



Agilent BioTek Gen5 Data Analysis Software

Getting Started Guide

ERRATA NOTICE: This document contains references to BioTek.
Please note that BioTek is now Agilent. For more information, go to
www.agilent.com/lifesciences/biotek.

BioTek Gen5

Getting Started Guide

Microplate Data Collection and Analysis Software

Agilent Technologies, Inc.

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Welcome to Gen5

BioTek Gen5 software for BioTek multimode and single mode microplate readers is an integrated tool for endpoint, kinetic, spectral scanning and well area scanning. It controls all the functions of the plate reader and has powerful data analysis capabilities for a broad range of applications. Gen5 microplate reader software is also used to integrate BioTek plate readers to BioTek BioStack and other automated systems.

BioTek Gen5 Software for imaging and microscopy offers automated image capture, processing and analysis for a broad range of samples, from whole organism imaging to high magnification subcellular imaging. Raw data and images are transformed into meaningful results with powerful built-in capture, process and analysis tools. The automated process, known as Augmented Microscopy, enables simple and straightforward imaging and analysis workflows.

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Revision History

| Rev | Date | Changes |
|-----|------|---|
| R | 2022 | Added support for Lionheart WFOV imagers. BioTek Instruments is now Agilent Technologies : Gen5 has been rebranded, including a new desktop icon, to support BioTek's integration with our new company, Agilent. Online Sample Files provided via the Task Manager are now accessed from an Agilent website: https://www.agilent.com/en/gen5-lhc-sample-files . |

Install Gen5

An "install wizard" guides you through the installation of Gen5—just follow the prompts. Before doing so, make sure your computer and Agilent instruments meet the system recommendations outlined in this section. You may also want to review the installation options, software warranty, and related information.

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Computer System Recommendations

To achieve the best Gen5 performance, Agilent offers the following recommendations.

Non-Imaging Use

- 64-bit version of Windows 10 Professional editions (or equivalent) version V1909, 2004, or 20H2
- Intel Celeron Dual Core Processor T1600 (1.66 GHz, 667 MHz FSB, 1 MB L2 cache) or equivalent
- 2 GB RAM or higher
- 100 GB free hard drive space or higher
- Monitor resolution 1280 x 800 or higher
- Keyboard/mouse
- Microsoft Internet Explorer v 9.0 or higher (for online Help); for Take3 XTML export, Internet Explorer v 10 or higher
- Microsoft Excel 2007 (32-bit only) or Microsoft Excel 2010 or higher (either 32- or 64-bit, install one edition only) for QuickExport and Power Export
- Serial or USB port for Agilent instrument

Imaging

Agilent provides an imaging controller for instruments that support imaging. The imaging controller is the recommended host computer. The system recommendations below are for host computers not supplied by Agilent.

- 64-bit version of Windows 10 Professional editions (or equivalent) version V1909, 2004, or 20H2



Windows N Editions and KN Editions, N for Europe and KN for Korea, require installation of the Media Feature Pack for N and KN versions available free from Microsoft Corporation.

- Intel® Core i5 (4th generation) processor or higher

- Intel 8 USB chipset or higher
- 8 GB RAM or higher
- 512 GB hard drive space or higher*
- Monitor resolution 1680 x 1050 or higher**
- Software:
 - Gen5 Image+, Gen5 Secure Image+, or Gen5 IVD Image+, or any edition of Gen5 Image Prime. Spot Counting and/or Auto ROI add-on modules are required for these advanced-level cellular analysis features.
 - Microsoft Internet Explorer v 9.0 or higher (for online Help)
 - Microsoft Excel for QuickExport and Power Export:
 - Gen5 3.0 or higher: Excel 2007 - 2016 (either 32- or 64-bit, install one edition only)
 - Keyboard and mouse
 - Connectivity:
 - USB3 port for camera
 - USB 2 or 3 port for instrument

* Each image is approximately 2 MB in size, and 7.8 MB when using a wide field-of-view (WFOV) camera. Image file management is the responsibility of the user. An RJ-45 Lan connector is recommended for network use. **Do not use a WiFi network connection.**

** Gen5 is not designed for use on very high resolution monitors, e.g., 4K or 5K monitors.

Example: Required Hard Drive Space for Imaging in standard FOV and WFOV/Confocal

| Imaging Parameters | Space Required | |
|---|----------------|-------------------|
| | Std FOV | WFOV ¹ |
| 1 image (standard FOV and WFOV) | 2.166 MB | 7.8 MB |
| 96-well plate, 1 image per well (96 images x 1 color) | 208 MB | 749 MB |

| Imaging Parameters | Space Required | |
|--|----------------|-------------------|
| | Std FOV | WFOV ¹ |
| 96-well plate, RGB, 1 field of view per well (96 wells x 3 colors) | 624 MB | 2.2 GB |
| 96-well plate, DAPI, z-stack, 10 slices per well (96 wells x 1 color x 10 z-planes) | 2.1 GB | 7.5 GB |
| 96-well plate, RGB, z-stack, 10 slices per well (96 wells x 3 colors x 10 z-planes) | 6.2 GB | 22 GB |
| 1 tissue section slide, RGB, 4x3 montage (1 sample x 4 x 3 x 3 colors (RGB)) | 78 MB | 281 MB |
| 6 H&E slides, whole sample, 15x15 montage (6 samples x 15 x 15 x 3 colors (RGB)) | 8.8 GB | 32 GB |
| Z-stack-Montage-Kinetic in 24-well plate, Green & DAPI (2 colors) 6 slices - 4x4 tiles - 20 time points (reads) (24 samples x 6 slices x 16 tiles x 2 colors x 20 reads) | 199.6 GB | 718.8 GB |

¹Confocal image file sizes match WFOV image file sizes.

Gen5 - Instrument Compatibility Chart

Verify that the basecode built into your Agilent instrument is compatible with Gen5.

Note: If your instrument reveals a basecode with a version number lower than those provided here, please contact TAC for instructions for downloading and installing updated software.

| Product | Basecode PN | Compatible Basecode Version | Compatible Gen5 Version ¹ | Notes |
|--------------|-------------|-----------------------------|--------------------------------------|---|
| 800 TS | 1560200 | All versions | 3.03 > | |
| Cytation 1 | 1650200 | All versions | 3.03 > | |
| Cytation 3 | 1220200 | 1.04 and 1.05 | 2.04 | |
| Cytation 3 | 1220200 | 1.14 | 2.05 > | Adds ability for 40x and 60x objectives and Gen5 Image+ |
| Cytation 5 | 1320200 | All versions | 2.07 > | |
| “W” Models | 1320200 | 2.05 | 3.06 > | |
| Cytation 7 | 1720200 | All versions | 3.09 > | |
| Cytation C10 | 1940200 | All versions | 3.11 > | |
| ELx800 | 7330202 | 3.07 | 1.00 > | |
| ELx808 | 7340201 | 3.15 | 1.00 > | |
| Eon | 1020200 | All versions (1.00) | 2.00 > | |
| Epoch | 7200200 | All versions (1.07) | 1.09 > | |
| Epoch 2 | 1330200 | All versions (1.06) | 2.06 > | |
| FLx800 | 7080207 | 1.15 | 1.00 > | |

¹ > this version and higher

| Product | Basecode PN | Compatible Basecode Version | Compatible Gen5 Version ¹ | Notes |
|------------------------|-------------|-----------------------------|--------------------------------------|--|
| Lionheart - LFX | 1760200 | All versions | 3.01 > | Basecode, PN 2020200, applies only to the "W" models: LFXW and LLXW. |
| LLX | 1760200 | All versions | 3.04 > | |
| LFXW | 2020200 | All versions | 3.12 | |
| LLXW | 2020200 | All versions | 3.12 | |
| LogPhase 600 | 1800200 | All versions | 3.09 > | Requires its own dedicated software app, in addition to Gen5. |
| PowerWave ² | 7280201 | 1.21.1 | 1.00 > | PowerWaveHT |
| PowerWaveXS | 7300200 | 1.06 | 1.00-3.03 | |
| PowerWaveXS2 | 7300205 | All versions (1.08) | 1.02 > | |
| Synergy 2 | 7130202 | 1.06 | 1.01 > | Use of Synergy 2/4 universal basecode 7160204 (version 1.12 or higher) requires Gen5 version 1.04 or higher. |
| Synergy 4 | 7160204 | All versions (1.08) | 1.04-3.03 | Synergy 2/4 basecode combined at version 1.12. |

¹ > this version and higher

²Several obsolete PowerWave models, with part numbers that began with PRW, are not compatible with any version of Gen5.

| Product | Basecode PN | Compatible Basecode Version | Compatible Gen5 Version ¹ | Notes |
|----------------|-------------|-----------------------------|--------------------------------------|---|
| Synergy H1 | 8040200 | All versions (1.01.1) | 1.11 > | Basecode 2.00 with S2-DIP2 toggled for gradient incubation requires Gen5 2.01 or higher. |
| Synergy H1 | 8040200 | 2.10 | 2.09 > | Adds dynamic extended range for fluorescence reads. Requires PMT calibration by service if upgrading from version 2.0 or earlier. |
| Synergy H1: M2 | 1910200 | 1.00 | 3.09 > | New in version 3.09, Synergy H1 M2 models support variable bandwidth monochromators. |
| Synergy H4 | 8030200 | All versions (1.00.1) | 1.10 > | |
| Synergy HT | 7090202 | 2.24 | 1.00 > | |
| Synergy HTX | 1340200 | All versions (1.02) | 2.06 > | |
| Synergy LX | 1500200 | All versions | 3.04 > | |

¹ > this version and higher

| Product | Basecode PN | Compatible Basecode Version | Compatible Gen5 Version ¹ | Notes |
|---------------------|-------------|-----------------------------|--------------------------------------|-------|
| Synergy Mx | 7190200 | All versions (1.01.0) | 1.07 > | |
| Synergy Neo | 1030200 | All versions (1.03) | 2.01 > | |
| Synergy Neo2 | 1350200 | All versions | 2.09 > | |
| “T” models | 1350200 | v.2.02 | 3.06 > | |
| μQuant (MicroQuant) | 7270201 | 2.02 | 1.00-2.06 | |

¹ > this version and higher

Gen5 Software Level Comparison

Agilent offers levels of Gen5, from the most basic Reader Control to the full-featured, imaging-capable, and FDA-compliant Gen5 Image+ IVD. In addition, Add-On Modules are available for advanced cellular analysis functions: Spot Counting and Auto ROI modules.

| Part Number | GEN5* | GEN5SECURE* | GEN5IVD | GEN5IPLUS* | GEN5SECUREIPLUS* | GEN5IVDIPLUS | GEN5IPRIME* | GEN5SECUREIPRIME* |
|-------------------------------------|-------|-------------|----------|-------------|--------------------|-----------------|------------------|-------------------------|
| | Gen5 | Gen5 Secure | Gen5 IVD | Gen5 Image+ | Gen5 Secure Image+ | Gen5 IVD Image+ | Gen5 Image Prime | Gen5 Secure Image Prime |
| Instrument Control | • | • | • | • | • | • | • | • |
| Data reporting and exporting | • | • | • | • | • | • | • | • |
| Analysis | | | | | | | | |
| Single and multi-mode data analysis | • | • | • | • | • | • | • | • |
| Image capture and basic analysis | • | • | • | • | • | • | • | • |
| Image capture and enhanced analysis | | | | • | • | • | • | • |
| Image capture and advanced analysis | | | | | | | • | • |
| Additional Features | | | | | | | | |
| 21 CFR Part 11 compliant features | | • | • | | • | • | | • |
| IVD com- | | | • | | | • | | |

| Part Number | GEN5* | GEN5SECURE* | GEN5IVD | GEN5IPLUS* | GEN5SECUREIPLUS* | GEN5IVDIPLUS | GEN5IPRIME* | GEN5SECUREIPRIME* |
|------------------------------------|-------|-------------|----------|-------------|--------------------|-----------------|------------------|-------------------------|
| | Gen5 | Gen5 Secure | Gen5 IVD | Gen5 Image+ | Gen5 Secure Image+ | Gen5 IVD Image+ | Gen5 Image Prime | Gen5 Secure Image Prime |
| pliant features | | | | | | | | |
| QC Trending | | | • | | | • | | |
| Gen5 Validation Package included** | | | • | | | • | | |

* For Research Use Only. Not for Diagnostic Use.

** Gen5 Validation Package is available for purchase separately for all other Gen5 editions.

Imaging-Specific Capabilities

| Features | Standard | Image+ | Image Prime |
|---|----------|--------|-------------|
| Supports all imaging instrument hardware functionality | • | • | • |
| Image Acquisition | | | |
| Image up to 4 color channels per image set | • | • | • |
| Fast kinetic imaging (up to 10 fps) | • | • | • |
| Long-term kinetic imaging (up to 7 days) | • | • | • |
| Z-stack imaging of up to 200 slices for thick samples | • | • | • |
| Montage imaging for large samples | • | • | • |
| Combined z-stack and montage imaging | • | • | • |
| Set multiple beacons per well or vessel in an experiment | • | • | • |
| Find ROIs: use low magnification to discover ROIs, capture ROIs with high mag | | • | • |
| Image Processing | | | |
| Background flattening to improve image signal to noise | | • | • |
| Add annotations, including text, shapes, call outs, to any image or graph | | • | • |
| Record movies of live samples and/or make movies of kinetic | | • | • |

| Features | Standard | Image+ | Image Prime |
|---|----------|--------|-------------|
| image series | | | |
| Image deconvolution to improve resolution | | • | • |
| Digital phase contrast to improve contrast in brightfield images | | • | • |
| Cellular Analysis | | | |
| Image Statistics (e.g., image total intensity, intensity above/- below a threshold) | • | • | • |
| Cell count and confluence on a live sample | • | • | • |
| Cell count and confluence on an acquired image | • | • | • |
| Cellular analysis using a single analysis mask (e.g., object size, shape, area, circularity, intensity) | | • | • |
| Analyze subpopulations of cells expressing certain criteria (e.g., size, shape, intensity) | | • | • |
| Two analysis masks for advanced measurements (e.g., cytoplasmic signal, signal translocation) | | | • |
| Advanced cellular analysis optimization tools | | | • |
| Scatterplots/histograms for visualizing and gating cell level data | | | • |
| Optional Add-On: Spot Counting Module for intracellular objects (e.g., mitochondria, steatosis) | | | • |
| Optional Add-On: Auto ROI Module for automatically detecting ROIs based on user-defined parameters. | | • | • |

Note: [Add-On Modules](#) are also available, for some Gen5 editions, to provide additional functionality.

Install the Software on a Computer

Note: Install Microsoft Office **before** installing Gen5 (if applicable).

Note: Recommended Installation Sequence for Imaging on page 23.

Prerequisite

Gen5 requires the user who is installing Gen5 to have Administrator privileges for the Windows operating system. If a user with restricted access attempts to install the software, errors may occur. Contact your organization's system administrator if you are uncertain about your privileges.

Note: Agilent strongly recommends running a Windows Update to ensure the latest Windows security fixes and critical updates are installed prior to installing Gen5.

Install Gen5 (all versions on a computer)

1. Start Windows.
 - Be sure you have administrative privileges.
2. Follow the instructions from the insert, **Installing Gen5 Software and USB Drivers**, found on the inside of the USB flash drive case.

Note:

- The “Typical” installation option is strongly recommended for most users (see below).
- Gen5 asks for the serial number shown on your product packaging. Enter it and click **Continue** to save time later. If the number is unavailable you can click **Cancel** and provide this information later.
- Be sure to register with Agilent for the fastest response from our support team, should the need arise.

Custom vs. Typical Installation

The Typical installation option is recommended for most users. It installs:

- Gen5 application (Gen5.exe and supporting files)
- Gen5 Diagnostic module
- Gen5 Take3 module

For Custom installations, click the arrow next to a feature to display the options menu. Select the desired option. When you opt to not install a feature, its disk icon is replaced with a red X.

Gen5 OLE Automation Toolkit

Custom installation is required during software installation to install the Gen5 OLE Automation Toolkit for programming robotic instruments to use Gen5. Instead of selecting the Install Wizard's default option for Typical installation, select **Custom**. Change the setting for OLE Automation Toolkit to install this feature.

Gen5 installs an OLE Automation folder when this option is selected. You'll find the *Gen5 Automation Programmer's Guide* (in PDF format), the BTIStatusCodes.h file, and a Samples folder containing several program samples in common programming languages.

