	ase object	•]		🔽 Clipboard		
V	/olume	◄	Datab	ase volume	•]				
L	.abel		Image	label		New				
Ot F	oject Visu Render	alisation	Image	object		New				Measured Object Features
	No	Vol	ume neter^3	SurfArea micrometer^2	MeanD grey	StdD grey		SurfAreaF micrometer^2	DS 🔺	
	1		3937.50	3550.00	81.90	2	28.94	3550.00		
	2		1187.50	975.00	129.58	7	71.89	975.00		
	3		687.50	825.00	66.09		9.21	825.00		
	4		2000.00	1387.50	94.16	3	38.45	1387.50		
	5		2125.00	1587.50	118.74	7	72.75	1587.50		
	6		562.50	675.00	65.33	1	11.77	675.00	-	Measured
	VolCo	ount 1060	VolVo microm 3946	olume VolVo eter^3 \$ 5437.50	lumeP VolSu % microm 3.26 1046	rfArea heter^2 0687.50	_			Volume Features
						ОК		Cancel	Apply	



The regions must be defined by an image sequence **Mask Image** (the objects must be separated from one another by black voxels with the grey value 0). This sequence is generated with the function **Segment**. If it is a multichannel sequence a single channel has to be chosen.

The image **Dens Image** is needed for the measurement of the densitometric features. Image sequence properties like scaling and unit are taken from **Dens Image**. A single channel of this sequence (if it is multichannel) must be selected with the buttons to the right of the parameter.

The measurement results can be stored to database files. These files are tab delimited ASCII files which can be easily imported to major Windows programs like text processing or spreat sheet application. Writing database files are independently supported for object and volume features. Activating the corresponding check boxes enables it. The name of the database is defined with the field **Database**. The files will be located in the subdirectory DATA of the main installation directory. The filename extension TXT will be added automatically.

If the check box **Label** is activated a single channel sequence will be generated. It contains all the measured objects, each object is coloured homogeneous but in different colours. To copy all measurement values to the clipboard activate the check box **Clipboard**.

A single object of interest can be visualized. Clicking on a specific row in the data grid chooses the object. By selecting a row in the data grid a new image is created with the object of interest visualized. The visualization depends on the settings in the **Object Visualisation** field. If **Render** is chosen, the object of interest is displayed with the Surface Rendering method. If Mask is chosen, the object is labelled in a pseudo colour in a new image stack.

Parameters:

Carl Zeiss

Mask Image	Single channel mask image sequence that defines the objects
Dens Image	Image sequence for densitometric measurement and property source
Object	Stores measurement values of objects, including database filename
Volume	Stores volume measurement values of objects, including database filename
Label	Generates an image sequence with all objects labelled in different pseudo colours
Clipboard	Measurement values are automatically written to the clipboard

3D FOR LSM 510