Setup for LanthaScreen® Europium Assays on SpectraMax® Paradigm® Microplate Detection Platform with SoftMax® Pro 6 software

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 Molecular Devices SpectraMax® Paradigm® Microplate Detection Platform 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 and provide representative data.

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 Molecular Devices instruments or software, please contact Molecular Devices at 1-800-635-5577 or www.moleculardevices.com.

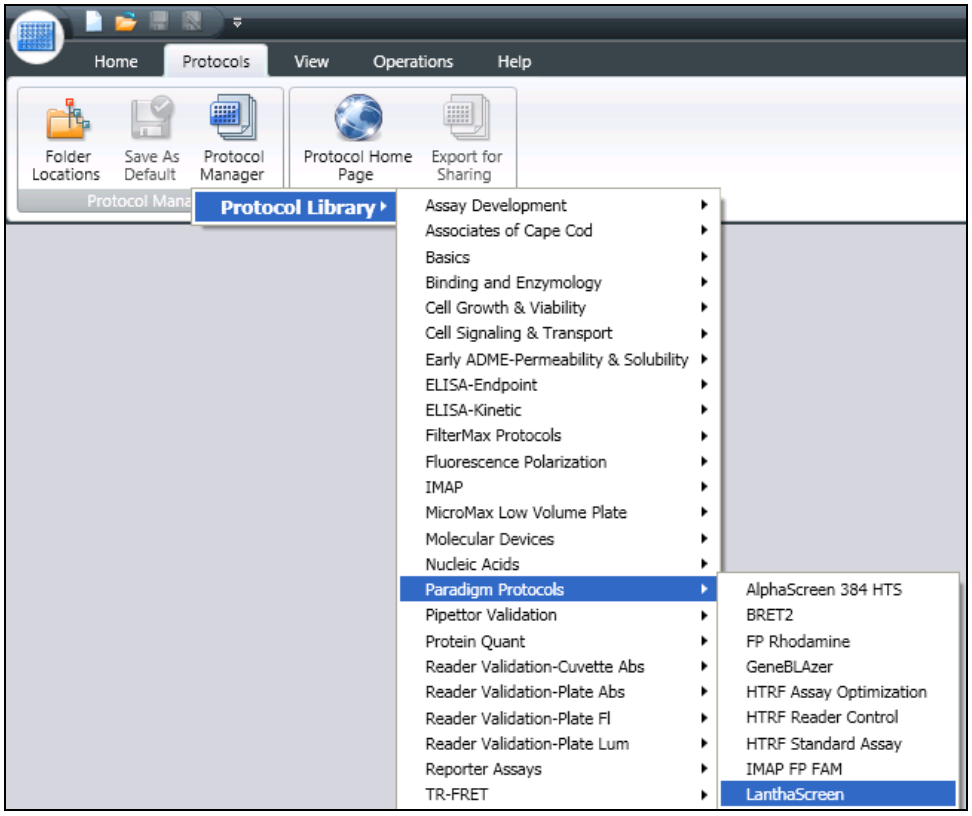
A. Recommended Optics

Parameter	Specification
Detection Cartridge Name	SpectraMax® Paradigm® HTRF Detection Cartridge
Part Number	0200-7011
Detection Technique	TR-FRET
Light Source	Xenon flash lamp
Filter Set	EX: 340 EM1: 616/10 EM2: 665/10

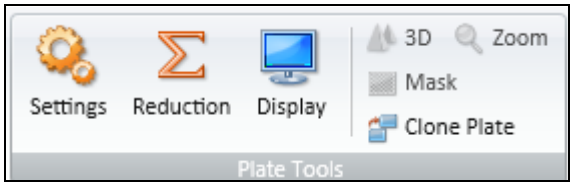
Note: LanthaScreen® Eu assays use the Molecular Devices HTRF filter cartridge described above. Make sure the HTRF detection cartridge is installed in the *top* cartridge drawer of the SpectraMax® Paradigm®.

B. Instrument Setup

1. Open SoftMax® Pro 6 software. Click on "Protocol Manager" to open the Protocol Library. Within the "Paradigm® Protocols" folder, locate the "LanthaScreen®" protocol and click to open.

