

V-Comp User Guide

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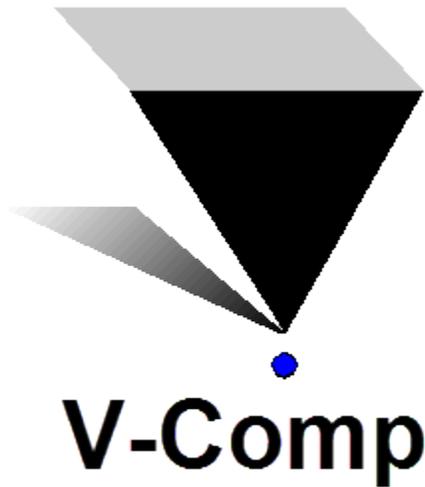
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V-Comp™ User Guide

This guide is intended for V-Comp™ application version 1.0 and later minor versions.

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Latest Versions

To find the latest versions for the software and documentation, visit the Verity Software House web site at www.vsh.com. This site provides a complete version of the latest revision of the online documentation, patches, and technical support information for the program.

You can also email us at verity@vsh.com to determine whether or not you have the latest version. Please include the version that appears on the start-up dialog box, and the file date for the program with your email.

The software also has a "Check for Updates" command in the Help menu. Use this command periodically to see if there are updates for your software. The command can download and install updates that are available, if you have administrative rights to your computer.

New Features in V-Comp™

Version 1.0

Hey, it's brand new, so ALL the features are new features, right?

System Requirements

Minimum System Configurations

PC:

Windows 7, Windows 8, Windows 10 or greater, 2GHz CPU or better, with 50MB free disk space for program files. Faster CPUs with more cores and greater RAM will improve performance.

Browser to view the user guide: Firefox 4+, Chrome 9+, or Internet Explorer 9+.

Macintosh:

OSX 10.7 or greater with 50MB free disk space for program files.

Browser to view the user guide: Safari 5+, Firefox 4+, Chrome 9+, or Internet Explorer 9+.

Compliance Mode Requirements

To operate the software in "Compliance Mode", administrators need to be able to set up user groups and add users to appropriate groups. User groups can be defined at the domain level or at the local machine level. See [Research and Compliance Modes](#) for details on setting up Compliance mode.

Installation and Setup of V-Comp™

Check [system requirements](#) to see if your computer will run the software.

You must have administrative rights to the computer during installation and registration of the software. After registration, the program can be run by users with restricted access to the computer.

- If your program is an update version, you will need the serial number for the previous version in order to register and unlock the software.
- You will need to register the software with Verity Software House in order to unlock the program on the computer. This can be done using the online registration process or by contacting technical support.

Windows Stand-alone Version

Installation

- Log on to your computer with an account that has Administrator privileges. You *must* have administrative rights during installation.
- If you have an installation CD, insert the CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.
- Double-click the **VComp.msi** file to launch the setup program. Follow the directions on the screen to complete the installation.
- See the section on [registering your software](#) for information on how to register your software.

Using the software

- Log on to your computer with your own user account. This should be the account that you normally use on the computer.
- Open the Start menu, and choose All Programs. Then navigate to the Verity folder, and choose the **V-Comp** application.

Macintosh Stand-alone Version

You will need to have administrative rights to install the software.

Installation on a Mac

V-Comp User Guide

- Log on to your computer with an account that has Administrator privileges. You *must* have administrative rights during installation.
- If you have an installation CD, insert the CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.
- Run the **VComp Installer**. Follow the directions on the screen to complete the installation.
- See the section on [registering your software](#) for information on how to register your software.

Using the software on a Mac

- Log on to your computer with your own user account. This should be the account that you normally use on the computer.
- Start the program by double clicking on the **V-Comp** application in the Applications/**VComp** folder.

Registering your new software

Why You Should Register

With this version of V-Comp, registration is more important than ever before. In fact, *your software will not be fully functional until you register*. Here's why.

This version of the program uses software security that allows it to be used without a hardware lock. Software security requires you to register your software in order to receive an **Unlock Code** to make the program fully functional. You can run the program for 5 days prior to registering the software.

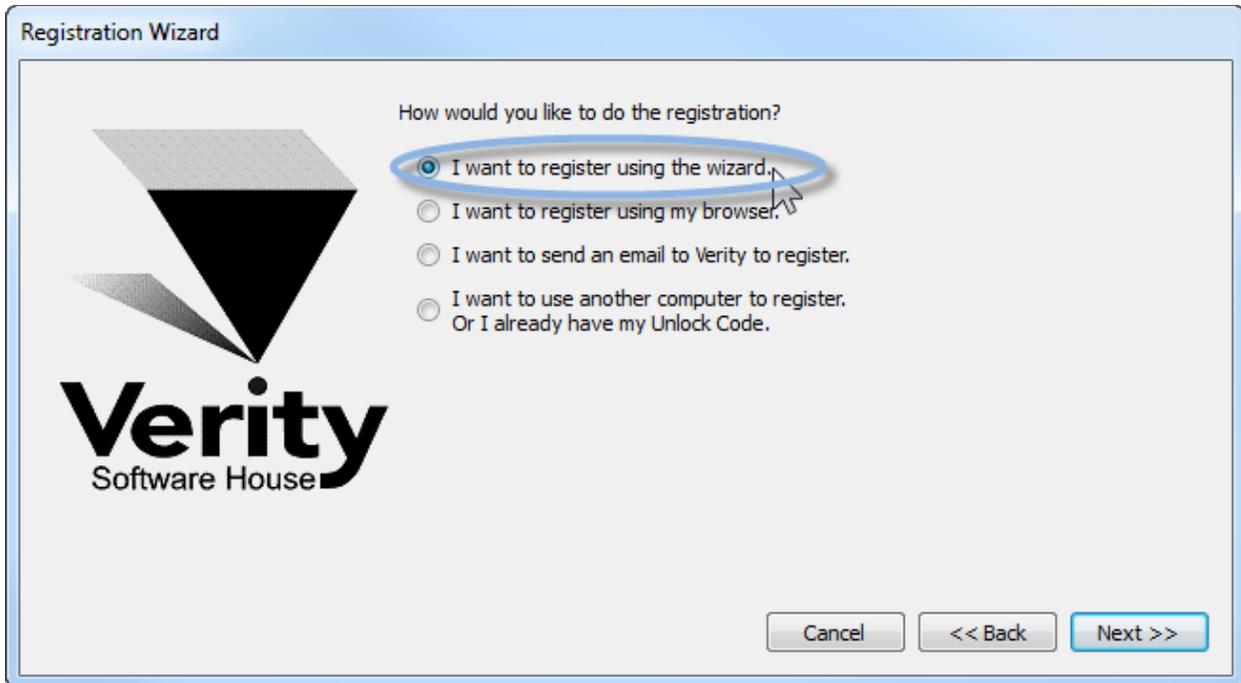
Of course, registration is useful for other reasons, too. We can inform you of bug fixes and updates, and provide you with better technical support.

How to Register

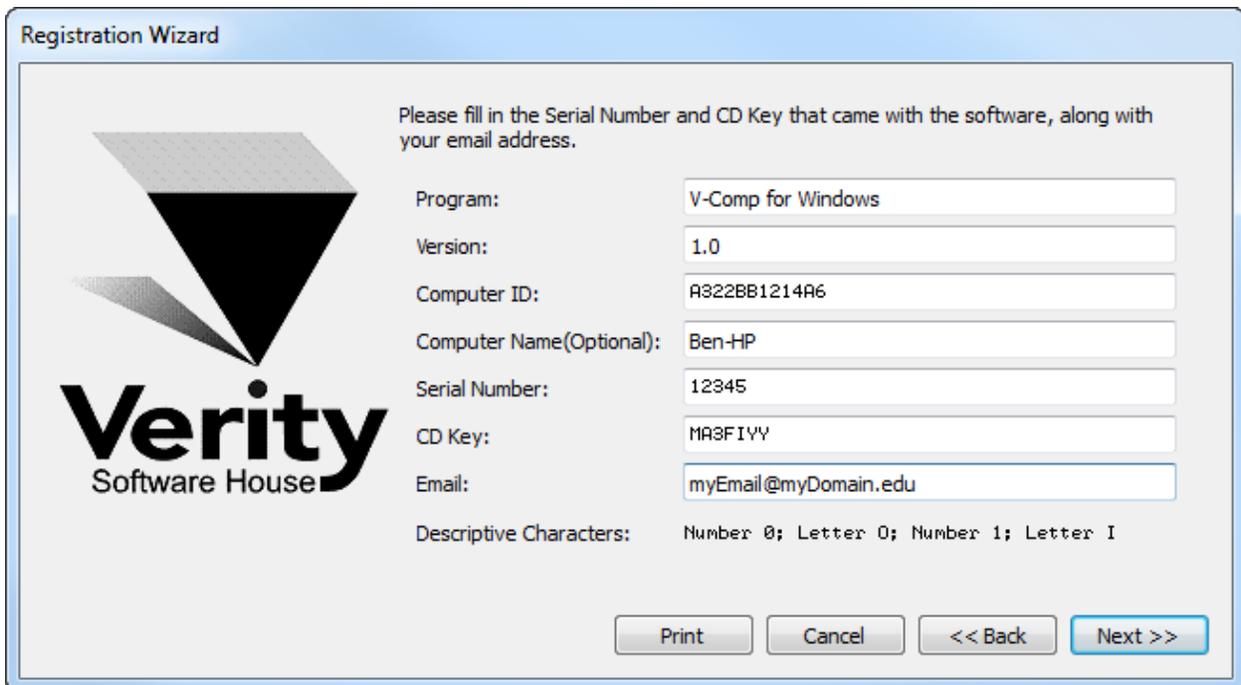
When you start the program for the first time, it will automatically start the Registration Wizard. You can access this dialog at any time by clicking the *Register* button on the dialog box that is displayed when the program starts up.



Click the **Next** button to begin the registration process.



The wizard will ask how you want to register the program. Select the appropriate option and click **Next**.



The next step in the wizard shows some information about the program. There are 3 fields that you need to fill in for this step: *Serial #*, *CD Key*, and *Email*. You can also enter or edit the *Computer Name* field to identify your computer.

The *Serial number* and *CD Key* can be found on the outside of the box the program came in, or in email if the program was provided to you by download. Enter these exactly as they appear; they are case-sensitive. If you have lost or misplaced the Serial number or CD Key, contact Verity technical support at tech@vsh.com or (207) 729 6767.

For the *Email* field, make sure to enter a valid email address. Otherwise, you may not receive the Unlock Code and the program will not be fully functional.

Click **Next** to continue the registration process.

If you have more than one user account at vsh.com, you will be presented with another step. This step *only* appears if you have more than one account.

Registration Wizard

In the list below are the user accounts that matched your email address. Please select the user account that you would like to use to perform the registration.

Email:

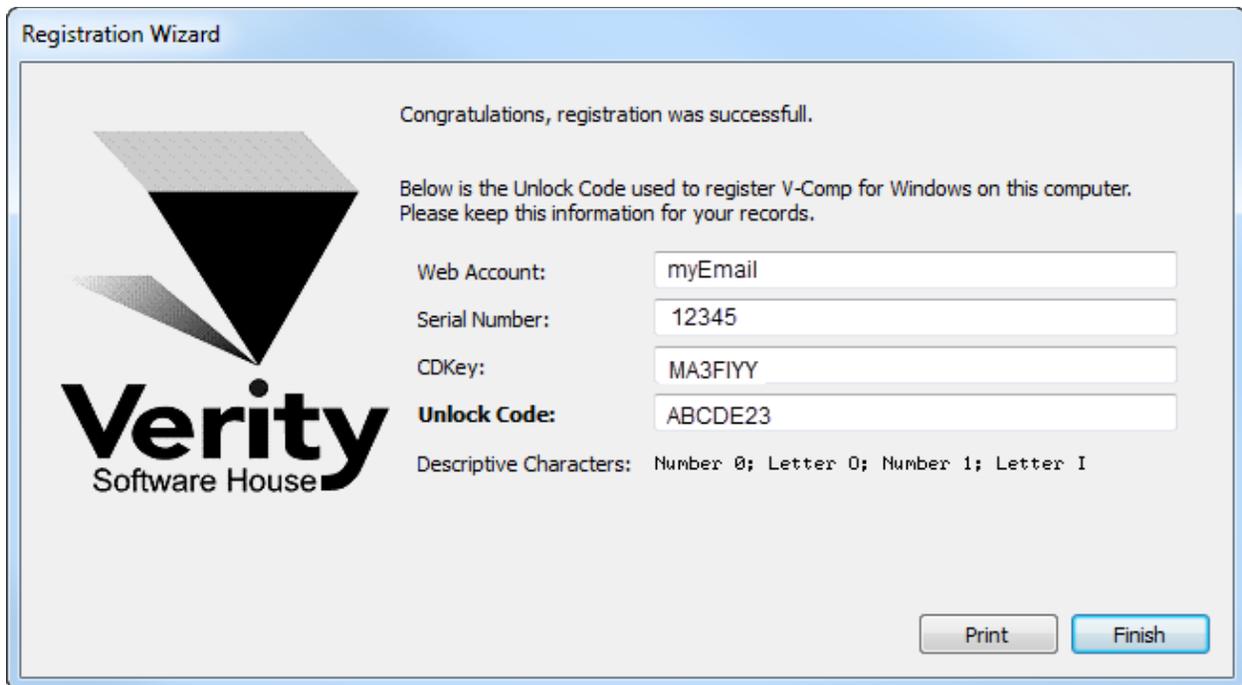
Matching Web accounts:

Verity
Software House

Cancel << Back Next >>

Choose the web account that you want to use for registration, and then click **Next**.

At this point, the program will request an unlock code from the registration server at vsh.com. If successful, you will see the confirmation step and the Unlock Code for your computer.



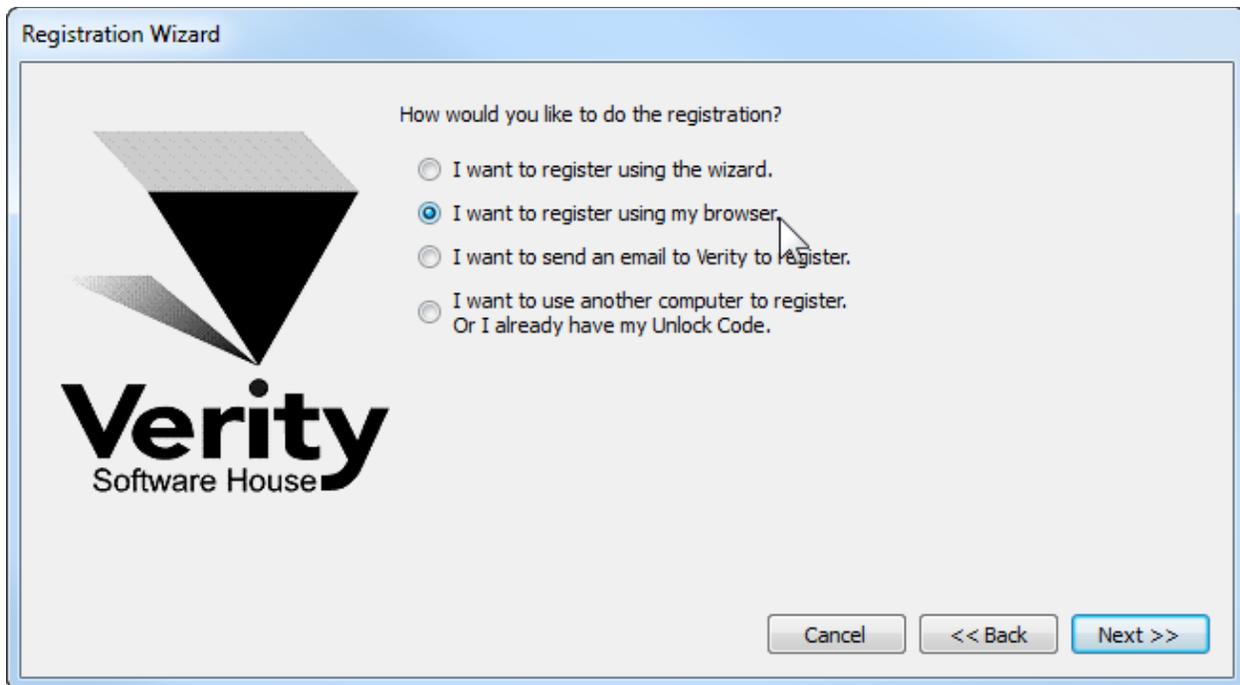
You can print the registration information by clicking the **Print** button. Click **Finish** to close the wizard and continue working with the program

If there was a problem with the registration, an informative message will explain the problem and steps to take to correct it.

