FUJIFILM

FLUORESCENT IMAGE ANALYZER

FLA-5000

Operation Guide

Science System, Industrial Materials Dept.

Fuji Photo Film Co., Ltd.

Version 1.0 October 2001

Thank you for purchasing our fluorescent image analyzer Model FLA-5000.
This Operator's Guide is intended to provide a brief description of the operating procedures for the image analyzer unit incorporated into your system together with the functional options available from the unit. For a detailed description of the operation of the unit, please refer to the Operating Manual provided.

Index

1	Features of the FLA-5000	3
2	Turning ON/OFF the FLA-5000	3
3	Startup/Shutdown the FLA-5000 Image Reader	4
4	Exposing IPs and Precautions	5
5	Reading IP	6
6	Reading Fluorescent Samples	9
7	Reading Chemiluminescent Samples	16
8	Reading digitize Samples	19

1 Features of the FLA-5000

* The Fluoro Image Analyzer FLA-5000 employs solid-state lasers of wavelengths suitable for the excitation of fluorescent pigments originally developed by FUJI PHOTO FILM Co., Ltd. It combines several lasers of different wavelengths with four types of filters and may read gel, membranes, etc. dyed with various fluorescent pigments. In addition, it may read imaging plates (IPs) originally developed as radiation energy sensors by FUJI PHOTO FILM Co., Ltd.

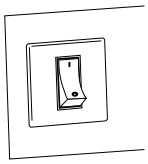
<Main features>

* Makes high-sensitivity, high-resolution images of gel, membranes, etc. dyed with fluorescent pigments.
* Produces ultra high-resolution images equivalent in the quality to X-ray film;
* Accommodates the IP featuring ultra-high sensitivity, wide dynamic range, reliable linearity, high resolution and capability of reuse;
* Ensures high resolution, high sharpness and linearity not attainable by existing filmless systems; and
* Obviates the need for a darkroom and automated processing lab for configuration of the system.

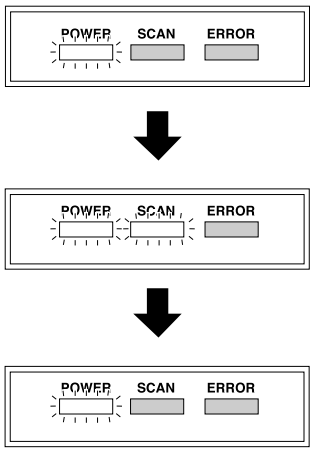
2 Turning ON/OFF the FLA-5000

1 Turning on procedures

1. Press the POWER switch on the right side of the unit to the "I" position.



2. The POWER indicator lamp should light up as shown below to indicate that the unit is correctly turned ON.



Note: If the alarm buzzer sounds during the power-ON sequence (with the ERROR lamp blinking), check if the door of the stage setting block is securely closed.

2 Turning off procedures

Check that scanning is not in progress in the unit, then press the POWER switch on the right side of the unit to the "0" position.

3 Startup/Shutdown the FLA-5000 Image Reader

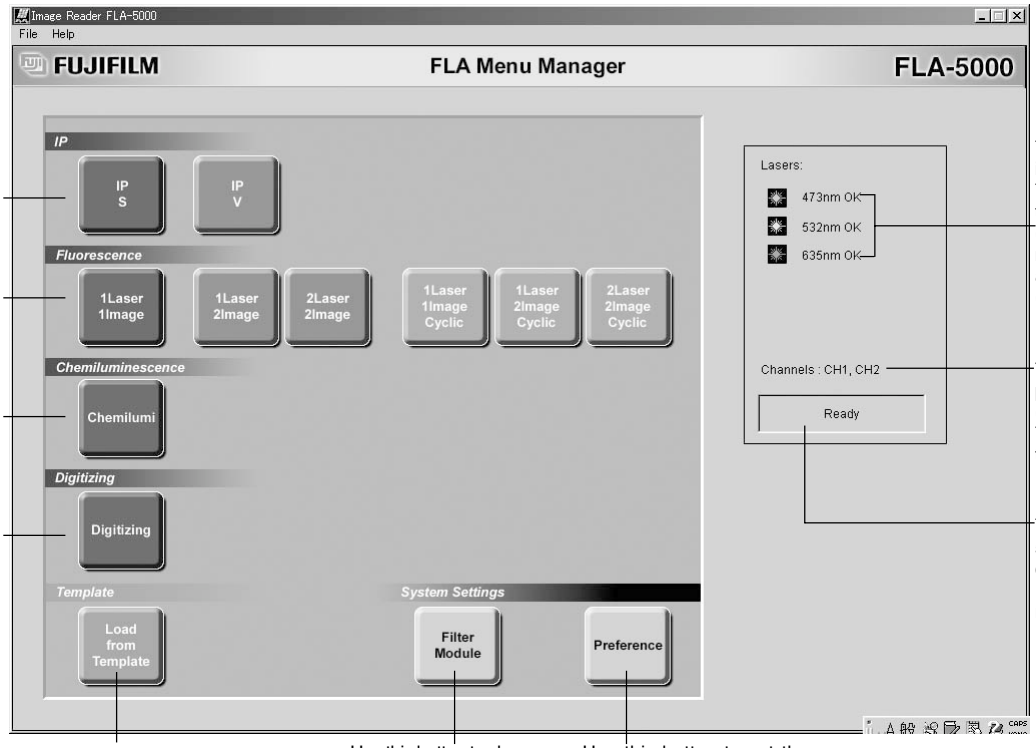
1 Startup procedures

1. Turn on the FLA-5000 and peripheral devices.

2. Turn on the computer (DOS/V PC or Macintosh).

3. Make sure that the FLA-5000 has warmed up. (Only the power lamp on the upper left panel on the front of the FLA-5000 is lit when warming-up is completed.) Start the FLA-5000 Image Reader from the startup menu or using the shortcut key. (On the Macintosh, double-click the alias to start the software.)

4. The main window of the FLA-5000 Image Reader is displayed.



IP mode
Click this button to open the screen for setting the IP reading conditions.