Note: To learn about security options when using OLE Automation, see **Login/Password Controls** in the Gen5 Help.

Printer Settings

When you install Gen5 on a computer with Windows 10, you may need to disable the **Let Windows manage my default printer** setting. Otherwise, your computer changes the default printer every time you use a different printer. Note that this option is not always present.

1. From the main screen, click **Settings > Devices > Printers & scanners**.
2. In the **Let Windows manage my default printer** box, turn off the option.

Recommended Installation Sequence for Imaging

After following the detailed installation instructions provided in the imaging instrument's user manual, perform the following steps for the best experience:

1. Set up the hardware components as applicable, including:
 - Removing the shipping hardware from the instrument
 - Connecting the gas controller, dispenser, and joystick
2. [Install Gen5 on the host computer.](#)
3. Install the USB driver software shipped on the Gen5 software USB flash drive.
4. [Connect the instrument](#) to the controller (host computer) with the USB cable.
5. Connect the USB3 camera cable to a USB3 port, and power on the instrument. Launch Gen5 and follow the prompts to configure your instrument.
6. [Install the Camera Driver.](#)

Install the USB3/Hamamatsu Camera Driver

Note: You must install the Gen5 software and connect the instrument to the controller (host computer) via both USB cable and USB3 camera cable before performing this procedure.

All Imagers Except Cytation C10 Models with Hamamatsu Camera

Follow these steps to install the USB3 camera driver:

1. Navigate to the Gen5 program files on your computer, for example, `C:\Program Files\Agilent\Gen5 <version>`.
2. Open the USB3 Drivers folder, the Windows_64 folder, and the PGRUSBCam folder.
3. Right-click **Install PGRDriver.bat**, and select **Run as Administrator** to run the driver installer.

```

C:\Windows\System32\cmd.exe
INFO: Option "/i"
INFO: Input file "C:\Program Files\BioTek\Gen5 3.00\USBCam\PGRUSBCAM.inf"
INFO: Flags 0x14 (20)
LOG Event: 1. Installing devices with Id "USB\VID_1E1F\PID_4042" using INF "C:\Windows\System32\DriverStore\FileRepository\cc3948416e204ca4\PGRUSBCAM.inf"
LOG Event: 1. ENTER UpdateDriverForPlugAndPlayDevices
LOG Event: 0. RETURN UpdateDriverForPlugAndPlayDevices
LOG Event: 1. Installation was successful
LOG Event: 0. Install completed
LOG Event: 1. RETURN: DriverPackageInstallW (0x0)
LOG Event: 1. RETURN: DriverPackageInstallA (0x0)
SUCCESS: Installed package C:\Program Files\BioTek\Gen5_64\PGRUSBCam\PGRUSBCAM.inf.
Press any key to continue . . .
  
```

Cytation C10 with Hamamatsu Camera: C10PHC2 and C10MPHC2 Models

1. Navigate to the Gen5 program files on your computer, for example, C:\Program Files\Agilent\Gen5 <version>.
2. Open the HamamatsuDrivers folder.
3. Right-click **setup.exe**, and select **Run as Administrator** to run the driver installer.

Restart the controller (host computer) after installing the camera driver.

Establish Communication with the Camera

1. From the main Gen5 screen, select **System > Instrument Configuration**, select your instrument, and then click **View/Modify**.
2. Click **Test Communication**.
3. Click **Camera Information**. If communication is successful, Gen5 displays information about the camera.

Note: Check the Bus Speed; it should be 5000 Mbits/sec. If a lower bus speed is reported, review the troubleshooting information next.

Troubleshooting Software Drivers

Some suggestions for troubleshooting either communication with the camera or a bus speed significantly lower than 5000 Mbits/sec follow:

- Reboot the host computer.
- Disconnect and reconnect the USB3 cable from/to the host computer.
- Rerun the batch (.bat) file as described in step 3 of [Install the USB3/Hamamatsu Camera Driver on the previous page](#).
- Make sure the computer meets the [Computer System Recommendations on page 12](#).

If problems persist, ask your IT group for support or See **Getting Technical Assistance** on page 56.

Defining the Image Library

System > Preferences > Image Save Options

Use the Image Save Options under **System > Preferences** to define the **Image Library** storage location for saving image files. The **Image Save Options** settings apply to all newly created experiments. Likewise, they apply to all computers/controllers sharing the same **Shared DB** on a network.

 Using an external hard drive for storing image files, i.e., the Image Library, is strongly recommended as image files are much larger than typical data files. See **Storing Gen5 Files on an External Hard Drive** on the facing page.

Note: To override these settings in an individual experiment, go to **Protocol > Protocol Options > Image Save Options**, and click **Select new image folder**. Navigate to the new image folder, then save the experiment file.

Change the Virtual Memory Settings

Note: Imaging controllers (host computers) supplied by Agilent have already been configured properly. Skip this step if you are running a Agilent-provided imaging controller.

For instruments with the imaging module, it is recommended that you prevent Windows from automatically manage paging file size.

1. From the Windows Start menu, go to **Control Panel**, and select **System**.
2. In the left pane, select **Advanced system settings**.
3. In the System Properties dialog, on the Advanced tab in the Performance area, click **Settings**.
4. In the Performance Options dialog, on the Advanced tab in the Virtual memory area, click **Change**.

5. Clear **Automatically manage paging file size for all drives**, if it is selected. This is the default setting for Windows 7 and higher.
6. Select **Custom Size**, enter the following minimum and maximum values, click **Set**, and then click **OK**:
 - Initial size: 20480 MB
 - Maximum size: ≥ 40 GB*
7. You will need to restart your computer for the change to take effect.

* The limit of 40 GB will allow you to work with 10,000 to 15,000 standard field-of-view images in memory at the same time. If you plan to open more images for processing at one time, consider increasing this maximum size.

Disable Sleep Mode

Note: Imaging controllers (host computers) supplied by Agilent have already been configured properly. Skip this step if you are running a Agilent-provided imaging controller.

Disabling a computer's sleep mode is recommended for all applications, but it is especially important when running kinetic or time-elapse assays.

1. Open the **Control Panel** and select **Power Options**.
2. Click **Change plan settings** for the power plan you are using.
3. Set **Put the computer to sleep** at **Never**.

Turn Off Automated Updates

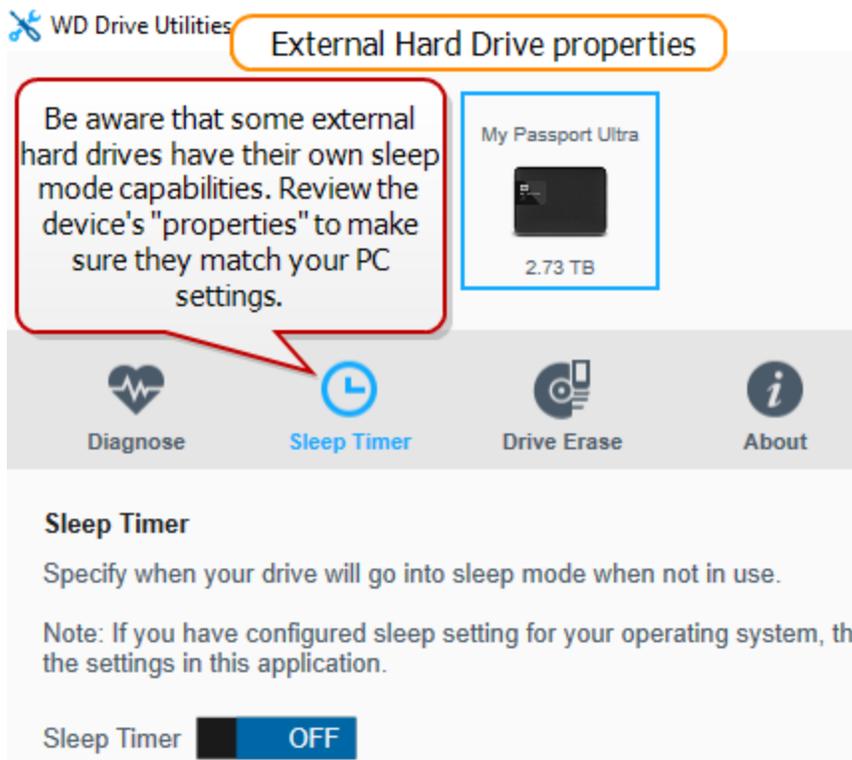
Consult your Information Technology (IT) experts and if possible, turn off the computer's auto-update routines, power-saving options, and virus scans that can interrupt a Gen5 experiment.

Storing Gen5 Files on an External Hard Drive

Here are some guidelines for using an external hard drive for storing Gen5 files, especially useful for imaging files and experiments:

- Use a USB3 drive for faster transfer rates.
 - Close the Gen5 experiment or manual mode session before ejecting the device.
 - Always use the eject utility when disconnecting the device from your computer.
- Do not disconnect the device by just pulling it out:

For example: right-click  or  in the lower-right corner of your task bar, and select **Eject <drive name>** or **Safely Remove Hardware and Eject Media**.



Register with Agilent

The Pre-Registration screen appears when you launch Gen5 until the software is registered. A trial-version serial number can be used for the specified number of days until a licensed version is purchased. Unless the purchased version of Gen5 is a higher version than the trial version there is no need to reinstall the software; simply register the software.

Note:

You must have administrative privileges to register with Agilent.

Generally, the user who logged in to Windows when installing Gen5 should be logged in when registering the software.

Windows 10 users: If prompted for administrative privileges, engage them before registering the software: Locate and right-click the Gen5 desktop icon, and select **Run as administrator**. At the User Account Control dialog, click **Allow**.

Launch Gen5 and Register the Software

1. Open Gen5 by clicking its desktop icon or by using the Windows Start button and selecting **Programs > Gen5 > Gen5**.
2. At the Pre-Registration dialog enter the product serial number (if it wasn't entered during installation or if you've been using a trial version).
3. Click **Register** to register the software and receive a password. The Registration dialog appears with the serial number and the site key; this is information provided by your computer.

Note: Click **Demo** to run Gen5 without registering it for the number of days displayed below the Demo button.

4. Click **Obtain Password**.
 - Alternatively, if your Internet browser and Gen5 are on different computers, enter www.biotek.com into your browser's address field and select **Request Support**. Make note of the serial number and site key for this process.
 - If you do not have access to the Internet, see [Getting Technical Assistance on page 56](#). Make note of the serial number and site key for this process.

- When using the same computer, you can copy and paste (Ctrl+C and Ctrl+V) the serial number and site key into the registration form.
5. At the Software Registration website page, enter or paste the serial number and site key information into the form and click **Submit**.

A registration form will be displayed containing any information Agilent already has about you and your organization.

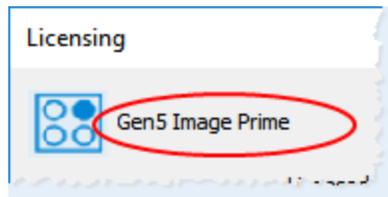
6. Review and edit the information as necessary, then click **Submit Registration Form**.
7. Your password will be displayed on screen. Copy or make note of it.
8. Return to the Gen5 Registration dialog to paste or enter it into the Password field.
9. Click **Validate Password**.

The software should now be registered and you will not see the Pre-Registration screen again.

Note: Gen5 stores the serial number and site key in the [Help > About Gen5](#) screen so you can log in to Agilent’s product registration site at any time.

Gen5 Licensing

Select [Help>Licensing](#) to display current status or activate an Add-On Module



From the Gen5 Licensing dialog, you can view the [Gen5 License Agreement and warranty information](#).

Your edition or level of software is displayed. Learn more: [Gen5 Software Level Comparison](#).



Installation Instructions

Add-On Modules:

You can see which add-on modules are available for your version of Gen5 and whether the modules are activated.

Gen5 Add-On Module

Advanced cellular analysis

REGISTER YOUR GEN5 ADD-ON MODULE



If you have purchased an add-on module, click **Enter serial number** and enter the dedicated serial number provided in the Gen5 Add-On module software package (not to be confused with the regular Gen5 software serial number). Your add-on module must be successfully activated, “Registered (*serial number*)” for its features to be usable.

Initial Setup

The first thing all users must do after installing Gen5 is connect an instrument to the computer and tell Gen5 how to communicate with it. Other initial setup steps can be performed to improve your experience using Gen5. When running Gen5 Secure, Gen5 Secure Image+, Gen5 IVD, Gen5 IVD Image+, and Gen5 Secure Image Prime, to meet FDA submission criteria, you must establish and maintain security conditions. You'll find instructions for performing these tasks in this section.

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Connect an Instrument

System > Instrument Configuration

Note: Before connecting the instrument: to the computer, install the USB driver provided on the Gen5 software USB flash drive. Connect the instrument after the driver installation is complete.

When you start Gen5 without an instrument connected to your computer, Gen5 prompts you to add one. **After its shipping hardware has been removed** and other installation steps completed, connect the instrument to the computer, power it on, and perform the following steps:

1. Click **Yes**.
2. Select an instrument from the list, then click **OK**.

You can also access the Instrument Configuration dialog by clicking **System > Instrument Configuration**.

3. Click **Add** to define the Instrument Settings:
 - Select either **Plug & Play** or **Com Port** as the communication type.
 - **Plug & Play:** Select an available instrument from the list and click **OK**.
 - **Com Port instruments:**
 - Select the Instrument Type from the list.
 - Enter the Com port number in the Com Port field.
 - If necessary, select a baud rate. **Retaining the default baud rate is recommended.**

Mismatched baud rate settings can cause “serial read” errors. When the baud rate is set to a non-default setting for non-keypad instruments, Gen5 is unable to communicate with the instruments if they are turned off and then turned on again while Gen5 is running. For keypad instruments, ensure that the instrument has the baud rate set to 9600. If the baud rate is changed, the instrument must be rebooted.

- If needed, click **Setup** to change the factory-tested and defined configuration values. Except for imaging and dispensing, this step is rarely required.
 - Click **OK** to save the settings.
4. Click **Test Comm**. Gen5 attempts to communicate with the instrument.

After you receive a passing message, “The reader is communicating,” you can save and **Close** the Instrument Configuration. If you receive any other message, look for a remedy: See **Troubleshooting** on page 75

Set Up Gen5, Gen5 Image+, and Gen5 Image Prime

Gen5 fulfills the instrument control and analytical needs for a wide range of laboratory settings. The degree to which you follow the recommendations provided here depends on the needs of your organization.

Recommended Tasks to Perform

1. Designate a System Administrator.
2. Install Gen5 on the Administrator’s computer.
3. Change the System Administrator’s password (default is **admin**).
4. Determine the optimal way to store Gen5’s protocol and experiment files. See **About File Storage** on page 90.
 - Organize the database or your Windows file structure.
5. Install Gen5 for other users and connect an instrument to each computer. See **Connect an Instrument** on the previous page.
6. If applicable, direct each user’s database configuration to point to the correct shared database.

Set Up Gen5 Secure, Gen5 Secure Image+, or Gen5 Secure Image Prime

1. Designate a System Administrator.
2. Complete the [System Administrator's To-Do List on page 38](#) (the Initial Setup tasks).
3. Organize the database. See **Organize Your Database Files** on page 43.
4. Review/modify Signature Reasons and other security controls (select **System > Security**).
5. Install Gen5 on each user's computer.
6. Set up each user's database configuration to point to the correct shared database. See **Move or Copy a Database to a Network** on page 44.
7. Connect an instrument to each user's computer.
8. Advise users to change their passwords.

Set Up Gen5 IVD or Gen5 IVD Image+

1. Designate a System Administrator.
2. Complete the [System Administrator's To-Do List on page 38](#) (the Initial Setup tasks).
3. Organize the database. See **Organize Your Database Files** on page 43.
4. Review/modify Signature Reasons and other security controls (select **System > Security**).
5. Install Gen5 on each user's computer.
6. Set up each user's database configuration to point to the correct shared database. See **Move or Copy a Database to a Network** on page 44.
7. Connect an instrument to each user's computer.
8. Advise users to change their passwords.

Set Up Gen5 Reader Control

1. Install Gen5 on the computer.
2. Determine the optimal way to store Gen5's protocol and experiment files.
 - Organize the database or your Windows file structure.
3. Connect an instrument to the computer. See **Connect an Instrument** on page 34.
4. Set user preferences. See the Gen5 Help for more information.