Once you have completed the registration successfully, the program will be fully functional.

If the registration wizard cannot connect to vsh.com...

Sometimes firewalls can prevent the registration wizard from connecting to vsh.com. In this case, you can try registering with your browser. Choose the second option in the list and click **Next** to launch your browser with the correct registration information.



Follow instructions on screen to complete the process.

To register your software using email...

The registration wizard can help you send email to Verity Software House with your registration information. Choose the third option in the list and click **Next** to create an email message.



Follow instructions on screen to complete the process.

If you do not have Internet access...

If you do not have access to the Internet, you can send the registration information below to Verity Software House by fax, mail, or telephone. Our technical support staff can register the software for you with the information you provide, and supply you with the Unlock Code to enter into the Registration Wizard.

Here is the information we need. You can print this page and fill it in.

These fields are found in the Registration dialog box.

- Program Name:
- Program Version:
- Computer ID:
- Serial number:
- CD Key:

User Information

- Name:
- Institution:
- Address:
- City:
- State/Province:
- Zip/Postal Code:
- Country:

Phone:

Fax:

Email:

Our technicians will use the information below to create a web user account for you on our web site.

Name to use on VSH web:

Password to use on VSH web:

A question we can ask to verify who you are:

The answer to the question to verify who you are:

If you are updating from a previous version, you will need to supply the serial number of the previous version.

Serial Number of previous version:

Contact Verity Software House

Verity Software House

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USA

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Phone: (207) 729 6767

Fax: (207) 729 5443

Frequently Asked Questions

V-Comp™ Setups

What is a V-Comp™ setup?

It defines a compensation setup for a specific panel run on a specific machine. The setup stores the names of single-color controls, one or more unstained controls, which detectors (parameters) you want to compensate, and other settings. Setups also store the crossover matrix that V-Comp™ creates.

Why do I have to create setups?

Most users have more than one panel that they run, and many have more than one instrument. Because each panel and each instrument can have different fluorochromes and instrument settings, they require different compensation settings, too. Each setup keeps track of a particular set of compensation settings.

Can I edit a setup?

Yes, it's easy. Select the setup in the [Setup List](#), and then click the Edit button. The [V-Comp™ Setup Wizard](#) will guide you through editing the setup. If you do not see this command in the menu, your V-Comp user group does not have permission to access it.

Where are my setups stored?

Setups are stored in folder names "Setups". You can find this folder easily by using the [Open Setups Folder](#) command in the Tools menu. If you do not see this command in the menu, your V-Comp user group does not have permission to access it.

How do I delete a setup I'm not using anymore?

Use the [Open Setups Folder](#) command in the Tools menu to go to the setups folder, then delete the file that has the same name as the setup. If you do not see this command in the menu, your V-Comp user group does not have permission to access it.

Can I copy setups to another computer?

Yes, you can copy setups to as many computers as you want. Use the [Open Setups Folder](#) command to locate the setups folder, and then copy the files for the setups you want. Paste those setup files into the setups folder of the other computer.

Exporting Files

Why does the program create a new FCS file instead of replacing my original file?

We believe it is important to preserve the original acquisition files so that they can always be used in their original form.

Will the program work with data files from my cytometer?

We have tested the V-Comp™ app with all major cytometer files at the time the program was created, and we are always adding files to our testing library. We would be happy to try reading files from your cytometer if you are not sure they will work. [Contact our tech support folks](#) to send files.

Where do the exported files go?

It depends on the settings on the [Options page](#) of the V-Comp™ setup. Files can be stored in the same folder as the original file, or in another folder that you specify. This can be set differently for each V-Comp™ setup that you create.

Compliance and Research modes

Why don't I see all of the commands?

When V-Comp runs in Compliance mode, the commands that are shown depend on the user group you are in. If there are things you need to do that are not available to you, talk with your V-Comp administrator. See [Research and Compliance Modes](#) for more information.

Why does the program show "Research Mode" on the ribbon bar?

"Research Mode" indicates that the program is running in research-use mode, and not in "Compliance mode". If you want it to run in Compliance mode, V-Comp must detect the one of its user groups when it starts up: **VCompAdmins**, **VCompEditors**, and **VCompUsers**. If any of the groups is defined, the program will run in Compliance mode. See [Setting Up Compliance Mode](#) for more information.

Why does the program say I don't belong to a V-Comp user group?

When the program runs in Compliance mode, it first checks to see that at least one of the required user groups is defined. Then it looks to see if the user logged into the computer is a member of one of those groups. If it doesn't find the logged-in user in one of the V-Comp user groups, it alerts the user to the problem and shuts the program down.

If this happens, it usually means one of two things.

The most common issue is that the computer user is not actually in one of the V-Comp user groups. Each computer user must be added to one of the V-Comp user groups to have access to the program when it is running in Compliance mode.

The second common issue relates to network access. If either the user groups or the user account are defined at the network level, the computer must have access to the network when the program starts up so that it can check the credentials of the user or user group. If it cannot access the network, it cannot verify the user group membership and it shuts down. Make sure the computer is connected to the network when the program starts up.

Getting started

Terminology

Let's clarify some terms that are used in this User Guide and in the software itself:

Spillover, Crossover

This term refers to the fluorescence from one signal that is detected by a secondary channel's detector. For example, if the FITC signal is detected in the PE detector, there is spillover from FITC into PE. The purpose of fluorescence compensation is to adjust the intensity values to account for the spillover of each signal into each detector.

Single-stained control file

A single-stained control file is an FCS data file collected from a tube that has only one fluorochrome added. Also known as single-color controls, you will need one for each of the fluorochromes in your panel when you create a V-Comp setup.

Unstained control file

This is an FCS data file from a tube that has no fluorochromes added to it. It may also be called a "no-stain" control or a "blank" control.

All-stained file

This is an FCS file that contains all of the fluorochromes used in a panel. The research and patient sample files that you acquire typically fall into this category. Some vendors refer to this as a "verify" file because it is used to verify the compensation settings.

Setup

A V-Comp setup defines the compensation for a panel. A setup is created and edited with the [V-Comp Setup Wizard](#). To create a setup, you need a set of single-stained controls and at least one unstained control. Once a setup is created, you can apply it to data files that you acquire with the same instrument settings.

Basic setup

A "basic" setup in V-Comp is probably the most common type of setup. It has one single-stained control for each of fluorochrome.

Label-specific setup

Sometimes the compensation required in a panel depends on the specific antibodies that are used. This is particularly true with some of the tandem dyes. In these cases, you can create a setup that contains several single-stained controls for the same fluorochrome. For example, suppose the CD3 PE-Cy7 conjugate you are

using has different emission characteristics than the CD19 PE-Cy7 conjugate. You can create a **Label-specific** setup that includes a single-stained control for both conjugates, and V-Comp will be able to compensate files with either one.

Spectral setup

Spectral cytometers such as Sony's SP6800 collect data in a fundamentally different way than traditional cytometers. These instruments have a set of detectors, each tuned to a different wavelength, and the values stored in the data file represent a "mixed" signal. Typically there are more detectors than fluorochromes for spectral systems. V-Comp can be used to "unmix" this type of data, creating a new file that can be analyzed with traditional flow cytometry analysis software.

What Files Do I Need?

1. Control Files

Each time you create a new compensation setup with V-Comp™ you will need a set of control files:

- One or more single-stained control file for each fluorochrome in your panel
- One or more unstained control file

These control files have to be run with the same instrument settings so that they have matching characteristics:

- Same number of parameters, in the same order
- Gain (\$PnG) for each compensation parameter must match across the set of files
- Range (\$PnR) for each compensation parameter must match across the set of files
- Log decades (\$PnE) for each compensation parameter must match across the set of files
- In Compliance mode, the voltage (\$PnV) for each compensation parameter must match across the set of files
- In Research mode, the voltage (\$PnV) for each compensation parameter must match its associated unstained control

The samples can be beads or cells, but use beads or cells for all controls.

2. All-color and "Verify" Files

When you prepare the single-color control tubes, you should also create an all-color (i.e. all-stained, "verify") tube containing all of the fluorochromes in your panel. The file for this tube is used to assess the V-Comp compensation that is created from the single-color controls.

3. Example

Suppose that you have a 5-color experiment that you want to compensate with V-Comp. The fluorochromes used are FITC, PE, PerCP, PE-Cy7, and APC. The control files that you would need for this experiment would be:

- FITC-only control
- PE-only control
- PerCP-only control

- PE-Cy7-only control
- APC-only control
- Unstained (or "Blank" or no-stain) control

The gain, range, log decades, and voltage for each detector has to match across all of the controls. So if the FITC detector has a voltage of 385 in the FITC-only control, it must also have a voltage of 385 in all other control files*. This is true for each detector that is selected for compensation.

If the one of the required characteristics doesn't match, V-Comp will alert you to the mismatch. The program will not proceed until the mismatch is corrected.

* In Research mode, the program has less restrictive requirements for matching.

Getting Started 1-2-3

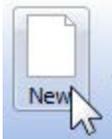
V-Comp™ is a quick and easy tool for automatically setting up perfect compensation for your FCS data files. Here's a quick start guide to using the program.

1. Start the V-Comp™ app

- If it's the first time you have run the software, you'll be asked to register it. Click [here](#) to see how.

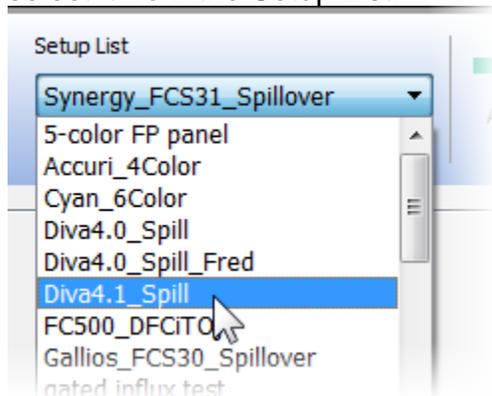
2. Create or select a V-Comp™ setup

- If you have never used the software before, the [V-Comp™ Setup Wizard](#) will launch automatically. Use the wizard to create a V-Comp™ compensation setup for one of your panels.
- Any time you want to create a new V-Comp™ setup, click the New button



to launch the [V-Comp™ Setup Wizard](#). This will guide you through the process of creating a setup from your single-color and unstained control files.

- If you have already created the V-Comp™ setup that you want to use, simply select it from the Setup List.



3. Apply the V-Comp™ setup to your files

With your V-Comp™ setup created and selected, you are ready to apply the setup to files acquired with the panel.

- Select the files you want to compensate with your new V-Comp™ setup. Drag and drop files onto the main window, or click the  button to navigate to your files.

- As files are added to the batch list, you should see a  symbol next to each one. This indicates that the files are compatible with the setup you have selected. If you see files with a  symbol, they are not compatible with the current setup and will not be processed.
- Click the  button to process the files in the list. The V-Comp™ app will export a new version of each one with the new compensation settings. Your original files are left untouched.

That's all there is to it!

See also:

[Example with 5-Color Panel](#)

[Example Label-Specific Setup](#)

[Example Spectral Setup](#)

[Workspace overview](#)

[V-Comp™ Setup Wizard](#)

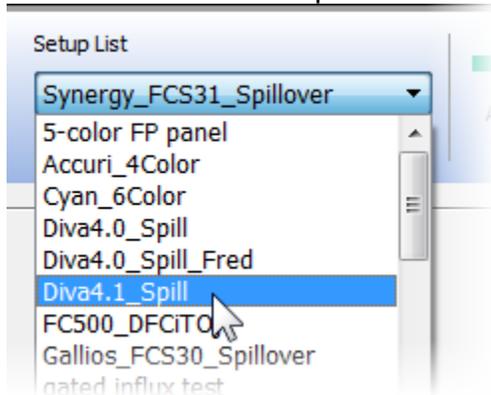
[Research and Compliance Modes](#)

Applying V-Comp Compensation

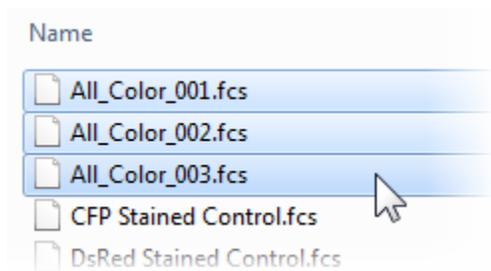
After you have created V-Comp™ setups with the wizard, you can use those setups to apply compensation settings to files acquired with the panel. This is probably the most common activity you will use V-Comp to do.

1. Processing files

- Start by selecting the V-Comp™ setup that you want to use. To do this, simply select it from the Setup List on the ribbon bar.



- Next, click the  button and in the Open dialog, navigate to the folder containing the files you want to compensate. Select the files you want and click Open.



- As files are added to the batch list, you should see a  symbol next to each one. This indicates that the files are compatible with the setup you have selected. If you see files with a  symbol, they are not compatible with the current setup and will not be processed.

"Compatible" files have the same **resolutions**, **gains**, number of **decades**, and **voltages** as the control files used to create the setup. If the files you select are not compatible with the setup, you will need to create a new setup or edit the existing setup with new control files.

- Click the  button to process the files in the list. The V-Comp™ app will export a new version of each one with the new compensation settings. Your original files are left untouched. [A report](#) of the export process is automatically created and stored in the Reports folder.

The files that the V-Comp™ app creates contain a new \$SPILLOVER keyword which defines the new compensation. Most software packages can read these files and interpret the new compensation settings, so you can perform your data analysis with your favorite software tools.

2. Special features

Using "Blank" to turn off compensation

Suppose that we have a 6-color setup that is designed for acute myeloid leukemia (AML) analysis. The fluorochromes in the panel are FITC, PE, PerCP, PE-Cy7, APC, and APC-H7.

- One tube uses all 6 colors: CD3 FITC, CD14 PE, HLADr PerCP, CD19 PE-Cy7, CD123 APC, and CD45 APC-H7.
- A second tube uses only 5 of the fluorochromes: CD38 FITC, CD10 PE, **Unused PerCP**, CD19 PE-Cy7, CD34 APC, and CD45 APC-H7.

Can we create *one* V-Comp setup that will work for both of these variants of the panels? Yes, we certainly can. However, to make this work correctly, you have to use special naming for the **Unused PerCP** parameter in the second tube. We want V-Comp to create a spill keyword that effectively turns off the PerCP spillover for files acquired from the second tube.

To accomplish this, you need to make the value of the \$PnS keyword for the PerCP parameter "**Blank**" in each of the files acquired from the second tube. So, for example, if PerCP is the 8th parameter in the file, the value of **\$P8S** would be set to "Blank" to turn it off. When V-Comp sees "Blank" (without quotation marks) as the \$PnS keyword value for a parameter, it sets the spillover values to "0.00" for that parameter spilling into others.

Signal	into CD38 FITC-A	into CD10 PE-A	into Blank PerCP-A	into CD19 PE-Cy7-A	into CD34 APC-A	into CD45 APC-H7-A
CD38 FITC-A	1.000000	0.190194	0.023139	0.003996	0.000000	0.000438
CD10 PE-A	0.010030	1.000000	0.178506	0.008773	0.001012	0.000000
Blank PerCP-A	0.000000	0.000000	0.000000	1.000000	0.000000	0.000000
CD19 PE-Cy7-A	0.000181	0.000000	1.000000	0.119471	0.006799	0.028590
CD34 APC-A	0.007502	0.006780	0.030677	0.000704	1.000000	0.092397
CD45 APC-H7-A	0.000000	0.000000	0.000000	0.014146	0.017705	1.000000

This technique of using "Blank" for the \$PnS keyword can be used to turn off spillover values for any number of parameters.

Using a Reference File

V-Comp has a great tool to allow you to compare its spillover matrix with another one from your instrument. Here's how it works.

1. Choose a Reference File

- Create a new setup or edit an existing setup using the V-Comp Setup Wizard.
- On the Options page of the wizard, locate the "Reference File" field near the bottom.

Other Options:

Drift Correction:

Reference File: 

Note: this option will only appear for Basic setups. It is not available for Label-Specific and Spectral setups.

- Click the folder icon to the right to display a file dialog box.
- Select an all-stained or "verify" FCS file that was acquired with the same settings as the control files and close the file dialog.

Other Options:

Drift Correction:

Reference File: 

- Click the Finish button to complete the wizard.

When you have a reference file selected and finish the wizard, V-Comp performs additional steps. It reads the reference FCS file and extracts its spillover matrix from its keywords. It then performs a comparison of the reference matrix with the V-Comp matrix, and it writes the comparison into a text file in the Setups folder.

