SpectroFlo VERSION 3.3.0 RELEASE NOTES

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OVERVIEW

SpectroFlo® 3.3.0 is a minor release to add support for conventional compensation workflows, allowing use of Aurora and Northern Lights as conventional cytometer in addition to existing full spectrum unmixing. This version also supports 384 well plate and operating systems other than English. In addition, some minor improvements and corrections of defects found in version 3.2.1 are also included.

SpectroFlo® 3.3.0 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 0C and Newer	FMB Firmware: 0.9.70 Aurora AFPGA: v1.0.2 Aurora Firmware (MFPGA): v1.0.1 LMB Firmware: v1.0.73
	Rev 0B	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 0C and Newer	FMB Firmware: 0.9.70 Aurora AFPGA: v1.0.2 Aurora Firmware (MFPGA): v1.0.1 LMB Firmware: v2.0.04
	Rev 0B	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.

IMPROVED FEATURES & DEFECT FIXES

Support Conventional Compensation Workflow: With this version, users have the flexibility of running Aurora and Northen Lights either in full spectrum mode or conventional compensation mode. In conventional compensation mode, users can either go with single stained controls or no controls (for simple experiments) at all for compensation.

Support for 384 Well Plate: This new version supports 384 well plate.

Support Operating Systems other than English Settings: In previous versions, the display language of the Windows operating system hosting SpectroFlo® software must be set to English. In this version, the restriction is lifted. User can set the display language to French, Spanish, German, or Italian.

Improved Gate Dot Size: To make it easier to see rare events, users can enable an option of a gate to make the dot size of gate events to be displayed bigger on all plots of the worksheet.

Improved Uniqueness of FCS File Name for Plate: In previous versions, the FCS file name of a well on a plate is a combination of the well ID and well name. In this version the plate name is concatenated at the end of the FCS file name to make it more unique.

Added a GUID in FCS File: In this version, a GUID is added as a keyword in each of the FCS files acquired by the software.

Added Option to Change Carrier Type in Reference Control Wizard: In previous versions, if users selected incorrectly the sample loading mode (either manual tube or plate loader) before going into reference control creation/update wizard, users must cancel the wizard to make the correction. In this new version, users can make the correction conveniently within the wizard.

Added Option to Hide Keywords Tab from Experiment Setup: A preference is added in this version to allow users to hide the Keywords tab in the Experiment Creation wizard.

Remove Compensation Unmixing Model: The compensation unmixing model is removed in this version.
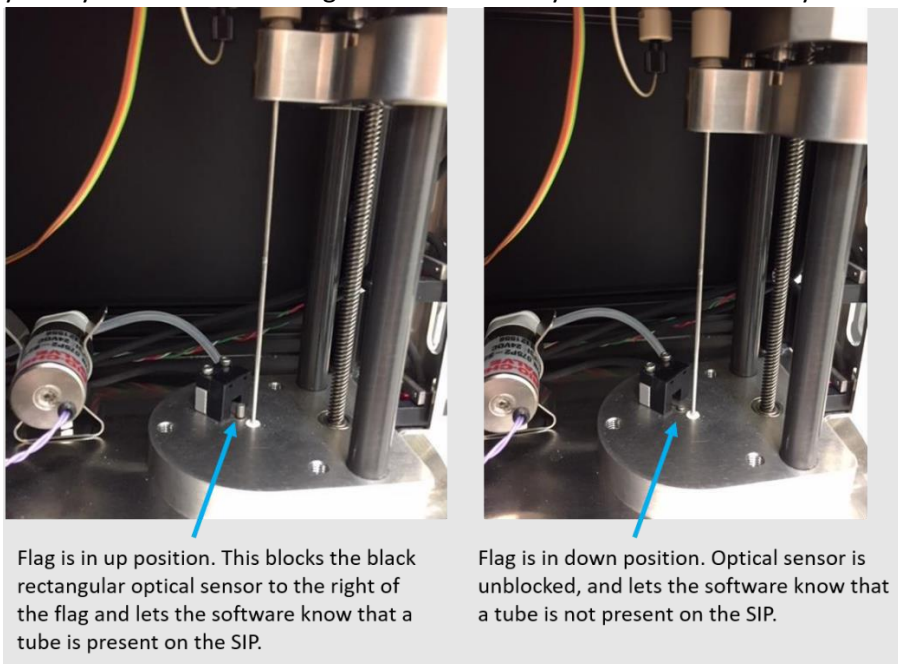
Area and Height MFI not Aligned Under CytekAssaySetting: In previous SpectroFlo® 3.x versions, the area and height signals are not aligned with the SpectroFlo® QC Beads under CytekAssaySetting. This defect is fixed.

