



Replacement of a Windows 10 OEM Hard Drive with an
Agilent SSD Overview

Installation Manual

Notices

© Agilent Technologies, Inc.

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent, Inc. as governed by United States and international copyright laws.

Manual Part Number

5994-4360EN

Edition

Printed in USA

Second edition, December 2022

Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610 USA

Warranty

The material contained in this document is provided “as is,” and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data -Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A CAUTION notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a CAUTION notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Replacement of a Windows 10 OEM Hard Drive with an Agilent SSD

Overview

Introduction 4

Required Materials 4

Part 1: Copy Application and System Files from the Agilent Seahorse XFe Controller to an External Storage Device 5

Part 2: Removal the XFe Controller Hard Drive and Install the SSD 11

Part 3: Transfer Seahorse XF Application and System Files to the XFe Controller After Hard Drive Replacement, Validate Your Windows 10 OS, then Install Seahorse XF Wave Controller Software and Seahorse XF Imaging and Cell Counting Software Applications 15

Part 4: Install Gen5 Software on the XFe Controller 22

Part 5: Perform XFe Analyzer Diagnostic Injection Test 27

Introduction

This document provides step-by-step instructions for upgrade the Agilent Seahorse XFe Controller hard drive (HD) to a 2TB solid state drive (SSD). It is recommended to have this upgrade performed by a trained Agilent Field Service Engineer. Failure to perform these steps correctly can result in an unusable Agilent Seahorse XFe Analyzer and Controller, resulting in extended downtime and potentially additional service cost.

Required Materials

- External USB storage device
 - Depending on the volume of information to be transferred (e.g., many image files and XF data files) then you must use an external storage device with a similar amount of storage as the original XFe Controller hard drive (500 GB).
 - The XFe Controller (computer) should not be used for storing large amounts of data (XF result files, images, etc.) or for archival purposes. It is strongly encouraged that you back up your data OFF the XFe Controller immediately after it is available. This good practice will avoid loss of data if the hard drive ever fails.
- Upgrade kit parts (purchased from Agilent)
 - Piece of paper shipped with hardware that provides the Windows activation code
 - Solid-state drive (SSD)
- Internet access on the Agilent Seahorse XFe Controller (for Microsoft Windows activation following the SSD upgrade).
- A soft cloth or piece of plastic slightly larger than the size of the Agilent Seahorse XFe Controller display. This will be used to protect the controller screen during hard drive removal and installation.
- Agilent Seahorse XFe FluxPak. This will be used to perform a diagnostic injection test to verify proper operation of your Seahorse XFe Analyzer after completing the hard drive upgrade.

Part 1: Copy Application and System Files from the Agilent Seahorse XFe Controller to an External Storage Device

The instructions below outline the application and system files to be copied off the original XFe Controller (computer) hard drive. These files must be transferred to the XFe Controller after completing the SSD upgrade as they are required for proper operation of the XFe Controller and Analyzer. Please note, we strongly advise assay result files are saved to a secure, shared network directory rather than saving on the XFe Controller hard drive.

- 1 Determine a suitable external storage device to use for this procedure. Depending on the amount of data you have acquired, you may need an external storage device as large as 500 GB.
- 2 Go to the XFe Controller and insert the external storage device.
- 3 Open Windows Explorer.
- 4 Go to: C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Data (Figure 1) and copy the **Instrumentconfiguration.xml** file to your external storage device.

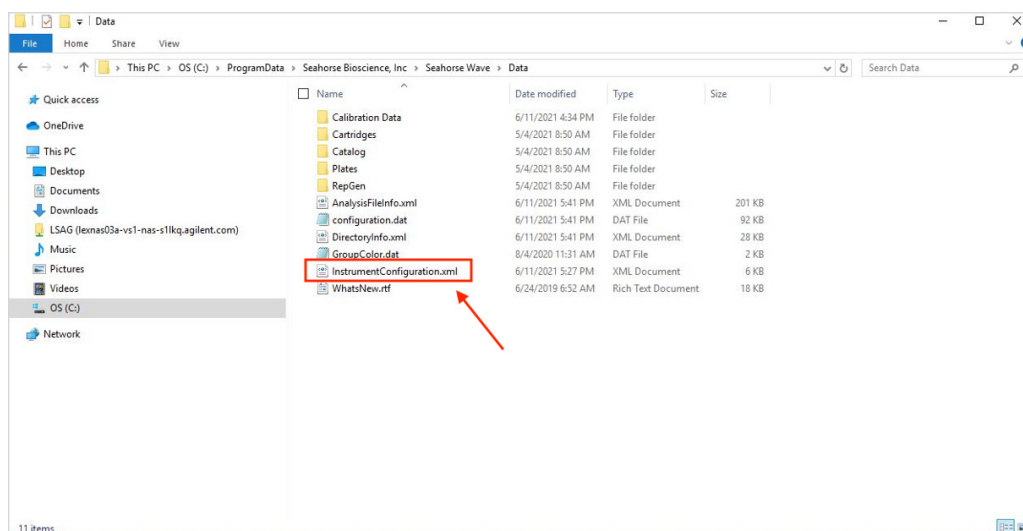


Figure 1. The **Instrumentconfiguration.xml** file location (C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Data).

- 5 In the same folder (C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Data), copy the **Configuration.dat** file to your external storage device (**Figure 2**).

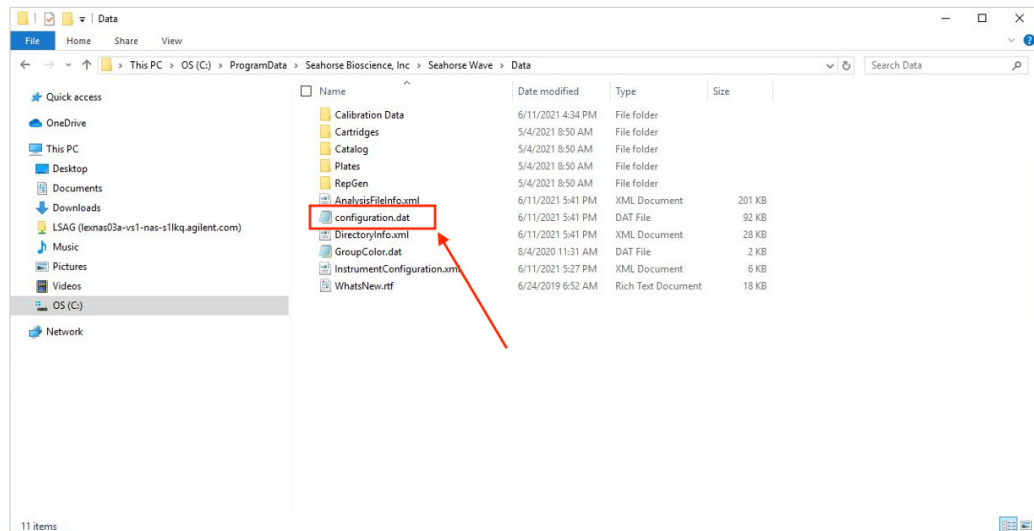


Figure 2. The **Configuration.dat** file location (C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Data).

- 6 Go to: C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Assays (**Figure 3**) and copy all assay result files to your external storage device.

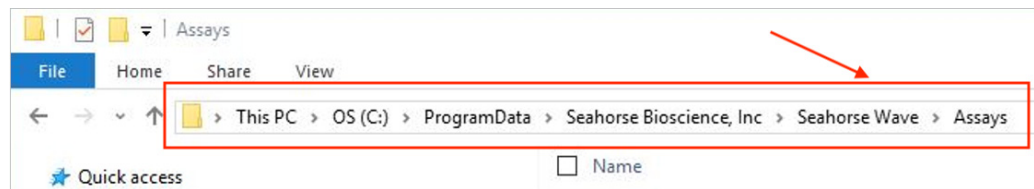


Figure 3. The assay results files location (C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Assays).

Do you have the Agilent Seahorse XF Imaging and Normalization System?

- If **yes**, continue reading the steps below.
- If **no**, skip ahead to **Part 2: Removal the XFe Controller Hard Drive and Install the SSD** on page 11.

- 7 Go to: C:\Program Files (x86)\Seahorse Bioscience\Cell Imaging (Figure 4) and copy the XF Cell Imaging and Normalization license file (**vortex.lic**) to your external storage device.

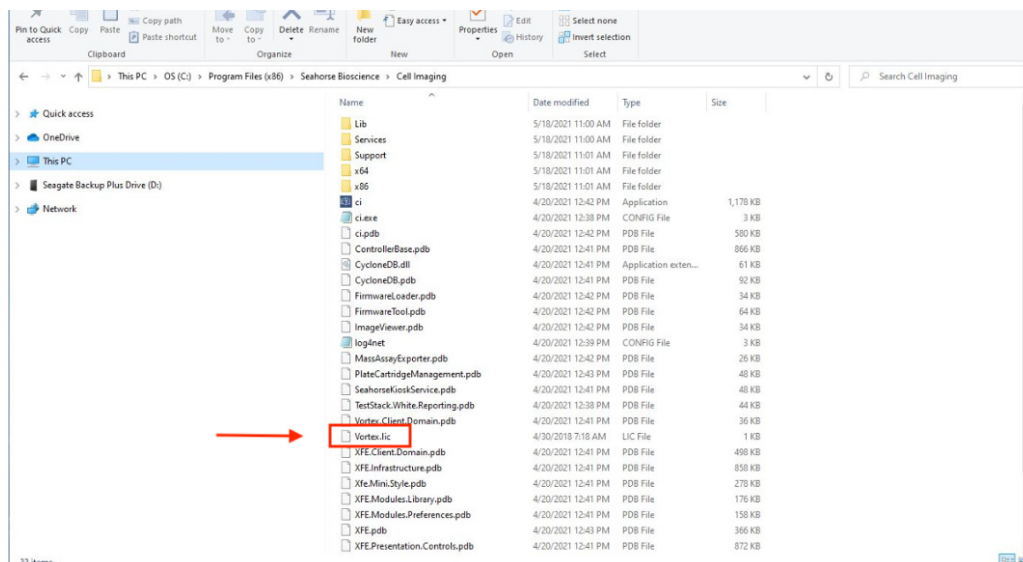


Figure 4. The XF Cell Imaging and Normalization license file (**vortex.lic**) location (C:\Program Files (x86)\Seahorse Bioscience\Cell Imaging).

- 8 Open the Biotech Gen 5 application on the XFe Controller. The **Task Manager** dialog will appear (Figure 5).

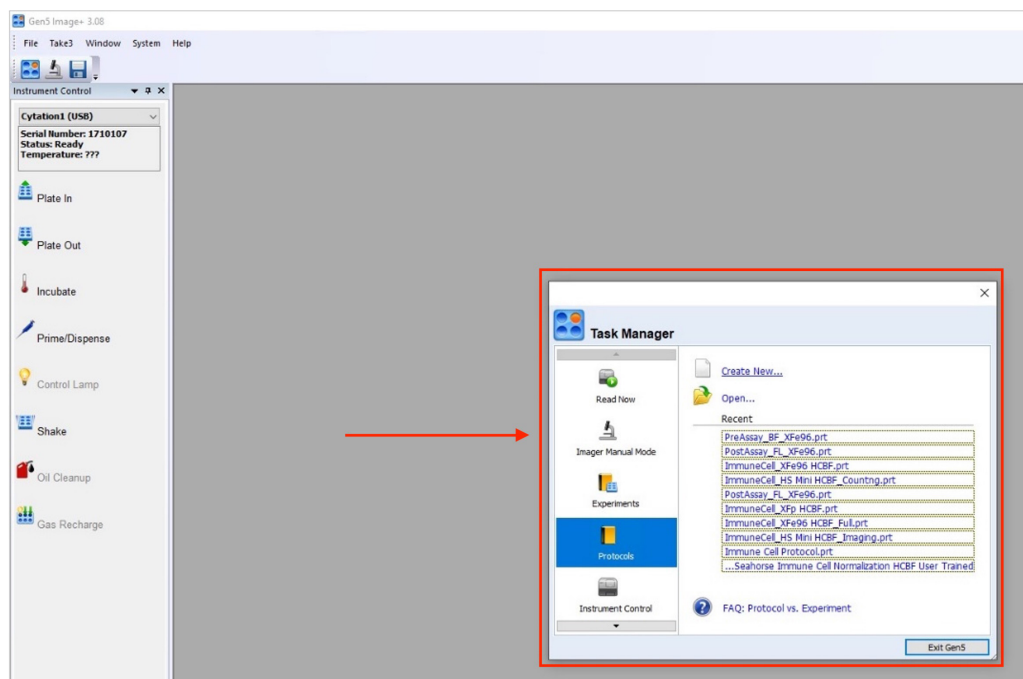


Figure 5. The **Task Manager** dialog in the Biotech Gen 5 application on the XFe Controller.