2. Review the Comparison

The file created with the comparison information has the same name as the setup, with "_RefComparison.txt" appended to it. So if the setup is named "Demo Setup", the reference comparison file will be named "Demo Setup_RefComparison.txt". The file is best viewed in a spreadsheet program, like Excel.

- Locate the comparison text file in the Setups folder.

- Use the *Open Setups Folder* command in the *Tools* button menu.
- Open the comparison text file with a spreadsheet program.

The screenshot shows a spreadsheet application window titled "Demo Setup_RefComparison.txt". The spreadsheet contains a table with 11 rows and 7 columns. The columns are labeled A through F. The data is as follows:

	A	B	C	D	E	F
1	Demo Setup Ref Comparison.txt					
2	Rank	Signal	Detector	Difference	VComp Value	Reference Value
3	1	HcRed-A	CFP-A	0.0371208	0.154679	0.1918
4	2	YFP-A	GFP-A	0.00728281	0.597517	0.6048
5	3	HcRed-A	GFP-A	0.00702102	0.012221	0.0052
6	4	GFP-A	YFP-A	0.00671741	0.374717	0.368
7	5	HcRed-A	DsRed-A	0.00449405	0.347306	0.3518
8	6	GFP-A	DsRed-A	0.00384459	0.0891446	0.0853
9	7	YFP-A	DsRed-A	0.00341841	0.297118	0.2937
10	8	HcRed-A	YFP-A	0.00209077	0.0107908	0.0087
11	9	DsRed-A	CFP-A	0.000919623	0.000919623	0

The file contains fields for Rank, Signal, Detector, Difference, VComp Value, and Reference Value. Rows are ordered from the highest difference to the lowest difference.

In the example show, the HcRed-A signal crossing over into the CFP-A detector has the highest difference: a value of 3.7%. The V-Comp value was ~15% and the reference value was ~19%. Note in this example that 3 of the top 5 differences relate to the HcRed signal. This comparison lets you quickly assess the differences between V-Comp and your acquisition software. Your next step might be to use your analysis software to open the all-color file and the V-Comp exported version of the same file.

Example with 5-Color Panel

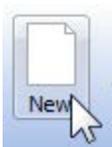
Here's a quick introduction with a set of demo files that will get you up and running.

1. Start the V-Comp™ app

- If it is the first time you have run the software, you will be asked to register it. Click [here](#) to see how.

2. Create a new V-Comp™ setup

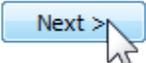
- If you have never used the software before, the [V-Comp™ Setup Wizard](#) will launch automatically. If you don't see the wizard dialog, click the New button to create a new setup.



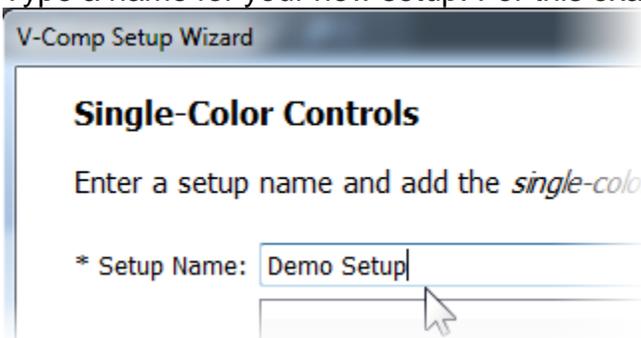
- On the Welcome page, select **Basic** for the setup type.



A **Basic** setup uses one single-stained control file for each of the fluorescence markers in the panel, which is the case for this demo. A **Label-specific** setup can have more than one single-stained file for the fluorescence markers, and a **Spectral** setup is used for unmixing data collected on a spectral cytometer.

- After selecting the setup type, click  to move to the next pane: [Single Color Controls](#).

- Type a name for your new setup. For this example, type "Demo Setup".

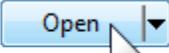


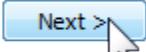
- Next we need to select the single-color control files. For this example, click the

 button and navigate to the location on your computer where the V-Comp™ app is installed. On the Mac, that's usually in the "Applications/V-Comp for Mac/Samples" folder. On the PC, look in "Program Files (x86)\Verity Software House\V-Comp for Window\Samples".

- Select the 5 single-color controls in the Samples folder as shown below. For this example, these are fluorescent protein controls.

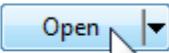
Name	Date
All_Color_001.fcs	10/6/2
All_Color_002.fcs	10/3/2
All_Color_003.fcs	10/3/2
CFP Stained Control.fcs	10/3/2
DsRed Stained Control.fcs	10/3/2
GFP Stained Control.fcs	10/3/2
HcRed Stained Control.fcs	10/3/2
Unstained Control.fcs	10/3/2
YFP Stained Control.fcs	10/3/2

- Click  to add the single-color control files. The order of the files does not matter. You can also drag-and-drop files onto the Single-Color Controls list to add them.

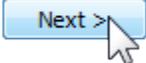
- Click  to move to the [Unstained Controls](#) pane. On this pane, you can drag and drop your unstained control file onto the wizard or click the folder icon to select one. You can set a different unstained for each file, but that's not usually needed.

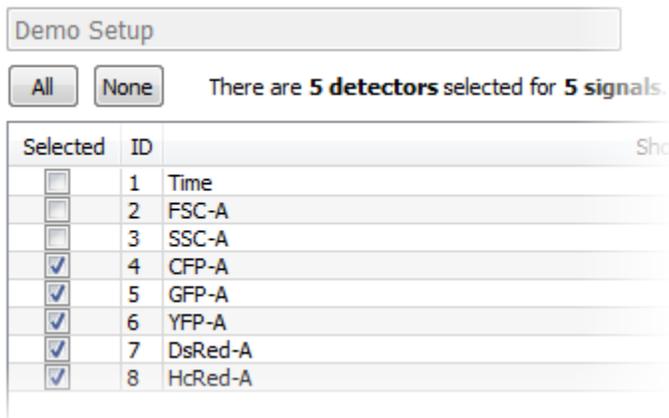
- Click  and select the file named "Unstained Control.fcs".

GFP Stained Control.fcs	10/
HcRed Stained Control.fcs	10/
Unstained Control.fcs	10/
YFP Stained Control.fcs	10/

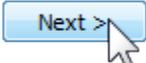
- Click  to add the file. Notice that the file is associated with all of the single-color controls. In working with your own files, you can specify a separate

unstained for any of the single-color files. For this example, we only need the one unstained file.

- Click  to move to the [Detectors](#) pane. The program will try to figure out which detectors you want to use for compensation automatically.

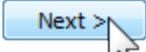


- In our demo, the correct detectors will be selected, but always review the selections and make sure the correct detectors are selected. These are the parameters that will be compensated.

- Click  to move to the [Match Controls with Detectors](#) pane. The program will assign a control file to the detector that was brightest for that control. Most of the time, this is correct, and it is correct for our demo files.

Detector ID	Name	Relative Brightness
4	CFP-A	
	CFP Stained Control.fcs	14502
5	GFP-A	
	GFP Stained Control.fcs	44630
6	YFP-A	
	YFP Stained Control.fcs	32941
7	DsRed-A	
	DsRed Stained Control.fcs	23213
8	HcRed-A	
	HcRed Stained Control.fcs	3424

- If you need to change assignments, drag the single-color controls to the correct detector in the list.

- Click  to move to the [Options](#) pane. On this page, you can edit the setup name and description and select several other options for the setup.

- Set the **Export Option** to "Export files to the Export Path". This means we want to use a specific location for the files we export.

Note: make sure to select the correct option here for this example. Choose the "Export files to the Export Path" option so that you can select a writable folder on your computer.

- Click the  icon to the right of **Export Path** and select a folder for which you have write-permissions.

Note: you must be able to create and write files in the path you select here.

- Set the other options as shown.

* Setup Name:

Setup Description:

* Control Type:

Output Options:

Export Option:

Export Path:

File Prefix:

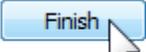
Overwriting:

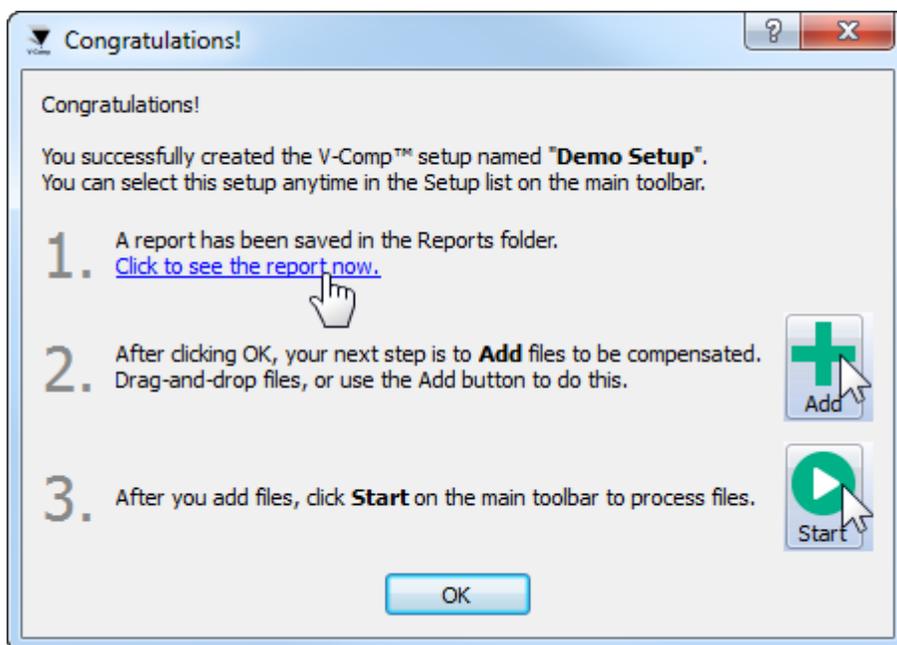
Note: '_vcomp' will be appended to all export file names

Other Options:

Drift Correction:

Reference File:

- Click  to complete the wizard. The wizard will analyze your control files and create a V-Comp™ compensation setup. You should see a Congratulations dialog when it finishes.

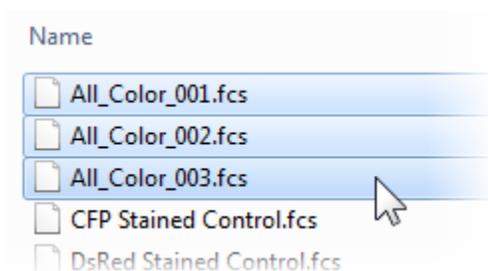


- Click the link to see the report and the program will show a [detailed report about the V-Comp™ compensation setup](#) you just created. This report is automatically stored in the Reports folder. Review the report and the on-screen instructions.
- Click OK to close the dialog. You have successfully created a V-Comp™ compensation setup from a set of control files.

3. Apply the V-Comp™ setup to your files

When you create a new V-Comp™ setup with the wizard, the new setup is automatically selected and you are ready to apply it to files acquired with the panel.

- Click the  button and in the Open dialog, navigate to the same folder where the control files for this example were located. Select the "All Color" files in the folder and click Open.



- As files are added to the batch list, you should see a  symbol next to each one. This indicates that the files are compatible with the setup you have selected. If you see files with a  symbol, they are not compatible with the current setup and will not be processed.

- Click the  button to process the files in the list. The V-Comp™ app will export a new version of each one with the new compensation settings. Your original files are left untouched. [A report](#) of the export process is automatically created and stored in the Reports folder.

The files that the V-Comp™ app creates contain a new \$SPILLOVER keyword which defines the new compensation. Most software packages can read these files and interpret the new compensation settings, so you can perform your data analysis with your favorite software tools.

Summary

To create a new V-Comp™ compensation setup, all you need is a set of single-color controls and one unstained control file created with the instrument settings for your panel. The unstained control needs to be the same particles (cells, beads, etc) as the single-stained controls. Use the [V-Comp™ Setup Wizard](#) to create a new setup. Once a setup is created, it can be reused with any data files that were acquired on the same instrument with the same fluorochromes and the same settings.

To apply V-Comp™ compensation to your all-color FCS files, first select the V-Comp setup, then add your all-color files to the batch list and click the  button. New files will be created with the new compensation keyword, ready to be analyzed with your favorite analysis tools.

Example Label-Specific Setup

Sometimes the compensation required in a panel depends on the specific antibodies that are used. This is particularly true with some of the tandem dyes. In these cases, you can create a setup that contains several single-stained controls for the same fluorochrome. For example, suppose the CD3 PE-Cy7 conjugate you are using has different emission characteristics than the CD19 PE-Cy7 conjugate. You can create a **Label-specific** setup that includes a single-stained control for both conjugates, and V-Comp will be able to compensate files with either one.

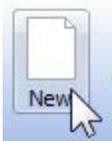
Please note, data files are not provided for this example.

These are the steps that you will use to create a label-specific setup.

1. Start the V-Comp™ app

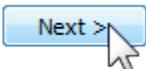
2. Create a new V-Comp™ setup

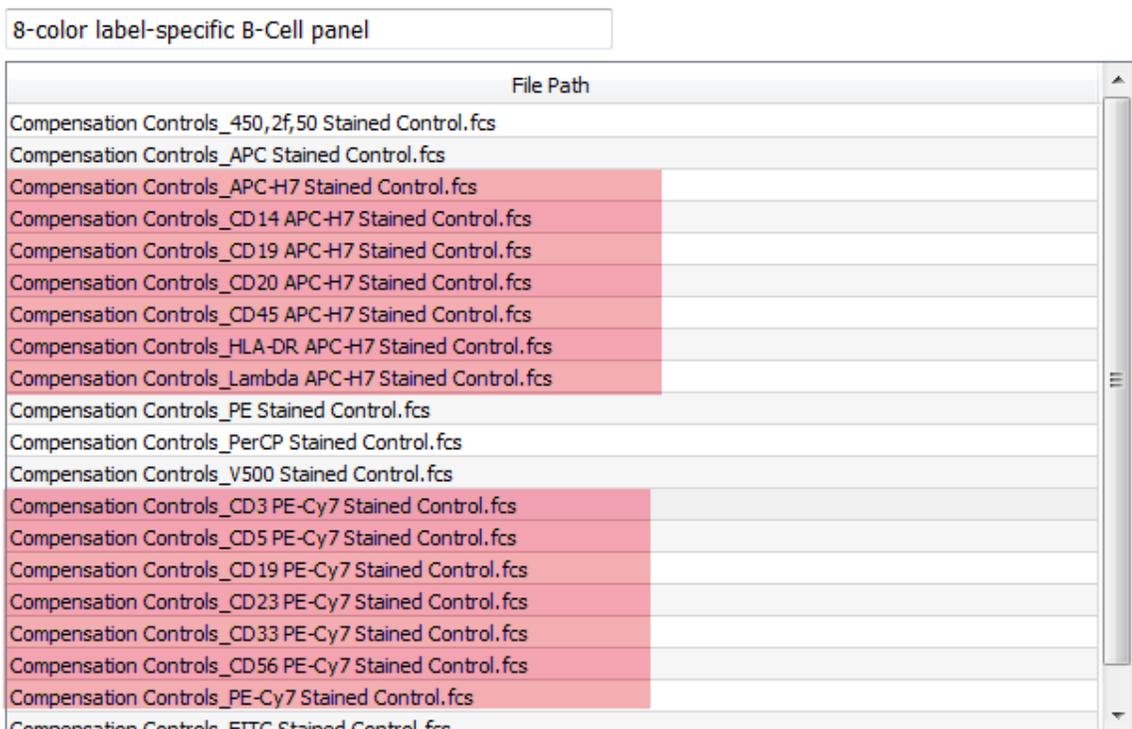
- Click the New button to create a new setup.



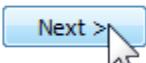
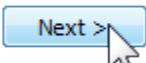
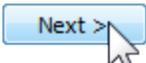
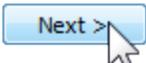
- On the Welcome page, select **Label-specific** for the setup type.



- Click  to move to the next pane: [Single Color Controls](#).
- Type a name for your new setup, and then add *all* of the single-stained controls. Typically, you will have more than one single-stained controls *only* for tandem dyes.
In the example below, we have highlighted the 7 files that are for APC-H7 and the 7 files that are for PE-Cy7 variants.



There are **20** single color controls.

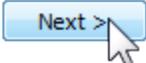
-  Click  to move to the [Unstained Controls](#) pane. Select the unstained control file (or files) to use for this setup.
-  Click  to move to the [Detectors](#) pane. The program will try to figure out which detectors you want to use for compensation automatically.