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.

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® v3.3.0, we recommend to first back up your data. This way, if anything goes wrong during the installation process, you can always revert back to the backup files. For a video overview of the SpectroFlo® Data Maintenance tool, please visit https://cytekbio.com/blogs/resources/tagged/video-tutorials and look for the "Data Maintenance" tutorial video.

Backing Up Your Data

1. Open the SpectroFlo® Data Maintenance tool.
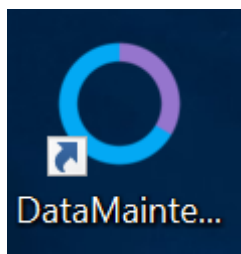
2. Click "Backup w/o FCS Files" button. Select a folder to export the backup ZIP file to and click OK. Depending how much data is in your software, this backup could take some time.
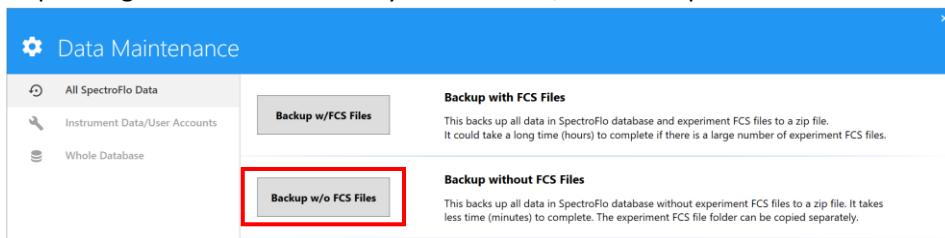
3. A message will appear when the backup has completed. Click "OK".
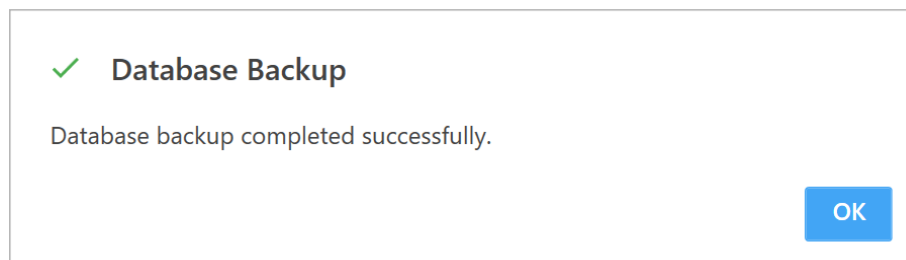
Now that you've backed up your system, continue with the software upgrade instructions that follow.

Upgrading SpectroFlo® from 3.2.x to 3.3.0

The upgrading procedure is as follows:

