Lumina Vision

USER'S MANUAL

Version 1

MITANI CORPOPATION

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* Macintosh is the registered trademark of Apple Computer, Inc.
* This Lumina Vision software is based on CodeWarrior (metrowerks, Inc.)

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Chapter 1

General Description

1. Software
2. Operating Environment

Chapter 1 General Description

1 Software

The **Lumina Vision** software is a fluorescent image processing software designed integrating all relevant programs from capturing of fluorescent image to its analysis. To execute all these programs, the software is provided with commands in plenty, but properly organized in a menu form so that they can be simply selected and executed using the mouse, making it very easy to operate. The software is thus designed to allow even beginners to easily proceed with highly sophisticated image analysis on a step-by-step basis in an interactive manner.

In particular, the software can be characterized by the following features:

(1)A screen window of Image Capturing Palette designed to allow fluorescent image to be easily captured from a camera into the system for analysis,

(2)A software function available to process monochrome image 16bit and color image 48bit data,

(3)A software function available to process image data for multicolor composition, and an optionally-available software function to time-lapse capture image (as an option), and

(4)A system design made to allow use of Macintosh's excellent user interface.

2 Operating Environment

2.1 Necessary Equipment

(1)Image capturing board/camera

The software requires the use of a camera designed to capture dark-field image. The software is designed to support Cool SNAP, SenSYS Series and ORCA Series.

The Cool SNAP and SenSYS Series are provided with image capturing boards designed for exclusive use with them.

The ORCA Series requires separate arrangement for Snapper (Data Cell)(Macintosh) as its image capturing board.

For the latest information about the image capturing boards available for use with this software, refer to “Instructions on Image Capturing Equipment” provided as a separate volume.

Image capturing equipment

LuminaVision

Image output equipment

(2) Computer

Macintosh (designed with a memory capacity of 64MB or above and a HD capacity of 6GB or above)
※The software requires the use of a Macintosh computer with a memory capacity of 128MB or above, if installed with an optional function "Time-lapse" image capturing.

(3) Keyboard

(4) Display

(5) Printer

2.2 Operating System (OS)

The software requires the use of Mac OS8.5 or its upgraded version for its operating system.

Chapter 2

Check before Power ON

Chapter 2 Check before Power ON

1 Checking Product Contents

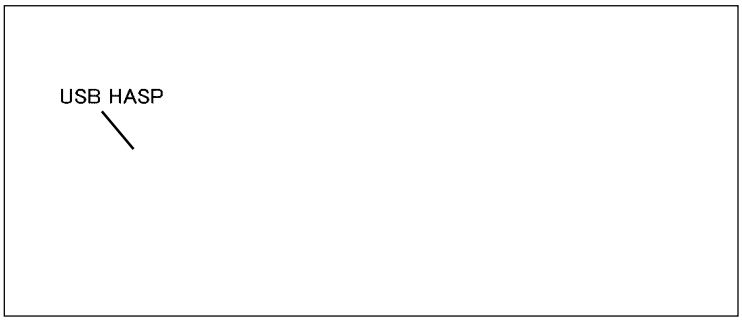
This product contains the following items. When you purchase the product, please check it to make sure that it contains all these items.

① LuminaVision CD-R	**1 floppy disk**
② User's Manual	**1 copy**
③ Instructions on Image Capturing Equipment	**1 copy**
④ User Registration Card	**1 copy**
⑤ Copy Protection Device	**1 unit**

2 Connecting Copy Protection Device

This software is designed so that even if installed in your computer, it cannot be executed unless the copy protection device provided as its accessory equipment is connected to the computer's USB port as illustrated in the following figure.

◆Macintosh



3 Connecting Image Capturing Board/Camera

The image capturing board to be used should be kept in an anti-static electric bag before being actually connected to the computer. When taking the image capturing board out of the bag for its connection to the computer, hold its card edge, never touching any of its components. When connecting the image capturing board to the computer, follow the procedure described in the manual provided with it.

For the latest information about the image capturing boards available for use with this software, refer to “Instructions on Image Capturing Equipment” provided as a separate volume.

Chapter 3

Software Installation

1. Setting up System Software

Chapter 3 Software Installation

1 Setting up System Software

1.1 How to Set up LuminaVision

①Close other application if opened on the screen.
②Set the LuminaVision CD-R disk in the drive unit.
③Open the CD-R program menu. From the menu, copy the LuminaVision folder onto the desktop screen of your computer using the drag-and-drop technique.

1.2 How to Set up HASP Driver

This software is designed so that it can only start its operation on your computer after making sure that the copy protection device has been properly connected to the computer's HASP. This requires the copy protection key (HASP) driver to be installed in your computer. The driver installation can be carried out as follows:

①Set the LuminaVision CD-R disk in the drive unit.
②Open the CD-R program menu. From the menu, copy MacHASPUsbDD into the system folder's function expansion folder.

1.3 How to Set up Image Capturing Device Driver

Install your computer with the driver attached to the image capturing board. The driver installation step can be omitted if the operation of the software will not involve any control of the image capturing equipment.

If the operation of the software involves any control of the image capturing equipment, be sure to install the driver in the computer. If the computer is not installed with the driver, you cannot capture your image from the device into the system.

1.4 Rebooting the Computer after Driver Setup

If the installation of the HASP driver and image capturing device driver is completed, be sure to reboot the computer, thus allowing it to become ready for the operation of the software.

Chapter 4

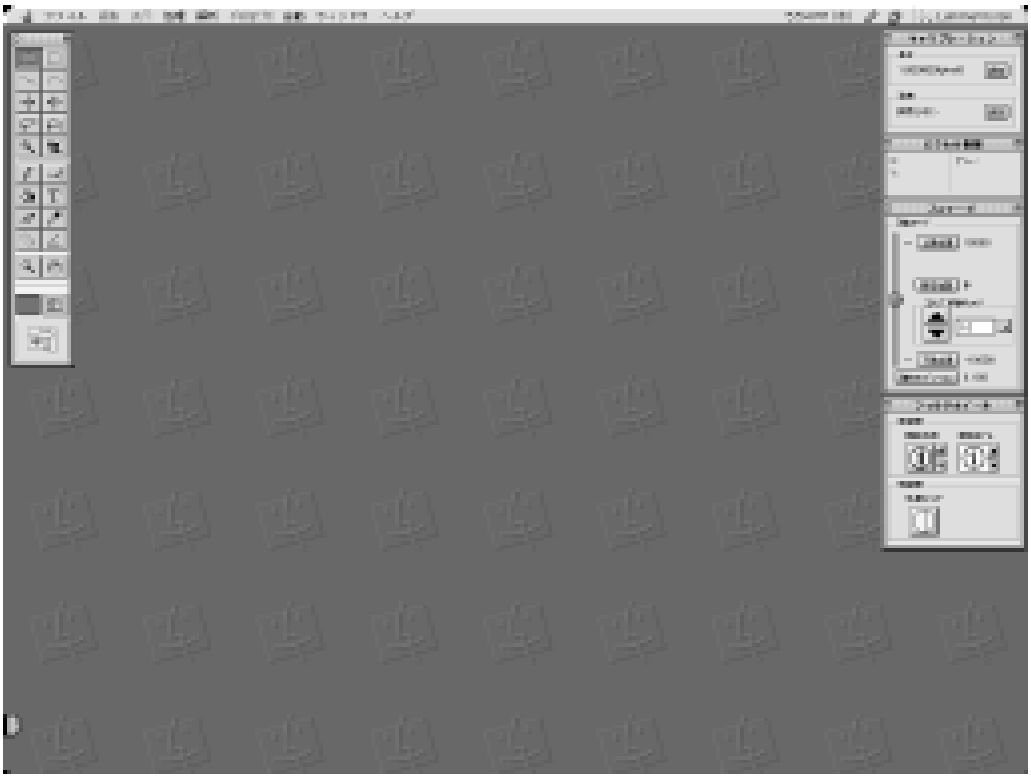
Basic Operation

Chapter 4 Basic Operation

1 Starting and Exiting

【Procedure for starting LuminaVision】

①On the desktop screen, open LuminaVision folder.
②Double-click on the LuminaVision application to start it. When the loading of the application onto the computer is completed, the computer display will show such a screen as shown in the following figure.