8-color label-specific B-Cell panel

All None **There are 8 detectors selected for 20 controls.**

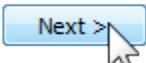
Selected	ID	Short Names	Long Names
<input type="checkbox"/>	1	FSC-A	
<input type="checkbox"/>	2	FSC-H	
<input type="checkbox"/>	3	FSC-W	
<input type="checkbox"/>	4	SSC-A	
<input checked="" type="checkbox"/>	5	FITC-A	
<input checked="" type="checkbox"/>	6	PE-A	
<input checked="" type="checkbox"/>	7	PerCP-A	
<input checked="" type="checkbox"/>	8	PE-Cy7-A	CD3; CD5; CD19; CD23; CD33; CD56
<input checked="" type="checkbox"/>	9	APC-A	
<input checked="" type="checkbox"/>	10	APC-H7-A	CD14; CD19; CD20; CD45; HLA-DR; Lambda
<input checked="" type="checkbox"/>	11	450/50-A	
<input checked="" type="checkbox"/>	12	V500-A	
<input type="checkbox"/>	13	Time	

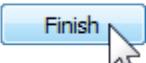
In this example, notice that we have fewer detectors selected (8) than we have single-color controls (20). We also see the different label-specific variant names for the tandem dyes.

- Click  to move to the [Match Controls with Detectors](#) pane. The program will assign a control file to the detector that was brightest for that control. If the automatic assignment is not correct, you can drag a control file to a different detector in the list to fix the mistake.

Detector ID	Name	Relative Bright
5	FITC-A	
	Compensation Controls_FITC Stained Control.fcs	10625
6	PE-A	
	Compensation Controls_PE Stained Control.fcs	82954
7	PerCP-A	
	Compensation Controls_PerCP Stained Control.fcs	29965
8	PE-Cy7-A CD3; CD5; CD19; CD23; CD33; CD56	
	Compensation Controls_CD3 PE-Cy7 Stained Control.fcs	110293
	Compensation Controls_CD5 PE-Cy7 Stained Control.fcs	34148
	Compensation Controls_CD19 PE-Cy7 Stained Control.fcs	69916
	Compensation Controls_CD23 PE-Cy7 Stained Control.fcs	51537
	Compensation Controls_CD33 PE-Cy7 Stained Control.fcs	76077
	Compensation Controls_CD56 PE-Cy7 Stained Control.fcs	85515
	Compensation Controls_PE-Cy7 Stained Control.fcs	61034
9	APC-A	
	Compensation Controls_APC Stained Control.fcs	56076
10	APC-H7-A CD14; CD19; CD20; CD45; HLA-DR; Lambda	

- Make sure that each detector has at least one control associated with it.

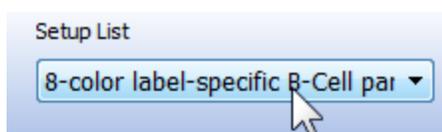
- Click  to move to the [Options](#) pane. Choose the output options you want

- Click  to complete the wizard. The wizard will analyze all of the control files and create a super V-Comp™ compensation setup. The setup is now ready to be used for creating compensation in your all-color files.

3. Apply the V-Comp™ setup to your files

Now you are ready to apply the setup to files acquired with the panel.

- Start by selecting the setup in the Setup List drop-down on the main ribbon bar.



- Next, click the  button and in the Open dialog, navigate to the same folder where the control files for this example were located. Select the "All Color" files in the folder and click Open.
- Click the  button to process the files in the list. The V-Comp™ app will export a new version of each one with the new compensation settings.

The files that the V-Comp™ app creates contain a new \$SPILLOVER keyword which defines the new compensation. Most software packages can read these files and interpret the new compensation settings, so you can perform your data analysis with your favorite software tools.

Summary

A **Label-specific** setup is just like a **Basic** setup, except that it allows more than one single-color control file to be associated with a detector. Label-specific setups allow you to create one setup that easily handles different combinations of antibodies in a single panel.

Once created, you can use a Label-specific setup just like a Basic setup. Simply select the setup, add files to the batch, and press the Start button.

Example Spectral Setup

Spectral cytometers such as Sony's SP6800 collect data in a fundamentally different way than traditional cytometers. These instruments have a set of detectors, each tuned to a different wavelength, and the values stored in the data file represent a "mixed" signal - the spectra of each event. Typically there are more detectors than fluorochromes for spectral systems, and most flow cytometry data analysis software cannot read and analyze spectral data. However, V-Comp can be used to "unmix" this type of data, creating new files that can be analyzed with your favorite flow cytometry analysis software.

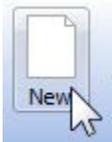
Please note, data files are not provided for this example.

These are the steps that you will use to create a spectral setup.

1. Start the V-Comp™ app

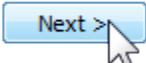
2. Create a new V-Comp™ setup

- Click the New button to create a new setup.

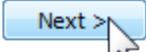


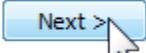
- On the Welcome page, select **Spectral** for the setup type.



- Click  to move to the next pane: [Single Color Controls](#).
- Type a name for your new setup, and then add of the single-stained controls. In this example, we have a 6-color panel, so there are 6 single-color controls.

6-Color Spectral	File Path
	CD8-ECD.LMD
	CD19-PC7.LMD
	CD45-FITC.LMD
	CD45-PC5.LMD
	CD3-PC5.LMD
	CD4-PE.LMD

- Click  to move to the [Unstained Controls](#) pane. Select the unstained control file (or files) to use for this setup.

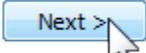
- Click  to move to the [Detectors](#) pane. The program will try to figure out which detectors you want to use for compensation automatically. You will probably have to make adjustments to select the correct fluorescence detectors. Only select detectors that relate to fluorescence in this pane.

6-Color Spectral

There are **32 detectors** selected for **6 controls**.

Selected	ID	Short Names
<input checked="" type="checkbox"/>	1	ch1
<input checked="" type="checkbox"/>	2	ch2
<input checked="" type="checkbox"/>	3	ch3
<input checked="" type="checkbox"/>	4	ch4
<input checked="" type="checkbox"/>	5	ch5
<input checked="" type="checkbox"/>	6	ch6
<input checked="" type="checkbox"/>	7	ch7
<input checked="" type="checkbox"/>	8	ch8
<input checked="" type="checkbox"/>	9	ch9
<input checked="" type="checkbox"/>	10	ch10
<input type="checkbox"/>

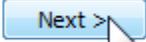
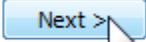
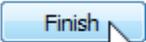
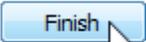
In this example, notice that we have many more detectors selected (32) than we have single-color controls (6).

- Click  to move to the [Signal Names Page](#) pane. Use this page to give meaningful names to the fluorochromes and targets in your panel. You can use the  and  arrows to re-order the parameters as they will appear in the unmixed output files.

6-Color Spectral

Single Color Control File	Fluor	Target
CD8-ECD.LMD	ECD	CD8
CD19-PC7.LMD	PC7	CD19
CD45-FITC.LMD	FITC	CD45
CD45-PC5.LMD	PC5	CD8
CD3-PC5.LMD	APC	CD3
CD4-PE.LMD	PE	CD4




- 
 Click  to move to the [Options](#) pane. Choose the output options you want
- 
 Click  to complete the wizard. The wizard will analyze all of the control files and create a V-Comp™ compensation setup. The setup is now ready to be used for creating compensation in your all-color files.

3. Apply the V-Comp™ setup to your files

Now you are ready to apply the setup to files acquired with the panel.

- Start by selecting the setup in the Setup List drop-down on the main ribbon bar.
- Next, click the  button and in the Open dialog, navigate to the same folder where the control files for this example were located. Select the "All Color" files in the folder and click Open.
- Click the  button to process the files in the list. The V-Comp™ app will export a new version of each one with the new compensation settings.

The files that the V-Comp™ app creates will contain "unmixed" or "compensated" data values. Most software packages can read these files so you can perform your data analysis with your favorite software tools.

Summary

A **Spectral** setup can be used to "unmix" FCS data acquired on spectral flow cytometers.

Once created, you can use a Spectral setup just like any other V-Comp setup. Simply select the setup, add files to the batch, and press the Start button.

Exploring the workspace

Workspace overview

The V-Comp™ workspace consists of a ribbon bar and a batch list. The ribbon bar contains the actions that you perform with V-Comp™, and the batch list is where you add all-color files that you want to process through V-Comp™.

Ribbon bar

What you see on the Ribbon bar will depend on whether you are using the program in "Compliance mode" or not. In Compliance mode, the user group to which you belong will determine what commands you see. In Research mode, all commands on the Ribbon bar will be displayed.

The left portion of the ribbon bar allows you to create, edit, and select compensation setups for different panels and instruments, if you have permissions to do so.

The center portion of the ribbon is used to add and remove files from the batch list, and to process the files in the batch list.

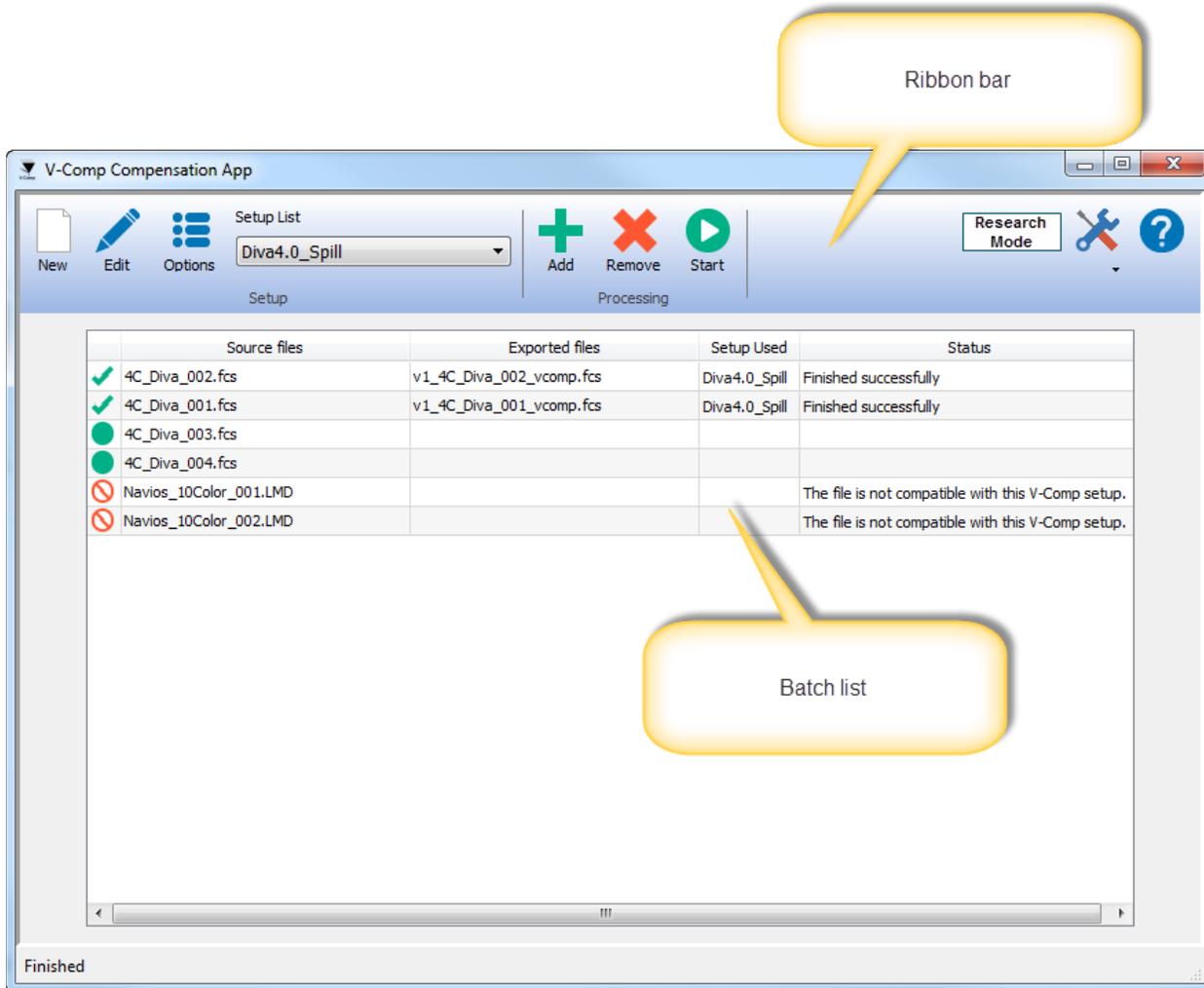
See [Ribbon bar commands](#) for additional details.

Batch list

You can use the Add button or drag-and-drop to add files to the batch list. This is where you put files to which you want to apply V-Comp™ compensation. Once files are added to the batch list, you can press the Start button to apply the current V-Comp™ setup.

The left column of the batch shows the status of the file:

-  The file is ready to be processed.
-  The file has been processed successfully.
-  The file is not ready to export. It is either not compatible with the current setup or can't be read.
-  The file failed to export. This usually means that the output file could not be created.



Finding File Locations

There is an easy way to find out where a file in a list is located. Mouse over the file name to see the full path to the file:

	Source files	Exported files	Setup Used
✓	All_Color_001.fcs	All_Color_001_vcomp.fcs	5-color FP
✓	All_Color_002.fcs	All_Color_002_vcomp.fcs	5-color FP
✓	All_Color_003.fcs	All_Color_003_vcomp.fcs	5-color FP

A tooltip is visible over the first row, displaying the full path: C:\NewDevelopmentSystem\V-Comp\Samples>All_Color_001.fcs

And if you would like to go that the folder, right-click the file name and choose **Open file location** from the menu.

	Source files	Exported files	Setup Used	Status
✓	All_Color_001.fcs	All_Color_001_vcomp.fcs	5-color FP	Finished successfully
✓	All_Color_002.fcs	All_Color_002_vcomp.fcs	5-color FP	Finished successfully
✓	All_Color_003.fcs	All_Color_003_vcomp.fcs	5-color FP par	Finished successfully

A context menu is visible over the first row, with the option "Open file location" selected.

Ribbon bar commands

The ribbon bar appears at the top of the workspace and contains command buttons that control the app. The bar is divided into several groups of related controls: Setup, Processing, and tools. This is what the bar looks like when running in research-use only mode.



If your lab runs V-Comp in "Compliance mode", the ribbon bar will show the Compliance icon on the right side.



If you are running in "Compliance mode" and your user group is "VCompUsers", you will see fewer options on the bar.



See [Research and Compliance Modes](#) for additional details about Compliance mode and user groups.

Setup

The Setup group is the left portion of the ribbon. It allows you to create, edit, and select compensation setups for different panels and instruments. You will want to create a setup for each panel and for different instruments.



New

Creates a new setup using the [V-Comp™ Setup Wizard](#). The wizard will guide you through the process of creating the setup.

Users in the VCompUsers group will not see this option.



Edit

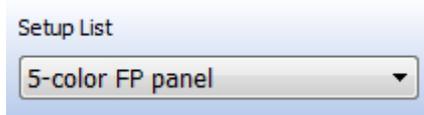
Edits the current setup using the [V-Comp™ Setup Wizard](#). You can make changes to an existing V-Comp setup, selecting different controls and output options.

Users in the VCompUsers group will not see this option.



Displays the [Edit Setup Options](#) dialog to edit options such as where output files are written.

Users in the VCompUsers group will not see this option.



Displays the current V-Comp setup. To select a different setup that has already been created, click the control and pick the setup from the list.

Processing

The center portion of the ribbon is used to add and remove files from the batch list, and to process the files in the batch list.



Adds files to the batch list. This command displays the Open dialog to select files. You can also drag-and-drop files into the batch list.



Remove

Removes the selected files from the batch list. If no files are selected, all files are removed.

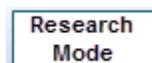


Start

Starts processing the files in the batch list with the current V-Comp™ setup. Files that are flagged as "Ready" in the batch list are processed.

Other Buttons

Several buttons are grouped on the right side of the ribbon bar.



or



Displays the current mode. Click the button to display more information. "Research Mode" means the program is operating in research-use only mode. "Compliance Mode" means the program is operating in a mode to support 20 CFR Part 11. See [Research and Compliance Modes](#) for additional details.



Tools

Opens the Tools menu to display additional commands.



User
Guide

Displays this User Guide in your browser.

Tools menu

The tools button expands to show a set of tools. Click the button to see the tools that are available.



Report a
Problem

Begins a new email message to Verity Software House tech support and displays the message for you to add comments before sending.



Application
Info

Displays the application info dialog, with information about your computer and the version of V-Comp™ you are running.