1. Download the setup package and make sure the installer is run with a Windows 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 it appears, click "Yes".
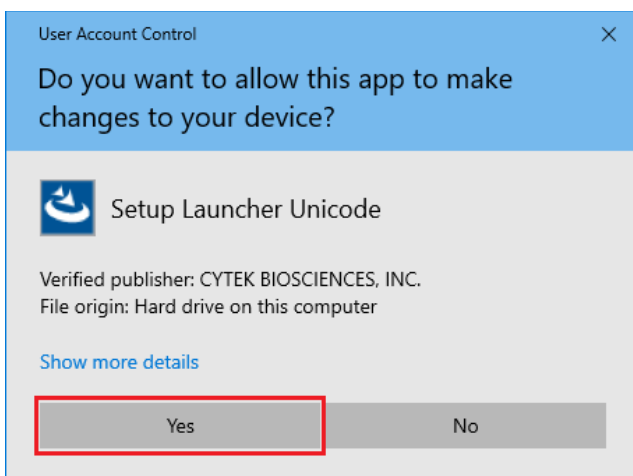
3. Confirm the version and then click "Next" to continue.
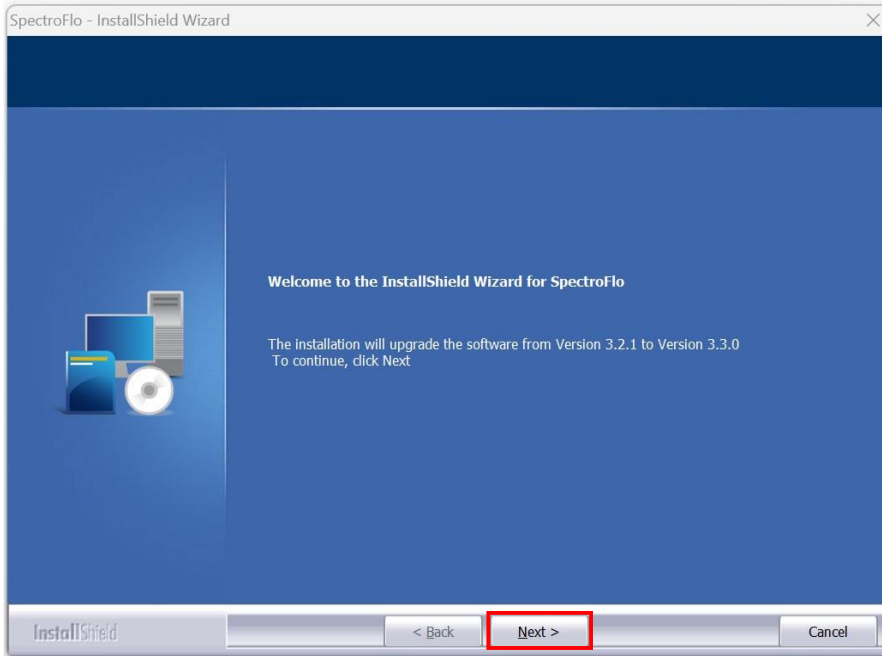
4. Click "Install" to begin the installation.
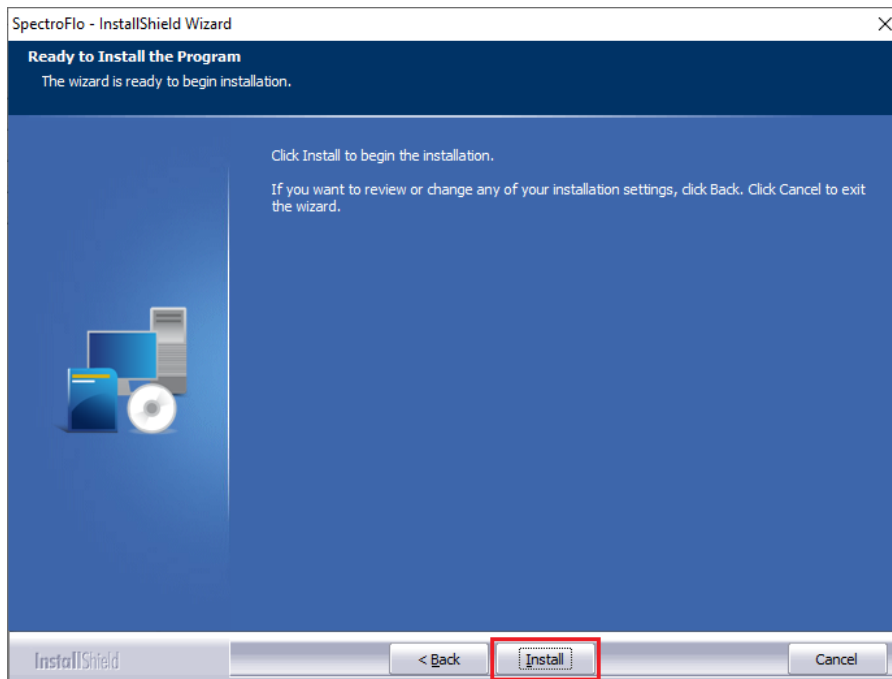
5. Click "Finish" to finish the installation.
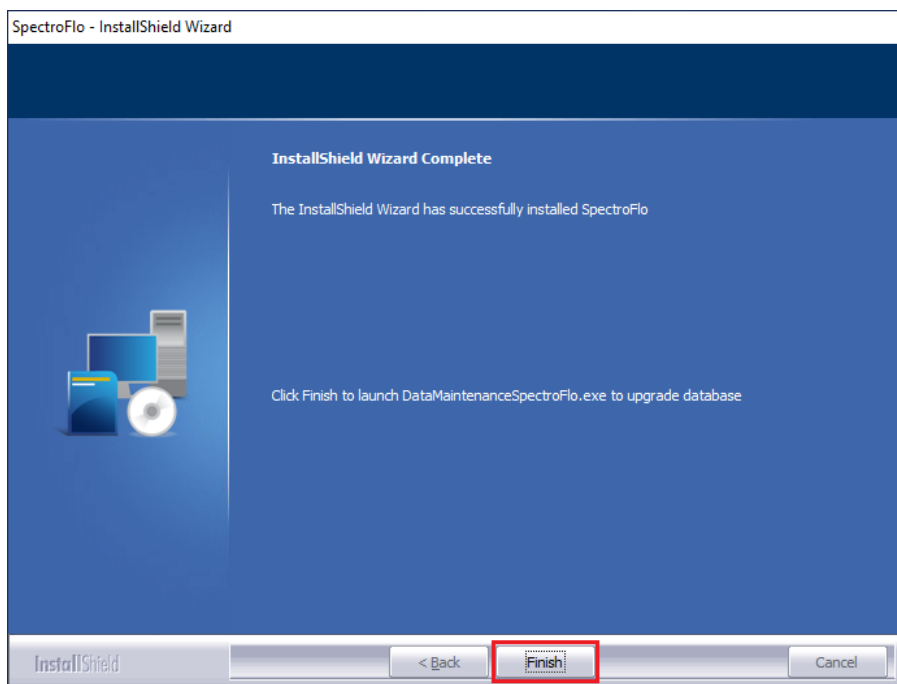
Installing SpectroFlo® 3.3.0 on a New PC

