Configuring the SpectraMax® i3x Multi-Mode Microplate Reader for ValitaTiter Assays

Setup Guide

2598A
October 2023

MOLECULAR DEVICES

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The SpectraMax i3x Multi-Mode Microplate Reader has been validated for use with the ValitaTiter and ValitaTiter Plus assays. The following detection cartridge is required:

- Fluorescence Polarization (FP-FLUO) Detection Cartridge (Molecular Devices cat. #0200-7009)

Optimal instrument settings for ValitaTiter (P/N VAL003 or VAL013, left table) and ValitaTiter Plus (P/N VAL004 or VAL014, right table) assays on the SpectraMax i3x reader are shown below. Read height should be optimized for each new assay. Microplate optimization should be performed the first time the assay is run, and subsequently for each new lot of plates, to ensure that the center of each well is read.

ValitaTiter settings

Parameter	Setting
Optical Configuration	FP-FLUO
Read Mode	FP
Read Type	Endpoint
Excitation	485 nm
Emission	535 nm
Plate Type	96-well: 96 Well Corning Half Area (must be optimized*)
G Factor	1.0
Integration time	400 ms
Read height (mm)	Optimize*
G factor (set in Data Reduction)	1.0

ValitaTiter Plus settings

Parameter	Setting
Optical Configuration	FP-FLUO
Read Mode	FP
Read Type	Endpoint
Excitation	485 nm
Emission	535 nm
Plate Type	96-well: 96 Well Corning Half Area (must be optimized*)
G Factor	1.0
Integration time	400 ms
Read height (mm)	Optimize*
G factor (set in Data Reduction)	1.0

*For more information, please see the section “Microplate & read height optimization” below.

Setting up standard curves for ValitaTiter and ValitaTiter Plus assays

The following is an example of how standard curves for the ValitaTiter assays can be set up and read on a SpectraMax i3x reader.

Materials

- ValitaTiter (Molecular Devices cat. #VAL003 or VAL013) or ValitaTiter Plus (Molecular Devices cat. #VAL004 or VAL014) 96-well assay plate
- Human IgG for use as standard (e.g., Sigma cat. #I2511)
- Assay diluent – may be cell culture medium

Note: this setup guide uses human IgG from serum as a standard, but when running your assay, you should select a standard that is as similar as possible to your test samples (e.g., same IgG isotype) in order to ensure accurate results.

Before starting, warm the ValitaTiter plate, assay diluent, and IgG stock solution to room temperature.

Standard curve setup

Make an 8-point standard curve starting at the highest recommended concentration for the assay (100 µg/mL for ValitaTiter, or 2000 µg/mL for ValitaTiter Plus) and including a standard with no IgG (standard 8, 0 µg/mL).

You will need enough of each standard concentration for 60 µL/well in triplicate wells per standard concentration, or 200 µL per standard concentration.

- ValitaTiter: Make up 600 µL of standard 1 at 100 µg/mL in assay diluent.
- ValitaTiter Plus: Make up 700 µL of standard 1 at 2000 µg/mL in assay diluent.

Make standards by following the tables below. Standards may be made in microfuge tubes, or in a multichannel reagent basin for ease of pipetting into the 96-well plate.

ValitaTiter

Standard	Concentration (µg/mL)	Standard 1 (µL)	Diluent (µL)	Assay plate wells
1	100	200	0	A1–A3
2	80	160	40	B1–B3
3	40	80	120	C1–C3
4	20	40	160	D1–D3
5	10	20	180	E1–E3
6	5.0	10	190	F1–F3
7	2.5	5	195	G1–G3
8	0	0	200	H1–H3

ValitaTiter Plus

Standard	Concentration (µg/mL)	Standard 1 (µL)	Diluent (µL)	Assay plate wells
1	2000	200	0	A1–A3
2	1600	160	40	B1–B3
3	1200	120	80	C1–C3
4	800	80	120	D1–D3
5	400	40	160	E1–E3
6	200	20	180	F1–F3
7	100	10	190	G1–G3
8	0	0	200	H1–H3

