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INTERACTIONS

# Tecan Spark: CMI Getting Started Guide to the Spark Multimode Plate Reader

Sample Preparation | Getting Started | Data Collection | Data Analysis | Shutdown | Instrument Specifications

## Instrument Overview

Multimode plate readers support a wide array of assays that measure fluorescence, absorbance or luminescence in a microplate. The CMI Tecan Spark Multimode Plate Reader is configured with Standard Absorbance and Luminescence Modes and Enhanced Fusion Optics Fluorescence Mode. Fusion Optics allows flexible set-up of fluorescence experiments using filter- or monochromator-based optics or a combination. Filters allow for higher sensitivity and speed, while monochromators allow for greater flexibility and specificity in wavelength selection.


### *Applications*

- Absorbance – *including UV/vis spectra*
- Fluorescence top and bottom – *including spectra*
- Time-resolved fluorescence (TRF) – *including spectra*
- Fluorescence Resonance Energy Transfer (FRET)
- Time-resolved fluorescence Resonance Energy Transfer (TR-FRET)
- Fluorescence Polarization (FP)
- Luminescence – glow, flash, multi-color, spectra
    - NOT configured for AlphaScreen or Fluorescence Imaging
    - Use with BSL2 materials (e.g. human cell lines) is limited and *requires CMI approval*

### *Key Features*

- Multimode plate reader: Fluorescence, Absorbance, Luminescence
- 96-well, 384-well, 1536-well compatibility
- Fluorescence
    - Fusion Optics for both filter-based and monochromator-based data collection
    - Monochromator spectral range: Ex. 230-900 nm; Em. 280 – 900 nm
    - Filters (wavelength/bandwidth [nm]):
        - Ex. 320/25, 340/35, 360/35, 465/35, 485/20, 495/10
        - Em. 520/10, 535/25, 540/25, 620/10, 635/35, 665/8.5
    - Mirrors: 50%, 510, 560, 625 nm
- Absorbance spectral range: 200 – 1000 nm
- Luminescence spectral range: 370 – 700 nm

### *Required Supplies*

- Black, White or Clear microplates (96-well or 384-well recommended)
- Samples and Assay Reagents

## Sample Preparation

*Assay Buffers*

- Many buffers are compatible with the Tecan Spark multimode plate reader. It is good practice to start with a buffer system in which your proteins are well behaved.
- Consider non-specific binding to the plate.
  - Polypropylene, polystyrene and treated polystyrene plates have different non-specific binding properties.
  - **Addition of detergent or BSA** to prevent sticking of proteins to the plates may be advisable.

*Samples*

- All samples should be diluted into matched buffer.
- Sample concentrations will be determined by your assay and may need to be optimized.
- Sample volumes will be determined by the plate format, assay type and read mode (top/bottom), starting recommendations:
  - 96-well 100 – 200 µl
  - 384-well 20 – 100 µl

## Getting Started

*Resources*

Additional resources are available at the instrument, including:
Tecan Spark Instructions for Use – SparkControl
Tecan Spark Instructions for Use – Basic Guide
Tecan Spark Instructions for Use – Reference Guide.

*Experimental Design Tips*

- Method Templates: Use or adapt pre-set templates for quick start.
- Blanking/Controls: Always include appropriate blanks and controls in your plate layout.
- Result Export: Data can be exported automatically or manually for further processing.
- Multiple Steps: Combine different measurement strips for multi-mode or multiplexed assays.

*General Care and Maintenance*

- Remove used plate after data collection.
- Clean up around instrument.
- Do not remove/change filters without contacting CMI staff.
- Turn off power when not in use.

## Data Collection

1. Book time on the PPMS calendar before you start.
2. Setup a plate reader experiment.
3. Turn on instrument power – back left (1/3 up, next to power cable).
4. Login to the computer using your PPMS credentials (eCommons ID and password).
5. Launch SparkControl Dashboard found on the Desktop.
   a. Open SparkControl Software on your connected PC.
   b. The Dashboard displays instrument status, recent methods, and shortcuts to core actions:
      i) Create new experiment
      ii) Load or edit existing methods
      iii) View system status
6. **Set/Check the Directory for data collection**
   a. Expand the left side panel in Dashboard (or Select Component in the Method Editor).
   b. Select Settings
   c. Select Directory
      i) Set Folder for data collection.
      ii) This will be the new default location.
7. Start a New Experiment or Method
   a. From the Dashboard,click "New Experiment" or go to the Method Editor.
   b. Enter an experiment name for easy identification (e.g., YourName_Assay_Date).
   c. Select your microplate type
      i) Choose the appropriate plate definition
         (1) exact match or
         (2) match well format and plate type (black, white, clear, 96-, 384-well).
8. Add and Configure Measurement "Strips" (SparkControl measurement step)
   a. Plate
      i) Plate; Part of Plate; Well
      ii) (optional) Plate Layout – define measurement types
      BL (Blank), SM (Sample), ST (Standard), PC (Positive control), NC (Negative control), LPC (Low positive control), HPC (High positive control), CL (Calibrator), RF (Reference), BF (Blank for polarization reference)
   b. Detection (choose from)
      i) Absorbance
      ii) Absorbance Scan
      iii) Fluorescence Intensity
      iv) Fluorescence Intensity Scan
      v) Fluorescence Polarization
      vi) TR Fluorescence Intensity (Time-Resolved)
      vii) Luminescence
      viii) Luminescence Multi Color
      ix) Luminescence Scan
   c. Action (less common)
      i) Wait, Shaking, Condition, User Intervention, Comment, Move Plate, Temperature
   d. Kinetic
      i) kinetic loop

