0

COMPACT MANUAL USE OF SPARK M10 PLATE READER

Room HG01.228 General Instrumentation

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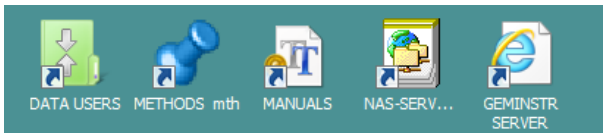
SPECIFICATIONS

The Tecan Spark M10 multimode plate reader has the following modules:

- **Multiple** types of **plate and wells**
- **Absorbance** reading with **monochromator** optics (200-1000nm)
- **Fluorescence** top / bottom reading with monochromator for Exc (230-900nm) and Em (280-900nm), also step-wise intensity scans over range
- **Fluorescence polarization** reading >390nm
- **Time-resolved fluorescence**
- **Luminescence** reading, single range, multicolor + scanning
- **Temperature control** including cooling option (range for measurement 18-42°C, not higher, not lower) and **shaking**
- **Spark and Magellan** programmable control and analysis software
- **Injector module 2x**, 1ml syringes with heating & stirrer option

ASSISTANCE - BOOKINGS

- **Liesbeth Pierson**, Tel. 024-3652199, e.pierson@science.ru.nl, Room HG01.222
- **Paul van der Ven**, Tel. 024-3652012, p.vanderVen@science.ru.nl, Room HG 01.212
- **Website**: http://www.ru.nl/science/gi/facilities/other-devices/plate-readers/
- **Bookings**: http://bookings.science.ru.nl/public/auth/login/ (4 days a week priority for the van Hest group)
- **Manuals**: paper manuals for Spark and Magellan in Room HG01.228
- Digital version on D drive of Spark computer (see desktop shortcuts) and geminstr server.



SWITCH ON

1. Switch on a) **Laird Cooling** unit if temperature below 28 °C is needed (right side Laird unit), b) **Spark M10** main power (rear side Spark) and c) function switch (front panel).

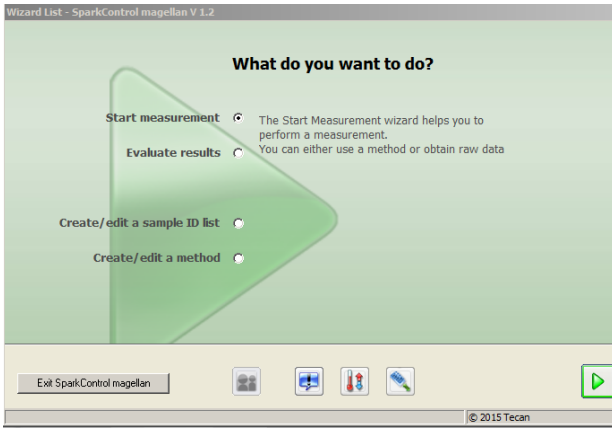
2. Switch on **computer and monitor**
   Blue: device on but not ready
   Magenta: Device ready
   Green: device measuring
   red flashing: error
   no light: off

3. **Login** under Windows as user “sparkm10”, password: sparkm10
4. As the Spark software has limitations for file handling, the powerful **Magellan** software will be utilized to make or load and save methods, to perform measurements and to save data and worksheets (workspace). **All method, data, worksheets have to be stored exclusively on the D drive!** Check out the subdivision of folders: D> DATA USERS> Institute > year> Group head> User> excel data / methods / misc / workspace

5. 

Click on the Magellan icon to open the program. A start screen opens with 4 options:

Start measurement
Evaluate results
Create/edit a sample ID list
Create / edit a method



6. The **slider** opens and the plate with samples can be placed on the tray. (Sample A1 as seen from above)
7. Close slide with ▲ knob (on front panel of the device)
8. Maximum filling of the wells

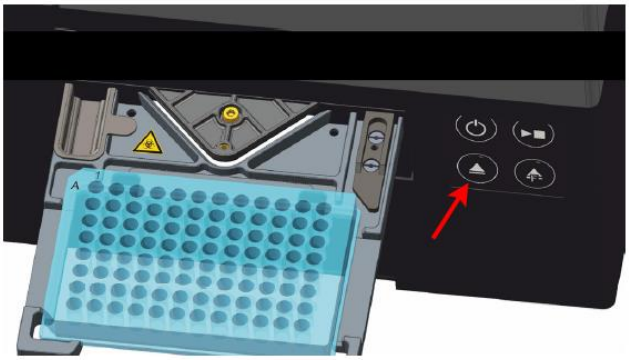


Figure 2: Microplate on the plate carrier with the A1 well in the upper left-hand corner

Maximum filling volumes for various plate sizes

CAUTION: The following microplates can be processed **only** with the subsequent filling volumes:

- 6-well plates: ≤ 2000 µl
- 12-well plates: ≤ 1200 µl
- 24-well plates: ≤ 1000 µl
- 48-well plates: ≤ 400 µl

Larger filling volumes can lead to an overflow of liquids, which can result in cross-contamination. Additionally, the spillover can cause damage to the device (e.g. contamination of the optics and the centering clamp).

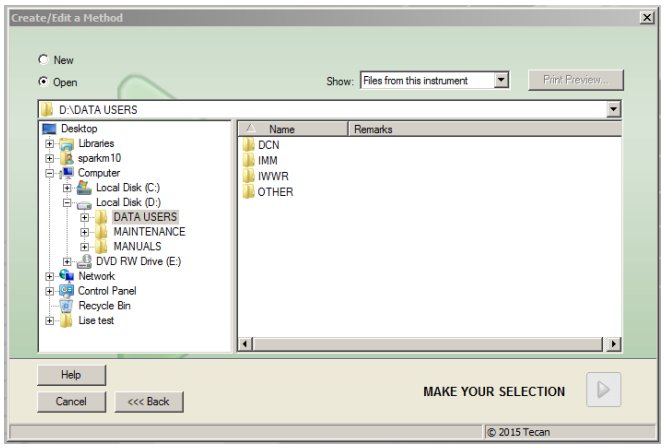
For fluids that have a lower viscosity than aqueous solutions, the filling volume should additionally be optimized during method validation.

Microplate types with less than 6 wells can be used with dry or solid substances only.

