LanthaScreen® Europium/Adapta® Assay Setup Guide on the PerkinElmer EnVision® Multilabel Reader

IMPORTANT INFORMATION

Test your plate reader set-up before using LanthaScreen® Terbium and Europium assays

We have developed two technical notes which provide a method for verifying that a fluorescent plate reader is able to detect a change in time-resolved fluorescence energy transfer (TR-FRET) signal, confirming proper instrument set-up and a suitable response. The method is independent of any biological reaction or equilibrium and uses reagents that are on-hand for the LanthaScreen® assay.

For complete instructions, visit www.lifetechnologies.com/instrumentsetup and click on "Download Terbium assay application note" or "Download Europium assay application note".

The PerkinElmer EnVision® Multilabel Reader was tested for compatibility with Life Technologies LanthaScreen® Kinase Binding and Adapta® Europium-based TR-FRET assays. The following document is intended to demonstrate setup of this instrument.

For more detailed information and technical support of Life Technologies assays including specific conditions for assay windows between 2-3 fold, please call 1-800-955-6288 and enter extension 40266 or email drugdiscoverytech@lifetech.com.

For more detailed information and technical support of PerkinElmer instruments or software, please call 1-800-762-4000 or by e-mail at productinfo@perkinelmer.com .

A. Recommended Optics

Life Technologies part number	Wavelength (nm)	Diameter (mm)
Excitation Filter (PV00215)	340/30 (or similar)	15
Emission Filter 1 (PV00315)	615/8.5	15
Emission Filter 2 (PV00315)	665/7.5	15
Dichroic Mirror *contact PerkinElmer	D400 or D400/D630 (LANCE or LANCE Dual)	

Note: Before beginning, you will need to install the correct filters in an appropriate slot. The slot number is not critical but the filters do need to be next to each other for the dual read. If they are not, the machine will give you an error message to switch them.

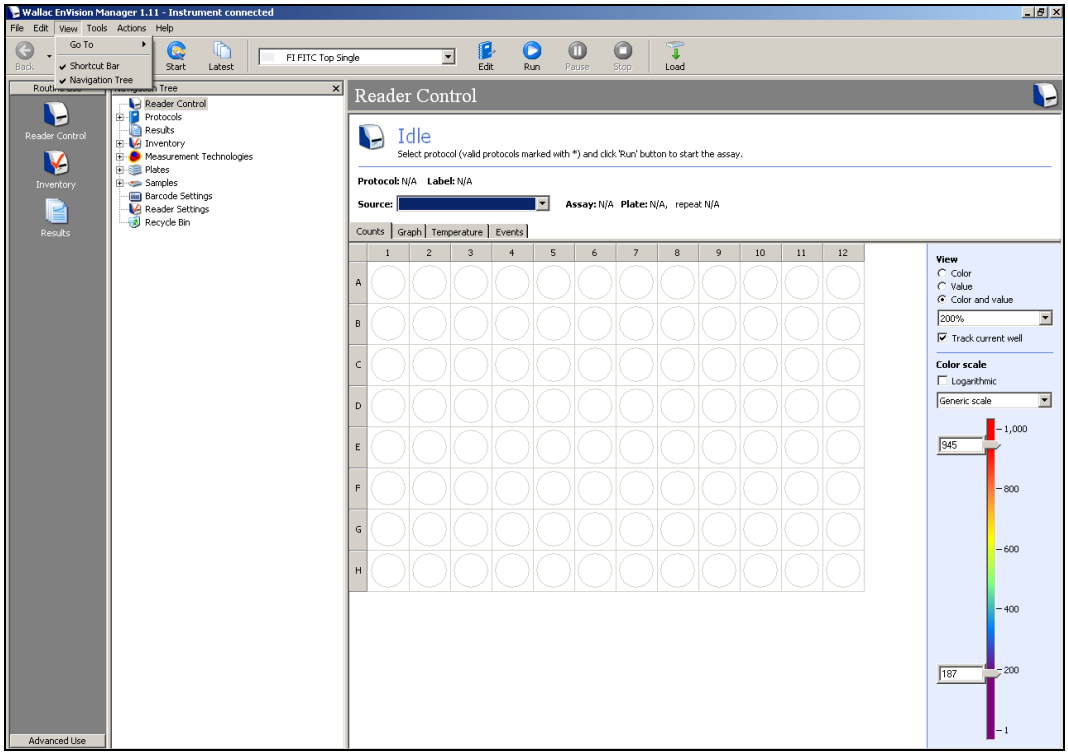
Note: When using laser-based excitation with the pre-installed 337nm laser, you will not need the excitation filter listed. However, you will also need a “Bias” dichroic mirror compatible with the laser.

Note: The PerkinElmer EnVision® software already contains programs for LANCE and other Eu-based TR-FRET assays. This document is intended to demonstrate how to set up an optimized protocol for Life Technologies's assays. Suitable results may be obtained by simply running the pre-defined LANCE program, however the following optimized setup conditions may provide an improvement in assay window over the default LANCE settings.

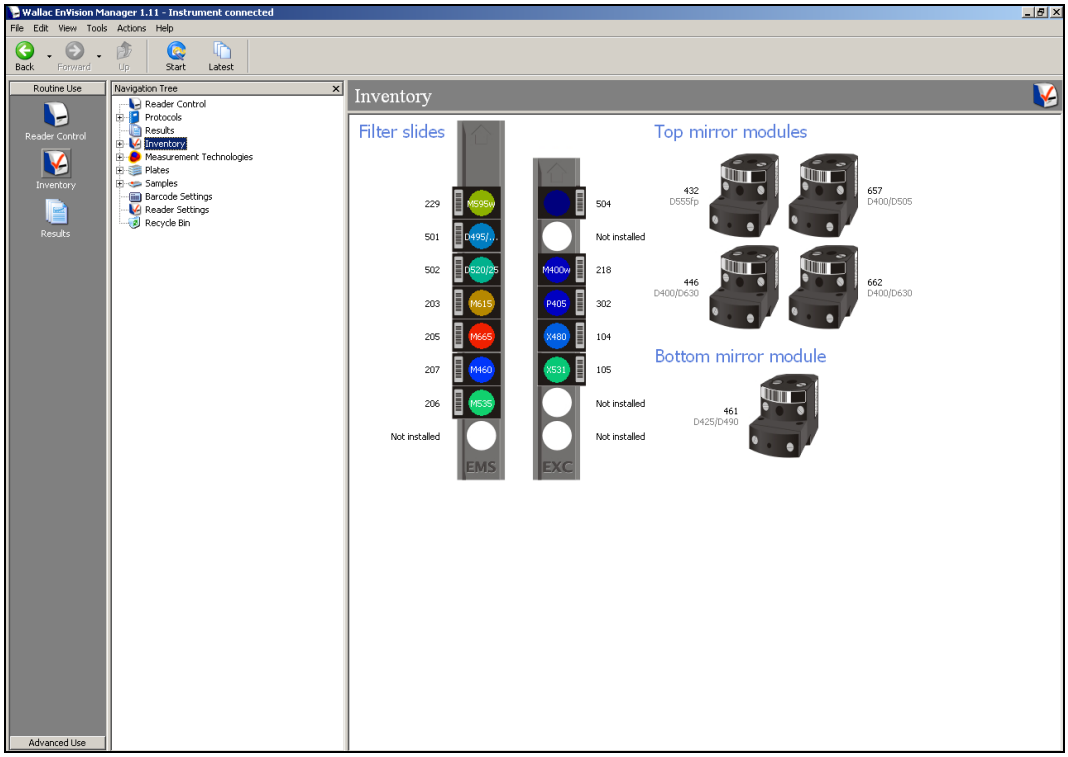
B1. Instrument Setup: Filters

Note: If your instrument does not already have Europium filters and definitions present, you may need to add them as follows. If not, skip to Section B2. Filters may be purchased from PE.

