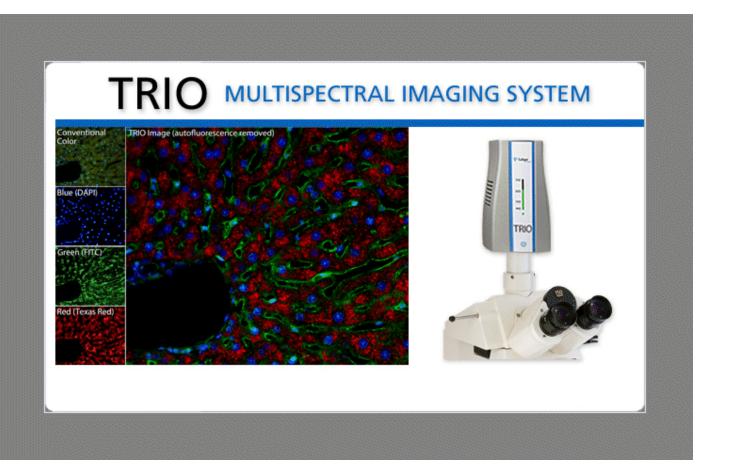
User's Manual for TRIO 1.0.2





Notice

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This manual describes system operation using Trio version 1.0.2 software.

For more information contact:

Caliper Life Sciences, Inc. 68 Elm Street, Hopkinton, MA, 01748, USA

(Phone) 508-435-9500 (Fax) 508-435-3439 Email: techsupport@CaliperLS.com Web site: http://www.CaliperLS.com

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Table of Contents

Part I	Introduction & Glossary	1
Part II	Getting Started	3
Part III	Transferring the License	5
Part IV	Trio Home Window	7
Part V	Software/System Settings	9
1	System Objectives Setup	
2	Lamp Setup	
3	Brightfield References	10
4	Stain Inventory	1
Part VI	Create An Experiment	13
1	Experiment Details	
2	Stains For The Experiment	
3	Layout For Acquired Pictures (Views)	
4	Export Settings For The Experiment	
5	Save the Current Experiment	2
Part VII	Run An Experiment	23
1	Live Window	
2	Take Multispectral Pictures	
3	Image Display Window	24
Part VIII	Take Color Pictures	27
Part IX	Review Captured Data	29
Part X	CRi Software EULA	31

1 Introduction & Glossary

Welcome to the TRIO Multispectral Imaging System. The following topics will help you create an experiment by choosing chromogens (brightfield) or fluorophores (fluorescence) for your slides, building a stains library, selecting image view options, and specifying how you want to export the image data. Next, it explains how to use your new experiment to capture images from slides (you can capture multispectral or simple color images), manipulate them to see the structures you want, and then save them for future analysis.

Before you begin, familiarize yourself with the following TRIO terminology:

Brightfield Reference

Also commonly known as "white reference." These reference images are pictures taken of an empty portion of the slide using each of the system objectives. All of these pictures must be taken using exactly the same lamp settings. TRIO uses these images to compensate for the background light and correctly unmix the signals it finds in your samples. Any changes to the lamp settings will require you to take new brightfield references using the System Setup function.

Component Image

A component image displays the spectral signature of one marker within the sample. For example, a complete multispectral picture might contain a Hematoxylin component and a DAB component. Each of these components can be displayed separately.

Composite Image

When the TRIO software overlays multiple component images to create a single image, this is called a composite image. TRIO lets you turn individual components ON or OFF, depending on which signals you want to see in the composite.

Control Slide

This is a slide that contains either only tissue (for obtaining background autofluorescence) or a single stain (for obtaining individual stains) for an experiment. For quantitative results, control slides should be prepared and mounted at the same time as the slides you intend to scan, with tissue cut to the same thickness on the same microtome, and using slides and coverslips from the same batch.

Experiment Record

Experiment records (.im4 files) can be opened in TRIO. They contain all of the original imagery data, so you can open them to review images and adjust their display settings. TRIO automatically saves an experiment record in the default TRIO data folder (setup during installation) each time you choose to keep a captured image.

Component Data tool

By clicking this tool and "mousing around" the image, you can examine the signal intensity of each component at any location throughout the images. For composite and component images, the signal intensity of each marker is given. For the color image, signal intensity is expressed as Red, Green, Blue.