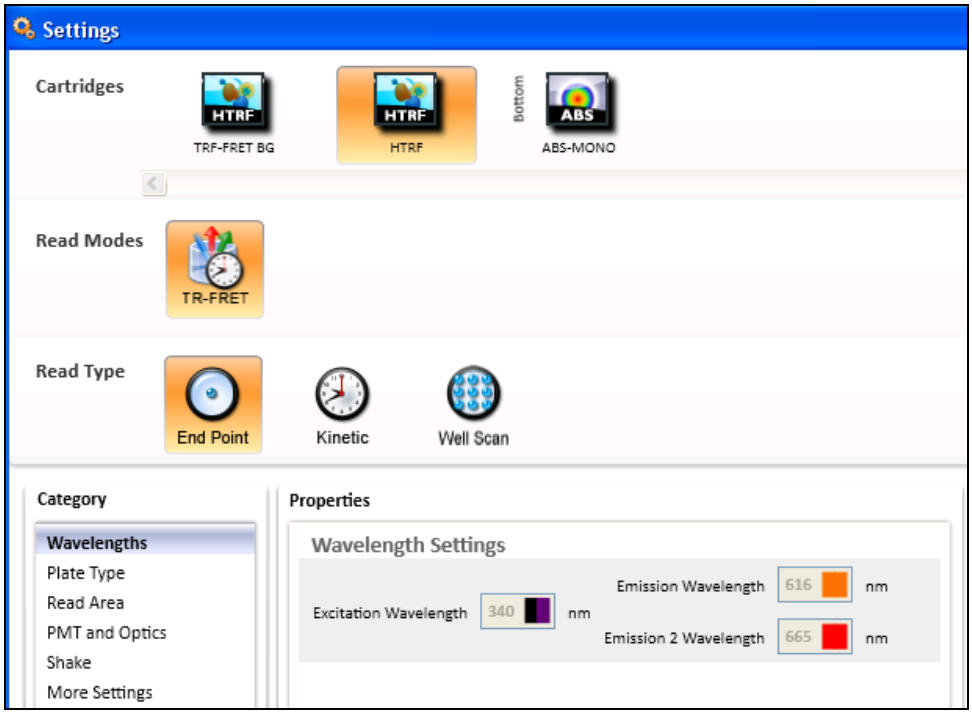


2. Click on "Plate01" in the Navigation Tree on the left side of the screen. Click on the Settings icon either in the toolbar at the top of the screen…

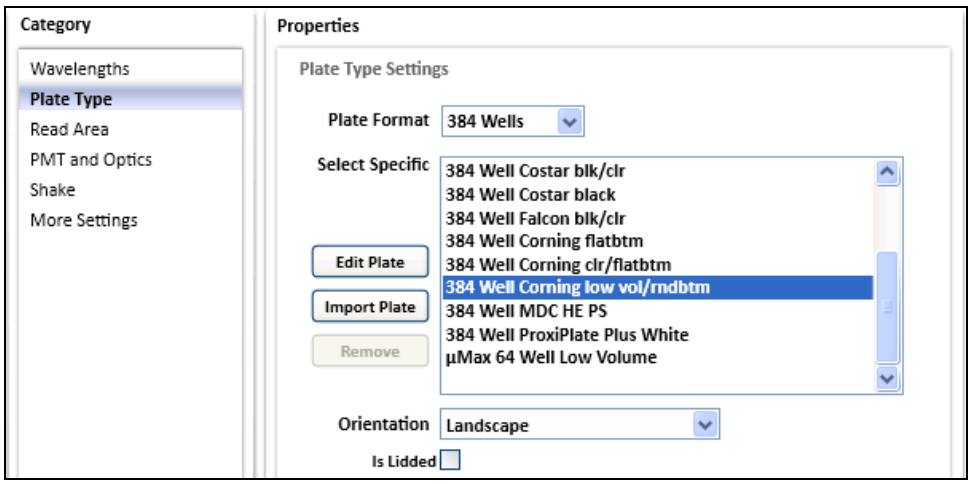


…or in the plate section header.

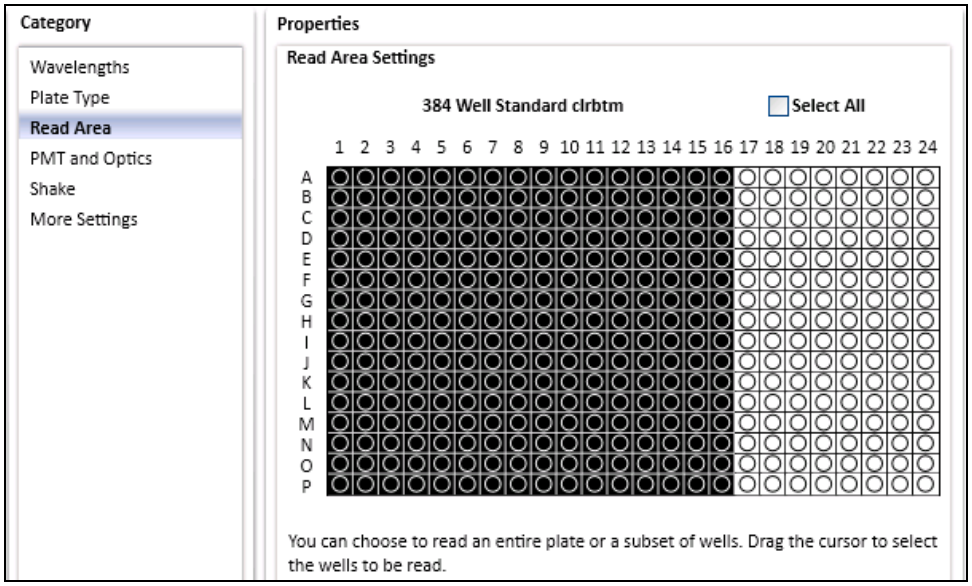
3. This opens the Settings window. Select the "HTRF" cartridge and TR-FRET read mode with End Point read type (already selected in the pre-configured LanthaScreen® protocol).