Fluorescent mode
Click this button to open the screen for setting the fluorescent sample reading conditions.

Chemiluminescence mode
Click this button to open the screen for setting the chemiluminescent sample reading conditions.

Digitize mode
Click this button to open the screen for setting the digitize sample reading conditions.

The conditions of the built-in laser units are displayed. This example shows that three types of laser units (473 nm, 532 nm and 685 nm) are built in and all units are ready for operation (OK).

The number of built-in photo-multiplier tubes (PMTs) is displayed. This example shows that two PMTs are built in.

The condition of the FLA-5000 is displayed. This example shows that the FLA-5000 is ready for reading.

Use this button to recall stored reading conditions.

Use this button to change or register the filter.

Use this button to set the display menu and file format.

2 Shutdown procedures

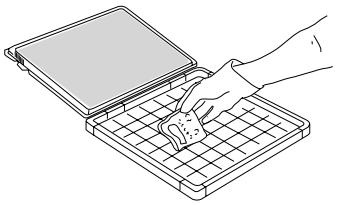
Click on Exit from the File menu.

(On the Macintosh, click on Quit from the File menu.)

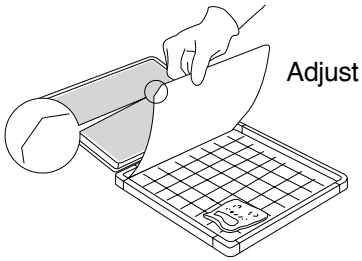
4 Exposing IPs and Precautions

<Procedures for exposure>

1. Immediately before the start of exposure, complete a thorough erasing process.
2. Wrap the sample in Saran Wrap® or other appropriate wrapping film.
3. Put the sample into the cassette with the surface to be scanned facing up.
   For proper position alignment between the sample and the IP, use the grids (inscribed at inter vals of 25 mm) as a guide.



4. Referring to the diagram below, load the IP into the cassette making sure that the surface of the IP for exposure faces the sample.
   Use the notch on the IP as a guide to identify the correct orientation of the sample, the IP and the loading of the sample-containing IP into the carrier.



Adjust the notch of the IP to the left front of the cassette.

5. Close the cassette lid fully until a click is heard.

<Precautions for handling the IP>

1. Keep the IP away from any moisture
   Although the IP is designed to be water resistant, moisture makes it susceptible to a lowering of sensitivity, causing incomplete linearity. Allow the sample to dry out completely before expo sure.
   Tightly wrap any sample that is not dried completely in Saran Wrap to ensure proper moisture tightness.
2. Keep the IP away from any volatile solvents
   Overnight or longer exposure of any sample containing dichloromethane, chloroform, acetone, acetic acid, or other solvent can cause shrinkage of the protective layer on the surface of the IP, leading to deformation of the plate. A deformed IP can cause jamming during the scanning. Wrap such samples with a double layer of Saran Wrap to ensure proper air tightness before exposure.
3. Wear cotton gloves
   When handling the IP, wear cotton gloves to protect the surface of the IP against any contami nation. When removing the IP from the cassette, use a suction disc. Using the bare hands (fingernails) can cause the edges to gradually peel off, eventually resulting in an unusable IP (due to the failure of scanning caused by jamming in the unit).
4. Use a dry cotton cloth for cleaning the IP
   Clean the surface of the IP with a dry cotton cloth or other appropriate material. Never use a water-dampened cloth. For any heavy contamination, use a cloth with a small amount of anhydrous ethanol (Extra Pure or Guaranteed Reagent). In actuality, however, it is necessary to ensure that such appropriate anhydrous ethanol to be used has been kept in a brown re agent bottle or stored in the proper environment recommended by the maker, since any anhy drous ethanol stored under incorrect storage conditions can cause deterioration of the IP.
5. Keep the IP away from any light source after completion of exposure
   After exposure, keep the IP strictly hidden from any direct light source. If the IP is exposed to light, the image information in the IP may be lost.

5 Reading IP

Set the reading conditions.

↓

Set an IP on the stage.

↓

Set the stage on the FLA-5000.

↓

Start reading.

1. Turn on the FLA-5000 and peripheral devices.
2. Turn on the computer (DOS/V PC or Macintosh).
3. Make sure that the FLA-5000 has warmed up. (Only the power lamp on the upper left panel on the front of the FLA-5000 is lit when warming-up is completed.) Start the FLA-5000 Image Reader from the startup menu or using the shortcut key. (On the Macintosh, double-click the alias to start the software.)
4. The main window of the FLA-5000 Image Reader is displayed.
5. Select a reading mode and set the reading conditions.

5.1 Selecting a reading mode

Two IP reading modes are available as shown below:

.....This is the standard reading mode, whose reading sensitivity is fixed. Values of read images are displayed in the PSL/mm2 unit system.

.....The sensitivity of this mode is variable. Use this mode if you need a low reading sensitivity. Values of read images are displayed in the LAU/mm2 unit system.

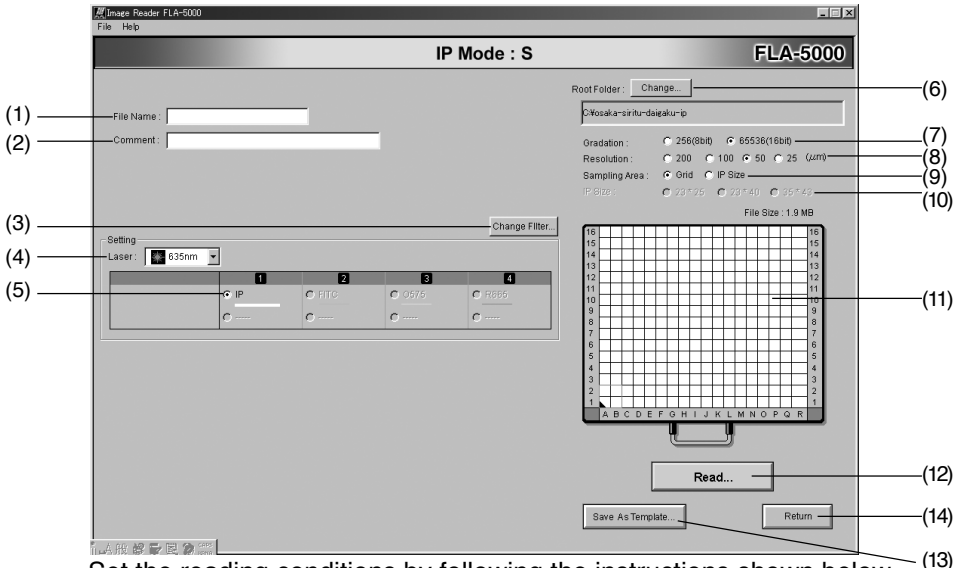
IP S mode

5-1-1 IP S mode

Click the 

button.