2 Getting Started

- 1. Follow all instructions in the "TRIO System Setup Guide" if you have not yet installed and set up your system. As explained in the setup guide, it is important that you install and activate the TRIO software first, then connect and power up the TRIO module, and then complete the initial configuration of the TRIO software. Here is an outline of the steps in the setup guide:
 - **a.** Unpack the system and make sure you have all the required components.
 - **b.** Install the TRIO software by inserting the installation CD. If the installation wizard doesn't start automatically, double-click **Setup.exe** to start the installation. Follow the prompts in the wizard.
 - **c.** Activate the TRIO software by selecting the **Activate** function on the **Home** window.
 - **d.** Connect the TRIO camera module's power and USB cables. Press the power button on the front panel of the TRIO camera module. During initialization, the LEDs on the front panel scroll through the full wavelength range while the filter is "exercising." The CAMERA and STATUS LEDs illuminate steady green when initialization is complete.
 - **e.** Setup the TRIO system software (i.e., add objectives, take brightfield references, adjust lamp brightness, and Koehler the microscope).
- 2. For subsequent use of the system after initial setup, simply turn on the computer and press the power button on the front panel of the TRIO module. Initialization is complete when the CAMERA and STATUS LEDs illuminate steady green.
- 3. Launch the TRIO software. The Home window 7 appears.

3 Transferring the License

You can transfer the TRIO software license from its current computer to another computer. When you transfer the license, it will be removed from the first computer, and the software will immediately shut down.

There are a few requirements for transferring the license:

- You must be an administrator on both machines.
- ➤ If the machine has a locked down install directory, this restriction must be removed.
- ➤ You need a USB thumb drive, and an available USB port on each machine.

To transfer the license, start at the unlicensed computer. Start up TRIO and select **Transfer In** instead of activating the software. Follow the instructions provided in the dialog boxes to complete the transfer process.

4 Trio Home Window

The TRIO Home window provides access to the four essentials of using the TRIO software. Each of the four functions are described below:

Run	an	expe	erim	ent
23		•		

Once you have created and saved your first experiment, select this function to run the experiment to capture multispectral pictures from slides.

Take color pictures 27

Choose this option if you want to take brightfield simple color imagery.

Review captured data 29

Click this function if you want to open and review images that are saved in experiment record.im4 files. (TRIO can only open images that are saved in experiment record files.)

Create an experiment 13

Click here when you are ready to create your first experiment. Naming the experiment, choosing stains and markers, and choosing export settings are all part of creating an experiment. You also use the *create experiment* function to open and modify previously saved experiments.

Set up my system

Click here to enter the system/microscope setup utility. This utility guides you through a setup procedure that includes entering system objectives, adjusting and Koehlering the lamp, and taking white references.

Activate/Upgrade/ Transfer my license

If you have not registered the software yet, click **Activate my license**. You can activate software via the Internet or by sending an activation request email to Caliper. Complete activation instructions are provided in the "TRIO System Setup Guide." (This is a printed setup guide that was shipped with your TRIO system.)

If you activated using a temporary license key, you can upgrade to a permanent license by clicking **Upgrade my license**.

Use the **Transfer my license** option if you want to transfer your license to a demo version of the software installed on another computer.

Note that if the TRIO camera was turned off or disconnected when you started the TRIO software, the acquisition functions remain disabled until you connect/turn on the TRIO camera, and then exit and restart the software.



Figure 1. TRIO Home Window

5 Software/System Settings

The information in this section is also given as a procedure in the separate "TRIO System Setup Guide," which was shipped with your TRIO system. The setup guide also includes instructions for installing and activating the TRIO software, and for connecting and powering up the TRIO module.

Some of the information in the setup guide is repeated here for handy reference in case you need to modify your software/system settings. These settings include naming your microscope objectives, adjusting and Koehlering the lamp, taking brightfield references, and creating a stains inventory.

Make sure the TRIO Imaging Module is on and ready. Then launch the TRIO software.

In the Home window, click Set up my system.

5.1 System Objectives Setup

The **System Objectives** window simply tells the TRIO software which objectives are installed on your microscope. (The software does not interface directly with the microscope.)

- 1. In the Turret Size box, enter the number of objective positions in your microscope's turret.
- 2. Enter the Name and Magnification of each objective.
- 3. You can also edit or delete existing objectives.
- **4.** Use the **Swap Objectives** feature if necessary so that the objective positions match their actual positions in the turret.



Figure 2. Objectives Setup

5. Click Next or Lamp to finish and proceed to Lamp Setup 10.

5.2 Lamp Setup

The Lamp Setup sequence helps you set up your microscope for TRIO use. This includes checking the Koehler alignment, setting the condenser NA, configuring the lamp, and taking "brightfield references." Once this is done, do not change any of these settings as this can invalidate these measurements and settings. You will only need to redo this setup if you change any of these settings at the microscope.