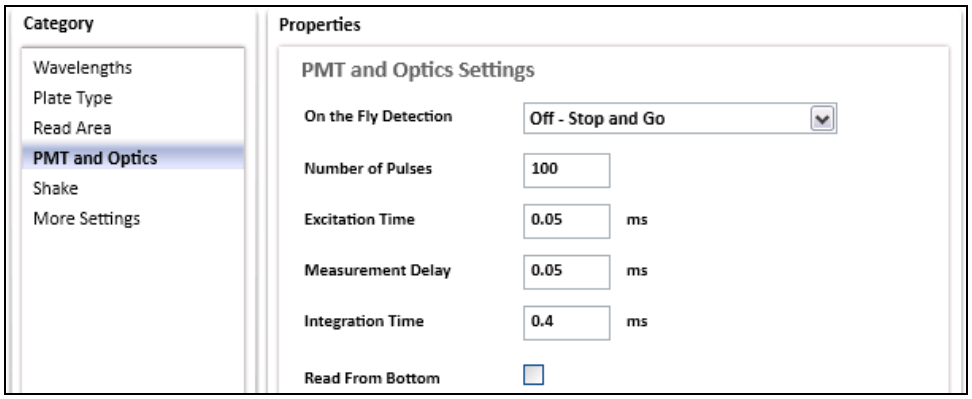
4. Choose the desired plate type, using the upper dropdown menu to choose plate format (96, 384, or 1536 wells) and the "Select Specific" menu to choose the specific plate type.



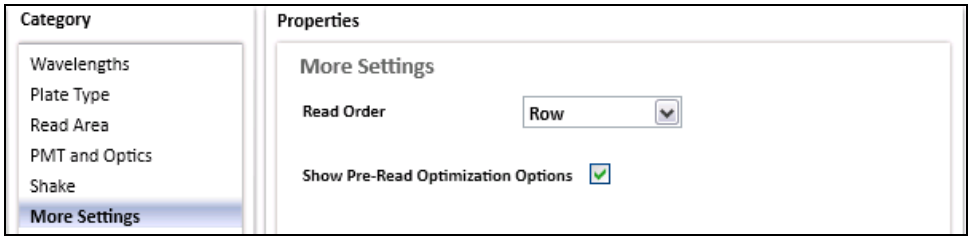
5. Now select the area of the plate to read.



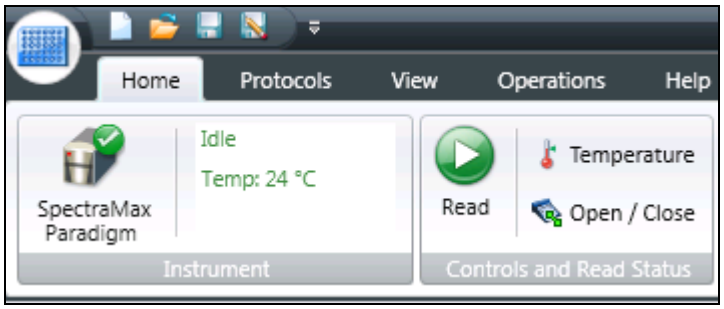
6. Select PMT and Optics settings. Optimized settings are 100 pulses, 0.05 ms excitation, 0.05 ms delay, and 0.4 ms integration (already selected in the pre-configured LanthaScreen® protocol). To select On the Fly for faster read times, use the dropdown menu to choose from Performance or Speed (faster) On the Fly options.



7. In "More Settings", choose the read order corresponding to how the assay plate is set up. If the entire plate is to be read, choose "Row". If entire rows of a partial plate are to be read, choose "Row"; if entire columns of a partial plate are to be read, choose "Column". Check the box "Show Pre-Read Optimization Options" to enable the Microplate Optimization and Read Height Adjustment options upon initiation of the plate read.



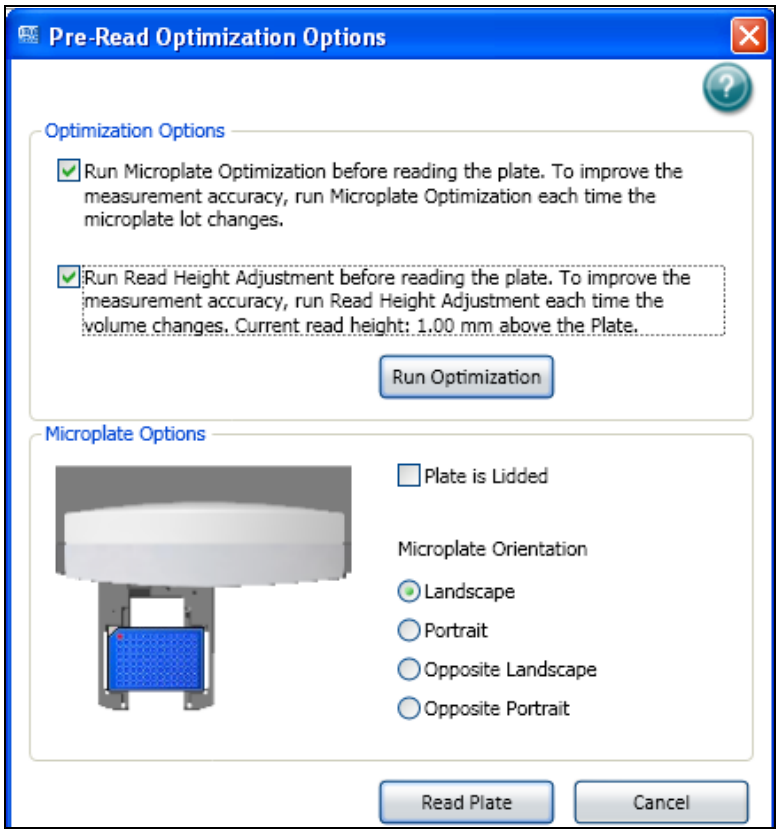
8. Click OK to close the Settings window. To read the plate, click the green "Read" button at the top of the screen.



