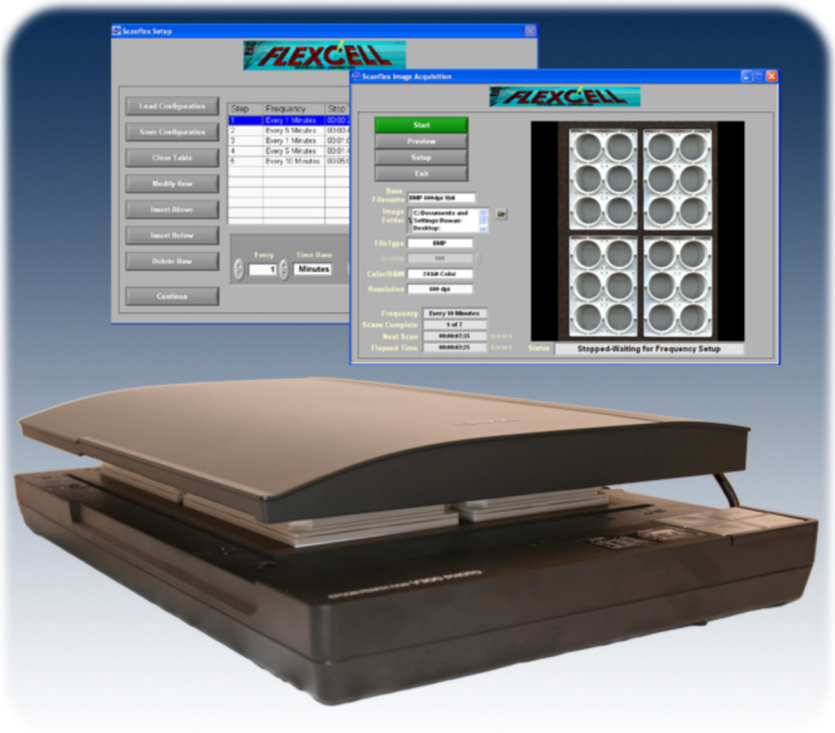
USER MANUAL

ScanFlex™

AUTOMATED SCANNING DEVICE



05-12-17
SCANFLEX 3.1.4
Rev 5.0

Culturing Cells in a Mechanically Active Environment™
Flexcell International Corporation • 2730 Tucker Street, Suite 200 • Burlington, NC 27215
800-728-3714 • (919) 732-1591 • FAX: (919) 732-5196 • www.flexcellint.com

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1. INTRODUCTION

1.1 USE OF SCANFLEX™ SOFTWARE WITH TISSUE TRAIN® CULTURE PLATES

ScanFlex™ software is designed to automate a repetitive scanning processes. It was initially designed to scan 3D bioartificial tissue constructs fabricated using the Flexcell® Tissue Train® Culture System. The ScanFlex™ scanner and software enable the user to create scanning regimens so bioartificial tissues in 6-well or 24-well Tissue Train® culture plates can be scanned periodically to determine the rate of matrix compaction by cells over time. Once the scanning is completed, the series of pictures can be analyzed for area measurement using the Flexcell® XyFlex™ software. The ScanFlex™ software allows the user to create scanning regimens and save the scanned images to a preferred location in a desktop or laptop computer.

1.2 USE OF SCANFLEX™ SOFTWARE FOR OTHER APPLICATIONS

ScanFlex™ software can also be used for any other application requiring periodic scanning. The component to be scanned can be placed in a flat container or on the bed of the scanner. The distance between the scanner bed and the item(s) to be scanned should be no more than 29 mm. It is not recommended to keep extremely hot or cold items on the scanner bed as it could damage the glass.

2. COMPONENTS

- ScanFlex™ software V3.1.4
- Epson Perfection V300 scanner
- Frame for 6-well culture plates
- Frame for 24-well culture plates
- Plate cover
- User manual
- Desktop computer or laptop computer (not provided). See Table 1 for more details.

NOTE: *Table 1 has the minimum system specifications for the computer to operate the scanner. More memory will be needed depending on scanner frequency and image resolution (see Table 3 for more information on image size corresponding to image parameter settings).*

Table 1. *Minimum system requirements for ScanFlex™ and scanner operation.*

Minimum system requirements: USB 2.0/1.1	**CPU**	**RAM**	**HDD**
Microsoft Windows XP, Vista, and Windows 7 (32-bit)	1 GHz	1 GB	95 MB
Microsoft Windows XP, Vista, and Windows 7 (64-bit)	1 GHz	1 GB	95 MB
Minimum screen resolution	1024 × 768 pixels		

3. INSTALLING THE SCANNER SOFTWARE

The scanner software is provided with the scanner. Follow the instructions below to install the scanner software on the computer.

1. It is recommended to supply power to the scanner and connect the USB cable between the computer and scanner before installing the scanner software.
2. Insert the CD that came with the scanner into the CD-ROM drive of the computer.
3. The installation wizard will automatically open. Choose the desirable language.
4. Make sure that antivirus software is disabled. Click *NEXT* in the message screen (Fig. 1).
5. Select the scanner model: Epson Perfection V300 Photo (Fig. 2).
6. Click *INSTALL* on the module selection screen (Fig. 3).
7. On the Epson license screen click *YES* to proceed with the installation (Fig. 4).
8. The scanner installation will be completed in a few minutes.

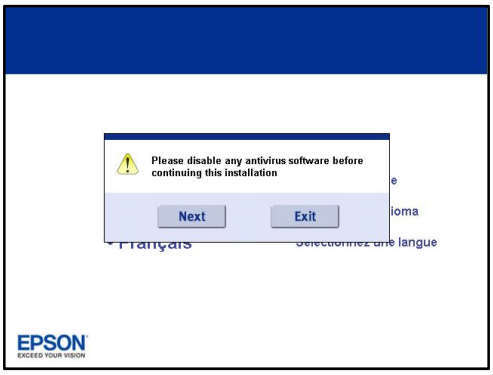


Figure 1. Antivirus software warning during scanner software installation.

Figure 2. Selection of scanner model.

Figure 3. Selection of modules.