System Administrator's To-Do List

Initial Setup Tasks: All editions of Gen5 Secure

- | | |
|--------------------------|---|
| <input type="checkbox"/> | 1. Make sure all designated computers and Agilent instruments meet the minimum requirements. See Computer System Recommendations on page 12. |
| <input type="checkbox"/> | 2. Install Gen5 Image+ Secure or Gen5 Secure on one computer. |
| <input type="checkbox"/> | 3. Start Gen5 and log in as the System Administrator. |
| <input type="checkbox"/> | 4. Change the System Administrator's password. |
| <input type="checkbox"/> | 5. Copy the database Shared.mdb to a secure network location. |
| <input type="checkbox"/> | 6. Test database configuration of the Shared.mdb on the network. |
| <input type="checkbox"/> | 7. Create/modify user groups, as needed, and assign user permissions to the groups. |
| <input type="checkbox"/> | 8. Create new user accounts and assign the users to a group.* |
| <input type="checkbox"/> | 9. Connect instrument(s) to the PC and establish communication. |
| <input type="checkbox"/> | Repeat Steps 2, 3, 6, and 8 for the remaining computers. |

Initial Setup Tasks: All editions of Gen5 IVD

- | | |
|--------------------------|---|
| <input type="checkbox"/> | 1. Make sure all designated computers and Agilent instruments meet the minimum requirements. See Computer System Recommendations on page 12. |
| <input type="checkbox"/> | 2. Install Gen5 IVD or Gen5 IVD Image+ on one computer. |
| <input type="checkbox"/> | 3. Start Gen5 and log in as the System Administrator. |
| <input type="checkbox"/> | 4. Change the System Administrator's password. |
| <input type="checkbox"/> | 5. Copy the database Shared.mdb to a secure network location. |
| <input type="checkbox"/> | 6. Test database configuration of the Shared.mdb on the network. |
| <input type="checkbox"/> | 7. Copy the database QCDB.mdb to a secure network location. |

- | | |
|--------------------------|---|
| <input type="checkbox"/> | 8. Test database configuration of the QCDB.mdb on the network. |
| <input type="checkbox"/> | 9. Create/modify user groups, as needed, and assign user permissions to the groups. |
| <input type="checkbox"/> | 10. Create new user accounts and assign the users to a group.* |
| <input type="checkbox"/> | 11. Connect instrument(s) to the PC and establish communication. |
| <input type="checkbox"/> | Repeat Steps 2, 3, 6, 8, and 10 for the remaining PCs. |

* For all editions of Gen5 Secure and Gen5 IVD, you can set up an alternative login method: Configure Windows Authentication on page 53.

Periodic/As Needed Tasks

- Customize the security features to accommodate your organization's needs.
- Organize your database files.
- Educate users on regulatory requirements and Gen5 best practices.
- Establish and implement a procedure and schedule for record retention and archival.
- Review records, including any training/user-qualification records.

Before modifying a user's account, make sure he/she is not logged in to the system. You can check the System Audit Trail to determine who is currently logged in.

Change the System Administrator's Password

[System > Security > Users](#)

[System > User Setup > Administrator tab](#)

You should change the System Administrator's password immediately following Gen5 installation to ensure a secure operating environment.

To change the password:

1. Log in as the System Administrator, if you haven't already done so.
 - Select **System > Login/Logout**.
 - Set the User to **Administrator**.

- Enter the default password: **admin**.

Note: Passwords are case-sensitive. For example, "Gen5admin" and "gen5admin" are two distinct passwords.

2. Select **System > Security > Users**.
3. Double-click the System Administrator user (to edit the record).
4. Define and confirm the new password. The System Audit Trail will open to log the change and accept your comments.

Note: Record and store the new password in a secure location. If you forget the password, [contact Customer Care](#) for assistance.

About Gen5 Databases

Gen5 installs two databases during regular installation: SharedDB and LocalDB. Only Gen5 Secure, Gen5 Secure Image+ , Gen5 Secure Image Prime, Gen5 IVD, and Gen5 IVD Image+ are initially set up to use the Gen5 Database for experiment and protocol file storage. All other levels of Gen5 must select to use the database to store experiment and protocol files at **System > Preferences > File Storage Mode**.

- SharedDB can be set up on a network for sharing information among multiple users. It contains all protocol and experiment data files and their associated audit trails, the plate types, and reader-diagnostic history data. In all editions of Gen5 Secure and Gen5 IVD, SharedDB also contains security settings, user accounts, and a system audit trail for shared events. This database can be moved, renamed, and copied. So, if desired, you can create a unique database for individual projects, teams, or other classification.



The SharedDB must reside in a location that presents the minimum risk of error. Before moving it to a networked drive, it is recommended that you analyze the network or disk and server to ensure that the

error rate is low. It is not recommended that you use WiFi network connections, external drives, or USB flash drives to hold the SharedDB.

Note: Gen5 Image+ IVD and Gen5 IVD also install the QC Trending database, QCDB.mdb. It can be set up on a network for sharing among multiple users, moved, renamed, and copied. It is initially installed in a QC folder in the default database location described below.

- **LocalDB** contains the local setup information, including the Instrument Configuration. For all editions of Gen5 Secure and Gen5 IVD, this database also contains an audit trail for local events. LocalDB is stored on the computer's hard drive, and it cannot be moved or renamed.
- **Default database location:** During normal installation, Gen5 installs its databases in Windows Common Application Data Folder:
 - **Windows 10:** C:\Program Data\Agilent\Gen5 (software level)\(version #)\SharedDB or LocalDB

Note: You may need to change your operating system settings to view the Application Data folder.

- **Database Names after Upgrade:** The Gen5 Upgrade Utility changes the names of the databases to help distinguish them:
 - **SharedDB:** The file name of the database selected for upgrade is not changed during the process, but the upgraded version is identified by this suffix: "Upgraded_<date_time>.mdb".

Note: The Gen5 Periodic Backup routine appends the filename with "Auto_Backup_<date_time>".

- **LocalDB:** The file name of the LocalDB selected for upgrade is changed because Gen5 requires the database stored on your hard drive to be named

LocalDB. After the upgrade the older version is named with this suffix:

"Before_<date>_<time>.mdb".

- **Max Size:** the maximum size of the database files is 2 gigabytes (GB). At startup, Gen5 checks the remaining size of the database. Warning messages are displayed when the database size exceeds 1536 MB. Use Gen5 maintenance and backup features to archive your database records.
- Gen5 has **built-in error recovery modes**. When your connection to the database is lost for any reason, Gen5 saves any unsaved files as Temporary Files. After a system failure, the next time you open an affected protocol or experiment file, Gen5 offers to replace the unsaved files with the Temporary Files. Select **Yes** to recover any changes made to the files before the system failure; select **No** to open the files as they were last saved, before the unsaved changes were made. Newly created files are also saved as Temporary Files. Following a system failure, you can rename these temporary files with the proper file name extension (.xpt or .prt) using Gen5 Maintain Files controls.
- **File locking:** When a file is opened in Gen5 it is “locked” to protect it from being modified (saved or renamed) by a different user. When a second user attempts to open the file, he or she receives a message stating: “File <filename> is already in use. Do you want to open it in read-only mode?”
- Gen5 offers **automatic backup**. You can define settings for regularly and automatically backing up and optimizing databases with Gen5 Auto-Optimize feature.

Organize Your Database Files

Note: During regular installation, all editions of Gen5 Secure and Gen5 IVD use the shared database to store experiment and protocol files. All other levels of Gen5 must elect to use the database at **System > Preferences > File Storage**.

All of your file management requirements can be fulfilled using Gen5 databases (except image files cannot be stored in the Gen5 databases). You'll be most satisfied with the final structure if you spend some time planning it up front. In a multiple-user environment, you can install a Gen5 database on a shared-network drive (LAN) so multiple users can access the same protocol and experiment files.

Backups: Performing backups on a regular schedule is highly recommended to preserve your data. And, Gen5 provides a tool to schedule backups to occur periodically. See below.

File Management Recommendations

- Put a **copy of the SharedDB on a shared-network drive** where all your Gen5 users can access it. Be sure to set each user's database configuration to point to the correct location.
- Before moving the SharedDB to a network location, make a copy of it to use as a template for future use:
 1. In the default SharedDB folder, highlight the original, right-click, and select **Copy**.
 2. Deselect the original (click elsewhere in the dialog), right-click, and select **Paste**.
 3. Highlight the copy, right-click, and select **Rename**.
 4. Give the copy a unique name, like SharedDB_original.mdb.
- Consider setting up **shared databases** for different projects or teams within your organization. You can follow the steps defined above to create multiple databases in the same folder (or directory), or you can move the unique databases to

a different network location/folder. Use Database Configuration to point users' Gen5 sessions to the correct database.

- **Regularly archive and back up the database** to preserve your records. Use Gen5 Optimize and Backup Settings to backup and clean small errors in your database. Agilent recommends following your organization's existing policy for securing data, for example, putting the shared database on the network to be backed up every night.
- For databases stored locally (not on a network drive), consider using Gen5's **automatic save feature**¹ to create new, date-stamped folders for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 organizes all that data by date. Define this kind of file management setting in the Initial Protocol Settings (**System > Preferences**) so it will apply to all newly created protocols.
- Gen5 handles multiple, simultaneous users performing database management tasks by giving precedence to the user with the greater administrative rights.

Move or Copy a Database to a Network

System > Database Configuration

Note: All editions of Gen5 Secure and Gen5 IVD install and enable the databases during regular installation. All other levels of Gen5 must elect to use the database to store protocol and experiment files at **System > Preferences > File Storage**.

In a multiple-user environment, you can set up the Gen5 database on a shared network drive so multiple users can access the same protocol and experiment files. This is a recommended step for System Administrators. You can also set up multiple databases, one for each team or project, for example. During a Gen5 session, access is provided to only one database at a time.

¹Click Protocol > Protocol Options > Save Options.

1. Select **System > Database Configuration**.
2. Select the **SharedDB** tab.
3. Next to the Source field, click **Browse**.
4. In the Open dialog, highlight and right-click the file **SharedDB.mdb**, and select **Copy** or **Cut**; use cut to move and copy to copy.

Note: SharedDB is the installed/original name for the shared database. Because you can change the name, it's possible it has already been changed.

5. Navigate to the desired location in the Look in field.
6. When the correct location is selected, right-click in the window and select **Paste**.
7. Click **Open** to save and close the window, and return to the Gen5 Database Configuration dialog.
8. Shut down and restart Gen5 to make the changes take effect.

About User Accounts

System > Security > Users

Prerequisite

This function is available only to the System Administrator. You must log in as the Administrator (**System Menu > LogIn/LogOut**) to access all the controls. Non-administrators are limited to changing their own password and selecting a Startup Mode, Startup Action and Protocol Folder.

How to Create, Modify, or Delete User Accounts

Only an Administrator can add, modify, or delete users. You can designate multiple individuals to be system administrators. Any user account can be changed or deleted, except there must always be at least one System Administrator:

- Click **New** to set up a new user.
- Double-click or highlight a user, and click **Edit** to modify its name, password, or Group assignment.
- Highlight a user, and click **Delete** to remove the user account.

About User Groups

System > Security > Groups

Prerequisite

This function is available only to the System Administrator. You must log in as the Administrator (**System > Login/Logout**) to access this control.

All editions of Gen5 Secure and Gen5 IVD use groups to manage the rights or permissions granted to users. When creating (or maintaining) a group, you define the level of access and the controls available to certain types of users and then assign actual users to the groups. Gen5 ships with three groups: Administrator, Power User, and Standard User.

The System Administrator and Power User groups are given access rights to all functions. The Administrator's rights cannot be changed and include additional rights to manage user accounts that are not extended to Power Users. When any edition of Gen5 Secure or Gen5 IVD is installed, the Standard User is limited to the following permissions. The System Administrator can change these rights as needed:

- Startup preferences
- Open a protocol
- Add a new plate
- Create/Edit Sample IDs
- Edit plate information
- Edit Plate Layout
- Edit Report/Export Builders
- Define test plates
- Create folder in database

Create New Groups and Modify Existing Groups

Only a System Administrator can add, modify, or delete groups. Except for the Administrator group, any group can be changed or deleted, and any group can be renamed.

- Click **New** to set up a new group.
- Highlight a group and click **Edit** to modify its name and permissions.
- Highlight a group and click **Delete** to remove it as an option. First you must reassign any users to another group. You cannot delete a group with users assigned to it.

Create/Maintain User Accounts

For Gen5 Secure or Gen5 IVD editions only

[System](#) > [Security](#) > [Users](#)

Prerequisite

Most options for user accounts are available only to the System Administrator. Non-administrators are limited to changing their own password and selecting a Startup Mode, Startup Action and Protocol Folder.

User ID

Enter a unique ID using 1 to 16 alphanumeric characters. The user will enter or select this ID when logging into Gen5 and when signing files.

Full Name

Enter the user's name. This name will be associated with events logged by this user's actions and with the digital signature applied by this user.

Group

Choose a Group membership to assign access rights and permissions to the user. See **About User Groups** on page 46 for information. Users receive the rights assigned to the Group.

Status

The check box shows whether the user's account is currently locked. The System Administrator can lock or unlock the account. When a user's account is locked, the user cannot log in to Gen5 and cannot sign files. A user's account may become locked due to one of three events:

- Intentional lock by the Administrator using this dialog
- Automatic lock if the user exceeded the number of successive failed login attempts
- Automatic lock if the user's password expired

Unlocking a user's account following an automatic lock resets its counter or clock. The reset is specific to the reason for the lockout: When it is caused by password expiration, the password expiration clock is reset; when it is caused by failed logins, the user's history of "successive failed login attempts" is reset to 0.

When lockout occurs due to an expired password, unlocking the account allows the user to log in to Gen5 with the same password, providing a chance to change it. Alternatively, as system administrator, you can simply change the password yourself (which will by default unlock the account) and tell the user to log in with the password you have assigned him or her.

Startup Mode

Gen5 offers slightly different workflow modes:

- **Simple:** a basic workflow designed for faster set up and execution of experiments; using limited choices and interactive dialogs. Simple Mode always starts Gen5 displaying the Simple Task Manager with Read Now in focus.
- **Standard:** classic Gen5 workflow with access to all potential parameters, settings, and options.

IQOQPQ Procedures: Choose **Standard** mode when performing Instrument Qualification Procedures supplied by Agilent for the best user experience.

Note: Administrators are set up by default to use Standard Mode.

Startup Action

Select the preferred method for starting Gen5:

- **Display Task Manager/Last used page** is the default setting. You can also specify a specific Task Manager page, such as Read Now page or Experiment page.
- **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected **Experiments > Create New**.
- **Start at system menu** opens Gen5 showing the File, Take3, Window, System, and Help menus only. Since neither a protocol or experiment is open, the workspace is blank.

Protocol and Experiment Folders

Browse to or enter the full path and directory to define the folder in which the current user will typically store protocol and experiment files. Gen5 defaults to the most recently accessed folder.

Password

Assign a password for the user to enter the first time he or she logs in to Gen5. Instruct users to change their password after the first login using the password you've assigned. Users can change only their own password. System Administrators can change any user's password.