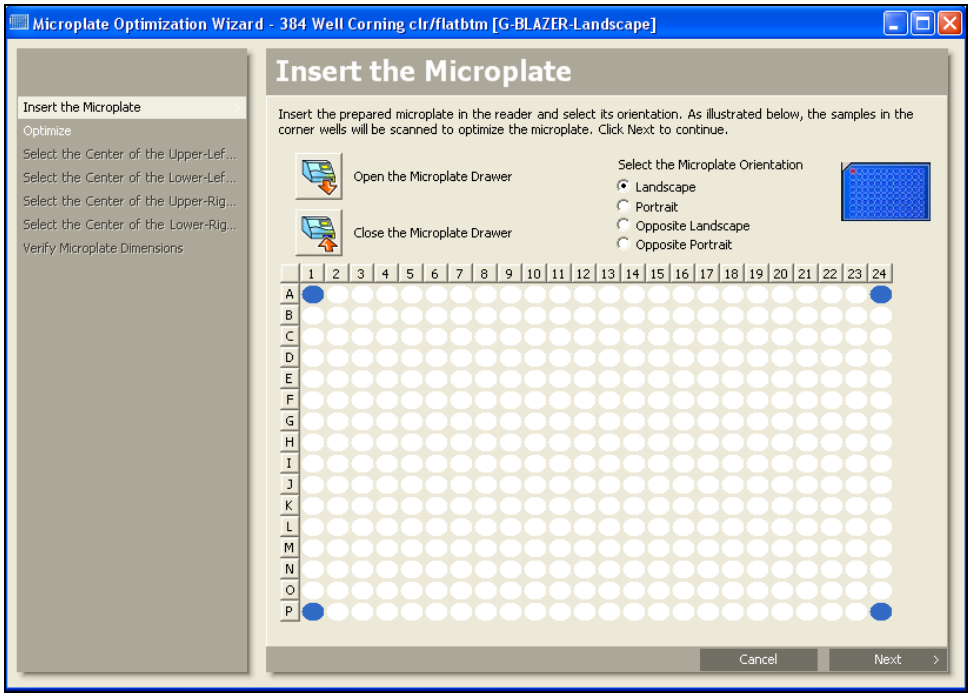
9. If selected, pre-read optimization options will appear:

- Microplate Optimization scans the four corner wells of the plate and adjusts the microplate dimensions if necessary to improve accuracy. It requires that all four corners of the microplate contain detectable fluorescent material (i.e. positive control samples).
- Read Height Adjustment determines the height above the plate at which the best signal is detected. It can be performed using any well in the plate with a relatively strong fluorescent signal (i.e. positive control sample).
- If the plate is lidded, check the box. Make sure that the selected microplate orientation matches the orientation of the actual assay plate.

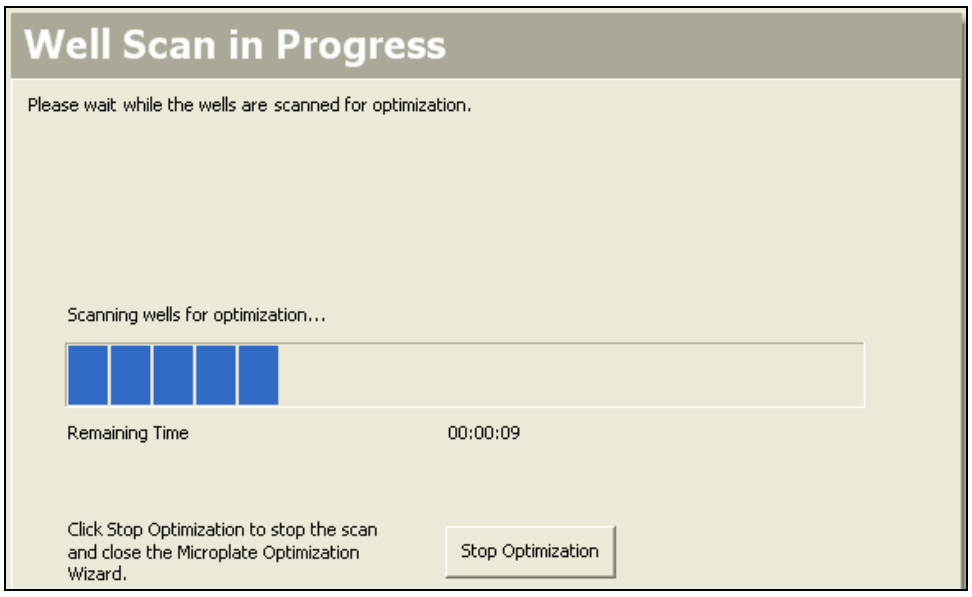
Click "Run Optimization" to proceed. Alternatively, if no optimization is desired, leave the boxes unchecked and click "Read Plate."