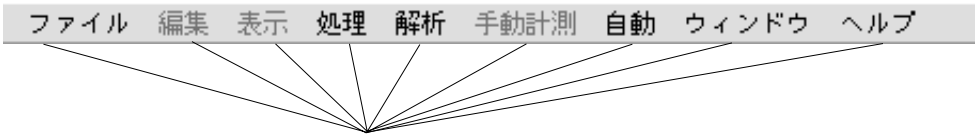
【Procedure for exiting LuminaVision】

You can cause the LuminaVision application to exit from the computer system by selecting command **Exit** in the **File** menu. Specifically, the **Exit** command can be selected; move the mouse cursor to **File** on the **Menu** bar at the top of the screen. Click on the **File** menu to pull it down. On the pull-down menu thus displayed, move the mouse cursor to command **Exit** and click on it.

2 Selecting Menu Items and Commands

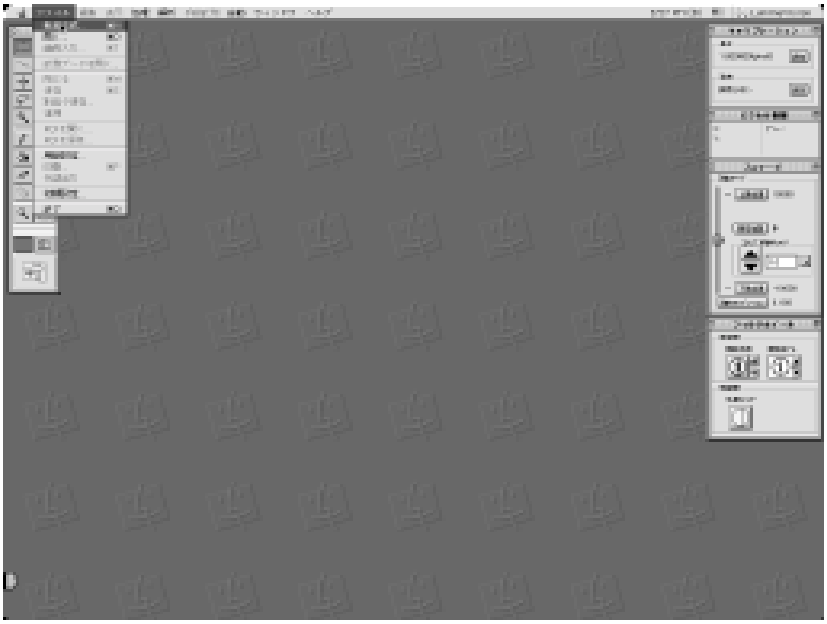
The LuminaVision software program is designed so that its menu items and commands can be selected using the mouse in various ways such as “Clicking (quickly pressing and releasing its left button) and dragging (pressing and holding down its left button while moving it).

(1)Menu bar



Clicking on one of these items on the **Menu** bar at its indicated position causes its corresponding menu to be displayed on the screen.

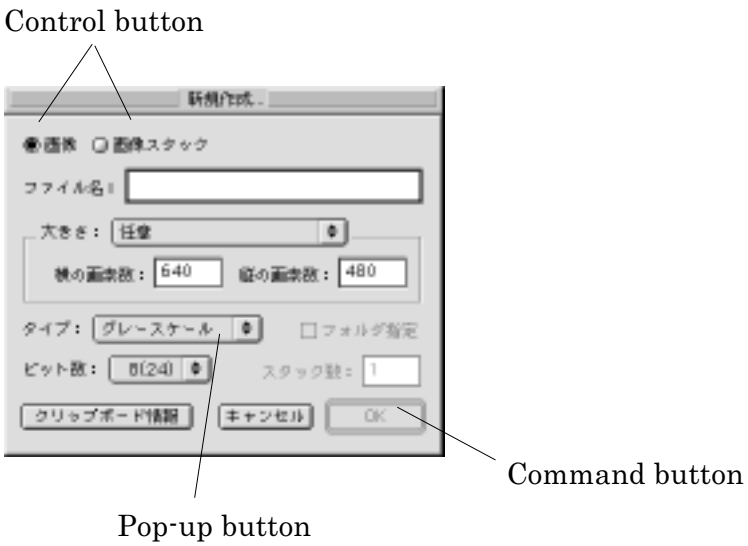
(2)Menu items



(3)Tool bar commands

Clicking on one of these tools on the Tool bar causes its corresponding tool command to be executed.

(4)Dialogue box



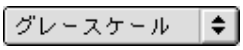
①Command buttons

Clicking on any of the command buttons causes its corresponding command to be executed.

②Option buttons

Option buttons are used for any multiple-choice item such as one shown above, providing more than one option from which to select one for its setting.
Clicking on the option button (◯) for the option you want causes a black dot to appear inside the circle of the button, turning it into ◉, which indicates that that option has been selected.

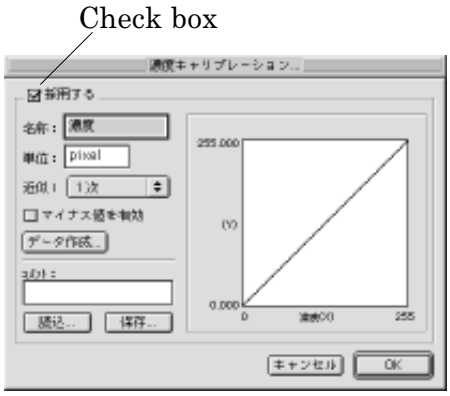
③Pop-up buttons



When any of the pop-up buttons is selected, it will display more than one option, from which to select one. In this case, for example, clicking on the Grayscale causes a list of more than one option to appear, from which you can select one you need by moving the cursor to it and releasing the clicking.

④Check box

A check box (☐) is used for any command item that is to be set activated (selected) or deactivated (unselected) by clicking on the box to put a check mark in it, turning it into☑, or leaving it unchecked, respectively.
Clicking on the checked box (☑) causes the selection of its corresponding command to be cleared with the check mark deleted from it, turning it into ☐ .



⑤Scroll bar

Appearing in the image window, the scroll bar can be used to scroll the display of the image in it. Clicking or dragging the position of the scroll bar allows the image display to be scrolled in the direction selected. You can also use the box portion of the scroll bar (▪) by dragging it to scroll the image display.

⑥Slider

In a dialogue box requiring input of a value selected from more than one, a slider appears to let you select the value by dragging the slider bar (▼) left or right to set it at that value.

3 Inputting Alphanumeric characters

The LuminaVision software is designed to allow any necessary alphanumeric character input to be made as described below:

(1)Inputting numeric characters

Clicking this area causes the cursor "|" to appear at the "I" position for numeric character input, allowing any numeric character to be entered from the keyboard with the entered numeric character appearing at this position.

(2)Inputting alphabetical characters

Clicking this area causes the cursor "|" to appear at the "I" position for alphabetical character input, allowing any alphabetical character to be entered from the keyboard with the entered alphabetical character appearing at this position.

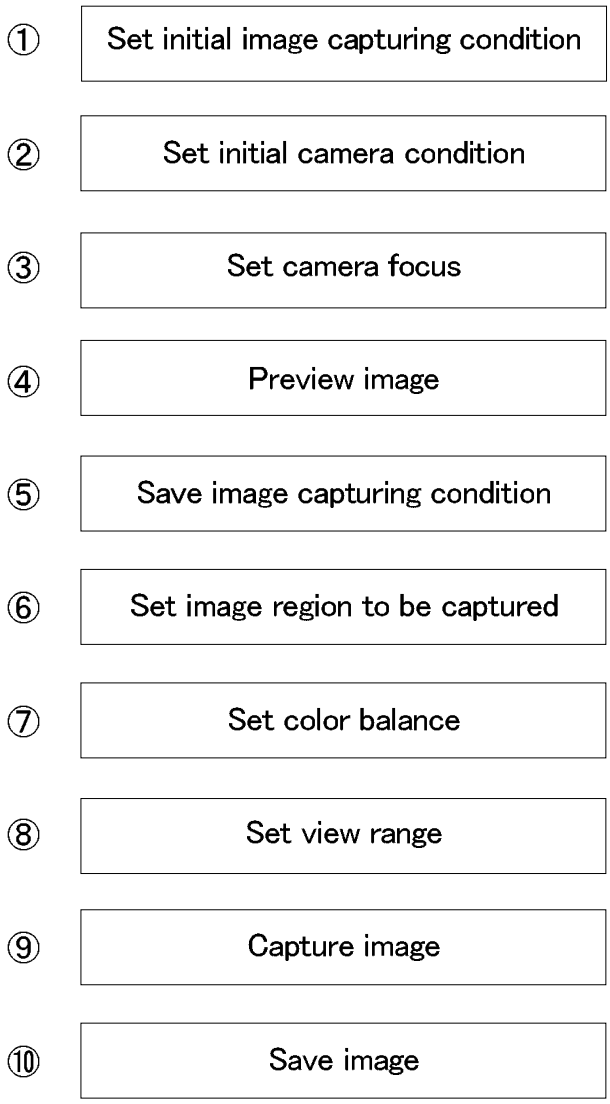
Chapter 5

Image Capturing Procedure

Chapter 5 Image Capturing Procedure

1 Standard Image Capturing Flowchart

In this chapter, let's operate the LuminaVision program actually by displaying the **Image Capturing Palette** window on the screen to learn how to use a fluorescence camera to capture a piece of image into the system according to the following flowchart:



2 Set Initial Image Capturing Condition

When you use a fluorescence camera to capture an image into the system, display the **Set initial image capturing condition** dialogue box on the screen as shown in the following figure. In the dialogue box, set the image capturing method by selecting a camera according to the procedure described below:

① From the tool bar, select  to display the **Set initial image capturing condition** dialogue box.