9. Mode Settings
    a. Fluorescence Intensity
        i) Use black plates
        ii) Configure measurement parameters
            (1) Name – assign measurement name
            (2) Mode – Top (default) or Bottom
            (3) Fluorophore – Pick from list or choose "other" to specify excitation/emission manually.
            (4) Excitation/Emission Wavelengths
                - Choose Monochromator or Filters
                - Set wavelength and bandwidth, for monochromator
            (5) Signal Integration – defaults or adjust, as needed
                - Lag time (delay time)
                - Integration time
            (6) Advanced Settings
                - Flashes – 30 (default)
                - Gain – Optimal (default) for automatic setting or choose "manual" for consistency.
                - Mirror – select, as needed
                - Z-position – Manual (default) set to 20000, or "Calculated from a well" (choose a high signal well).
                - Multiple Reads – Not used (default)
    b. Fluorescence Polarization
        i) Use black plates
        ii) Configure measurement parameters as for Fluorescence Intensity
        iii) (Recommended) Perform G-factor calibration to correct optical bias between parallel and perpendicular channels.
            (1) Perform for each new fluorophore, wavelength, or filter
            (2) Include a reference fluorophore (RF) with low polarization
                - Use a standard solution (e.g., 1 nM fluorescein in NaOH)
            (3) Include a non-fluorescent blank (BL or BF)
            (4) Use replicates for more accuracy
            (5) This step is essential for accurate FP calculation
    c. TR Fluorescence Intensity (Time-Resolved Fluorescence)
        i) Use white plates
        ii) For TR-FRET, create two TR fluorescence strips
            (1) Donor excitation, Donor emission
            (2) Donor excitation, acceptor emission
        iii) Configure measurement parameters
            (1) Name – assign measurement name
            (2) Mode – Top (default) for most assays
            (3) Fluorophore – Pick from list or choose "other" to specify excitation/emission manually.
            (4) Excitation/Emission Wavelengths
                - Choose Monochromator or Filters
                - Set wavelength for excitation and bandwidth

(5) Signal Integration
- Lag time (delay time) – 100 µs (50-100)
- Integration time – 400 µs (200-400)

(6) Advanced Settings
- Flashes – 30 (default)
- Gain – Optimal (default) for automatic setting or choose "manual" for consistency.
- Mirror – select, as needed
- Z-position – Manual (default) set to 20000, or "Calculated from a well" (choose a high signal well).
- Multiple Reads – Not used (default)

d. Absorbance
   i) Use clear or clear-bottom plates
   ii) Configure measurement parameters
      (1) Wavelength Selection – single or multiple wavelengths.
      (2) Reference Wavelength – optional; can help correct background noise.
      (3) Scan Range – set scan (e.g., 200–1000 nm) or select fixed wavelength(s) for your assay.
      (4) Pathlength Correction – for quantitation, especially in microplate formats.
         - If you are not using a Greiner/Tecan specialized plate or specific software settings, then measurement at 977 nm and 900 nm (the water peak) is required to normalize to a 1 cm pathlength.
      (5) Number of Reads – set for averaging, especially for heterogeneous samples.
      (6) Kinetic or Endpoint – choose if measuring change over time or at a single time point.

e. Luminescence
   i) Use white plates
   ii) Configure measurement parameters.
      (1) Reading Mode – select Standard or Enhanced, as per your installed modules.
      (2) Wavelength Selection – choose "Integral" for total light or set wavelength range for multi-color assays (if applicable).
      (3) Integration Time – set longer for weak signals or specific assay requirements.
      (4) Attenuation – use attenuation filters if your signal is very strong.
      (5) Multiple Reads, Kinetics, or Endpoint – select according to assay format (e.g., flash, glow, kinetic).

f. Advanced Settings (Optional Across Modes)
   i) Temperature Control: ambient+3°C (~25-26) – 42°C.
   ii) Shaking: For sample mixing prior to measurement.