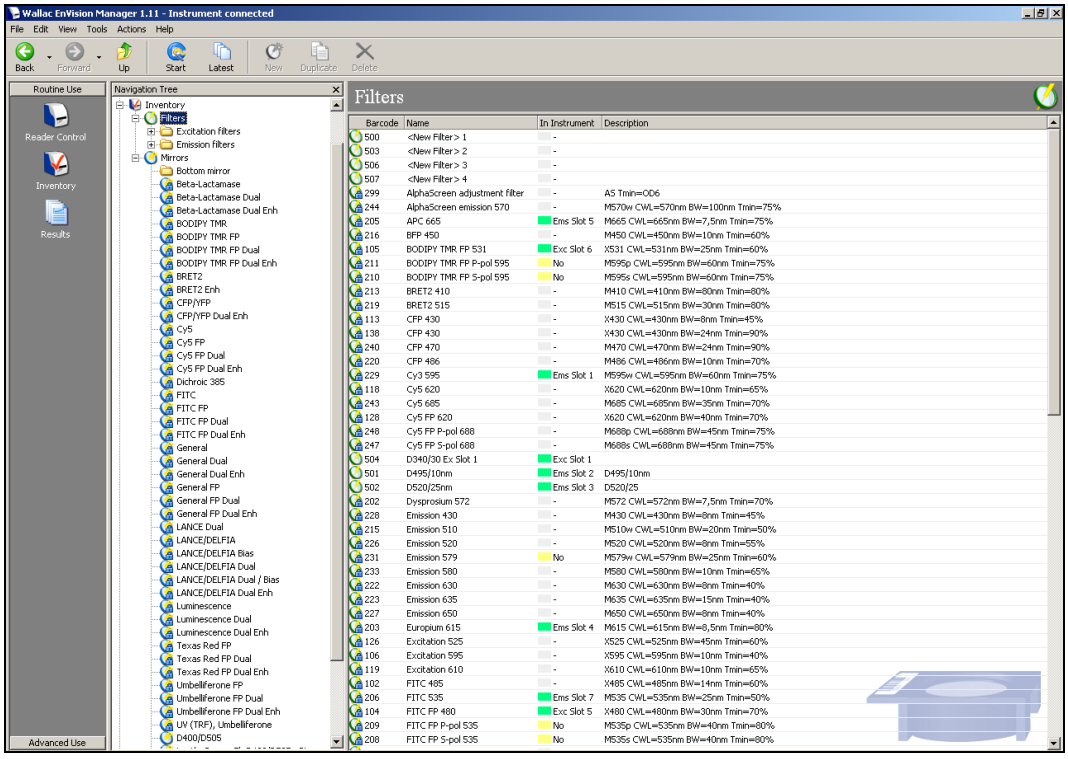
1. Make certain plate reader is turned on, and open up Wallac EnVision® Manager software on computer. **Note:** You may have to go to the "View" tab at the top and select both "Shortcut Bar" and/or "Navigation Tree" to make sure both options are open to the left of the Reader Control window.



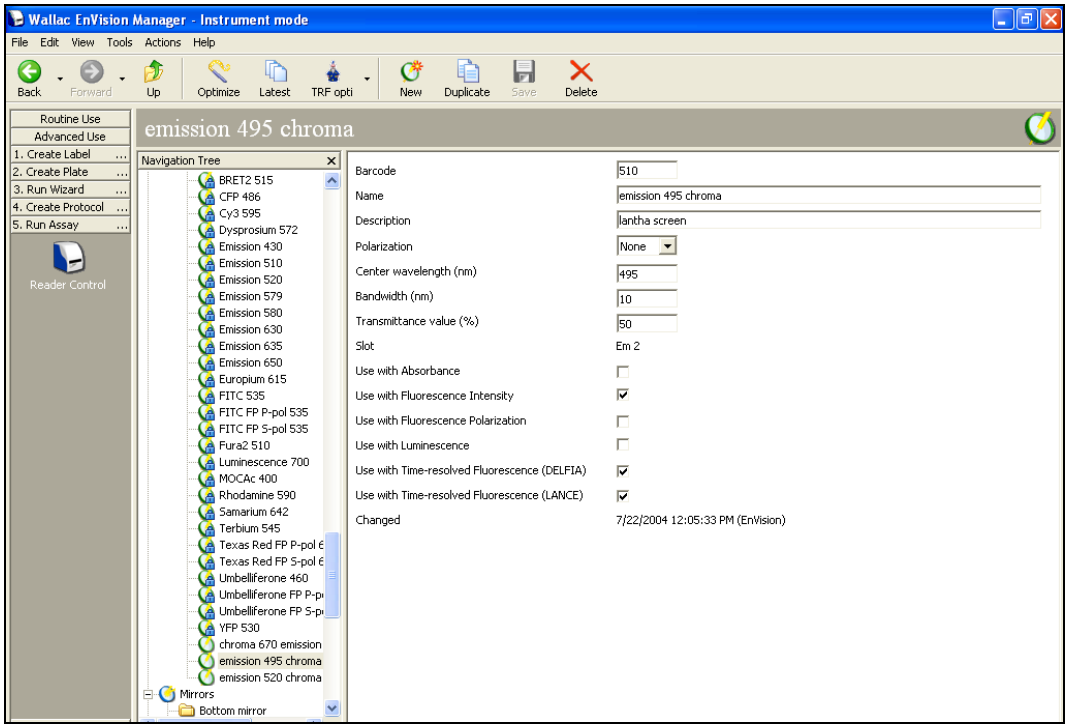
2. Double-clicking on the "Inventory" icon at the far left will open the following window, where you can view the filters currently available in your reader. If you do not have the appropriate filters or dichroic mirror, you can change them as described below. **Note**: PE filters and mirrors are bar-coded and recognized by the machine, and can be simply added/removed. Non-PE filters of the correct size can be placed in PE filter holders and then need to be identified for the instrument.



3. If filters need to be added, add as follows: Click on the "Filters" tab in the Navigation Tree menu. This will open the folder. Select "Excitation filters" or "Emission filters" and change as shown in Step 4. If the correct filters are present, proceed to section B2 of this document.