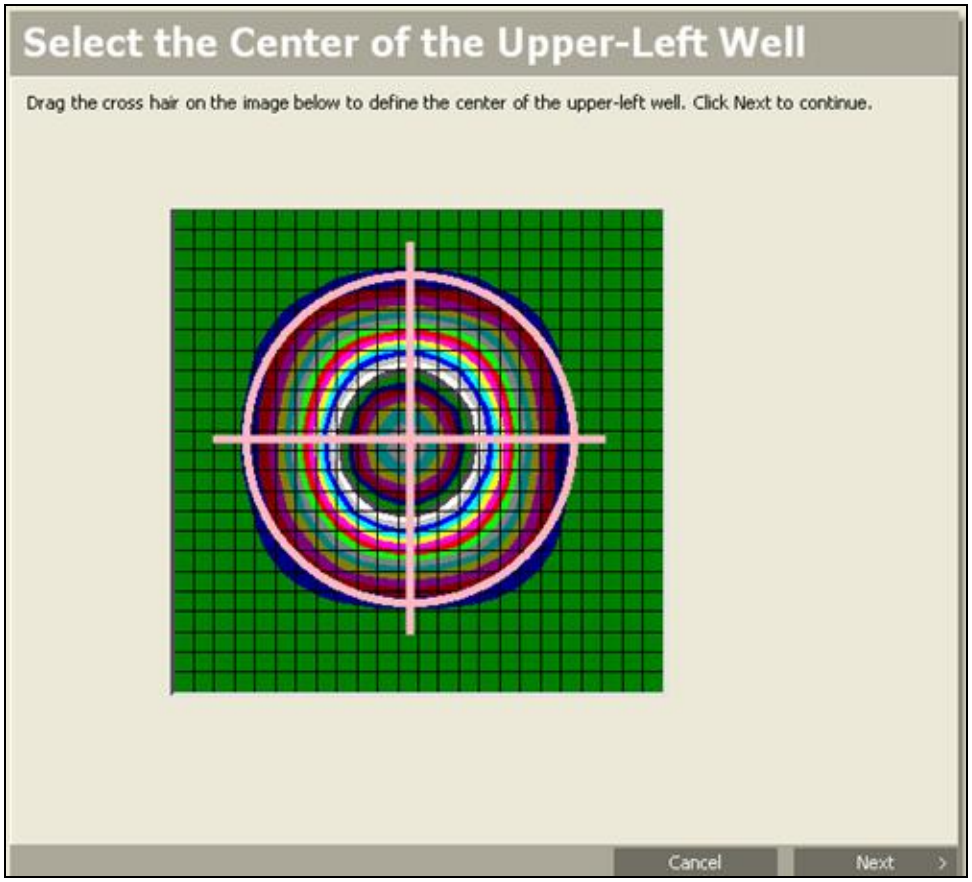
10. If optimization was selected, a wizard will pop up. Follow the steps outlined in the wizard.



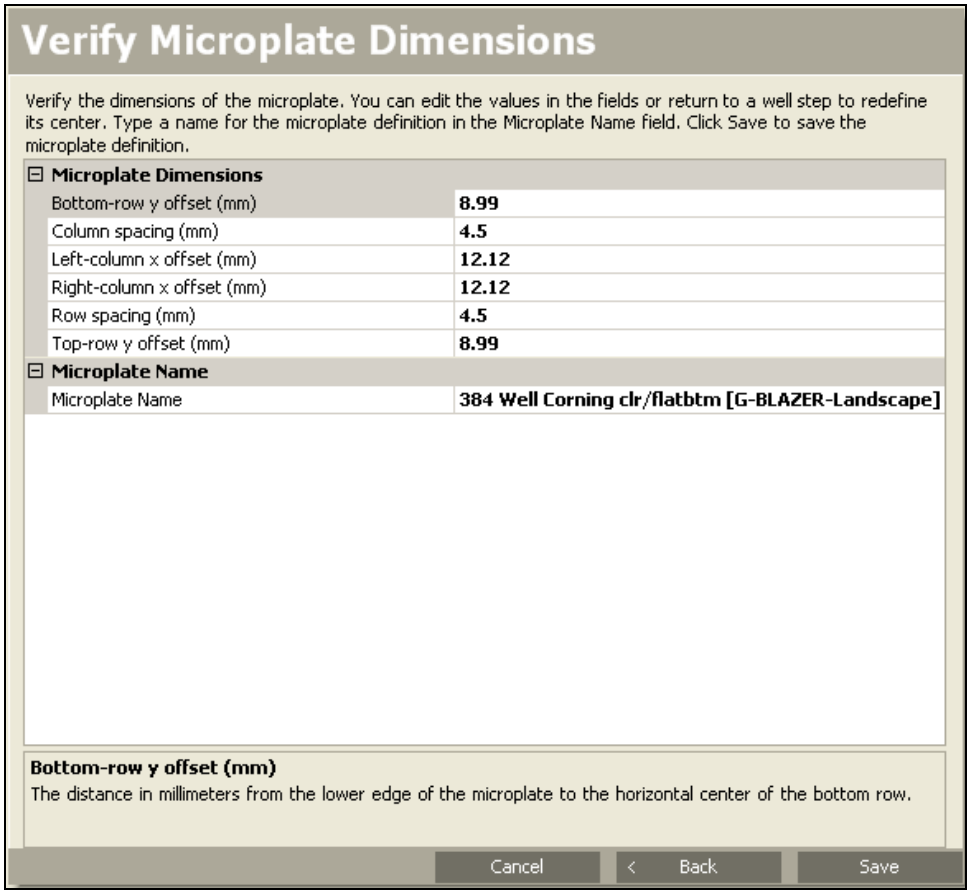
11. When you select read plate, a progress screen will appear as the plate is read.