The following window appears.



Set the reading conditions by following the instructions shown below.

(1) File Name
Assign a file name to the read image data for saving.

(2) Comments
Enter any comments required for saving with the assigned file name.

(3) Change Filter...
Use this button when changing any filter in formation. (No need to use this option in the normal IP reading operation.)

(4) Laser
Select the laser to be used. (In the IP reading mode, the 635nm laser will be automatically selected.)

(5) Filter
Select the filter to be used for reading. (In the IP reading mode, the IP filter will be automatically selected.)

(6) Root Folder Change
Specify where to save the file for saving the image data.

(7) Gradations
Click to select the number of gradations of a read image.

(8) Resolution
Click to select the pixel size for reading.

(9) Sample Area
Click to select the method for setting the reading area.

(10) IP Size
Remember to select the size of the IP when IP Size has been selected for Sample Area in (a).

(11) Reading Area
The actual reading area will be shown in the form of a red-colored box. As neces sary, move the cursor onto the red frame or to any point in the red box and drag the box to change the size and/or position of the reading area.

(12) Read
Click on this button to start the reading operation.

(13) Save Template
Use this button to save the reading conditions in a file. (For details, see the Operation Manual.)

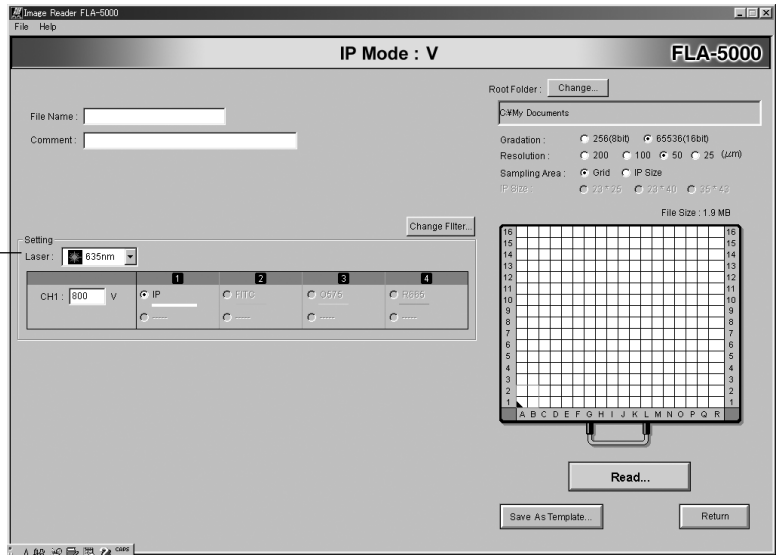
(14) Return
Click on this button to return to the Main window.

IP V mode

5-1-2 IP V mode

Click the  button.

The following window appears.



(1)

For the setting of any additional conditions other than those described below, refer to the instructions for Setting Conditions in the IP S Mode (page 6).

(1) 

This voltage is applied to the first PMT.

Input a voltage value directly in this box. (You may input a value between 250 and 1000.) The greater the value is, the higher the voltage to be applied to the PMT and the reading sensitivity are.

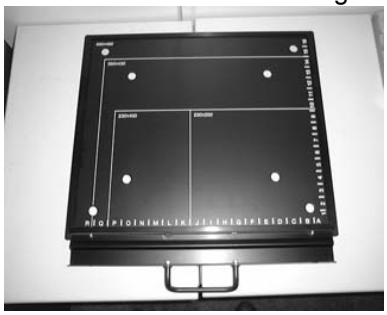
Small ◄—— Value ——► Large

Low ◄—— Sensitivity ——► High

6. Place the IP on the IP stage.

* You may use only IPs with magnetic absorption layers on the back on the IP stage. Since an IP is stuck on the IP stage by the magnetic force, any IPs without magnetic absorption layers may not be attached to the IP stage. Such IPs are unusable.

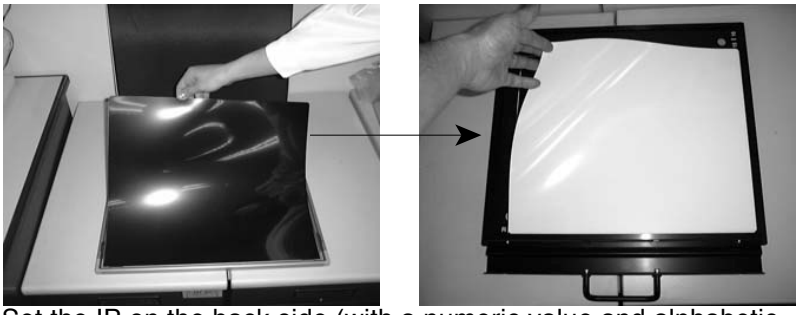
6-1 Put the IP cassette with an exposed IP on the side of the IP stage.



6-2 Turn down the room lighting (below 20 luxes).

(Keep the room lighting turned down until the IP stage is set and the main cover is closed.)

6-3 Take the IP out of the IP cassette and set it on the IP stage immediately.



Set the IP on the back side (with a numeric value and alphabetic characters) of the IP stage so that the IP reading surface (white or blue side with fluorescent material applied) faces up.