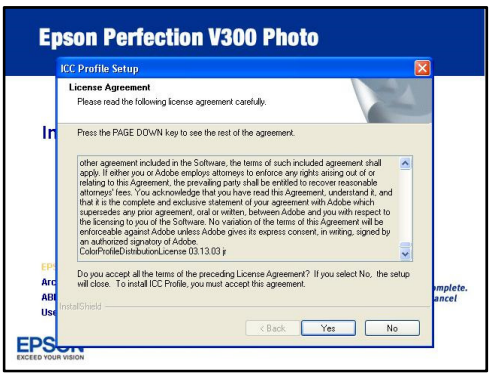


Figure 4. Epson license agreement.

4. INSTALLING SCANFLEX™ SOFTWARE

1. Insert the ScanFlex™ CD into the CD-ROM drive of the computer.
2. The ScanFlex™ installation process should start automatically.
3. Select the destination directory to save all program files (Fig. 5) and click NEXT to continue.
4. Click *NEXT* on the start installation screen (Fig. 6).
5. Once installation is completed, click on *NEXT* in the "installation complete" screen (Fig. 7).
6. In the NI activation wizard screen, select the first activation option (Automatically activate through a secure internet connection) and click on *NEXT* (Fig. 8)
7. Enter the NI Vision Development Module Run-Time License number, which is included with the equipment documentation, in the white box and click *NEXT* (Fig. 9).
8. Select language in the next screen and click *OK*.
9. The EZTwainX setup wizard will start installing. On the EZTwainX setup screen, click *NEXT* to proceed to installation (Fig. 10).
10. On the EZTwainX license agreement screen, select "*I accept the agreement*" and click on *NEXT* (Fig. 11).
11. Select destination location for the EZTwainX files to be saved (Fig. 12).
12. Click on *INSTALL* on the next screen to start installation (Fig. 13).
13. Once installation is completed, click on *FINISH*.

