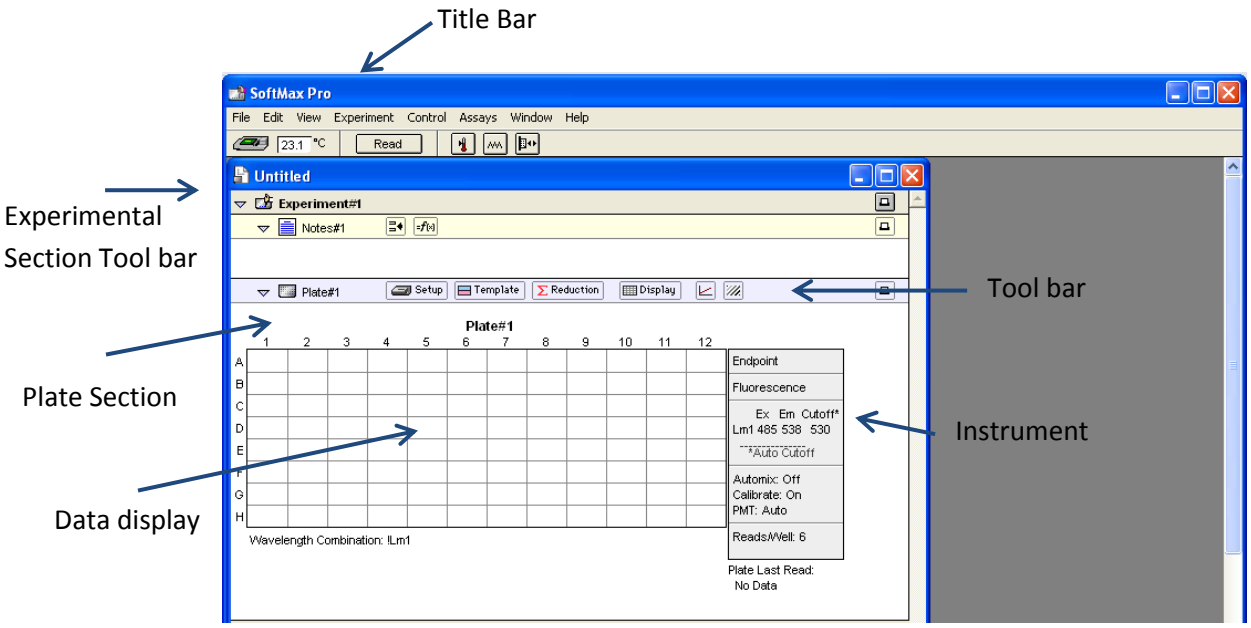
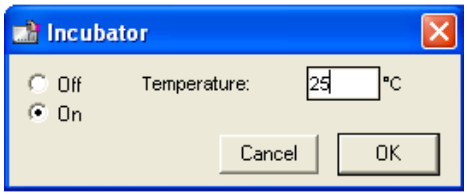
Fluorescence Plate Reader (Molecular Device, SpectraMax GeminiXS)

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…



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

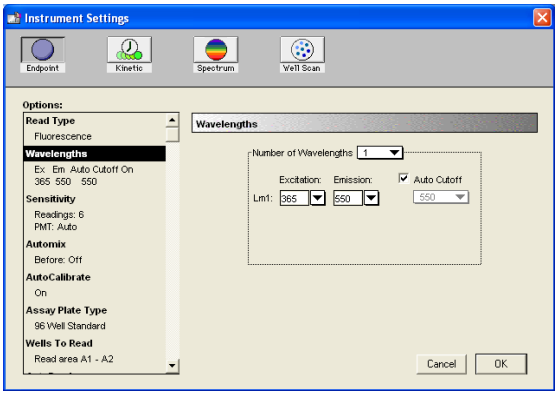
Reading ---> Fast, Normal, Precise

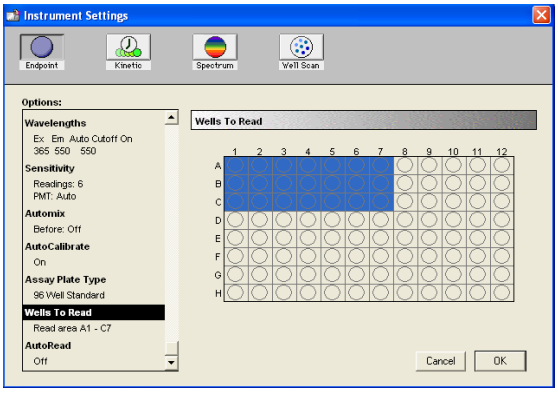
PMT sensitivity ---> Automatic, Fast, Medium, Low

Automix: You can choose to mix the sample before first read.