- a Scroll down and select the **Setup** option (Figure 6).

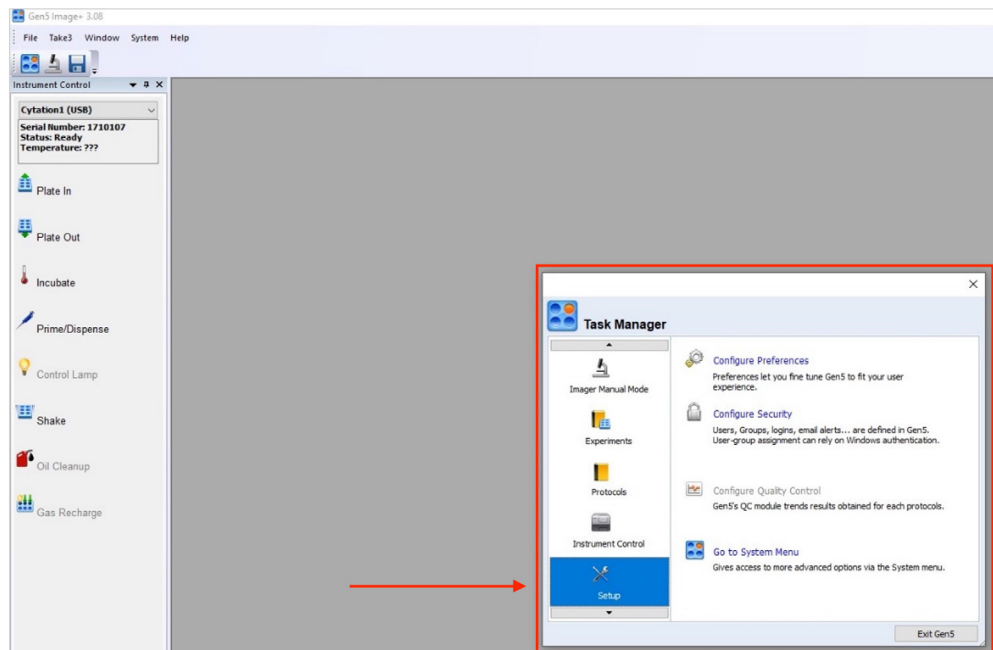


Figure 6. The **Setup** option in **Task Manager**.

- b Click **Configure Preferences** (Figure 7).

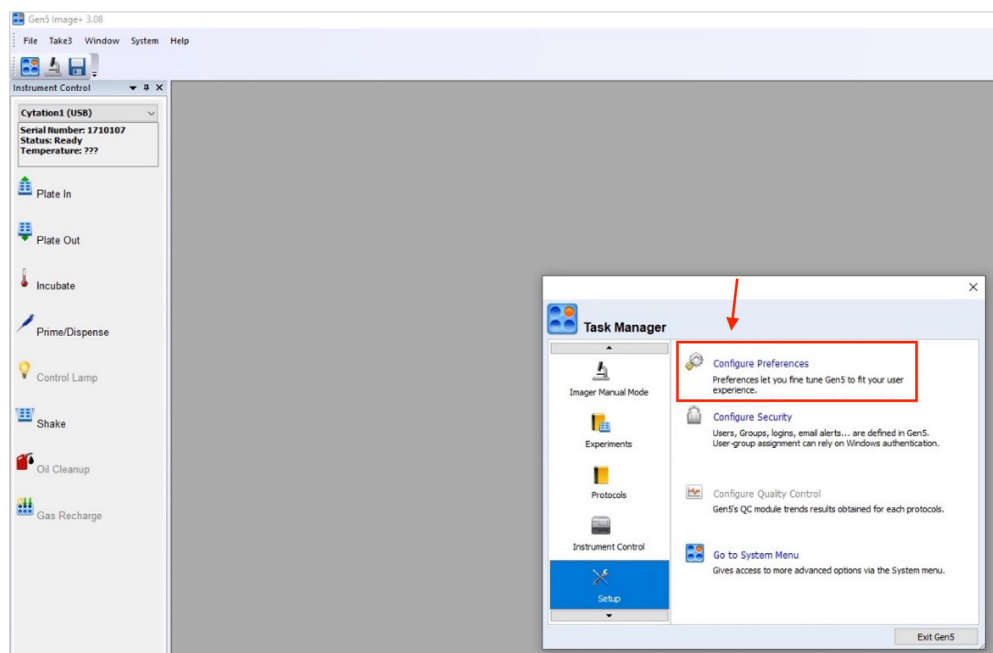


Figure 7. **Configure Preferences** in the **Setup** option in the **Task Manager**.

- c Select the **Preferred File Locations** option (Figure 8) and record the folder location where the Biotek Cytation image files are stored. If the Cytation image files are saved on the hard drive to be replaced, copy the folder where the images are stored off the hard drive to your external storage device.

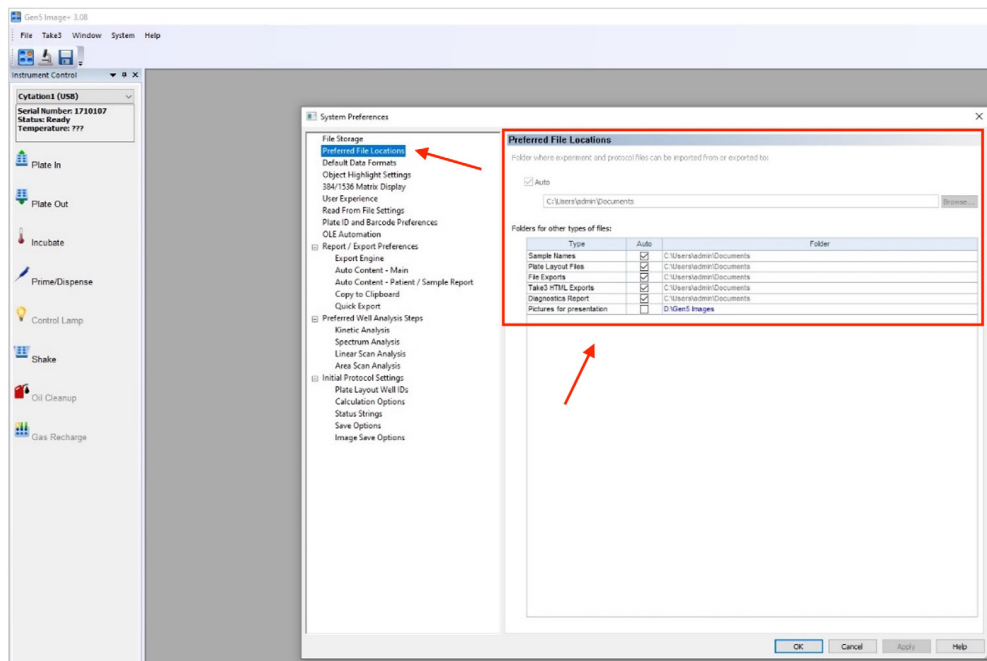


Figure 8. The **Preferred File Locations** option in **System Preferences**.

- 9 Record your Gen5 serial number from the XFe Controller. Navigate to **System > About Gen5** from within the software. The serial number is typically a 14-digit code comprised of all letters.

- 10 Open the XF Imaging and Cell Counting application and go to the **Help** view and record the path to the **Image Database** file location (**Figure 9**).

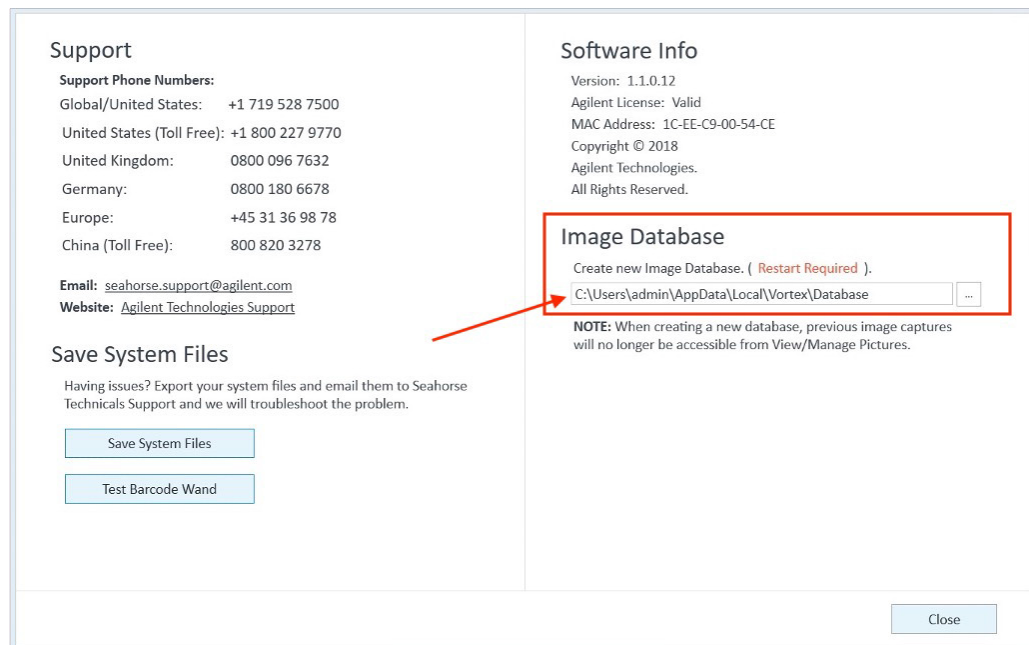


Figure 9. The **Image Database** file location in the **Help** view.

- 11 Open Windows Explorer and go to the Image Database file location recorded in the previous step.
- 12 Copy the Image Database file (called **vortex.db**) off the hard drive to your external storage device.
- 13 Shut down the XFe Controller. Once the controller is powered OFF, unplug the power cable from the controller.

Part 2: Removal the XFe Controller Hard Drive and Install the SSD

The instructions in Part 2 outline the physical steps to remove the original XFe Controller (computer) hard drive and install the new solid-state drive. After completing Part 2, the application and system files copied from the original XFe Controller hard drive must be transferred to the new SSD. These steps are described in **Part 3: Transfer Seahorse XF Application and System Files to the XFe Controller After Hard Drive Replacement, Validate Your Windows 10 OS, then Install Seahorse XF Wave Controller Software and Seahorse XF Imaging and Cell Counting Software Applications** on page 15.