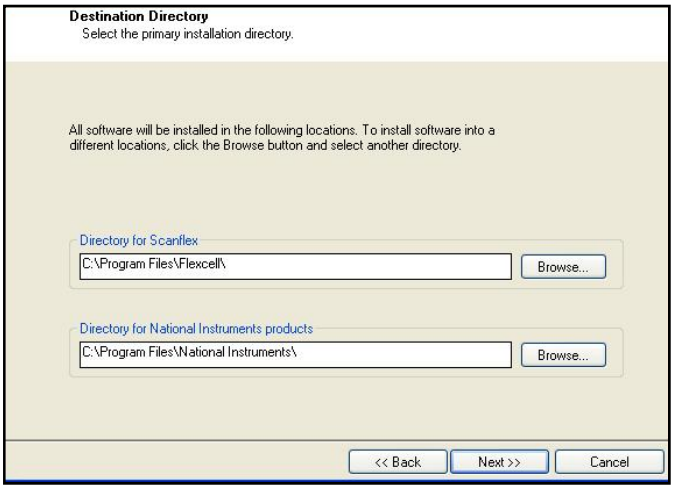


Figure 5. *Selecting destination directory.*

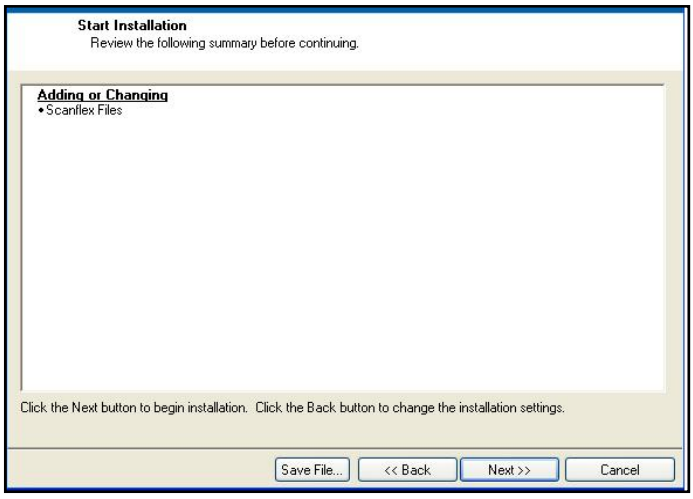


Figure 6. *Installation start screen.*

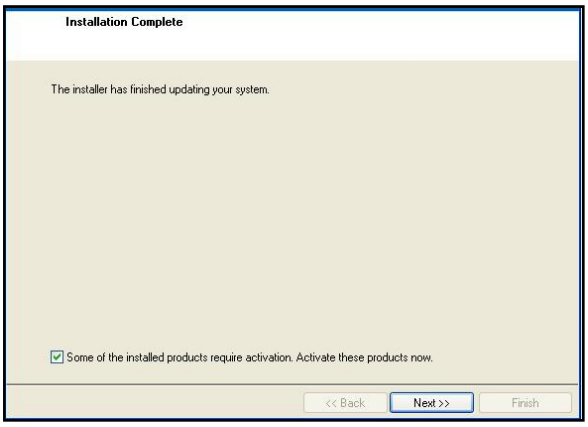


Figure 7. *ScanFlex™ installation complete screen.*

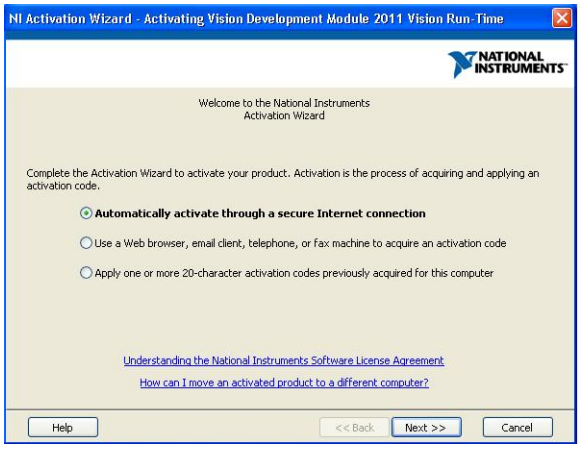


Figure 8. *Options for vision module license activation.*

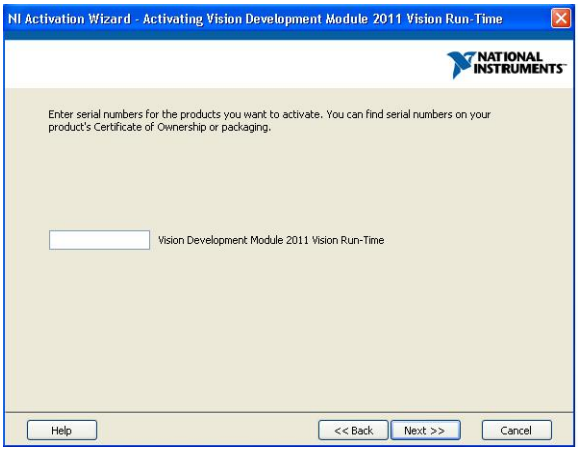


Figure 9. *Screen for entering vision module license.*

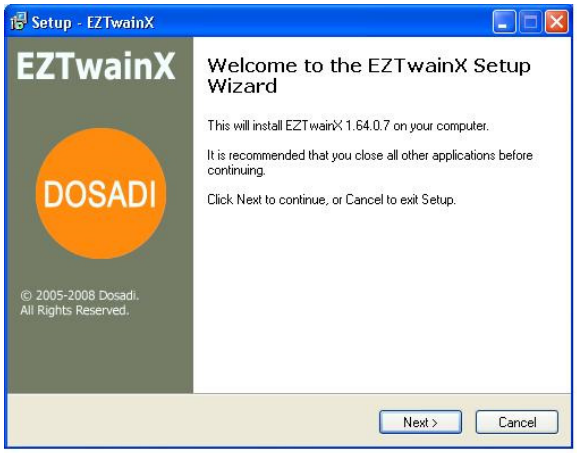


Figure 10. *EZTwainX setup screen.*

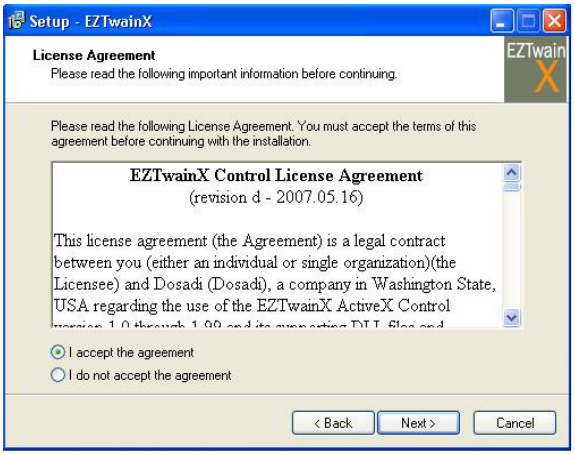


Figure 11. *EZTwainX license agreement.*

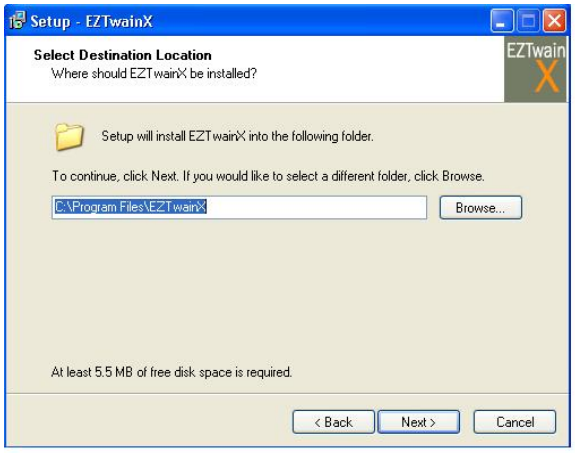


Figure 12. *Selecting location to store the EZTwainX files.*

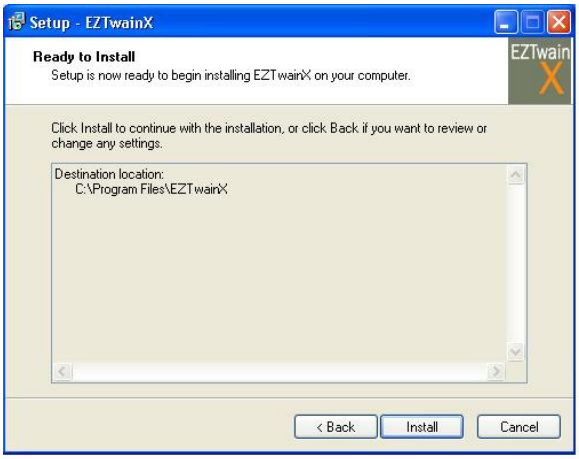


Figure 13. *EZTwainX ready to install screen.*

5. ACTIVATION OF SCANFLEX™ SOFTWARE

1. Click on the ScanFlex™ icon on the desktop or go to program files and open ScanFlex™ software.
2. When the ScanFlex™ activation screen opens up, select either *ACTIVATE SCANFLEX* or *EVALUATE SCANFLEX* (Fig. 14).
3. If *ACTIVATION* is selected, two user codes will be displayed on the next screen (Fig. 15). Call (1-800-728-3714 or 919-732-1591) or email (*activation@flexcellint.com*) Flexcell® International with the user codes to obtain an activation code.

Note: *Do **NOT** close the ScanFlex™ software until the activation codes have been received from Flexcell® International. Doing so will generate new user codes, voiding the previous user codes and the activation code.*

4. Enter the activation code in *ACTIVATION CODE 1* box and click on *CONTINUE*.
5. If *EVALUATE SCANFLEX* is selected, you will have 30 days to use the ScanFlex™ software after which it will automatically deactivate.

