

SpectroFlo® VERSION 3.2.1 RELEASE NOTES

TABLE OF CONTENTS

OVERVIEW	2
DEFECT FIXES	2
KNOWN SOFTWARE DEFECTS	3
KNOWN HARDWARE DEFECTS	3
DATA BACKUP AND SOFTWARE INSTALLATION INSTRUCTIONS	4
Backing Up Your Data	4
Upgrading SpectroFlo [®] from 3.1.x or 3.2.0 to 3.2.1	5
Installing SpectroFlo [®] 3.2.1 on a New PC	7
Installing SpectroFlo [®] on a PC Inside a Network	14

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OVERVIEW

SpectroFlo[®] 3.2.1 is a patch release to fix a couple of critical defects for multiple autofluorescence extraction and fluidics shutdown using tube rack. In addition, corrections of several other minor defects for multiple autofluorescence extraction are also included.

SpectroFlo[®] 3.2.1 should only be installed on instruments with the following versions of firmware (or higher). If these versions are not present, the firmware must be updated at the time of software installation:

Paired Loader	Fluidic Motherboard Hardware Revision	SpectroFlo [®] 3.X
Loader 1.5 'AMS'	Rev OC and Newer	FMB Firmware: 0.9.70 Aurora AFPGA: v1.0.2 Aurora Firmware (MFPGA): v1.0.1 LMB Firmware: v1.0.73
	Rev OB	FMB Firmware: 0.9.63.20200312 Aurora AFPGA: v1.0.2 Aurora Firmware (MFPGA): v1.0.1 LMB Firmware: v1.0.73
Loader 2.0 'ASL'	Rev OC and Newer	FMB Firmware: 0.9.70 Aurora AFPGA: v1.0.2 Aurora Firmware (MFPGA): v1.0.1 LMB Firmware: v2.0.04
	Rev OB	FMB Firmware: 0.9.63.20200312 Aurora AFPGA: v1.0.2 Aurora Firmware (MFPGA): v1.0.1 LMB Firmware: v2.0.04

Software installation instructions are included at the end of this document.

DEFECT FIXES

Unmixed Result for Multiple Autofluorescence Extraction Incorrect Sometimes: In certain special scenarios, it was discovered that the unmixed result for multiple autofluorescence was incorrect. This defect is corrected in this new version.

Incorrect SIT Position after Fluidics Shutdown Using Tube Rack: It was found that the SIT position was not maintained as intended after fluidics shutdown using tube rack. Instead of being kept down and immersed in the DI water tube to prevent drying out, the SIT was lifted. This issue is corrected.

Edited Multiple Autofluorescence Extraction Not Applied: After initial creation of the multiple AF controls, when Edit Multiple AF to modify some of the gates, only the changes made to the leaf gate were applied, while parent gates were not updated. This issue has been resolved. Now any modifications to both leaf and parent gates will be properly applied to the already generated AF controls.

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Unable to Extract AF from Gate If No Area Parameters are available: Exception occurs when attempting to extract AF from a gate if the unstained control tube did not have area signal parameters. This defect is fixed.

Exceptions When Esc Key is Pressed in Some Specific Rare Scenarios: In some specific rare scenarios, pressing the Esc key during loading of the Autofluorescence Explorer or the unmixing wizard could result in exceptions. These issues have been addressed in this version.

Drag and Drop Autofluorescence Control: In version 3.2.0, it was possible to drag and drop the generated AF controls to a sample group. In this new version, the drag and drop of the AF controls has been locked to prevent possible operator errors.