- 1 Before beginning Part 2:
 - a Ensure the XFe Controller is powered **OFF**.
 - b Ensure the XFe Analyzer is powered **OFF**.
- 2 To perform this upgrade, put a piece of cloth (or plastic) on the table. This will be used to protect the controller screen when swapping the hard drive.
- 3 Place the screen face onto the cloth, so the cable cover is available. Remove the cable cover. Some sites may have this removed already.
- 4 Take a clear picture of the cable setup. This can be used when reinstalling the cables later in the process.
- 5 Remove the external barcode wand USB cable and remove the two USB 3.0 cables from the USB ports that lead to the BioTek Imager. If needed, remove the other cables if they seem to get in the way of the hard drive replacement.
- 6 Locate the two tabs that secure the rear cover to the controller. Gently lift the two tabs to unlatch the rear cover and pull it away from the controller to expose the cable area (**Figure 10**).

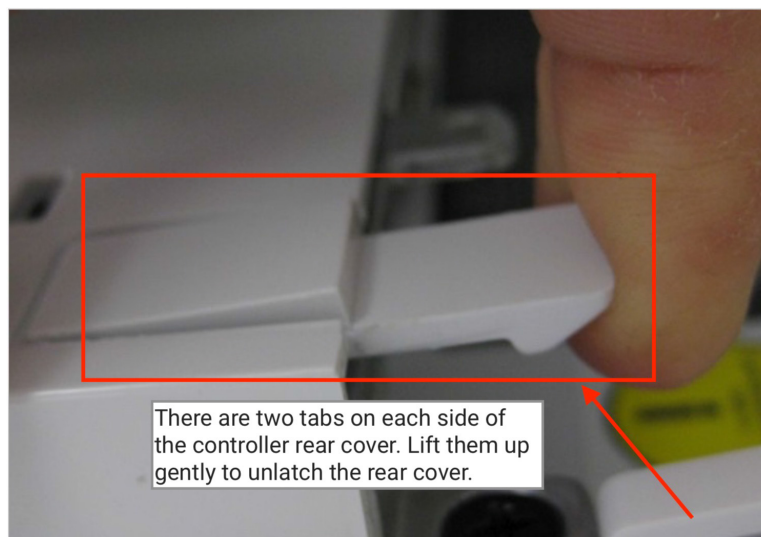


Figure 10. Gently lift the two tabs to unlatch the rear cover.

- 7 With the rear cover removed, the hard drive can be found in the bottom slot position (**Figure 11**).

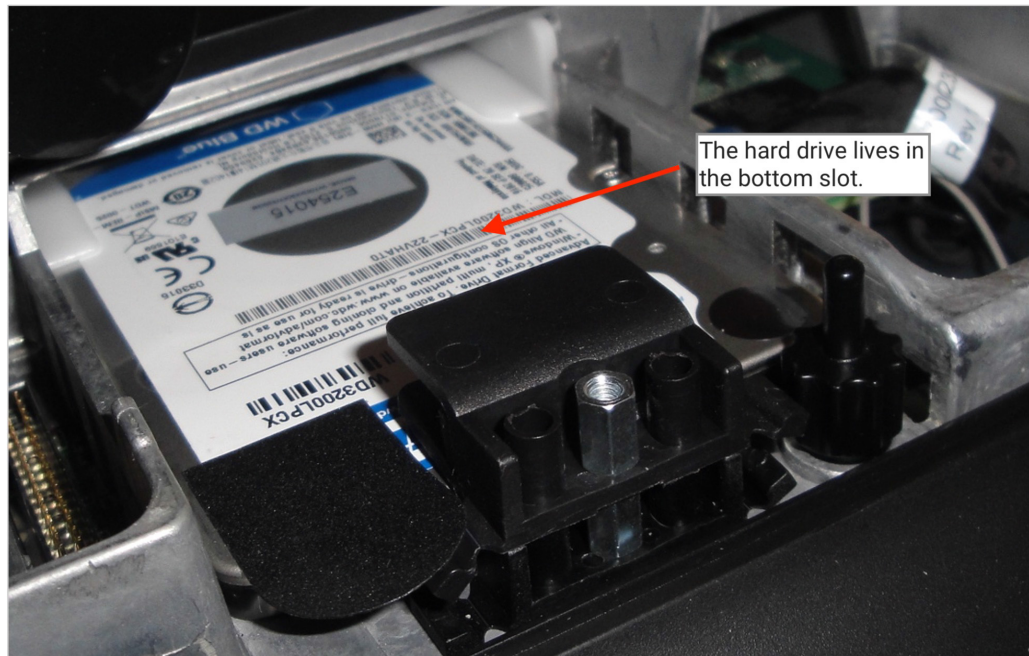


Figure 11. The hard drive in the bottom slot position.

- 8 There is a small wrench next to the hard drive (inside the XFe Controller), which is used to remove the standoffs holding the hard drive in position (**Figure 12**).

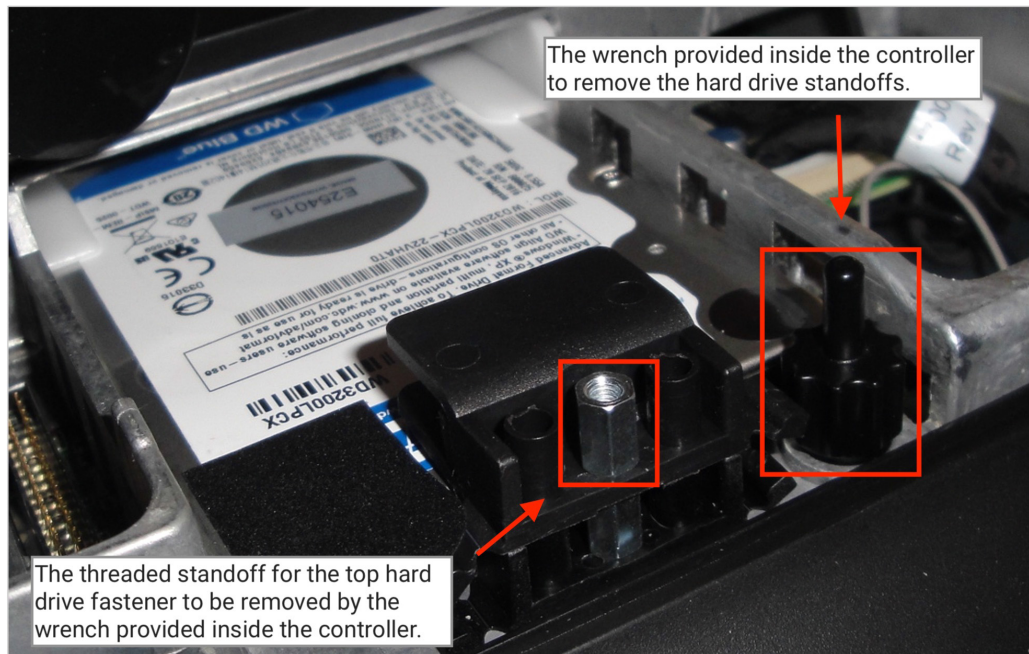


Figure 12. The wrench provided inside the controller, and the threaded standoff for the top hard drive fastener to be removed by the wrench.

- 9 Remove the top position standoff fastener and set it aside (Figure 13).

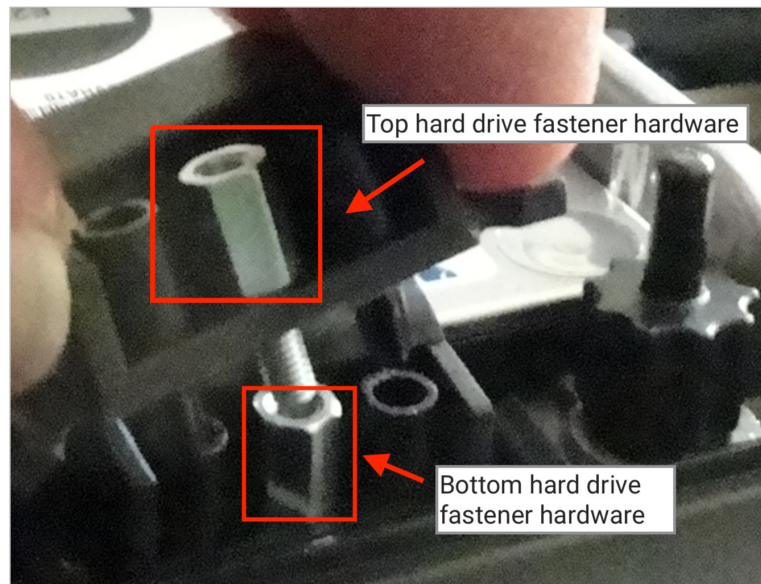


Figure 13. The top and bottom hard drive fastener hardware.

- 10 Remove the bottom position fastener holding the hard drive in place (Figure 13).
- 11 Remove the hard drive by pulling laterally on the finger tab (Figure 14).



Figure 14. Gently pulling the hard drive finger tab.

- 12 Install the new solid-state drive into the same bottom position. Ensure the new SSD seats properly into the connectors (**Figure 15**).



Figure 15. Installing the new solid-state drive into the same bottom position.

- 13 Using the wrench provided inside the Controller (seen in **Figure 12**), reinstall the bottom position standoff fastener (seen in **Figure 13**), then install the top position standoff hard drive fastener (seen in **Figure 13**).

NOTE

Ensure you do **NOT** over-tighten.

- 14 The only cables to be installed are the 9 Pin serial cable from the XFe Analyzer, the USB cable from the XFe Analyzer and the XFe Controller power cable from the AC/DC converter. The Biotek imager and external barcode wand must not be connected to the XFe Controller for the next several steps.
- 15 Replace the rear cover, securing it with the two tabs seen in **Figure 11**. Leave the cable cover off.
- 16 Place the Seahorse XFe Controller (computer) into the upright position.
- 17 Power **ON** the XFe Analyzer.

NOTE

Power **ON** the XFe Analyzer *first*, before moving on to **step 18** (Power **ON** the XFe Controller).

- 18 Power **ON** the XFe Controller. The Windows operating system should boot up without any issues.

Part 3: Transfer Seahorse XF Application and System Files to the XFe Controller After Hard Drive Replacement, Validate Your Windows 10 OS, then Install Seahorse XF Wave Controller Software and Seahorse XF Imaging and Cell Counting Software Applications

The instructions below outline the steps to replace the original application and system files on the XFe Controller after completing the hard drive upgrade (**Part 2: Removal the XFe Controller Hard Drive and Install the SSD** on page 11). These files are required for proper operation of the XFe Controller and Analyzer, failure to perform these steps will result in an unusable Agilent Seahorse XFe Analyzer and Controller. Once this is complete, you will install the appropriate Seahorse XF Wave Controller software (for XFe96 or XFe24), install Seahorse XF Imaging and Cell Counting software (if applicable), and validate your Windows 10 OS on the XFe Controller.

- 1 Plug in the external storage device containing files saved from the original XFe Controller hard drive.
- 2 Open Windows Explorer.

- 3 Go to: C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Data (Figure 16) and copy the **Instrumentconfiguration.xml** file and the **Configuration.dat** file from your external storage device to this location.

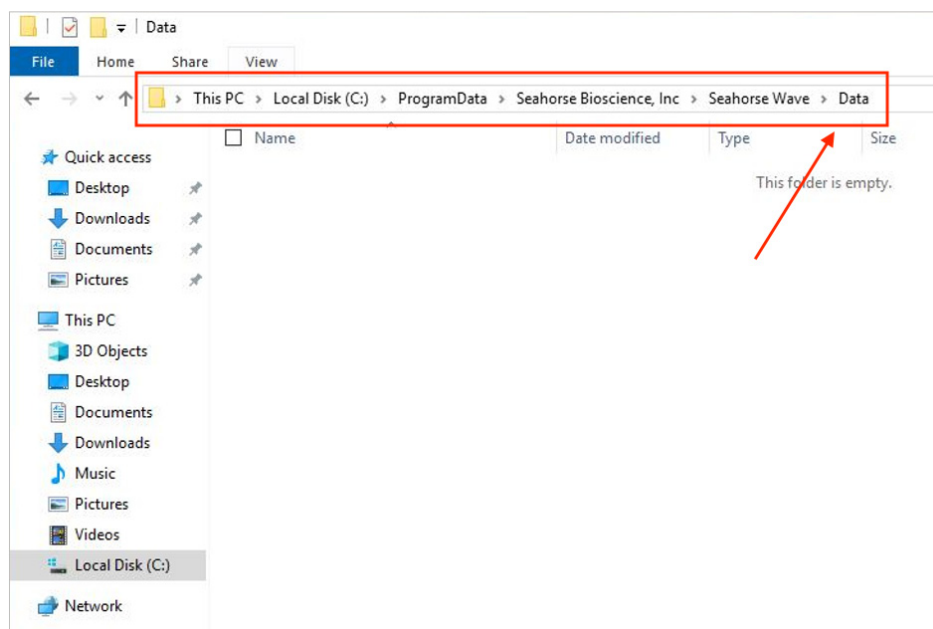


Figure 16. The **Instrumentconfiguration.xml** and **Configuration.dat** file location (C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Data).