Login/Password Controls

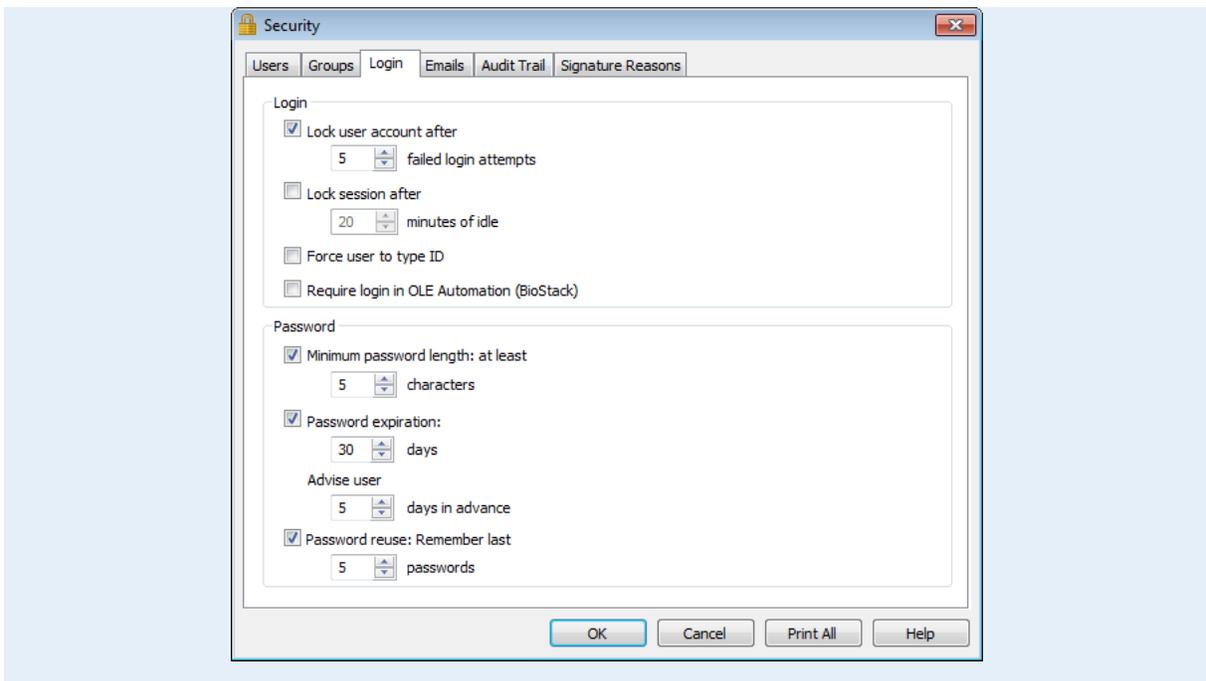
For Gen5 Secure or Gen5 IVD editions only

[System](#) > [Security](#) > [Login](#)

Prerequisite

Only the System Administrator can access these controls. You must log in as the Administrator ([System](#) > [LogIn/LogOut](#)) to change the settings.

The default settings shipped with all editions of Gen5 Secure and Gen5 IVD, as shown in the screenshot below, comply with the FDA's 21 CFR Part 11 requirements for controls for identification codes/passwords.



Login

- **Lock user account after:** Specify the number of successive failed login attempts a user may make before being locked out of Gen5. This feature does not apply to System Administrator accounts, and only a System Administrator can reinstate a locked-out account. Valid entry range: 2-10. When this feature is not enabled, users' login attempts are unlimited. Compliance with 21 CFR Part 11 requires setting a limit for failed login attempts.
- **Lock session after:** Specify the number of minutes that a Gen5 session can be idle before it is locked and requires successful user login to reactivate. A session is considered idle when there is no keyboard or mouse activity and Gen5 is not controlling a reader activity. Valid entry range: 1-1440 minutes. Compliance with 21 CFR Part 11 requires setting an idle-time limit.
- **Force user to type ID:** Apply this control if your security rules require users to enter their ID at login and to apply their Signature. When this feature is not selected, the last user's ID is displayed in the login and signature screens, and users can select an ID from a drop-down list of users.
- **Require login in OLE Automation (BioStack):** Select this option to ensure that Gen5 security permissions are enabled when Gen5 Secure, Gen5 Secure Image+,

Gen5 Secure Image Prime, Gen5 IVD, and Gen5 IVD Image+ is run as an OLE Automation server, for example, for using the BioStack. When [Windows Authentication](#) is enabled, the login process may take place automatically. When Windows Authentication is disabled, Gen5 prompts the user to enter a login ID and password. Logins performed while this option is selected are tracked in the Audit Log.

Password

- **Minimum password length:** Specify the minimum number of alphanumeric characters required for a valid password. Valid entry range: 2-10 characters.
- **Password expiration:** Specify the number of days a password can be used before users are required to change it. When users let their password expire without changing it, their accounts are locked out and only a System Administrator can reinstate a locked-out account. Valid entry range: 1-10000 days. If this feature is not selected, passwords do not expire. Compliance with 21 CFR Part 11 requires an expiration period.
- **Lock out:** When a user's password has expired, the system administrator has two choices:
 - Manually remove the Locked out flag. This resets the password expiration period allowing the user to log in using his/her current password.
 - Enter a new password for the user (which unlocks the account) and tell the user to log in with the password you have assigned him/her. Advise the user to change the password after logging in.
- **Advise user:** If password expiration is set, specify the number of days before passwords expire to alert users to change their password. Valid entry range: 1-30 days, but cannot exceed the number of days to Password Expiration.
- **Password reuse:** Specify the number of passwords Gen5 will remember for each user's account to prevent a recently used password from being reused. Valid entry range: 2-20.

Configure Windows Authentication

For Gen5 Secure or Gen5 IVD editions only

Gen5 Secure, Gen5 Secure Image+, Gen5 Secure Image Prime, Gen5 IVD, and Gen5 IVD Image+ provide an option to allow users to sign in to Gen5 using Microsoft Windows Authentication instead of user accounts defined in Gen5. Through Windows Authentication, users log in to their workstations once and have access to Gen5 without having to log in again. The Windows Authentication feature benefits system administrators as well by providing a single location for the management of user settings.

To use Windows Authentication, client workstations must be running Windows 10 or higher; and LDAP Servers are required. Before Windows Authentication can be activated in Gen5, both the Gen5 Administrator and the IT administrator must perform setup tasks.

Gen5 Administrator Tasks

Gen5 Secure, Gen5 Secure Image+, Gen5 Secure Image Prime, Gen5 IVD, and Gen5 IVD Image+ use groups to manage the rights or permissions granted to users. The Group Names are defined in Gen5 by the Gen5 Administrator.

When creating or maintaining a group, the Gen5 Administrator defines the level of access and the controls available to certain types of users, and then assigns actual users to the groups. Gen5 ships with three default groups: Administrator, Power User, and Standard User.

The System Administrator group is given access rights to all functions. The Administrator's rights cannot be changed. When Gen5 Secure, Gen5 Secure Image+, Gen5 Secure Image Prime, Gen5 IVD, and Gen5 IVD Image+ is installed, the Standard User is limited to the following permissions (the System Administrator can change these controls as needed):

- Open Protocol
- Use Gen5 Protocols (requires Gen5 Native)

- Add a New Plate
- Create/Edit Sample IDs
- Edit Plate Information
- Edit Report/Export Builders
- Create folder in database
- Edit Paneled protocols
- View protected/read-only protocol items

IT Administrator Tasks

The IT administrator must create user groups on the server to mirror the Gen5 User Groups defined in Gen5. The user groups on the server must be named Gen5_<Gen5 Group Name>, where <Gen5 Group Name> is the name of a group in Gen5, such as Gen5_System Administrators. The IT administrator can also define permissions that apply to Windows resources, such as access to folders, printers, and so on.

Each user who has permission to run Gen5 must be defined as a member of a single Gen5 group in Windows. If a user is defined as a member of more than one Gen5 group, an error message will appear when the user attempts to log in to Gen5. If the user is not assigned to a Gen5 group, a warning message will appear, indicating that the user is not a valid Gen5 user. The user can then log in with another, valid Gen5 account.

Configure the Email Notification Feature

For Gen5 Secure or Gen5 IVD editions only

All editions of Gen5 Secure and Gen5 IVD can be set up to send an email notification to specified recipients when a predefined event, such as a reader error or failed login, occurs. The email server settings must be configured to support this feature. The Gen5 Administrator must test these settings to verify that the Email Notification feature is functioning correctly.

To verify that the email server settings are configured correctly to support this feature, check with your IT administrator.

Defining a Custom Email Template

By default, Gen5 generates the title and body of the email notification messages, but you can define a custom template that includes specific variables.

1. On the Email tab of the Security dialog, click **Auto** in the Template column.
2. In the Email Definition dialog, select **Custom** as the mode and either **Text** or **HTML** as the format.
3. Modify the Subject and Body text as necessary. Variables associated with the defined event are listed in the Variables text box. To insert them in the Subject or Body of the email notification, place the cursor where you want the variable to occur, then double-click the variable in the Variable text box.
4. Click **OK**.

Configure the Email Server

For Gen5 Secure or Gen5 IVD editions only

[System > Preferences > Email Server](#)

[System > Security > Emails tab > Email Server](#)

The email server must be configured correctly to support the Gen5 Email Notification feature. Check with your IT administrator to verify your configuration settings.

In the **Send Emails from** area, you can define the email addresses used in the Email Notification feature.

- **Display name:** This field is required. By default, Gen5 displays Gen5_Notice_Do_Not_Reply@biotek.com as the address from which the notification message is sent. You can change this address, if desired.
- **Forward errors to:** The address specified in this field will receive any mail delivery error messages generated by the server related to the Email Notification feature. If no address is entered, error messages will be sent to the address in the Display Name field.

- **Reply to:** The address specified in this field will receive messages sent as a reply to an Email Notification message. If no email address is entered in this field, the address in the Display Name field will receive any reply emails.

Getting Technical Assistance

You can contact Technical Support using email, telephone or Agilent's Worldwide Support website.

Please be prepared to provide the following information:

- The software version and revision numbers displayed at **Help > About Gen5**
- The license type or software level
- The specific steps that produce your problem
- Any error codes displayed
- A daytime phone number
- Your name and company information
- An email address

Agilent

Service Toll-Free: (800) 227-9770

Customer Care Email: bio.CustomerCare@agilent.com

Service Email: bio.tac@agilent.com

Obtain a Password Email: bio.password@agilent.com

Worldwide Support Web site: <https://www.agilent.com/en/contact-us/page>

Getting Started

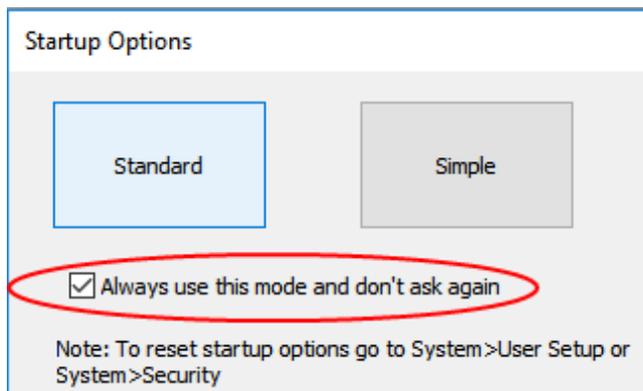
Gen5 software provides a logical interface to all automated Agilent plate reads. It is designed to flow from reading parameters, to plate layout, to data reduction, and finally to flexible data output options.

| | |
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Launching Gen5



During software installation, Gen5 installs a desktop icon and adds itself to the Windows Start menu. For most users, the first launch of Gen5 presents **Startup Options**:



Fill the checkbox to stop this prompt from opening every time you launch Gen5. To reset the prompt:

- Gen5: [Change Your Startup Preferences below](#)
- Gen5 Secure/IVD: [Change Your Startup Preferences in Gen5 Secure and Gen5 IVD on page 60](#)

Startup Mode

Gen5 offers slightly different workflow modes:

- **Simple**: a basic workflow designed for faster set up and execution of experiments; using limited choices and interactive dialogs. Simple Mode always starts Gen5 displaying the Simple Task Manager with Read Now in focus.
- **Standard**: classic Gen5 workflow with access to all potential parameters, settings, and options.

IQOQPQ Procedures: Choose **Standard** mode when performing Instrument Qualification Procedures supplied by Agilent for the best user experience.

Simple Mode is an interactive work flow that limits user options to make accomplishing tasks easier. Only one file may be open at a time. **Standard Mode** represents Gen5 behavior since version 2.09. Standard mode enables a choice of “Startup Action” options and the full range of Gen5 features for every Gen5 edition.

Change Your Startup Preferences

For all editions of Gen5 except Secure and IVD.

System > User Setup

1. Click **System > User Setup**.
2. Select the preferred **Startup Mode**: Simple, Standard, or Prompt at startup.
3. If Standard Mode is selected above, choose the preferred **Startup Action**, for example:
 - **Display Task Manager** is the default setting. It opens Gen5 with a screen that offers several common tasks including creating a new item or opening a recently used item. (In Simple Mode this option, in Read Now focus, is fixed.)
 - **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected Experiments > Create New based on an existing protocol.
 - **Start at system menu** opens Gen5 showing the File, Take3, System, and Help menus only. Since neither a protocol or experiment is open, the workspace is blank.
4. Click **Browse** to change your Protocol and Experiment folders: browse to the full path and directory to define the folder where you will typically store protocol and experiment files. Gen5 points to these folders when you save and open a protocol or experiment.
5. Click **OK**.

The changes will take effect the next time you log in to Gen5.

Contact your System Administrator if you need assistance.

Change Your Startup Preferences in Gen5 Secure and Gen5 IVD

For Gen5 Secure and Gen5 IVD only.

System > Security > Users

Users other than the System Administrator are limited to changing their own password and startup options.

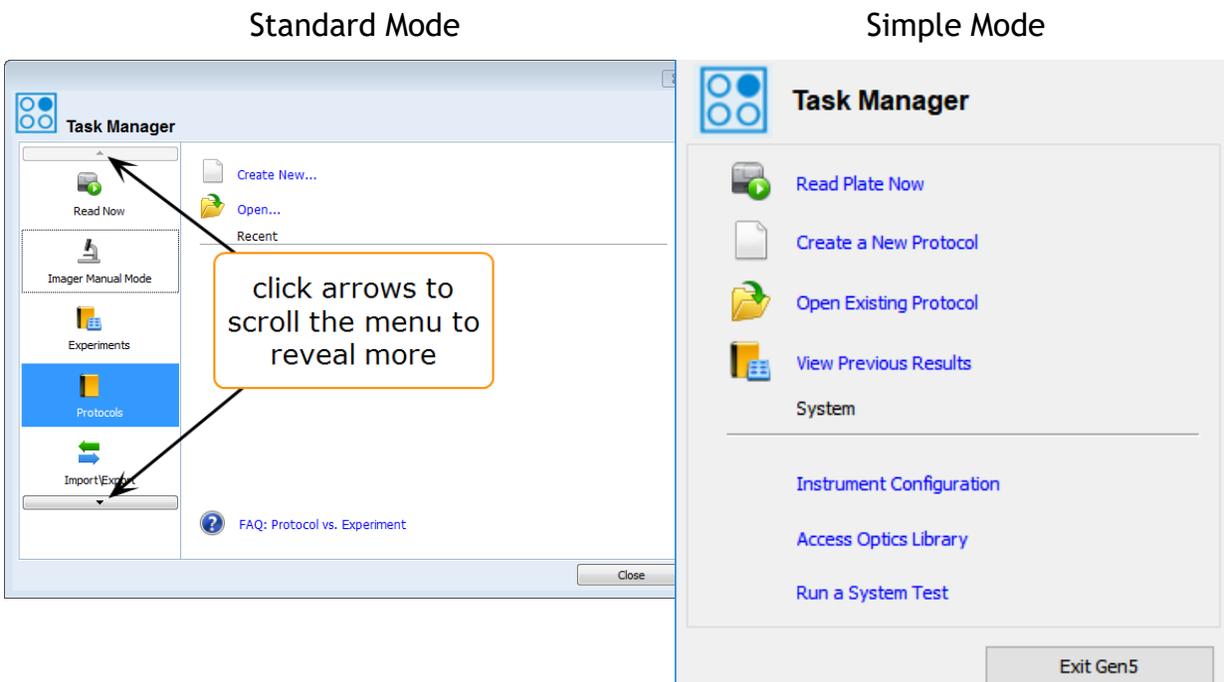
1. Click **System > Security > Users**.
2. Highlight your user account and click **Edit**.
3. Select the preferred **Startup Mode**: Simple, Standard, or Prompt at startup.
4. If Standard mode is selected in the previous step, choose a **Startup Action**, for example:
 - **Display Task Manager** is the default setting. It opens Gen5 with a screen that offers several common tasks including creating a new item or opening a recently used item.
 - **Create new experiment** opens Gen5 with the Protocol selection dialog open, as if the user had selected Experiments > Create New based on an existing protocol.
 - **Start at system menu** opens Gen5 showing the File, Take3, System, and Help menus only. Since neither a protocol or experiment is open, the workspace is blank.
5. Click **Browse** to change your Protocol and Experiment folders: Browse to the full path and directory to define the folder where you will typically store protocol and experiment files. Gen5 points to these folders when you save and open a protocol or experiment.
6. [Change your password](#), if desired.
7. Click **OK**.

The changes will take effect the next time you log in to Gen5.

Contact your System Administrator if you need assistance.