② In the dialogue box, select the **Capture image** tab and set **Method** by selecting **Camera**.

③ In the **Cameras or capture cards available for use with the system** group box, select the camera or capture card connected to the computer.

④ Select the  button after confirmation that all the setting is correct.

⑤ Move the cursor to the tool bar and click on  to display the Image Capturing Palette as shown on the next page.

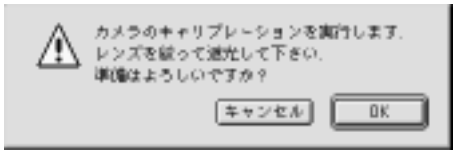
The camera selection in the **Set initial image capturing condition** dialogue box does not involve its physical access to the camera connected to the computer, but no more than its software setting. Therefore, in the **Cameras or capture cards available for use with the system** group box, you can select ORCA Series even if you have CoolSNAP, SenSYS Series connected to the computer, and the reverse is also true. Make sure that your camera selection is correct in this respect.

3 Set Initial Camera Condition

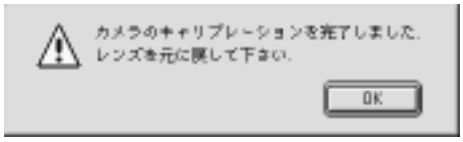
In the **Image Capturing Palette**, you can set the initial camera condition such as the black level, maximum/minimum exposure time, auto exposure condition and use/disuse of the shutter in focus setting mode.

① From the **Image Capturing Palette**, select  to display the **Set Initial Camera Condition** as shown in the following figure:

② Set the black level of the camera.
Any camera captures some image if its lens opening is minimized. Therefore, the system must be set so as to exclude any image data captured from the camera with its lens opening minimized from the image data captured from it under normal condition for analysis. Such setting of the system can be achieved by capturing image data from the camera with its lens opening minimized and setting it as black according to the procedure described below.
In the **Set initial camera condition** dialogue box, activate the **Calibration** check box and select the **Measure** button. Then, minimize the camera's lens opening for light shutoff.
If All preparations for the camera's black level setting are completed, follow the message displayed on the screen to click on the  button.



③ Return the camera's lens to its original opening and follow the message displayed on the screen as shown in the following figure to click on the OK button .



＊ The execution of steps ② and ③ mentioned above completes the setting of the camera's black level. Be sure to perform this process if you use a camera to capture image data into the system, irrespective of whether the data is a color or monochrome image.

④ Set the maximum/minimum exposure time of the camera.

This setting can be made using the **Exposure time** group box in the **Set initial camera condition** dialogue box to enter your desired

※This setting is effective only for the CoolSNAP/SenSYS Series. In the case of the ORCA Series, the maximum/minimum exposure time is to be set in three modes, each of which has a certain allowable range for the exposure time setting.

⑤ Set the camera lens exposure time determining condition for the auto exposure mode, in which the system can automatically determine the exposure time while capturing image data from the camera. (Refer to Section 5 "Preview Image".)

This setting can be made using the **Auto exposure condition** group box in the **Set initial camera condition** dialogue box to enter 0 to 4095 in the **Level** text box as a level for the exposure time determining condition. This level can be regarded as the intensity of the image data to be captured from the camera.
The **Upper limit** text box in the **Auto exposure condition** group box is used to enter the upper limit on the exposure time in the auto exposure mode. When, in the **Image Capturing Palette**, the **Select auto exposure for image capturing** check box is activated to perform image capturing operation in the auto exposure mode, the system will increase the exposure time from the **Min.** value to the value set in the **Upper limit** text box to determine the exposure time, at which the maximum intensity of the image data exceeds the value set in the **Level** text box, as the exposure time in the auto exposure mode.

For example, when you capture a 12bit monochrome image (which ranges in intensity from 0 to 1023) with 800 entered in the **Level** text box, the system will determine the exposure time, at which the maximum intensity of the image data first exceeds 800, as the exposure time in the auto exposure mode.

⑥ Set whether to use the shutter in the focus setting mode by activating or deactivating the **Use shutter in focus setting mode** check box.

This setting is effective only for CoolSNAP/SenSYS Series.

4 Set Camera Focus

When you capture a fluorescent image into the system, you are required to focus your camera on the image in a short time because it may undergo quick discoloration. The LuminaVision software is designed to set the camera focus on an image to be captured into the system, always using its monochrome image data with a binning of 2×2.

Binning is a technique by which to allow a camera to capture an image by using more than one pixel of the camera for one pixel of the image to increase its sensitivity. With a binning of 2×2, for example, the camera can capture the image with the image's pixel size reduced to 1/4 of the camera's pixel resolution, thereby shortening the time required for transmission of the image data from the camera to the personal computer. The binning is also effective in increasing the sensitivity of the image to light, allowing its exposure time to be shortened with resultant control of its discoloration that may otherwise occur during its focus setting process.

①Set your fluorescent sample in a microscope.

②From the tool bar, select  to display the **Image Capturing Palette** as shown in the following figure.

③In the **Image Capturing Palette**, set No. of bits to that of the camera connected to the computer.

④Set **Exposure time** and select the button. When this button is selected, the system will cause the camera to start capturing the image, displaying the captured image data in the image window continuously.

You can change the exposure time, even while the system is operating for continuous display of the captured image data in the image window. The exposure time can be shortened by shifting the slider bar leftward and lengthened by shifting it rightward. If the image is dark, lengthen the exposure time. Conversely, if the image is too bright, shorten the exposure time.

※When you use CoolSNAP/SenSYS series, you can select whether or not to output the signal to actuate the shutter in the focus setting mode by activating or deactivating the **Use shutter in focus setting mode** check box in the **Set initial camera condition** dialogue box.

⑤ Adjust the Z stage to optimize the camera focus on the image.

⑥When the camera focus setting is completed, select again to stop the image capturing operation.

＊When the ORCA Series is selected, the exposure time can be set in three modes as described in the following table. The camera focus can only be set with a binning of 2×2.

ORCA exposure time setting modes

Mode	Description
Normal	Fixed at 55.6mSEC, the exposure time cannot be changed.
Shutter mode	With the operation of the electronic shutter, the exposure and image loading are repeated. The exposure time can be set in the following ranges depending on the pinning. No pinning **Exposure time = 132.1μSEC+(n-1)×106.9μSEC(n=1-1039)** Pinning 2×2 **Exposure time = 132.1μSEC+(n-1)×106.9μSEC(n=1-519)** Pinning 4×4 **Exposure time = 132.07μSEC, Exposure time = 238.95μSEC** **Exposure time = 238.95μSEC+(n-2)×118.27μSEC(n=3-260)**
Frame blanking	With frame blanking, the exposure and image loading are repeated. The exposure time can be set in the following ranges depending on the pinning. No pinning **Exposure time = n×111.2mSEC(n=1-90)** Pinning 2×2 **Exposure time = n×55.6mSEC(n=1-180)** Pinning 4×4 **Exposure time = n×31.5mSEC(n=1-325)**

5 Preview Image

After the camera focus setting is completed, making sure that the camera focus has been set properly, select the **Preview image** function to finalize the camera condition such as the exposure time and pinning. In addition, this function allows you to preview a color image captured into the system. This function can be also used to make the final adjustment of the camera focus.

①When the image data to be captured is RGB color, activate the **RGB color** check box in the **Image Capturing Palette**.
＊If your camera connected to the system is a monochrome one, this check box cannot be selected.

②Set **Pinning** by pressing its corresponding pop-up button in the **Image Capturing Palette**.