7. Load the IP stage into the FLA-5000 body.

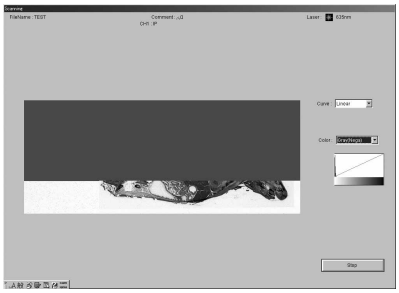
7-1 Open the door of the stage setting block, and set the IP stage with the stuck IP facedown.

7-2 Push the IP stage to the very end (until it butts).

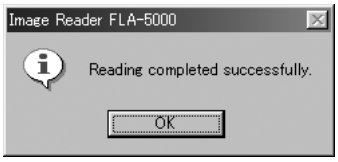
7-3 Close the door of the stage setting block.

8. Starting reading

8-1 Click the Read... button, and reading starts.



8-2 When reading finishes normally, the following dialog box appears. Click the OK button.

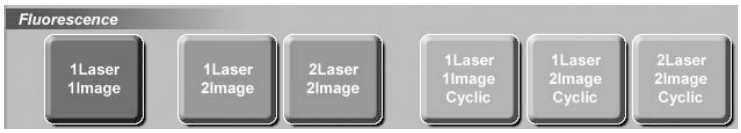


8-3 You may finish reading at any time before the whole reading area is read. Click the Stop button when you want to finish reading.

6 Reading Fluorescent Samples

Set the reading conditions.
↓
Set a Fluorescent Samples on the stage.
↓
Set the stage on the FLA-5000.
↓
Start reading.

1. Turn on the FLA-5000 and peripheral devices.
2. Turn on the computer (DOS/V PC or Macintosh).
3. Make sure that the FLA-5000 has warmed up. (Only the power lamp on the upper left panel on the front of the FLA-5000 is lit when warming-up is completed.) Start the FLA-5000 Image Reader from the startup menu or using the shortcut key. (On the Macintosh, double-click the alias to start the software.)
4. The main window of the FLA-5000 Image Reader is displayed.
5. Select a reading mode and set the reading conditions.



5-1 The number of modes available for reading fluorescent samples depends on the number of photo-multiplier tubes (PMTs) built in the FLA-5000 as shown below.

One PMT is built in the FLA-5000 → Two reading modes are available.
Two PMTs are built in the FLA-5000 → Six reading modes are available.

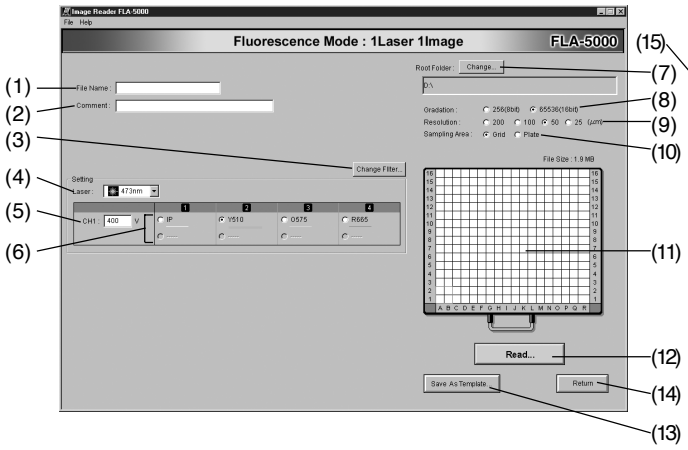
Available when one PMT is built in.

- 1 laser 1 image ... One laser is used to excite fluorescent samples. Fluorescent light from a sample passes through a single filter and forms a single image.
- 1 laser 1 image Cyclic ... 1-laser, 1-image reading is repeated (up to four times).

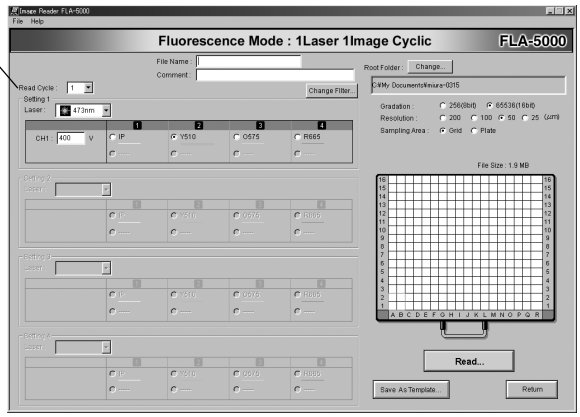
Available when two PMTs are built in.

- 1 laser 2 image ... One laser is used to excite fluorescent samples. Fluorescent light from a sample is divided and filtrated through two filters to form two images.
- 1 laser 2 image Cyclic ... 1-laser, 2-image reading is repeated (up to two times).
- 2 laser 2 image ... Two lasers are used to excite fluorescent samples simultaneously. Fluorescent light from a sample is divided and filtrated through two filters to form two images.
- 2 laser 2 image Cyclic ...2-laser, 2-image reading is repeated (up to two times).