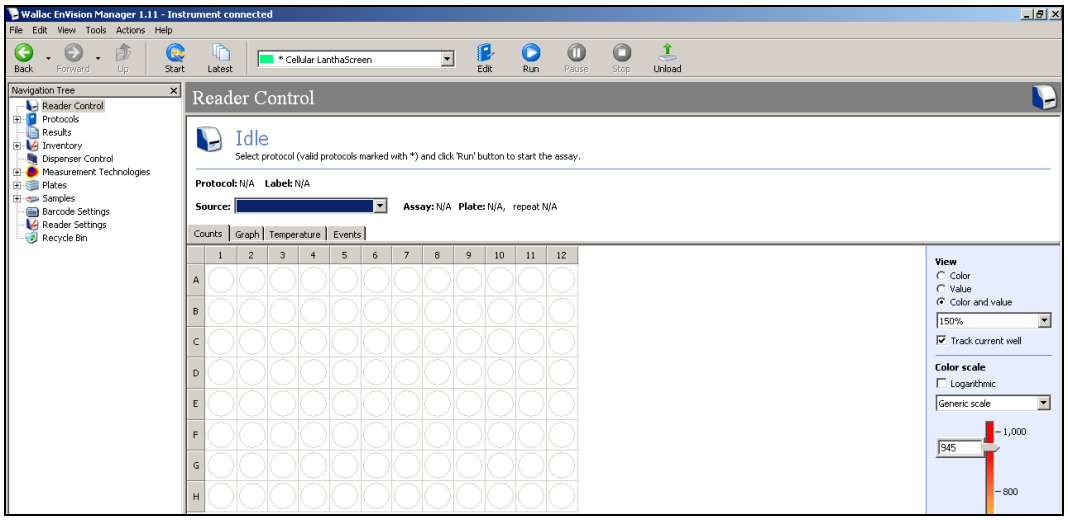


4. In the Navigation Tree area, scroll down to "Filters, emission." Select "New" from the tabs at the top of the screen and enter the filter settings as shown in the example screen shot below. **Note**: The barcode may differ, depending on the filter holder you are using. Do this for any filters that need to be added, or for the dichroic by repeating this step in the "Mirrors" tab shown in Step 3.

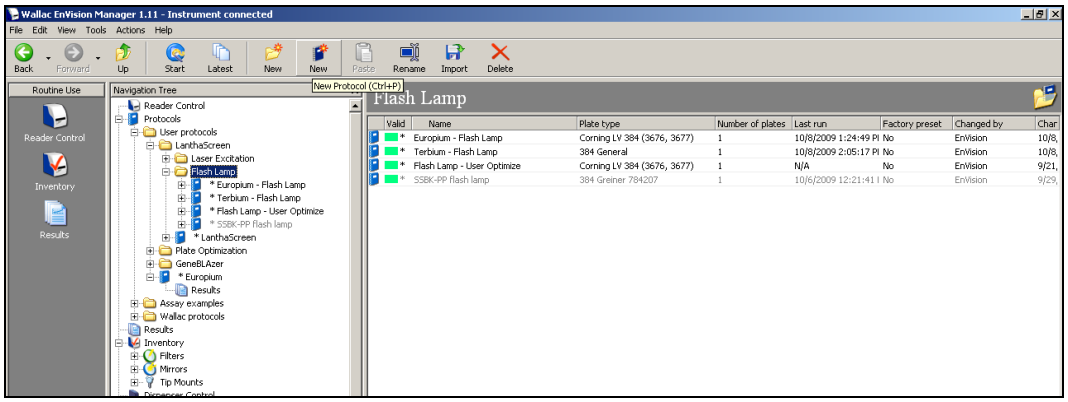


B2. Instrument Setup

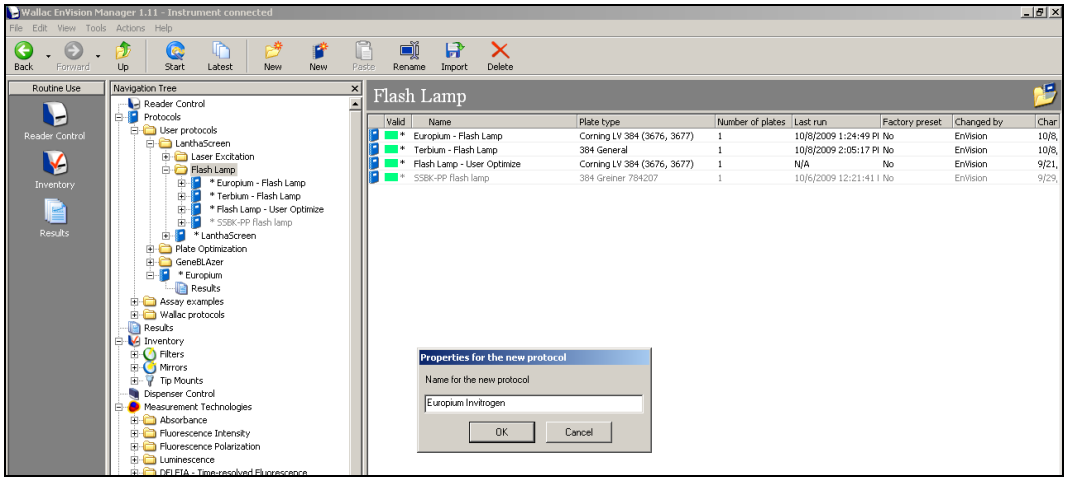
1. When Wallac EnVision® Manager opens, select "Protocols" in the "Navigation Tree" menu near the upper left corner of the screen.



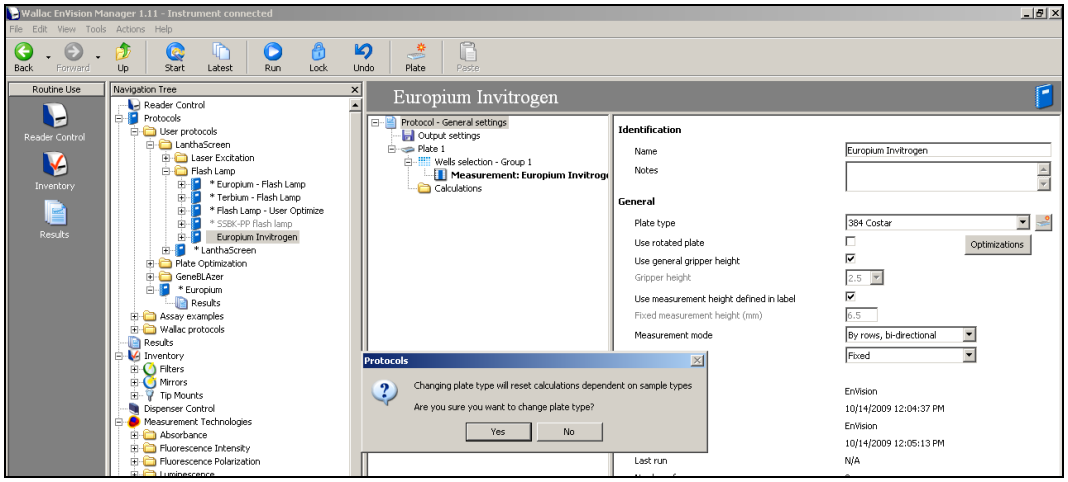
2. At this point, a protocol list will open. Open the “User Protocols” folder. Click on the blue “New Protocol” button on the toolbar at the top of the screen (second “New” button).