- 4 On the XFe Controller Desktop, there are two folders called **XFe24** (Figure 17) and **XFe96** (Figure 18). These folders contain the software installer (executable) file to install Wave Controller software application. Select the appropriate folder based on your Seahorse XFe Analyzer type and run the installer.

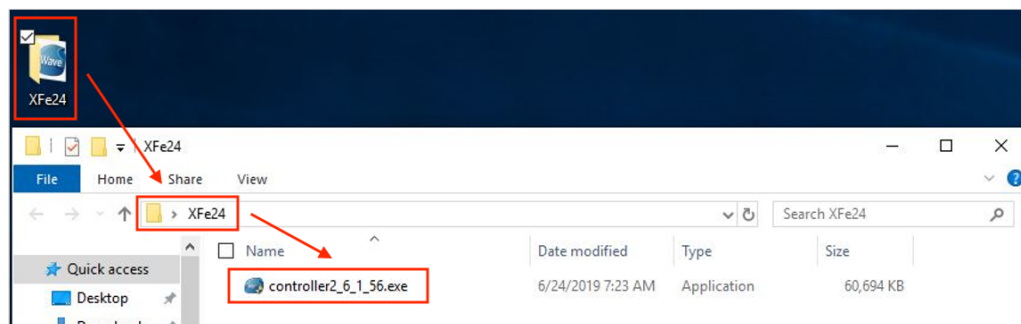


Figure 17. Select the appropriate folder based on your Seahorse XFe Analyzer type (in this example, **XFe24**).

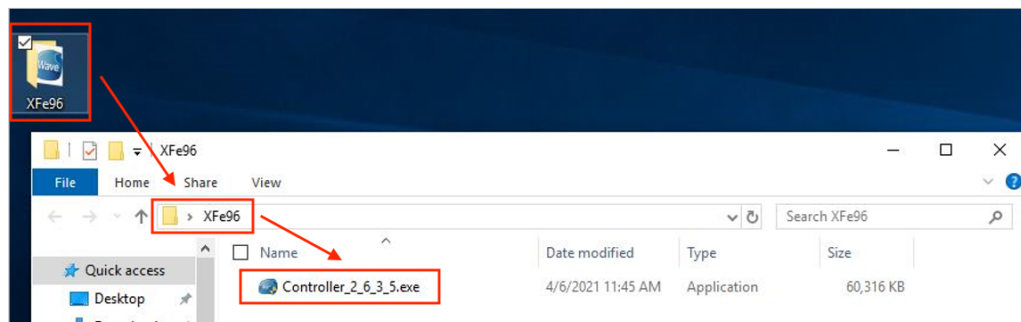


Figure 18. Select the appropriate folder based on your Seahorse XFe Analyzer type (in this example, **XFe96**).

- 5 Go to: C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Assays and copy all XF assay result files from your external storage device to this location (**Figure 19**).

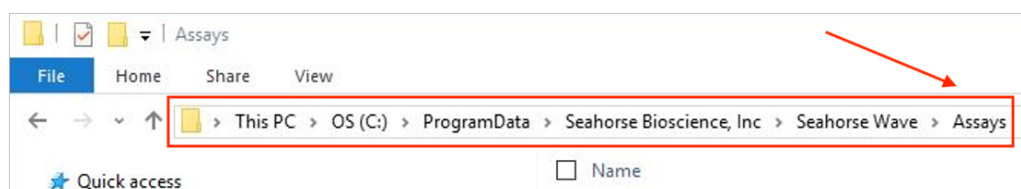


Figure 19. The file location for all assay result files (C:\ProgramData\Seahorse Bioscience, Inc\Seahorse Wave\Assays).

- 6 Connect the XFe Controller the internet. To activate Windows 10 on the XFe Controller, it must have an active internet connection.
- 7 Using the task bar (search bar) shown in **Figure 20**, type *Activate* and press **Enter** to present the **Settings > Activation view** (not pictured).

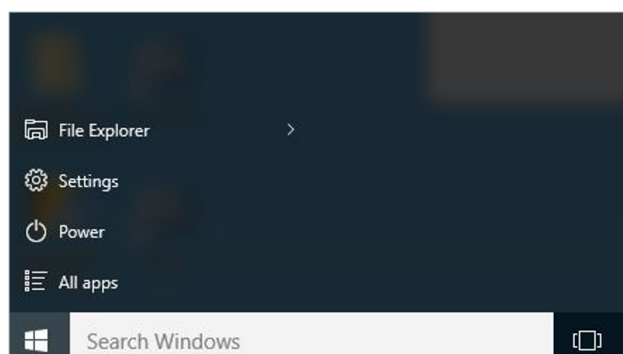


Figure 20. The task bar search feature. (Type *Activate* and press the **Enter** key).

- 8 Follow the on-screen instructions from the **Settings > Activation** view. When presented with the **Enter a product key** dialog, enter the Windows Activation code provided with the upgrade kit (**Figure 21**).

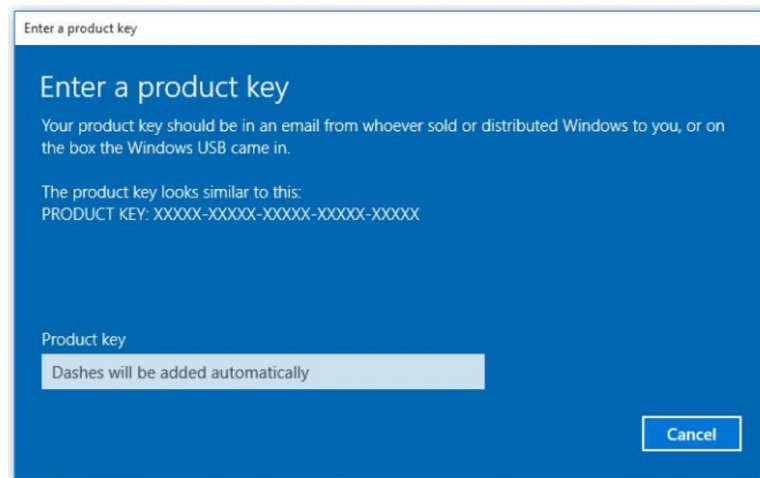


Figure 21. Enter the Windows Activation code provided with the upgrade kit.

- 9 Press the **Start** (Windows button) on the task bar, then select **Settings** (**Figure 22**).

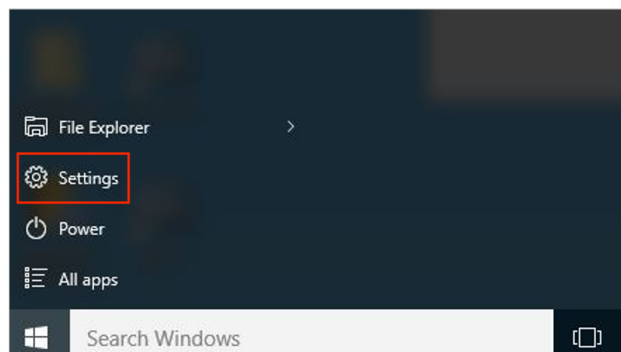


Figure 22. Select **Settings** from the start menu.

- 10 Open the **Power & sleep** settings (Figure 23). Configure this setting to **Never** go to sleep when plugged in. (Select **Never** for both **Screen** and **Sleep** settings).

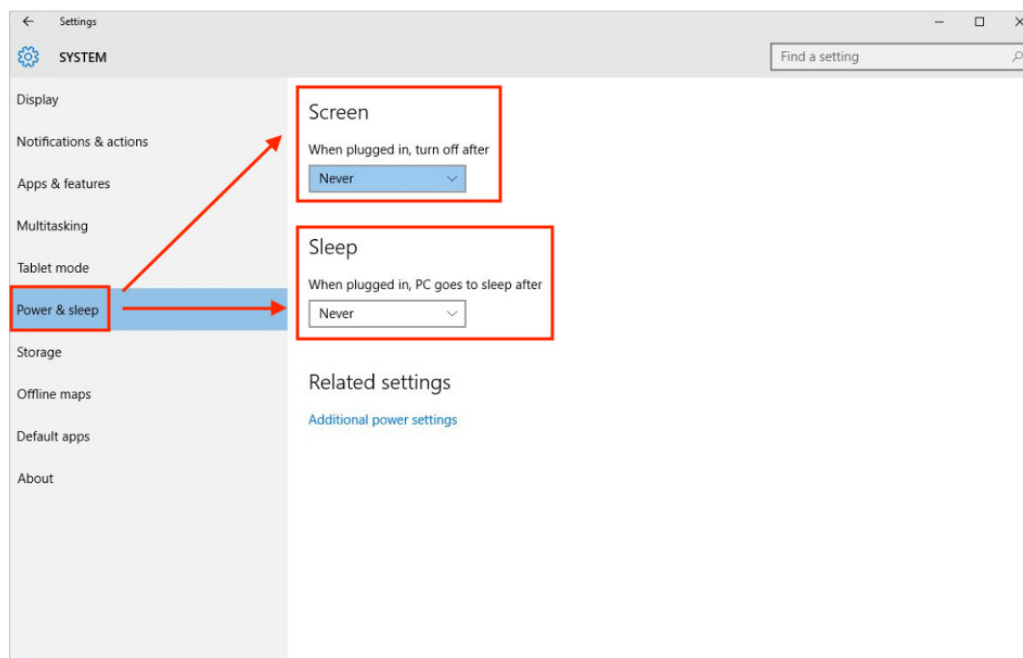


Figure 23. In the **Power & sleep** settings, configure both **Screen** and **Sleep** to **Never**.

NOTE

Failure to complete this step can cause interruptions in data acquisition if the XFe Controller goes to sleep while running an assay and can result in loss of data.

Do you have the Agilent Seahorse XF Imaging and Normalization System?

- If **no**, then you have completed the upgrade procedure.
- If **yes**, continue reading through **Part 4: Install Gen5 Software on the XFe Controller** on page 22.

- 11 Go to: C:\Program Files (x86)\Seahorse Bioscience\Cell Imaging (Figure 24).

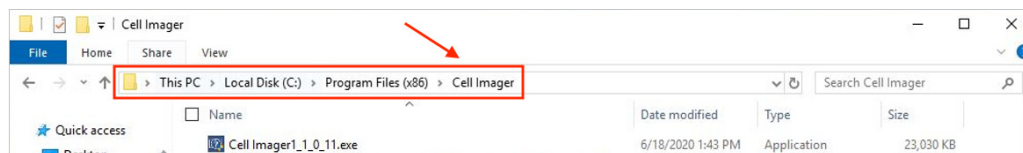


Figure 24. Go to: C:\Program Files (x86)\Seahorse Bioscience\Cell Imaging.

- 12 Run the installer file (**Cell Imager1_1_0_17.exe**) shown in **Figure 25**.