1. Download the setup package and make sure the installer is run with a Windows 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 it appears, click "Yes".
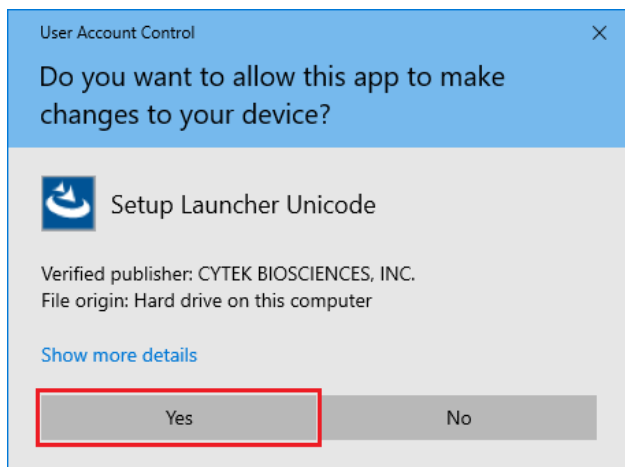
3. Click "Install" to install Microsoft SQL Server 2019 Express RTM.
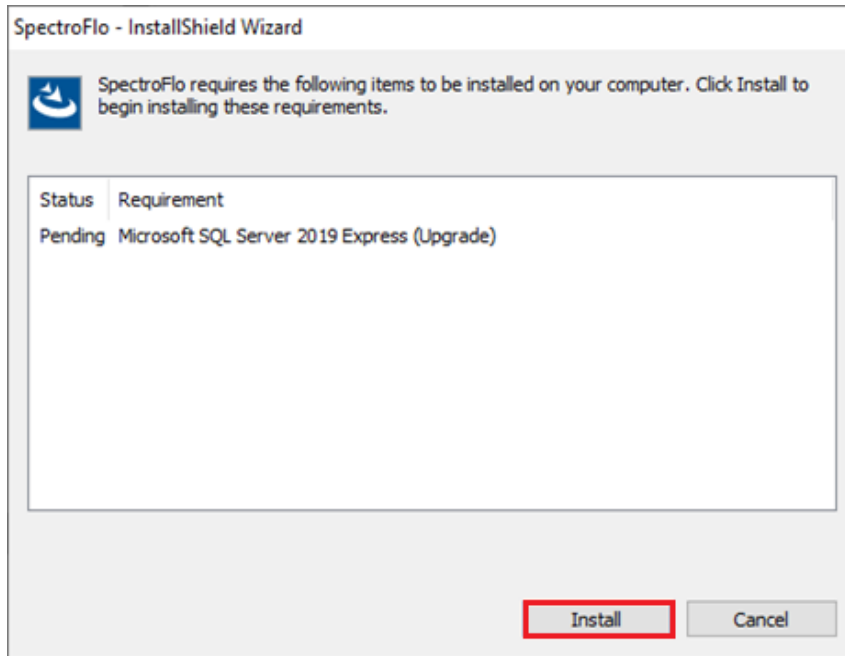
4. Select "I accept the terms of the license agreement" radio button, and then click "Next".
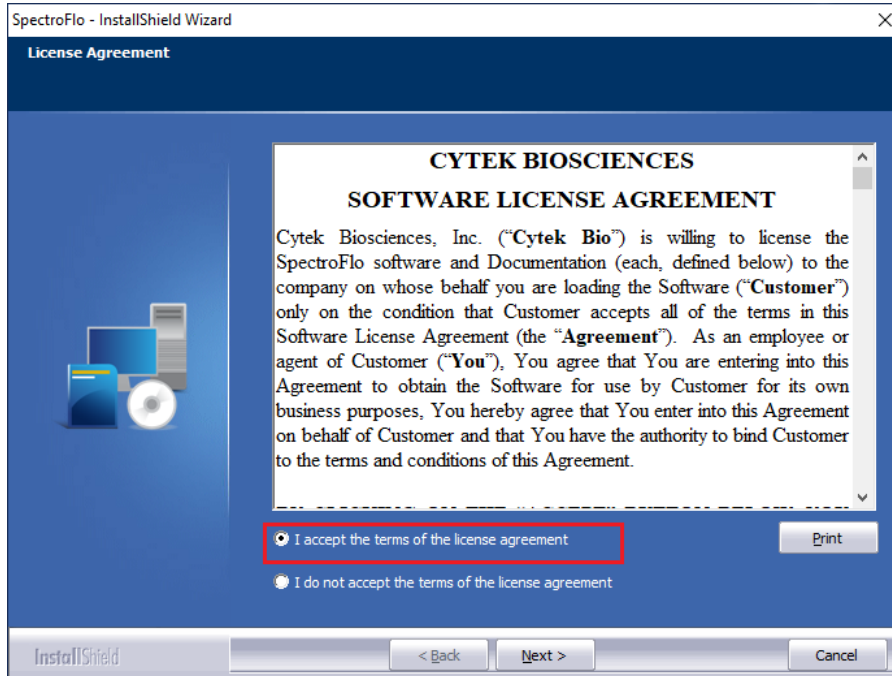
5. Click "Next" to continue.
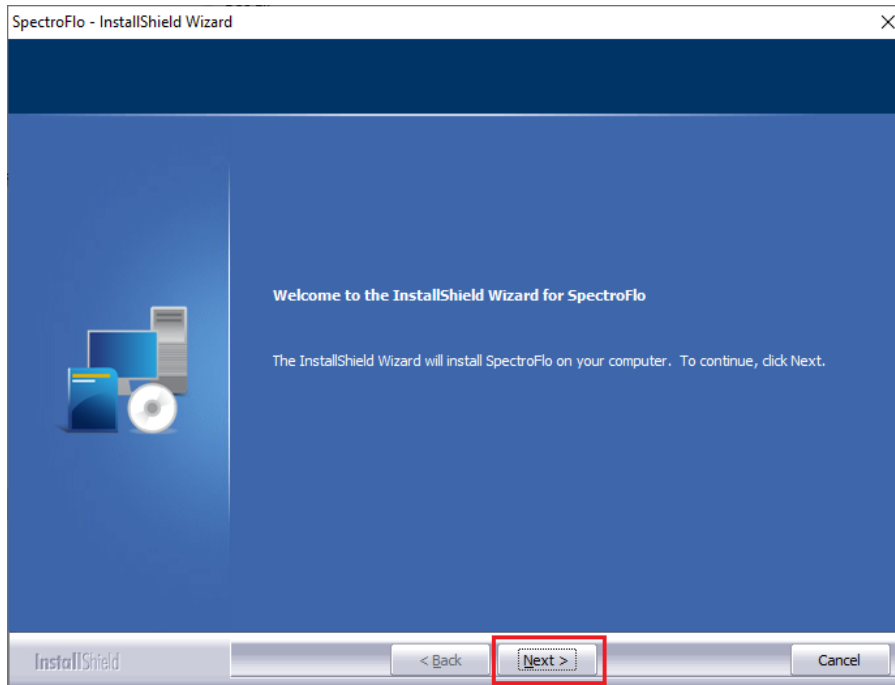