- 1. Click **Next** to begin. At the **Sample Setup** prompt, select a brightfield specimen slide and follow the instructions on the screen.
- 2. Click **Next** when you are ready to continue. At the **Koehler Adjustment** prompt, follow the instructions to complete the Koehler adjustment.
- 3. Click **Next** to proceed to the **Condenser NA Adjustment** prompt. Follow the instructions on the screen.
- **4.** Click **Next** to begin the **Lamp Brightness Adjustment**. Follow each set of instructions to complete this step.

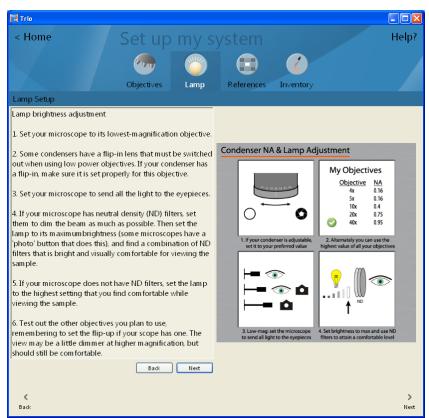


Figure 3. Lamp Setup

5. Click Next when finished. You are now ready to take your Brightfield References 10.

5.3 Brightfield References

In order to take pictures in brightfield, a **Brightfield Reference** image needs to be acquired and saved for each of the installed objectives.

- Click Next or References to view the panel shown below. Each of the installed objectives will be listed.
- **2.** Click **Take Reference** next to the first objective. A prompt will remind you to set your microscope's objective before proceeding. Click **OK** when ready.
- 3. Find a place on the slide that has no sample on it. Make sure light is diverted to the camera and click **Take First Reference**. The system will take the first of three pictures. When prompted, move the slide to the left or right and click **Take Second Reference**. Take a third reference after moving the slide up or down.
- **4.** If the picture looks like a good brightfield reference image, click **Accept Picture**. If not, reject it and start over.

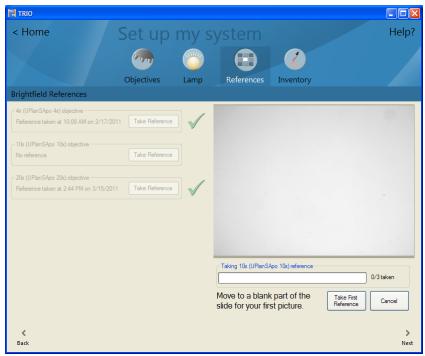


Figure 4. References Setup

5. Repeat the procedure for each of the objectives. When all objectives have green check marks, click **Next** or **Inventory** to customize your Stain Inventory 11.

5.4 Stain Inventory

- 1. The **Stain Inventory** panel lets you manage the number of stain examples that you have saved. Simply select the samples you no longer wish to keep and click the **Remove Selected Example** button. Removing a stain from this list does not affect existing experiments; it only removes the stain from the list of available stains when making new experiments.
- 2. Use the options in the **Show box** to select the kinds of examples you want to see: brightfield stains, fluorescent stains (fluorophores), and tissue examples.

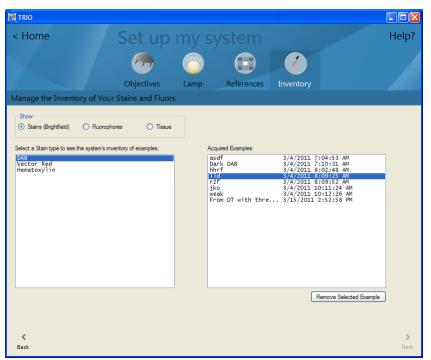


Figure 5. Stain Inventory Setup

3. System setup is now complete. Click the **Home** button to exit setup.

6 Create An Experiment

This section provides instruction on how to configure and save experiments. Click on **Create an experiment**. The **Experiment Details** 133 window appears.

Note: Creating an experiment is separate from your regular data collection process. You don't need to do this every time you acquire images with TRIO. Most users create an experiment for the first set of slides they want to image, and then copy and adapt that experiment for future experiments. Experiments that you create here can be used many times over to take pictures of compatible slides.