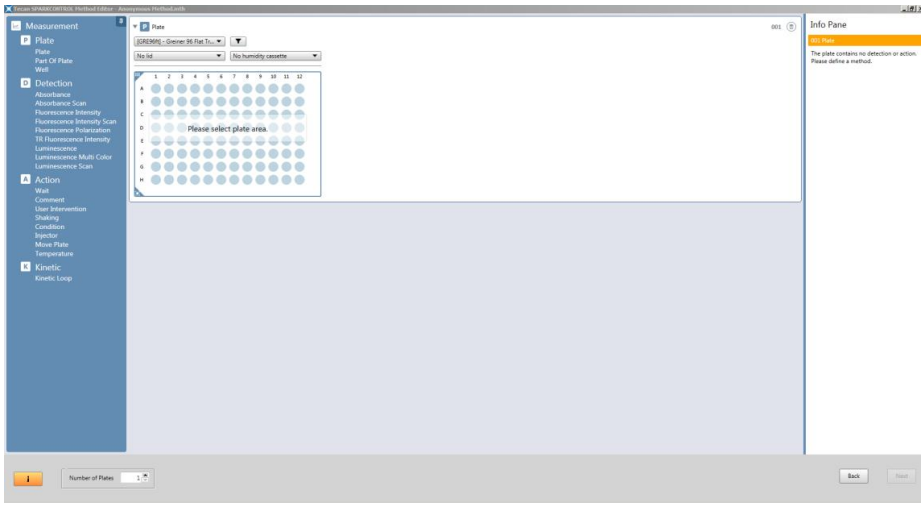
CREATE/EDIT METHODS (IN MAGELLAN)

9. **To make a new method** select “Tecan confirmed us that there is no direct function” in the Magellan start screen and choose “New”. (To **change an existing method, choose “Open**” and then search

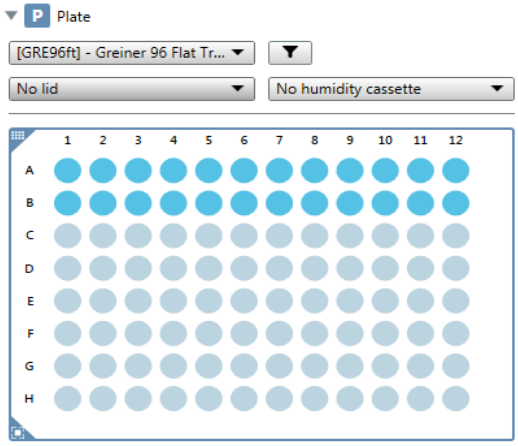
the method in your folder on the D > DATA USERS drive).



10. **MAKE YOUR SELECTION** ►
11. Method Editor Screen appears.



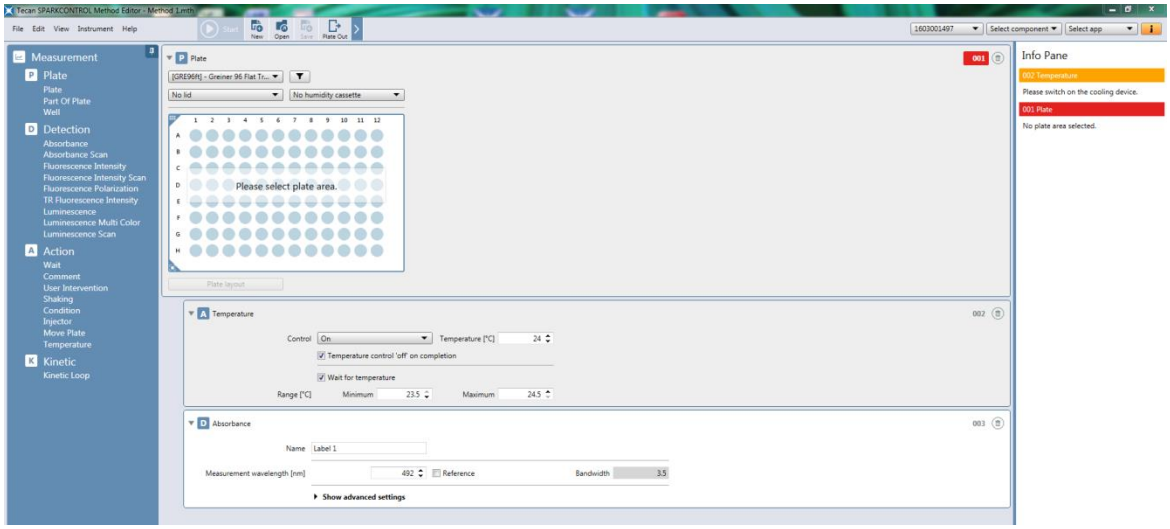
12. **Select** with the mouse cursor the **wells to measure** (clicking in left upper corner selects all).



13. Select the type of **plate** to use
    Accepted types of plates:

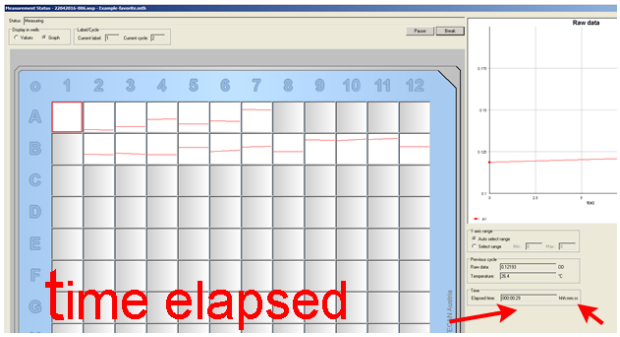
14. Select if you measure with or without **lid** (Important: select always *without lid* for luminescence!)
15. Select **No humidity cassette** (humidity cassette option not purchased)

16. Select a detection method in the left menu and double click. A **menu** (e.g. absorbance) is **added to the workspace**. Various options can be selected. (Check out the “Detection methods” and “Action” chapter further up). Any item from that left menu can be imported by double click to the central activity field. The sequence of items in this activity field can be adjusted by selecting blocks and **dragging** them up or down in order to create a **chronological** list according to which