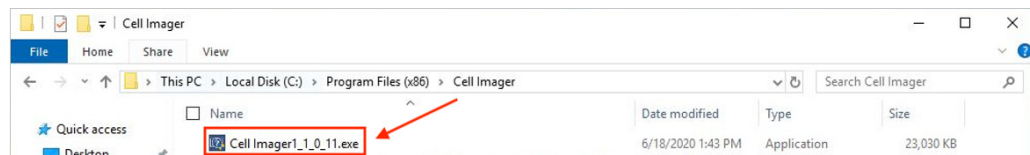


Figure 25. The installer file (**Cell Imager1_1_0_17.exe**) location.

- 13 Once installation of the XF Imaging and Cell Counting software application complete, go to: C:\Program Files (x86)\Seahorse Bioscience\Cell Imaging (**Figure 26**).

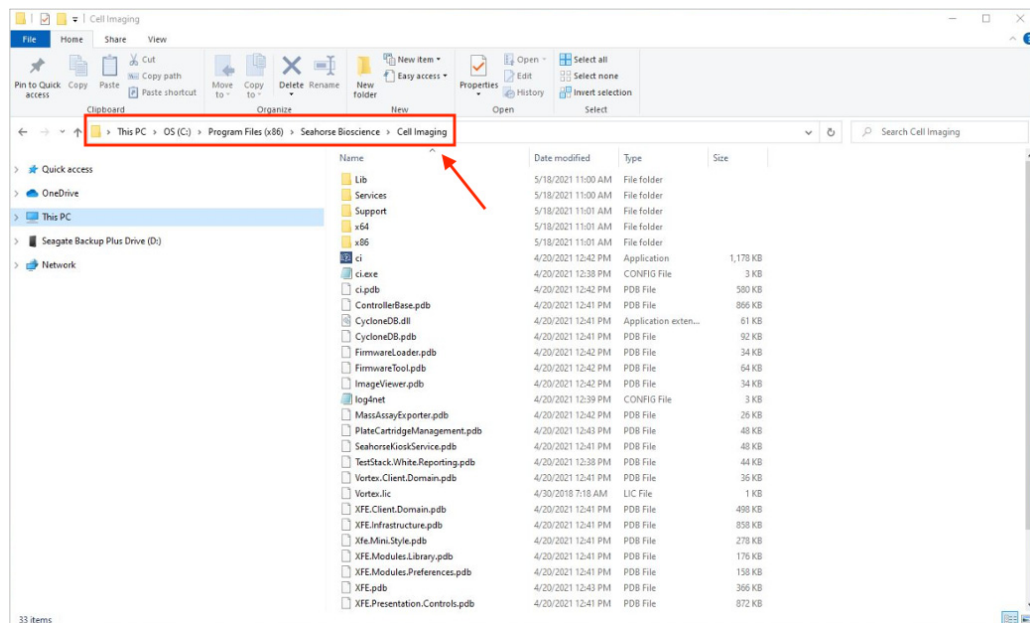


Figure 26. C:\Program Files (x86)\Seahorse Bioscience\Cell Imaging file location.

- 14 Copy the **vortex.lic** license file to this location from your external storage device (Figure 27).

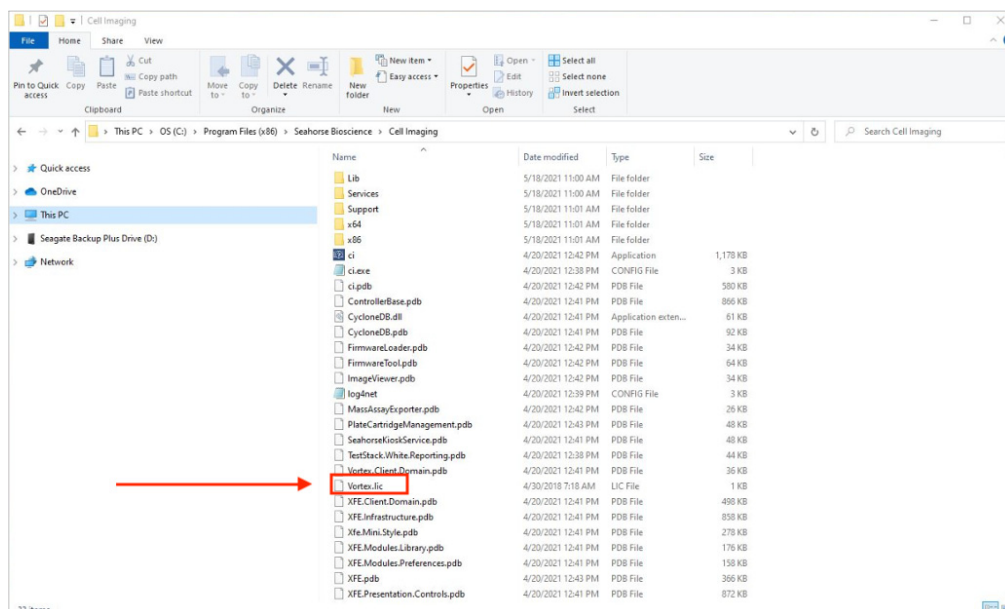


Figure 27. Copy the **vortex.lic** license file to this location from your external storage device.

- 15 Open the XF Imaging and Cell Counting software application; this will automatically generate the **Image Database** file path and will confirm the license validity. You can verify this information on the **Help** view in the XF Imaging and Cell Counting software application (Figure 28).

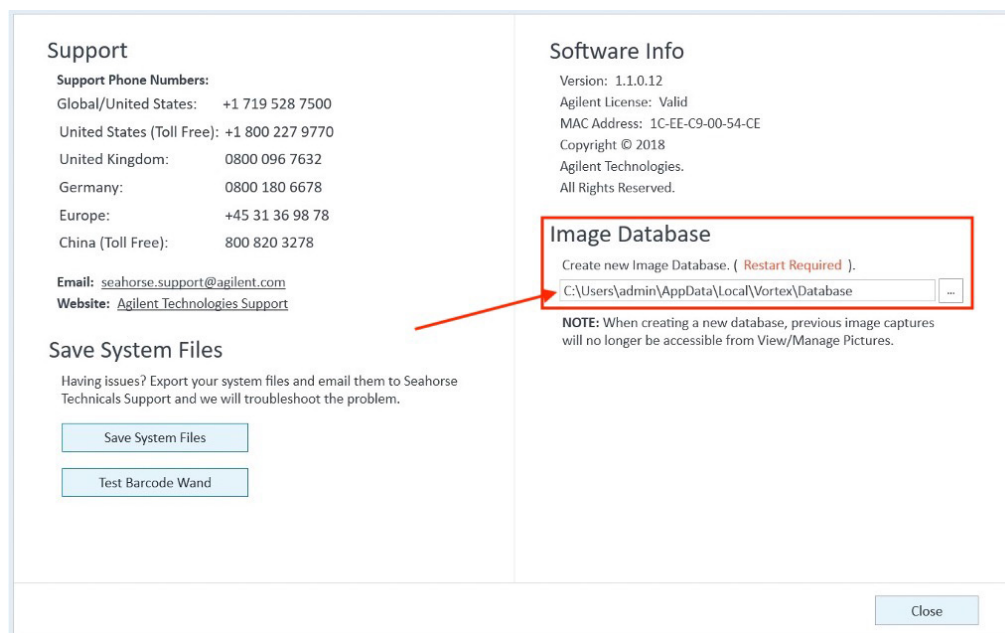


Figure 28. Verification of the **Image Database** information on the **Help** view in the XF Imaging and Cell Counting software application.

Part 4: Install Gen5 Software on the XFe Controller

The instructions below outline the steps to install and configure the Biotek Gen5 software application on the XFe Controller after installing the solid-state drive (**Part 2: Removal the XFe Controller Hard Drive and Install the SSD**), installing Seahorse software applications, and transferring Seahorse XF system and applications files (**Part 3: Transfer Seahorse XF Application and System Files to the XFe Controller After Hard Drive Replacement, Validate Your Windows 10 OS, then Install Seahorse XF Wave Controller Software and Seahorse XF Imaging and Cell Counting Software Applications**).

NOTE

If you need any assistance with this process:

- contact bio.tac@agilent.com or,
- call (800) 242-4685, option 3.

Important information before you start

- Ensure the Biotek Cytation USB 3.0 cables are unplugged from the Seahorse XFe Controller (computer).
- If you intend to continue saving images to the XFe Controller (computer) hard drive, after you complete the SSD installation, you must set the same image file save path recorded in **Part 1: Copy Application and System Files from the Agilent Seahorse XFe Controller to an External Storage Device, step 8(c)**.
- Get your Gen5 serial number. Registering the newly installed Gen5 software will require your Gen5 serial number. This can be found on the USB case received with your reader or from the Gen5 installation on the old computer by navigating to **System > About Gen5** from within the software. The serial number is typically a 14-digit code comprised of all letters.
- You will also need to install a USB driver. Before you install the driver, ensure the Biotek Cytation USB 3.0 cables are unplugged from the Seahorse XFe Controller. Use the link(s) below to download the driver directly to the XFe Controller (computer) for installation (internet access required), or download the driver to another PC and transfer to the XFe Controller for installation.
 - Open an internet browser and go to: <https://www.ftdichip.com/Drivers/VCP.htm>.
 - Find the driver for the Windows operating system and click the **setup executable** link to download the driver (**Figure 29**)

Operating System	Release Date	X86 (32-Bit)	X64 (64-Bit)	PPC	ARM	MIPSII	MIPSIV	SH4	Comments
Windows 11 (Desktop)	2021-10-05	–	2.14.1.2	–	–	–	–	–	WHQL Certified. Includes VCP and D2XX. Available as a setup executable . Please read the Release Notes and Installation Guides .
Windows 11 (Universal)	2021-10-05	–	2.14.1.2	–	–	–	–	–	WHQL Certified. Includes VCP and D2XX. Available as a setup executable . Please read the Release Notes and Installation Guides .
Windows*	2021-07-15	2.12.36.4	2.12.36.4	–	–	–	–	–	WHQL Certified. Includes VCP and D2XX. Available as a setup executable . Please read the Release Notes and Installation Guides .

Figure 29. Click the setup executable link to download the driver.

NOTE

If you cannot locate your serial number, have any questions about available software licenses, or require a password for software registration, contact bio.password@agilent.com.

Installing Biotek Gen5 software

- 1 The easiest way to re-install Gen5 is by using the USB flash drives originally sent with your Cytation instrument. A Windows user account with administrator rights must be logged in to the PC for Gen5 installation.

NOTE

If you are unable to locate the Gen5 USB flash drive, contact bio.password@agilent.com for download instructions.

- 2 Once downloaded, right-click the **Gen5.exe** file and **Run As Administrator**. Follow the onscreen prompts.
- 3 When installation is complete, you will be prompted to enter your serial number.
- 4 Enter your serial number, click **Register**, and then **Obtain Password** to complete the registration process.
- 5 Close Gen5 software.

Install the USB driver

- 1 Download the .zip file containing the FTDI VCP driver (<https://www.ftdichip.com/Drivers/VCP.htm>) and extract the zip file content.
- 2 Right-click the **CDM21xxx_Setup.exe** file and select **Run As Administrator**.
- 3 Follow the onscreen prompts.

Connect the Cytation and install camera driver

- 1 Plug in the USB cable for the external handheld barcode wand to the XFe Controller.
- 2 The Cytation connects to the Seahorse XFe Controller via two USB 3.0 cables, one for the Cytation instrument and another for the camera. Connect both USB cables to available USB 3.0 ports on the Seahorse XFe Controller and then install the cable cover.
- 3 On the Seahorse XFe Controller, navigate to the folder containing the camera driver: C:\Program Files\BioTek\Gen5 3.11\USB3 Drivers\Windows_64\PGRUSBCam
- 4 Right-click on the **InstallPGRDriver.bat** file and select **Run As Administrator**.