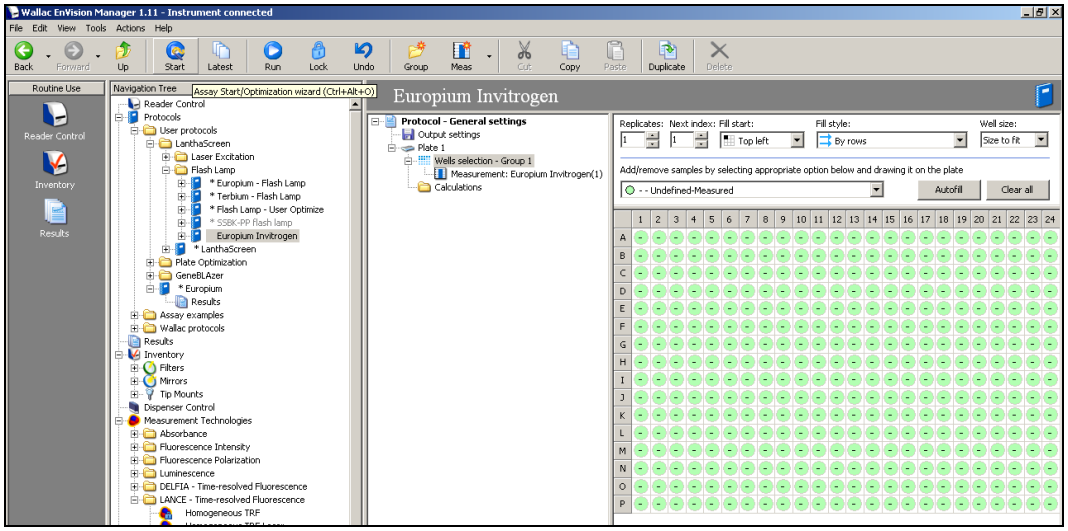
3. Follow the prompt and enter a name for the protocol, then select "OK". **Note**: Because the protocol is empty, you will initially get an error message stating that the operation is invalid. This is ok. Proceed to Step 4.



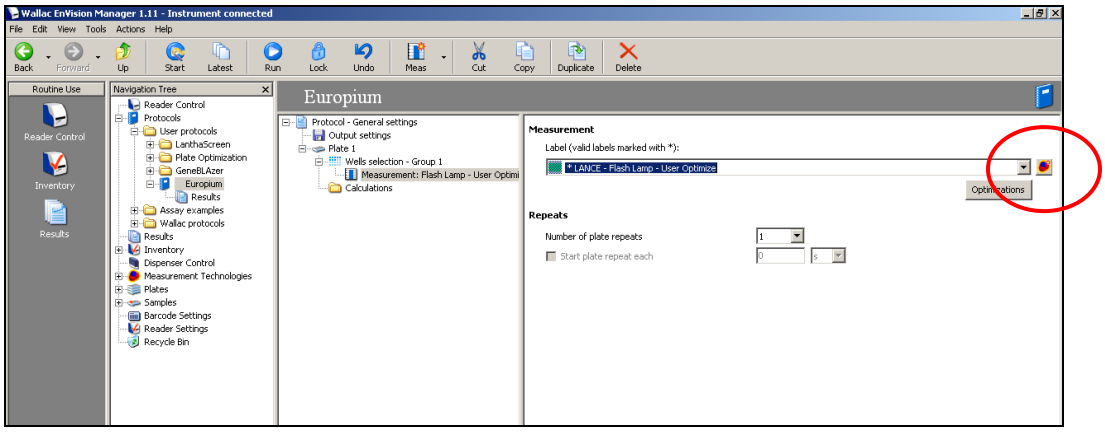
4. Your new protocol will open automatically. From the new Protocol menu near the center of the screen select "Protocol General settings". Choose your plate type. **Note**: For this protocol we selected "384 Costar." A warning prompt may appear, confirming that you wish to change plate type. If this appears, select "Yes".



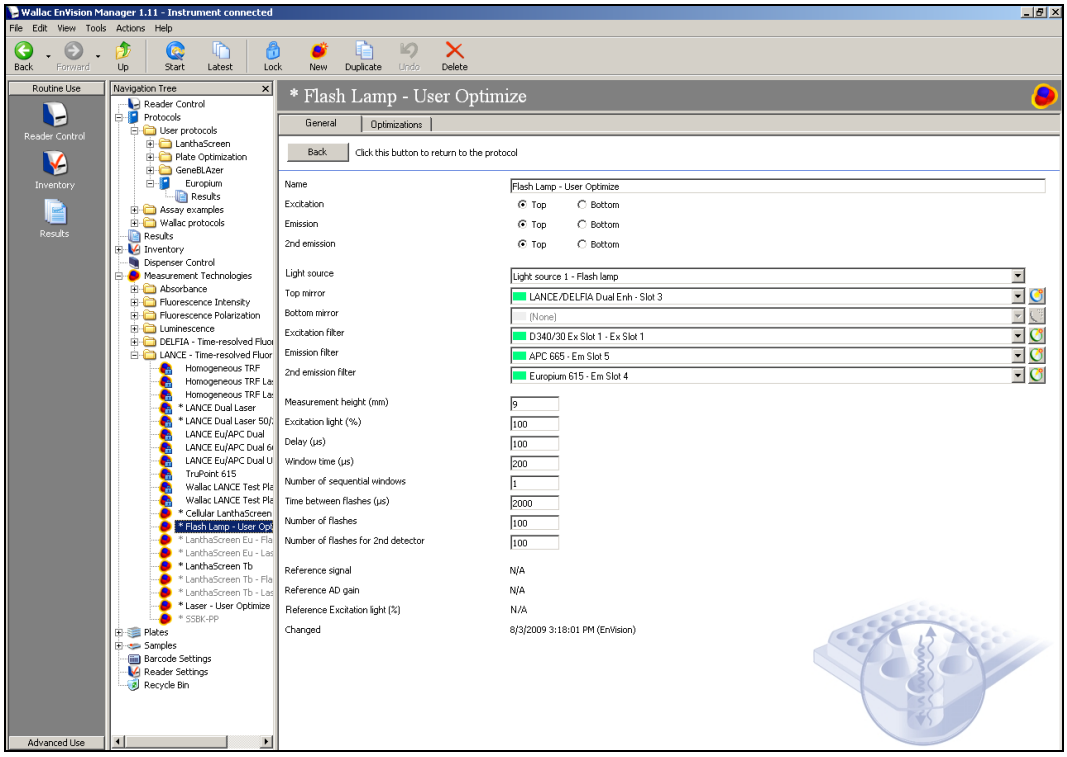
5. Now select "Well Selection". A new screen will appear with a plate schematic, allowing you to use the drop-down toolbar to define your wells as needed. **Note:** in this example all wells are simply selected for reading and controls for auto-subtraction are not indicated.