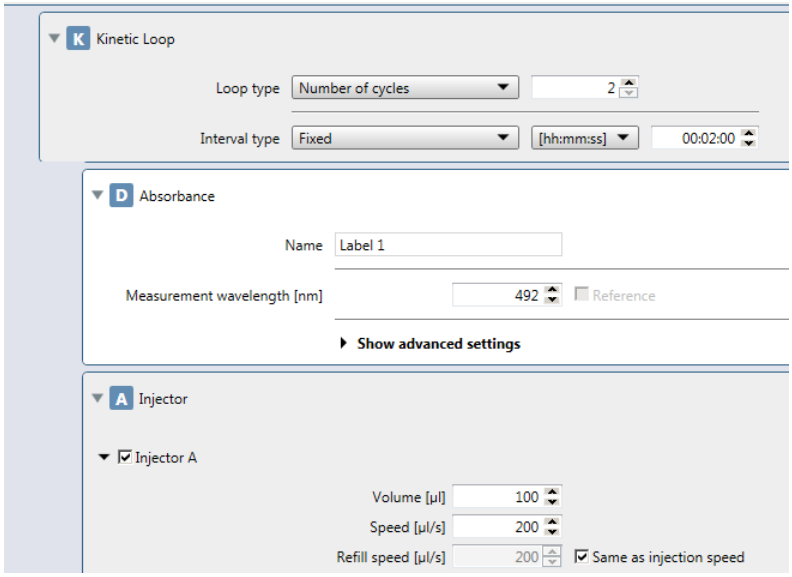


measurements/functions will be performed.

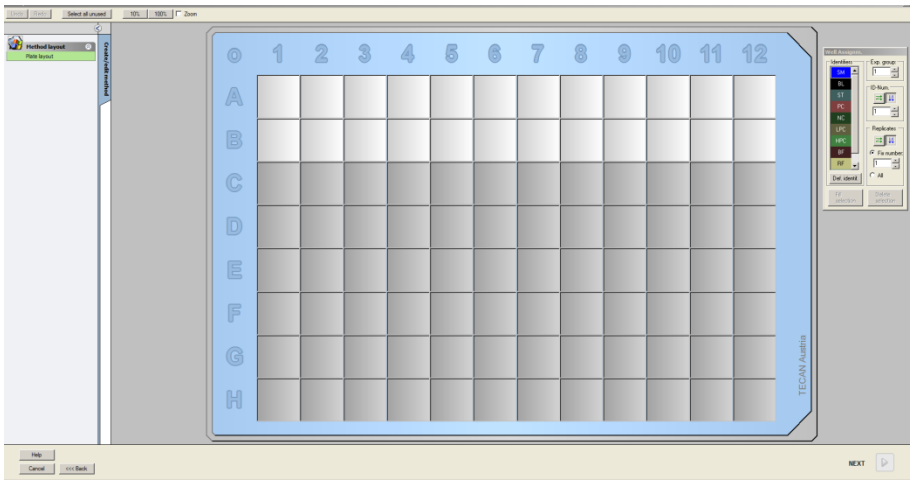
17. To repeat measurements a number of times or to insert a time interval between repeated cycles of measurements, double click “**Kinetic Loop**”. (For details on options see chapter Kinetic loop in this mini manual). Note: It is possible to determine the **duration of a cycle of measurement** by following the timing from the right panel when performing a measurement (or a tryout measurement in the process of establishing a method). Tecan confirmed us that there is no other, direct function.

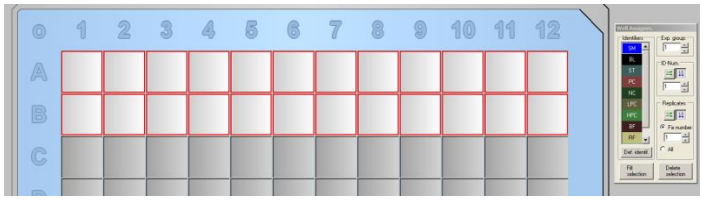


18. Drag the “kinetic loop menu” to a position above the menu to repeat (here absorbance).



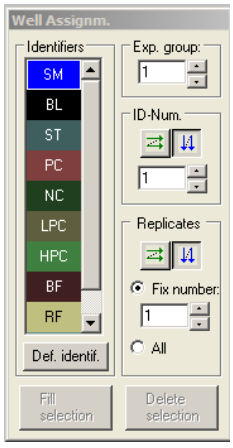
19. To insert an action, for example “Wait” or “Temperature” (More details further on), drag the item to the moment in the list of events when this action should be performed.
20. Check for info remarks (orange) or error messages (red) in the Info pane (right column).
21. When all ready, click on **Next**
22. A new window with the plate lay out appears. **Select the wells to measure** again by dragging with the mouse cursor over the positions