- 5 A message will appear indicating the installation was successful (Figure 30).

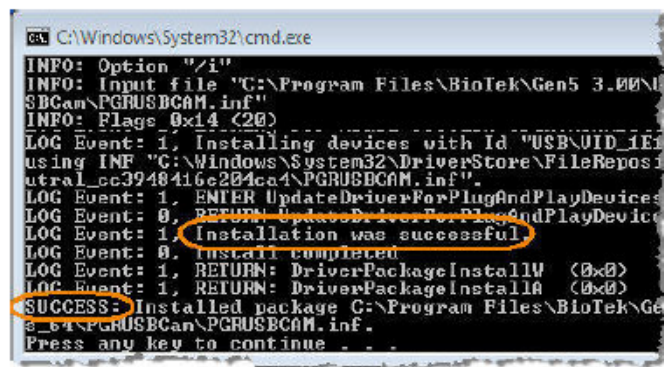


Figure 30. A message indicating that the installation was successful.

Open Gen5 and configure the Cytation Instrument

- 1 Turn on the Cytation instrument and allow it to complete the power up sequence.
- 2 Open Gen5, you will be prompted to **Add a New Reader**.
- 3 From the drop-down menu select your instrument from the list, either **Cytation 1** or **Cytation 5**.
- 4 Using the **Reader Settings** dialog (Figure 31), select **Plug & Play** and then confirm the instrument is properly connected by clicking the **Test Communications** button. If working properly a success message will be displayed.

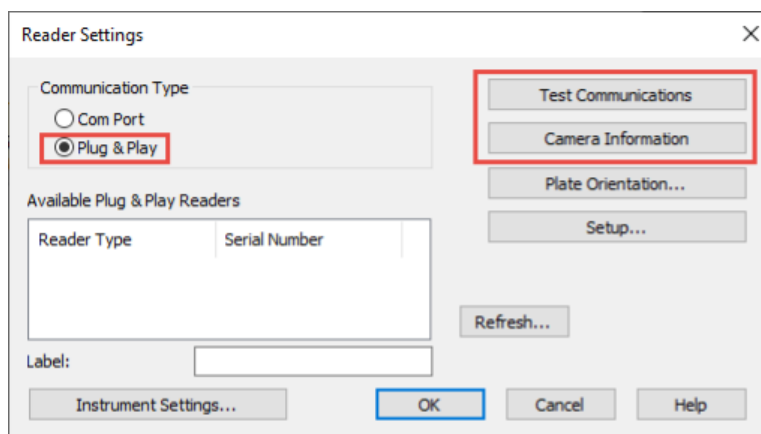


Figure 31. The **Reader Settings** dialog.

- 5 Click the **Camera Information** button. If successful, camera information will be displayed as seen in **Figure 30**. Confirm the **Bus Speed** is **5,000 Mbits/sec** (**Figure 32**). If it is significantly lower than this, contact BioTek TAC (bio.tac@agilent.com) for troubleshooting.

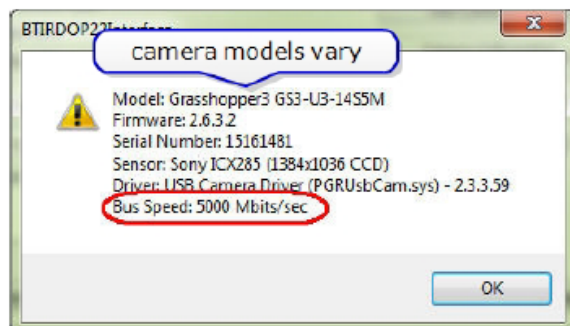


Figure 32. **Bus Speed** shown in the Camera Information mode.

- 6 Choose **OK** to confirm the **Reader Settings** dialog (see **Figure 31**).
- 7 You will then be prompted to select an **Image Save** location and other image save preferences (**Figure 33**). This will be the folder where all image data collected with the **Seahorse** or **Cytation** will be stored.

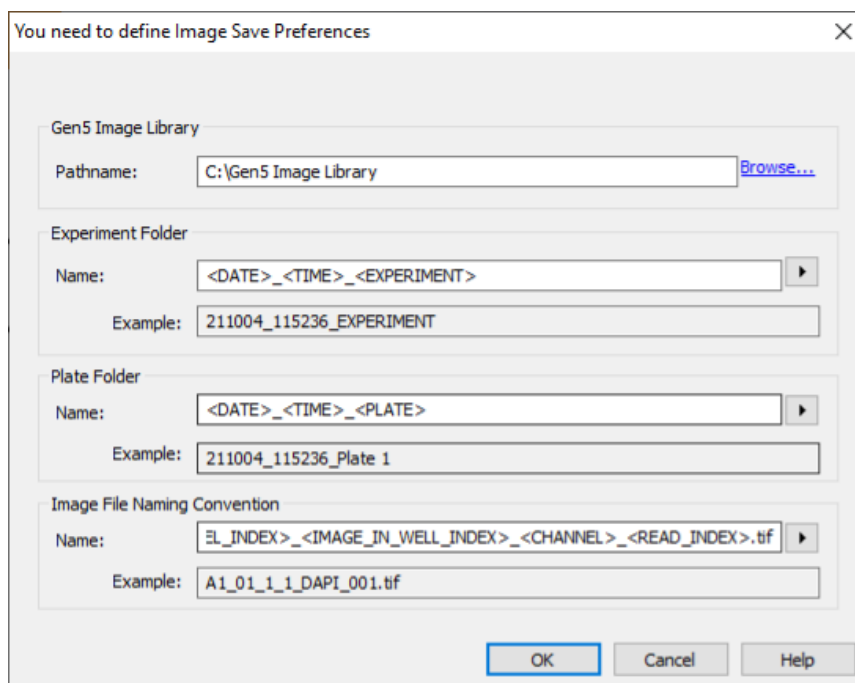


Figure 33. Define an image save location (**Pathname**), **Experiment Folder**, **Plate Folder**, and **Image File Naming Convention** in **Image Save Preferences**.

- 8 Browse to set the previously recorded path for image files. Select an appropriate location with adequate storage space. It is recommended to save images *off* the XFe Controller (computer).

- 9 Click **OK** on the **Image Save Preferences** dialog.
- 10 Close Gen5 software.
- 11 Open the **XF Imaging and Cell Counting** application and confirm the connection to the Cytation instrument.
- 12 Go to the **Help** view and note the path of the **Image Database** file (**Figure 34**).

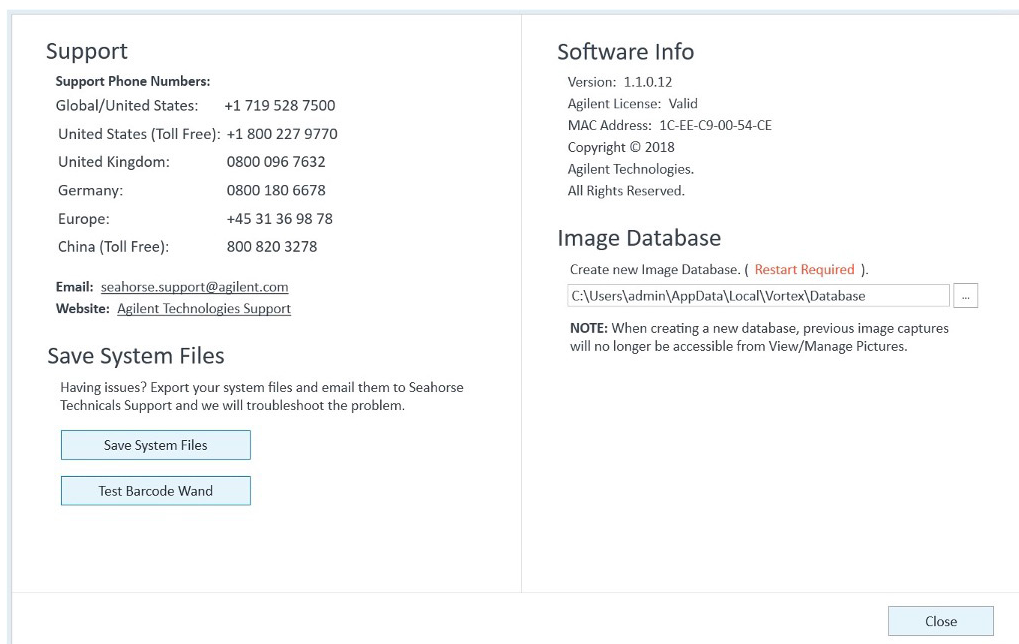


Figure 34. The path of the **Image Database** file shown in the **Help** view.

- 13 Copy the **Image Database** file, **vortex.db**, from your external storage device to the image database path on the new hard drive.

The hard drive upgrade procedure is now complete.

Part 5: Perform XFe Analyzer Diagnostic Injection Test

The **final step** after completing the hard drive upgrade on an XFe Analyzer is performing a diagnostic injection test. The diagnostic injection test procedure is required to verify proper operation of the XFe96 and XFe24 Analyzer.

Diagnostic Injection Test Procedure: Seahorse XFe96 Analyzer

Required Materials

Agilent Seahorse XFe96 FluxPak containing:

- 1 Seahorse XFe96/XF Pro Extracellular Flux Assay kit:
 - Cartridge lid
 - Sensor cartridge
 - Hydrobooster
 - Utility plate
- 2 Seahorse XF96/XF Pro cell culture microplates
- 3 Seahorse XF calibrant solution

Also required, but not included:

- 1 200 µL pipettor and tips
- 2 5 mL pipette
- 3 50 mL conical tube
- 4 Non-CO₂ 37 °C incubator
- 5 Phenol red (preferred)

Day prior to test

Turn on the instrument

- 1 Go to the Agilent Seahorse XFe96 Analyzer and verify the instrument is powered **ON** and connected to the XFe Controller (computer). You can verify the instrument connection status in the widget panel in the lower-left corner of Wave Controller software.

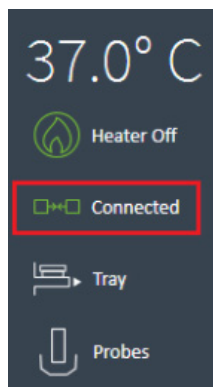


Figure 35. Verification of instrument connection status.

Hydrate Sensor Cartridge

- 1 Open the Agilent Seahorse XFe96/XF Pro Extracellular Flux Assay kit and remove the contents.
- 2 Place the sensor cartridge, upside down, next to the utility plate.

NOTE

Make sure the cartridge is not expired.

- 3 Fill each well of the utility plate with 200 μ L of XF calibrant solution.
- 4 Place the XF Hydrobooster on top of the utility plate and push the XF Hydrobooster downward to make sure it has tightly assembled with the utility plate.

NOTE

Make sure not to scratch the sensors.

- 5 Verify the XF calibrant solution level is high enough to keep the sensors submerged.
- 6 Place the sensor cartridge in a non-CO₂ 37 °C incubator overnight (minimum of 12 hours). To prevent evaporation of the XF Calibrant, the incubator should be humidified.
- 7 Open the Agilent Seahorse XF96/XF Pro Cell Culture Microplate packaging.
- 8 Place an empty cell culture microplate in a non-CO₂ 37 °C incubator overnight (minimum of 12 hours).
- 9 Aliquot at least 30 mL XF Calibrant Solution into a 50 mL conical tube and place in a non-CO₂ 37 °C incubator overnight to prewarm the solution (minimum of 12 hours).

Create diagnostic test protocol

Create a diagnostic test protocol template using Wave Desktop, Wave Controller, or upload the pre-made Diagnostic Injection Test protocol template provided by Cell Analysis Technical Support (cellanalysis.support@agilent.com).

- 1 Double-click to open the **Blank** assay template file from the **Templates** view (Alternatively transfer the pre-made template using a shared network drive or USB flash drive).