10. Save and Review
    a. Save your method/protocol so it can be reused or adapted.
    b. Review the Summary to ensure all your settings match the assay's requirements.
11. Running the Measurement
    a. Load your plate as prompted.
    b. Start the measurement via the Dashboard or Method Editor.
    c. Monitor progress and ensure correct plate/well orientation (A1 upper left).
    d. View and export raw data or trigger automated calculations/analysis if set up.

12. Data saved to excel format

## Data Analysis

1. Spark Control exports data to an excel file, which opens automatically (Office 365 account required).
2. For non-linear curve fitting, use GraphPad Prism or other software.
3. **Fluorescence Intensity**
   a. Raw data output: Relative Fluorescence Units (RFU) for each well.
   b. Initial processing (blank subtraction): Subtract the mean RFU of the blank control wells (ex. buffer) from the RFU of all sample and standard wells.
   c. Standard curve generation (for concentration determination):
      i) Plot the blank-subtracted RFU vs. the known concentration of the standards.
      ii) Determine the standard curve using an appropriate mathematical fit (linear fit, typically).
      iii) Calculate the unknown sample concentrations using the standard curve.
   d. Kinetic Analysis (for time-course experiments):
      i) Plot the RFU vs. Time for each well.
      ii) Calculate the reaction rate (ex. *d*(RFU)/*dt*) over a specific time interval or fit the data to an appropriate kinetic model.
4. **Fluorescence Polarization (FP)**
   a. Raw Data Output: Parallel intensity ($I_{||}$) and perpendicular intensity ($I_{\perp}$) measurements.
      i) Polarization (mP or P):
      $$P = \frac{I_{||} - I_{\perp}}{I_{||} + I_{\perp}}$$
      ii) Anisotropy (r):
      $$r = \frac{I_{||} - I_{\perp}}{I_{||} + 2I_{\perp}}$$
      iii) Where $I_{||}$ is intensity of parallel and $I_{\perp}$ is perpendicular emission.
   b. Initial Processing (blank correction): Subtract the background parallel and perpendicular intensities (from blank wells) from the raw $I_{||}$ and $I_{\perp}$ of the samples.
   c. Polarization (P) Calibration:
      i) Calculate the grating factor (G factor) from a free dye control to correct for instrumental bias toward polarized light detection:
      $$G = \frac{I_{||,\,free\ dye}}{I_{\perp,\,free\ dye}}$$
      ii) Calculate the Polarization (P) for all samples:
      $$P = \frac{(I_{||} - G \cdot I_{\perp})}{(I_{||} + G \cdot I_{\perp})}$$

d. Graphing (for binding assays):
   i) Plot the Polarization (P) or Anisotropy (r) value vs. the concentration of the binding partner.
   ii) Fit the data to an appropriate binding model to determine dissociation constant ($K_d$).

5. Time-resolved Fluorescence
   a. Raw Data Output: Signal(s) measured after a delay time following excitation.
      i) For TR-FRET assays, at two different emission wavelengths, $\boldsymbol{\lambda}_{donor}$ and $\boldsymbol{\lambda}_{acceptor}$.
   b. Initial Processing (blank subtraction): Subtract the mean signal of the negative control (blank) wells from the signals at each wavelength for all samples.
   c. Ratio Calculation (for TR-FRET): Calculate the TRF Ratio (typically the acceptor signal divided by the donor signal):

$$TRF\ ratio = \frac{FL_{\text{acceptor}}}{FL_{\text{donor}}}$$

   d. Graphing and model fitting:
      i) Plot the TRF Ratio vs. the concentration of the analyte/ligand.
      ii) Fit data to an appropriate binding model to determine equilibrium constants, $K_d$, $EC_{50}$, $IC_{50}$.

6. Absorbance
   a. Raw Data Output: Optical Density (OD) at measured wavelength ($\boldsymbol{\lambda}$) in absorbance units (AU).
   b. Initial Processing (blank subtraction): subtract the OD of a reference blank (ex. buffer) from the raw OD of the samples.
   c. Additional quantitative analysis (ELISA or Nucleic Acid Quantification):
      i) Plot the corrected OD vs. the known concentration of the standards.
      ii) Determine the standard curve (ex. linear fit for DNA/RNA or Four-Parameter Logistic model for ELISA) to calculate the unknown sample concentrations.