Open Setups
Folder

Opens the Setups folder on your computer. This is where V-Comp™ stores all of the setups that you create. You can copy files from this folder to other computers with V-Comp™ installed to share setups across computers. Setups can be deleted from this folder when they are no longer needed.



Open
Reports
Folder

Opens the Reports folder on your computer. V-Comp™ stores two types of reports in this folder: setup reports and export reports. Setup reports are generated each time you create or edit a V-Comp™ setup using the [V-Comp™ Setup wizard](#). Export reports are create whenever you click the Start button to process a batch of files. Reports are stored in PDF format and are named automatically based on the setup name and the date and time the report was created. Reports can be deleted from this folder when they are no longer needed.



Configuration

Displays a dialog to set the location for V-Comp setups, reports, and logs. This command only available to Compliance Mode users in the VCompAdmins group.



Logging

Displays the [Logging dialog](#). This dialog allows you to turn logging on and off, view the log, and change how large the log can get.



Check for

Checks www.vsh.com to see if any updates for the program are available. If updates are found, you can choose whether or not to download and install them.

Updates

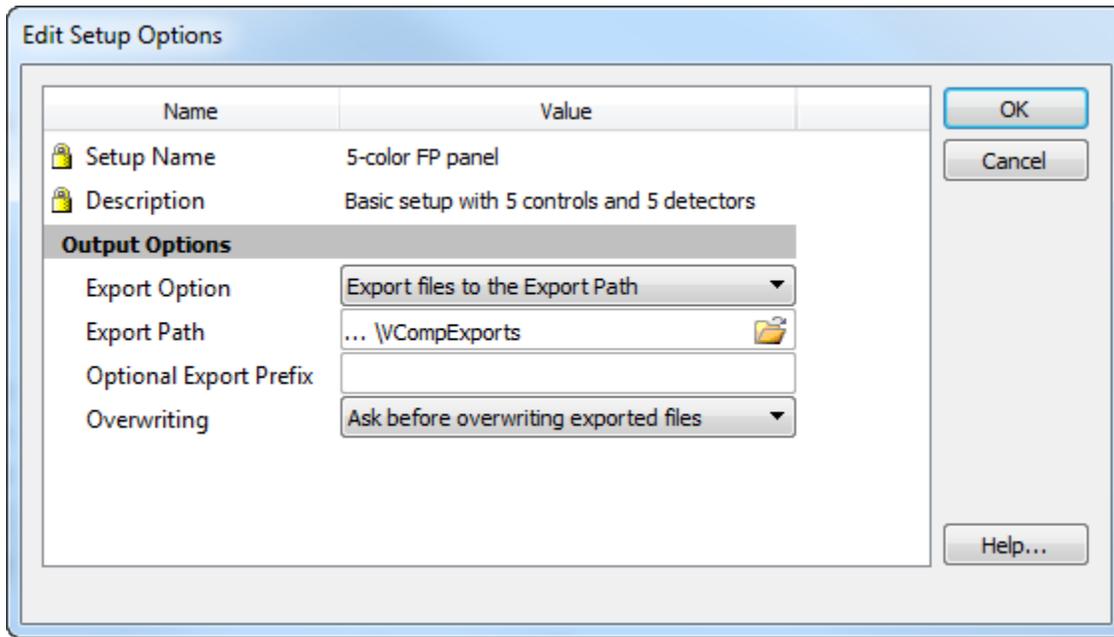


About

Displays the About dialog. This dialog shows program version and registration information.

Options

This dialog is displayed when the Options button is clicked on the ribbon bar. It allows some of the output options of the current V-Comp™ setup to be edited. The options in this dialog can be edited without requiring the program to re-compute the spillover.



Setup Name:

This displays the name of this setup from the Setup Wizard..

Setup Description:

This displays the description entered in the Setup Wizard.

Export Option:

When files are processed to apply V-Comp™ compensation, the new files can either be created in the same folder as the original file or into a user-specified export path. This option determines which location will be used. When the option is set for "Export files to the Export Path", the program will use the path specified in the next field of the dialog: Export Path.

Export Path:

This option is enabled when the Export Option is set for "Export files to the Export Path". Click the path editor to choose a path for the export.

Optional Export Prefix:

When files are processed and exported, they preserve the original file name and "_vcomp" is appended to that name. In addition, you can specify a prefix for the export file names. This will be added to the beginning of the exported file name.

Overwriting:

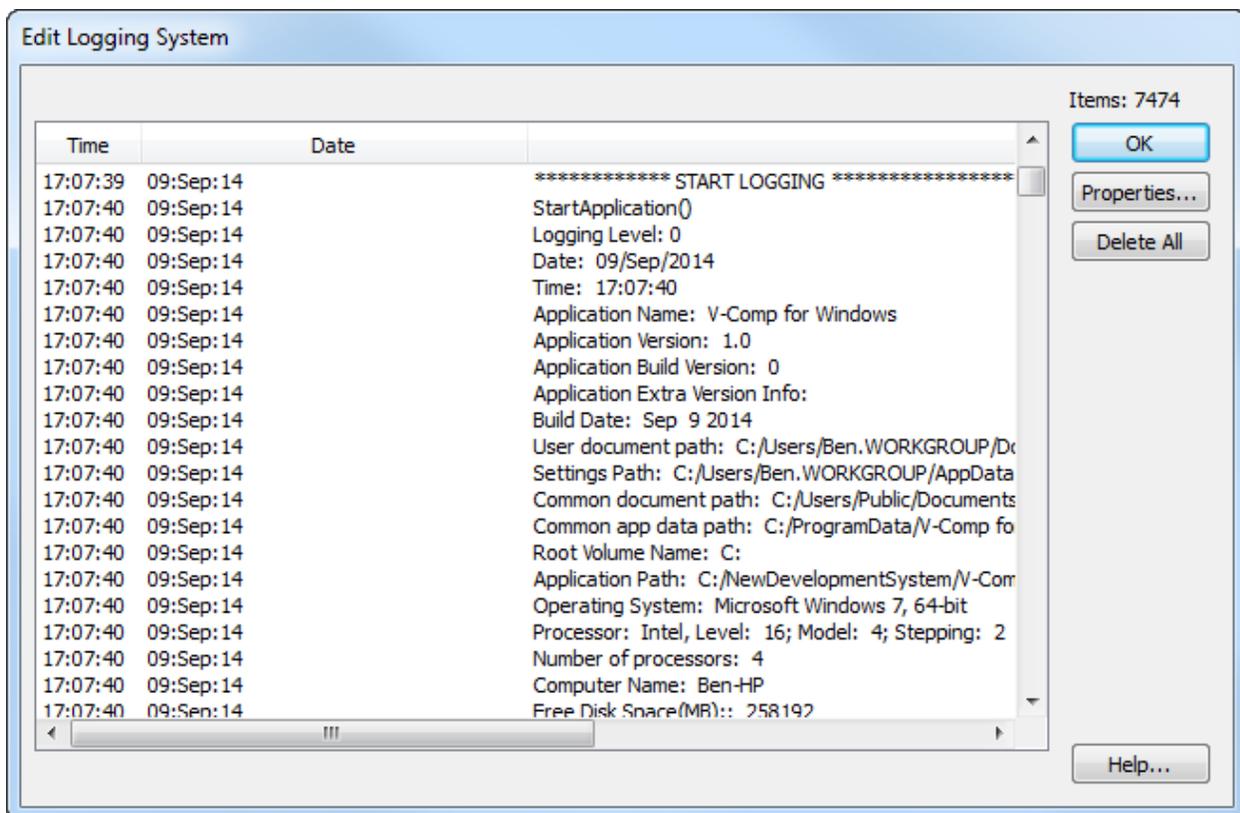
If there is already a file with the same export name in the export path, the program can ask whether you want to overwrite the file, or it can overwrite the file automatically. Choose the option that best fits your requirements.

Logging

This dialog is accessed from the Tools button menu.

The V-Comp™ application can create a log of the actions you perform with it, as well as other useful information. The log is a valuable record of the program's activity and provides an audit trail for QC/QA purposes.

The logging dialog displays entries in the current log file, with the most recent activity at the end of the log. In Compliance mode, the log file is stored in a location set by the VCompAdmins; by default this is in the V-Comp folder under the shared users (public, all users) folder. In Research mode, the log file is stored in the V-Comp folder of the user's documents folder.

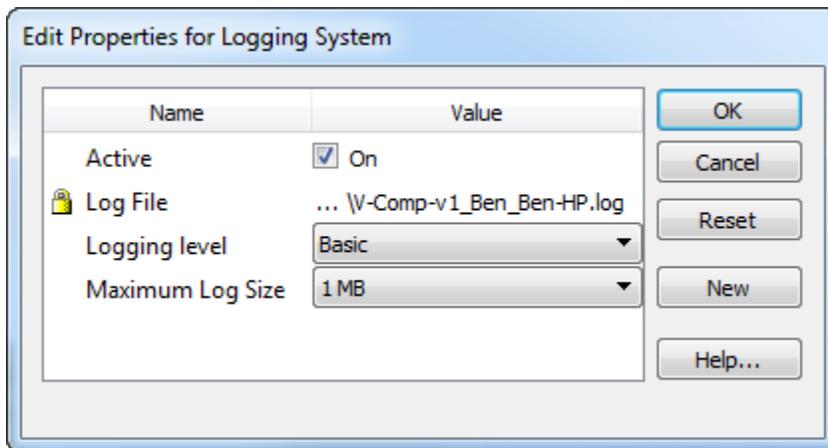


The [Logging Properties](#) dialog is used to turn logging on or off and to set the size limit for the log file.

Logging Properties

This dialog is accessed from the Logging dialog, which is displayed in the Tools button menu.

Logging properties determine whether the log is active, the type of logging to perform, and how large the log file is allowed to grow.



Active:

Check this property to turn logging on, or clear the check to turn logging off. In Compliance mode, logging is *always* active and cannot be turned off.

Log File:

The field shows the path to the log file. Hover the mouse over the field to see the full path. You can also right-click the path to locate the file.

Logging Level:

Typically, the logging level should be set to Basic. This provides a detailed log of user and program activities. In some case, tech support may ask you to put the log into Comprehensive mode, which provides additional detail that can be used to help debug problems.

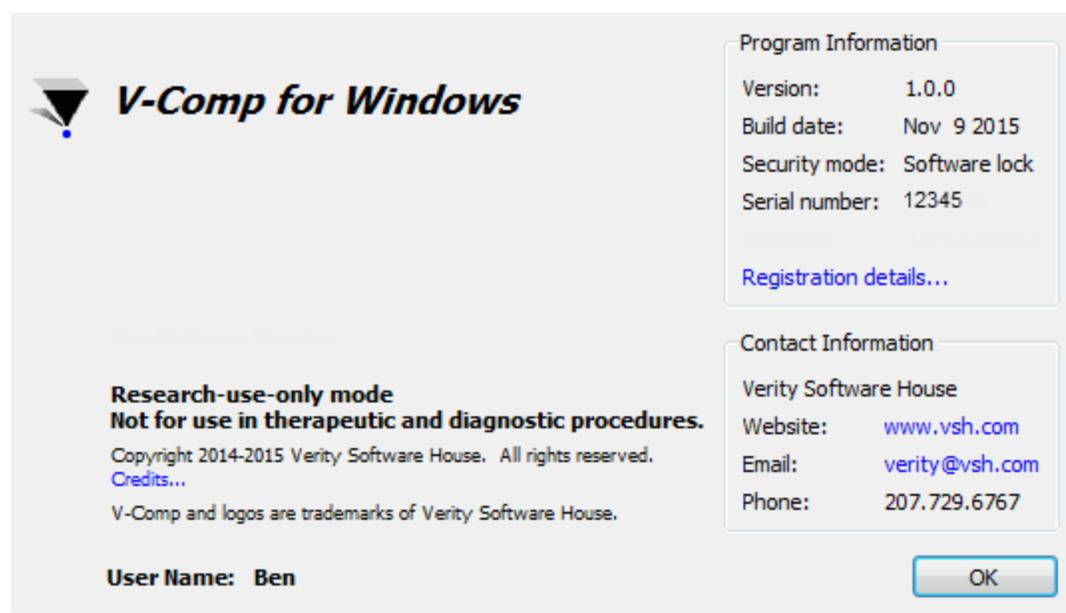
Maximum Log Size:

If you set a maximum log file size, the V-Comp™ application will create a new log file whenever the limit is reached. The previous log file will be renamed with a number appended to its name. If this property is set to "Unlimited", the log file is allowed to grow without restriction. We recommend using the 1MB log size.

About

When the program starts up, the About dialog is displayed with information about the program version, registration, and contact information. The user name is shown at the bottom of the dialog.

The mode of program operation is shown: either **Compliance mode enabled** or **For research Use Only**. If the program is running in Compliance mode, the user group for the current user is also displayed.



V-Comp Setup Wizard

V-Comp™ Setup Wizard

Note: If your institution is using V-Comp in [Compliance-mode](#), your V-Comp user group determines whether you have permission to access V-Comp Setup Wizard.

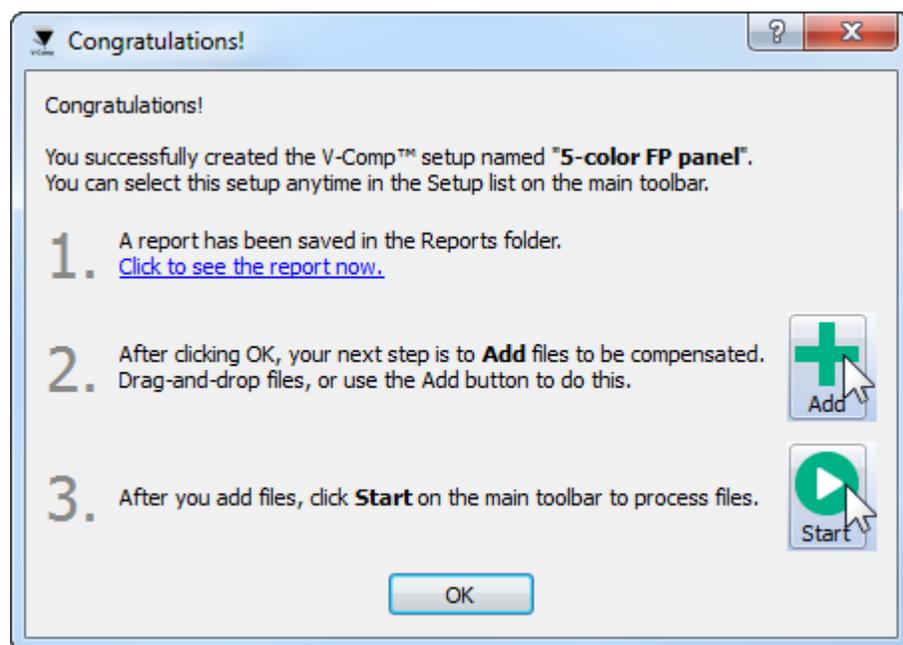
This wizard is used to create and edit V-Comp™ setups. Each setup defines a V-Comp™ compensation based on single-color and unstained controls. You will typically create a V-Comp™ setup for each panel and each instrument, and edit the setup when setting change on the instrument or reagents change in the panel.

The wizard is displayed when the [New](#) or [Edit](#) button is clicked on the ribbon bar. It is also displayed automatically when the program starts up if there are no setups defined.

Move through the wizard by filling in the required information on each page and clicking Next to move to the next page.

When you complete the wizard by clicking the Finish button, the V-Comp™ wizard will read and analyze the control files you selected and generate a V-Comp™ compensation that can then be used to apply V-Comp™ compensation to FCS files that match the setup you have created.

After the wizard creates the new setup, the Congratulations dialog is displayed. This dialog allows you to view the report of the V-Comp™ setup, and explains what the next steps are.



Typically, after completing the wizard to create a setup, you will [add files to the batch list](#) and [process them to create new files](#) with V-Comp™ compensation.