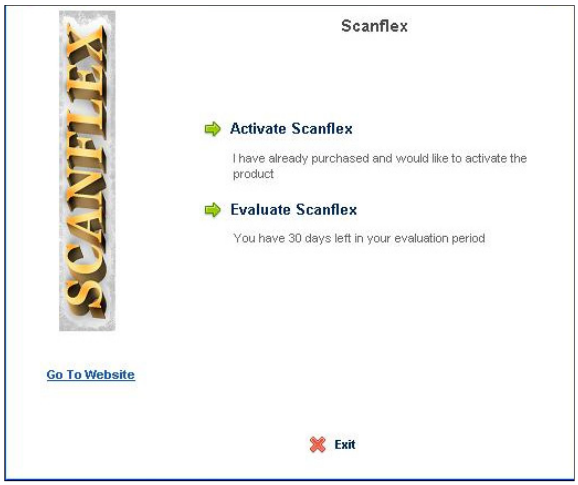


Figure 14. *Options for activating ScanFlex™ software.*

Figure 15. *User code and activation code for ScanFlex™ activation.*

6. SCANNER SETUP

6.1 GENERAL SETTINGS

Plug in the scanner power cable to a 100-240 VAC power outlet. Connect the USB cable that is provided with scanner between the scanner and the computer (Fig. 16). A dedicated computer is recommended to use with the ScanFlex™ software. The computer should also have the following settings turned OFF for long term experiments:

- Auto updates
- Virus scanning
- Sleep mode
- Automatic defragmentation.

The screen saver *should* be turned ON such that a screen burn-in or ghost imaging will not occur if the scanner is used for especially long time periods. The maximum number of scans that can be generated by the ScanFlex™

software is only limited by the memory capacity of the computer to store the scanned images. The program will give an error message and pause scanning if there is no longer space to store the scanned images. Always be certain that you have sufficient memory capacity for all scanned images. The maximum number of images that can be stored for a given amount of memory capacity can be increased by choosing a lower image resolution (see Table 3).

6.2 SCANNING TISSUE TRAIN® CULTURE PLATES

Two different plate frames, one frame for four 6-well plates and one frame for four 24-well plates, are provided with the ScanFlex™ software package (Fig. 17). Place the corresponding plate frame on the scanner making sure that it firmly fits within the scanner area. The frame has markings on its side to identify the location of each culture plate (see Note 1 below). The culture plate location in the frame corresponds to the numbering of the plates and wells in the XyFlex™ software. If the plates are placed according to the instructions, the wells will be named as shown in Figures 18-21 in the XyFlex™ software. See the Appendix for an illustration to identify the corresponding location of wells on the scanned image with reference to their physical orientation on the scanner bed.

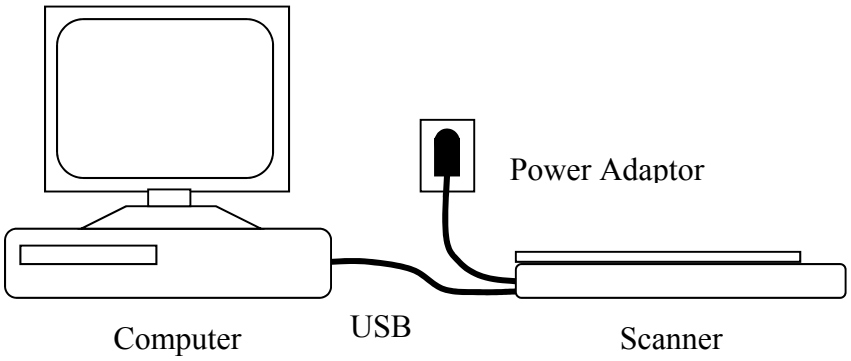
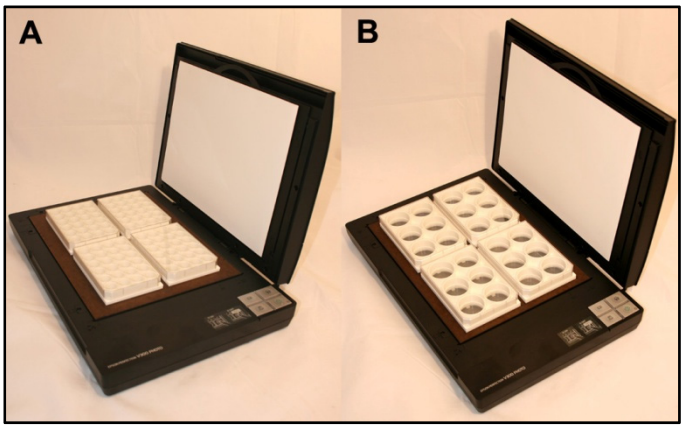


Figure 16. *Scanner and computer setup.*



NOTE 1: *The position of the plate frame triangles must be positioned closest to the power button on the scanner for the well locations defined in Figures 18-21 to be accurate.*

Figure 17. *Arrangement of Flexcell®'s A) 24-well and B) 6-well culture plates on the scanner bed.*

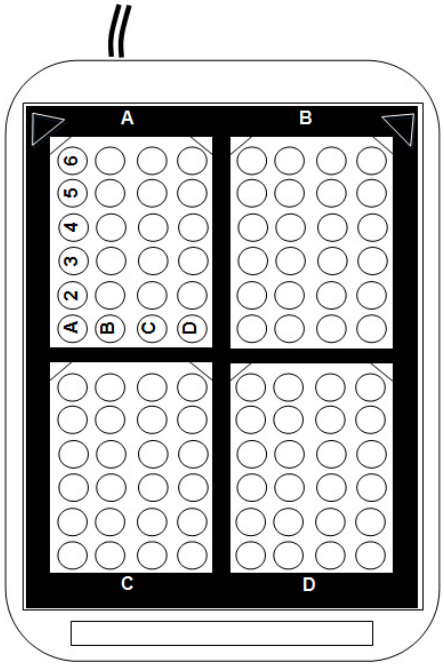


Figure 18. *The orientation and numbering of 24-well culture plates on the scanner when the frame provided is used for placing the culture plates on the scanner. The black area is the frame used to hold the plates on the scanner surface.*