Assay Plate Type: 96 well plate standard

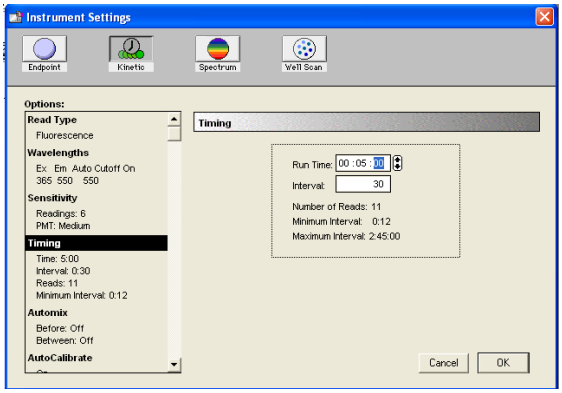
Choose Wells to read: Click and drag on the plate





4.2) Kinetic

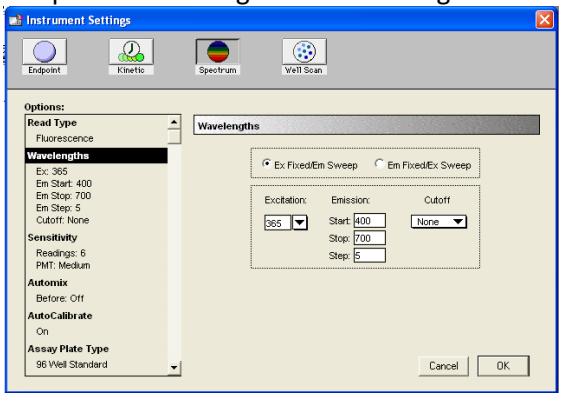
In a kinetic read, the data are collected over time with multiple reading taken at regular intervals. To achieve the shortest possible interval for kinetic reading, choose wavelengths in ascending order.



4.3) Spectrum

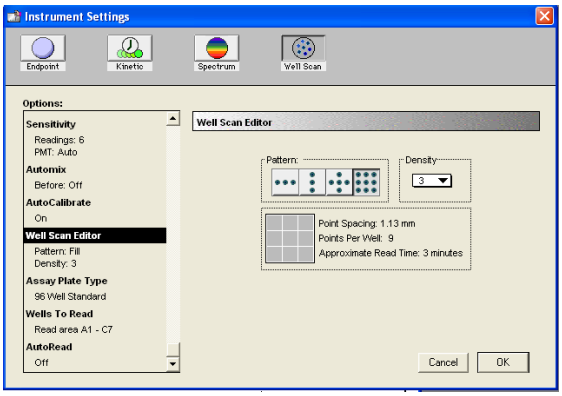
A spectrum read measures relative fluorescence units (RFU) or relative luminescence units (RLU) across a spectrum of wavelengths.

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).



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)

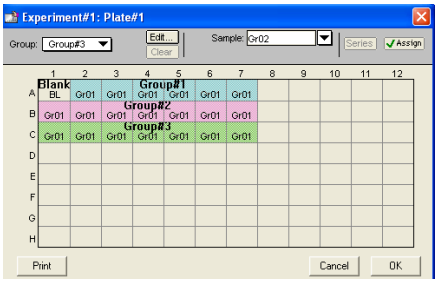
Most fluorescence substances are not suitable for this type of reading. However, the fluorescence emitted by lanthanide dyes is delayed long enough to measure fluorescence after the lamp is turned off. Time resolved fluorescence is used to reduce the amount of background noise that interferes with fluorescence. 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.



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.

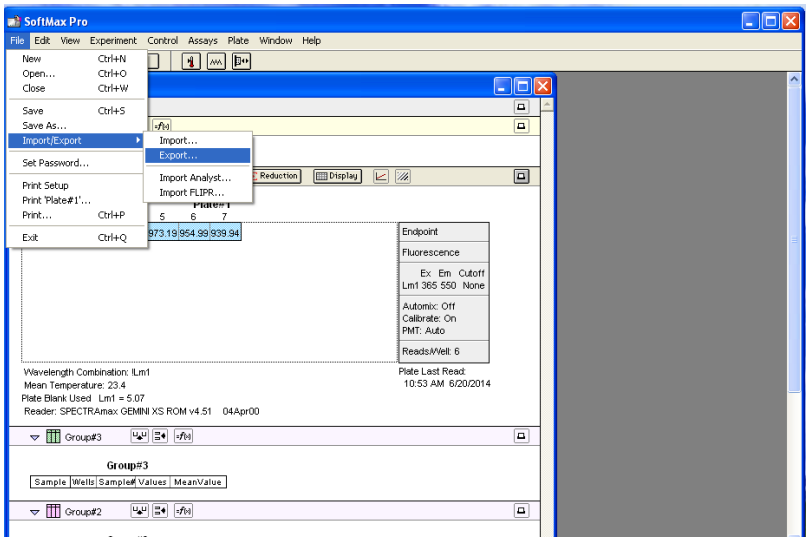
7) Reading a microplate

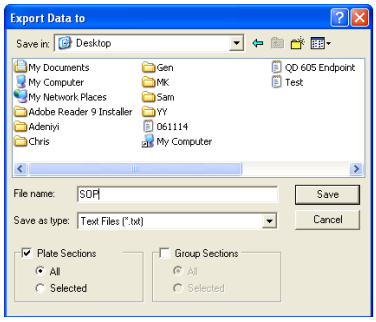
To read a microplate:

- 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.





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