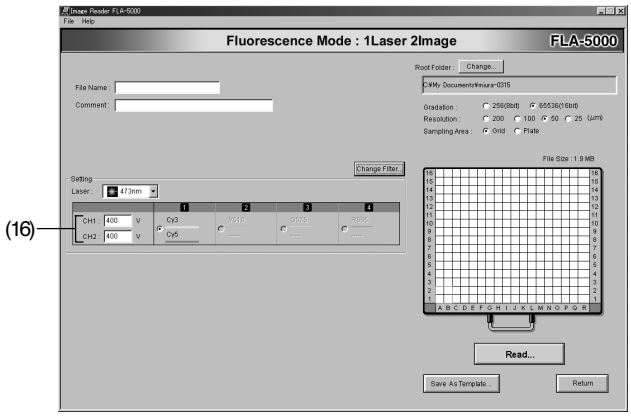
1laser 1image



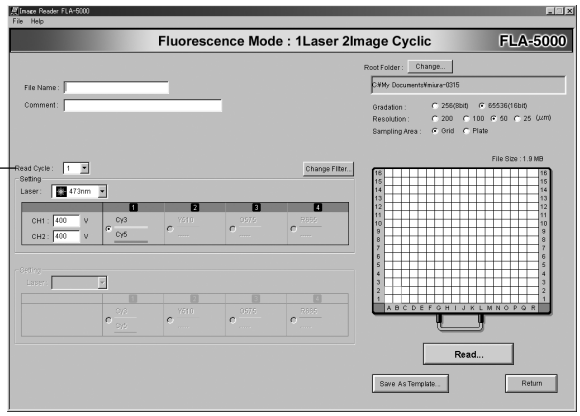
1laser 1image Cyclic



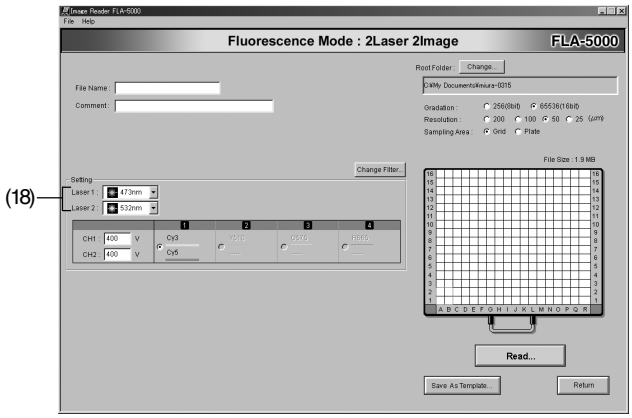
1laser 2image



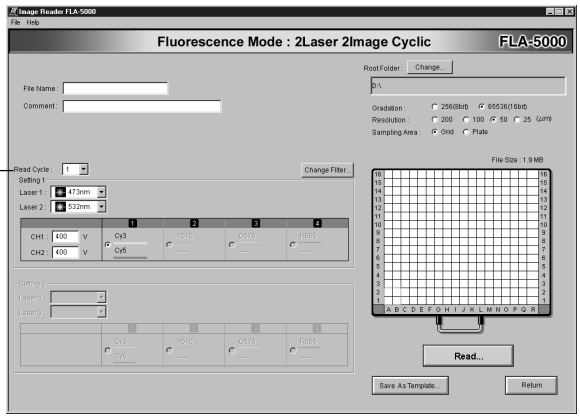
1laser 2image Cyclic



2laser 2image



2laser 2image Cyclic



(1) File Name
Assign a file name to the read image data for saving.

(2) Comments
Enter any comments required for saving with the assigned file name.

(3) Change Filter...
Use this button when changing any filter in formation. (No need to use this option in the normal IP reading operation.)

(4) Laser
Select the laser to be used from the pull-down menu.

(5) 

Enter the voltage value to be applied to the first PMT. (Voltage values must be an integer between 250 and 1000.)

(6) Filter
Select the filter to be used for reading.

(7) Root Folder Change
Specify where to save the file for saving the image data.

(8) Gradations
Click to select the number of gradations of a read image.

(9) Resolution
Click to select the pixel size for reading.

(10) Sample Area
Click to select the method for setting the reading area.

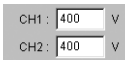
(11) Reading Area
The actual reading area will be shown in the form of a red-colored box.
As necessary, move the cursor onto the red frame or to any point in the red box and drag the box to change the size and/or position of the reading area.

(12) Read
Click on this button to start the reading operation.

(13) Save Template
Use this button to save the reading conditions in a file. (For details, see the Operation Manual.)

(14) Return
Click on this button to return to the Main window.

(15) Read Cycle (a)
Specify how many times to repeat the reading cycle. It is possible to repeat reading up to four (4) times in the “1 laser 1 image Cyclic” mode.

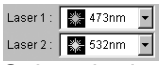
(16) 

→Voltage to be applied to the first PMT

→Voltage to be applied to the second PMT

Enter the voltage values. (The voltage values must be an integer between 250 and 1000.)

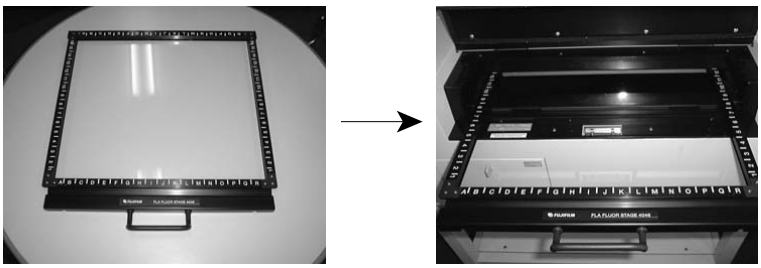
(17) Read Cycle (b)
Specify how many times to repeat the reading cycle. It is possible to repeat reading up to two (2) times in the “1 laser 2 image Cyclic” or “2 laser 2 image Cyclic” mode.

(18) 

Select the laser to be used from the pull-down menu.

6. Set a fluorescent sample on the fluorescent sample stage or multi-stage.
   Setting a gel sample:
   6-1 Set a fluorescent gel sample on fluorescent sample stage.
   When reading a fluorescent gel sample, set the fluorescent sample stage on the FLA-5000 first and set the fluorescent gel sample on the fluorescent sample stage then as shown below. This is an easy way.

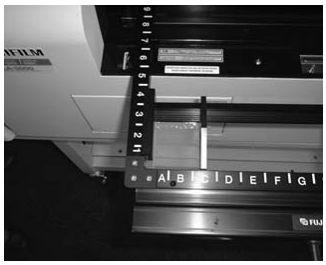
   6.1.1 Open the door of the stage setting block, and set the fluorescent sample stage with the printed frame side faceup on the FLA-5000.



Set the stage in the front position.
Do not pull it to the very end.