Task Manager

Gen5's Task Manager varies depending on the mode you are in:



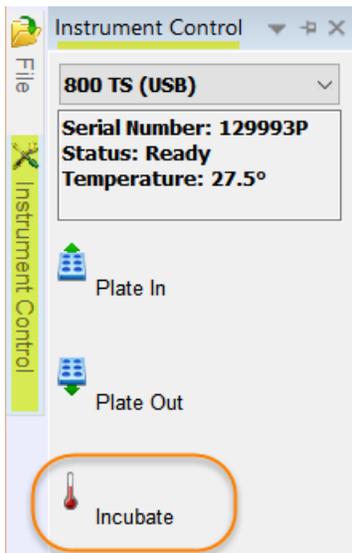
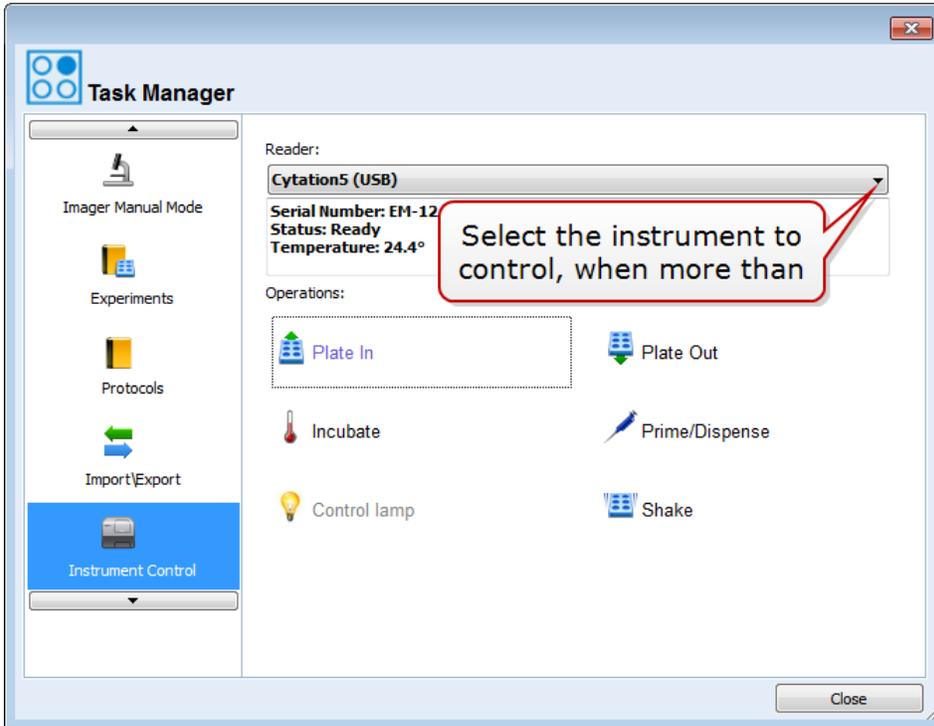
Click an option ([blue link](#)) to open it.

Whenever you start Gen5, the Task Manager opens. You can reopen the Task

Manager by clicking the toolbar button: . Likewise, in the Task Manager,

clicking  closes the Task Manager, returns to the main screen.

Instrument Control options are available from the Standard Mode Task Manager:



Gen5 also provides fly-out tabs on the left side or in the bottom left corner of the workspace in all modes. Click the **Instrument Control** tab to access the controls.

Instant Access

The Task Manager provides quick links to give you instant access to:

- Executing a new read
- Using the Imager Manual Mode (imaging only)

- Opening or creating an experiment or protocol; the most recently used protocols and experiments are listed
- Control several of the instrument's operations (e.g., incubation, shaking, plate in, stacker control)
- Accessing configuration and security settings
- Accessing the Gen5 Help system, FAQs, and sample files

Standard Mode Gen5 Workspace

Gen5 offers several controls and workspaces for developing protocols, running experiments, and viewing and reporting results:

The screenshot shows the Gen5 Image Prime 3.12 software interface. The main window is titled "Plate 1" and displays a data matrix. The "File" tab is selected, showing a menu tree of Protocol and Experiment controls. A callout box points to the "File" tab, stating: "Open the File tab for a menu tree of Protocol and Experiment controls that mirror toolbar buttons." Another callout box points to a pick list in the "Data:" field, stating: "Use the pick list to choose the data set to display in the matrix." The matrix displays data for rows A through G and columns 1 through 12. A caption at the bottom of the screenshot reads: "Gen5 experiment workspace with File tab and plate Matrix displayed".

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| A | 3064 | 3134 | 25046 | 25589 | 6697 | 5598 | 55689 | 59876 | 10001 | 12145 | 56879 | 56887 |
| B | 3936 | 4064 | 40847 | 41197 | 4001 | 4258 | 55598 | 58879 | 33659 | 34587 | 4005 | 4256 |
| C | 7 | 81637 | 85389 | 55698 | 57894 | 4567 | 5001 | 56668 | 55498 | 3369 | 3456 | |
| D | 9 | 54789 | 55687 | 66943 | 67894 | 23569 | 25698 | 5002 | 5148 | 14556 | 15998 | |
| E | 4 | 33691 | 35987 | 15589 | 16987 | 19876 | 20115 | 69878 | 66787 | 71559 | 69115 | |
| F | 9669 | 9946 | 6647 | 6879 | 7889 | 8005 | 69871 | 65478 | 8006 | 8456 | 5692 | 5879 |
| G | 13159 | 13754 | 45897 | 46897 | 36987 | 34987 | 56899 | 58794 | 9876 | 9997 | 25468 | 26540 |

- Protocol

Every experiment is based on a [protocol](#). Learn the differences between a protocol and an experiment in Gen5: [Experiment vs. Protocol on page 88](#).

- Toolbars and Menus

See **Buttons and Icons** on page 68 to learn about Gen5 toolbars, buttons, and menus.

- Plate View

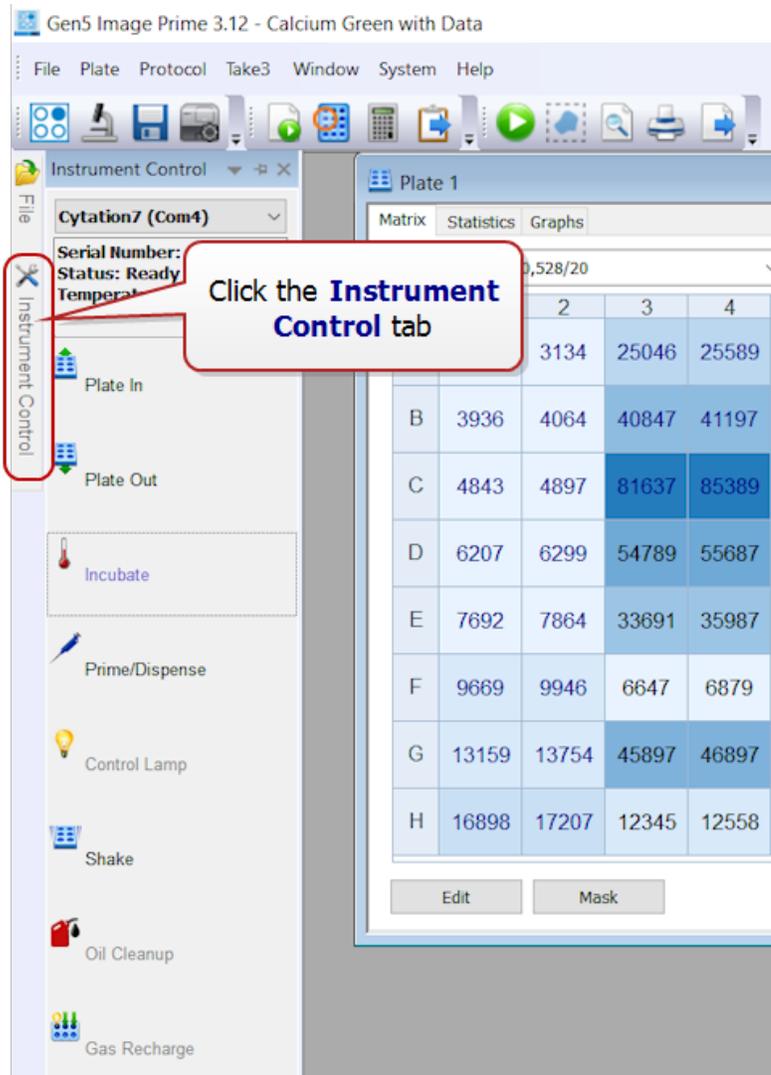
In an experiment, Gen5 provides a view or matrix for each plate processed (or to be processed). Protocols do not have plates, so you must have an experiment open to have a plate view. Gen5 displays reading and data reduction results in the matrix.

To open the plate view: double-click the plate icon in the menu tree or select **Plate > View**.



Gen5 offers several ways to modify and customize the Plate View for on-screen display and reporting/outputting results. See **View Results** on page 98 to learn more.

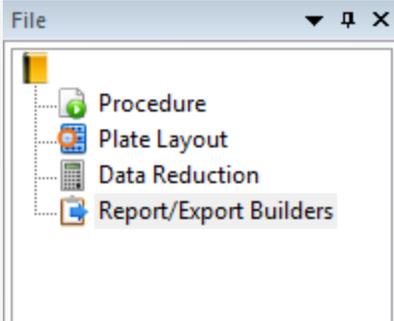
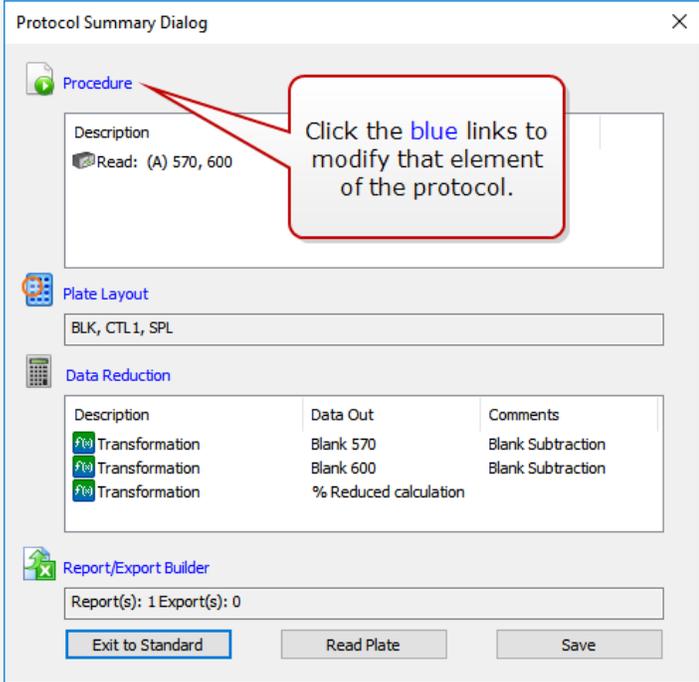
- Instrument Control Panel



In the Gen5 workspace, click the **Instrument Control** panel to access commands and actions for the attached readers and stacker. Select the instrument to control from the list at the top of the panel.

Protocol Workspace

When you create a new protocol, Gen5 opens a special workspace limited to the protocol's components.

| Standard Mode | Simple Mode |
|---|---|
|  <p>The workspace is made up of the menu tree with a branch for each of the protocol's elements. The order of the protocol elements reflects the order to follow when defining most protocols.</p> |  |



Defining the **Procedure** or reading parameters is the most important step to Gen5. The Procedure describes the data sets that are used in most subsequent steps to generate results output. The Plate Layout is the only other protocol element that is not affected by the Procedure; it is affected by the selected plate size.



For most protocols, it's best to define the **Plate Layout** in your second step. Gen5 automatically performs a blank-subtraction calculation when Blanks are defined in the plate layout. (You'll see this Transformation in the Data Reduction workspace.) Defining the standards and their concentrations in the plate layout is a prerequisite to generating a standard curve.

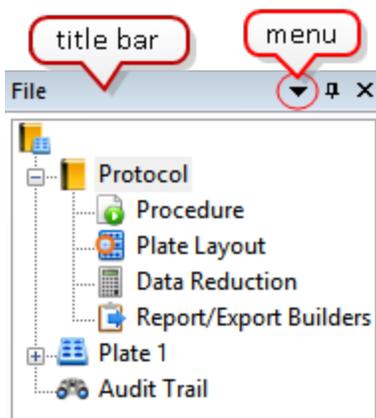


Data Reduction is one of the most powerful features in Gen5, and it requires the information provided by the two previous steps to logically offer you its capabilities. Automatically generated transformations, like path length correction and the ability to conduct well analysis, for example, depend on the Procedure. To plot a standard or titer curve and to validate Transformation formulas requires knowing the Plate Layout.



Report/Export Builders is a tool for selecting and customizing the appearance of data sets that are then available for printing or exporting.

The Menu Tree(Standard Mode)



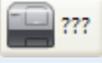
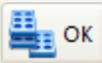
- To keep the menu tree open in a protocol or experiment, click  to dock it in place (click thumbtack again to collapse the menu tree). When working with a protocol file, the menu tree, like the toolbar, is limited to related operations.
 - Click  and  next to an item to reveal or hide its components.
 - The menu tree provides a visual cue of the steps to follow when creating a protocol.
- All of the controls available from the menu tree can be accessed using toolbar buttons or menus.
 - Highlight an item in the menu tree and right-click for a context-sensitive menu of options, including **Read** when a plate is selected, for example.
 - Asterisks (*) are displayed next to plate icons (and in the title bar) of an experiment when a change is made or an action is taken but the file has not yet been saved.
 - You can move the menu tree to another corner of the workspace or let it float undocked like the Plate workspace: select Floating from the menu, drag the title

bar, and using the temporarily displayed placement guides, drop it in the desired location.

- When you add multiple plates to an experiment, highlight a plate and right-click for menu options to delete and renumber plates.

Buttons and Icons

| Button | Description |
|---|---|
|  | Open the Task Manager |
|  | Reader Setup |
|  | Save the protocol or experiment |
|  | Read the plate |
|  | Print preview |
|  | Print results (formatted via the Report Builder) |
|  | Export results |
|  | Define the Procedure |
|  | Define the Plate Layout |
|  | Create Data Reduction steps |
|  | Open the Report/Export Builders to design reports |

| Button | Description |
|---|--|
|  | Paneled protocols |
|  | <i>(Imaging only)</i> Go to manual mode |
|  | <i>(Imaging only)</i> ROI Manager |
|  | Set Reader Optics: Opens the Set Reader Optics dialog in which you can update the reader with new definitions for filters and mirrors. |
|  | Instrument Control: Check the status, open the control panel |
|  | Stacker icon: Opens the Stacker control panel, if a Stacker is attached. |
|  | Export results to QC (Gen5 Image+ IVD and Gen5 IVD only) |
|  | Edit trended protocols (Gen5 Image+ IVD and Gen5 IVD only) |
|  | Pin the current plate view to the workspace |
|  | Duplicate a coincident display of the plate's results |

| Menu Tree Icons | |
|---|---|
|  | Plate—not read: Put the plate in the reader and click  |
|  | Plate read successful |
|  | Plate read paused by Stop/Resume step: When you're ready, put the plate in the reader, click  , and select Resume Plate x to continue |

| Menu Tree Icons | |
|---|---|
|  | Plate read aborted: To begin again, put the plate in the reader, click  , and select Re-Read Plate x |
|  | Plate read in progress |
|  | Plate read error, which is always preceded by an error message. The error code and message are recorded in the plate data audit trail. It is the user's responsibility to verify the integrity of the data after an error occurs. |
|  | Protocol |
|  | Experiment – See Experiment vs. Protocol on page 88 |
|  | Procedure: Define the reading parameters |
|  | Plate Layout: Assign location of samples |
|  | Data Reduction: Set up calculations |
|  | Report/Export Builders |
|  | Plate Information: Information obtained at runtime |
|  | Sample IDs: User-defined names or IDs assigned to samples |
|  | Calculation Warning Log: Data Reduction-related errors issued by unexpected curve or calculation results |
|  | Audit trail displays any logged events |
|  | Multi-Plate protocol view of data reduction statistics and curves |

| Menu Tree Icons | |
|---|---|
|  | Panel: Multi-protocol experiment performed on one plate |
|  | Paneled Protocols: Lists the protocols run (or to be run) in the panel experiment |

Imaging : Manual Mode

The following sections **briefly** describe how to use the Gen5 imaging controls in manual mode. See the Gen5 Help for more complete instructions and descriptions of these features.