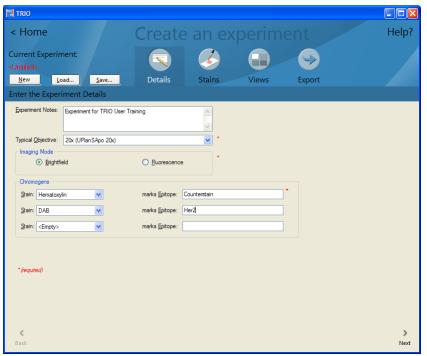


Figure 6. Experiment Details

6.1 Experiment Details

Enter the Experiment Details:

- 1. Enter any notes that will help to further identify this experiment.
- **2.** Select the **Typical Objective**. This is the objective you will most likely use when taking pictures. You can still select a different objective later, but this sets the default for this experiment.
- **3.** For the **Imaging Mode**, select "*Brightfield*" if you want to take pictures in brightfield. Select "*Fluorescence*" if you want to take pictures of slides in fluorescence mode.

For taking pictures in Brightfield:

- **4.** For the **Imaging Mode**, select "*Brightfield*".
- 5. Select the **Chromogens** in use on your

For taking pictures in Fluorescence:

- **4.** For the **Imaging Mode**, select "Fluorescence."
- **5.** Choose a **Tissue Type**. You can choose

slides. To do this, first select a **Stain** from the list, then specify the **Epitope** the stain marks. (For example, you might start with "Hematoxylin" and enter "counterstain" as the associated epitope. Then you might select DAB and enter "Her 2" as the epitope.) You can select up to three chromogen/epitope pairs.

from any of the listed tissue types, or create a new one. Any new tissue type you create will be added to the list.

6. Select the Fluorophores in use on your slides. To do this, first select a Fluorophore from the list, then specify the Epitope it marks. You can select up to three fluorophore/epitope pairs.

Click **Next** to proceed to stains selection 14.

6.2 Stains For The Experiment

Selecting Stains for Taking Pictures in Brightfield

- Click Select [stain] Example. If the necessary stains have already been acquired and saved in your stains inventory, then you can choose from the list of available samples. (Or, if you decide not to use any of the saved stain examples, click the Acquire New button to acquire and save a new stain, as described in the next step.)
- **2.** From your control slides, place a singly-stained sample of the requested stain on the microscope stage and click **OK**.
- **3.** Select the objective you want to use and confirm that the same objective is selected at the microscope. Focus the image. Find a region on the slide that exhibits a strong stain signal. Make sure light is diverted to the camera port.

Important Note! When searching for stains, make sure you do not change the lamp brightness, ND filters, Koehler alignment, etc, as this will invalidate the white references that were acquired during microscope setup.

- **4.** Click **Take Picture**. TRIO will acquire an image.
- **5.** Click **Find Example** and the system will find areas that best represent the stain. These areas will be highlighted in a bright color.
- **6.** If necessary, adjust the threshold of the found stain.
- 7. Accept or reject the stain example after examining the stain regions. Tip: There are buttons located above the image to help with this. You can use the zoom buttons to zoom in/out, or click the Mask button to view or hide the regions mask. If you accept the example, enter a name and any notes you want to make about the example. Make sure you enter enough information so you can easily identify this stain example later. When you click OK, TRIO saves the stain example in your stains inventory.

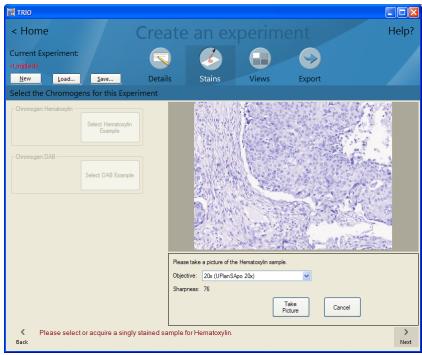


Figure 7. Select Brightfield Stains

- a. If you see a "Multiple stains present..." message, TRIO has found more than one stain species in the image. You may have accidentally used a slide that contained more than one stain. If this is the case, do not use it. Reject the stain and start over using a different singly-stained slide. If the system found dust or some other endogenous chromagen (like red blood cells) as a stain, then it is ok to pick the correct one. To choose a species, use the Show Species and Keep Valid Species boxes. Adjust the threshold as needed. Then click Accept Example and enter the Stain Details. Click OK to save the stain example.
- **b.** If you see a "Wide variation in stain signals present..." message, TRIO has found a wider than expected range of possible stain targets present in the image. This could be normal for your stain, but there is a lot of variability and you need to tell TRIO which signals are the correct target. Adjust the stains one at a time, choosing which ones to add to (or exclude from) the true stain. This helps for stains like DAB, which may have distinct specific and non-specific signals (both of which you might want to include in the complete stain example).
- **8.** Repeat this process to acquire the remaining stain examples from your other control slides.