   6.1.2 Set the gel stopper as shown below.

   6.1.3 Put a gel sample so that the side with charging holes faces up. Fix it with the gel stopper.



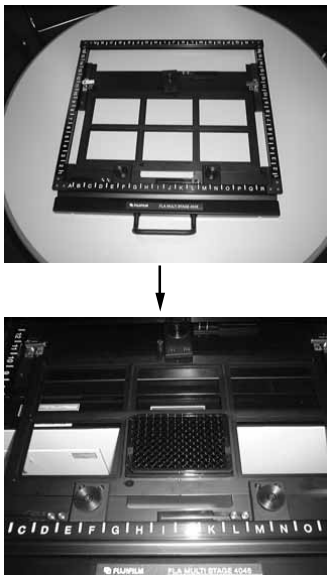
   6.1.4 Press the fluorescent sample stage slowly to the very end (until it butts).

   6.1.5 Close the door of the stage setting block.

Setting the tighter plates:

6.2 Set a tighter plate on the multi-stage.

6.2.1 Set a tighter plate in any position of the tighter plate frame set on the multi-stage.



6.2.2 Open the door of the stage setting block, and set the multi-stage with the printed frame side faceup on the FLA-5000. Push the multi-stage to the very end.

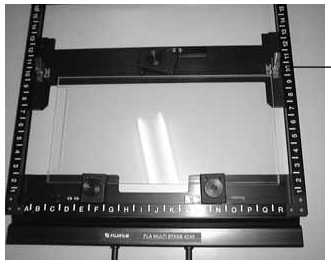
6.2.3 Close the door of the stage setting block.

Setting gel with glass:

6.3 Set a gel sample with glass on the multi-stage.

6.3.1 Detach the tighter plate plug-in as shown below, if it has been set.

6.3.2 Insert one or two gel samples with glass from the front side. Adjust the movable stays to the glass edges (by moving them from the back to the front).



6.3.3 Turn the levers on both sides horizontally to lock them.

6.3.4 Fix the glass with two glass holders in the front position and one glass holder in the rear position. (Turn the dial of each glass holder clockwise to fix the glass.)

There are spacers on the side of the glass holders. Each spacer has two levels. Slide the spacers to adjust the balance of the glass holders.

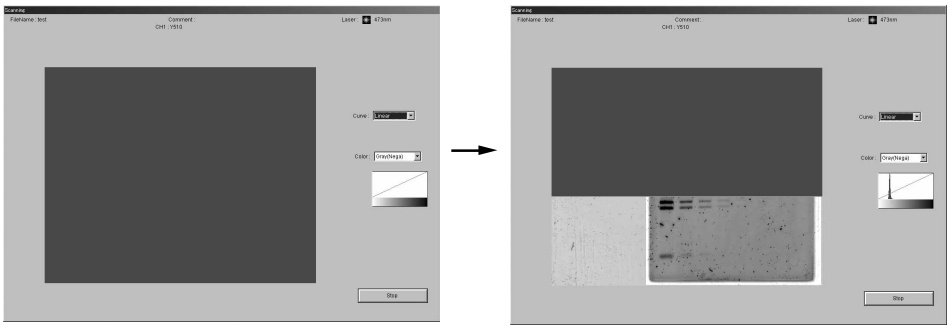
6.3.5 Open the door of the stage setting block, and set the multi-stage with the printed frame side faceup on the FLA-5000. Push the multi-stage to the very end (until it butts).

6.3.6 Close the door of the stage setting block.

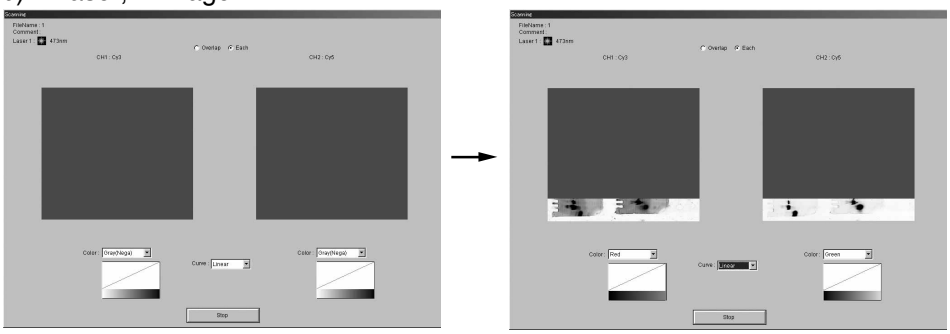
7. Start reading.

7,1 Click the Read... button, and reading starts.
The condition of the preset reading area, where reading is completed, is displayed in the reading status real-time display window. Reading is executed from the front side of the stage to the back. On the monitor screen, read data is displayed from below to above.

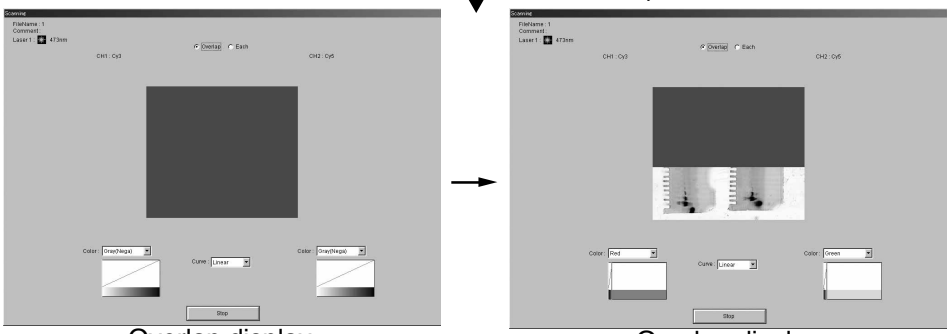
a) 1-laser, 1-image



b) 1-laser, 2-image



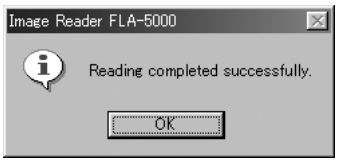
Clicking the radio button on the left alternates Overlap and Each.



Overlap display

Overlap display

7.2 When reading finishes normally, the following dialog box appears.
Click the OK button.



7.3 You may finish reading at any time before the whole reading area is read.
Click the Stop button when you want to finish reading.