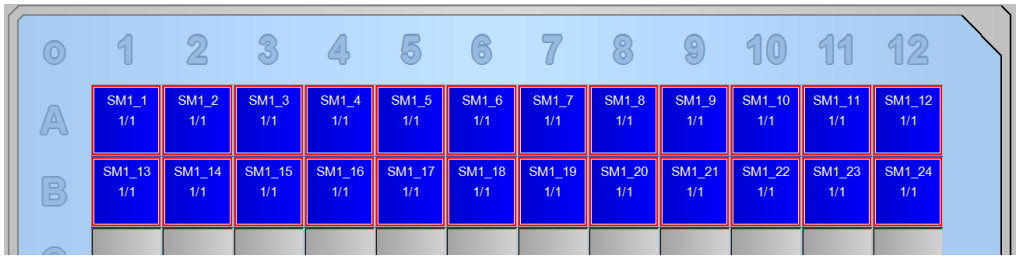


Positions are now highlighted

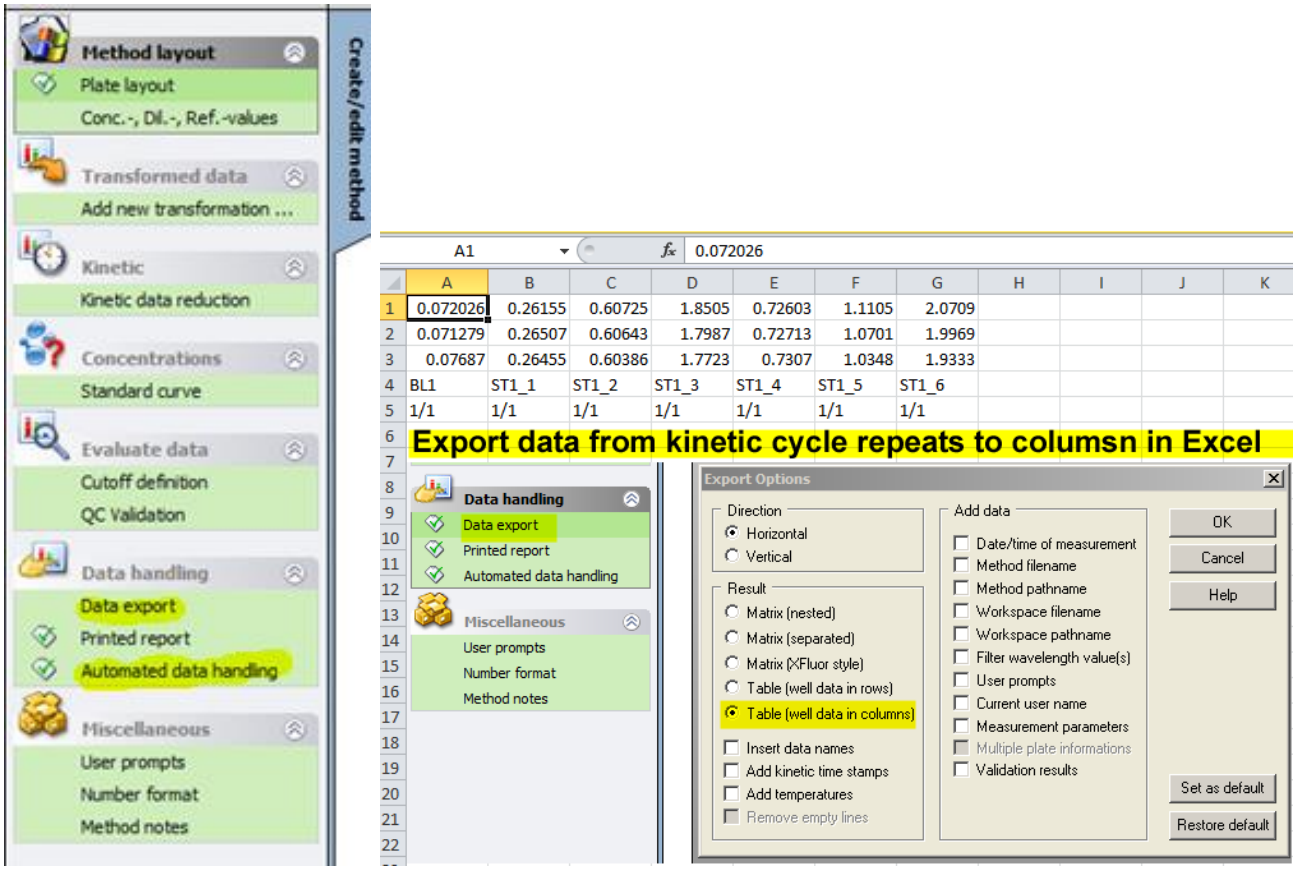
23. Click on a **color code** in Identifiers.
    Note: It is possible to insert different identifiers on one plate by selecting the single idenfier with the mouse and double clicking them in the well/place where they need to be filled.



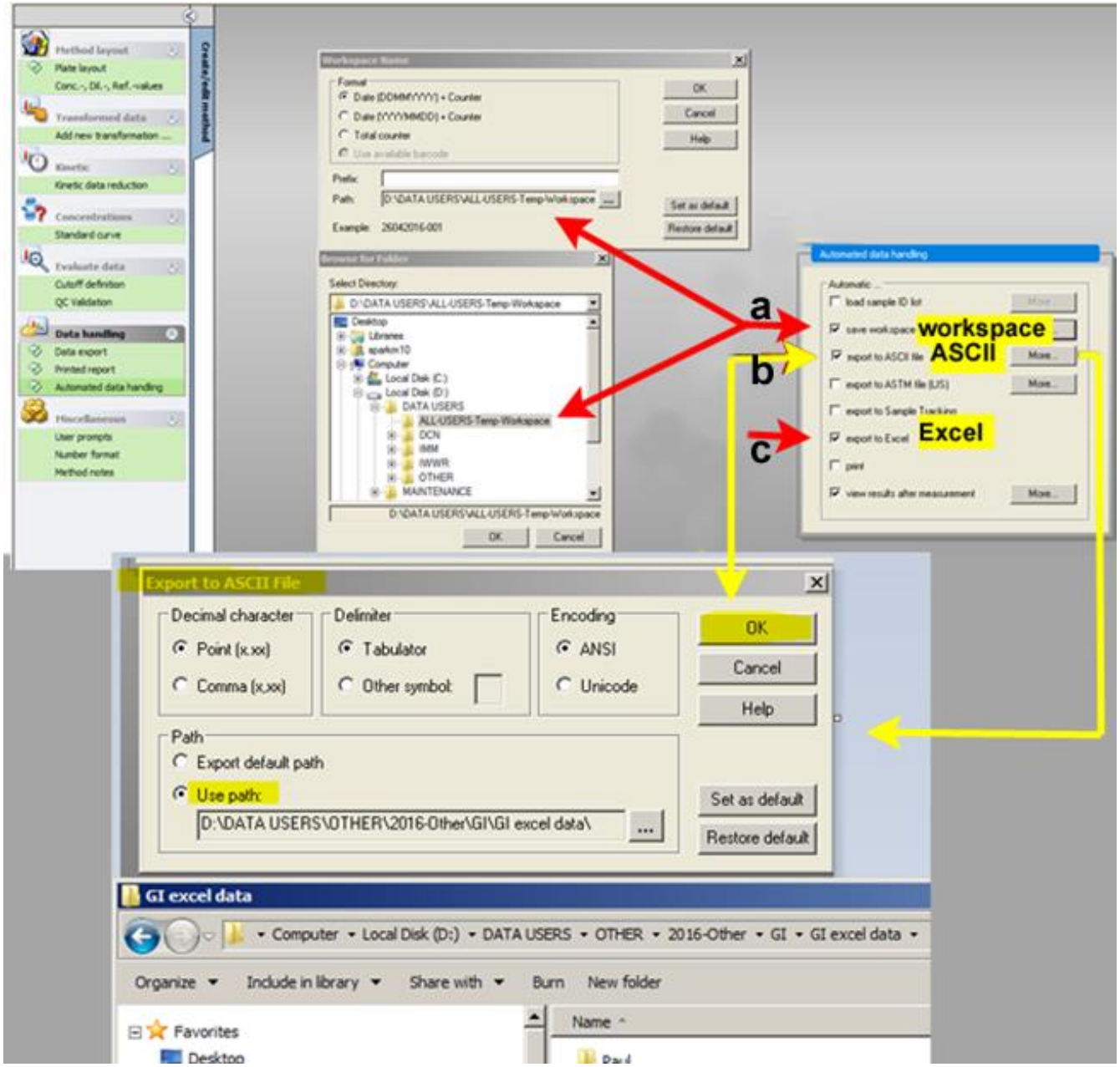
24. Click on **ID-Num**:  either horizontal or vertical order and numbering of measurement
25. Click fill selection and check out if the filling is correct.