6. Select instrument type that is "Northern Lights" or "Aurora" or "Special/None-standard Aurora".
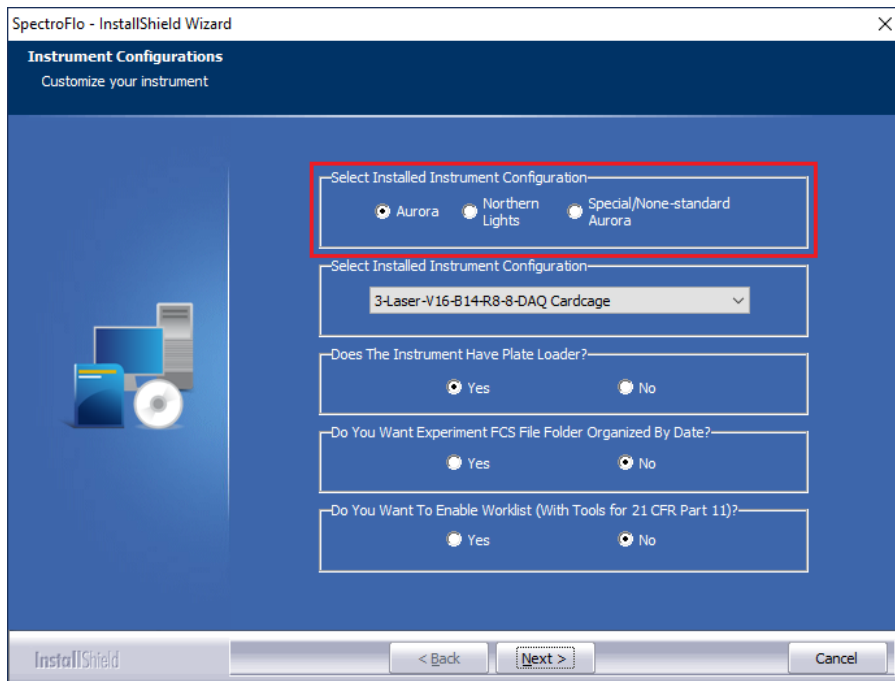
7. Select the configuration for your installed instrument.
    For Aurora:
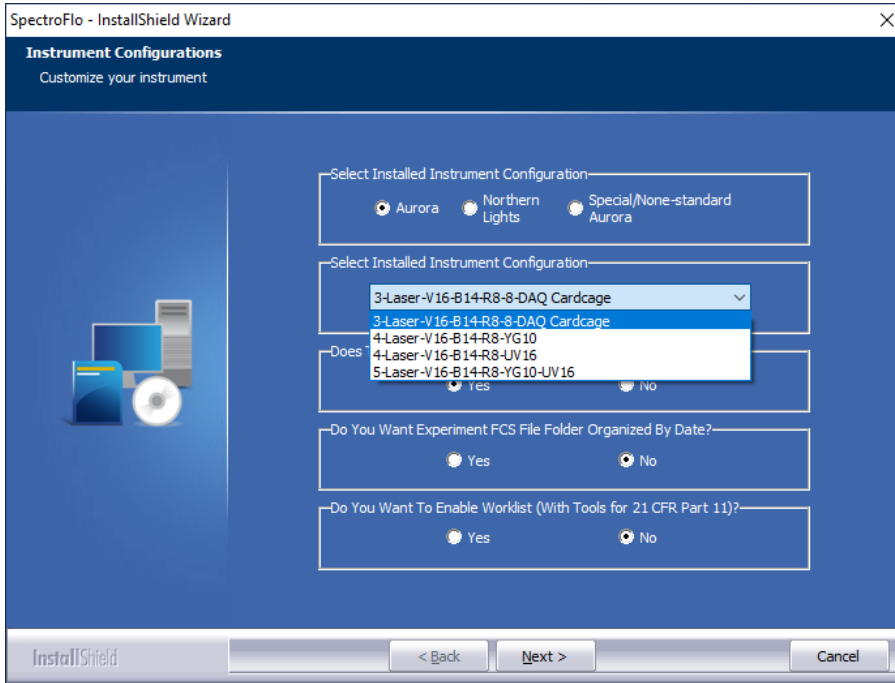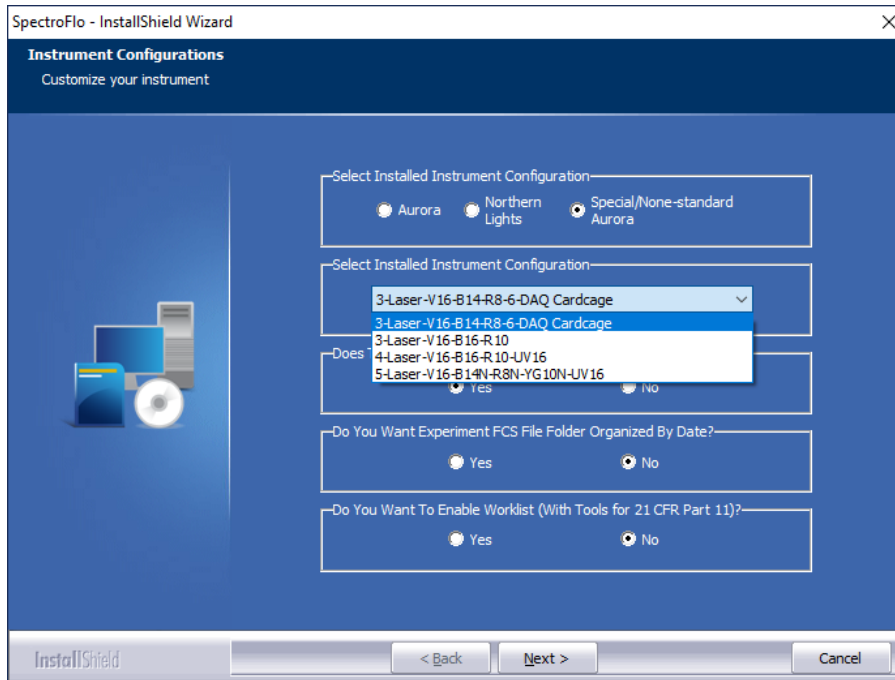
For Northern Lights:

For Special/None-standard Aurora:

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

9. Choose "No" if you don't want experiment FCS file folders automatically organized by date. Otherwise select "Yes". Then click "Next".
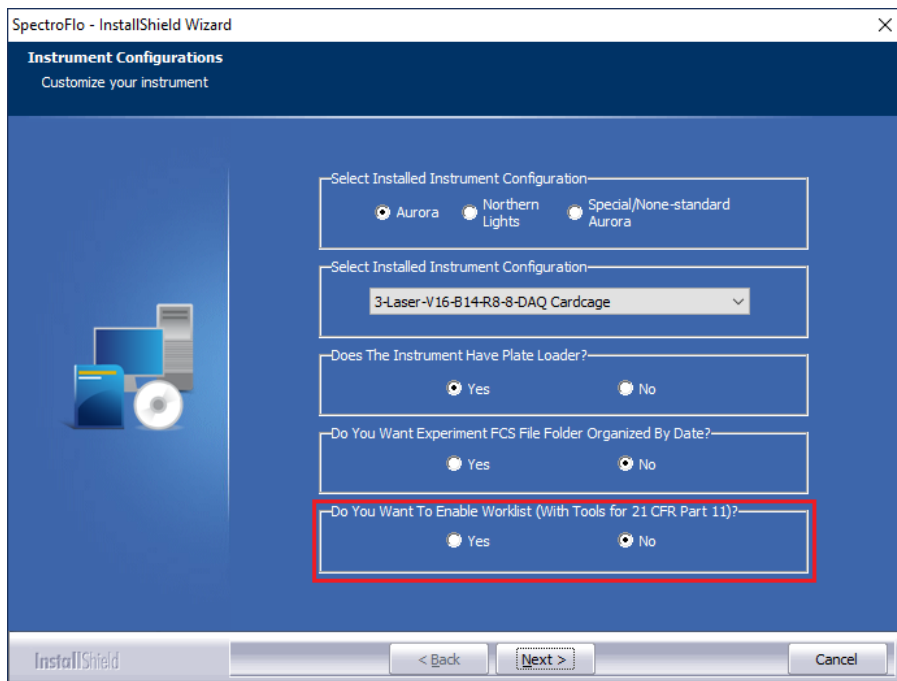
10. Choose "No" if you don't want to include the worklist module. Otherwise select "Yes". Then click "Next".