7 Reading Chemiluminescent Samples

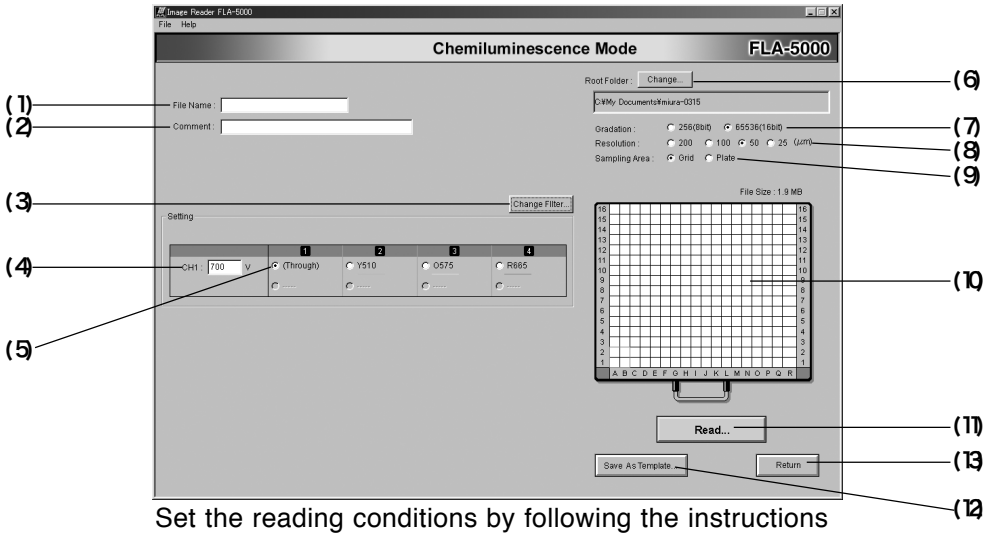
Set the reading conditions.
↓
Set the stage on the FLA-5000.
↓
Set a Chemiluminescent Sample on the stage.
↓
Start reading.

1. Turn on the FLA-5000 and peripheral devices.
2. Turn on the computer (DOS/V PC or Macintosh).
3. Make sure that the FLA-5000 has warmed up. (Only the power lamp on the upper left panel on the front of the FLA-5000 is lit when warming-up is completed.) Start the FLA-5000 Image Reader from the startup menu or using the shortcut key. (On the Macintosh, double-click the alias to start the software.)
4. The main window of the FLA-5000 Image Reader is displayed.
5. Setting the reading conditions

5-1 Click the 

button.

5-2 The following window opens.



Set the reading conditions by following the instructions shown below.

(1) File Name
Assign a file name to the read image data for saving.

(2) Comments
Enter any comments required for saving with the assigned file name.

(3) Change Filter...
Use this button when changing any filter information. (No need to use this option in the normal chemiluminescent sample reading operation.)

(4) 

Enter the voltage value to be applied to the first PMT. (Voltage values must be an integer between 250 and 1000.)

(5) Filter
Select the filter to be used for reading. In the chemiluminescent sample reading mode, no filter is used as a general rule. In the chemiluminscence mode, the filter tray must contain at least one through-position where no filter module is installed. (For details, refer to the Operation Manual.)

(6) Root Folder Change
Specify where to save the file for saving the image data.

(7) Gradations
Click to select the number of gradations of a read image.

(8) Resolution
Click to select the pixel size for reading.

(9) Sample Area
Click to select the method for setting the reading area.

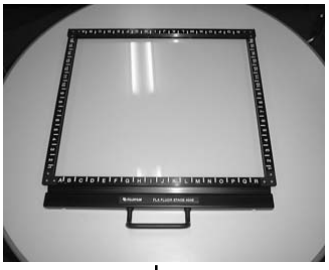
(10) Reading Area
The actual reading area will be shown in the form of a red-colored box.
As necessary, move the cursor onto the red frame or to any point in the red box and drag the box to change the size and/or position of the reading area.

(11) Read
Click on this button to start the reading operation.

(12) Save Template
Use this button to save the reading conditions in a file. (For details, see the Operation Manual.)

(13) Return
Click on this button to return to the Main window.

6. Set the fluorescent sample stage on the FLA-5000.
   6.1 Open the door of the stage setting block, and set the fluorescent sample stage with the printed frame side faceup on the FLA-5000.



Set the stage in the front position. Do not pull it to the very end.

7. Set the chemiluminescent sample on the fluorescent sample stage.
   The following descriptions show an example of setting a membrane sample.
   7.1 Set a membrane sample with the chemiluminescent side facedown on the fluorescent sample stage.

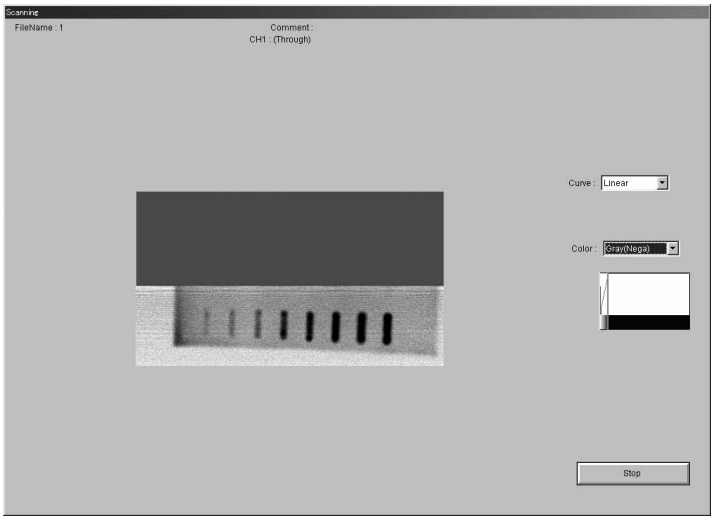
Tape the corners of the membrane to reduce influences of physical vibrations upon the image.

   7.2 Press the fluorescent sample stage slowly to the very end (until it butts).

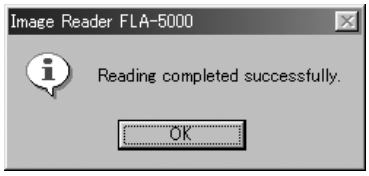
   7-3 Close the door of the stage setting block.