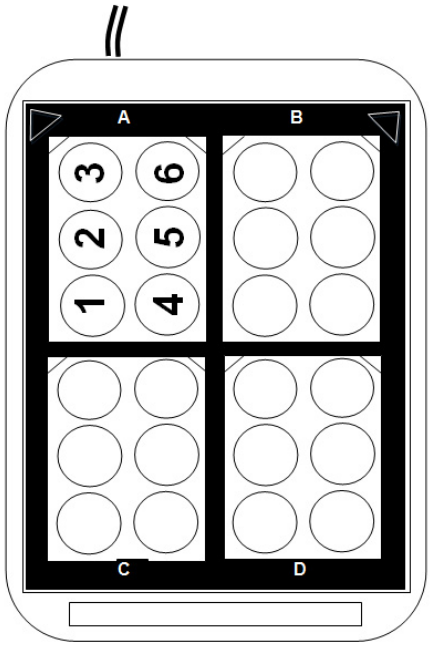


Figure 19. *The orientation and numbering of 6-well culture plates on the scanner when the frame provided is used for placing the culture plates on the scanner. The black area is the frame used to hold the plates on the scanner surface.*

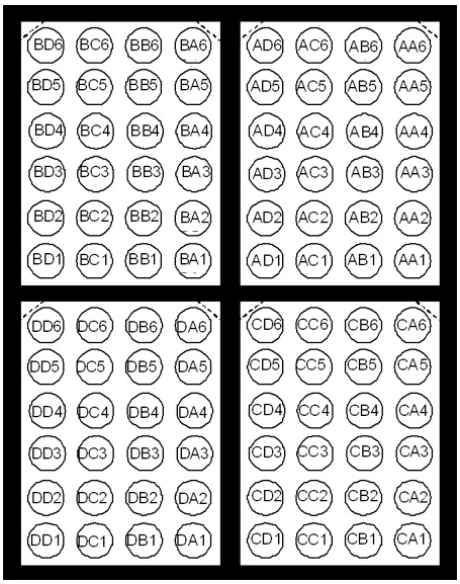


Figure 20. *The orientation and numbering of 24-well culture plate wells in the scanned picture. The black area is the frame used to hold the plates on the scanner surface. This numbering system is used in the XyFlex™ software for identification of wells.*

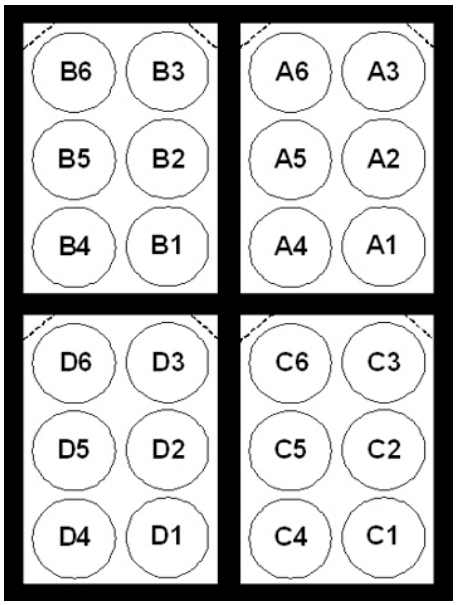


Figure 21. *The orientation and numbering of 6-well culture plate wells in the scanned picture. The black area is the frame used to hold the plates on the scanner surface. This numbering system is used in the XyFlex™ software for identification of wells.*

7. SETTING UP SCANFLEX™ SOFTWARE

7.1 OPENING SCANFLEX™ SOFTWARE

When the ScanFlex™ software is completely installed, the ScanFlex™ icon (Fig. 22) is displayed on the desktop. Click on the icon to open the ScanFlex™ software. The ScanFlex™ start-up screen (Fig. 23) will open up.

Figure 22. ScanFlex™ icon.

Figure 23. *ScanFlex™ start-up screen.*

7.2 CONFIGURING SCANNER

1. To configure the scanner, click on the CONFIGURE SCANNER button in the ScanFlex™ start-up screen (Fig. 23).
2. If the scanner is not already displayed (Fig. 24), click on the SELECT button to open-up the *Select Source* screen.

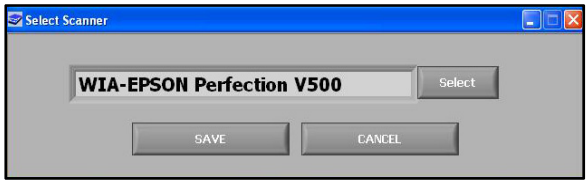


Figure 24. Scanner selection screen.

3. If the scanner is properly installed, it should be displayed in the *Select Source* screen. Click on the correct scanner and click SELECT (Fig. 25).

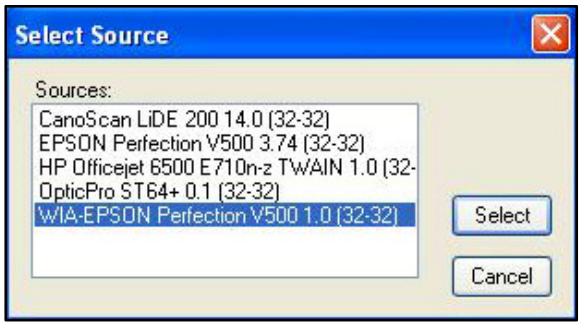


Figure 25. Selection of scanner model screen.

4. If you do not see the scanner in the *Select Source* screen, check the USB cable for proper connection and power to the scanner. You may also have to reinstall the scanner software.
5. On the *Select Scanner* screen, click SAVE to save the selected scanner for scanning.

7.3 CREATING A USER LOGIN

1. To access the ScanFlex™ *User Login* screen, click on *START* on the ScanFlex™ Start-up screen (Fig. 23).
2. Click on *CHOOSE USER* to open-up a drop down menu (Fig. 26).

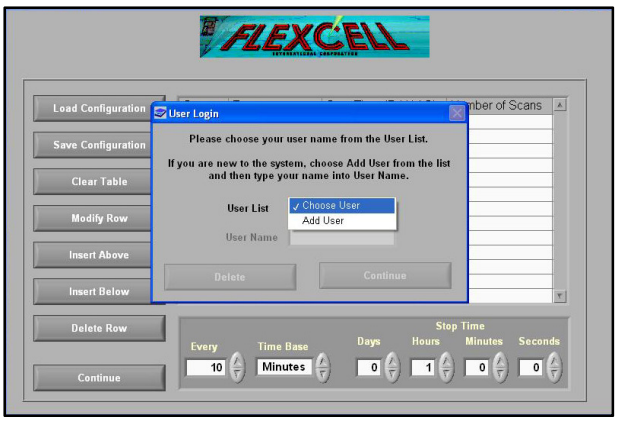


Figure 26. Choosing a user.