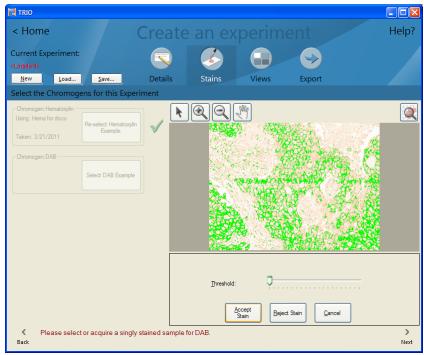


Figure 8. Finished Selecting Brightfield Stains

9. When you have finished saving stains to your library (indicated by the checkmarks shown above), click **Next** to set up a layout for pictures 19.

Selecting Stains for Taking Pictures in Fluorescence

- 1. Start with your autofluorescence control slide (i.e., contains no fluorophores). Click **Select** [tissue] Example.
- 2. If the necessary tissue sample has already been acquired and saved in your tissue samples inventory, then you can choose from the list of available samples of tissue. (Or, if you decide not to use any of the saved tissue samples, click the **Acquire New** button to acquire and save a new tissue sample, as described in the next step.)
- **3.** Place an unstained sample of the tissue on the microscope stage and click **OK** when ready.
- **4.** At the microscope, select the TRIO filter cube.
- **5.** Also at the microscope, select the objective you want to use and confirm that the same objective is selected in the TRIO software. Focus the image. Find a region on the slide that exhibits a strong autofluorescence signal. Make sure light is diverted to the camera port and click **Take Picture**. TRIO will acquire an image.
- **6.** Click **Find Example** and the system will find areas that best represent the tissue. These areas will be highlighted in a bright color. An orange mask showing the autofluorescence will typically cover most of the image.
- 7. If necessary, adjust the threshold of the found tissue.

8. If you think this is a good representation of the autofluorescence, click Accept Example. (Or you can reject the image and start over.) If you accept it, enter a name and any notes you want to make about the tissue in the Tissue Details dialog box. Make sure you enter enough information so you can easily identify this tissue later. When you click OK, TRIO saves the tissue in your tissue samples inventory.

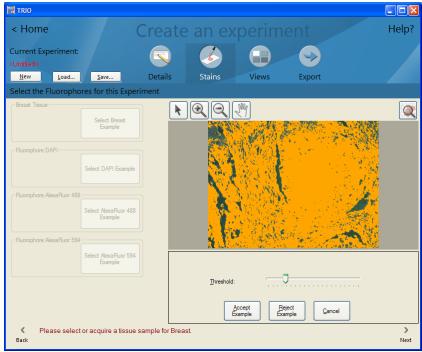


Figure 9. Select Fluorescent Stains

- **9.** Now repeat this process for each of the fluorophores used in this experiment, using your other control slides to acquire each fluorophore. Click on the **Select** button to select or acquire each fluorophore.
- 10. If the necessary fluorophores have already been acquired and saved in your stains inventory, then you can choose from the list of available fluorophore samples. (Or, if you decide not to use any of these fluorophores, click the Acquire New button to acquire and save a new stain, as described in the next step.)
- 11. Have your control slides ready and click **Select [fluorophore] Example** and follow the prompts:
 - **a.** From your control slides, place a singly-stained sample of the requested fluorophore on the microscope stage and click **OK**.
 - **b.** After you take each picture, either accept or reject each stain after examining the stain regions. **Tip:** There are buttons located above the image to help with this. You can use the zoom buttons to zoom in/out, or click the **Mask** button to view or hide the regions mask.
 - **c.** If you see a "Multiple stains present..." message, TRIO has found more than one stain species in the image. You may have accidentally used a slide that contained more than one stain. If this is the case, do not use it. Reject the stain and start over using a different singly-stained slide. If the system found dust or some other endogenous chromagen (like red blood cells) as a stain, then it is ok to pick the correct one. To choose a species, use

- the **Show Species** and **Keep Valid Species** boxes. Adjust the threshold as needed. Then click **Accept Example** and enter the **Stain Details**. Click **OK** to save the stain example.
- **d.** If you see a "Wide variation in stain signals present..." message, TRIO has found a wider than expected range of possible stain targets present in the image. This could be normal for your stain, but there is a lot of variability and you need to tell TRIO which signals are the correct target. Adjust the stains one at a time, choosing which ones to add to (or exclude from) the true stain.