8. Starting reading

8.1 Click the Read... button, and reading starts.



8.2 When reading finishes normally, the following dialog box appears. Click the OK button.



8.3 You may finish reading at any time before the whole reading area is read. Click the Stop button when you want to finish reading.

8 Reading Digitize Samples

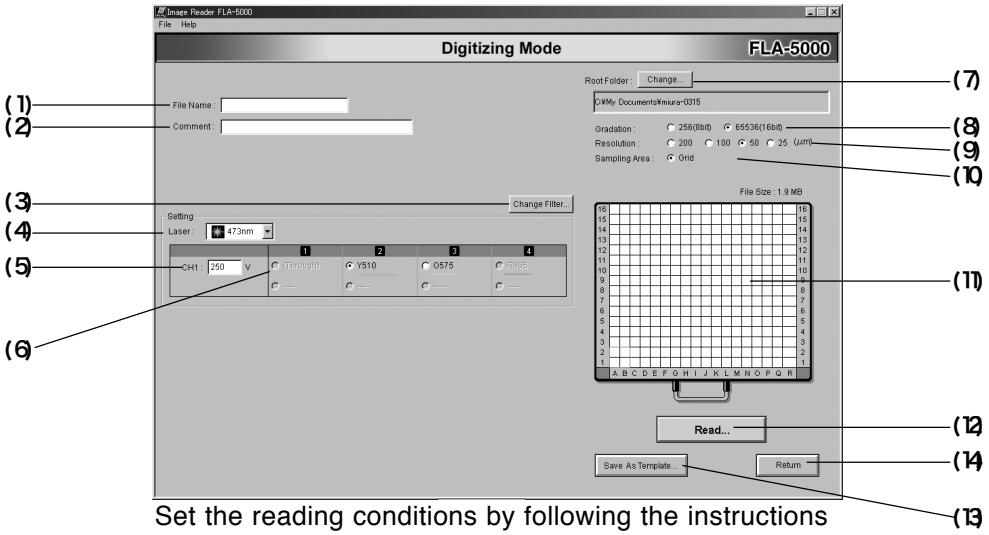
Set the reading conditions.
↓
Set the stage on the FLA-5000.
↓
Set a Digitize Samples on the stage.
↓
Start reading.

1. Turn on the FLA-5000 and peripheral devices.
2. Turn on the computer (DOS/V PC or Macintosh).
3. Make sure that the FLA-5000 has warmed up. (Only the power lamp on the upper left panel on the front of the FLA-5000 is lit when warming-up is completed.) Start the FLA-5000 Image Reader from the startup menu or using the shortcut key. (On the Macintosh, double-click the alias to start the software.)
4. The main window of the FLA-5000 Image Reader is displayed.
5. Setting the reading conditions

   5.1 Click the 

button.

   5.2 The following window opens.



Set the reading conditions by following the instructions shown below.

(1) File Name
Assign a file name to the read image data for saving.

(2) Comments
Enter any comments required for saving with the assigned file name.

(3) Change Filter...
Use this button when changing any filter information. (No need to use this option in the normal chemiluminescent sample reading operation.)

(4) Laser
Select a laser unit to be used for reading from the pull-down menu. (In the digitizing mode, two options are available, 473nm and 532nm.)

(5) 

Enter the voltage value to be applied to the first PMT. (Voltage values must be an integer between 250 and 1000.)

(6) Filter
Click to select the filter to be used for reading. (In the digitizing mode, two options are available, Y510 and O575.)

(7) Root Folder Change
Specify where to save the file for saving the image data.

(8) Gradations
Click to select the number of gradations of a read image.

(9) Resolution
Click to select the pixel size for reading.

(10) Sample Area
Specify the reading area. (in the digitizing mode, the available option is Grid.)

(11) Reading Area
The actual reading area will be shown in the form of a red-colored box.
As necessary, move the cursor onto the red frame or to any point in the red box and drag the box to change the size and/or position of the reading area.

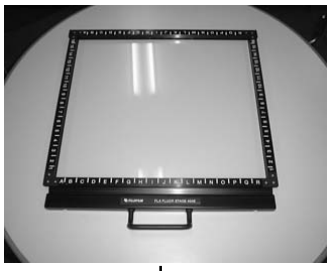
(12) Read
Click on this button to start the reading operation.

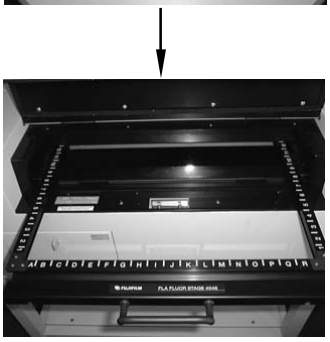
(13) Save Template
Use this button to save the reading conditions in a file. (For details, see the Operation Manual.)

(14) Return
Click on this button to return to the Main window.

6. Set the fluorescent sample stage on the FLA-5000.

6.1 Open the door of the stage setting block, and set the fluorescent sample stage with the printed frame side faceup on the FLA-5000.





Set the stage in the front position. Do not pull it to the very end.

7. Set the fluorescent sample stage on the FLA-5000.

7.1 Set a sample with the migration side facedown on the fluorescent sample stage.

7.2 Put the fluorescent plate for digitizing supplied with the fluorescent sample stage on the digitize sample.
* Put the fluorescent plate with the mat (lusterless) side facedown.

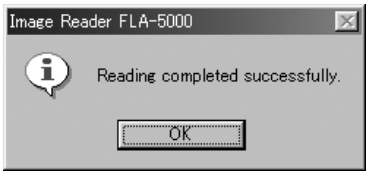
7.3 Press the fluorescent sample stage slowly to the very end (until it butts).

7-4 Close the door of the stage setting block.

8. Starting reading

8.1 Click the Read... button, and reading starts.

8.2 When reading finishes normally, the following dialog box appears. Click the OK button.



8.3 You may finish reading at any time before the whole reading area is read. Click the Stop button when you want to finish reading.

Science System, Industrial Materials Dept.

Fuji Photo Film Co., Ltd.

Version 1.0 October 2001