11. Click "Install" to begin the installation.
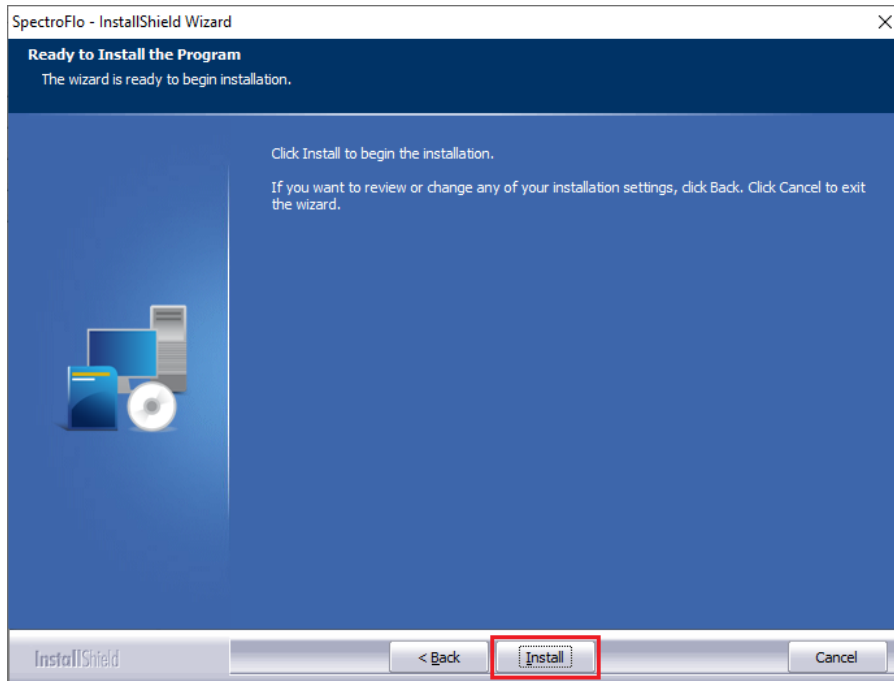
12. Click "Finish" to finish the installation.

Installing SpectroFlo® on a PC Inside a Network

Step 1: Remove security policies around the devices needed to run the cytometer
There are 2 devices that are needed. One is a port that controls the fluidics, and another is a USB needed for data acquisition.
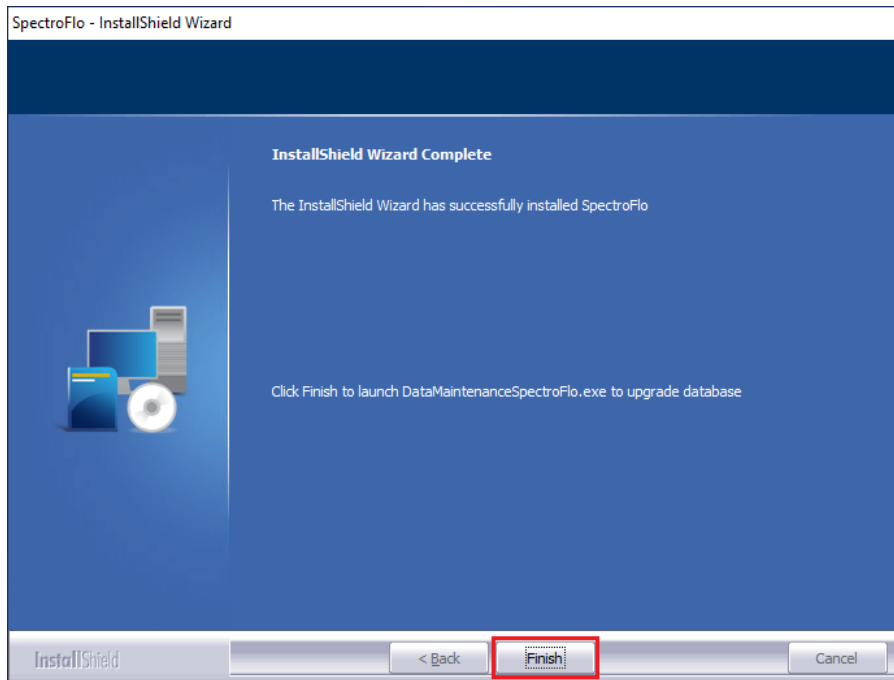
1. Find the fluidics port:
   a. Open Windows 10 or 11 Device Manager:
   b. Open Ports: see list of Ports
   c. Disconnect the USB cable that goes from the instrument to the computer (there is only one cable going from the instrument to the computer, that is the one you need to disconnect)
   d. As soon as you disconnect, you will see one of the Ports originally listed disappear (it varies across systems, it can be COM 3 or COM 6, for example). That is how you identify the first port for which you need to allow user access.
2. Next, you need to identify the second device:
   a. Still under Windows 10 Device Manager, go to Universal Serial Bus Controller: in there, find Cytek Aurora DAQ. This is the other device you need to allow user access.
   b. Once user access has been allowed for these 2 devices, open SpectroFlo® software, login as Admin, turn on the cytometer and see if you can connect to the cytometer.

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

Step 3: If step 2 fails, then we would like to get a log file that will allow us to see what prevented the connection. The log file can be found under C:\CytekbioExport\ApplicationLog_MM YYYY.txt. If there are several of these 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.