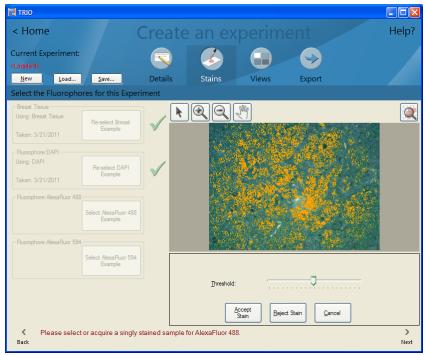


Figure 10. Finished Selecting Fluorescent Stains

12. When you have finished saving stains to your library (indicated by the check marks shown above), click **Next** to set up a layout for pictures 19.

6.3 Layout For Acquired Pictures (Views)

Set up a **layout for acquired pictures**. This determines the image view (i.e., how the images will be displayed when you run the experiment to take or review pictures).

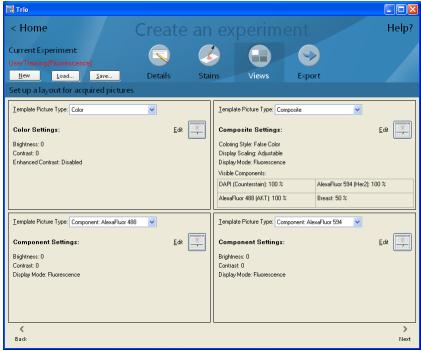


Figure 11. Select View Types

- 1. Select a **Template Picture Type** for each box in the grid. For example, as shown above, you might want to select "Color" for the upper-left image. Depending on the number of stains, you might want to select "Composite" for the next picture type. Then select a different "Component" for the remaining two boxes in the grid.
- 2. Color images can be edited: You can change the brightness, contrast, and whether or not to use enhanced contrast, which will sharpen up images that are weakly stained, but will oversaturate strongly stained ones.
- **3.** Components can also be edited: You can change the brightness and contrast as well as toggle the display mode between brightfield and fluorescence.
- **4.** For a composite view, you can click its **Edit** button to select the following settings in the **View Editor**:

- **a.** Show it as "Brightfield" or "Fluorescence."
- b. Choose the Component Display Color: Selecting "True Color" displays each component in its original color (i.e., the color you see when looking through the microscope eyepiece). Selecting "False Color" assigns either red, green, or blue to each of the components, which can often help with the clarity of each displayed component. (Autofluorescence is always displayed in greyscale.)
- **c.** In the **Component Visibility** box, select "*True Strength*" or "*Adjustable*." Selecting "Adjustable" lets you adjust the display strength of each component (i.e., dim overpowering signals and/or brighten weaker signals).
- **d.** The **Reset to Default** button returns the display of any image back to the defaults that were selected when the experiment was created.



Figure 12. View Editor

5. When finished with layout setup, click **Next** to select export settings 20 l.

6.4 Export Settings For The Experiment

Select Export Settings:

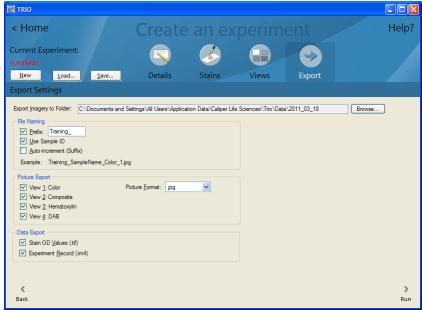


Figure 13. Select Export Settings

Note: TRIO automatically saves all acquired data as Experiment Record (.im4) files in the default TRIO data folder, which was selected during installation of the TRIO software. Experiment records can be opened in TRIO; they contain all of the original imagery data needed to review images. The export settings you select in this window set the defaults —of the current experiment—for exporting *additional* pictures and data to *any* folder of your choice.

- 1. Export Imagery to Folder: Click the Browse button to select the drive location to which you want to export the images and data acquired when you run this experiment. (This can be any location of your choice, including a thumb drive or network folder.)
- 2. Select File Naming options:
 - **a.** Check the **Prefix** box and enter a prefix if you want each exported file name to begin with a prefix.
 - **b.** Check the **Use Sample ID** box if you want to include the sample ID in the exported file names. You will be prompted to enter the sample ID later when you run the experiment.
 - **c.** Check the **Auto-increment (suffix)** box if you want to add a sequential number to the end of each exported image.
- **3.** Select **Picture Export** options: Choose which pictures you want to export, and choose a **Picture Format**. You can select any combination (or none) of the pictures to export.
- **4.** There are two **Data Export** options:
 - a. Stain OD Values: Select this option if you want to save a tif stack of component data for analysis using third party analysis software. In Fluorescence mode, this is called Fluorophore Signal Values and the values are counts normalized for exposure times.
 - **b.** Export Experiment Record (.im4): Every picture TRIO captures gets saved automatically in the TRIO data directory. Select this option if you want to save a duplicate set of images in some other location that you specify. This is useful if you want to send a duplicate copy of your images to a thumb drive or network folder for use at another computer that also has the TRIO software. You can also import im4 files into inForm for further analysis.