3. If a new user has to be added, select *ADD USER* from the drop down menu and type the user's name in the *USER NAME* box (Fig. 27). Then click on *CONTINUE* to go to the setup screen.
4. If the user's name is already in the list, click on the user's name and then click *CONTINUE*. You will be directed to the folder which contains all previously created configuration files (regimens).

Figure 27. Adding a new user.

NOTE 2: Deleting a user from USER LIST.
If a user's name needs to be deleted from the user list, click on the user name and then click DELETE. Click YES or NO on the popup warning message to select whether you want to delete all regimens that belong to this user. If you select NO, the user's name will not be deleted from the list. If YES is selected, all regimens saved under that user will be deleted with the user name.

7.4 CREATING SCANNING REGIMENS

7.4.1 Creating a New Regimen for a New User

1. All new users will be directed to the ScanFlex™ Setup screen. Follow the instructions below to setup a scanning regimen.
2. Set the scanning frequency time under *EVERY*, and select units in *TIME BASE* (Fig. 28, white circle). ***Important: The maximum scanning frequency is one scan per every 2 minutes. The minimum frequency is one scan per every 200 hours.***

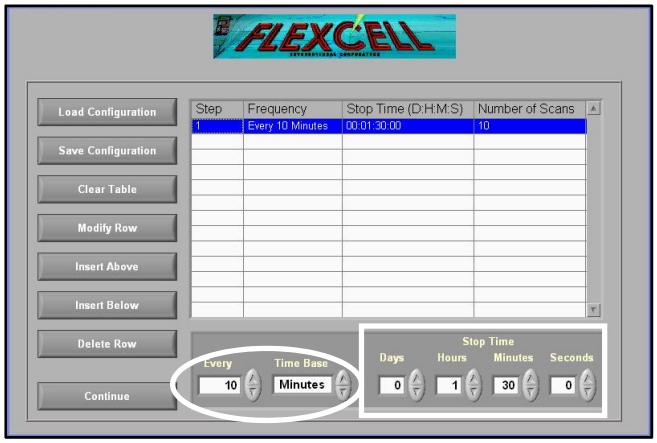


Figure 28. Creating a new regimen.

3. Set *STOP TIME* by setting *days*, *hours*, *minutes* and *seconds* (Fig. 28, white rectangle).
4. Click *INSERT BELOW* to insert frequency data to *Step #1* in the table.
5. If another step needs to be setup, enter the required parameters in *EVERY, TIME BASE* and *STOP TIME* and then click *INSERT BELOW*. The *Step #2* will be displayed in the table. Refer to **Note 3** for an explanation on *STOP TIME* calculation.
6. When all steps have been added, save regimen by clicking on *SAVE CONFIGURATION*. A folder with the

user's name as the title will be opened (Fig. 29).

NOTE 3: Calculating STOP TIME
The Stop Time in the table displays the cumulative time. To calculate the time parameters that need to be entered under STOP TIME, add the absolute time for the next subsequent step to the time displayed under the current step.

For example, assume that another step needs to be added in addition to the Step #1 shown in Fig. 28 with a scanning frequency of every 2 minutes and a stop time of 30 minutes. Since Step #1 has 1 hr and 30 minutes, the stop time that needs to be entered for Step #2 is 2 hrs (1 hr 30min + 30 min). There will be 15 scans for Step #2.

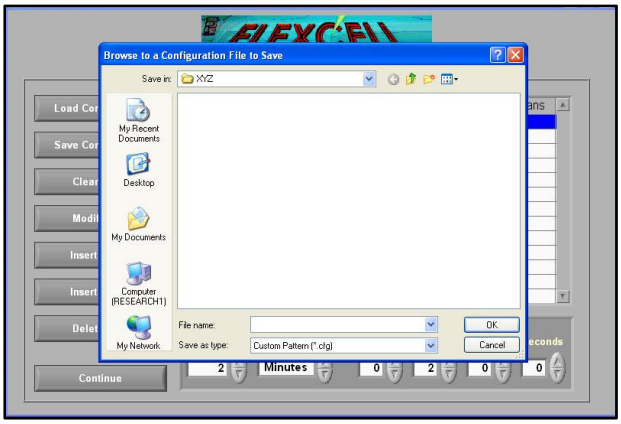


Figure 29. Saving a new regimen.

7. Type the desired file name in the *FILE NAME* box and click on *OK*.
8. On the ScanFlex™ Setup screen (Fig. 28), click on *CONTINUE* to proceed to the image acquisition screen (Fig. 30).

*Refer to **Note 4** to access the location where the configuration files are saved in the computer.*

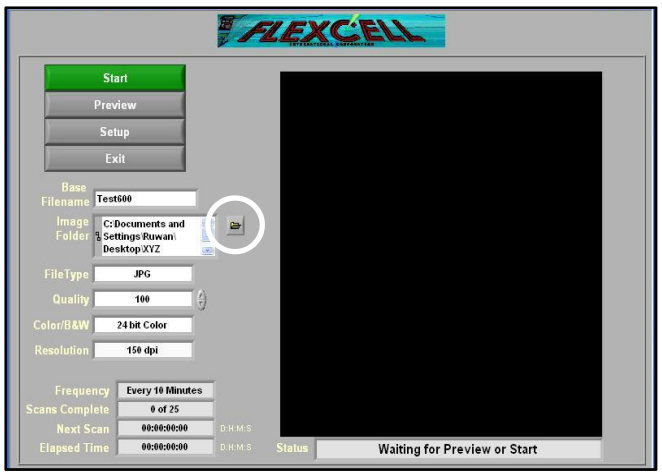


Figure 30. *Image acquisition screen.*

NOTE 4: Access to Configuration Files
The configuration file is saved in the "config" folder in the C: drive. You may use the following address to locate the configuration files. To access, click START on your desktop and click on "My Computer". In the top address bar type in the following address:
C:\Documents and Settings\All Users\Application Data\Flexcell_Scanflex\config

7.4.2 Creating a New Regimen for an Existing User

Use the following instructions to create a new regimen for an existing user.