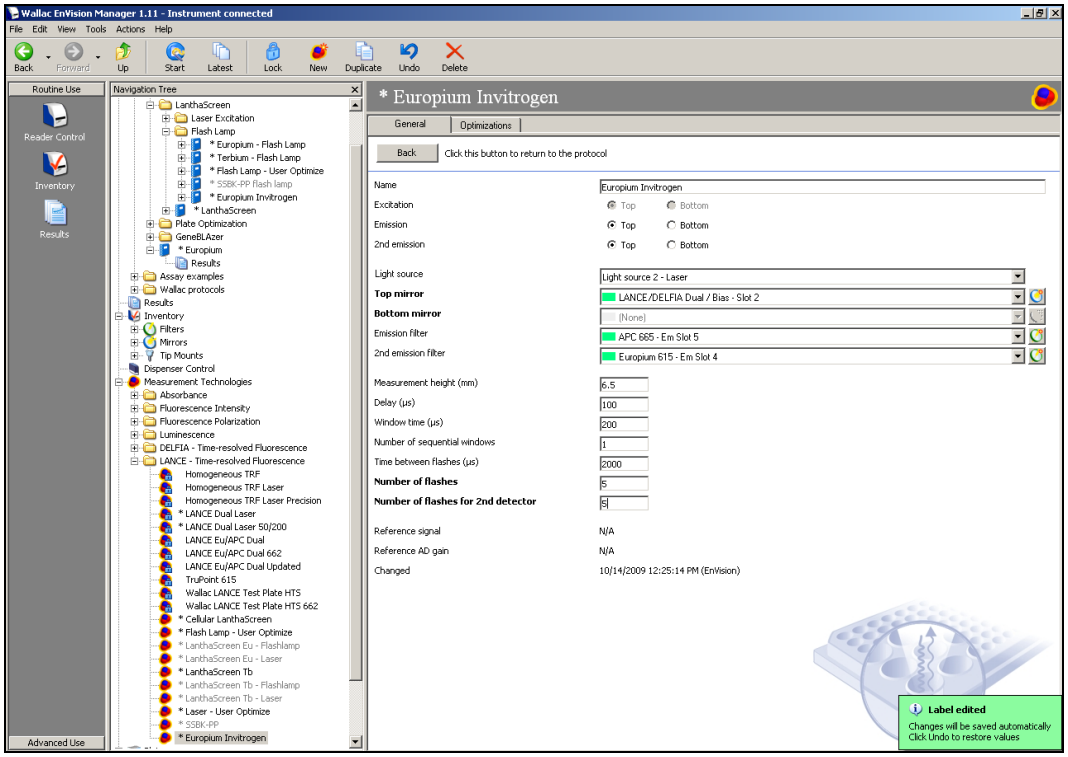
6. Select "Measurement" in the Protocol window, and select the correct filter set. Select the small icon to the right of the drop-down assay list to do this (circled).



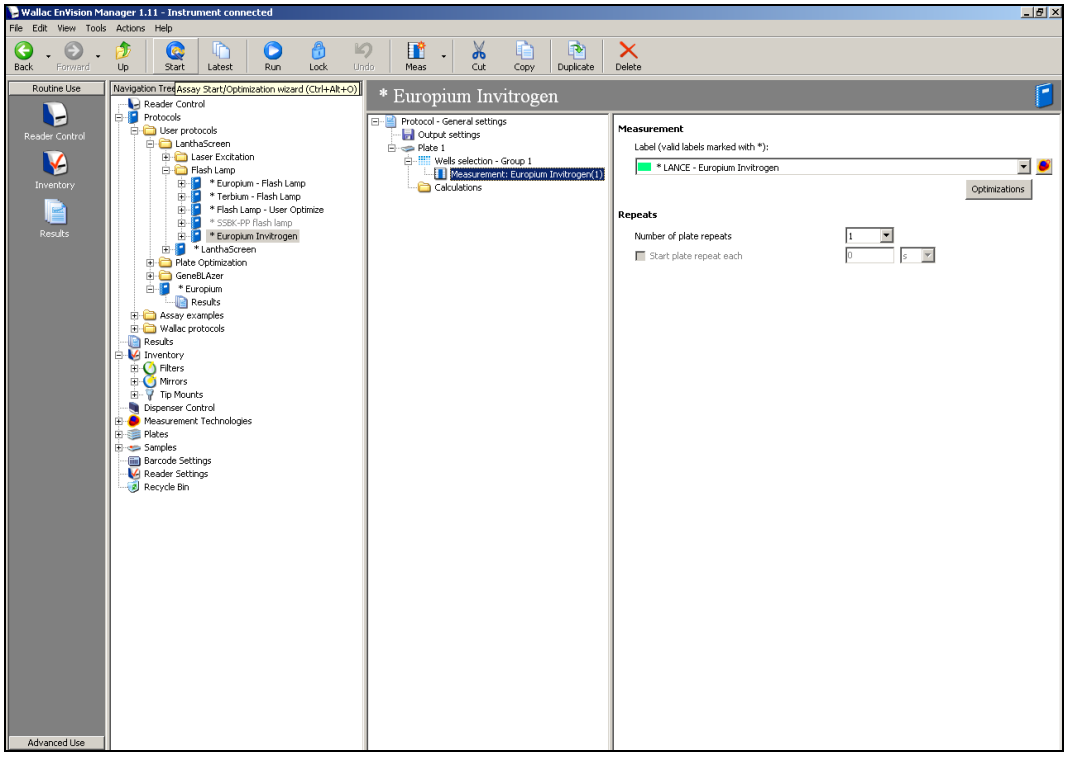
7. Select your labels for Light-Source, op Mirror, Excitation Filter, Emission Filter, and 2nd Emission Filter from the drop-down tabs as shown below. Set Delay, Window, and Number of Flashes as below also. When finished click the "Back" tab to return to your protocol.



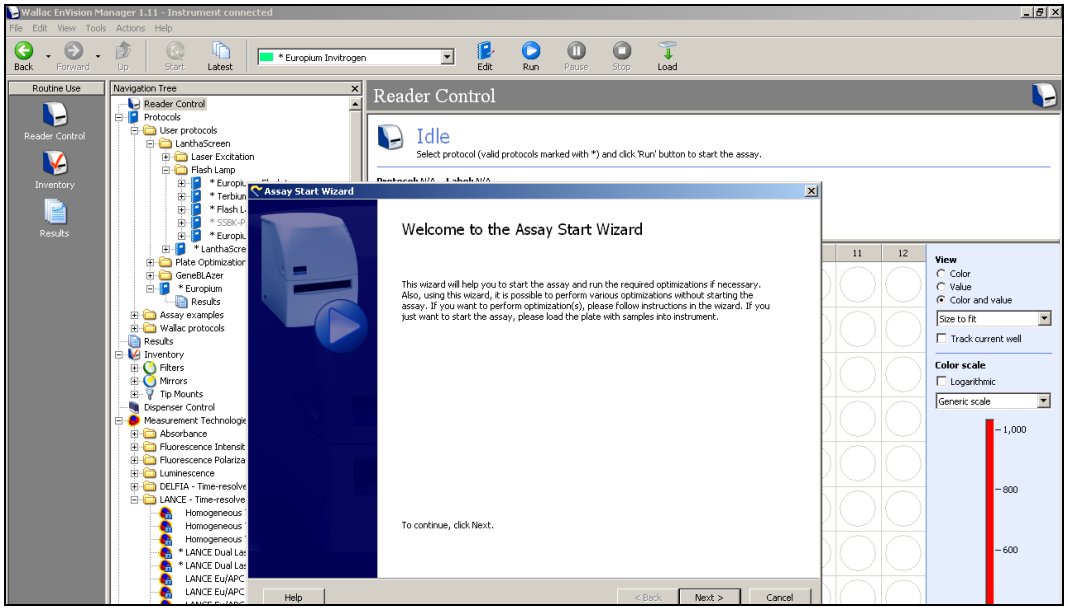
7B. PerkinElmer offers a 337nm laser for Europium excitation as well. If your instrument is equipped with a laser, it generally offers a significant improvement in assay window to use this. From the drop-down options, select the laser for your light source, and make certain to select the BIAS mirror for this application, as the laser only works with the bias mirrors. Again, select the "Back" tab when finished to return to the original protocol.