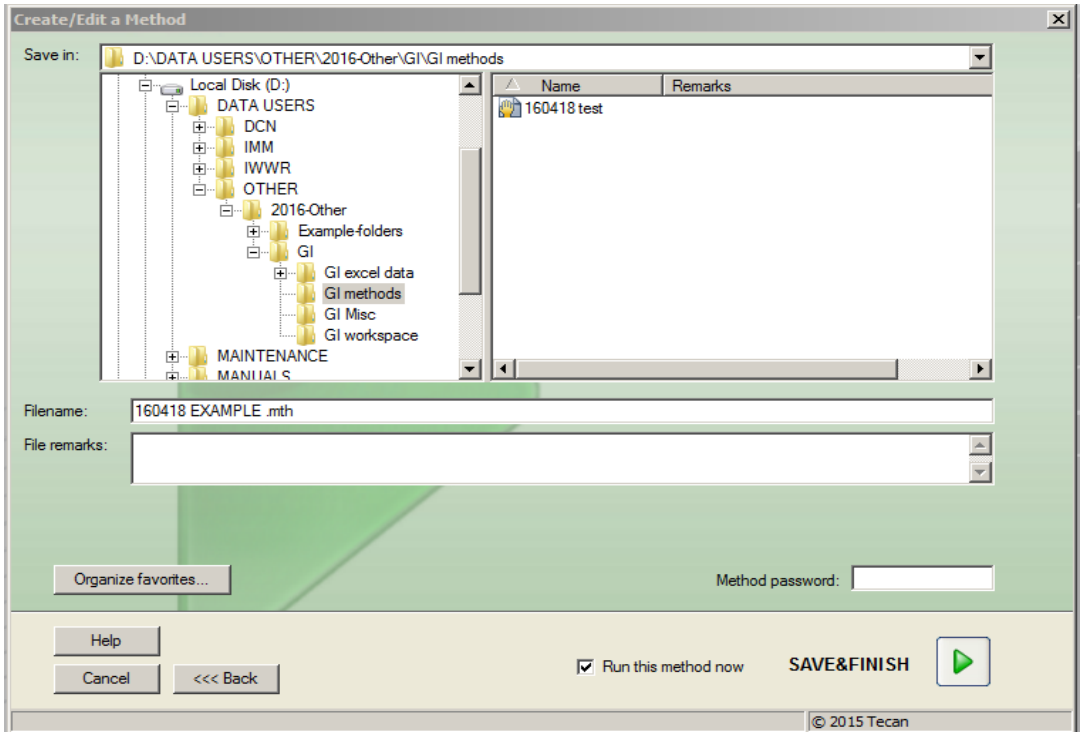
26. A menu appears on the left side of the screen, in which **concentrations, references, values, type of standard curve** etc. can be given
27. Click **Next** ▶
    A new window opens with on the left a menu



28. Select **Data Export** in this menu. A popup window opens. Bring at least Well positions, Layout and Raw data to the “Selected data” wing by selecting them and clicking -> (or by a double click)
29. From the above vertical menu, go to “**Automated data handling**”. A new popup opens.
    a) Check “save workspace” (→Strongly recommended: this will automatically save the data with a DDMMYYYY + counter name to the default path
    D>DATA USERS> >ALL-USERS-Temp-Workspace.
    b) Check ASCII and define path (→optional; with “More”, define path to your own folder)
    c) Check export to excel (then data are temporarily dumped after measurement to an Excel file which can be accessed through an icon. This excel file needs to be saved manually to your own excel folder!).



30. **Next ►**
31. A window entitled “Create/Edit a method” opens. Now **select your own path**
    D: DATA USERS ► Institute ►year ►Department group ► your name ► your method folder
    Preferably : Date+Name+Methods as name



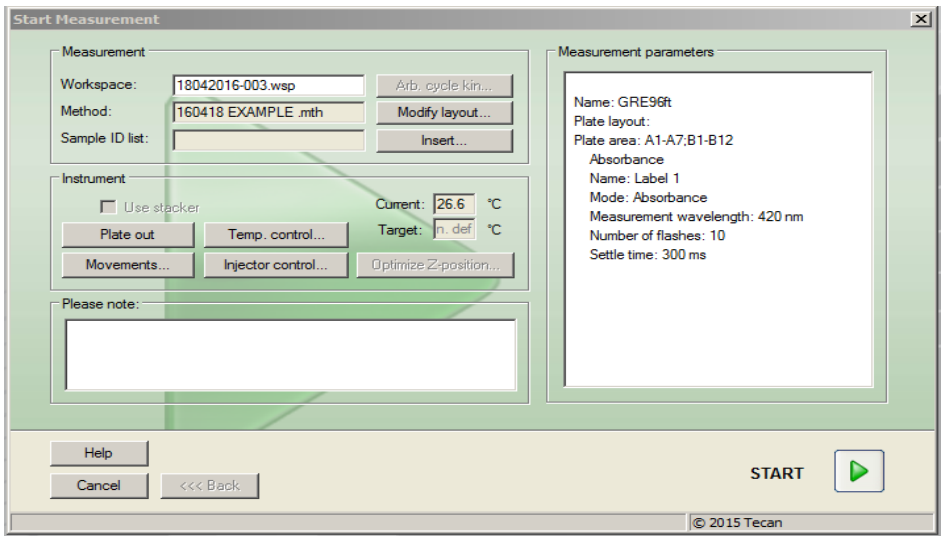
(Note: the first 15 users have also received a default **favorite** under ALL-USERS-Favorites> your name-favorite.mht. You can overwrite your own dummy default method with the currently created method. Always make also a backup of this favorite method).

32. **SAVE&FINISH ►**

Note: unfortunately the Spark Dashboard function does not communicate with the Magellan software. So there is no way yet to import methods in the Spark dashboard.