You can set the pinning by selection from the three choices – No, 2×2 and 4×4, in which order the sensitivity increases, allowing the exposure time to be shortened. It is therefore recommended that if your image is subject to severe discoloration in a short time, the binning should be set larger to allow it to be captured into the system in a time as short as possible.
It should be noted, however, that a larger pinning setting results in reduced resolution of the image – the resolution of the image captured with a pinning of 2×2 is reduced to 1/2 of that with no pinning. The same is true when the image resolution achieved with a pinning of 2×2 is compared to that with a pinning of 4×4.

＊The pinning setting varies with the specification of the camera you use with the system. Carefully read the instruction manual for the camera.

③ Set **Exposure time** and select  in the **Image Capturing Palette**.

When this button is selected, the system will cause the camera to start capturing the image, displaying the captured image data in the image window continuously. You can change the exposure time, even while the system is operating for continuous display of the captured image data in the image window. The exposure time can be shortened by shifting the slider bar leftward and lengthened by shifting it rightward. If the image is dark, lengthen the exposure time. Conversely, if the image is too bright, shorten the exposure time.

＊When the **Preview image** function is executed with the CoolSNAP/SenSYS Series selected as your camera, the system outputs the signal to actuate the shutter during the image capturing.

④Preview your image data to make sure that they are OK before clicking on  again to stop the image capturing operation.

6 Save Image Capturing Condition

Save the data about the image capturing condition set using the **Camera** tab in the Image Capturing Palette (**No. of bits**, **Binning**, **RGB color**, **Select auto exposure for image capturing**, **Exposure time** as shown in the following diagram).

①Click on the **Set** pop-up button to display a list of items. From the list, select **Create a new camera condition** to save the data on the initial image capturing condition set in the **Image Capturing Palette**.
The image capturing condition thus saved will be added to the items of the list that is displayed by pressing the **Set** pop-up button.

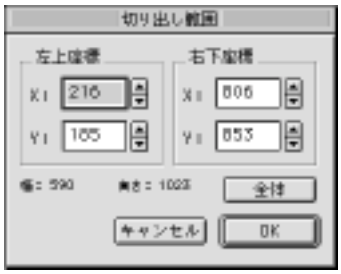
＊Next time when you capture the image of a sample of the same type as the one you captured previously, you can press the Set pop-up button to display a list of items, including the previously registered image capturing condition(s), from which you can select the one suitable for capturing the image of your current sample, loading it onto the screen for its prompt preview.

7 Set Image Region to be Captured

At the stage of the camera focus setting and image previewing, you can set a ROI on the image to be captured in order to allow the camera to capture only the ROI image region into the system, thereby accelerating the image capturing operation.

①Select the  button in the **Image Capturing Palette**.

The selection of this button causes the screen to display the **Set image region to be captured dialogue** box as shown in the following figure.



②In the dialogue box, set the region (ROI) of the image you want to capture into the system by specifying its top left and bottom right coordinates as exemplified in the above figure.

The ROI setting can be made not only by the above-mentioned method, which involves the use of the dialogue box for numerical setting, but also by the method which involves the direct use of the image window on which to drag the cursor to create a desired rectangular ROI on the image. If the ROI setting is completed, press the  button.

8 Set Color Balance

When the camera captures a color image, it is necessary to set the image so that it can be captured into the system with its color balance optimized. This image's color balance optimization can be achieved using the **Set color balance** function to select its black and white areas on the image window for its black and white component specification, based on which the system is to make its color correction upon its capturing.

The **Set color balance** function cannot be selected when your camera connected to the system is a monochrome one.

①From the **Image Capturing Palette**, select  to display **Set color balance** dialogue box as shown in the following figures.

②In the **Set color balance** dialogue box, select the **Black balance** tab to Select the Specify black area by clicking button and use the mouse to click your selected black area in the image window. This black balance setting allows the system to set the camera focus, preview the image and capture it so that the clicked area will become black. If, after the clicking, you want to further adjust the black balance of the image, you can make the adjustment by using the **R/G/B** component adjusting sliders in the dialogue box. Each slider has its center set at "0", the right and left sides of which represent its plus and minus values, respectively. The value thus set will be added to its corresponding color component of the image data.

③In the **Set color balance** dialogue box, select the **White balance** tab to set the white balance of the image.
Select the Specify white area by clicking button and use the mouse to click your selected white area in the image window. This white balance setting allows the system to set the camera focus, preview the image and capture it so that the clicked area will become white. If, after the clicking, you want to further adjust the white balance of the image, you can make the adjustment by using the **R/G/B** component adjusting sliders in the dialogue box. Each slider has its left end set at "1", increasing its value when shifted rightward. The value thus set will be added to its corresponding color component of the image data.

9 Set View Range

If the camera captures a 10bit(30bit)-or-above image, you can use the **Set view range** function. This function allows you to specify the intensity range, within which you want to have your image data displayed in the image window. Any image data whose intensity is out of the specified range will be set black or white.
The view range setting can be also achieved automatically during the camera focus setting, image previewing and capturing processes by selecting the **Auto scaling** function provided in the **Image Capturing Palette** as shown in the following figure.

①From the **Image Capturing Palette**, select  to display the **Set view range** dialogue box as shown in the following figure.

②When you want to select the **Auto scaling** function to cause the system to set the view range automatically, deactivate the **No auto scaling** check box with no check mark put in it.

③When you want to set the view range manually, activate the **No auto scaling** check box with a check mark put in it. Then, enter the minimum and maximum values of the view range you need in the **Minimum** and **Maximum** text boxes, respectively.

The view range you can set varies with the number of bits of the image to be captured in the system.
If you want the system to optimize the maximum and minimum values of the view range you set manually, select the **Optimize** button.

10 Capture Image

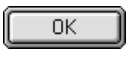
①Select the format in which you want to have your image captured into the system.
From the **Image Capturing Palette**, select  to display a list of options. From these options, select **Image window** in this case as a typical format in which to capture your image into the system. The **Image window** format allows your captured images to be created as one image window. If you select **File** from the options list, displaying the **Save file** dialogue box, you can save your captured image data as an image file.

②From the **Image Capturing Palette**, select  to capture your image into the system in the **Image window** format selected in ① above.

11 Save Image

Save your image window, created in 10 above, onto your disk as an image file.

①From the **File** menu, select the **Save As** command.

②In the **Save As** dialogue box thus displayed, specify the name of the file in which you want to save the image window by, in this case, entering TEST in the **File name** text box. Then, press the 

button.