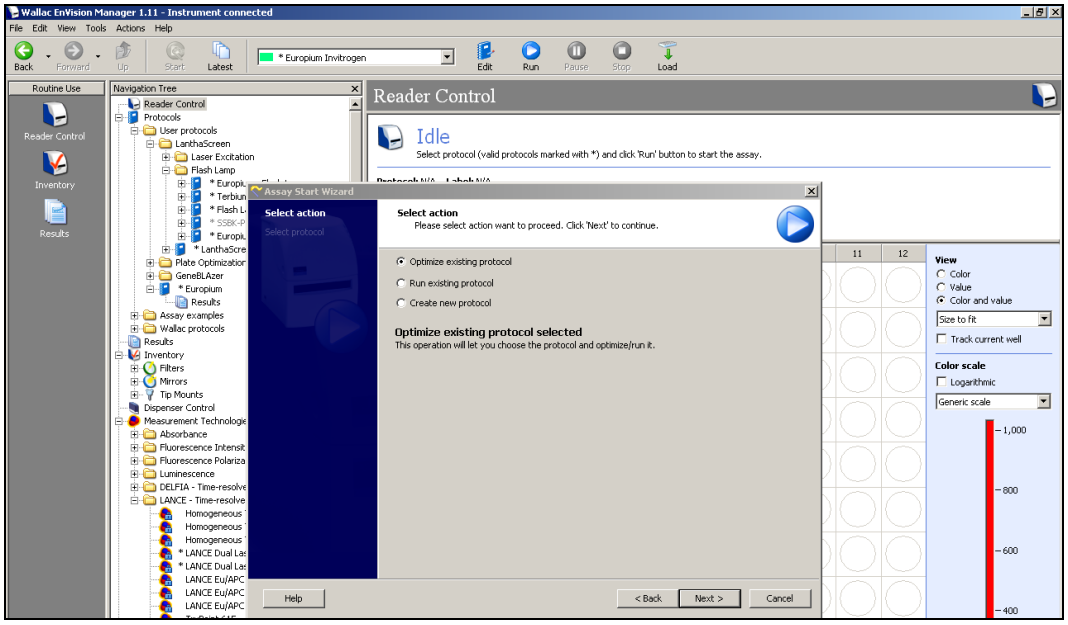
8. When finished, select the "Start" button at the top of the screen. This will take you through the optimization wizard. **Note:** Selecting "Run" will run the assay directly, without optimization. We recommend optimizing before running the assay.



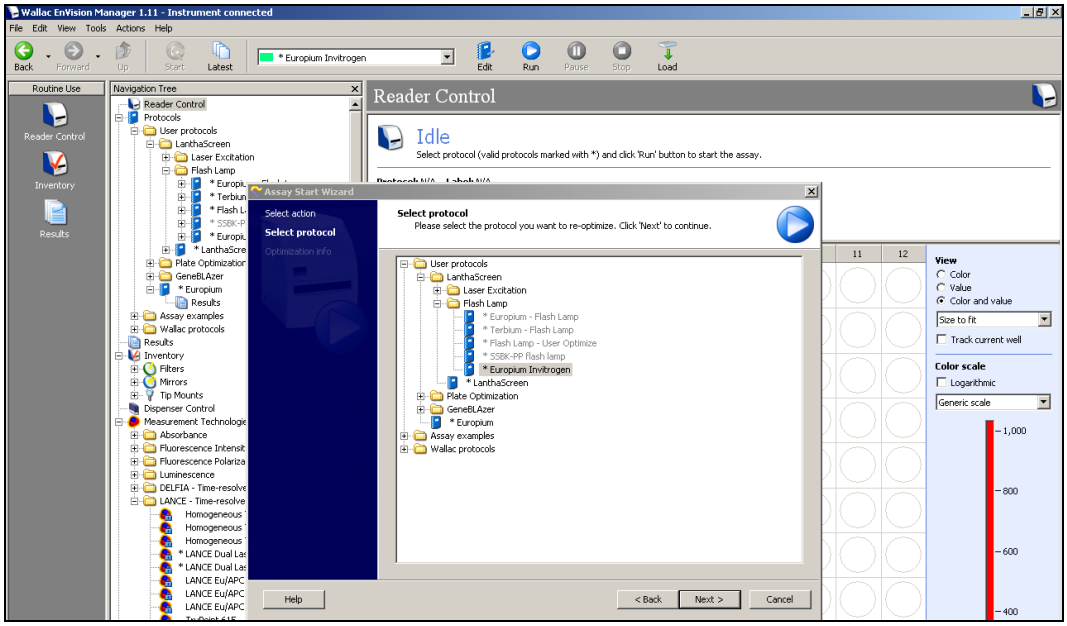
9. The Assay Start Wizard will open automatically. Select Next.



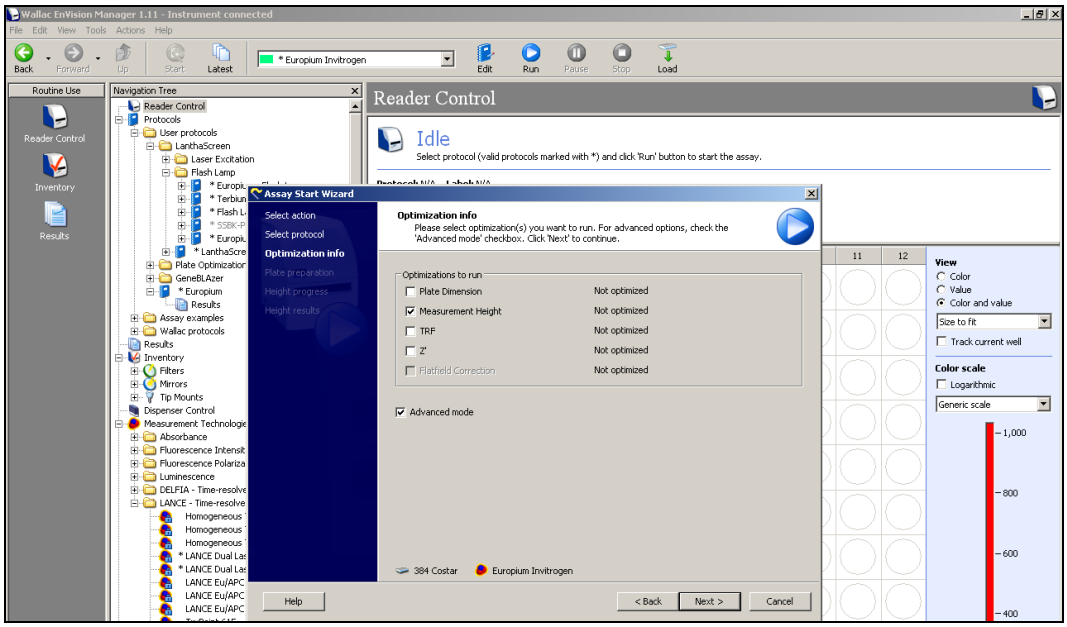
10. Select "Optimize Existing Protocol," then select Next.