Important Prerequisites

After the imager has been installed and configured (as described in its operator's manual), some basic setup steps are required to prepare the instrument to successfully capture images:

- [Plate Type](#): tell Gen5 the precise characteristics of the vessel you are using. Be sure to [define the Bottom Elevation for your plate or vessel](#). When using high power objectives (20X and higher): [Adjust the Correction Collar](#).
- [Saving Images](#): tell Gen5 where and how to save your image captures and analysis results. An external hard drive is recommended for most users because image files are significantly larger than other data files, making storage an important issue to address. Seek help from your IT group, if necessary.



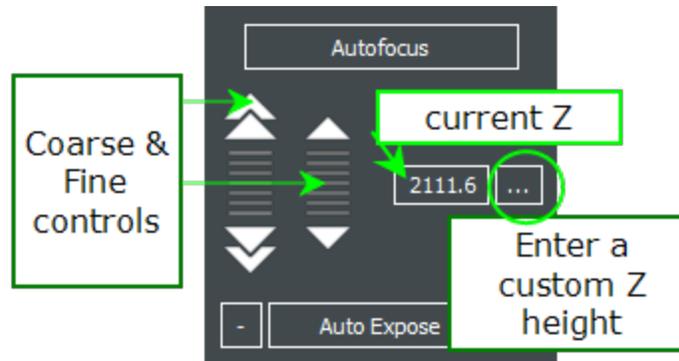
Automate your imaging assay by converting a manual mode session into an experiment. Use manual mode to determine and fine-tune settings like autofocus and image processing, then save those settings to process multiple plates.

Focusing and Capture

1. From the Task Manager, select **Imager Manual Mode** and click .

Options vary based on the type of imager. Generally, you need to choose the objective and color channel.

- Click **Find Image**. Gen5 automatically sets the exposure and focuses on the image.



If the displayed image requires additional fine-tuning:

- Click **Auto Expose**, or expand the Exposure panel to manually adjust the settings.
- Click **Autofocus**, or use the focus controls until the image is in focus.
 - Repeat these two steps until the image is exposed and focused to your liking.

Gen5 overlays up to 6 channels in the same capture (same X & Y coordinates) - color squares show channels acquired.



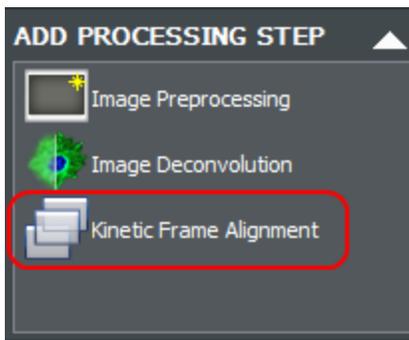
-  Click the camera button to capture the image.

Change the channel, if desired, to capture the same image in another color.
 Note: if you move to a different place in the current well or a different well, or change the objective, Gen5 will create a new image capture.

Process and Analyze

PROCESS / ANALYZE

After you capture one or more images, click .



Gen5 presents the image processing options applicable to the type of capture with default settings based on the objective and color channel. You can modify the processing settings to improve the image for analysis.

 Use the [Line Profile](#) or other tools to determine optimal settings like threshold and object size.

In the Color Channel panel, use the checkboxes to show or hide individual channels in the image for review purposes.

Note: Any adjustments made using the B&C (brightness and contrast) and the Channel Shift tools are for display purposes only; the changes do not affect the data measurements from the images.



Gen5 adds a prefix to the data set created by image processing:

Image Preprocessing: "Tsf"

Deconvolution: "Deconvolved"

Digital Phase Contrast: "Dig.Ph.Cont"

Z-Projection: "ZProj"

Image Stitching: "Stitched"

Kinetic Frame Alignment: "Aligned"

Edit the Image Processing Step

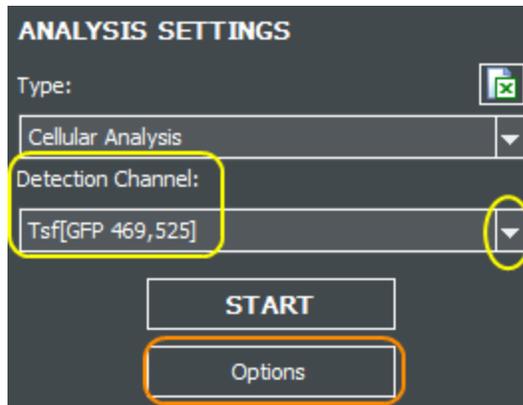
In manual mode, you can modify or add imaging process steps: Select the data set in the **Image History** box and click **Edit step** or choose another processing step to apply to it, such as or , that are relevant for your image.

Note: To display the raw image or a previously processed image, select its data set in the **Image History** box.

Analyze Captured and Processed Images



1. Click **Analyze** tab.



2. In the Analysis Settings box, select the **Detection Channel** and click **Start**. The results are displayed in the right pane.
3. Click **Options** to modify and/or expand the analysis, e.g., to add a Secondary mask or subpopulation (if supported).

Troubleshooting

- **First Response:** Running a System Test is the best first response to an instrument error. The test may restore the instrument's initial settings and computer communication capability. **Note:** To stop the alarm, **press the carrier eject button**; except touch screen readers: tap the screen to acknowledge the error.
- **Reboot the Computer and Instrument:** When you can't run a system test, for example, Gen5 is not responding, or when running a system test doesn't resolve the issue, turn off your computer and instrument, check all the cabling (i.e., make sure your serial or USB cable is in good condition and is properly connected to the computer and instrument), and then, power on your computer and

instrument. These steps should refresh the devices and reset communication parameters.

Visit Agilent's website for useful suggestions on getting the most from your reader.

- **Degrading performance:** Consider changing the [Multi-Read Calculations](#) setting if system performance slows significantly.
- **Incompatible protocols:** Protocols created with one instrument are not instantly compatible with other instruments. To correct the error: "Procedure was defined for a different instrument," open the Procedure and click **Validate**. If this does not correct the error, open each step in the Procedure and review it for compatibility with the current instrument.
- **Windows 10 Missing Files:** The Gen5 installation routine attempts to avert potential file sharing issues but when multiple users share a computer, Windows may use its "VirtualStore" as the default location for file storage.

Communication Errors

If Gen5 fails to save a file

- Ensure you are using a wired connection between the computer running Gen5 and the drive on which you are trying to save the files. Using a WiFi connection when running experiments is strongly discouraged. External hard drives connected via USB to the computer are recommended for storing imaging experiments.

When the computer won't communicate with the instrument

- **Confirm that the instrument passes its system self-test.** All Agilent instruments perform a self-test when turned on. Refer to the instrument's operator's manual for more details. The instrument will not communicate if it fails an internal system test. Non-keypad instruments beep continuously when the system test fails. (Press the plate-carrier button to stop the alarm.) Keypad instruments display an error message when the test fails. Refer to the operator's

manual to resolve the failure or [contact Technical Support](#).

- **Confirm the Instrument COM Port settings.** The instrument COM port must be configured correctly for successful communication. This is set in the **Reader Settings** window under **Instrument Configuration**. COM1 is reserved for serial cable connections while USB connections are assigned COM2 and higher. The COM port assignment can be identified through **Windows Device Manger** under the 'Ports (COM & LPT)' subheading. Confirm the Gen5 COM setting matches that found in device manager. **Test Communication**.
- **Confirm the correct USB drivers are installed.** Instruments connected to the computer via a USB cable or adapter require drivers to be installed. These drivers can be found with the original Gen5 installation media. Please contact Technical Support for driver download or installation assistance.
- **Make sure the serial or USB cable is in perfect condition and properly attached** to the port defined in the Instrument Configuration dialog (e.g., COM 1 or Plug & Play). Correct and reboot both computer and reader. Test communication.
- **Confirm that the serial/USB cable was obtained from Agilent.** Serial/USB cables are not universal. Consult the instrument's operator's manual for proper cable configuration or [contact Customer Care](#) to purchase a factory-tested cable. After installing a known good cable, reboot both computer and instrument. Test communication.
- **Make sure computer processes are not using excessive memory.** CPU intensive tasks, such as virus scans and complex Gen5 data analysis, can interrupt communication between the computer and the instrument. Check your computer's Task Manager for memory intensive processes. Review current settings: **System>Preferences>Calculation Options**.

Serial Cable Connected and Keypad Readers

- To prevent damage to the instrument, always **turn off the instrument or the computer** before removing or inserting a serial communications cable. USB cables do not have the same threat.

- **Confirm the baud rate** (or transmission speed) defined in the Gen5 Instrument Configuration dialog matches the instrument's settings. Consult your instrument's operator's manual for the correct rate. Correct the Gen5 Instrument Settings to match and reboot both computer and instrument. Test communication.
- **Confirm** with your computer supplier or a local technician **that the serial port has been enabled**. For example, the IBM Thinkpad was originally shipped with the serial port disabled. Correct and reboot both computer and reader. Test communication.
- For advanced computer users, the serial port of the instrument and computer can be independently tested using an independent serial-communication software package such as Windows Terminal, Hyper Terminal, or ProCom. Agilent does not support or sell these programs.
 - Select flow control for "XON/XOFF" and send an ASCII asterisk symbol (*) to the reader. The instrument should initiate a self-test and return the results to the computer. If the instrument fails to communicate, and Steps 1 through 5 do not resolve the problem, test the instrument on an alternative computer to confirm which device is at fault. Please contact Technical Support if the instrument is diagnosed to be faulty.

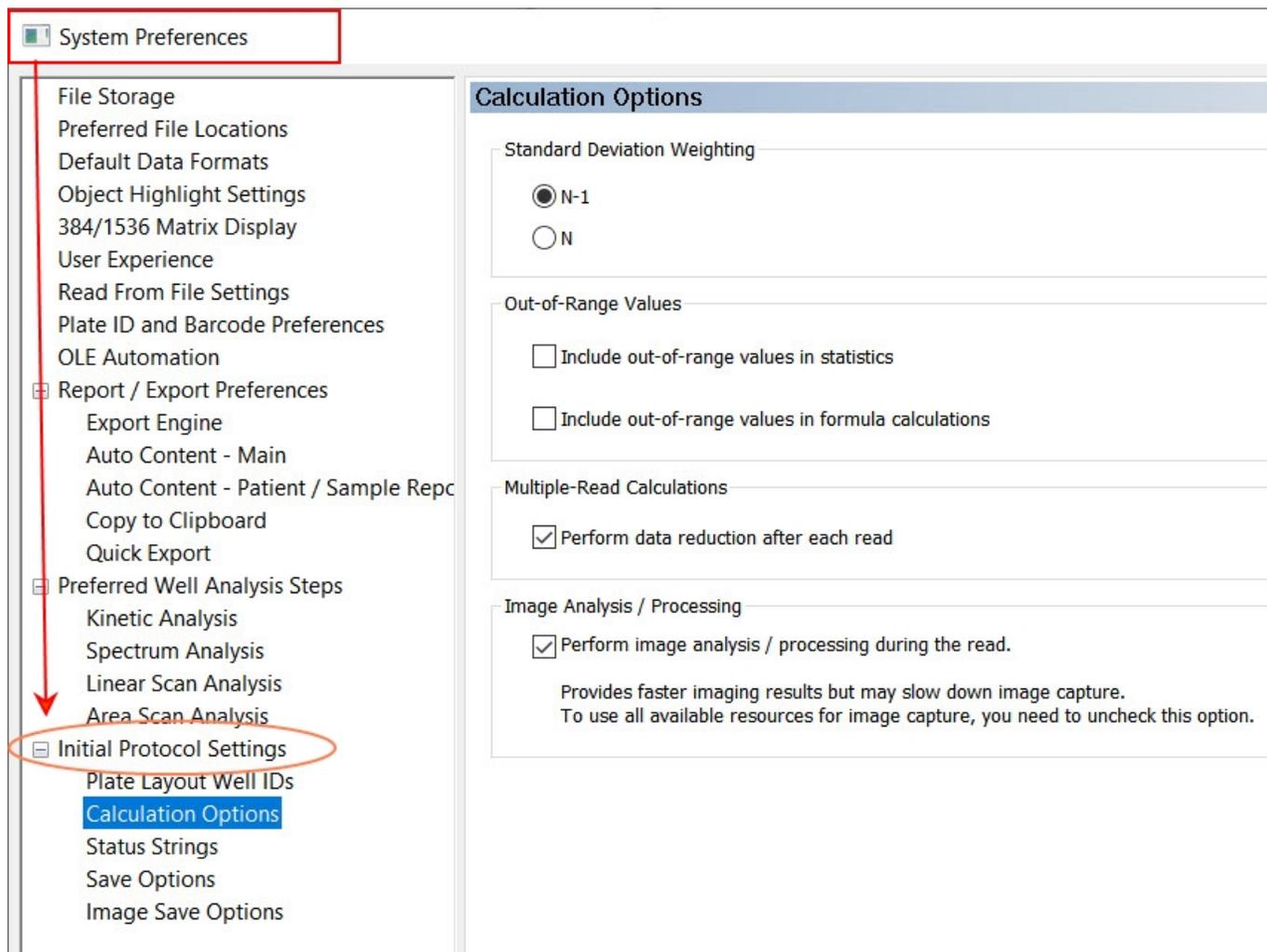
Restore Optimal Performance

Numerous factors can affect your computer's performance. If you notice a slowdown in Gen5's performance, follow these suggestions:

- Close all other applications, including Internet browsers, when running Gen5.
- Do not display Gen5's Curves data in the Plate View while performing a kinetic analysis. Wait until the read step is finished before viewing the Curves data set. Displaying the Curves data during a Kinetic read can consume excessive resources resulting in performance degradation. You can drill down to a Well Zoom to monitor the progress of one well, then, leaving the Well Zoom open, change the Matrix Data to a numeric view.

- Disable the Calculation option **Perform data reduction after each read** to give Gen5 sufficient time between obtaining measurements to perform calculations. Select **Protocol > Protocol Options > Calculation Options**.
- Disable the auto-Save option: Save when: Interim read completed. Change the Save Options to free up resources. Select **Protocol > Protocol Options > Save Options**.

To apply these settings universally, to all future experiments, make the changes in **System>Preferences>Initial Protocol Options**.



Fluorescence/Luminescence Measurements

Here's a list of potential problems, the possible cause, and a remedy:

Fluorescence/Luminescence Readings Too Low

- Possible cause: The fixed Gain in the Read Step dialog is currently set too low
Raise the Gain to an appropriate level. For fluorescence, the Gain is usually set between 45 and 130. For luminescence it is usually set between 100 and 200. Learn more in the Gen5 Help.

Try Automatic Gain Adjustment, using the Scale to High Well option and setting the target value to be between 20,000 and 80,000 for standard range, or 1,000,000-3,500,000 for extended range (if supported by your reader).

Note: Some readers have extended range capabilities with flash fluorescence. These models are auto-ranging up to 10,000,000.

- Possible cause: The wrong filters are selected in the Read Step dialog (for filter-based reads only)

Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.

- Possible cause: Top probe setting is not optimized

Gen5 generally positions the top probe at the optimal height for fluorescence reads, based on the plate type selected; it focuses the beam above the well. Refer to the Gen5 Help and use the Read Height option in the Read Step dialog to make adjustments. If supported by your reader, try the Auto-Adjust feature for the probe height. The reader's user manual may contain additional suggestions.

Fluorescence Background Too High

- Possible cause: Using incorrect microplates

Solid black plates for top probe reading lower the background. Black plates with clear bottoms lower the background if bottom reading is necessary. Corning 3615 or 3614 (for cell culture) are appropriate.

- Possible cause: The wrong filters are selected in the Read Step dialog (for filter-based reads)

Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.

- Possible cause: Phenol red is used in the media when exciting at 485 nm and reading at 528-530 nm

Eliminate or replace the phenol red.

- Possible cause: Cells, media and other contents fluoresce

Use deionized-water blank wells as a diagnostic tool. The blank-well reading will help you determine the background value contributed by the instrument, labware, and media.

- Possible cause: The top and/or bottom probe needs cleaning

Refer to the operator's manual for guidance; not all readers have user-accessible internal components.

- Possible cause: The instrument has fluorescing material spilled inside

Refer to the operator's manual for guidance; not all readers have user-accessible internal components.

- Possible cause: The Gain in the Reading parameters dialog is currently set too high

Lower the Gain setting. The background should still read higher than zero. 200 is a recommended minimum and a value of 1000 takes advantage of the system's five-digit resolution.