KNOWN SOFTWARE DEFECTS

- **Post-Acquisition Unmixing File Size Constraint:** We recommend only unmixing multicolor FCS files that contain fewer than 10,000,000 total events. Files that are bigger than 10,000,000 events may take a long time to unmix.
- Events to Display & Live Unmixing: If you want to display a lot of events (e.g., 1,000,000) while live unmixing a tube, the software will become very slow. We recommend reducing your events to display to 50,000 events or less when live unmixing your data. After the acquisition is finished, you can display 5-100% of your recorded events without any negative performance effects.
- Auto-Scaling of Axes for Unmixed Files: Auto-scaling of plot axes (found in Plot Properties) does not always apply properly for an unmixed tube. You may need to toggle to Manual Scale and then back to Auto Axis Scale to get the scaling to update and apply correctly.
- Limitation for the Name of User Defined Keyword: "Name" cannot be used as the key of a user defined keyword. The software will not prevent users from doing so. However, if users define such a keyword and this keyword is added in the statistics table, batch analysis cannot be completed due to a conflict with a reserved string used in the statistics table of SpectroFlo[®].
- Inconsistent Format of Displayed Week in Exported Use Time File: When exporting the use time log by week, the week number for weeks 1-12 is displayed with an incorrect format in Excel by default.
- **Disabled Laser Delay UI**: In some rare scenarios the Laser Delay fields are not selectable from the Instrument Control Window. They are always accessible if the Instrument Control Window is floated.
- Data Maintenance App and SpectroFlo[®] can be Opened at the Same Time: In some rare scenarios, both the Data Maintenance App and SpectroFlo[®] can be opened at the same time. When this happens, please close one program before proceeding.

KNOWN HARDWARE DEFECTS

• Automatic SIT Flush: SIT flush does not always occur automatically between sample tubes. Occasionally, the mechanical flag that blocks and unblocks the tube sensor gets stuck in the tube present position. If you do not hear a SIT flush after unloading your tube, open the SIT door and check the position of the flag. If it is stuck in the up position when no tube is present, press the flag down. The SIT flush will then execute. If you notice a lot of salt buildup around the flag, let your Cytek Field Service Engineer know so they can clean it out for you.

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Flag is in up position. This blocks the black rectangular optical sensor to the right of the flag and lets the software know that a tube is present on the SIP.

Flag is in down position. Optical sensor is unblocked, and lets the software know that a tube is not present on the SIP.

DATA BACKUP AND SOFTWARE INSTALLATION INSTRUCTIONS

Before upgrading your system to SpectroFlo[®] v3.2.1, we recommend that you back up your data. If anything goes wrong during the installation process, you can always revert to the backup files.

Backing Up Your Data

1. Open the SpectroFlo[®] Data Maintenance tool.



2. Click **Backup w/o FCS Files** button. Select a folder to export the backup ZIP file to and click OK. Depending how much data is in your software, this backup could take some time.

۵	Data Maintenance		
Ð	All SpectroFlo Data		Backup with FCS Files
9,	Instrument Data/User Accounts	Backup w/FCS Files	This backs up all data in SpectroFlo database and experiment FCS files to a zip file. It could take a long time (hours) to complete if there is a large number of experiment FCS files.
	Whole Database		
		Backup w/o FCS Files	Backup without FCS Files This backs up all data in SpectroFlo database without experiment FCS files to a zip file. It takes less time (minutes) to complete. The experiment FCS file folder can be copied separately.

Depending how much data is in your software, this backup could take some time.

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3. A message appears when the backup has completed. Click **OK**.



Now that you've backed up your system, continue with the following software upgrade instructions.

Upgrading SpectroFlo® from 3.1.x or 3.2.0 to 3.2.1

The upgrading procedure is as follows:

- 1. Download the setup package. Log into the host computer using an account with administrative rights.
- 2. Start the installer.

Note: this screen may not show if your PC's user account control setting is set to "Never notify". If the following message appears, click **Yes**.