1. When the user name is selected in the login screen (Fig. 27), the folder containing saved regimen files (Configuration files) will be opened as a popup screen (Fig. 31).
2. Click on *CANCEL* to exit from the popup screen.
3. Set the scanning frequency time under *EVERY*, and select units in *TIME BASE* (Fig. 28, white circle).
4. Set *STOP TIME* by setting days, hours, minutes and seconds (Fig. 28, white rectangle).

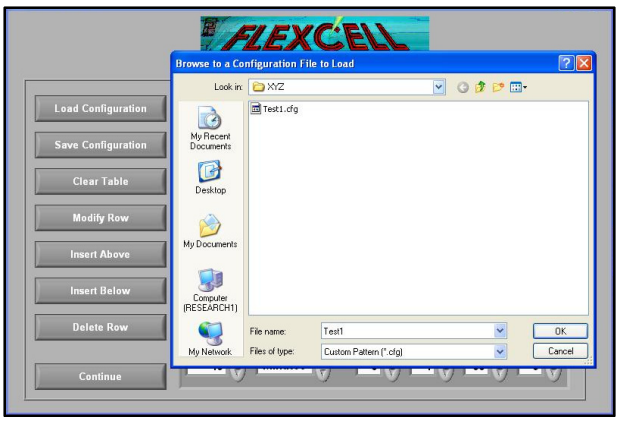


Figure 31. Opening existing regimen files.

5. Click *INSERT BELOW* to insert frequency data to *Step #1* in the table.
6. If another step needs to be setup, enter the required parameters in *EVERY, TIME BASE* and *STOP TIME* and then click *INSERT BELOW*. The Step #2 will be displayed in the table. Refer to **Note 3** above for explanation on *STOP TIME* calculation.
7. When all steps have been added, save regimen by clicking on *SAVE CONFIGURATION*. A folder with user's name as the title will be opened (Fig. 31).
8. Type the desired file name in the *FILE NAME* box and click on *OK*.
9. On the ScanFlex™ Setup screen, click on *CONTINUE* to proceed to the image acquisition screen (Fig. 30).

*Refer to **Note 4** to access the location where the configuration files are saved in the computer.*

7.4.3 Using an Existing Regimen

1. For an existing user, when the name of the user is selected from the User list (Fig. 27), the folder containing all regimen files (Fig. 31) that belong to the user will open automatically.
2. To load the desirable regimen file onto the ScanFlex™ setup screen, click on the desired Regimen File and then click on *OK*.
3. The selected regimen data should then be displayed in the parameter table.
4. If the displayed data needs to be changed or more data needs to be added, use the appropriate buttons displayed on the left and bottom of the setup screen. A description of button functions is shown in Table 2.
5. If another regimen file needs to be uploaded, click on the *LOAD CONFIGURATION* button on the setup screen.
6. In the popup user folder screen, select the appropriate file and click *OK* or double click on the appropriate file.
7. Click on *CONTINUE* to proceed to the image acquisition screen (Fig. 30).

*Refer to **Note 4** to access the location where the configuration files are saved in the computer.*

***Table 2.** Functions of the buttons in ScanFlex™ setup screen.*

Button	Function
Load Configuration	Opens a regimen (configuration) file from a user specified location.
Save Configuration	Saves a regimen file in the user configuration folder.
Clear Table	Clears all data in the regimen table.
Modify Row	Modifies current data in the regimen table according to the frequency and stop time data set in the current setup screen.
Insert Above	Inserts regimen data above the selected row in the table.
Insert Below	Inserts regimen data below the selected row in the table.
Delete Row	Deletes the selected raw in the table.
Continue	Continues to the next screen which is the image acquisition screen.

7.5 IMAGE ACQUISITION

The image acquisition screen (**Fig. 30**) enables the user to set several parameters such as *image file type, quality, color scheme,* and *resolution*. It also displays real time scanning information such as the *number of scans completed, next scanning time,* and *elapsed time*. The image of the last scan is displayed on the right side of the screen. Follow the instructions shown below to set each parameter.

1. Base file name
Enter a file name that will be used as the base file name for all your stored images. The images will be named using this text as the base with a time stamp. For example, if the base file name is *Test*, all images will be saved in the format "Test-0000hr00min" sequentially according to the scanned time.

NOTE: *A new image folder needs to be opened if the same base file name needs to be used for images in a new scanning regimen. Change the base file name if the new images need to be stored in the same folder. Otherwise, the images will not be stored, and the ScanFlex™ software will display an error message.*

2. Image folder
Click on the image folder icon (Fig. 30, white circle) to specify a location for the scanned images to be stored. The file path of the selected location will be displayed in the image folder box.

3. File type
Click on the FILE TYPE box to select either BMP or JPG for the file type of the scanned image.

4. Quality
The quality of the image can be varied from 0 to 100 in increments of 5. The higher the quality of the image, the larger the file size will be. The relationship between the quality setting and image file size is shown in Table 3. Use the up/down buttons to set the quality of the image.

Table 3. *Relationship between image file size and image parameter setting.*

Image file type	Resolution setting (dpi)	Quality setting (%)	Image size (MB)
BMP	600	N/A	102
JPG	600	100	19.2
		75	2.96
		50	1.96
		25	1.29
BMP	300	N/A	25.6
JPG	300	100	5.53
		75	0.93
		50	0.62
		25	0.39
BMP	150	N/A	6.4
JPG	150	100	1.44
		75	0.28
		50	0.19
		25	0.13

5. Color scheme
The color scheme of the images can be set to one of the following:
1 bit B&W
8 bit Grayscale
24 bit color
Click on the box and choose the desired color setting.

6. Resolution
The resolution of the scanned image can be set to one of the following:
150 dpi
300 dpi
600 dpi
The size of the image file and the scan time will depend on the selected resolution (see Tables 3 and 4). Click on the box to choose the desired image resolution.

7. Frequency
The scanning frequency set in the ScanFlex™ setup screen will be displayed in the frequency display.

8. Scans complete
This box will display the total number of scans and the number of scans completed at a given time.

9. Next scan
This box will display the time left for the next scan. This time is based on the scanning frequency value set in the setup screen. It will count down the time for the next scan once the *START* button is activated.