Reader Not Achieving Desired Fluorescence Detection Limit

- Possible cause: The wrong filters are selected in the Read Step dialog
Examine the current filter settings and make any corrections. If the filter settings appear to be correct, check the locations of the actual filters in the instrument.
- Possible cause: Using incorrect microplates
Refer to the reader's user manual for information on supported plate types.
- Possible cause: The fixed Gain is currently set too low
Raise the Gain setting until the background wells read at least 200 RFU (1000 RFU is preferred).
- Possible cause: Readings are taken using the bottom probe
Try switching to the top probe, if applicable/appropriate for your reader and assay.
- Possible cause: The solution volume is 50 μ L or less
Increase the solution volume to 150-200 μ L, if possible.
- Possible cause: Wrong pH
Fluorescence is very pH dependent. Use the appropriate pH.
- Possible cause: Phenol red is used in the media when exciting at 485 nm and reading at 528-530 nm
Eliminate or replace the phenol red.
- Possible cause: Top probe setting is not optimized
Gen5 generally positions the top probe at the optimal height for fluorescence reads, based on the plate type selected; it focuses the beam above the well. Refer to the Gen5 Help and use the Read Height option in the Read Step dialog to make adjustments. If supported by your reader, try the Auto-Adjust feature

for the probe height. The reader's user manual may contain additional suggestions.

- Possible cause: Transfection efficiency in gene expression is too low
Use more cells, or improve the transfection efficiency.
- Possible cause: DNA is old or of poor quality
Use high quality, new DNA.
- Possible cause: Not using nuclease-free buffer in DNA quantitation
Use nuclease-free buffer.
- Possible cause: Poor dilution methods
Use appropriate dilution method in tubes.

Reader Over-ranging in Fluorescence

- Possible cause: The Gain in the Read Step dialog is currently set too high
Lower the Gain setting. If using Automatic Gain Adjustment, try the Scale to High Well option and set the High Value in the range of 50,000 to 70,000.

Bandwidth Verification Failed

- Error or warning messages are issued when Gen5 detects overlapping wavelengths or bandwidth
Select/enter Filter Set wavelengths that do not overlap. Learn more about Gen5 bandwidth verification in the Gen5 Help.

Error during Auto-Sensitivity Determination

- Reader cannot fulfill request to determine optimal Gain
Gen5 displays an error message when the reader cannot determine the optimal Gain based on the defined reading parameters.

Luminescence integration time should be ≤ 1 sec and > 1 ms, especially when scaling to low wells.

Manually enter a Gain value or use an alternative method to determine the optimal sensitivity, if the error persists. Learn more in Gen5 Help.

Optimizing Imaging Performance

Performing imaging reads requires much more computer resources than a non-imaging read. If you are experiencing problems with imaging reads, such as an automatic abortion of the read with a memory error message, consider the following recommendations:

Ensure that your computer is fast enough to avoid accumulating too many images in memory. [Computer System Recommendations on page 12](#). An extended delay in the display of image data reduction results or very high CPU activity during the read may indicate that the performance of the computer or network is not high enough to execute such a procedure.

 Image files are significantly larger than typical data files. Consider using an external hard drive to store images: [Image File Management on page 92](#).

Change the virtual memory settings.

1. From the Windows Start menu, go to **Control Panel** and select **System**.
2. In the left pane, select **Advanced system settings**.
3. In the System Properties dialog, on the Advanced tab in the Performance area, click **Settings**.
4. In the Performance Options dialog, on the Advanced tab, in the Virtual Memory area, click **Change**.
5. Clear **Automatically manage paging file size for all drives**, if it is selected.
6. Select **Custom Size**, enter the following minimum and maximum values, and then click **OK**:
 - Minimum: 10 GB
 - Maximum: 20 GB
7. You may need to restart your computer for the change to take effect.

Run a complete chkdsk on the hard drive and defragment the hard drive.

- Accessible at:
[<Drive> > Properties > Tools > Error checking](#) (include the recovery of bad
-

sectors)

<Drive> > Properties > Tools > Defragmentation.

Close and restart Gen5 before running a protocol with which you have had trouble in the past.

Rebooting your computer can also be helpful.

If you keep experiencing problems during imaging experiments, consider the following options:

- Reduce the number of images collected during the read.
- Use lower montage dimensions by using lower magnifications.
- Limit the read to channels that are absolutely necessary.
- Use fewer kinetic points and a larger interval.

System Administrator's Password

[Contact](#) Customer Care if you have lost or forgotten the System Administrator's password. Gen5 ships with the System Administrator's password set to **admin**.

Essential Concepts

This section will give you a good basis of information for understanding the structure and terminology of Gen5. You can find more details and answers to specific questions by using the online Help system. Select [Help > Help Topics](#) from the menu.

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Experiment vs. Protocol

Gen5 uses two common terms to define distinct elements of its toolkit. The distinction is subtle and will have more or less importance depending on how you use Gen5. In any case, you'll work most efficiently by understanding each role and making them work for you.

|  Protocol (*.prt) |  Experiment (*.xpt) |
|--|---|
| <p>A protocol is a set of instructions designed to capture, transform, and report and/or export data.</p> | <p>An experiment has a copy of the protocol and at least one plate. It executes the instructions provided by the protocol to produce results.</p> |
| <p>Protocols are created and saved as stand-alone files. They function as a template; an unlimited number of experiments can be based on one protocol.</p> | <p>Although an experiment is created using an existing protocol, that protocol can be modified within the experiment and saved as a new protocol.</p> |
| <p>A protocol consists of reading requirements, like detection method and wavelength, and reading-related actions, like shaking and incubation, plate layout, data reduction, and data viewing, reporting, and exporting parameters.</p> | <p>Running an experiment is the only way to process a protocol.</p> <p> The Read Now button is available after a read step has been defined in a protocol, but this action creates an experiment based on the protocol.</p> |
| <p>A protocol can be used repeatedly (as is or modified) within experiments. By itself, a protocol does not produce results. Protocols do not have plates associated with them.</p> | <p>Multiple plates can be processed in an experiment, each one considered a unique assay with independently reported or exported results. The exception is multi-plate protocols, described</p> |

|  Protocol (*.prt) |  Experiment (*.xpt) |
|--|---|
| | later. |
| .prt is the protocol's file name extension. | .xpt is the experiment's file name extension. |
| A copy of the protocol is saved within an experiment or as a stand-alone .prt file. Since protocols do not have plates, they cannot generate data outside of an experiment. | An experiment is saved as the full collection of procedures, formulas, reporting definitions, and other details. The non-imaging plate data are recalculated when the file is opened in Gen5. |
| Gen5 Secure and Gen5 IVD maintain an audit trail of all activity and changes related to a protocol. All other Gen5 software levels do not support this feature. | Data acquired and transformed in an experiment is protected by an audit trail in Gen5 Secure, Gen5 IVD, and Gen5. The Reader Control edition does not support this feature. |
| Changes made to a stand-alone protocol are not reflected in any previously created experiments based on that protocol. A new experiment must be created to apply the revised protocol. | Within an experiment, you can select Save Protocol As to capture the current details of the protocol and save them as either a new protocol or as an overwrite of the original protocol. |

Gen5 also supports more complex multi-plate protocols that are not covered in this introductory material. See **Design a Multi-Plate Protocol** in the Gen5 Help system.

About File Storage

File Types

Gen5 creates multiple file types:

- Protocol = .prt
- Experiment = .xpt
- Panel = .pnl
- Imaging = .tif
- Imager manual mode session = .imm

System file types include:

- Plate type (vessel) = .ptf
- *.xml = several components, like objectives and filter cubes, are managed with their own unique XML file.

The Gen5 executable file (.exe) and numerous other types of supporting files, like a Microsoft Excel template, are also installed on the computer.

Databases

Gen5 installs two databases on your system called LocalDB and SharedDB. While the databases are always used for critical, internally used files, Gen5 offers you the choice of using the Windows File System or the Gen5 (SharedDB) database for storing Gen5 protocol (.prt) and experiment (.xpt) files (excluding image files, which cannot be stored in the SharedDB). This option, combined with the ability to create multiple databases, allows you to structure file storage according to your organization's requirements.

- Files may be stored on the computer's hard drive, on a network, or on a portable medium. Windows Explorer or a similar application can be used to view the file names and locations, and to move, copy, rename, and delete files.
- Alternatively, protocol and experiment files may be stored in a secure, shared-access database. This database, initially named SharedDB.mdb, can be stored on a user's computer or on a shared-access network/computer (LAN). Gen5 provides a special file maintenance utility for viewing the file names and their

locations, and for moving, copying, renaming, deleting, importing, and exporting files.

- Select the preferred method of storing protocol and experiment files at **System > Preferences > File Storage**.

Note: When upgrading to a higher version of Gen5, you will be prompted to also upgrade your databases (or not). Gen5 does a good job during a database upgrade to both preserve your legacy data and to apply changes to files to make them compatible with new and improved features.

Gen5 IVD and Gen5 IVD Image+ also install the QC Trending database, QCDB.mdb. It can be set up on a network for sharing among multiple users, moved, renamed, and copied. It is initially installed in a QC folder in the default database location described below. See the Gen5 Help system for more information.

File Location

During a typical installation:

- the program files are stored in this default location: C:\Program Files\Agilent\Gen5 (software edition)
- the databases are stored in this default location: C:\Program Data\Agilent\Gen5 (software edition)\(version #)\SharedDB or LocalDB
- Gen5 installs Protocol and Experiment folders in the respective File Storage locations, for example: C:\Users\Public\Documents\Protocol
- Gen5 prompts you to define the location for the **Image Library**, when applicable. Then, Gen5 maintains a connection between an imaging experiment or manual mode session and the images acquired in the session.

The databases are critical to Gen5 functionality. Make sure they are not deleted from your system.

 **Image Save Options** are defined globally in the **System>Preferences** and locally in **Protocol>Protocol Options**.

Image File Management

Each saved image is saved as a TIF file. The TIF files will contain metadata, pertaining to the instrument, experiment, plate, well, and image, though this data will likely not be accessible by other TIF file readers. With [Quick Export for Imaging](#) (described in the Gen5 Help) you can save and report the metadata.

Defining the Gen5 Image Library

The first time you connect to an instrument with imaging capability, Gen5 prompts you to define an Image Library location where images will automatically be saved when you run an experiment. The Image Library location can be changed at any time in the Image Save Options dialog.

Storing Images



Warning! Each image is at least 2 MB in standard field-of-view (FOV). When using a wide field-of-view (WFOV) camera, e.g., Cytation 5 W-models, or a confocal imager, e.g., Cytation C10, images are almost four times the size of standard FOV images. Imaging microplates can quickly generate very large amounts of data: reading only one image per well of a 96-well microplate in standard FOV results in 200 MB of images. Image montages, z-stacks and processed image files, such as [stitched images](#), can be much larger. Review your data storage requirements with your IT department.

Within the image files folder, Gen5 creates an experiment folder for each imaging experiment executed. Within the experiment folder are subfolders for captured images of each plate run in the experiment. It is important to not rename the folders that contain the images. Doing so may break the link between the experiment or IMM session from its images. See also [Relinking an Experiment](#) (described in the Gen5 Help).

You may find that you need more hard disk space than is allocated by default. For example, running a multi-plate experiment, imaging all 96 wells in a 4x4 montage in

three colors will require an increase to virtual memory. Without this change, the message, "This procedure may require that you increase the size of the virtual memory in Windows," may appear. In this case, please consult with your IT group to increase: [Change the Virtual Memory Settings on page 26](#).

Binning: Autofocus binning

Capture binning (affects exposure)

Reduce file size by applying [binning](#) during image capture.

Best Practices

Like most software tools, Gen5 is flexible and offers several ways to accomplish a task. Here are some recommendations for saving time and using it most efficiently.

Efficiencies

- For an assay or experiment that you will run numerous times, develop a protocol to define the Procedure, Data Reduction, Data Views, and Reports required. Then you can run an experiment (from the Task Manager, [Experiments > Create using an existing protocol](#)) based on the protocol whenever necessary. You can fine-tune the protocol within an experiment, but remember to select [File > Save Protocol As](#) to update the original protocol with your improvements.
- Use [File > Save As](#) to give you a head start creating a new protocol based on an existing one that contains the same or similar plate layout, reading parameters, or other elements that will be repeated in your new protocol.
- Define and customize Data Views before selecting what to include in reports or export files. All the on-screen data (i.e., data views) can be reported or exported. If you use on-screen views and paper reports equally, it is most efficient to first fine-tune the Data Views, and then include them in reports/exports.
- When appropriate, assign Blanks to the plate. Blanks can be deionized (DI) water, buffer, reagent without analyte, substrate, and so on. When running fluorescence cellular assays, a DI-water blank illustrates the background contributed by the instrument and labware as separate from the cells and media.

Identify the location of the Blanks in the Plate Layout, and Gen5 will automatically create the blank-subtraction data reductions.

- Back up your database regularly: once per week is recommended for most organizations. If you're using a Gen5 Database for protocol and experiment file storage, use the built-in Periodic Optimization feature.
- Take action if you get a warning message about the remaining size of your databases; see **Maintaining Files** in Gen5 Help for instructions on archiving and deleting records.
- Turn off the Multi-Read Calculation option to improve Gen5 performance. Calculation results will be the same, but your computer's resources will not be diverted for performing interim calculations. Find this option at **Protocol > Protocol Options > Calculation Options**.

Time-Savers

- **Partial Plate:** For assays using strips or partially filled plates, especially if the read steps are long or complicated, you can save time by telling the reader exactly which adjacent wells or portion of the plate to read.
- Use the Gen5 automatic Save feature to create a new, date-stamped folder for storing experiment records. This is an especially good practice for large labs with multiple users who run hundreds of plates per day. Gen5 will keep all the data organized by date. Define this kind of file management setting in the Initial Protocol Settings so it will apply to all newly created protocols. Select **System > Preferences > Initial Protocol Settings > Save Options**.
- **Print Preview:** Save time and paper by viewing reports on screen before sending them to the printer.

Basic Tasks

This section provides instructions for some basic tasks.

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Quick Read



You can perform a quick read using the microplate reader connected to the computer to read a plate and report the results. It's called quick because it is accomplished without taking the time to set up a full protocol.

Perform a Quick Read:

1. Click **Read Now** from the Task Manager, and either select an existing protocol or create a new one for this read.
2. After you select a protocol, or define a reading procedure for a new protocol, the reader reads the plate.

When the reading is done you can report the results. If you have a full-level edition of Gen5 (any version other than Gen5 RC), you can perform data analysis.

Create a Standard Curve

Gen5 lets you create one or more standard curves for determining the concentration of test samples:

1.  Select **Read Now > New**.
2. In the Procedure dialog, define the **Read** step (and any other required steps), then click **OK**.

Gen5 performs the read and exports your results.

3.  Select **Plate Layout:**

- **Standard Curves** Select **Standard Curves** as one of the well types.
- Define the concentrations of the Standards.
- Assign the location of the standards, samples, and blanks (if any) on the plate.

Conc. \Dil. values Colors

Define dilutions/concentrations

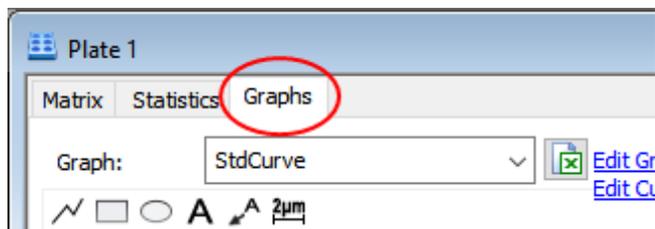
Type: Concentrations

| | |
|------|----|
| STD1 | 10 |
| STD2 | 20 |
| STD3 | 30 |
| STD4 | 40 |
| STD5 | 50 |
| STD6 | 60 |
| STD7 | |
| STD8 | |
| STD9 | |

4.  Select **Data Reduction > Standard Curve**.

Note: Gen5 may have generated a “corrected” data set: if you assigned blanks to the plate or selected Path length Correction or Polarization in the Read step, you may want to select these data sets for **Data In** for the Y-Axis Data to plot the curve.

5. On the Data In tab, select the **Y-Axis Data**.
6. On the Curve Fit tab, choose a curve fit method.



7. After you save the data reduction step, the plate matrix will display a **Graphs** tab to display the standard curve.
8. Define the reporting or export requirements and save the protocol using **File > Save Protocol As**.

Other options and requirements when defining multiple curves:

Curve Name: Replace the default “Curve” with a more meaningful or unique name.