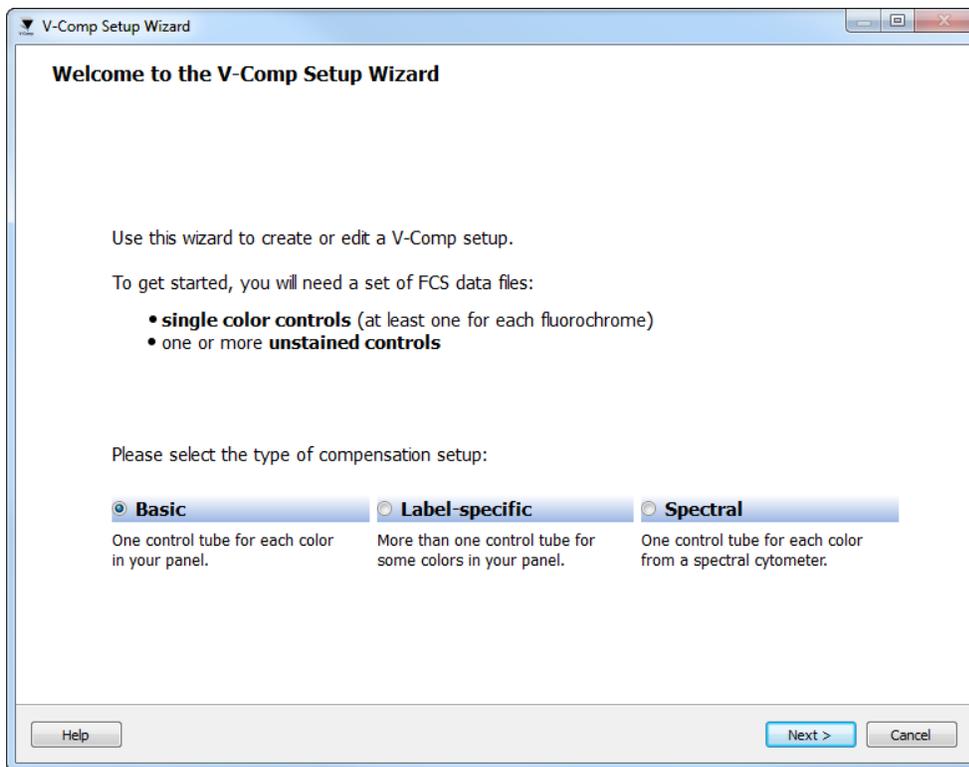
Welcome page

The initial page of the wizard is shown when creating a new setup. It provides a brief overview of what is required by the wizard to create a V-Comp™ setup.

There is one decision to make on this page: what kind of compensation setup do you want to create.

- **Basic** is used for the typical compensation setup, where you have one single-stained control file for each fluorochrome.
- **Label-specific** is used typically with tandem dyes where you might have more than one single-stained control for some fluorochrome. This is commonly used with Becton Dickinson fluorochromes.
- **Spectral** is used to set up "unmixing" of files acquired on a spectral cytometer, e.g. Sony's SP6800.

Select the type of setup you want to create, and then click Next to advance to the next page.



Single-Color Controls Page

On this page of the wizard, give your V-Comp™ setup a name and select the single-color control files that relate to the compensation setup you are creating.

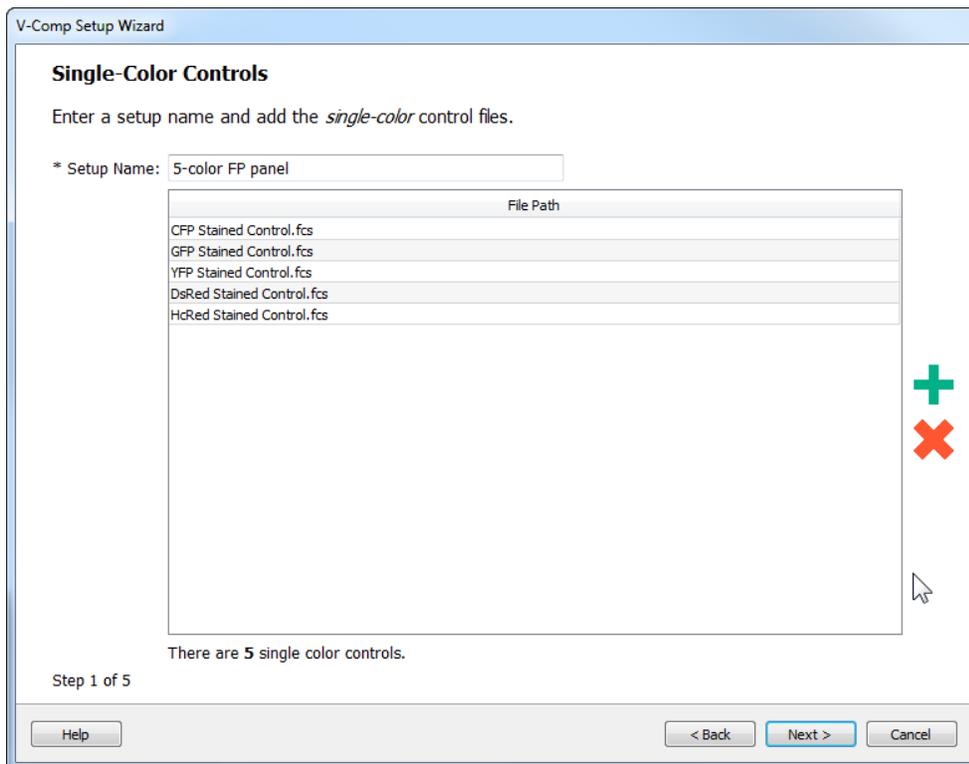
The setup name can be an informative phrase to help describe the use for this V-Comp™ setup. Since the setup name will also be used as a file name, you can only use characters in the name that are allowed in file names on your computer.

Single-color control files can be added to the list using the  button on the right side of the list, or by dragging and dropping files onto the list. You can add the controls in any order.

To remove a file, select it in the list and click the  button on the right side of the list.

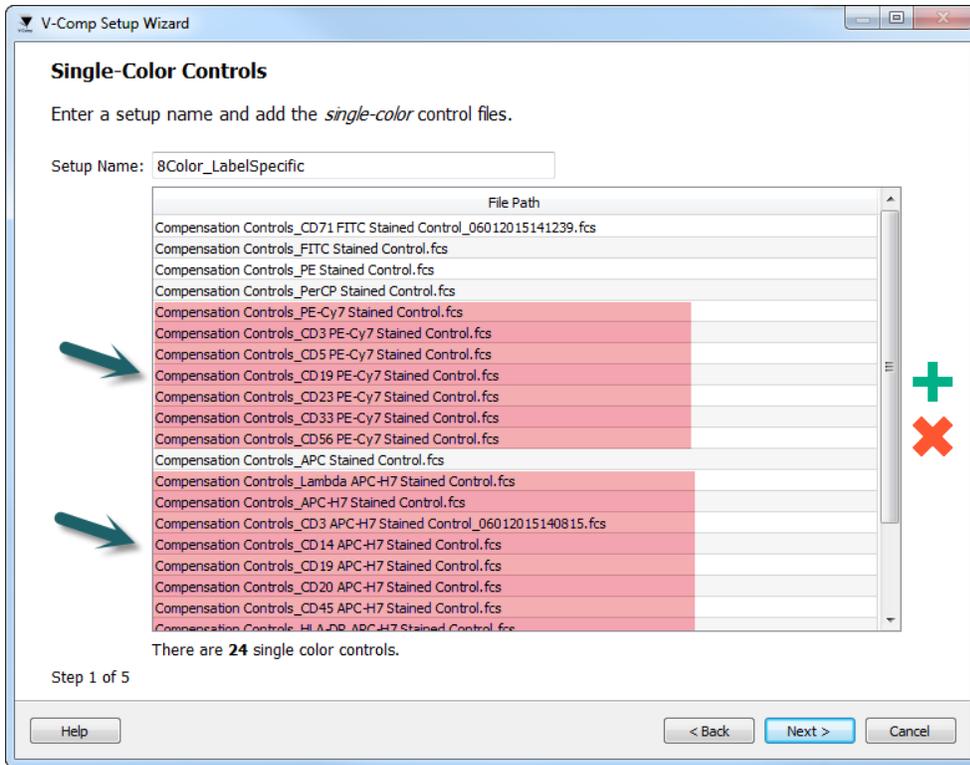
All of the single-color controls must have matching instrument settings for voltage and gains. The wizard will not advance to the next page if any of the controls do not match the others.

For **Basic** and **Spectral** setups, there should be one single-stained control file for each fluorochrome in your panel.



For **Label-specific** setups, you can have additional single-stained control files for label- or antibody-specific variants in your panel. In the example shown below, there are several label-specific variants for PE-Cy7 and APC-H7.

V-Comp User Guide



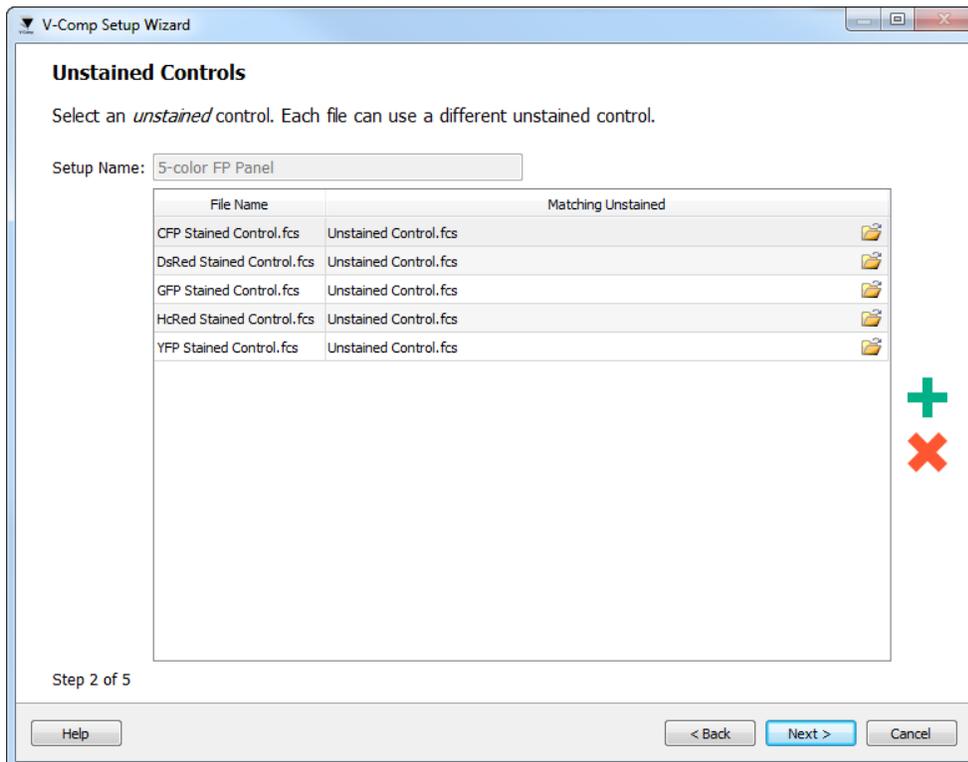
Unstained Controls Page

On this page of the wizard, select the unstained control file that relates to the compensation setup you are creating.

You only need one unstained control for the panel, and that is typically what you would add - just one unstained control. However, each single-color file can be associated with a different unstained control. This is useful, for example, if one of the single-color controls has to be re-run because of a new lot of reagent. In that case, you would also collect an additional unstained file so that it can be associated with the new single-color file.

The unstained control needs to be the same particles (cells, beads, etc) as the associated single-stained control(s). The only difference between the unstained and associated single-color control files is that the single-color control has one fluorochrome added to the tube.

To add an unstained file, you can click the  button on the right, or use the  button on the right side of the list, or drag and drop a file onto the list. Use the  button to remove an unstained control file.

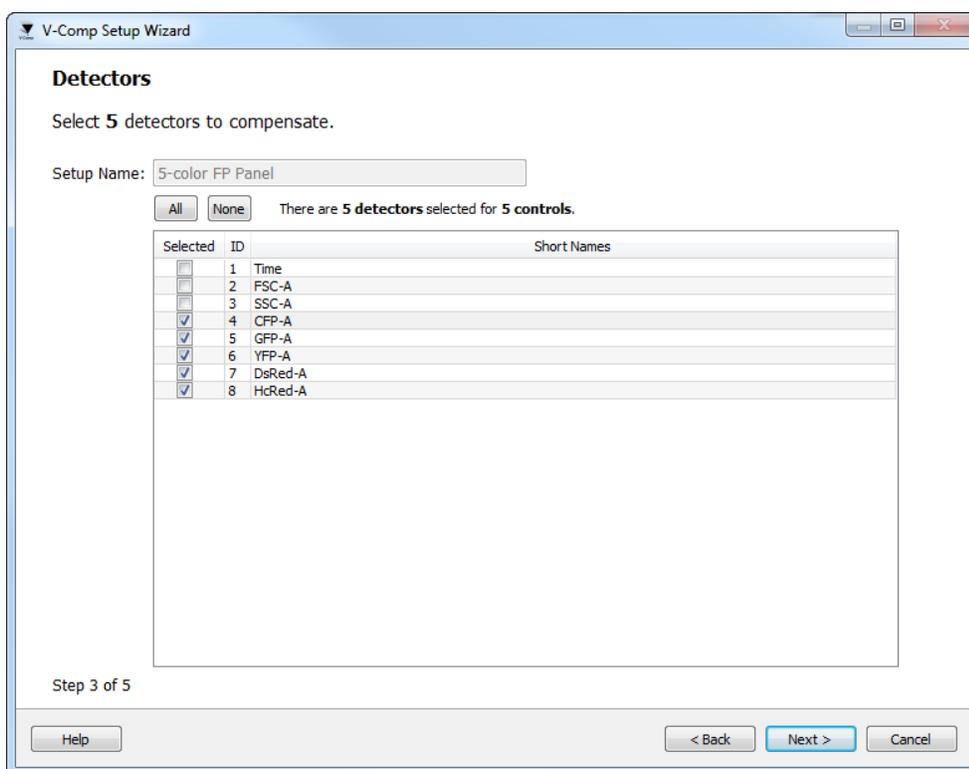


Detectors Page

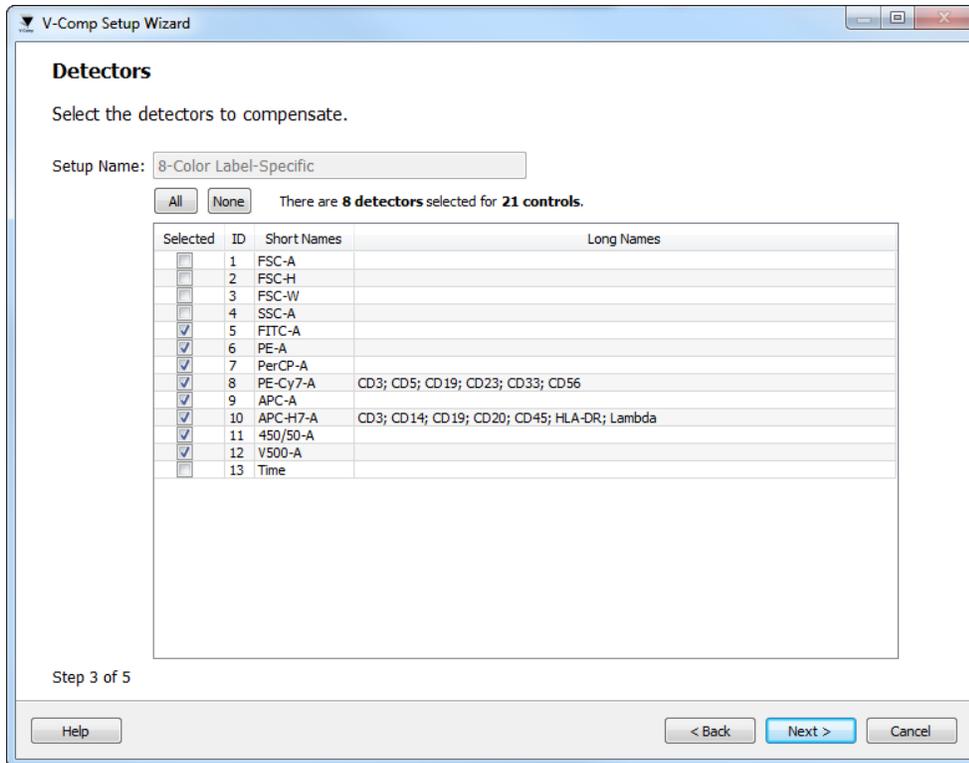
On this page of the wizard, select the fluorescence detectors that you want to compensate with your V-Comp™ compensation. Typically you should select the Area or Integrated fluorescence detectors, not the Height or Width. Do *not* select Scatter detectors or time measurements.

The program will automatically attempt to select the fluorescence detectors. This works in most cases to select the correct detectors, but you should always review the selected detectors.

For **Basic** setups, the wizard will require that you choose at the same number of detectors as you have single-color controls.