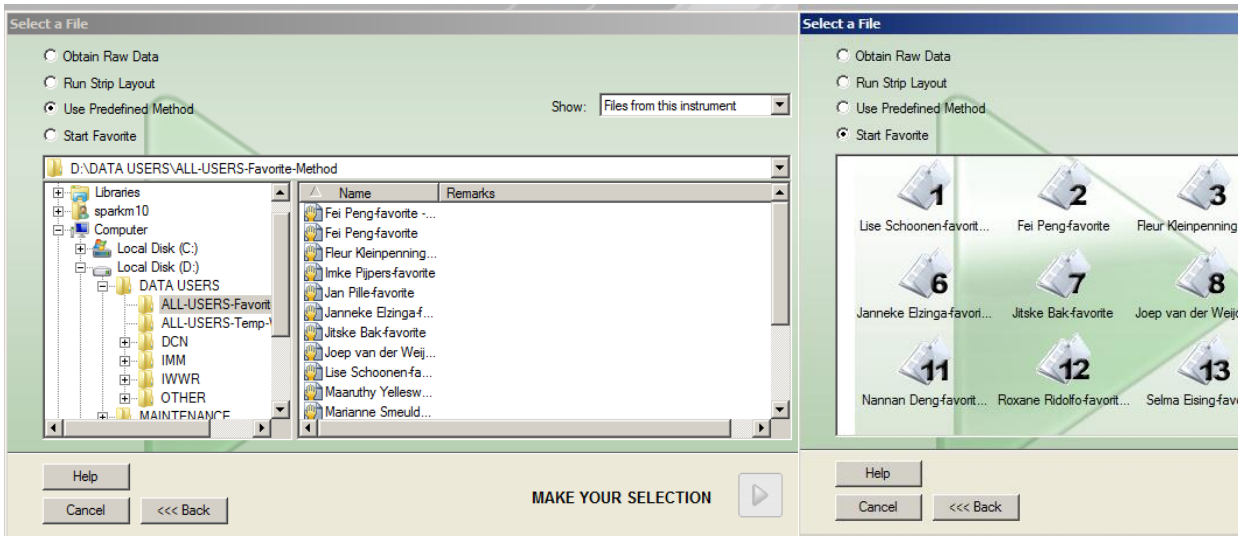
MEASUREMENT

33. Option **immediately after saving a created/edited method**. In that case, the following menu opens. Check the method and workspace and Click on **START** ▶

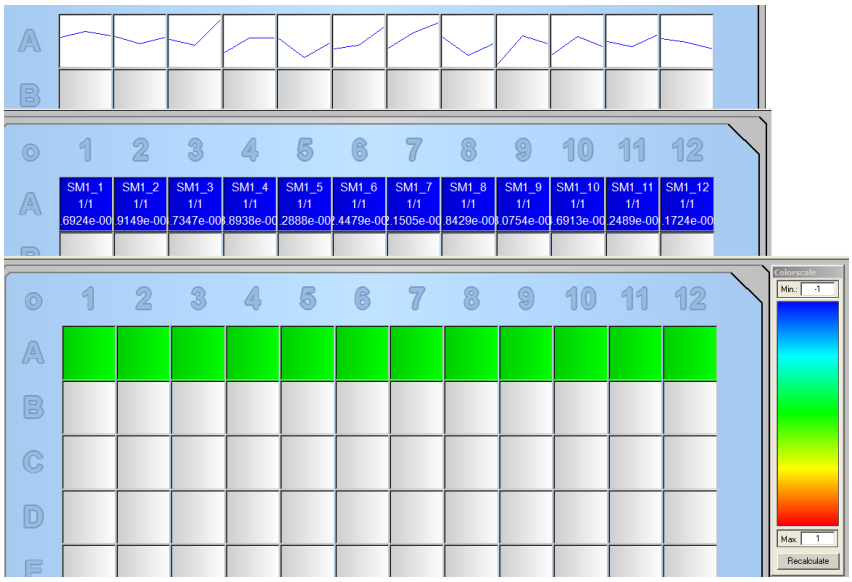


Note: in the above menu entitled “Start measurement” it is still possible to modify the method by selecting “Modify layout. Then, once in the Modify layout window, at the left top Edit Conc./Dil. Edit Meas. para. provides access to the method editor. Click OK (at the right) when ready.

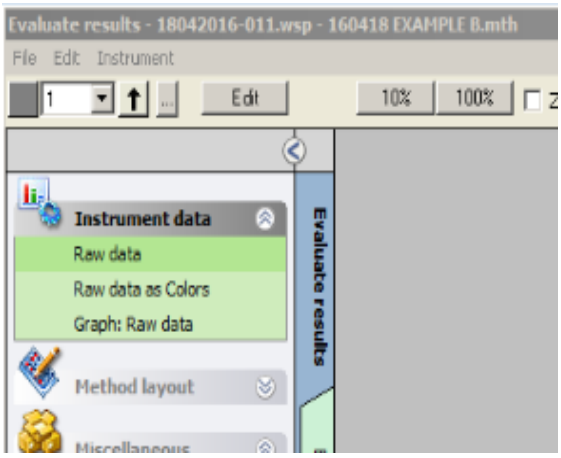
34. Option **when starting measurement from scratch**. Then, open first Magellan. In the initial “**Start measurement screen**” click on Start measurement.
35. Choose either “**Use Predefined Method**” or “**Start favorite**“ (your own favorite) and **MAKE YOUR SELECTION**



36. The chosen measurement can begin. During measurement one can choose either values or graph view.



After measurement the “Evaluate results” screen automatically appears.



Data can be displayed as values, colors or graphs

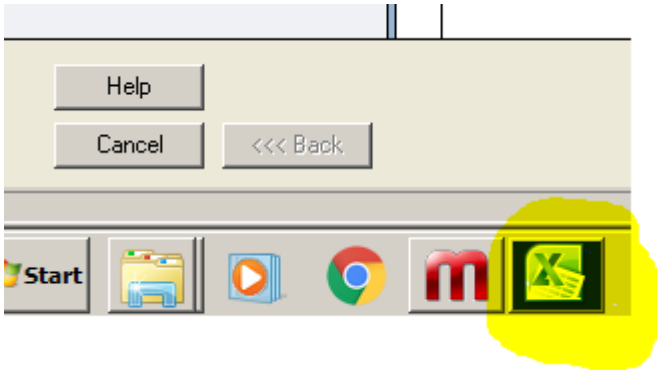
STORAGE DATA USERS AND METHODS

37. Three type of data files can be saved:
When chosen in “Automated data handling” in the method definition ,
- **the workspace file (wsp) which contains data and method is automatically saved** when previously selected in “Automated data handling”, as DDMMYYYY + counter name (incremental name) to the default path, that is:
D>DATA USERS> >ALL-USERS-Temp-Workspace.
Move this file to your own workspace folder. Please, do not leave wsp files in the ALL-USERS-Temp-Workspace folder.