Set up assay plate

1. Pipet 60 µL diluent to each assay well. Use reverse pipetting to avoid bubbles, and do not mix yet.
2. Pipet 60 µL standard to triplicate assay wells containing diluent. Do not mix yet.
3. After all standards are added to wells, mix contents of wells very gently by pipetting up and down 3X using a multi-channel pipettor. Avoid introducing any bubbles.
4. Incubate at room temperature for 5 minutes (ValitaTiter) or 15 minutes (ValitaTiter Plus).
5. Read the plate on the SpectraMax i3x reader equipped with a Fluorescence Polarization (FP-FLUO) Detection Cartridge and using the optimized settings shown above.

Microplate & read height optimization

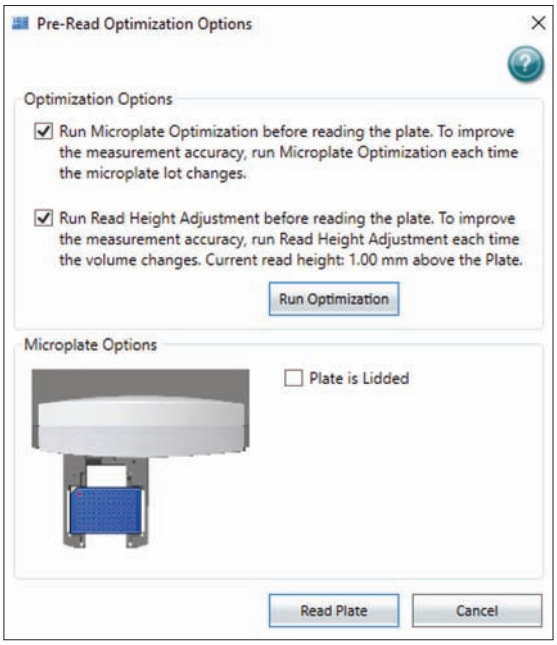
Microplate optimization ensures that the center of each well of the microplate is detected, providing more optimal results. This optimization should be run for each microplate reader, detection cartridge, or optical configuration (e.g., FP filters) used, as well as with each new lot of assay plates.

Read height optimization ensures that maximal signal is detected from the assay wells. It can be run for each new assay set up, but it need not be re-run as long as the plate type, plate lot number, and assay volume remain the same.

For microplate optimization, make sure all four corner wells (A1, H1, A12, and H12) contain material that will produce a fluorescent signal – for the ValitaTiter plates, assay wells containing diluent and sample, or wells containing diluent, are sufficient.

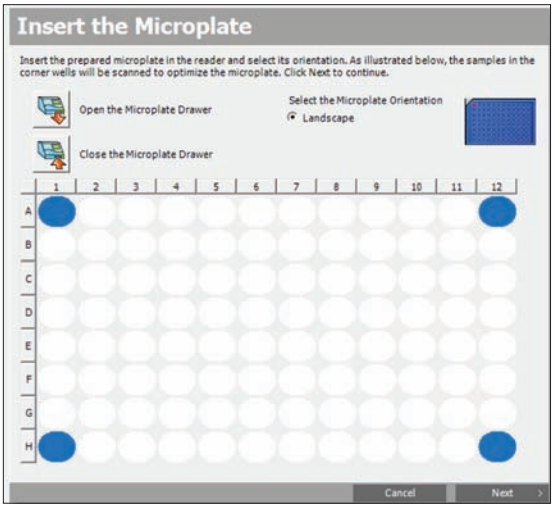
To perform microplate and read height optimization, make sure the box “Show Pre-Read Optimization Options” in the Settings dialog box in SoftMax Pro Software is checked:

When you are ready to read the plate, click “Read”, and the following dialog box will appear:

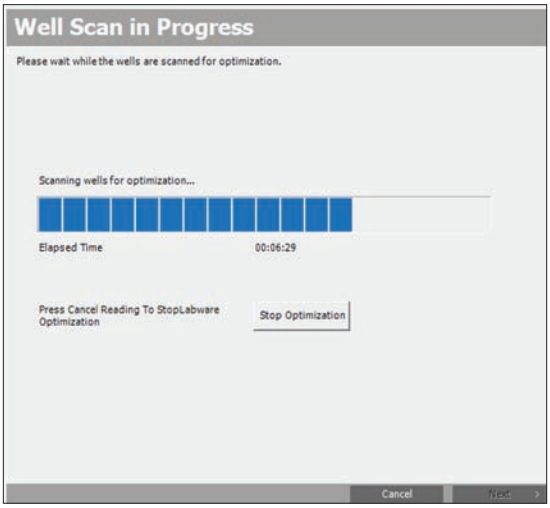


Make sure both boxes are checked, and then click “Run Optimization”.

Follow the instructions that appear:

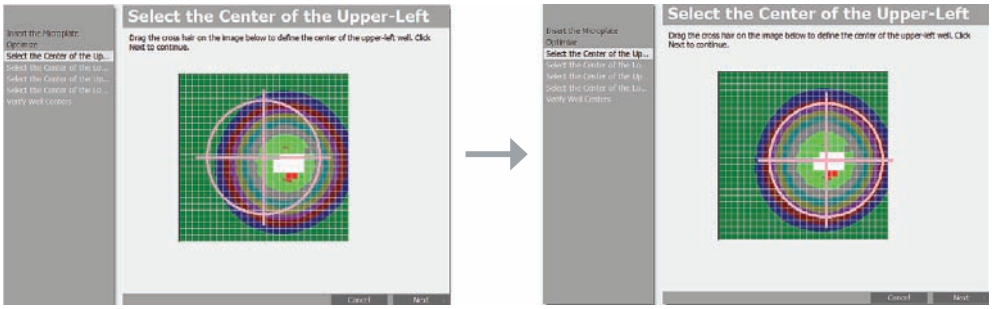


Click “Next”, and the reader will start scanning the four corner wells; a progress indicator will appear:

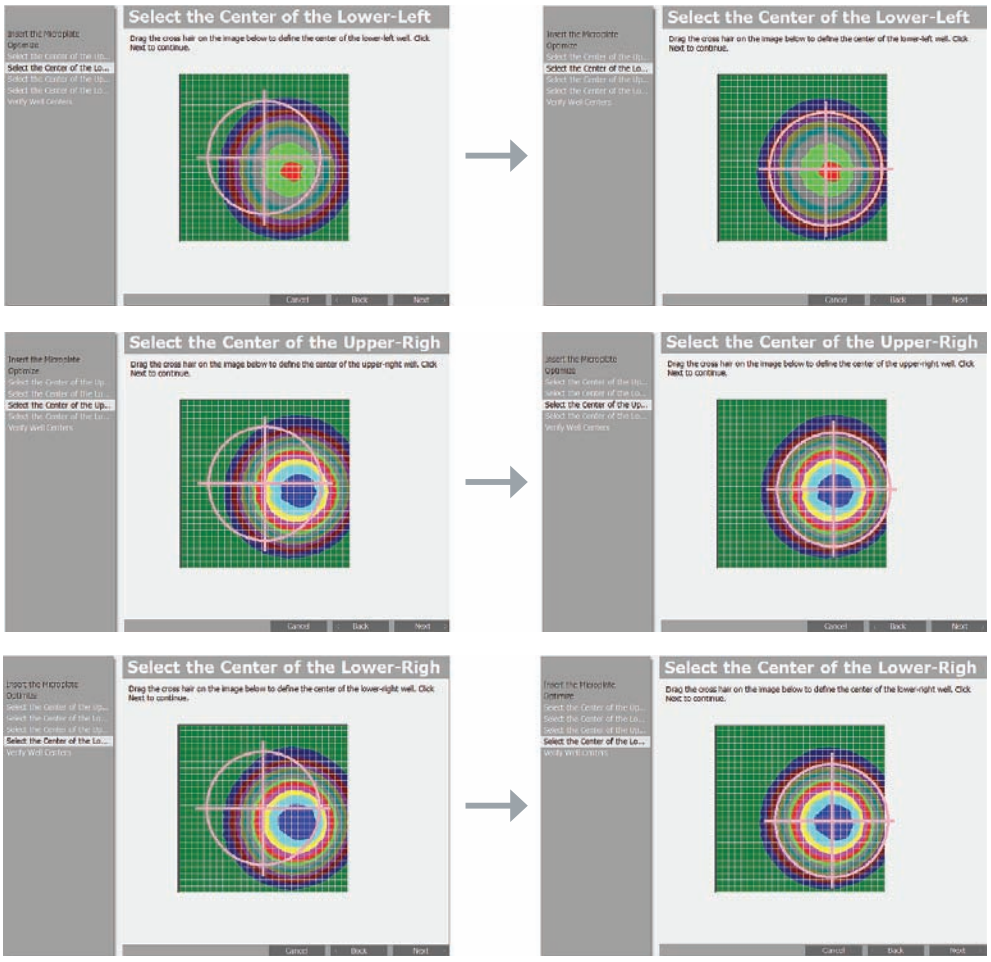


After the reader finishes scanning the four corner wells, you will see a screen with an image of the upper left corner well and a pink crosshair. Click on the crosshair and move it so that it is centered over the well image. Make sure to align the crosshair to the outermost ring, or overall ring pattern, but not necessarily to the centermost spot.

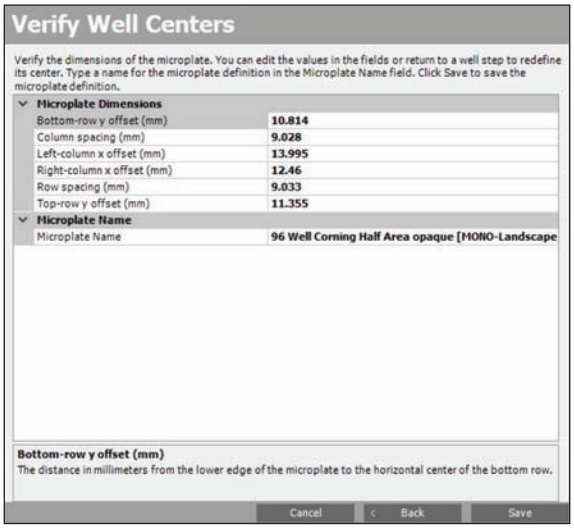
Here is an example of the initial image, followed by the crosshair-adjusted image:



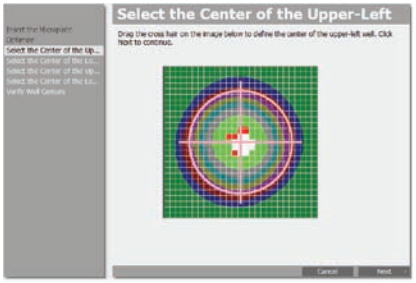
Click ‘Next’ and repeat for the remaining three corner wells:

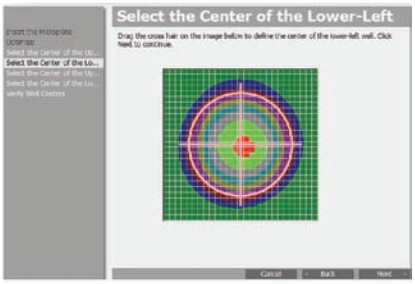


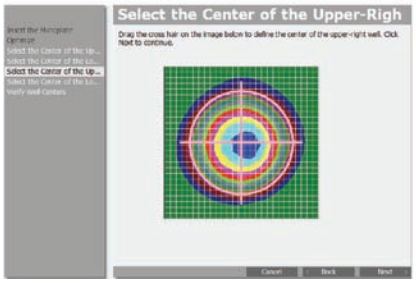
Save the optimized microplate via the dialog box that appears. Note that a bracketed addition is made to the original microplate name to indicate that it has been optimized. Here you may also change the name of the plate:

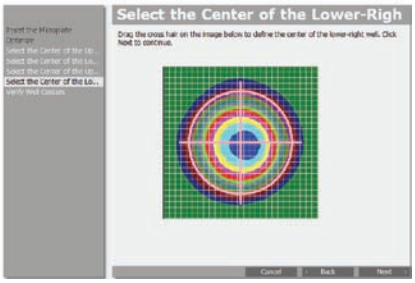


The new, optimized microplate dimensions will now ensure that each well is read in its center. Thus, if the microplate optimization were re-run, the images would now appear centered and would require no further adjustment:

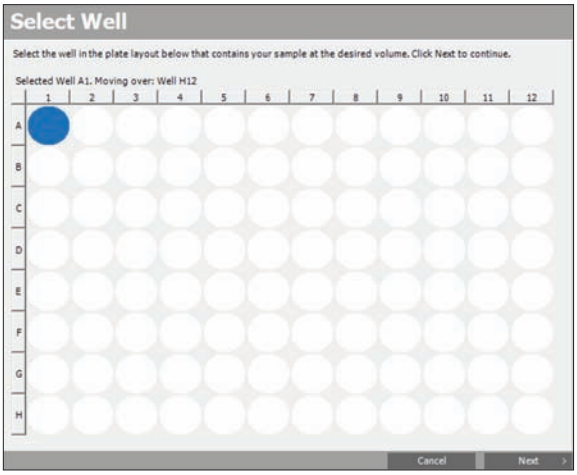




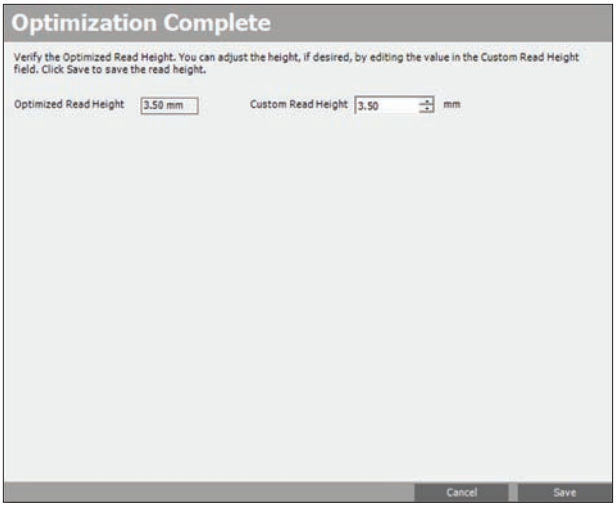




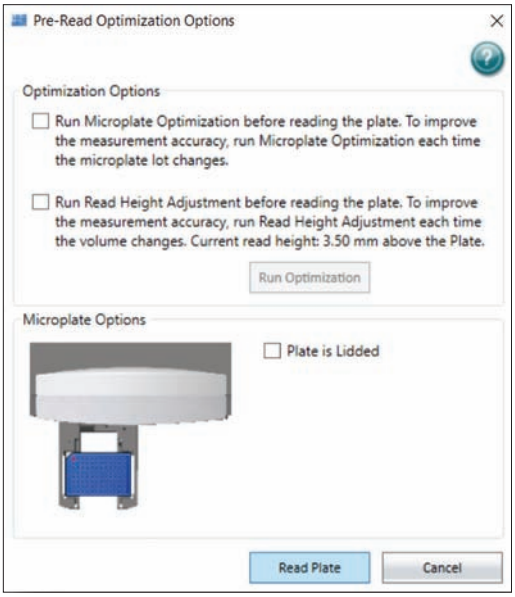
Next, perform read height optimization. First, select the well to use for optimization. Any assay well containing diluent and sample is sufficient.



Click next, and optimization will proceed. A progress indicator will appear. Once optimization is complete, you can save the optimized read height; you also have the option to customize the read height.



Once both optimizations are complete, click “Read Plate” to read using your optimized microplate definition and optimized read height.



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10/23 2598A
Printed in USA