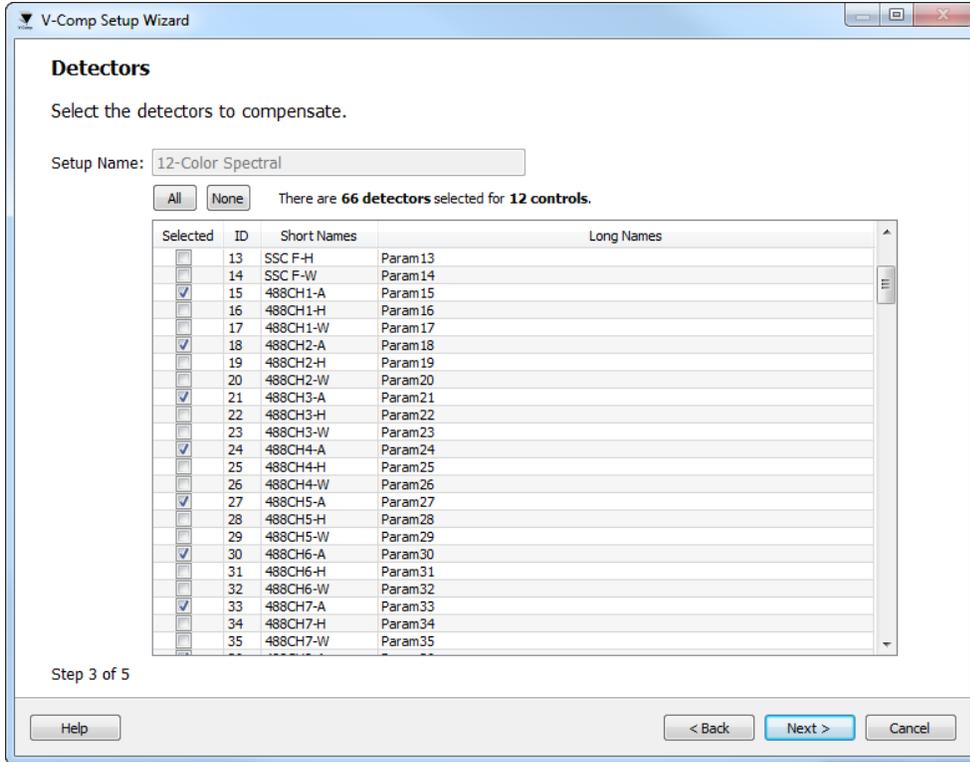
For **Label-specific** setups, you will generally select *fewer* detectors than the number of single-color controls. In this example, 8 detectors are selected for 21 single-stained control files. The variants of PE-Cy7-A and APC-H7-A are shown in the column on the right.



For **Spectral** setups, choose *at least* as many detectors as you have single-color controls. With spectral cytometers, the detectors represent a portion of the wavelength spectrum and there are typically more detectors than single-color controls. The program can "unmix" spectral data using special features in the V-Comp™ compensation logic.

In the spectral example below, there are 66 detectors selected for 12 single-stained controls.

V-Comp User Guide



Match Controls with Detectors Page

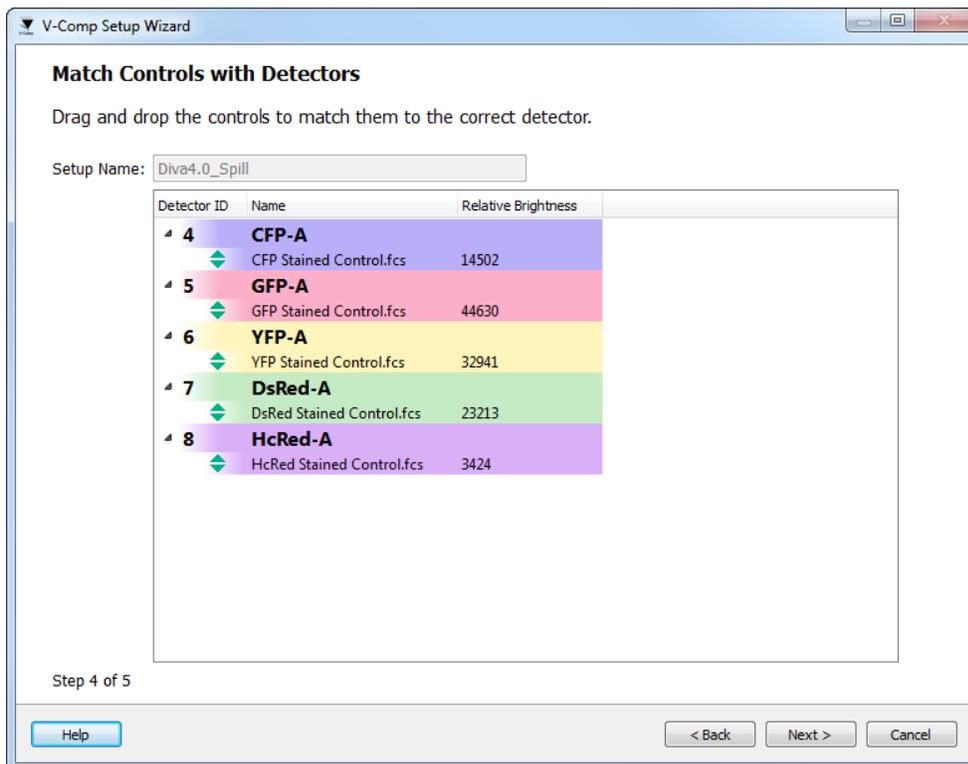
On this page of the wizard, match the control files with the detectors that you have selected on previous panes of the wizard. You will see this page if you are creating a **Basic** or **Label-specific** setup.

If you are creating a **Spectral** setup, you will see the [Signals Names page](#) instead of this one.

The wizard will automatically associate a single-color control with a detector based on the intensity of the events in the control file. In most cases, this will associate the correct control with each detector. Always review the list carefully to ensure the correct detector-control matches, and make corrections as needed.

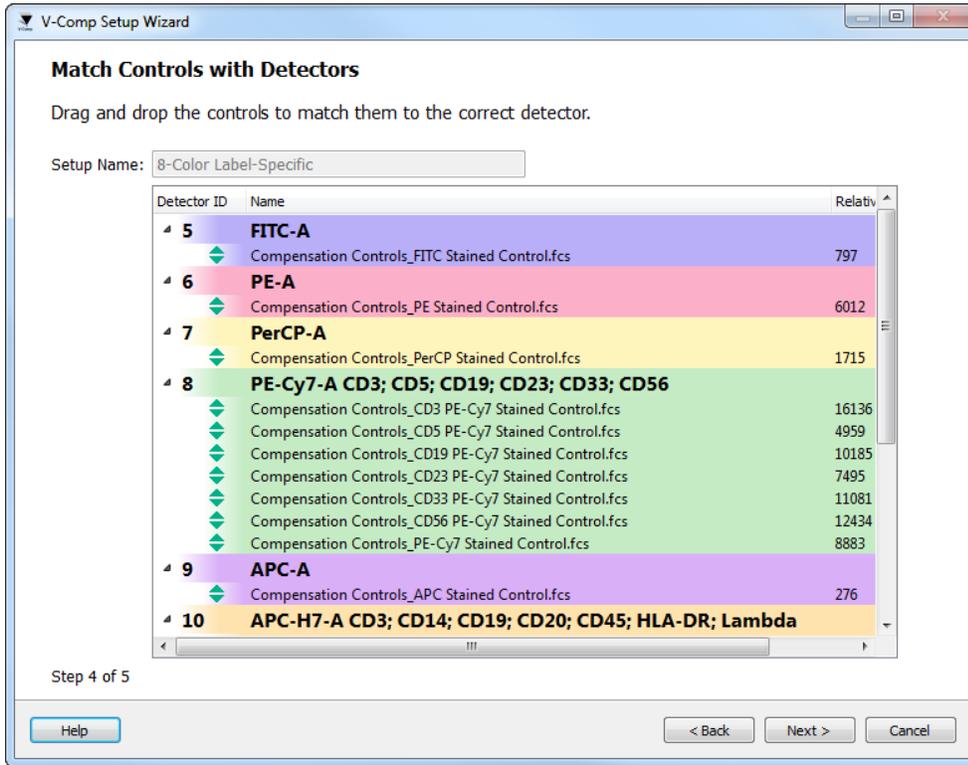
To move a control file to a different detector, simply click and drag the control file in the list to the correct detector.

For **Basic** setups, each detector needs to have one control file associated with it in order to move to the next page. The **Relative Brightness** is an indicator of how "good" a control is. The larger the relative brightness, the better the control. If the relative brightness is too low, the wizard will not continue to the next page.



For **Label-specific** setups, each detector has to have at least one control file associated with it. The color grouping helps identify the files associated with each

detector. Again, you can move a control file to a different detector by clicking on the file and dragging it to the correct detector.



Signal Names Page

On this page of the wizard, edit the fluorochrome in the Fluor column and the target antibody in the Target column to describe each of the single-color control files. You will see this page if you have more detectors than controls.

If you are creating a **Basic** or **Label-specific** setup, you will see the [Match Control with Detectors Page](#) instead of this one.

The values that you enter will be stored in the exported FCS file. The Fluor is stored in the \$PnN keyword for each parameter; the Target is stored in \$PnS.

You can control the order of parameters in the output file by moving items up and down in the list. Use the  and  arrows on the right to move selected items in the list.

V-Comp Setup Wizard

Signal Names

Edit the Fluor (e.g. FITC) and Target (e.g. CD3) for each signal. This setup has **12** signals and **66** detectors.

Setup Name:

Single Color Control File	Fluor	Target
Single Stained (Alexa Fluor 532).fcs	Single Stained (Alexa Fluor 532)	CD11c
Single Stained (Alexa Fluor 700).fcs	Single Stained (Alexa Fluor 700)	CD25
Single Stained (APC).fcs	Single Stained (APC)	CD11b
Single Stained (APC-Cy7).fcs	Single Stained (APC-Cy7)	HLADR
Single Stained (Cy2).fcs	Single Stained (Cy2)	CD34
Single Stained (FITC).fcs	Single Stained (FITC)	CD7
Single Stained (PE).fcs	Single Stained (PE)	CD13
Single Stained (PE-AF610).fcs	Single Stained (PE-AF610)	CD45
Single Stained (PE-Cy5).fcs	Single Stained (PE-Cy5)	CD33
Single Stained (PE-Cy7).fcs	Single Stained (PE-Cy7)	CD117
Single Stained (PE-eF610).fcs	Single Stained (PE-eF610)	CD38
Single Stained (PerCP-Cy5.5).fcs	Single Stained (PerCP-Cy5)	CD16

Step 4 of 5

Help < Back Next > Cancel

Options Page

On this page of the wizard, you can edit the name, description and several other options for the V-Comp™ setup.

Setup Name:

Enter the name that you want to use for this setup. The name will appear in the Setup List on the ribbon bar and is used to name the setup file that is automatically stored in the Setups folder.

Setup Description:

Edit text to describe what the setup is used for. The description is used as a tool tip when you hover the mouse of the Setup List in the ribbon bar.

Control Type:

Select the sample type for the controls. This setting determines how V-Comp analyzes the controls to create the spillover matrix.

Export Option:

When files are processed to apply V-Comp™ compensation, the new files can either be created in the same folder as the original file or into a user-specified export path. This option determines which location will be used. When the option is set for "Export files to the Export Path", the program will use the path specified in the next field of the dialog: Export Path.

Export Path:

This option is enabled when the Export Option is set for "Export files to the Export Path". Click the path editor to choose a path for the export.

Export Prefix:

When files are processed and exported, they preserve the original file name and "_vcomp" is appended to that name. Optionally, you can specify a prefix for the export file names. This will be added to the beginning of the exported file name.

Overwriting:

If there is already a file with the same export name in the export path, the program can ask whether you want to overwrite the file, or it can overwrite the file automatically. Choose the option that best fits your requirements.

Drift Correction:

The V-Comp™ wizard can apply sophisticated drift correction logic when it is processing the control files, so that only the stable events in the listmode file are considered when determining the best compensation settings. With this option enabled, you will see an additional "cleaning" step in the status as the wizard analyzes to create the V-Comp™ setup, and the setup report will include additional information to report how much drift was detected in each control.

Reference File:

This is an optional field. The V-Comp™ wizard allows you to select an FCS file that contains a spillover matrix to be used as a reference to compare with the V-Comp spillover. If you select a file as a reference, it should be an all-color file related to this setup. When a reference file is selected, V-Comp will create a comparison file showing the differences between the reference matrix and the V-Comp matrix.

Reports

Setup Reports

When you create or edit a V-Comp™ setup with the [V-Comp™ Setup Wizard](#), the program creates a PDF report for you and stores it in the Reports folder. You can display the Reports folder using the [Open Reports Folder](#) in the Tools menu on the ribbon bar.

Setup reports are automatically named based on the name of the V-Comp™ setup and the current date and time, preserving a history of changes made to a setup. The report's name begins with "VComp_Setup_Report" followed by the name of the setup and the current date.

The setup report is divided into several sections of information.

Setup Information

This section describes the V-Comp™ setup, where it is stored, and when it was last modified.

Setup Information

Research-use-only mode

Setup name: My Setup
Setup type: Basic
Description: Setup with 5 signals and 5 detectors
Control sample type: Cells

Configuration: 5 signals, 5 detectors
Setup Path: C:\Users\Ben.WORKGROUP\Documents\V-Comp for Windows\Setups\
V-Comp Engine Version: 29
Last modified: 09-Nov-2015 10:32
Modified by: Ben

Exporting path: C:\Users\Ben.WORKGROUP\Documents\V-Comp for Windows\Export
Overwriting: ask before overwriting export files
Drift correction: enabled for control files
Output type: export files with a new \$SPILLOVER keyword and the original data

QA/QC

This section contains information about the quality of the control files and any problems that were found by the V-Comp™ wizard. If problems are reported for any of the controls, review the controls to determine if they need to be re-run.

QA/QC	
Controls	Remarks
CFP Stained Control.fcs	High drifting was detected (12.42%) for detector CFP-A in the middle of the file. -+-----++-----
GFP Stained Control.fcs	High drifting was detected (12.42%) for detector GFP-A at the end of the file. -----+--+
YFP Stained Control.fcs	High drifting was detected (12.44%) for detector YFP-A at the end of the file. -----+--+
DsRed Stained Control.fcs	No problems detected.
HcRed Stained Control.fcs	Minor drifting was detected (4.16%) for detector HcRed-A in the middle of the file. -----+-----
Unstained Control.fcs	Modest drifting was detected (8.28%) for detector CFP-A in the beginning of the file. --+-----

Control Files

This section summarizes what events were used in each of the control files. The V-Comp™ wizard removes events that are "pegged" at the maximum value, outlier events, and if drift-correction is enabled, any events that are out of drift boundaries.

Control Files					
Controls	Total	Pegged*	Outlier**	Drift***	Events Used
CFP Stained Control.fcs	5000	0.00%	0.54%	12.42%	4352
GFP Stained Control.fcs	5000	0.00%	0.56%	12.42%	4351
YFP Stained Control.fcs	5000	0.00%	0.52%	12.44%	4352
DsRed Stained Control.fcs	5000	0.00%	0.50%	0.00%	4975
HcRed Stained Control.fcs	5000	0.00%	0.14%	4.16%	4785
Unstained Control.fcs	5000	0.30%	0.22%	8.28%	4560

V-Comp Crossover Matrix

The V-Comp™ crossover matrix is displayed in this section, if it will fit on the report. It also shows the location of a text file that contains the matrix. The rows in the matrix represent the signals, and columns represent detectors. This text file can be used if you need to enter the matrix manually into other software.

V-Comp Crossover Matrix

Crossover matrix file: My Setup.txt
 Location: C:\Users\Ben.WORKGROUPE\Documents\V-Comp for Windows\Setups\

Signals are in rows; detectors are in columns.

	CFP-A	GFP-A	YFP-A	DsRed-A	HcRed-A
CFP-A	100.00	2.75	1.37	0.54	0.02
GFP-A	0.52	100.00	37.47	8.91	0.01
YFP-A	0.04	59.75	100.00	29.71	0.02
DsRed-A	0.09	1.12	10.92	100.00	0.09
HcRed-A	15.47	1.22	1.08	34.73	100.00

Export Reports

When you process a batch of files by pressing the start button, the program creates a PDF report for you and stores it in the Reports folder. You can display the Reports folder using the [Open Reports Folder](#) in the Tools menu on the ribbon bar.

Export reports are automatically named based on the name of the V-Comp™ setup and the current date and time, creating a history of all of the batches you process with each V-Comp™ setup. The report's name begins with "VComp_Export_Report" followed by the name of the setup and the current date.