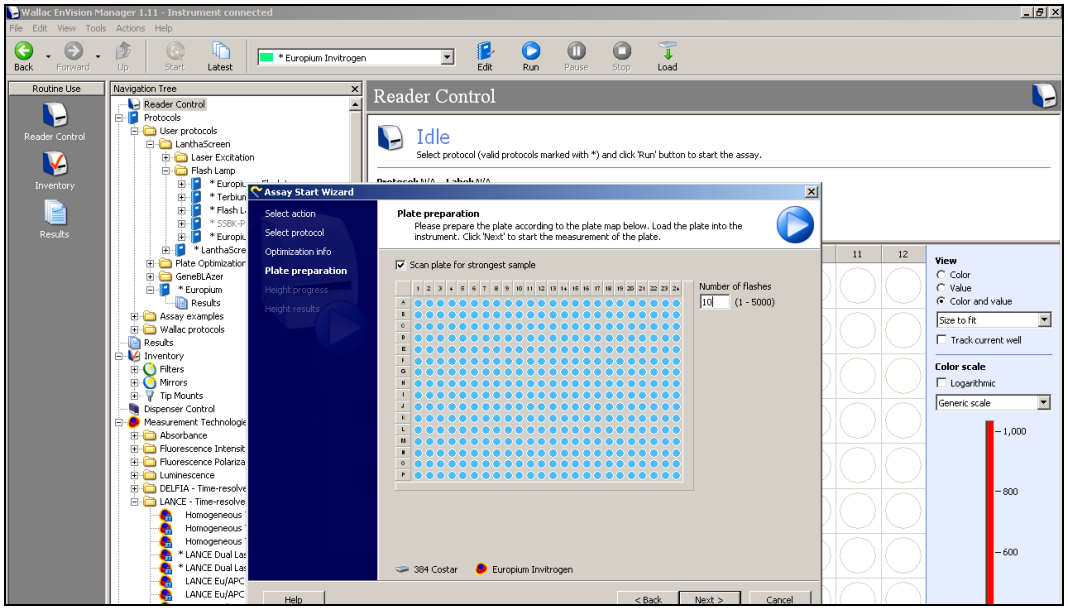
11. Select the protocol you wish to optimize and again select Next.



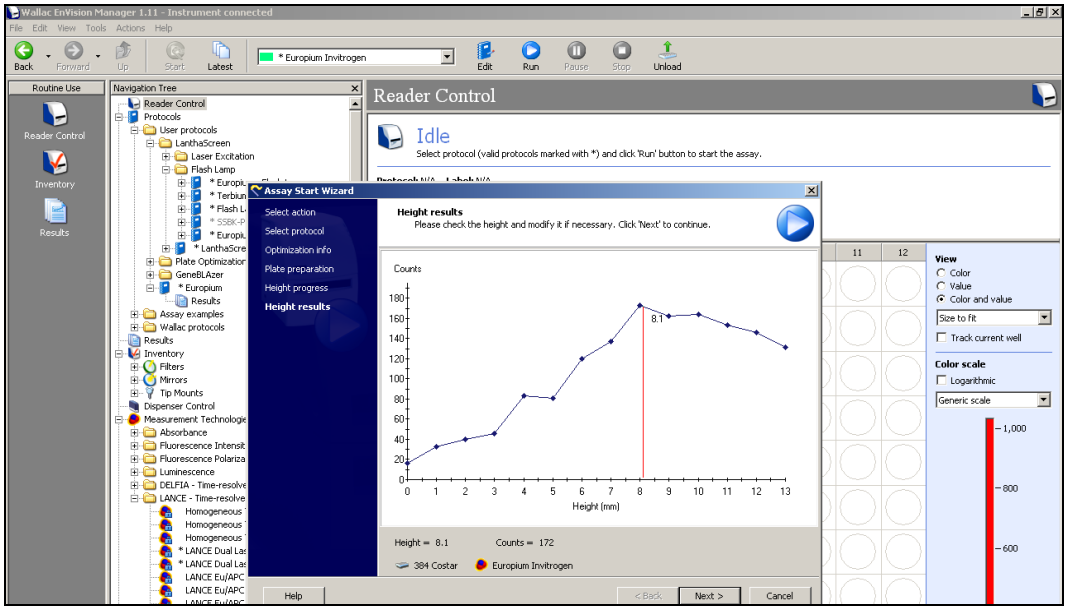
12. In this case we only chose to optimize Measurement Height. Check the boxes next to Measurement Height and Advanced mode then click Next.



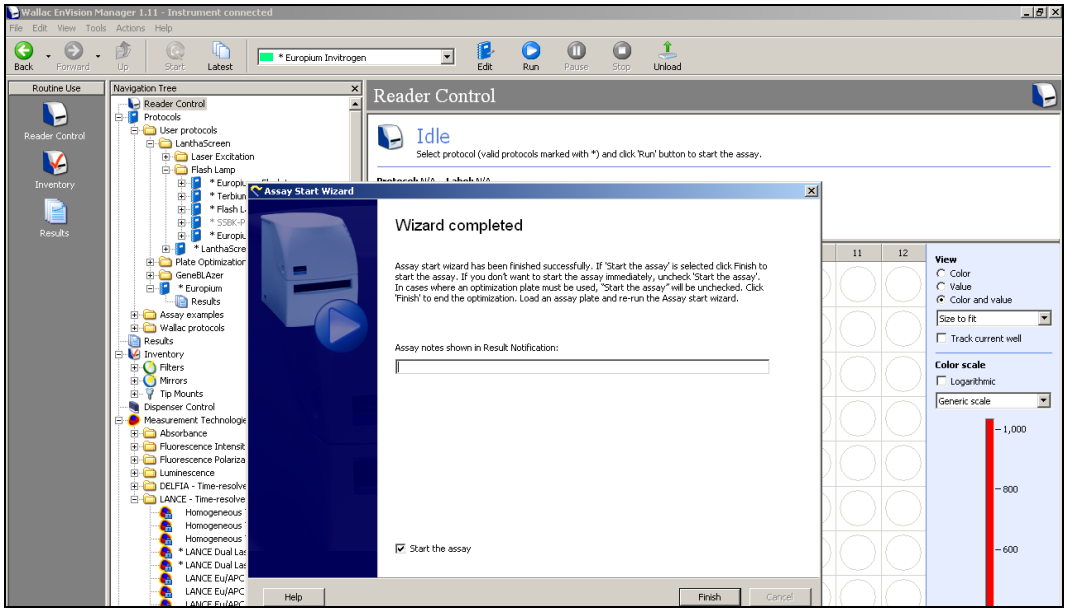
13. Check the "Scan plate for strongest sample" button and click Next.