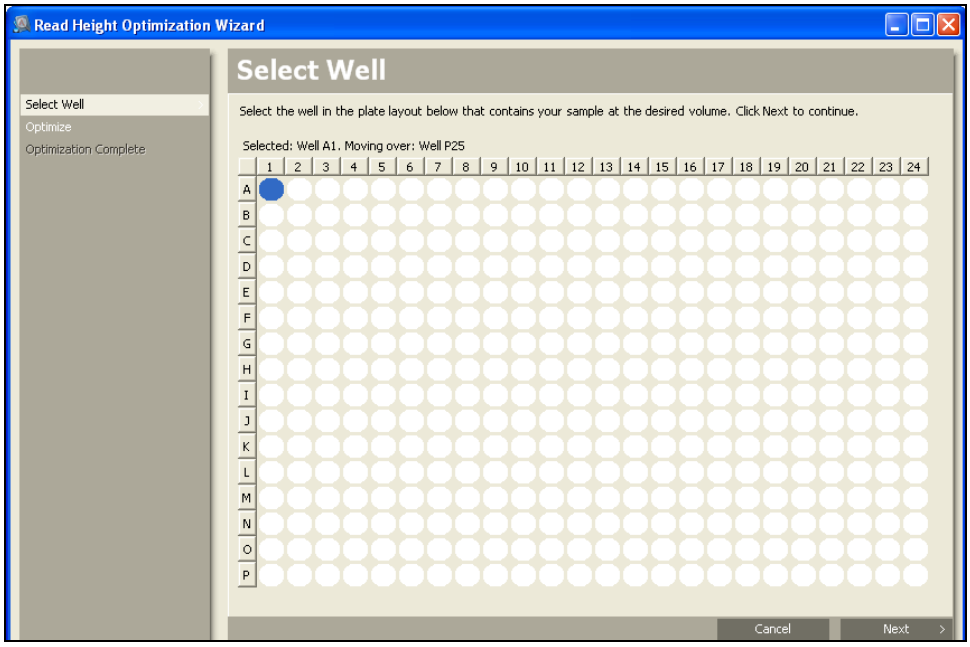
12. Center the pink target over the image of the scanned well. Click "Next" and repeat for the remaining three wells. This adjusts the microplate definition to match the actual plate.



13. Click "Save" to save the modified plate dimensions with the Microplate Name as shown. This optimized microplate type will be available in the Settings for future use.