6.5 Save the Current Experiment

- 1. Click the **Save** button located on the button bar. Or click the **Run** button at the bottom of the window if you want to save and run the experiment right away.
- 2. In Windows XP, the default directory for saving experiments is C:\Documents and Settings\All Users\Application Data\Caliper Life Sciences\Trio\Experiments. In Windows 7, the default directory is C:\ProgramData\Caliper Life Sciences\Trio.
- 3. Name the experiment and click Save.

7 Run An Experiment

This section provides instruction on how to run experiments. Click on Run an experiment.

If you haven't loaded an experiment yet this session, the file open dialog box opens for you to choose the experiment you want to run. If you just created an experiment, it will load automatically in the **Live** Window [23].

7.1 Live Window

- 1. If you are using dual monitors, you can maximize the **Live Window** on the secondary monitor. If the **Live Window** is closed, clicking the **Live** button will open it again.
- 2. Place the slide from which you want to take pictures on the microscope stage. Choose your objective and focus the image. Make sure you set the objective in the **Live Window** to match the objective you selected on the microscope.

Important Note! When searching the slide, make sure you do not change the lamp brightness, ND filters, Koehler alignment, etc, as this will invalidate the white references that were acquired during microscope setup.

3. Make sure light is diverted to the camera port. The live view of the slide should appear.

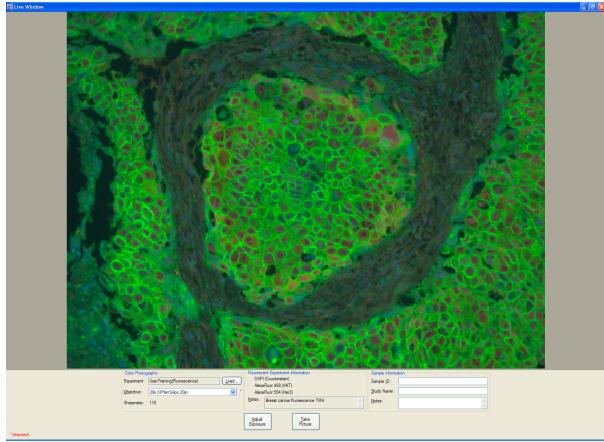


Figure 14. Live Window

- **4.** Make sure the correct **Objective** is selected and enter any desired **Sample Information**.
- 5. In *Fluorescence* mode, if you see saturated regions (areas covered by a red mask) in the image, or the image is very dark, the live view is over- or under-exposed. Click the **Adjust Exposure** button to correct the exposure level. Note that TRIO automatically exposes the real multispectral image regardless of the live view exposure.

7.2 Take Multispectral Pictures

- To take a multispectral picture, click Take Picture. The system will acquire and display a
 multispectral image in the Live Window. It also sends color, composite, and component images
 (based on the preferences you selected earlier) to the image display view.
- 2. If you like this picture, fill in the Sample Information fields and click Keep Picture. Or if you want to try again, click Discard Picture.
- 3. When you keep a picture, the Export Settings 20 window reappears in case you want to adjust the export settings for this multispectral image information.

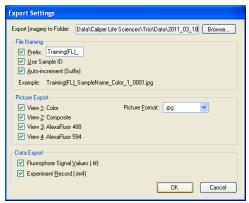


Figure 15. Picture Export Settings

4. When you click **OK** to export, the images are saved and exported to the selected folder. The images are cleared from the **Image Display Window** 24, but the live image remains for you to take more pictures.

7.3 Image Display Window

The Image Display Window can display either "all views" or "single view" of the captured image. In all views, you can see four different representations of the current image. For example, View 1 (upper-left) might show the color (RGB) image; View 2 could show a composite image; View 3 and View 4 could each show a different fluorophore component.