The export report is divided into several sections of information.

Setup Information

This section describes the V-Comp™ setup, where it is stored, and when it was last modified.

Setup Information

Research-use-only mode

Setup Name: 5-color FP panel

Setup type: Basic

Description: Basic setup with 5 controls and 5 detectors

Control sample type: Cells

Configuration: 5 signals, 5 detectors

Setup Path: C:\Users\Ben.WORKGROUP\Documents\V-Comp for Windows\Setups\

V-Comp Engine Version: 29

Last modified: 09-Nov-2015 14:49

Modified by: Ben

Exporting path: C:\Temp\VCompExports

Overwriting: ask before overwriting export files

Output type: export files with a new \$SPILLOVER keyword and the original data

Files Processed

This section contains information about the files in the batch list and the result of the exporting process. If there were errors, they are reported in this section.

Files Processed

Source file: All_Diva4.0.fcs
Export file: All_Diva4.0_vcomp.fcs
Location: C:\Temp\VCompExports\All_Diva4.0_vcomp.fcs
Status: Finished successfully

Source file: All_FC500.LMD
Export file:
Location:
Status: Range of detector 4 does not match.

Summary

This section summarizes the batch of files and indicates the total number of errors, if any.

Summary

End Time: 14:53:18
Files processed: 2
Files with errors: 1

Research and Compliance Modes

Research and Compliance Modes

V-Comp can be set up to run in either a Research-Use-Only mode, or in Compliance mode. There are some differences in how the program operates in each mode.

- Compliance mode depends on operating system user groups to control the level of access to program features. Research mode allows any user to perform any operation.
- Compliance mode displays a login dialog when the V-Comp is starting up to verify the user and determine the group to which the user belongs.
- Compliance mode enables logging so that there is a record of the activities of each user. Research mode can be run with logging enabled or disabled.
- Compliance mode is designed to support clinical labs and can be part of a compliance plan for the US-FDA's 21 CFR Part 11 regulations. Research mode does not provide compliance with 21 CFR Part 11.
- Both modes use the same intuitive interface and V-Comp engine.

Setting Up Research Use Only Mode

After initial installation, V-Comp will automatically be in Research mode. There is no further configuration required.

To revert to Research mode after setting up Compliance mode, the user groups that were created for Compliance mode must be removed. Once the user groups are removed, the program will revert to Research mode.

Setting Up Compliance Mode

Setting up Compliance mode is something that should be performed by IT personnel.

While it is not a complicated task, it requires administrative access rights and a clear understanding of user groups and permissions. It is therefore something that we strongly recommend be performed by the IT team at your institution. The task involves creating several *required* V-Comp user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode. [Click here for details.](#)

21 CFR Part 11 Compliance Matrix

This matrix provides a simple summary of how V-Comp in Compliance mode helps you with 21 CFR Part 11 compliance. [Click here for details.](#)

Setting Up Compliance Mode

Setting up Compliance mode should be performed by your IT team. It requires administrative rights and a clear understanding of your network operating system.

The task involves creating several *required* V-Comp user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode.

V-Comp User Groups

There are three (3) user groups for Compliance mode: **VCompAdmins**, **VCompEditors**, and **VCompUsers**. Optionally, you can define an additional user group, **VCompResearchers**, for research users within the Compliance setting who are not restricted by Compliance requirements.

The table below shows the features that group members can access.

Feature	VCompAdmins	VCompEditors	VCompUser	VCompResearchers
Run a batch of files	Yes	Yes	Yes	Yes
Add & remove files from batch	Yes	Yes	Yes	Yes
Select setups	Yes	Yes	Yes	Yes
Edit options	Yes	Yes		Yes
View & manage report folders	Yes	Yes		Yes
View & manage setup folders	Yes	Yes		Yes
Create & edit setups	Yes	Yes		Yes
Edit logging options	Yes			Yes
Check for program updates	Yes			Yes
Edit configuration	Yes			

Creating User Groups

The program requires at least one of the V-Comp user groups to be defined in order to run in Compliance mode. V-Comp user groups can be defined for your computer or for your entire domain using tools provided by the operating system. This is typically done by information technology personnel because it requires administrative rights.

The advantage of defining the user groups at the domain (network) level is that the groups are defined *for all computers* on the domain at the same time. If you define the user groups on an individual computer, those groups are available *only on that computer*.

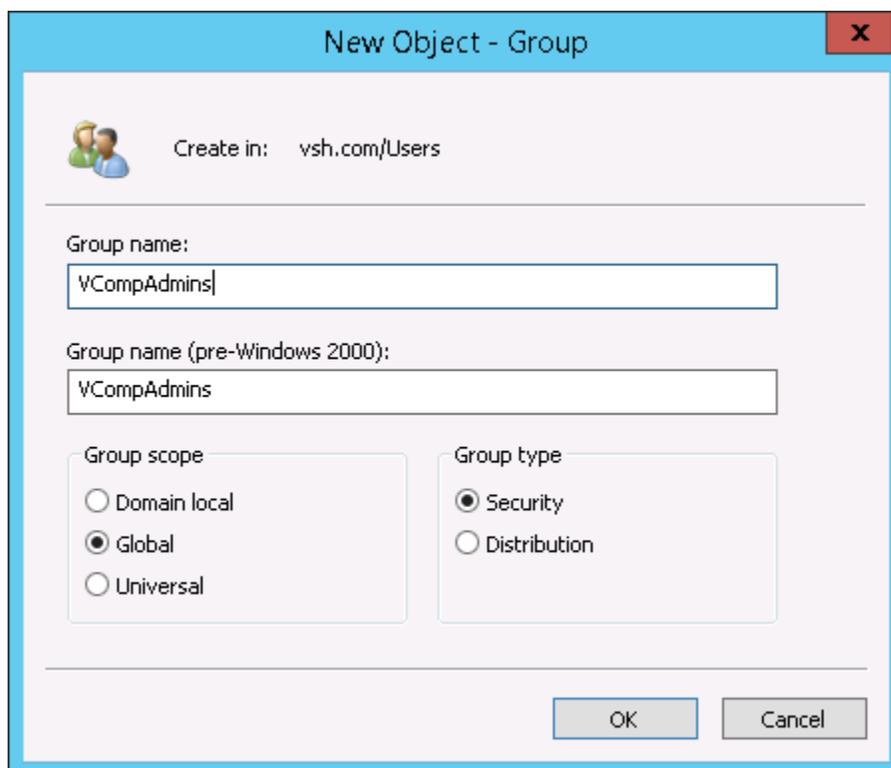
Below we describe how to create user groups on a Windows server, on a Windows computer, and on a Mac computer as examples. The specific methods you need to use may differ depending on the version of the operating system you are working with.

After you have defined the user groups and added users to them, V-Comp will detect the groups when it starts up and operate in Compliance mode automatically.

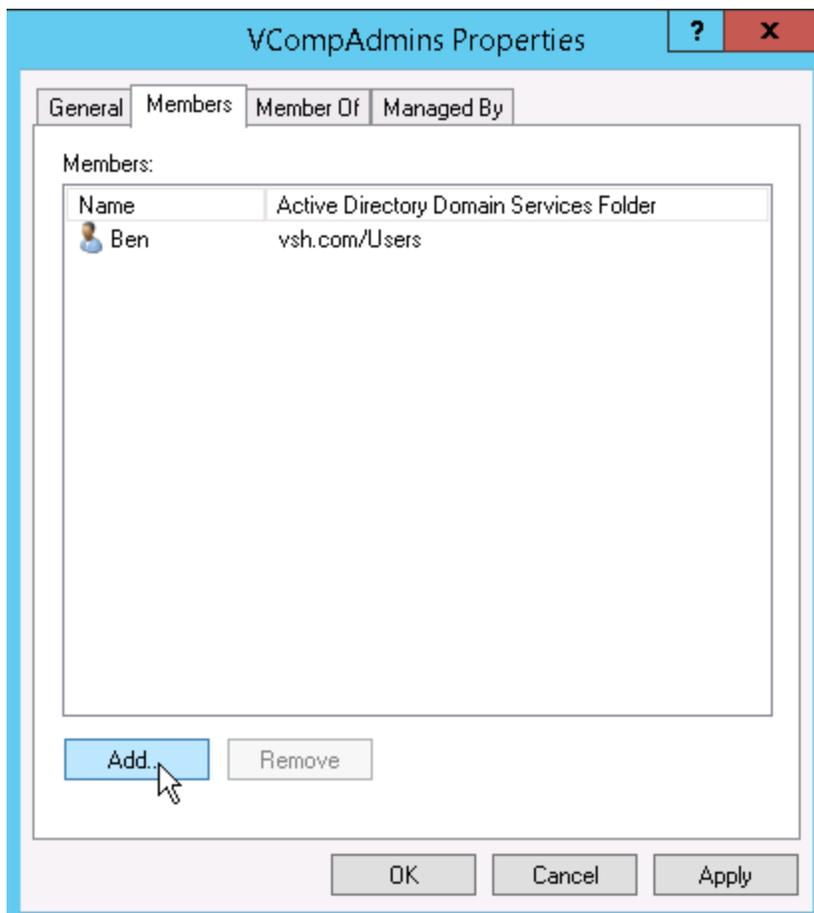
Windows Server User Groups

Follow these steps to define user groups for an entire network or domain. **Note: if you use this technique, you do not need to define user groups on each individual computer.**

- On a domain server, launch the "Active Directory Users and Computers" administrative tool.
- Right click the "Users" folder in the left panel and choose New->Group. The New Group dialog is displayed.



- Type **VCompAdmins** for the group name and click OK.
- Once the group is created, double-click the group in the list to display the Properties for the group.
- Click the **Members** tab and click **Add** to select members for the VCompAdmins group. You should have at least one user in the VCompAdmins group. Members can be added by typing user names, or by using the dialog's search features.



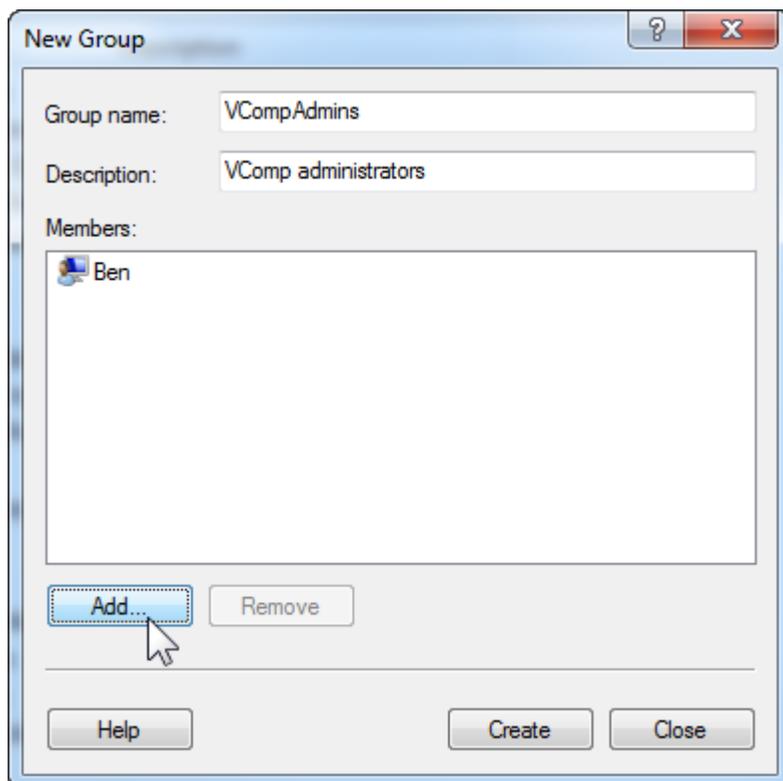
- Repeat this process for the **VCompEditors**, **VCompUsers**, and **VCompResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up and is connected to the domain.

Windows Computer User Groups

Follow these steps to define user groups for an individual Windows computer. **Note, if you already defined user groups for your domain, you do not need to define the groups again for each computer.**

- On a Windows 7 computer, right click Computer and choose "Manage". For Windows 8 and newer, right click the Windows button (lower left) and choose "Computer Management". The Computer Management app will display.
- Expand "System Tools" and "Local Users and Groups" in the left panel.

- Right click the "Groups" folder and choose "New Group". The New Group dialog is displayed.



- Type **VCompAdmins** for the group name and enter a description for the group.
- Click **Add** to select members for the VCompAdmins group. You should have at least one user in the VCompAdmins group. Members can be added by typing user names, or by using the dialog's search features.
 - Click **Create** to create the new local user group.
- Repeat this process for the **VCompEditors**, **VCompUsers**, and **VCompResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up on that computer.

Mac OS X User Groups

Follow these steps to define user groups for an individual Mac OS computer.

- On a Mac OS computer, launch System Preferences. Choose "Accounts" or "Users and Groups", depending on the version of the OS.
- Click the lock icon to authenticate to make changes. This will require administrative rights.

- Click the "+" button under the list of users and groups. The "New Account" dialog is displayed.
- Change the New Account type to "Group".
- Type **VCompAdmins** for the group name and click "Create Group".
 - Select the users you want to be members of the VCompAdmins group. You should have at least one user in the VCompAdmins group.
- Repeat this process for the **VCompEditors**, **VCompUsers**, and **VCompResearchers** groups, adding appropriate users to each group.
- V-Comp will run in Compliance mode the next time it starts.

See also:

[21 CFR Part 11 Compliance](#)

21 CFR Part 11 Compliance

V-Comp can be part of your lab's compliance strategy for 21 CFR Part 11 when it is setup and operated in [Compliance mode](#). It has been developed under a quality management system and validated with a suite of quality assurance tests. The table below summarizes how the key elements of Part 11 relate to V-Comp and its use in Compliance mode settings.

Section	Summary of Rule	Compliance Strategy
11.10	Controls for closed systems	V-Comp can be accessed only through the user interface; it is a closed system.
11.10 (a)	Validation and accuracy.	V-Comp uses externally-generated data to validate its results and employs proprietary binary format, static PDF, and/or CRC methods to ensure valid files.
11.10 (b)	Record generation and readability	V-Comp generates setup and export reports in a designated pathway. The reports are human-readable and machine-readable.
11.10 (c)	Record protection and retrieval	Reports and setups are automatically archived. Record retention and retrieval plan is managed by the laboratory's file management practices.
11.10 (d)	Authorized access	V-Comp uses the operating system's user login validation, and user group membership determines permissions within the software.
11.10 (e)	Audit trails	V-Comp has a logging system with time stamps, and log files are preserved. Log files cannot be deleted within the program.
11.10 (f)	Operational system checks	V-Comp uses a guided sequence of user actions to enforce permitted sequencing of steps and events.
11.10 (g)	Authority checks	V-Comp uses the operating system user groups and login validation to assure authorized access.
11.10 (h)	Device checks and validity	V-Comp performs validation checks on the source data files.
11.10 (i)	Education, training and experience	V-Comp provides a User Guide, guided analysis steps, and tutorial videos to assist with the proper, intended use of V-Comp. Additional on-site training may be arranged.
11.10 (j)	Electronic signatures	This is an individual laboratory requirement beyond the scope of use of V-Comp.
11.10 (k)	Systems documentation	V-Comp version releases are accompanied by an updated User Guide. Verity employs document control procedures.

11.30	Controls for open systems	V-Comp is designed as a closed system.
11.50	Signature manifestations	V-Comp does not require or offer electronic signatures.
11.70	Signature linking	V-Comp does not require or offer electronic signatures.
11.100 (a)	Unique electronic signatures	Your laboratory must ensure that electronic signatures are unique to each user on the network.
11.100 (b)	Identity verification	Each laboratory must verify its own employment records and IDs.
11.100 (c)	Signature verification	Each laboratory must verify electronic signature eligibility with the FDA.
11.200 (a)(1)	Two-component signing	V-Comp does not require or offer electronic signatures.
11.200 (a)(2,3)	Authenticity and falsification	V-Comp does not require or offer electronic signatures.
11.200 (b)	Biometric signatures	V-Comp does not utilize biometric IDs.
11.300 (a)	Controls for identification	V-Comp uses the operating system user groups and login validation to assure unique user identities.
11.300 (b)	Identification aging	The individual laboratory IT structure must control password checks and aging requirements.
11.300 (c)	External ID validation	Not applicable. V-Comp does not utilize external tokens, cards, RFID, or other physical identity-confirming devices.
11.300 (d)	Unauthorized use	The individual laboratory IT structure must control login authenticity and safeguards.
11.300 (e)	External device testing	Not applicable. V-Comp does not utilize external tokens, cards, RFID, or other physical identity-confirming devices.

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