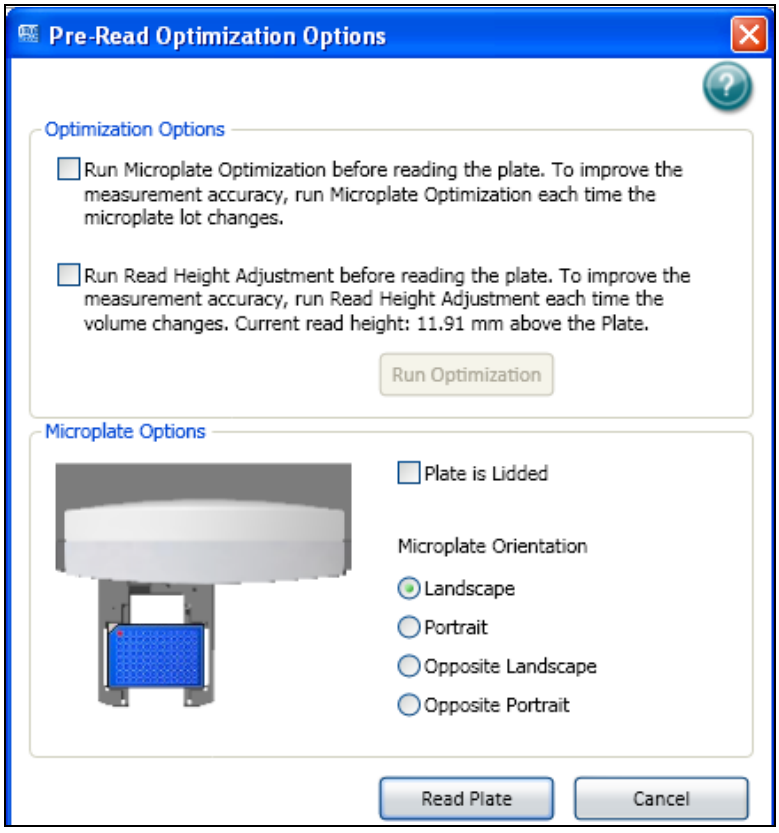


14. If you chose to perform Read Height Adjustment, this wizard will now appear. Select the well you want to use for read height adjustment. This should be a relatively bright well, e.g. a positive control. Click "Next" to read.

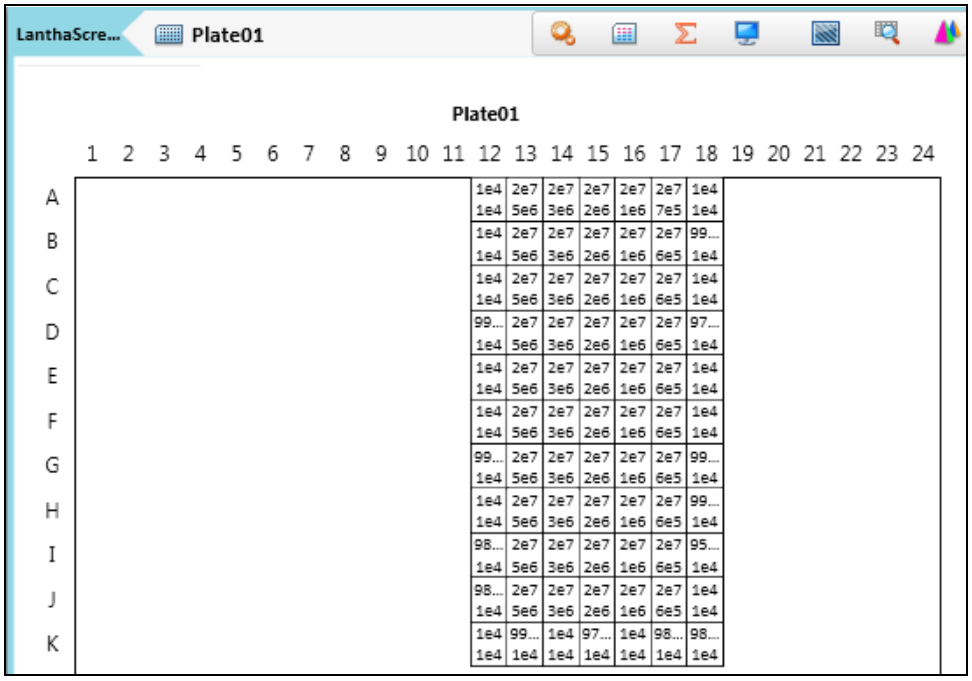


15. The instrument will calculate and report optimized read height. Click "Save".

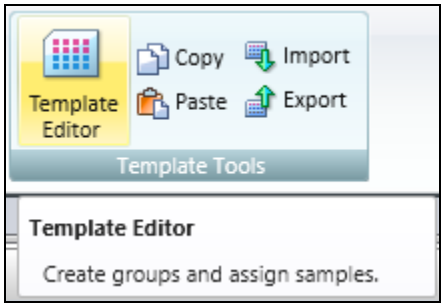
16. After optimization is complete, click on "Read Plate" to proceed.



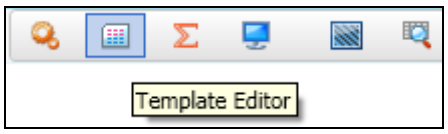
17. After plate read is complete, data will appear in the plate section:



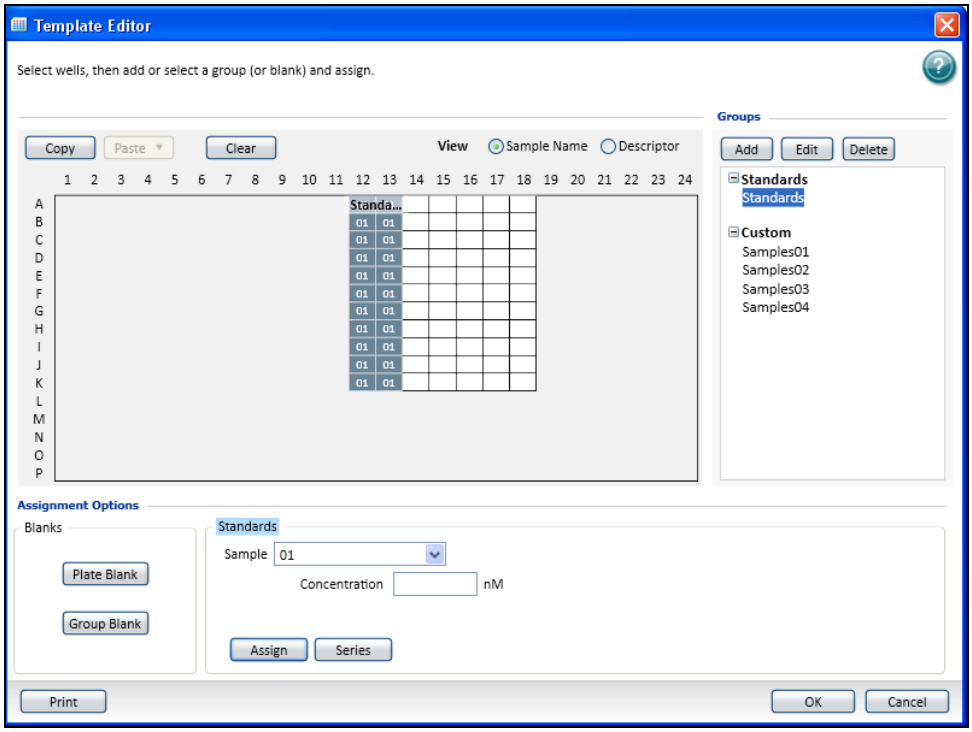
18. To set up the template for data analysis, click on Template Editor icon in the top toolbar…



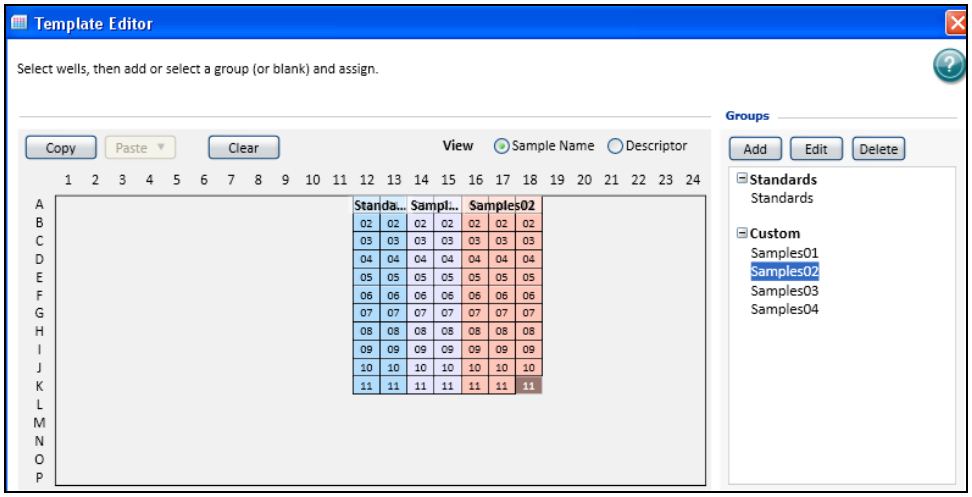
…or on the plate section header.



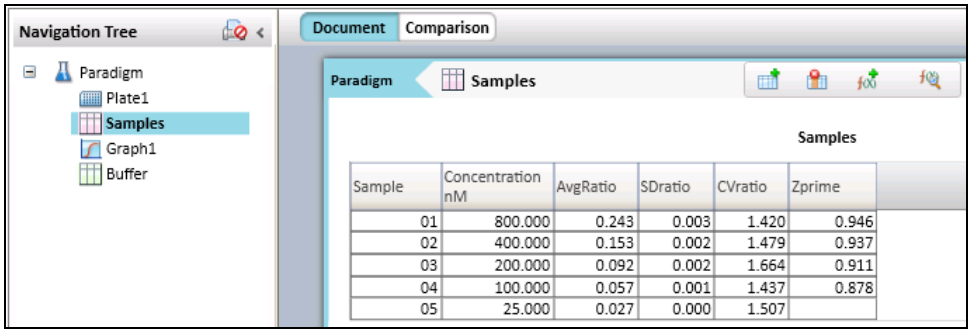
19. Select wells and choose the template group you want to assign them to; click Assign. Repeat for each sample type.



Template with wells assigned to different template groups:



20. When wells are assigned to template groups, data will populate group tables where analysis can be done:



Sample	Concentration nM	AvgRatio	SDratio	CVratio	Zprime
01	800.000	0.243	0.003	1.420	0.946
02	400.000	0.153	0.002	1.479	0.937
03	200.000	0.092	0.002	1.664	0.911
04	100.000	0.057	0.001	1.437	0.878
05	25.000	0.027	0.000	1.507	

C. Results:

Table 1. ® Europium TR-FRET testing on the SpectraMax® Paradigm®. 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 25nM acceptor), and Z' values at each concentration are shown.

Acceptor (nM)	TR-FRET Ratio	RR	Z'
800	0.243	9.00	0.95
400	0.153	5.67	0.94
200	0.092	3.41	0.91
100	0.057	2.11	0.88
25	0.027		