7. Luminescence
   a. Raw Data Output: Relative Light Units (RLU) or counts for each well.
   b. Initial Processing
      i) Background Subtraction: subtract the mean RLU of the background/blank wells (containing no luminescent reagent or sample) from all other sample wells.
      ii) Ratio Calculation: for assays requiring two readings (ex. BRET or dual-Luciferase), calculate the ratio of the two signals (ex. Acceptor RLU / Donor RLU for BRET) after background subtraction.
   c. Graphing and model fitting:
      i) Plot the background-subtracted RLU (or the Ratio) vs. the known standard concentration (for standard curve generation) or a relevant kinetic parameter (e.g. enzyme concentration).
      ii) Determine the standard curve using a suitable fit (e.g. linear) to calculate the unknown sample activities or concentrations.
   d. Kinetic Analysis (for Flash Luminescence): Plot RLU vs. Time to determine peak height, integral (total light output), or decay kinetics.

## Shutdown

1. Clean up in and around the instrument. Do not leave any plates inside the instrument.
2. Close the control and analysis software.
3. Turn off the instrument power.
4. Logoff from PPMS!

Book time and Report Problems through the PPMS system: https://ppms.us/hms-cmi

- rates are based on *booked and real-time usage.*

Contact cmi@hms.harvard.edu with questions.

## Instrument Specifications

| | |
|---|---|
| **Fluorescence - enhanced** | |
| Light source | High energy xenon flash lamp |
| Spectral range | Ex: 230-900 nm<br>Em: 280-900 nm |
| Wavelength accuracy | Ex: <0.5 nm; Em: <0.5 nm |
| Wavelength reproducibility | <0.5 nm |
| Bandwidth | Adjustable from 5-50 nm |
| Optical mirrors | 50%, 510, 560, 625 nm built-in |
| Well scanning | Up to 100 x 100 data points |
| **FI (fluorescence intensity)** | Limit of detection[1] |
| Filter – top | ≤8 amol/well (10 µl; 1,536 wells). |
| Fusion – top | ≤15 amol/well (10 µl; 1,536 wells) |
| Mono – top | ≤20 amol/well (10 µl; 1,536 wells) |
| Filter – bottom | ≤180 amol/well (10 µl; 1,536 wells) |
| Fusion – bottom | ≤200 amol/well (10 µl; 1,536 wells) |
| Mono – bottom | ≤220 amol/well (10 µl; 1,536 wells) |
| **FP (fluorescence polarization)[2]** | |
| Spectral range | 300-850 nm |
| Precision Filter | ≤1.25 mP2 |
| Precision Fusion | ≤2.0 mP |
| Precision Mono | ≤2.5 mP |
| **TRF (time-resolved fluorescence)[3]** | |
| Limit of detection Filter | ≤0.5 amol/well (20 µl; 384 sv wells)[3] |
| Limit of detection Fusion | ≤0.6 amol/well (20 µl; 384 sv wells) |
| Limit of detection | Mono ≤0.7 amol/well (20 µl; 384 well) |
| **Fastest read time** | |
| 384-well plate (FI) | ≤22 sec |
| 1,536-well plate (FI) | ≤34 sec |
| **Filters** wavelength/bandwidth (nm) | 320/25, 340/35, 360/35, 465/35, 485/20, 495/10, 520/10, 535/25, 540/25, 620/10, 635/35, 665/8.5 |

| | |
|---|---|
| **Absorbance – standard** | |
| Light source | Dedicated xenon flash lamp |
| Spectral range | 200-1,000 nm |
| OD range | 0–4 OD |
| Scan speed (200–1,000 nm) | ≤5 sec |
| Wavelength accuracy | <0.3 nm |
| Wavelength reproducibility | ≤0.3 nm |
| **λ** ratio accuracy (260/230) | <0.08 |
| **λ** ratio accuracy (260/280) | <0.07 |
| Precision @ 260 nm | <0.2 % |
| Accuracy @ 260 nm | <0.5 % |
| Limit of detection (nucleic acids) | <1 ng/µl |
| **Luminescence – standard** | |
| Spectral range | 370-700 nm |
| Luminescence (glow) – Limit of detection[4] | ≤225 amol/well |
| Luminescence (glow) – | Limit of detection[4] |
| Luminescence (flash) – Limit of detection[5] | ≤12 amol/well |
| Limit of detection[5] | (55 µl; 384 wells)[5] |
| Dynamic range | >9 orders of magnitude |
| Multi-color luminescence | 38 spectral filters |
| Multi-color luminescence | Multi-color luminescence |
| **Temperature Control** | |
| Ambient+3C – 42C | |

[1] Detection limit for fluorescein
[2] FP detection limit @ 1 nM fluorescein
[3] Detection limit for europium
[4] Detection limit for ATP (144-041 ATP kit SL (BioThema))
[5] Detection limit for ATP (ENLITEN. Kit)

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