NOTE: *The timer will also count the time spent for scanning. Table 4 illustrates the scan time for different image resolution settings.*

Table 4. *Scan time for different image resolutions.*

Image resolution (dpi)	Scan time (sec)
150	27
300	33
600	57

10. Elapsed time
The box will display the amount of time passed, counting up from the first scan. It will also take into account the time spent on scanning the image. If multiple steps are setup in the regimen table, the elapsed time will display the total time passed once the *START* button is activated.

11. Once all scanner parameters are correctly set, click on *PREVIEW* to generate a preview scan of the items. Make sure the items to be scanned are in the correct orientation on the scanner bed, and the scanner lid is placed on the items prior to generating a preview.

12. Review the preview image. If the image is correct, click on *START* to begin the scanning process. The scanner will start generating images according to the set regimen.

13. If a preview is not necessary, click *START* to begin the scanning process.

14. If any changes need to be done to the scanner parameters once the scanning process has started, click on *STOP* and then make necessary changes to the parameters. Repeat steps 11-13 to restart scanning process.

15. If any changes need to be done to the scanning regimen, click on *STOP* and then *SETUP* to go back to the ScanFlex™ setup screen. Repeat procedures shown under Section 7.4.1, 7.4.2, or 7.4.3 as necessary to setup a scan regimen. Recheck the scanner parameters on the image acquisition screen prior to starting scanning.

APPENDIX

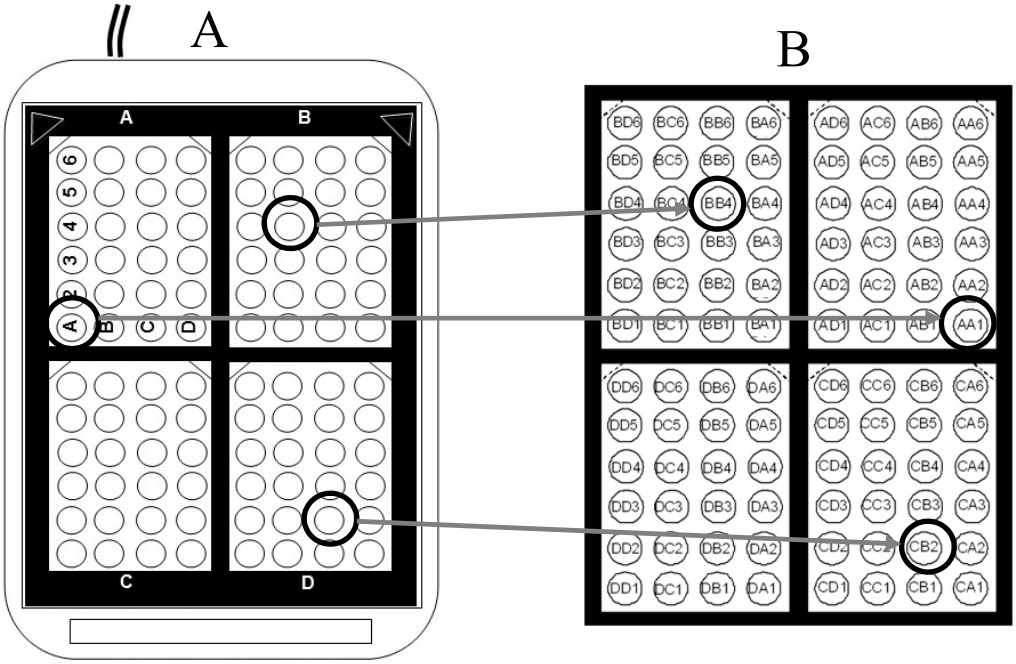


Figure 32. *Physical orientation of the wells in Flexcell®'s 24-well plates on the scanner bed (A) and their corresponding locations in the scanned image (B).*

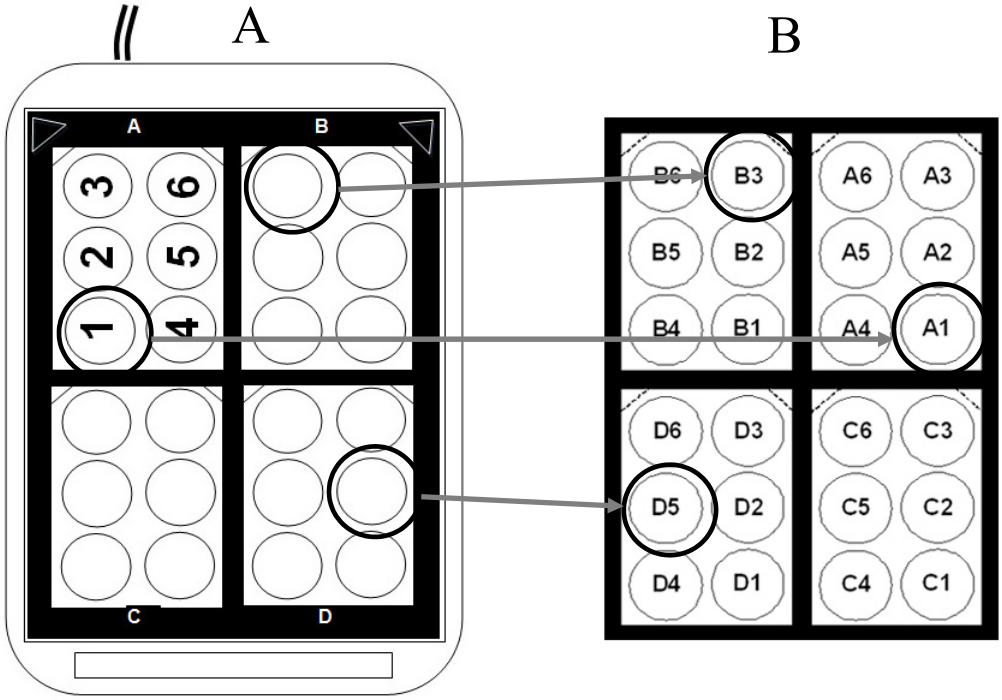


Figure 33. *Physical orientation of the wells in Flexcell®'s 6-well plates on the scanner bed (A) and their corresponding locations in the scanned image (B).*