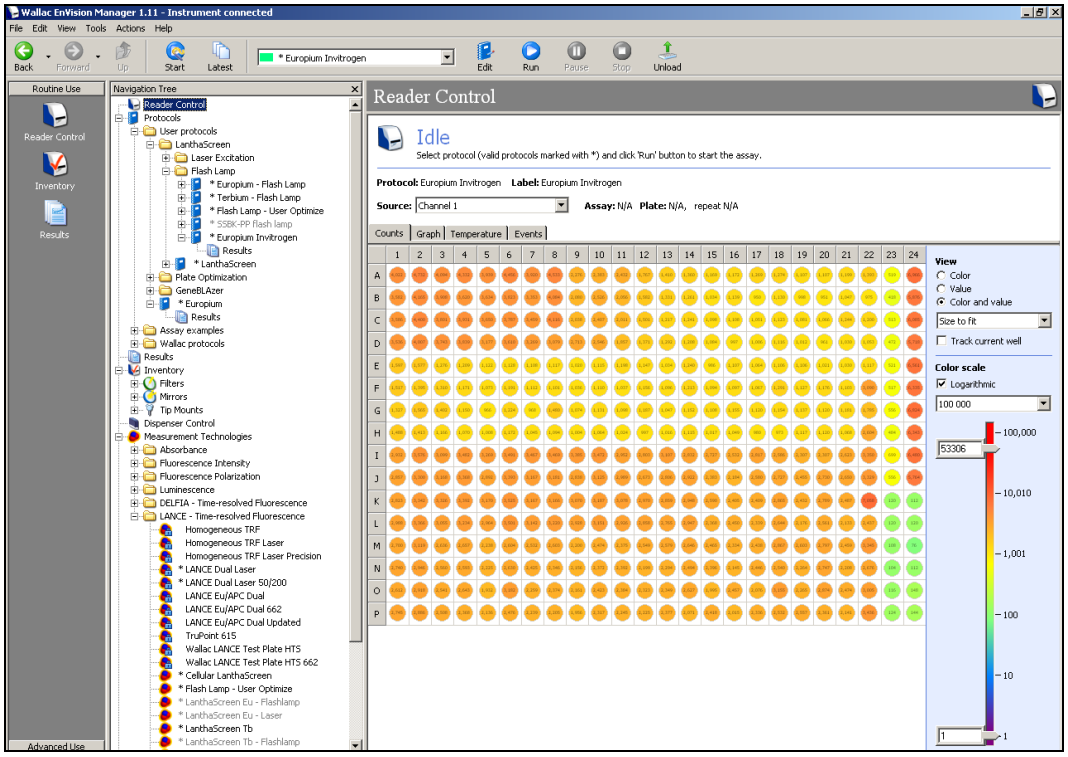
14. The Wizard will select a well and optimize focal height. When finished, it will present a graph as shown below. Select Next, or if needed, move the red line to a suitable spot under the curve where maximal signal intensity is observed and then click Next.



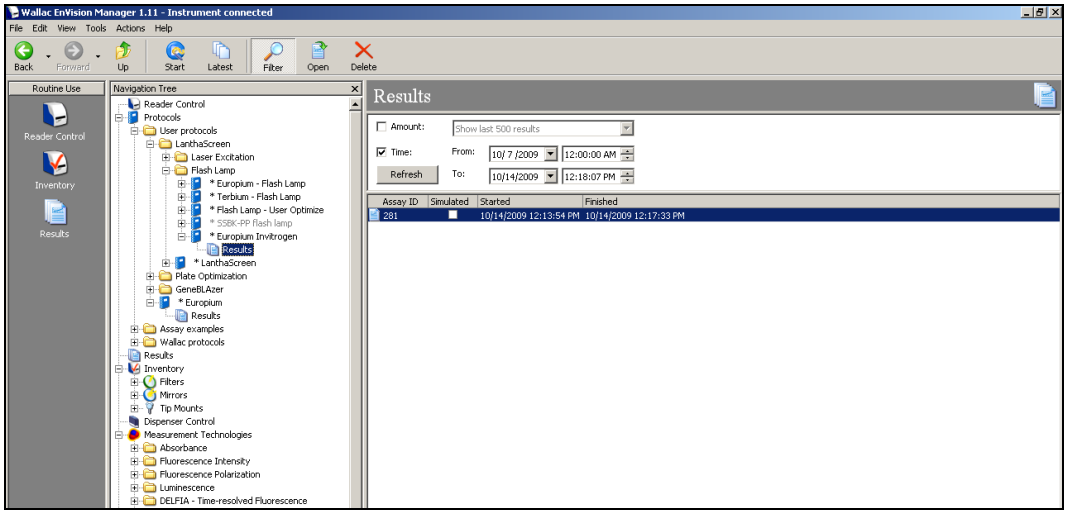
15. The Wizard is now completed. Make sure the "Start the assay" box is checked, and click on Finish to read the plate.



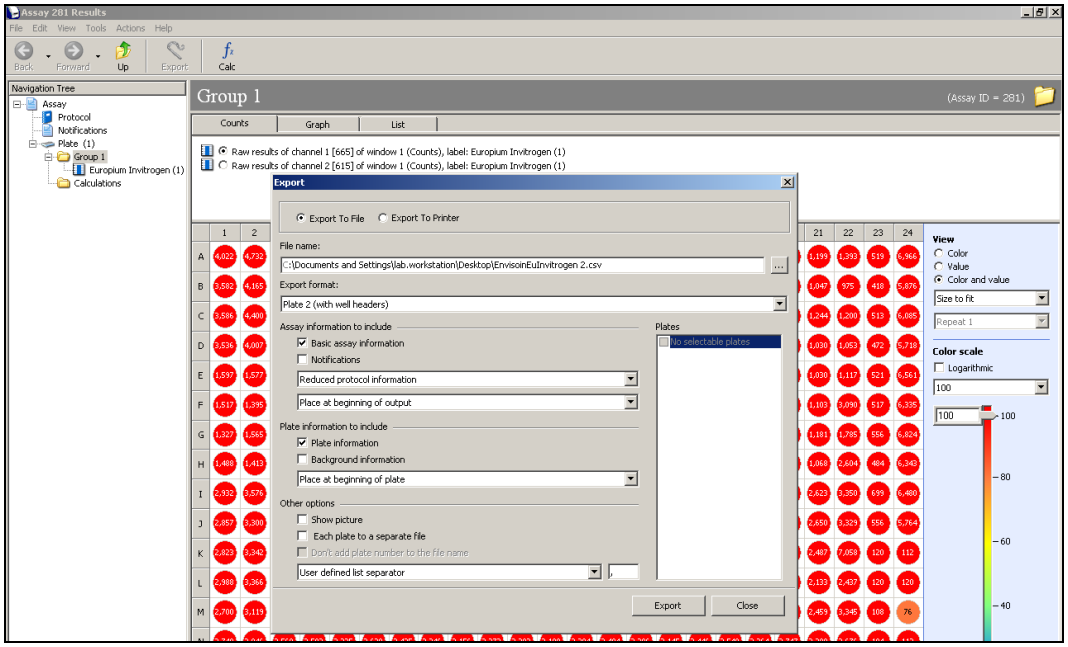
16. The EnVision® will read the assay plate. During the read, a hot map of the assay wells is shown as below.



17. When the assay is finished, find the protocol you prepared under the Navigation tree on the left side of the screen. Below it there should be a "Results" tab. Clicking on the Results tab will take you to the screen below. Click on the assay run (in dark blue) to open.



18. The results tab will open. You can export your data and analyze at this time.



C. Results

Acceptor (nM)	TR-FRET Ratio	RR	Z'
800	0.039	4.22	0.83
400	0.026	2.76	0.76
200	0.018	1.94	0.59
100	0.013	1.45	0.22
25	0.009		

Table 1. LanthaScreen® TR-FRET testing on the PerkinElmer EnVision®. Data obtained from running the diffusion-based TR-FRET instrument test available at Life Technologies Instrument Portal (www.lifetechnologies.com/instrumentsetup) under "Download Europium assay Application Note." Ratios obtained, response ratio (RR = ratio at a given high concentration of acceptor divided by the TR-FRET ratio obtained at 400nM acceptor), and Z' values at each concentration are shown.