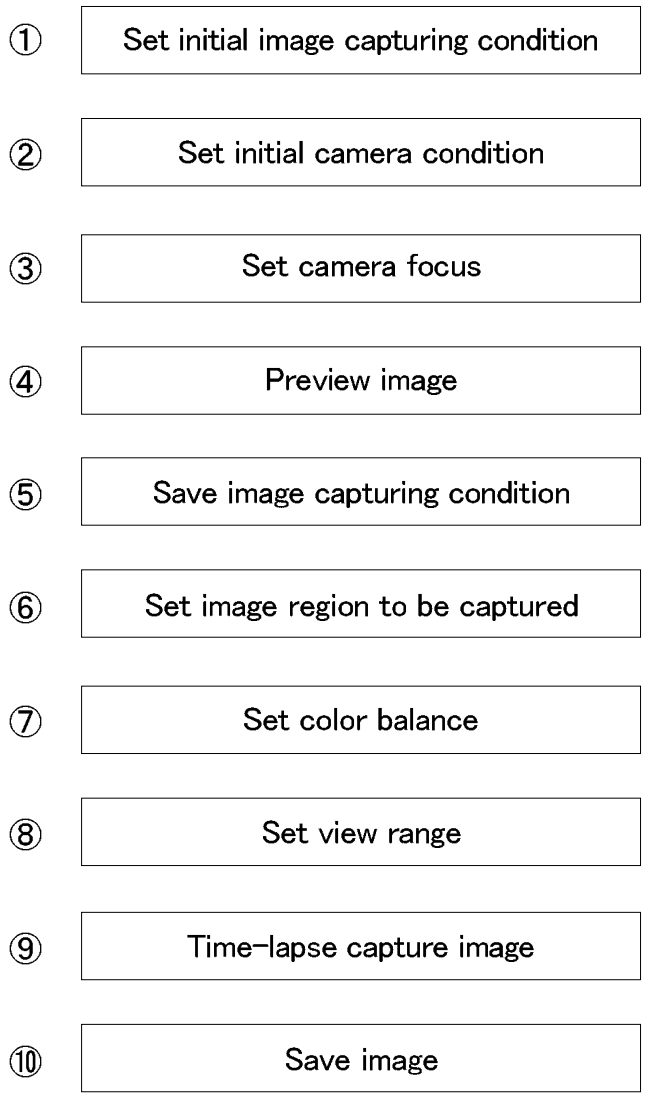
Chapter 6

Time-lapse Image Capturing (Optional) Procedure

Chapter 6 Time-lapse Image Capturing Procedure

1 Standard Image Capturing Flowchart

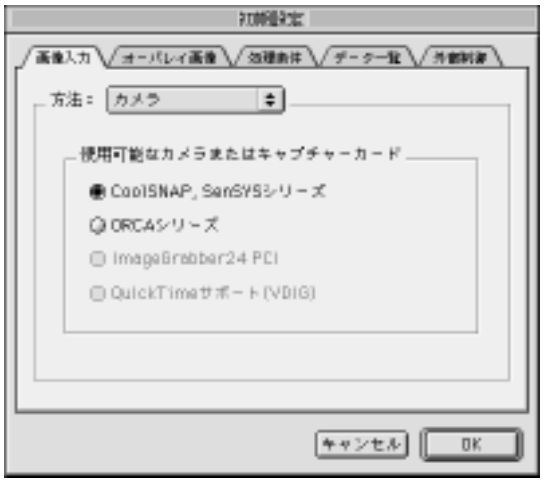
In this chapter, let's operate the LuminaVision's program in a similar manner to that done in Chapter 5 to display the **Image Capturing Palette** window on the screen, but this time, to learn how to use a fluorescence camera to capture more than one piece of image into the system by specifying the time for capturing each of the images according to the following flowchart:



2 Set Initial Image Capturing Condition

When you use a fluorescence camera to capture an image into the system, display the **Set initial image capturing condition** dialogue box on the screen as shown in the following figure. In the dialogue box, set the image capturing method by selecting a camera according to the procedure described below:

①From the tool bar, select  to display the **Set initial image capturing condition** dialogue box.



②In the dialogue box, select the **Capture image** tab and set **Method** by selecting **Camera**.

③In the **Cameras or capture cards available for use with the system** group box, select the camera or capture card connected to the computer.

④Select the  button after confirmation that all the setting is correct.

⑤Move the cursor to the tool bar and click on  to display the Image Capturing Palette as shown on the next page.

The camera selection in the **Set initial image capturing condition** dialogue box does not involve its physical access to the camera connected to the computer, but no more than its software setting. Therefore, in the **Cameras or capture cards available for use with the system** group box, you can select ORCA Series even if you have CoolSNAP, SenSYS Series connected to the computer, and the reverse is also true. Make sure that your camera selection is correct in this respect.

3 Set Initial Camera Condition

In the **Image Capturing Palette**, you can set the initial camera condition such as the black level, maximum/minimum exposure time, auto exposure condition and use/disuse of the shutter in focus setting mode.

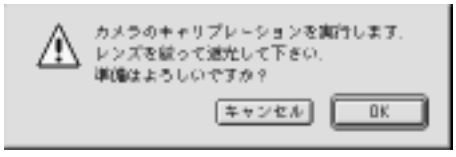
①From the **Image Capturing Palette**, select  to display the **Set Initial Camera Condition** as shown in the following figure:

②Set the black level of the camera.

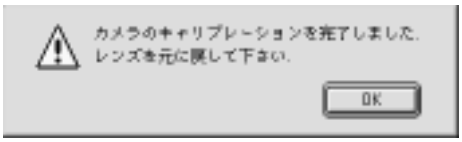
Any camera captures some image if its lens opening is minimized. Therefore, the system must be set so as to exclude any image data captured from the camera with its lens opening minimized from the image data captured from it under normal condition for analysis. Such setting of the system can be achieved by capturing image data from the camera with its lens opening minimized and setting it as black according to the procedure described below.

In the **Set initial camera condition** dialogue box, activate the **Calibration** check box and select the **Measure** button. Then, minimize the camera's lens opening for light shutoff.

If All preparations for the camera's black level setting are completed, follow the message displayed on the screen to click on the OK button.



③Return the camera's lens to its original opening and follow the message displayed on the screen as shown in the following figure to click on the OK button .



＊The execution of steps ② and ③ mentioned above completes the setting of the camera's black level. Be sure to perform this process if you use a camera to capture image data into the system, irrespective of whether the data is a color or monochrome image.

④ Set the maximum/minimum exposure time of the camera.
This setting can be made using the **Exposure time** group box in the **Set initial camera condition** dialogue box to enter your desired values in the **Min** and **Max** text boxes.

＊This setting is effective only for the CoolSNAP/SenSYS Series. In the case of the ORCA Series, the maximum/minimum exposure time is to be set in three modes, each of which has a certain allowable range for the exposure time setting.

⑤Set the camera lens exposure time determining condition for the auto exposure mode, in which the system can automatically determine the exposure time while capturing image data from the camera. (Refer to Section 5 "Preview Image".)

This setting can be made using the **Auto exposure condition** group box in the **Set initial camera condition** dialogue box to enter 0 to 4095 in the **Level** text box as a level for the exposure time determining condition. This level can be regarded as the intensity of the image data to be captured from the camera.
The **Upper limit** text box in the **Auto exposure condition** group box is used to enter the upper limit on the exposure time in the auto exposure mode.
When, in the **Image Capturing Palette**, the **Select auto exposure for image capturing** check box is activated to perform image capturing operation in the auto exposure mode, the system will increase the exposure time from the **Min.** value to the value set in the **Upper limit** text box to determine the exposure time, at which the maximum intensity of the image data exceeds the value set in the **Level** text box, as the exposure time in the auto exposure mode.

For example, when you capture a 12bit monochrome image (which ranges in intensity from 0 to 1023) with 800 entered in the **Level** text box, the system will determine the exposure time, at which the maximum intensity of the image data first exceeds 800, as the exposure time in the auto exposure mode.

⑥Set whether to use the shutter in the focus setting mode by activating or deactivating the **Use shutter in focus setting mode** check box.

※This setting is effective only for CoolSNAP/SenSYS Series.

4 Set Camera Focus

When you capture a fluorescent image into the system, you are required to focus your camera on the image in a short time because it may undergo quick discoloration. The LuminaVision software is designed to set the camera focus on an image to be captured into the system, always using its monochrome image data with a binning of 2×2.
Binning is a technique by which to allow a camera to capture an image by using more than one pixel of the camera for one pixel of the image to increase its sensitivity. With a binning of 2×2, for example, the camera can capture the image with the image's pixel size reduced to 1/4 of the camera's pixel resolution, thereby shortening the time required for transmission of the image data from the camera to the personal computer. The binning is also effective in increasing the sensitivity of the image to light, allowing its exposure time to be shortened with resultant control of its discoloration that may otherwise occur during its focus setting process.

①Set your fluorescent sample in a microscope.

②From the tool bar, select  to display the **Image Capturing Palette** as shown in the following figure.

③In the **Image Capturing Palette**, set No. of bits to that of the camera connected to the computer.

④Set **Exposure time** and select the  button. When this button is selected, the system will cause the camera to start capturing the image, displaying the captured image data in the image window continuously.

You can change the exposure time, even while the system is operating for continuous display of the captured image data in the image window. The exposure time can be shortened by shifting the slider bar leftward and lengthened by shifting it rightward. If the image is dark, lengthen the exposure time. Conversely, if the image is too bright, shorten the exposure time.

＊When you use CoolSNAP/SenSYS series, you can select whether or not to output the signal to actuate the shutter in the focus setting mode by activating or deactivating the **Use shutter in focus setting mode** check box in the **Set initial camera condition** dialogue box.

⑤Adjust the Z stage to optimize the camera focus on the image.

⑥When the camera focus setting is completed, select again to stop the image capturing operation.

＊When the ORCA Series is selected, the exposure time can be set in three modes as described in the following table. The camera focus can only be set with a binning of 2×2.

ORCA exposure time setting modes

Mode	Description
Normal	Fixed at 55.6mSEC, the exposure time cannot be changed.
Shutter mode	With the operation of the electronic shutter, the exposure and image loading are repeated. The exposure time can be set in the following ranges depending on the pinning. No pinning Exposure time = 132.1 μSEC+(n−1)×106.9 μSEC(n=1−1039) Pinning 2×2 Exposure time = 132.1 μSEC+(n−1)×106.9 μSEC(n=1−519) Pinning 4×4 Exposure time = 132.07 μSEC, Exposure time = 238.95 μSEC Exposure time = 238.95 μSEC+(n−2)×118.27 μSEC(n=3−260)
Frame blanking	With frame blanking, the exposure and image loading are repeated. The exposure time can be set in the following ranges depending on the pinning. No pinning Exposure time = n×111.2mSEC(n=1−90) Pinning 2×2 Exposure time = n×55.6mSEC(n=1−180) Pinning 4×4 Exposure time = n×31.5mSEC(n=1−325)

5 Preview Image

After the camera focus setting is completed, making sure that the camera focus has been set properly, select the **Preview image** function to finalize the camera condition such as the exposure time and pinning. In addition, this function allows you to preview a color image captured into the system. This function can be also used to make the final adjustment of the camera focus.