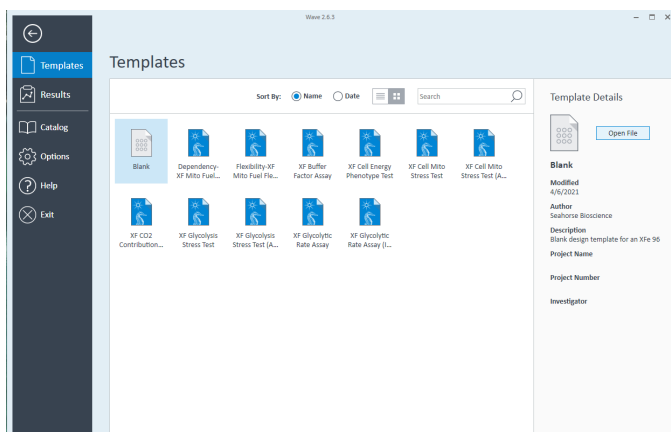


Figure 36. Templates view.

- 2 On the **Group Definitions** view, create 6 groups.

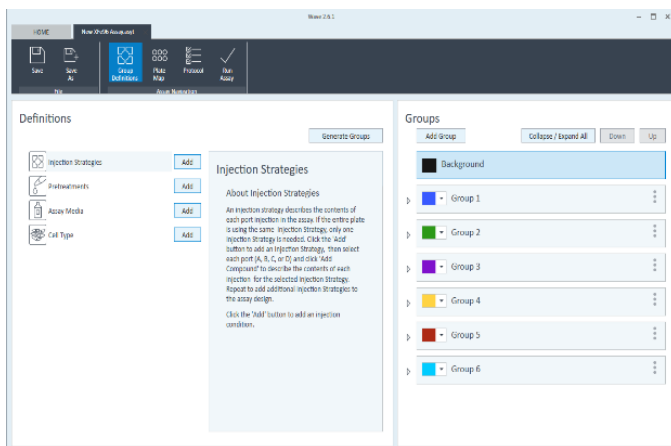


Figure 37. Group Definitions view.

- 3 Click **Plate Map** in the functions ribbon (under "Assay Navigation").
- 4 Unassign the background wells by clicking on each of the 4 default background wells (black) to make the wells gray.

- 5 Click on **Distribute Groups**.

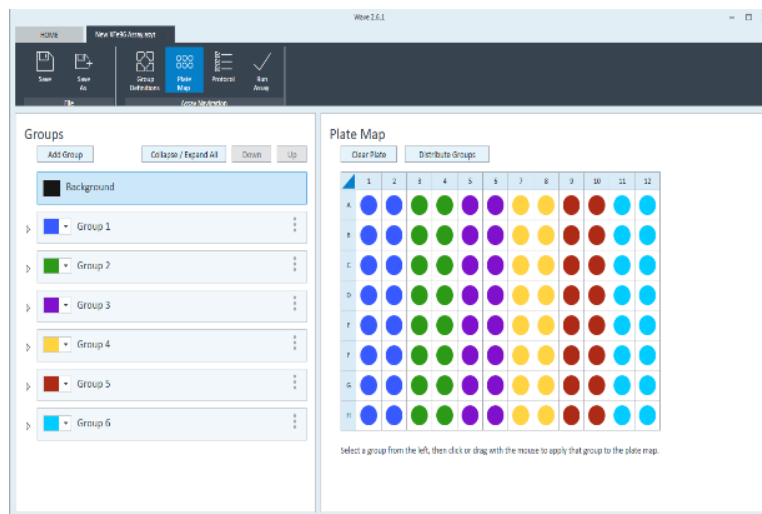


Figure 38. Distribute Groups.

- 6 Click on **Protocol** in the functions ribbon.
- 7 Expand the **Edit Measurement Details** drop-down.
- 8 Increase the number of cycles from 3 to 5.

NOTE

Important: Do not change the Mix, Wait and Measure times.

- 9 Add 4 injections.
- 10 Expand the **Edit Measurement Details** drop-downs in each of the 4 injections.

- 11 Decrease the number of cycles in the injections 1 through 4 from 3 to 1.

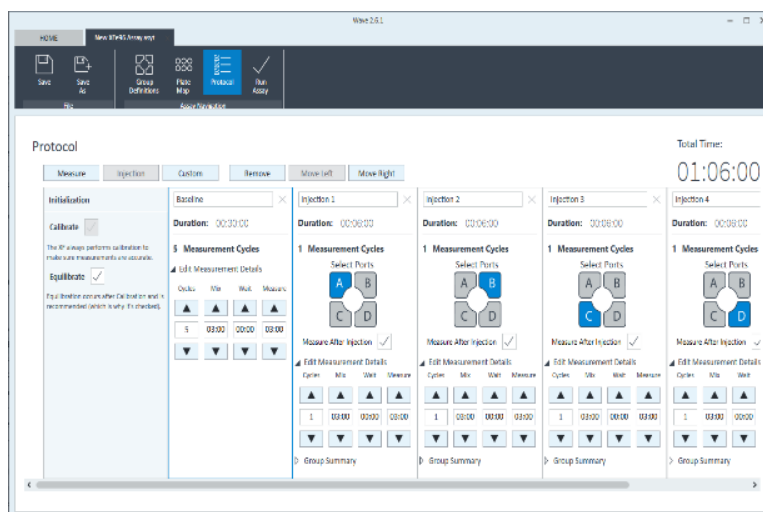


Figure 39. Decrease the number of cycles in the injections.

Day of test

- 1 Remove the empty XF Cell Culture Microplate from the incubator.
- 2 Fill each well in the XF Cell Culture Microplate with 200 μ L prewarmed XF Calibrant solution.
- 3 Place the XF Cell Culture Microplate back into the non-CO₂ 37 °C incubator.
- 4 Remove the sensor cartridge from the utility plate with the Hydrobooster and place the sensor cartridge next to the utility plate upside down with the sensors up into the air.

NOTE

Make sure not to scratch the sensors.

- 5 Remove the Hydrobooster between the XF sensor cartridge and the utility plate.

NOTE

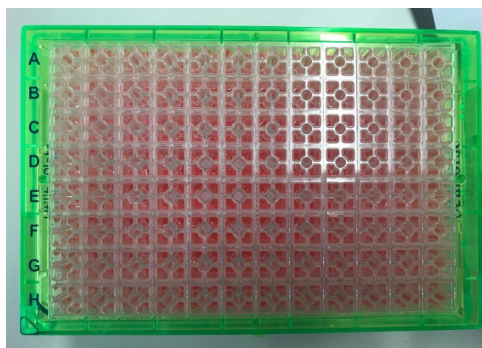
The Hydrobooster is well attached to the utility plate.

- 6 Place the sensor cartridge back onto the utility plate, and pipette 25 μ L XF calibrant solution containing phenol red (30 mg/L) into each injection port (A, B, C, D).
 - For 5 mL Calibrant solution containing phenol red (30 mg/L), using 0.5% phenol red solution (P0290 from Sigma Aldrich) is recommended:

Solution	Volume (μ L)
Agilent Seahorse XF Calibrant	4,970
0.5% Phenol red solution	30

- For correct loading of the XF Sensor cartridge, please refer to the **Loading guide**.
- Take a picture of the XF Sensor cartridge from above and from the side that includes all wells (see **Figure 40**). Make sure not to lift the cartridge for the pictures.

Example from above:



Example from the side:

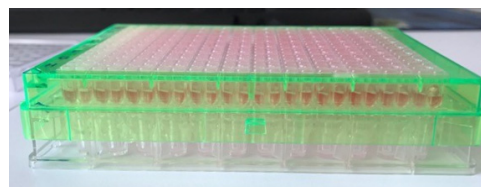


Figure 40. Examples of pictures of cartridges.

- 7 Optional:** Place the loaded XF Sensor Cartridge back into the non-CO₂ 37 °C incubator for 10 minutes to make sure it is 37 °C before starting the calibration.
- 8** Open the **Diagnostic Test** protocol template on the Agilent Seahorse XFe96 Analyzer.
- 9** Start the calibration of the XF sensor cartridge.
 - Time to complete calibration is approximately 10 to 20 minutes.
 - Once sensor cartridge calibration is complete, Wave Controller will display the **Load Cell Plate** dialog.
- 10** Click **Open Tray** to eject the utility plate. Remove the utility plate and place the cell culture microplate containing XF calibrant on the tray.

NOTE

Important: ensure the lid is removed from the cell plate.

- Ensure the proper orientation (direction) of the cell plate on the tray.
- Click **Load Cell Plate** to initiate the test protocol.

- Take a picture of the utility plate after calibration (see **Figure 41**).

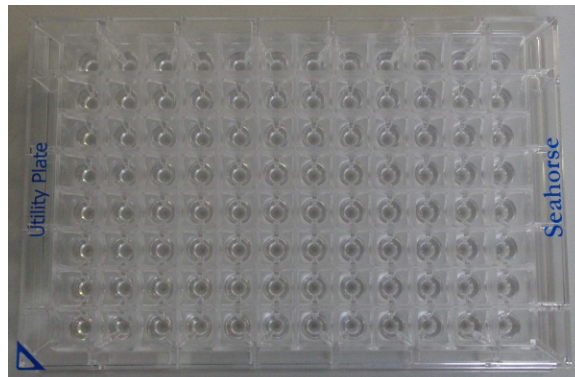
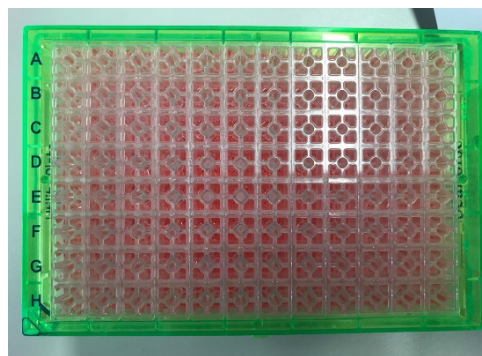


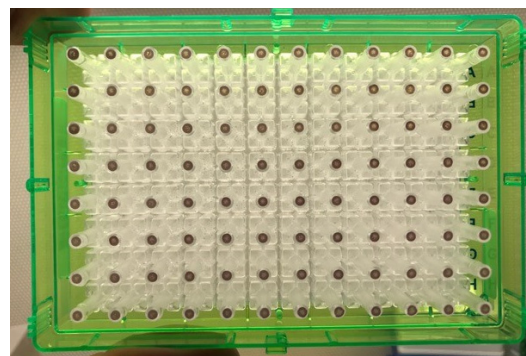
Figure 41. Example of utility plate.

- 11 Upon completion of the diagnostic injection test, take a picture of the cartridge from above, from below and from the side that includes all wells and make sure all injection ports are visible (see **Figure 42**).

Example from above:



Example from below:



Example from the side:



Figure 42. Example pictures of cartridges.

- 12 E-mail the data file and pictures to Cell Analysis Technical Support:
cellanalysis.support@agilent.com

Diagnostic Injection Test Procedure: XFe24 Analyzer

Required Materials

Agilent Seahorse XFe24 FluxPak containing:

- 1 Seahorse XFe24 Extracellular Flux Assay Kit:
 - Cartridge lid
 - Sensor cartridge
 - Hydrobooster
 - Utility plate
- 2 Seahorse XF24 Cell Culture Microplates
- 3 Seahorse XF Calibrant Solution

Also required, but not included:

- 1 200 μ L and/or 1,000 μ L pipettor and tips
- 2 5 mL pipette
- 3 50 mL conical tube
- 1 Non-CO₂ 37 °C incubator
- 2 Phenol red (preferred)

Day prior to test

Turn on the instrument

- 1 Go to the Agilent Seahorse XFe24 Analyzer and verify the instrument is powered **ON** and connected to the XFe Controller (computer). You can verify the instrument connection status in the widget panel in the lower-left corner of Wave Controller software.