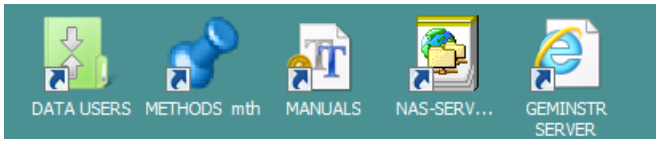
- **the ascii file** is directed to the path (your own folder) that you choose before though “More”

- **the excel file** is opened (an icon appears at the bottom of the monitor screen). It can be retrieved at the bottom of the monitor screen view. Expand the excel sheet and save it on the D disk: D>

DATA USERS> Institute (IMM, IWWR, DCN, OTHERS)> year> Group head> User> your excel folder Save with format : yymmdd Name (your filename).
Example: 160418 User (Tray 1 180416)
Users can store their data for max 1 year on that D drive.



38. Path for users files is indicated by shortcut on the desktop



Default excel format

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	<>	1	2	3	4	5	6	7	8	9	10	11	12
2	A	0.13789	0.3816	0.63154	0.89153	0.67034	0.76483	1.3265					
3	B	0.61195	0.68637	0.71753	0.6626	1.0703	0.76912	0.8125	0.82016	1.3305	1.247	1.3564	1.2613
4	C												
5	D												
6	E												
7	F												
8	G												
9	H												
10	<>	1	2	3	4	5	6	7	8	9	10	11	12
11	A	0.13616	0.38184	0.63121	0.87996	0.67375	0.76539	1.3532					
12	B	0.60874	0.70127	0.73415	0.65781	1.0992	0.81776	0.83151	0.85963	1.3395	1.2443	1.3684	1.2577
13	C												
14	D												
15	E												
16	F												
17	G												
18	H												

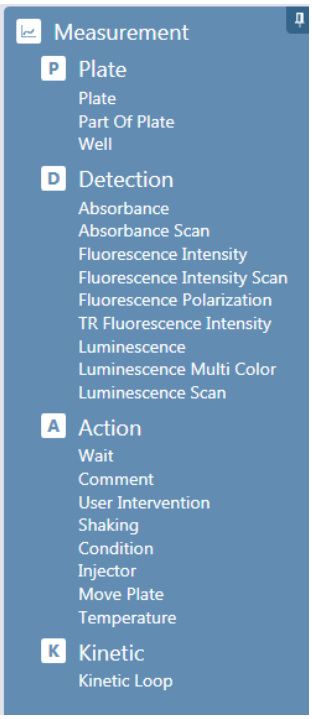
39. **Please, use servers to transfer your data. Choice of servers: your own department domain, the geminstr server, the NAS server of General Instrumentation (Ask your assistant for help, if unclear).**
DO NOT USE USB STICK OR EXTERNAL HARD DRIVE, as they are a source of virus spreading.

SWITCH OFF

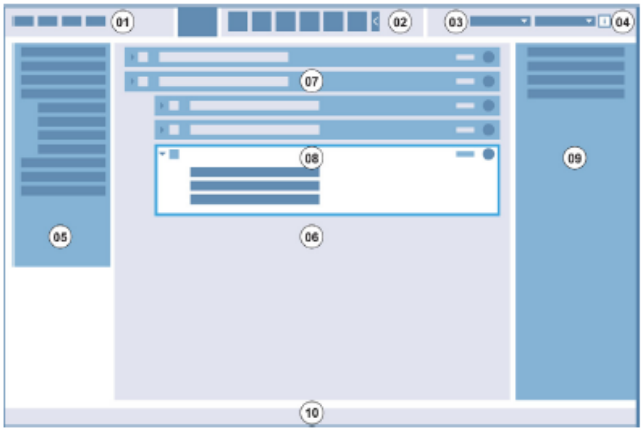
40. Rinse syringe with milli-Q
41. Remove injector rod
42. Remove plates
43. Press front panel main switch for a few seconds
44. Switch off rear panel main power Spark M10 and Cooling unit main power.

OPTIONS FOR DETECTION, ACTION AND KINETIC

45. Menu



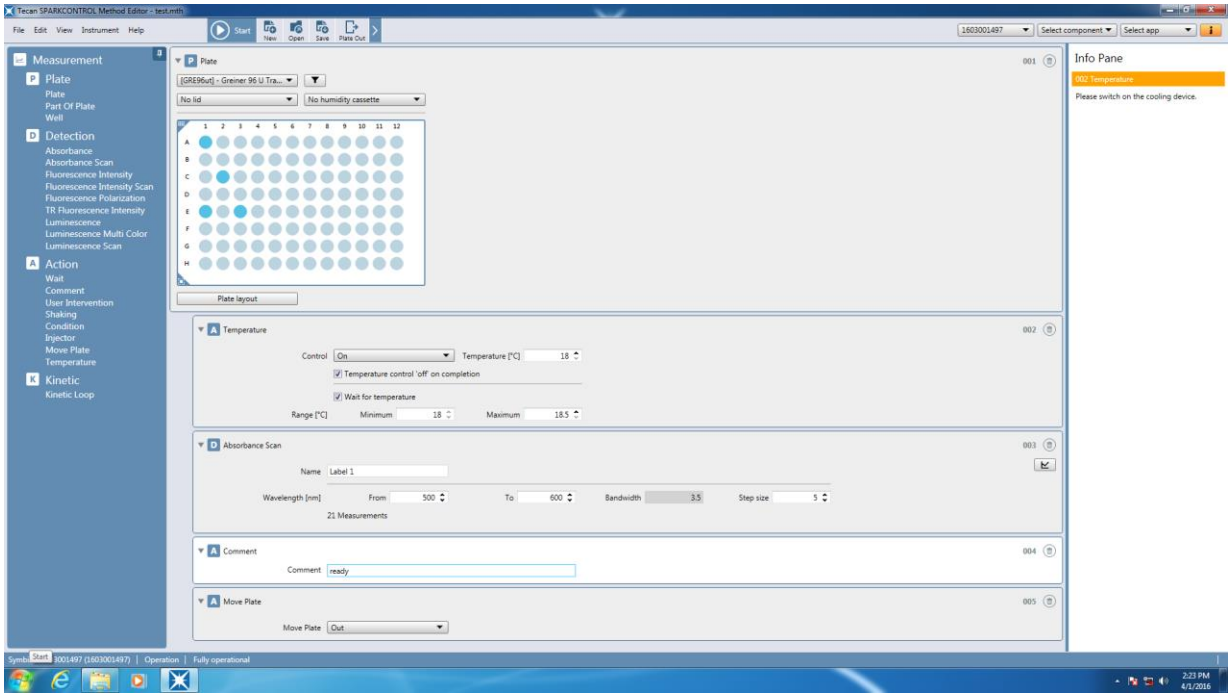
The Method Editor is used to set up workflows.