①When the image data to be captured is RGB color, activate the **RGB color** check box in the **Image Capturing Palette**.

＊If your camera connected to the system is a monochrome one, this check box cannot be selected.

②Set **Pinning** by pressing its corresponding pop-up button in the **Image Capturing Palette**.

You can set the pinning by selection from the three choices – No, 2×2 and 4×4, in which order the sensitivity increases, allowing the exposure time to be shortened. It is therefore recommended that if your image is subject to severe discoloration in a short time, the binning should be set larger to allow it to be captured into the system in a time as short as possible.
It should be noted, however, that a larger pinning setting results in reduced resolution of the image – the resolution of the image captured with a pinning of 2×2 is reduced to 1/2 of that with no pinning. The same is true when the image resolution achieved with a pinning of 2×2 is compared to that with a pinning of 4×4.

＊The pinning setting varies with the specification of the camera you use with the system. Carefully read the instruction manual for the camera.

③Set **Exposure time** and select  in the **Image Capturing Palette**.

When this button is selected, the system will cause the camera to start capturing the image, displaying the captured image data in the image window continuously. You can change the exposure time, even while the system is operating for continuous display of the captured image data in the image window. The exposure time can be shortened by shifting the slider bar leftward and lengthened by shifting it rightward. If the image is dark, lengthen the exposure time. Conversely, if the image is too bright, shorten the exposure time.

＊When the **Preview image** function is executed with the CoolSNAP/SenSYS Series selected as your camera, the system outputs the signal to actuate the shutter during the image capturing.

④Preview your image data to make sure that they are OK before clicking on  again to stop the image capturing operation.

6 Save Image Capturing Condition

Save the data about the image capturing condition set using the **Camera** tab in the Image Capturing Palette (**No. of bits**, **Binning**, **RGB color**, **Select auto exposure for image capturing**, **Exposure time** as shown in the following diagram).

①Click on the **Set** pop-up button to display a list of items. From the list, select **Create a new camera condition** to save the data on the initial image capturing condition set in the **Image Capturing Palette**. The image capturing condition thus saved will be added to the items of the list that is displayed by pressing the **Set** pop-up button.

＊Next time when you capture the image of a sample of the same type as the one you captured previously, you can press the Set pop-up button to display a list of items, including the previously registered image capturing condition(s), from which you can select the one suitable for capturing the image of your current sample, loading it onto the screen for its prompt preview.

7 Set Image Region to be Captured

At the stage of the camera focus setting and image previewing, you can set a ROI on the image to be captured in order to allow the camera to capture only the ROI image region into the system, thereby accelerating the image capturing operation.

①Select the  button in the **Image Capturing Palette**.
The selection of this button causes the screen to display the **Set image region to be captured dialogue** box as shown in the following figure.

②In the dialogue box, set the region (ROI) of the image you want to capture into the system by specifying its top left and bottom right coordinates as exemplified in the above figure.

The ROI setting can be made not only by the above-mentioned method, which involves the use of the dialogue box for numerical setting, but also by the method which involves the direct use of the image window on which to drag the cursor to create a desired rectangular ROI on the image. If the ROI setting is completed, press the  button.

8 Set Color Balance

When the camera captures a color image, it is necessary to set the image so that it can be captured into the system with its color balance optimized. This image's color balance optimization can be achieved using the **Set color balance** function to select its black and white areas on the image window for its black and white component specification, based on which the system is to make its color correction upon its capturing.
The **Set color balance** function cannot be selected when your camera connected to the system is a monochrome one.

①From the **Image Capturing Palette**, select  to display **Set color balance** dialogue box as shown in the following figures.

②In the **Set color balance** dialogue box, select the **Black balance** tab to set the black balance of the image.
Select the Specify black area by clicking button and use the mouse to click your selected black area in the image window. This black balance setting allows the system to set the camera focus, preview the image and capture it so that the clicked area will become black. If, after the clicking, you want to further adjust the black balance of the image, you can make the adjustment by using the **R/G/B** component adjusting sliders in the dialogue box. Each slider has its center set at "0", the right and left sides of which represent its plus and minus values, respectively. The value thus set will be added to its corresponding color component of the image data.

③In the **Set color balance** dialogue box, select the **White balance** tab to set the white balance of the image.

Select the Specify white area by clicking button and use the mouse to click your selected white area in the image window. This white balance setting allows the system to set the camera focus, preview the image and capture it so that the clicked area will become white. If, after the clicking, you want to further adjust the white balance of the image, you can make the adjustment by using the **R/G/B** component adjusting sliders in the dialogue box. Each slider has its left end set at "1", increasing its value when shifted rightward. The value thus set will be added to its corresponding color compo-

9 Set View Range

If the camera captures a 10bit(30bit)-or-above image, you can use the **Set view range** function. This function allows you to specify the intensity range, within which you want to have your image data displayed in the image window. Any image data whose intensity is out of the specified range will be set black or white.
The view range setting can be also achieved automatically during the camera focus setting, image previewing and capturing processes by selecting the **Auto scaling** function provided in the **Image Capturing Palette** as shown in the following figure.

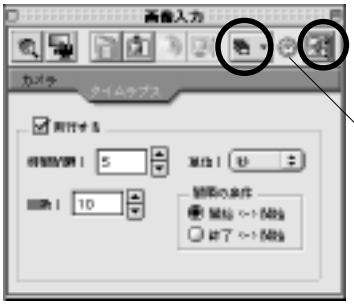
①From the **Image Capturing Palette**, select  to display the **Set view range** dialogue box as shown in the following figure.

②When you want to select the **Auto scaling** function to cause the system to set the view range automatically, deactivate the **No auto scaling** check box with no check mark put in it.

③When you want to set the view range manually, activate the **No auto scaling** check box with a check mark put in it. Then, enter the minimum and maximum values of the view range you need in the **Minimum** and **Maximum** text boxes, respectively.

The view range you can set varies with the number of bits of the image to be captured in the system.
If you want the system to optimize the maximum and minimum values of the view range you set manually, select the **Optimize** button.

10 Time-lapse Capture Image



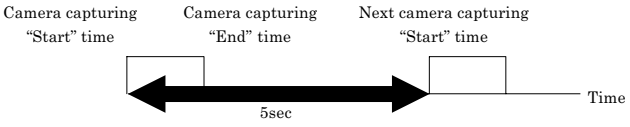
When the **Time-lapse** tab is selected, appears in the **Image Capturing Palette** at this position.

①From the **Image Capturing Palette**, select the **Time-lapse** tab. The **Image Capturing Palette** will then change to such one as shown in the above figure.

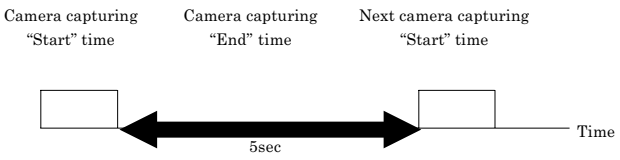
In the **Image Capturing Palette**, activate the **Execute** check box with a check mark put in it. Set parameter **Time interval** by selection from 1 to 32,767 to specify the time interval at which to capture your image data into the system. Parameter **Unit** can be set by selection from second, minute and day to specify the unit of time for the value entered in the **Time interval** text box. Parameter **No. of cycles** is used to set the number of times you want the camera to capture your image data at the specified time interval. The **No. of cycles** parameter can be set by selection from 1 to 10,000. The **Interval condition** group box with two options – **Start <-> Start** and **End <-> Start** is used to select whether to define the **Time interval** as the time duration from the camera capturing "Start" time to the next camera capturing "Start" time or the time duration from the camera capturing "End" time to the next camera capturing "Start" time as described in the following figures.

< Description of **Interval condition** group box >

・When option Start <-> Start is selected, the Time interval will be defined as follows:



・When option End <-> Start is selected, the Time interval will be defined as follows:



②Select the format in which you want to have your image data captured into the system.

From the **Image Capturing Palette**, select  to display a list of options. From these options, select **Stack window** in this case as a typical format in which to capture your time-lapse image data into the system. The **Stack window** format allows your time-lapse captured images to be created as one stack window, which can display these multiple image windows collectively at one time.

