# Fluorescence Plate Reader (Molecular Device, SpectraMax GeminiXS)

1) Turn on the instrument (SpectraMax GeminiXS) and allow the instrument for self-calibration and initialization.

2) Turn on the computer and open the software "SoftMax Pro 4.7.1" Under "Start" menu ---> All programs ---> SoftMax Pro 4.7.1



3) Temperature of the plate (Incubator) can be set Under "Control" menu ---> Incubator...

📑 Incuba	ator	×
⊙ Off ⊙ On	Temperature: 25 °C	
	Cancel OK	

4) Set up the parameters for measurement.

## Read Type

4.1) Endpoint

In an endpoint read, a reading of each microplate well is taken at a single or at multiple wavelengths (up to 4 wavelegths). The value is reported as relative fluorescence units (RFU).

Sensitivity

Reading ---> Fast, Normal, Precise PMT sensitivity ---> Automatic, Fast, Medium, Low Automix: You can choose to mix the sample before first read. Assay Plate Type: 96 well plate standard Choose Wells to read: Click and drag on the plate



📸 Instrument Settings	
Endpoint Kinetio	Spectrum Well Scan
Options:	
Wavelengths	Wells To Read
Ex Em Auto Cutoff On 365 550 550	<u>1 2 3 4 5 6 7 8 9 10 11 12</u>
Sensitivity	
Readings: 6 PMT: Auto	
Automix	
Before: Off	
AutoCalibrate	
On	
Assay Plate Type	
96 Well Standard	
Read area #1_C7	
AutoPead	
off	Cancel OK

#### 4.2) Kinetic

In a kinetic read, the data are collected over time with multiple reading taken at regular intervals. To achieve the shortest possible interval for kinetic reading, choose wavelengths in ascending order.



#### 4.3) Spectrum

A spectrum read measures relative fluorescence units (RFU) or relative luminescence units (RLU) across a spectrum of wavelengths.

All spectrum readings are made using the scanning monochromator of the instrument.



## 4.4) Well Scan

A Well Scan read takes one or more readings of a single well of a microplate at single or multiple wavelengths. Every option available for Endpoint reads is available for Well Scans. Values reported are relative fluorescence units (RFU), or relative luminescence units (RLU).

instrument settings		
Endpoint Kinetic	Spectrum Well Scan	
Options:		
Sensitivity	Well Scan Editor	
Readings: 6 PMT: Auto		
Automix	Pattern. Density	
Before: Off	3 🔻	
AutoCalibrate		
On	Point Spacing: 1.13 mm	
Well Scan Editor	Points Per Well: 9	
Pattern: Fill Density: 3	Approximate Read Time: 3 minutes	
Assay Plate Type		
96 Well Standard		
Wells To Read		
Read area A1 - C7		
AutoRead		
Off	▼ Cancel	OK

## 5) Set up Read Mode for measurement

The read mode is the form of detection used by the instrument. All read types except Well Scan are available for all read modes.

## 5.1) Fluorescence (RFUs)

Fluorescence is light emitted by a substance resulting from the absorption of incident radiation. The governing equation for fluorescence is:

Fluorescence = (extinction coefficient) × (concentration) × (quantum yield) × (excitation intensity) × (pathlength) × (emission collection efficiency)

## 5.2) Luminescence (RLUs)

Luminescence is the emission of light by processes that derive energy from essentially non-thermal changes, the motion of subatomic particles, or the excitation of an atomic system by radiation.

## 5.3) Time Resolved Fluorescence (RFUs)

Most fluorescence substances are not suitable for this type of reading. However, the fluorescence emitted by lanthanide dyes is delayed long enough to measure fluorescence after the lamp is turned off. Time resolved fluorescence is used to reduce the amount of background noise that interferes with fluorescence. The excitation lamp flashes, and after it is off, the delayed emission is collected for a set period of time before the lamp is flashed again.

## 6) Template editor

The Template Editor is used to describe the location of samples in a microplate, and thus provides the link between raw data and analysis groups.

Each template is composed of samples and groups:

---> A set of one or more replicate wells makes up a sample.

---> A set of related samples forms a group.

For example, you may have a group named "Standards" that consists of seven samples named STD01, STD02, ..., STD07, and a group named "Unknowns" that consists of five samples names UNK01, UNK02, ..., UNK05.

🗈 Experiment#1: Plate#1 🛛 🔀													
Group:	Broup: Group#3 ▼ Edit Clear			Sample: Gr02				Series Assign					
	1	2	3	4	5	6	7	8	9	10	11	12	
A	Blank BL	Gr01	Gr01	Gr01 Gr01	Gr01	Gr01	Gr01						
в	Gr01	Gr01	Gr01	Gr01	Gr01	Gr01	Gr01						
с	Gr01	Gr01	Gr01	Gr01	Gr01	Gr01	Gr01						
D													
E													
F													
G													
н													
P	rint								Г	Cancel	1	OK	

## 6.1) Blanking

Wells can be assigned to a "Blank" group in the Template Editor to create a plate blank:

- Use plate blanks when all samples on the plate have been prepared in the same way, and therefore can be corrected from a single blank reading.
- Plate Blank subtraction can be turned off in the "Reduction" dialog box so that the data can be reviewed with or without plate blank subtraction.

# 7) Reading a microplate

To read a microplate:

- Open the drawer of the instrument by clicking the drawer button is the status bar, or selecting the "control" ---> "Open Drawer" command.
- Insert the prepared microplate.
- Click the "Read" button in the status bar or choose the "Control" ---> "Read" command.
- When a reading begins, the "Read" button changes to "Stop", allowing you to terminate a reading if desired.

8) Export data to Excel spreadsheet

When the reading is done, the data can be exported to Excel spreadsheet by click :
File ---> Import/Export ---> Export
Put the file name, the exported file will be text file (.txt); however, it can be opened as Excel file.

📸 SoftMax Pro		
File Edit View Experiment Cor	Control Assays Plate Window Help	Export D
New CH-N Open CH-N Close CH-N Save CH-S Save Ac Frid Set Password Print Setup Print Piete#1	Addu Wagy Fake Whom Kep	Save in: My Do My Co My Co Adobi Adobi Adobi
Print Ctrl+P	5 6 7 873.10/864.09/030.34 Fluorescence Ex. Em. Cutorf Ln1 365.550 None Automic: Of PMT: Auto Resds/Veit. 6	File name Save as I
Wavelength Combination: ILm1 Mean Temperature: 23.4 Plate Blank Used Lm1 = 5.07 Reader: SPECTRAmax GEMINI XS Group#3	Pade Last Reat 10:53 AM 6/20/2014 IXS ROM v4:51 04Apr00	
Group#3 Sample  Wells Sample# Value ⊽ III Group#2 □	iter MeanValue	

Export Data	to			? 🛛
Save in: 🚺	Desktop	1	• 🗢 📾	💣 🎟 •
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<				>
File name:	SOP			Save
Save as type:	Text Files (*.b	d)	•	Cancel
Plate Ser All C Selec	ctions	C Selected	8	

9) Exit the program by click File ---> Exit and turn off the instrument.