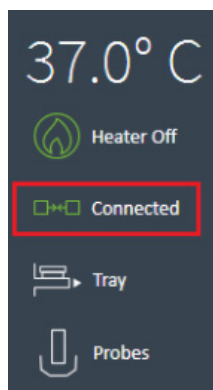


Figure 43. Verification of instrument connection status.

Hydrate Sensor Cartridge

- 1 Open the Agilent Seahorse XFe24 Extracellular Flux Assay kit and remove the contents.
- 2 Place the sensor cartridge, upside down, next to the utility plate.

NOTE

Make sure the cartridge is not expired.

- 3 Fill each well of the utility plate with 1 mL of XF calibrant solution.
- 4 Place the XF Hydrobooster on top of the utility plate and push the XF Hydrobooster downward to make sure it has tightly assembled with the utility plate.

NOTE

Make sure not to scratch the sensors.

- 5 Verify the XF calibrant solution level is high enough to keep the sensors submerged.
- 6 Place the Sensor Cartridge in a non-CO₂ 37 °C incubator overnight (minimum of 12 hours). To prevent evaporation of the XF Calibrant, the incubator should be humidified.
- 7 Open the Agilent Seahorse XFe24 Cell Culture Microplate packaging.
- 8 Aliquot minimum 30 mL Agilent Seahorse XF calibrant into a 50 mL tube.
- 9 Place the tube with XF calibrant in a non-CO₂ 37 °C incubator overnight to prewarm the solution (minimum of 12 hours).

Create diagnostic test protocol

Create a diagnostic test protocol template using Wave Desktop, Wave Controller or upload the pre-made Diagnostic Injection Test protocol template provided by Cell Analysis Technical Support (cellanalysis.support@agilent.com).

- 1 Double-click to open the **Blank** assay template file from the **Templates** view (Alternatively transfer the pre-made template using a shared network drive or USB flash drive).

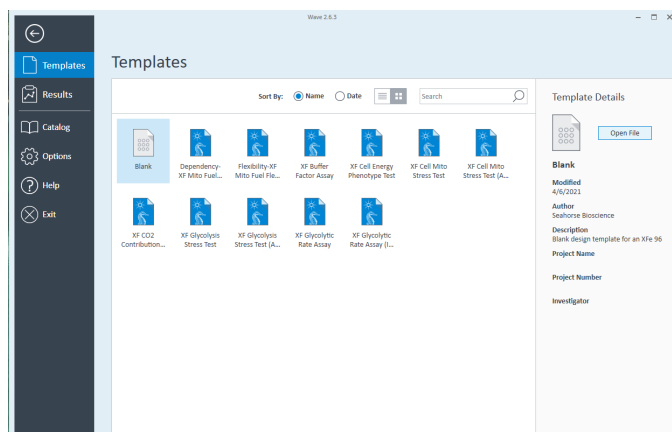


Figure 44. Templates view.

- 2 On the **Group Definitions** view, create 6 groups.

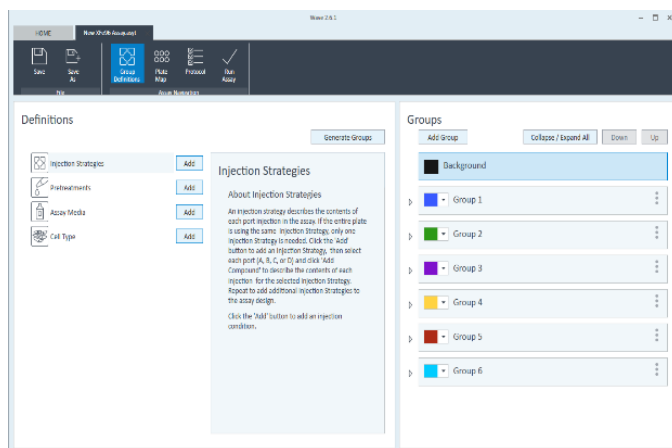


Figure 45. Group Definitions view.

- 3 Click **Plate Map** in the functions ribbon (under "Assay Navigation").
- 4 Unassign the background wells by clicking on each of the 4 default background wells (black) to make the wells gray.

- 5 Click on **Distribute Groups**.

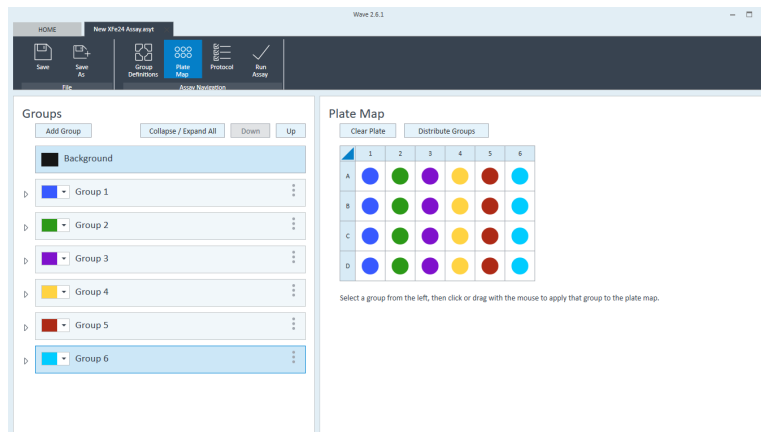


Figure 46. Distribute Groups.

- 6 Click on **Protocol** in the functions ribbon.
- 7 Expand the **Edit Measurement Details** drop-down.
- 8 Increase the number of cycles from 3 to 5.

NOTE

Important: Do not change the Mix, Wait and Measure times.

- 9 Add 4 injections.
- 10 Expand the Edit Measurement Details drop-downs in each of the 4 injections
- 11 Decrease the number of cycles in the injections 1 through 4 from 3 to 1.

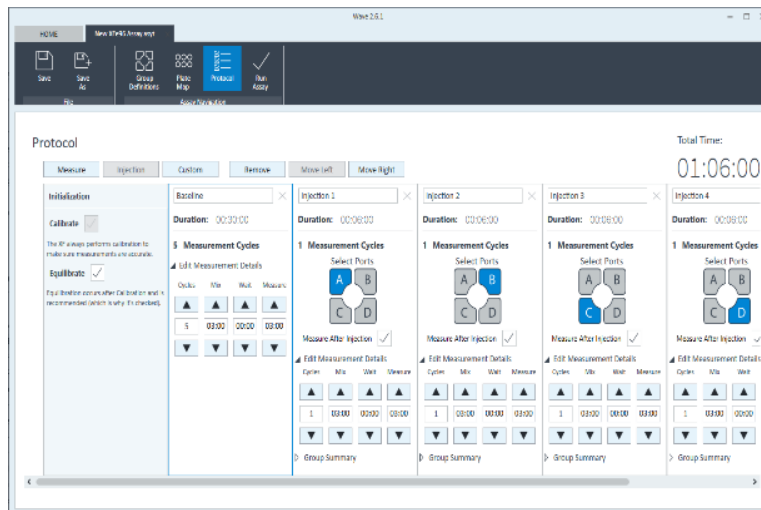


Figure 47. Decrease the number of cycles in the injections.

Day of test

- 1 Remove the empty XF Cell Culture Microplate from the incubator.
- 2 Fill each of the wells in the XF Cell Culture Microplate with 1 mL prewarmed XF calibrant solution.
- 3 Place the XF Cell Culture Microplate back into the non-CO₂ 37 °C incubator.
- 4 Remove the sensor cartridge from the utility plate with the Hydrobooster and place the sensor cartridge next to the utility plate upside down with the sensors up into the air.

NOTE**Make sure not to scratch the sensors.**

- 5 Remove the Hydrobooster between the XF sensor cartridge and the utility plate
- 6 Place the sensor cartridge back onto the utility plate, and pipette 75 µL XF calibrant solution containing phenol red (30 mg/L) into each injection port (A, B, C, D).
 - For 5 mL calibrant solution containing phenol red (30 mg/L) using 0.5% phenol red solution (P0290 from Sigma Aldrich) is recommended:

Solution	Volume (µL)
Agilent Seahorse XF Calibrant	4,970
0.5% Phenol red solution	30

- For correct loading of the XF Sensor cartridge, please refer to the [Loading guide](#).
- Take a picture of the XF Sensor cartridge from above and from the side that includes all wells (see [Figure 48](#)). Make sure not to lift the cartridge for the pictures.

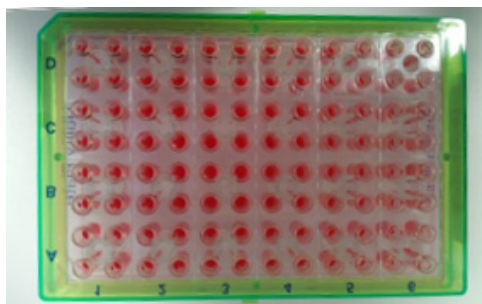
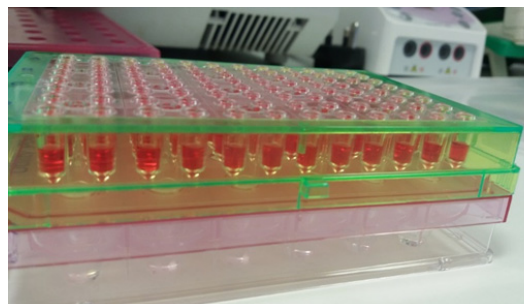
Example from above:**Example from the side:**

Figure 48. Examples of pictures of cartridges.

- 7 **Optional:** Place the loaded XF sensor cartridge back into the non-CO₂ 37 °C incubator for 10 minutes to make sure it is 37 °C before starting the calibration.
- 8 Open Diagnostic Test protocol template on the Agilent Seahorse XFe24 Analyzer.
- 9 Start the calibration of the XF sensor cartridge.
 - Time to complete calibration is approximately 10 to 20 minutes.

- Once sensor cartridge calibration is complete, Wave Controller will display the **Load Cell Plate** dialog.

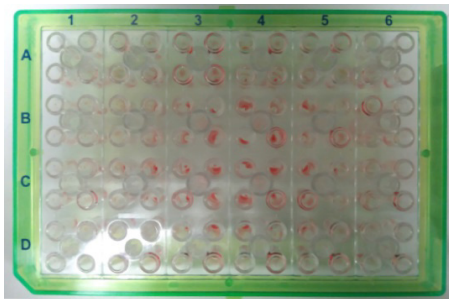
10 Click **Open Tray** to eject the utility plate. Remove the utility plate and place the cell culture microplate containing XF Calibrant on the tray.

NOTE

Important: Ensure the lid is removed from the cell plate.

- Ensure the proper orientation (direction) of the cell plate on the tray.
 - Click **Load Cell Plate** to initiate the test protocol.
 - Take a picture of the utility plate after calibration (see **Figure 49**).
- 11** Upon completion of the diagnostic injection test, take a picture of the cartridge from above, from below, and from the side that includes all wells and make sure all injection ports are visible (see **Figure 49**).

Example from above:



Example from the side:

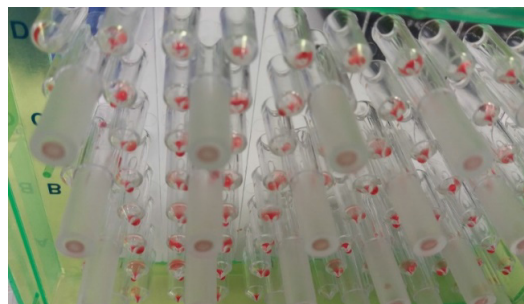


Figure 49. Examples of pictures of cartridges.

12 E-mail the data file and pictures to Cell Analysis Technical Support:
cellanalysis.support@agilent.com

This page intentionally left blank.

This page intentionally left blank.

www.agilent.com

© Agilent Technologies, Inc. 2022

Second edition, December 2022



5994-4360EN