If you select **File** from the options list, displaying the **Save file** dialogue box, you can save your time-lapse captured image data as files by specifying the file name in the dialogue box.
For example, if you enter TEST in the file name text box in the dialogue box, your time-lapse captured images will be saved as files with file names “TEST00000”, “TEST00001” and so on. It should be noted in this connection that if, after this time-lapse image capturing process is completed, another time-lapse image capturing is performed with the same file name entered in the **Save file** dialogue box, the newly time-lapse captured image data will overwrite the previously stored image data.

③From the **Image Capturing Palette**, select  to capture your image into the system in the **Stack window** format selected in ② above.

11 Save Image

Save your time-lapse captured stack window, created in 10 above, onto your disk as a file.

①From the **File** menu, select the **Save** command.

②In the **Save** dialogue box thus displayed, specify the name of the file in which you want to save the stack window by, in this case, entering STACK TEST in the **File name** text box. Then, press the OK button.

Chapter 7

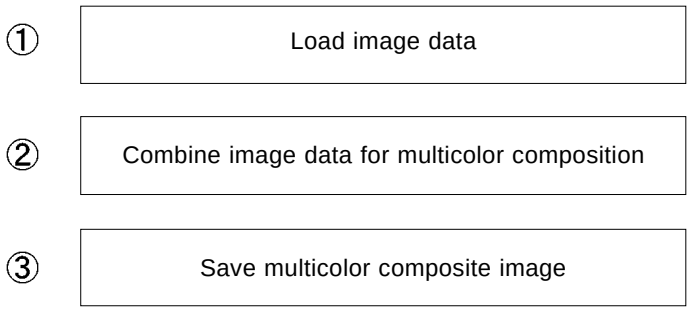
Image Analysis Procedure 1

1.Analysis Flowchart
2.Capture Image
3.Combine Image Data for Multicolor Composition
4.Save Multicolor Composite Image

Chapter 7 Image Analysis Procedure 1

1 Analysis Flowchart

In this chapter, let's operate LuminaVision program to combine more than one image captured from a fluorescence camera into the system using a different excitation light from the one used in Chapter 5 to create one piece of image data according to the following flowchart:



2 Load Image Data

Load the image data captured into the system according to the procedure described in Chapter 5 using a different excitation light to the one used in Chapter 5. In this case, use a certain sample image data saved in the particular folder in the hard disk as described below.

①In the **File** menu, click on the **Open** command to display the **Open** dialogue box on the screen.

②From the **LuminaVision** folder displayed in the dialogue box, select **Img** folder. From this folder, select image files **sample_r**, **sample_g** and **sample_b** to load them.

3 Combine Image Data for Multicolor Composition

①Open the **Process** menu. From the menu, select the **Combine image data for multicolor composition** command.

②Specify the resolution of the image to be obtained by processing the three sample images for multicolor composition.
In the **Combine image data for multicolor composition** dialogue box as shown in the following figure, select the **Set** tab. In the **Size** group box, click on the pop-up button to select the resolution of the multicolor composite image you want as the numbers of its horizontal and vertical pixels. If you want to use the **No. of pixels (H)** and **No. of pixels (V)** text boxes to set your selected image resolution, click on the **Size** pop-up button to select **Optional**. In this case, set the **Size** pop-up button to select 640(H)×480(V).

③Then, in the **Process image for multicolor composition** dialogue box, select the **Select** tab. From the image windows thus displayed in the dialogue box, select the images to be combined for multicolor composition.

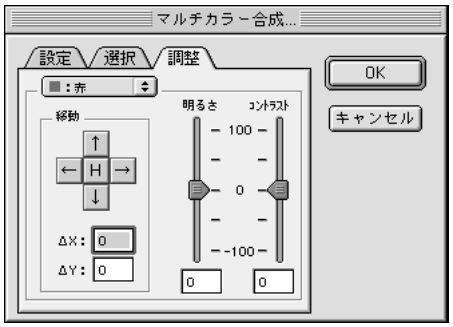
④Click on the [Red] pop-up button to select image file **sample_r** as a red component to be combined with the other two components to create a multicolor composite image.

⑤Click on the [Green] pop-up button to select image file **sample_g** as a green component to be combined with the other two components to create a multicolor composite image.

⑥Click on the [Blue] pop-up button to select image file **sample_b** as a blue component to be combined with the other two components to create a multicolor composite image.

＊This case involves the use of only three colors for multicolor composition. However, the software has been designed to allow the use of yellow, magenta, cyan and grayscale, providing for seven-channel multicolor composition.
＊When steps ④～⑥ are completed to select the three color components for multicolor composition, the resultant multicolor composite image will be previewed.

⑦When you want to adjust the positions of the three images combined, their brightness and/or contrast, select the **Adjust** tab in the dialogue box.



⑧Click on the **Select component** pop-up button to select the color component of the image you want to adjust for its position, brightness and/or contrast. In this case, set the pop-up button to select the Red component.

⑨To adjust the position of the color component, use the ↑ ↓ ← → buttons in the **Move** group box. If you want to have the color component returned to its original position, select the H button.

⑩To adjust the brightness and contrast of the color component, use their respective longitudinally-arranged sliders in the dialogue box.

⑪If all the necessary adjustment is completed and confirmed proper, select OK. The three color images will be combined as set to create a multicolor composite image window.

＊The multicolor composite image window is a 24bit color-fixed image.

❹ Save Multicolor Composite Image

Save the multicolor composite image window created in ❸ .

①In the File menu, select the **Save As** command.

②In the **Save As** dialogue box thus displayed, specify the name of the file in which you want to save the multicolor composite image window by, in this case, entering MULTI in the **File name** text box. Then, press the OK button.

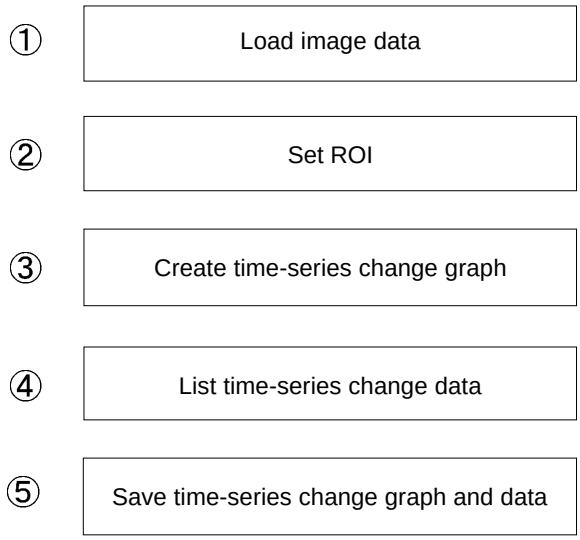
Chapter 8

Image Analysis Procedure 2

Chapter 8 Image Analysis Procedure 2

1 Analysis Flowchart

In this chapter, let's operate LuminaVision program to set a ROI in the stack image window loaded onto the screen and measure the ROI in the image for its intensity according to the following flowchart:



2 Load Image Data

Load the image data time-lapse captured into the system according to the procedure described in Chapter 6. In this case, use a certain sample image data saved in the particular folder in the hard disk as described below.

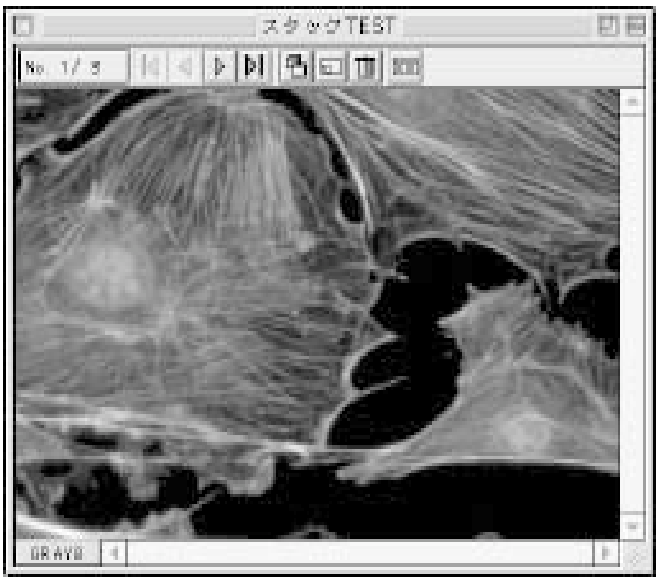
①In the **File** menu, click on the **Open** command to display the **Open** dialogue box on the screen.

②From the **LuminaVision** folder displayed in the dialogue box, select **Img** folder. From this folder, select image files **sample_t1**, **sample_t2** and **sample_t3** to load them.

③In the window menu, select **Stack** to display the **Stack** dialogue box as shown in the following figure.