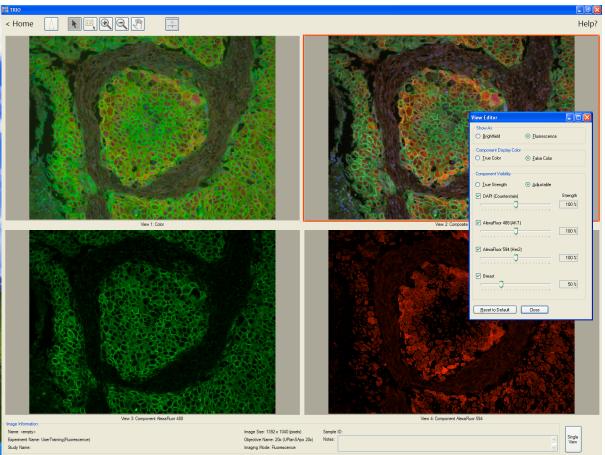


Figure 16. Displayed Images

You can switch any image to single view by double-clicking on the image or clicking the **Single View** button. Cycle through the images by clicking **Next->**. Click the **All Views** button or double-click again to return to *all views*.

If you want to adjust an image's display, select the image and click the View Editor [20] button.

You can also access many image functions by right-clicking on an image. These functions include Save, Copy to Clipboard, Zooming, toggle Single View and All Views, and Adjust View Settings.



Click the **Live Window** tool to display the live window.



Click the **Component Data** tool to examine the signal intensity at specific locations throughout the images. For composite and component images, the signal intensity of each marker is given. For the color image, signal intensity is expressed as Red, Green, Blue.



Click the **View Editor** tool to adjust the display of any image. For composite images, try toggling between the brightfield and fluorescence views for a higher definition view of certain structures, or try switching between true color and false color. For component colors, adjusting the contrast and brightness can help improve the image. And you can always click **Reset to Default** to undo your changes.







Click the **Export** tool if you have modified the image display settings and you want to save the new images. This will save the images as well as a new experiment record.

Click the **Load** tool (available in review mode only) whenever you want to load an im4 file to view images.

The **Zoom** and **Pan** tools can be synchronized in all views mode by holding down the shift key.

8 Take Color Pictures

- 1. If you only want to take a color picture (not a multispectral image), return to the **Home** window and select the **Take color pictures** option.
- 2. The live window opens. Focus the image. Choose the objective you are using.
- 3. You can now click the Take Picture button.
- **4.** TRIO will take a color picture and display it in the **Live Window** and the **Image Display Window** shown below. (If the **Live Window** is closed, you can open it again by clicking the **Live** button.)

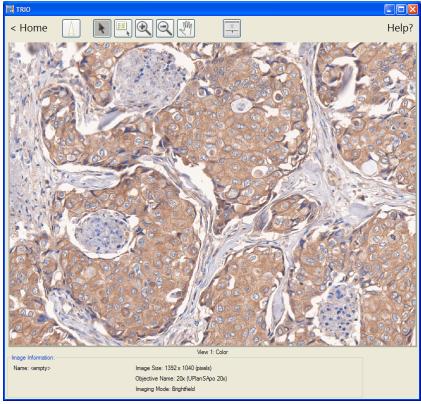


Figure 17. Live Color Photo

- 5. If you like this picture, click Keep Picture in the Live Window. Or if you want to try again, click Discard Picture.
- **6.** When you click **Keep Picture**, the **Save Image As** dialog opens for you to name the file and select its location. The image is cleared from the **Image Display Window**, but the live image remains in case you want to take more pictures.

9 Review Captured Data

- 1. At the Home window, click Review Captured Data to open the Image Display Window.
- 2. In the Select Experiment Record dialog box, locate the experiment record im4 file that contains the images you want to open. (TRIO can only open images that are saved in experiment record files.)
- 3. Select the file and click Open. The images will display in the Image Display Window, just as they were saved.
- **4.** You can adjust and export the images that you see.

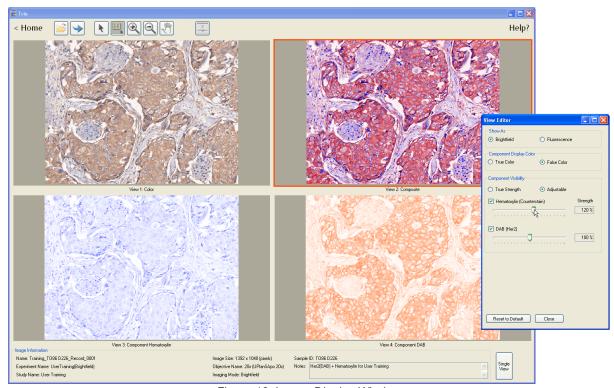


Figure 18. Image Display Window

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