User Account Control	×
Do you want to allow th changes to your device	nis app to make ?
Setup Launcher Un	icode
Verified publisher: CYTEK BIOSCII File origin: Hard drive on this con	ENCES, INC. nputer
Show more details	
Yes	No



3. Confirm the version and then click **Next** to continue.

SpectroFlo - InstallShield Wizard	×
Welcome to the InstallShield Wizard for SpectroFlo The installation will upgrade the software from Version 3.1.0 to Version 3.2.1 To continue, click Next	
InstallShield < Back	Cancel

4. Click **Install** to begin the installation.

SpectroFlo - InstallShield Wizard					×
Ready to Install the Program The wizard is ready to begin insta	lation.				
	Click Install to begin th If you want to review i the wizard.	e installation. or change any of yo	ur installation setti	ngs, click Back. Click Can	cel to exit
InstallShield		< <u>B</u> ack	nstall		Cancel

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5. Click **Finish** to finish the installation.

SpectroFlo - InstallShield Wizard	
	InstallShield Wizard Complete
	The InstallShield Wizard has successfully installed SpectroFlo
	Click Finish to launch DataMaintenanceSpectroFlo.exe to upgrade database
InstallShield	< Back Finish Cancel

Installing SpectroFlo® 3.2.1 on a New PC

- 1. Download the setup package. Log into the host computer using an account with administrative rights.
- 2. Start the installer.

Note: this screen may not show if your PC's user account control setting is set to "Never notify". If the following appears, click **Yes**.



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3. Click Install to install Microsoft SQL Server 2019 Express RTM.

alled on your computer. Click Install to
Install Cancel

4. Select I accept the terms of the license agreement, and then click Next.



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5. Click Next to continue.

SpectroFlo - InstallShield Wizard		×
SpectroFlo - InstallShield Wizard	Welcome to the InstallShield Wizard for SpectroFlo The InstallShield Wizard will install SpectroFlo on your computer. To continue, dick Next.	×
InstallShield	< <u>Back</u> Cance	

6. Select instrument type that is "Northern Lights" or "Aurora" or "Special/None-standard Aurora".

SpectroFlo - InstallShield Wizard				×
Instrument Configurations Customize your instrument				
	Select Installed Inst Aurora Select Installed Inst -Does The Instrumer Do You Want Exper	rument Configuration Northern Lights rument Configuration B14-R8-8-DAQ Card at Have Plate Loader Yes iment FCS File Folder Yes able Worklist (With T Yes	n	
InstallShield	< <u>B</u> ad	k Next >		Cancel

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7. Select the configuration for your installed instrument. For Aurora:

Instrument Configurations	
Customize your instrument	
	Select Installed Instrument Configuration Aurora Northern Special/None-standard Aurora Select Installed Instrument Configuration Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select Installed Instrument FCS File Folder Organized By Date? Select
InstallShield	< Back Next > Cancel

For Northern Lights:

pectroFlo - InstallShield Wizard		×
Instrument Configurations Customize your instrument		
	Select Installed Instrument Configuration Aurora Northern Special/None-standard Lights Aurora Select Installed Instrument Configuration Select Install	
	🗢 Yes 🔍 No	
	Do You Want To Enable Worklist (With Tools for 21 CFR Part 11)?	
	🔍 Yes 🔍 No	
InstallShield	< Back Next > Can	:el 🛛

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For Special/None-standard Aurora:

SpectroFlo - InstallShield Wizard		×
Instrument Configurations Customize your instrument		
	Select Installed Instrument Configuration Aurora Northern Special/None-standard Lights Special/None-standard Aurora Select Installed Instrument Configuration Select Installed Instrument Configuration 34.aser-V16-B14-R8-6-DAQ Cardcage 34.aser-V16-B14-R8-6-DAQ Cardcage 74.aser-V16-B14-R8-6-DAQ Cardcage 74.aser-V16-B14-R8-6	
InstallShield	< Back Next > Car	ncel

8. Choose "Yes" if there is a plate loader. Otherwise select "No". Then click **Next**.

SpectroFlo - InstallShield Wizard		×
Instrument Configurations Customize your instrument		
	-Select Installed Instrument Configuration Aurora Aurora -Select Installed Instrument Configuration -Select Installed Instrument Configuration -Select Installed Instrument Configuration -Select Installed Instrument Have Plate Loader? -Select Instrument Have Plate Loader? -Select Instrument Experiment FCS File Folder Orga -Do You Want Experiment FCS File Folder Orga Yes -Do You Want To Enable Worklist (With Tools f	ial/None-standard ora
InstallShield	< Back	Cancel