④Enter STACK TEST in the **file name** text box and activate the **Stack all applicable windows** check box with a check mark put in it.

⑤If all the setting is completed, select the OK button. The three image files will be stacked to create a stack window with a file name of STACK TEST as illustrated in the following figure.



❸ Set ROI

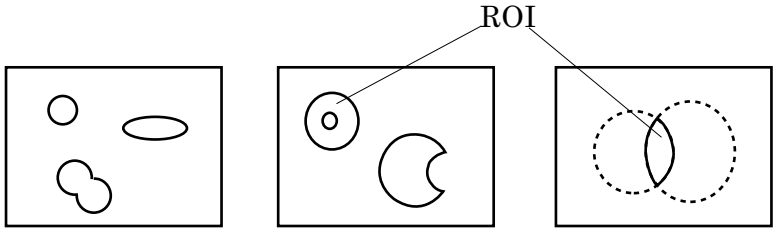
①From the **Tool** bar, select the  button.

② Set a circular ROI in the stack window created in ❷.
The setting of the ROI can be achieved by placing the mouse cursor at its starting point and dragging the mouse to its ending point.

<Further information about ROI>
You can use  in the **Tool** bar for ROI setting.
When you select one of these buttons by double-clicking the mouse button, the **Set ROI** dialogue box will appear on the screen as shown in the following figure.

When you click on either  or  , the dialogue box gives you three options from which you can choose one as the method of setting the size of your rectangular or circular ROI – **Set standard size** which allows you to use the mouse to set the ROI size optionally, **Set size with aspect ratio fixed** and **Fix size** which allows you to set the ROI size in pixels. When you select  , the dialogue box gives you four options from which you can choose one as the method of setting the length of your linear ROI – **Set standard length** which allows you to use the mouse to set the ROI length optionally, **Fix length as horizontal line** and **Fix length as vertical line** which both allow you to set the ROI length in pixels, and **Fix length and angle**.

When you select one of  for ROI setting, you can use ⌘ or option to set such ROI as illustrate in the following figures:



(1) (2) (3)

(1) When you click on ⌘ , the mouse cursor will have + added to it at its lower right position to allow you to create more than one ROI, which can be connected to each other.

(2) When you click on option , the mouse cursor will have – added to it at its lower right position to allow you to specify the area you want to delete from your ROI, for example, to create a doughnut-shaped ROI.

(3) When you click on ⌘ + option , the mouse cursor will have × added to it at its lower right position to allow you to create the overlapping ROI area as a new ROI.

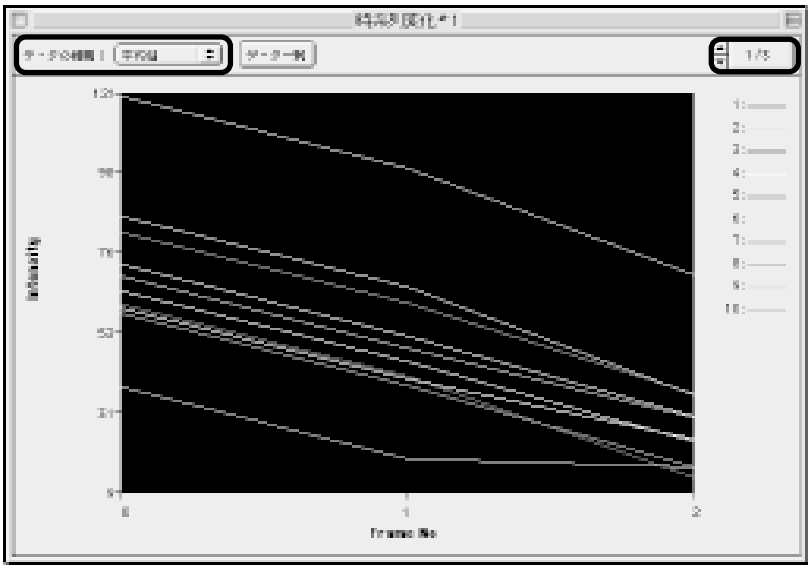
❹ Create Time-series Change Graph

Measure the intensity of the ROI set in the stack window in ❸ .

①From the **Analyze** menu, select **Create time-series change graph**.

②The **Create time-series change graph** window will appear to display the measurement data on the intensity of the ROI in the stack window, the type of which can be selected by clicking on the **Type of data** button from **Minimum**, **Maximum**, **Total**, **Average** and **Standard deviation**.

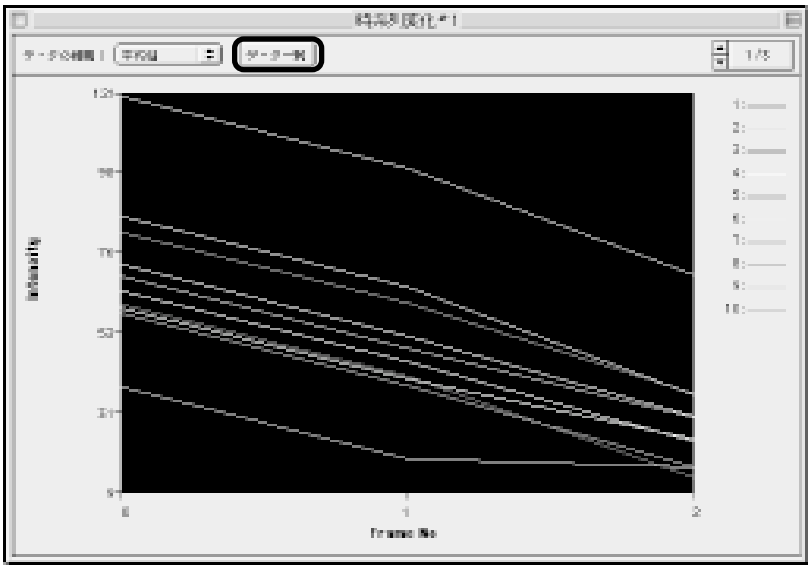
The **Create time-series change graph** window can normally accommodate up to 10 ROIs for display of their intensity measurement data. If you want to display the measurement data for more than 10 ROIs in the window, you can use the 1/2 button.

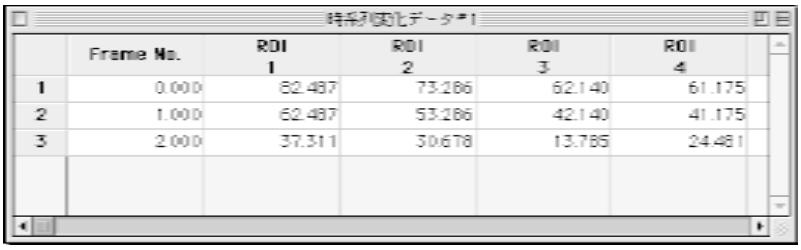


5 List Time-series Change Data

Process the data displayed in 4 for numerical representation in a tabular form.

①In the **Create time-series change** graph window, select the List data button.





	Frame No.	ROI 1	ROI 2	ROI 3	ROI 4
1	0.000	62.487	73.286	62.140	61.175
2	1.000	62.487	53.286	42.140	41.175
3	2.000	37.311	30.678	13.785	24.481

When the List data button is selected, the **List time-series change data** window will appear as shown in the above figure.

❻ Save Time-series Change Graph and Data

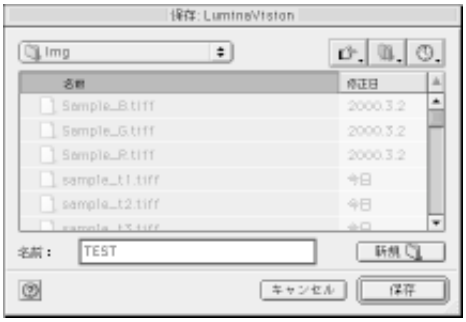
Save the time-series change graph and data obtained in ❹ and ❺ , respectively.

①Select the **Create time-series change graph** window.

②In the **File** menu, select **Save** to display the **Save** dialogue box as shown in the following figure.

③In the **Save** dialogue box, specify the name of the file in which you want to save the time-series change graph by, in this case, entering TEST in the **File name** text box. Then, press the OK button.

※ In this saving, only the graph displayed in the window will be saved as an image data file.



④Select the **List time-series change data** window.

⑤In the **File** menu, select **Save** to display the **Save** dialogue box as shown in the following figure.

⑥In the **Save** dialogue box, specify the name of the file in which you want to save the time-series change graph by, in this case, entering TEST_DATA in the **File name** text box. Then, press the OK button.

※ In this saving, the data displayed in the window will be saved as an text data file.