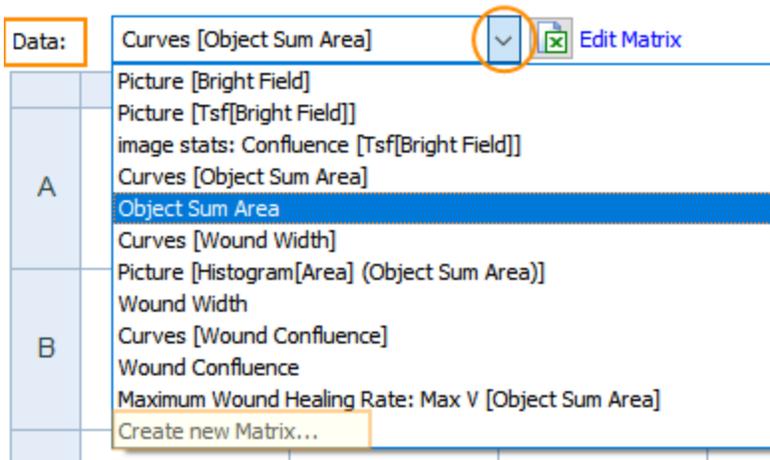
On the Data Out tab: Replace the default “Conc” for the Data Set Name with a more meaningful or unique name.

On the Data Out tab: Define interpolations to plot on the curve.

View Results

You can instantly view the results of an experiment in the Gen5 main workspace:

[Plate View \(Workspace\) on page 100.](#)



- After reading the plate (or otherwise acquiring data), in the plate view (Matrix) use the Data list to a data set to display. You can also select **Create new Matrix** to define a new view.

- Click **Edit Matrix** next to a data set to customize the view’s appearance. This feature is also available in the [Data Views](#).
- Click  to instantly open the current view in Microsoft Excel.
- Asterisks (**) are used to signal a change: In the Gen5 title bar an asterisk indicates the current file has been changed but not yet saved. When a data set is enclosed by asterisks, it has become invalid. Generally this is because a Read step or Data Reduction step has been altered. Edit custom-made data views to select valid data sets.
-  384- and 1536-well plates require resizing to effectively see the data. Gen5 adds a button to the Plate View to zoom in on the top-left quadrant of the plate and zoom out to view the entire plate. After zooming in, use the scroll bars to bring the other quadrants into focus.

- Multi-index readings offer another viewing option. Kinetic and scanning reads generate views based on the number of intervals, wavelengths, or positions defined. Use the buttons or enter the desired read index and click **Show** to display it. Gen5 displays the time, wavelength, or position of the selected read number.
- Kinetic and Scanning protocols can generate Well Analysis data sets labeled Curves. In the Matrix tab, open the Curves data set and click on a well for a [Well Zoom](#). (384- and 1536-well plates show a magnifying glass in the well in lieu of a curve.)



Starting at the Curves data set, you can display multiple well zooms simultaneously by holding Ctrl while selecting wells.

- Select the **Statistics** tab to view a table of data reduction results.
- Select the **Graphs** tab (when available) to view any standard curves.
- Select the Results List tab (when available) to view the values or results of the cutoff or validation formulas.
- Review the description of the Gen5 naming convention for the raw data/results.
- In the upper-right corner, click  to pin a plate view in the workspace. Pinning a plate view allows you to have multiple plates' plate views open simultaneously. If a plate view is not pinned, when you open another plate, it opens in the same plate view.
- In the upper-right corner, click  to open another instance of the Plate View. Use this feature to view the raw data results of a reading in one window and simultaneously display a curve plotted from the results in another window, for example.

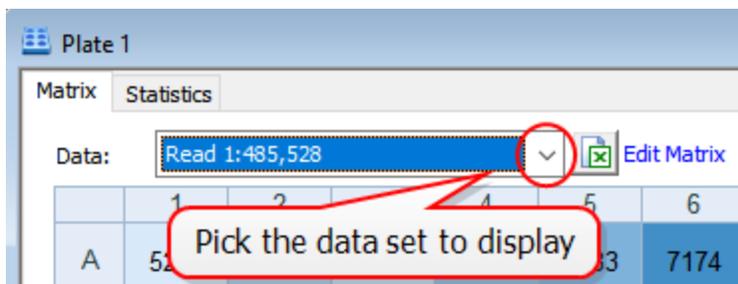
Important Notes

- Gen5 may not display some data points by default; to see them you must create your own Data Views. If you expected to see certain results that are not currently displayed, try creating your own views.

- All data views are also available for reporting and/or exporting.
- Modify a data view to change the way results are reported, including the number of decimal places and significant digits.
- Gen5 always uses your computer's regional settings to display and input data.

Plate View (Workspace)

Plate > View



Use the Plate View to view the results of an experiment and, if needed, to mask or alter the results.

- [Well Zoom](#) - drilling into individual wells - is available in imaging and multi-read assays.
- Gen5 uses [icons](#) in the menu tree to indicate plate read status (successful, aborted, in progress, error).

Print Results



Click the **Print** button to print the results of an experiment.

Prerequisite

First, you must select the specific content for the report using the Gen5 Report/Export Builder (**Protocol > Report/Export Builders**).

Reporting in an experiment is done on a per-plate basis:

- Highlight a plate in the menu tree and select **Print/Print Preview**.
- In a multi-plate experiment: You can select multiple plates by holding the Ctrl key while highlighting them, or to select contiguous plates, highlight the first plate, hold the Shift key, and select the last plate. Then, click **Print**.



Gen5 offers enormous flexibility in report output. After defining the report elements, use the Print Preview option to view the report on screen before printing it to paper. Unneeded columns and other individual report elements can be removed or modified to improve the appearance and usefulness of the report.

Note: In Gen5 Help you can find step-by-step instructions for creating and customizing reports.

Test the Instrument

System > Diagnostics > Run System Test

Most Agilent instruments perform a self-test every time they're turned on, but when you want to view and/or print the results of a system test:

1. From the Gen5 main window, select **System > Diagnostics > Run System Test**.
When there is more than one instrument attached to the computer, select the desired one and click **OK**.
2. When the test is completed, fill in the text fields—User, Company, Comments—to be included in the report of the test results, then click **OK**.
3. Print the report to retain a hard copy for your records.
4. Click **Save As** to convert the results to a text file. This is especially useful when troubleshooting an instrument. You can email the text file to Agilent.

Test History

Gen5 keeps the results of System Tests when they are performed using the menu controls.

Note: To review or print the results, select **System > Diagnostics > Test History**.

Set Up a Protocol

This section walks through the basic steps to creating a protocol. You'll find more details in the Gen5 Help. If you haven't already done so, read the differences between experiments and protocols in Gen5 in the Essential Concepts section.

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Design a Protocol

In the Task Manager, click **Read Now > New** to get started.

1.  Define the **Procedure**, then click **OK**. Gen5 performs the read and displays the resulting data.
2.  Define the plate layout (for all except Gen5 Reader Control).
3.  Define the data reduction requirements (for all except Gen5 Reader Control).
4.  Define the reporting and exporting requirements.
5. Save the protocol (**File > Save Protocol As**).

Note: Follow this sequence of tasks when developing a protocol to take advantage of the automatically created Gen5 data reduction events. For example, when you add Blanks to the Plate Layout, Gen5 automatically creates a Blank-Subtraction data set.

You can find instructions for developing specific types of protocols in the Gen5 Help.

Define the Imaging or Reading Procedure

Protocol > Procedure

Note: Procedure grayed out? When one or more plates have been read in an experiment, the procedure cannot be changed for the current experiment. If this isn't the case, your System Administrator may have restricted your ability to [modify](#) the protocol elements.

Note: Grayed-out buttons mean the action cannot be performed by the current reader or because previously defined steps—for example, a kinetic loop—limit the function or your level of software limits the feature.

For all procedures, define the [Plate Type](#) and then:

1. Click a link to add that step to the procedure. Most links open a screen for defining the parameters of that step, for example, **Read** lets you define wavelengths.

When defining a kinetic or synchronized well/plate mode analysis, add the [Kinetic](#) or [Synchronized Mode](#) steps **first**. Kinetic and Synchronized Mode steps form a loop or block. Put the Read and other valid steps to be performed inside the loop, between the Start and End. Monitor Well is similar: First add the [Monitor Well step](#) and then add a Read step inside the Monitor Well loop.

2. Define the details of the step and click **OK**.
3. Click **Validate** to check the selection and sequence of the steps.

Your instrument must be communicating with Gen5 for it to fully validate the procedure: Make sure it is turned on, not busy, and properly connected to the computer.

StepWise Procedure Features

- You can drag and drop steps in the procedure to change their sequence order.
- You can also copy (Ctrl+C) and paste (Ctrl+V) a procedure step or a block of steps from one location to another in the same procedure.
- Highlight a step in the procedure, and then click an action button to add a step before it.
- Double-click a step to open it for editing.
- Select a step in the sequence and right-click for additional options.
- Click **Validate** at any time to verify the reader's ability to perform the current sequence of steps.
- Highlight a step and press **Delete** to remove it from the procedure.

- Kinetic analysis, Synchronized Mode processing, and Monitor Well functions are set up in a loop or block. First define the function, for example, add the Kinetic step, and put the read and other steps inside the loop

Drag and drop is limited in Synchronized Modes. For example, you cannot drag and drop a step into or out of a Well Mode block.

Define the Plate Layout

Protocol > Plate Layout

Note: The Plate Layout Wizard appears when the plate layout has not yet been defined (and custom Well IDs have not been created and the wizard is not disabled in System Preferences).

1. In the Plate Layout Wizard, select the well types you want to define for the plate, then click **Next**. The Wizard prompts you to define each type of well you have selected. When all well types are defined, the Plate Layout dialog opens.
2. Select a well ID in the left pane, then click in a well, or drag over contiguous wells, to assign the selected well type to the plate layout.

Note: Some readers support random well selection.

- The well assignment starts with the well ID you select in the left pane. For example, if you select Well SPL1:4 in the left pane, when you click in a cell in the plate layout, that cell will be assigned SPL1:4.
- Use the Auto Select and Replicates options to speed up your work: set the options and click and drag to fill multiple wells at once. Click a column or row header to fill it.
- Use the Serial Assignment tools (, , and ) to quickly assign replicates to the plate layout in a horizontal or vertical line or in square groupings. Select one

of the directions (toggle through to access horizontal, vertical, or square) then click or drag in the plate layout.

- You can export a plate layout for use in multiple experiments, or import an existing plate layout.

Note: The type of plate, such as 96-well, is defined in the procedure and displayed in a representative matrix or grid format in the Layout and Transformation screens.

Helpful Hints

- Set up your preferred default Well IDs at **System > Preferences > Plate Layout Well IDs**. For example, you can define PC (for Positive Control) instead of CTL1 for Assay Controls. Well IDs defined in the System Preferences are available when defining the plate layout for all newly created protocols/experiments.
- Click **Undo** at the bottom of the screen to undo the last action. Up to 10 previous actions can be undone.
- To clear the grid and start over, right-click and select **Empty Layout**. To clear selected cells, set the Type of Well Settings to **Empty** and select the cells you want to clear.
- You can print the plate layout. 384-well plates print out in two sections, columns 1-12 and 13-24. 1536-well plates print in eight sections to fit all 48 columns and rows from A to Z and AA to AF.
- For Samples (unknown test specimen) Gen5 lets you assign and track data points, such as age or gender, in addition to the Sample ID. You can create additional identification fields.
- To copy the contents of the grid to the Windows virtual clipboard to paste into a text/external file, right-click and select **Copy Layout**. Open the receiving file, for example, Microsoft Word or Excel, then right-click and select **Paste**. Generally, plates larger than 96 wells do not fit completely in a standard-size Word or text file; a spreadsheet is required.

- Each instance of a Sample and Sample Control Well ID and each Assay Control group can have a unique concentration/dilution value. Gen5 assigns a dilution index to the Well ID to keep track each instance.
- Well selection must be compatible with the Replicate, Auto Select, and Filling option settings.
- You can resize the plate view in the standard Microsoft Windows way: click and drag the outer borders of the view, or click the maximize button in the top-right corner.
- You can resize the rows and columns: hover your mouse over a grid line between two numbered columns or alpha-labeled rows until the cursor changes to a separator, then click and drag.
- When running an experiment with a cuvette, the plate layout is mapped on a 96-well plate.

Set Up Data Reduction

Protocol > Data Reduction

 For the best experience using Gen5's data reduction  options, first define the read or imaging parameters  and the plate layout , e.g., assign blanks or standards, if applicable.

Gen5 uses a logical approach to presenting data reduction steps. Based on the defined read step and plate layout, Gen5 either creates the most commonly applied data reduction steps, like blank subtraction, or presents applicable data reduction options for definition. For example, you cannot create a standard curve until standards have been assigned in the plate layout.

To analyze complex imaging captures, a montage, z-stack, and kinetic timeline, requires image processing before data reduction steps can be applied:

| Image capture | Processing required |
|------------------|---------------------------------------|
| Montage | Stitching |
| Z-stack | Z Projection |
| Kinetic timeline | Image Statistics or Cellular Analysis |

Gen5 presents only the data reduction options that can be performed with the currently available data sets. [See also Dynamic Data Reduction Explained.](#)

Example

When the raw data is a montage-Z-stack image capture, Gen5 presents only image preprocessing, deconvolution, and stitching, because the montage must be stitched before a Z Projection can be created. Likewise, the Z Projection must be created before Image Statistics or Cellular Analysis can be performed. Thus, these data reduction options are not presented until that step has been defined.

Likewise with kinetic analysis, montages must be stitched, z-stacks made into z projections, and either a statistics or analysis step defined before kinetic analysis is offered.

Run image analysis on:

All Wells / All Reads

[Imaging Process and Analysis Optimization Control](#) button at

bottom of dialog.

[Top Things to Know about Data Reduction Workspace](#)



Make sure none of the Data Reduction step icons display the red invalid symbol. The invalid symbol may indicate that a previously selected “data in” set has changed, e.g., it has been renamed. Open the step and reselect the data in to fix the problem.



Important note about Cellular Analysis improvements: With each release of Gen5, we make improvements to the software. When data reduction processes are improved, results can be altered. Gen5 uses the Calculation Warning Log to alert users that their data may have changed. For example, users may see this message: “Failed to calculate cell count due to improved edge object exclusion when using plugs” when they open an experiment created in a previous version of Gen5.

About Exporting Results

Gen5 provides the following exporting tools:

- Quick Export to instantly export the current view to a Microsoft Excel worksheet
- [Export to Excel](#): to export selected data to Excel using the Power Export feature
- [Export to File](#): to export selected data, excluding curves, to a text file (for use in another software application) using the File Export feature
- [Right-Click Menu Options](#): **Copy to Clipboard** and **Save As**; to copy or save the current selection for use in another software application
- to instantly export an image to an Excel spreadsheet.
- to export an image's metadata to an Excel spreadsheet.

Prerequisites

For the QuickExport and Power Export features, you must have Excel 2007 or higher installed on your computer. Use File Export or right-click options if you do not have Excel.

About the Export Tools

- The Power Export and File Export methods require selecting the content you want included in the output file **before** executing the export for a designated plate.
- You can save your export selections with the protocol to reuse them every time you run an experiment based on that protocol.

- Exporting data is like generating a report; it is done individually for each plate*. Although you can select the export content in a protocol, you must run (or execute) the export in an experiment (after selecting a plate or [multiple plates¹](#)).
[* except in multi-plate assays]
- In an experiment, to run the export, you can select a plate in the menu tree and right-click for a menu that offers the **Export** option.

Export Multiple Plates to One File

When you run multiple plates in an experiment you can export all the data to one file:

1. In the menu tree, select/highlight multiple plates (by holding down the Ctrl key).
2. Right-click and select **Export**.

Make sure the [File Export Settings](#) are defined to automatically append the data.

About Reports

[Protocol](#) > [Report/Export Builders](#) > [New Report](#)

You can use the Report/Export Builders to define exactly:

- What to include in the report
- How to format an item in the report
- Where to place the item in the report

Defining Report Elements

1. When setting up the protocol, after customizing the Data Views, select **Report/Export Builders** from the Protocol menu tree.
2. From the Report/Export Builders dialog, click **New Report**.

¹Hold the CTRL key while selecting multiple plates.

3. Define your settings in the Properties, Content, and Options screens, then click **OK**. If you are running Gen5 Secure, Gen5 Secure Image+, Gen5 Secure Image Prime, Gen5 IVD, or Gen5 IVD Image+, Gen5 prompts you to add a comment to the Audit Trail event.

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