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9. Choose "No" if you don't want experiment FCS file folders automatically organized by date. Otherwise select "Yes". Then click **Next**.

ectroFlo - InstallShield Wizard		×
Instrument Configurations Customize your instrument		
	Select Installed Instrument Configuration Aurora Northern Special/None-standard Lights Aurora Select Installed Instrument Configuration 3-Laser-V16-814-R8-8-DAQ Cardcage	
	Do You Want To Enable Worklist (With Tools for 21 CFR Part 11)?	
inster[[Chin]d]	C Back	cel

10. Choose "No" if you don't want to include the worklist module. Otherwise select "Yes". Then click **Next**.

SpectroFlo - InstallShield Wizard		×
Instrument Configurations Customize your instrument		
	Select Installed Instrument Configuration Aurora Northern Special/None-standard Select Installed Instrument Configuration Aurora Select Installed Instrument Configuration Staser-V16-B14-R8-8-DAQ Cardcage J-Laser-V16-B14-R8-8-DAQ Cardcage Select Instrument Have Plate Loader? Oces The Instrument Have Plate Loader? No Over Yes No Do You Want Experiment FCS File Folder Organized By Date? Yes No Do You Want To Enable Worklist (With Tools for 21 CFR Part 11)? Yes No	
InstallShield	< Back [Next >] Cancel]

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11. Click **Install** to begin the installation.

SpectroFlo - InstallShield Wizard		\times
Ready to Install the Program The wizard is ready to begin insta	silation.	
	Click Install to begin the installation. If you want to review or change any of your installation settings, click Back. Click Cancel to exi the wizard.	t
InstallShield	< Back Install Cancel	

12. Click Finish to finish the installation.

SpectroFlo - InstallShield Wizard	1
	InstallShield Wizard Complete
	The InstallShield Wizard has successfully installed SpectroFlo
	Click Finish to launch DataMaintenanceSpectroFlo.exe to upgrade database
InstallShield	< Back Finish Cancel

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Installing SpectroFlo® on a PC Inside a Network

Step 1: Remove security policies around the devices needed to run the cytometer

Two USB ports are required. One is the port that controls the fluidics, and the other is a USB needed for data acquisition (DAQ).

- 1. Find the fluidics port:
 - a. Open Windows 10 or 11 Device Manager:
 - b. Open Ports: see list of Ports
 - c. Disconnect the USB cable that goes from the instrument to the computer

There is only one cable going from the instrument to the computer. That is the one you need to disconnect.

- d. As soon as you disconnect, one of the ports listed initially disappears. The port that disappears varies across systems, so, for example, it can be COM 3 or COM 6.
- 2. Identify the DAQ port:
 - a. In Windows 10 or 11 Device Manager, go to Universal Serial Bus Controller. Find the port labeled **Cytek Aurora DAQ**.

This is the other device you need to allow user access.

- b. Write down the port.
- 3. Allow user access for both devises.
- 4. Open SpectroFlo[®] software. Log in as Admin.
- 5. Turn on the instrument and wait for it to connect.

Step 2: If step 1 fails, and you still cannot connect the cytometer, try removing the computer from the network and running it as a local machine. Ideally, we would like to log into the computer as we originally did. <u>User Name: Aurora User, Password: Welcome#1</u>. Make sure that you log into SpectroFlo[®] as Admin.

Step 3: If step 2 fails, download the log file. Go to C:\CytekbioExport\. The file name is ApplicationLog_MM YYYY.txt. If there are several log files, please copy the latest one and send it to Cytek for assistance.

Have Questions? Comments? Want to work with us? Visit us at www.cytekbio.com.