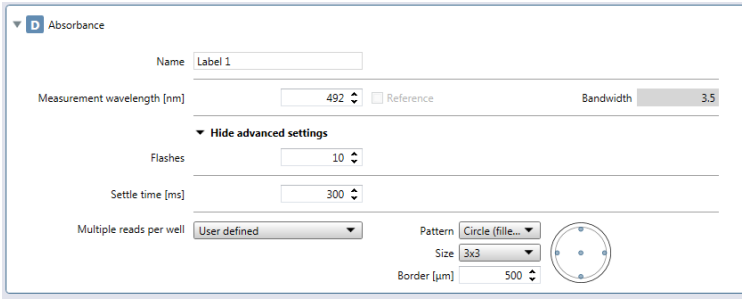
01 Menu bar; 02 Toolbar; 03 Drop-down list; 04 Button for opening the Info pane; 05 Control bar; 06 Workflow pane; 07 Collapsed strip; 08 Expanded strip; 09 Info pane; 10 Status bar

Menu bar	01	Contains a drop-down menu of editor and reader functions (e.g. File, Edit, Settings)
Toolbar	02	Contains icons for commonly used editor functions (e.g. New, Save)
Drop-down lists	03	Select and start functions related to the respective software application or instrument connected (e.g. Select app)
Control bar	05	Contains strips for defining workflows
Workflow pane	06	Insert strips into this pane to define the workflow. Default settings can also be adjusted here
Info pane	09	Displays additional information about the workflow
Status bar	10	Displays information about the connected instrument (e.g. name, temperature)

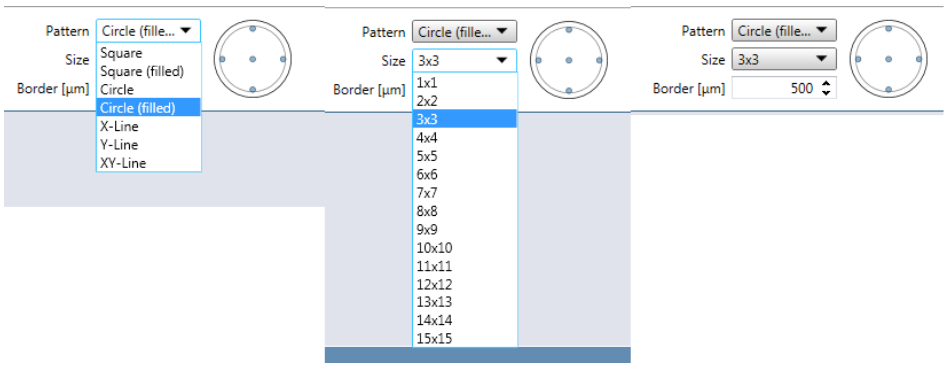
Each workflow can be created easily by dragging and dropping the process steps into a sequence according to the application. The application workflow is then visible to the user in the Workflow pane and can be saved for future use.



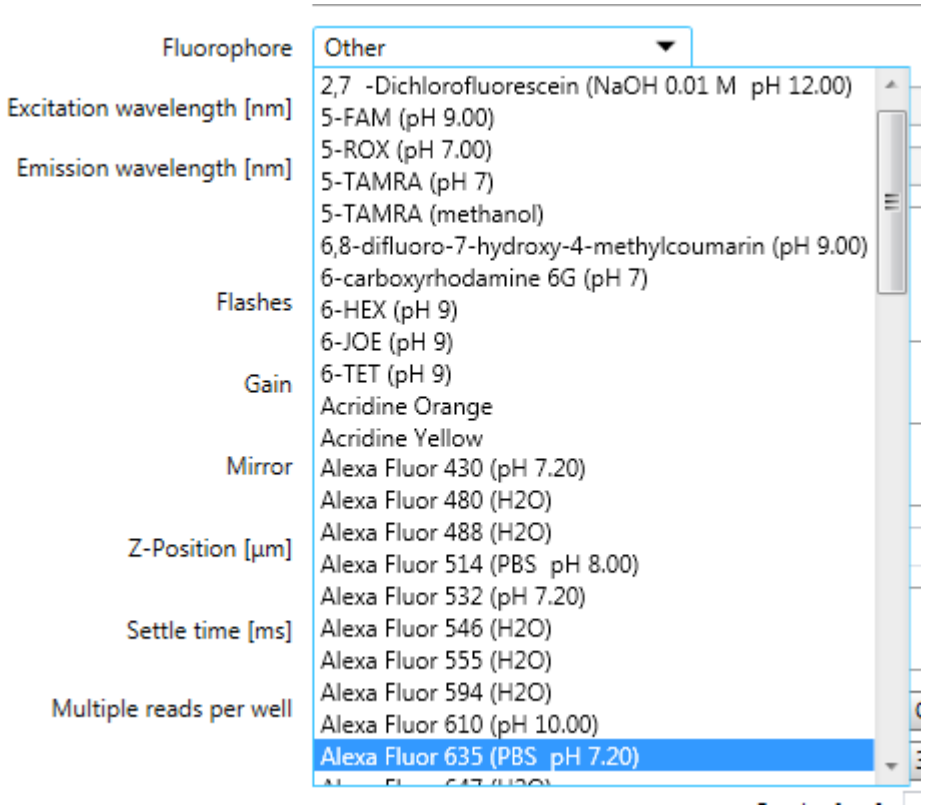
46. **Plate.** Define:
    Type of plate
    Lid or no lid (ALWAYS CHOOSE **NO** when applying luminescence, as the detector is placed deep over the wells).
    No humidity cover
    Select the wells to analyze by clicking or dragging regions (clicking on upper left angle of plate diagram selects all positions). Selected positions appear in blue.
47. Select the type of **Detection** from the menu in left column and drag it to the workflow pane. Note: dragged items can be moved to change the order of performance.
48. **Absorbance** at fixed wavelength is the first mode in the list of detection. The following options can be performed:
    - reference wavelength
    - bandwidth
    - number of flashes
    - settle time in ms
    - multiple reads per wells
    - pattern of measurements of multiple reads
    - size of read region

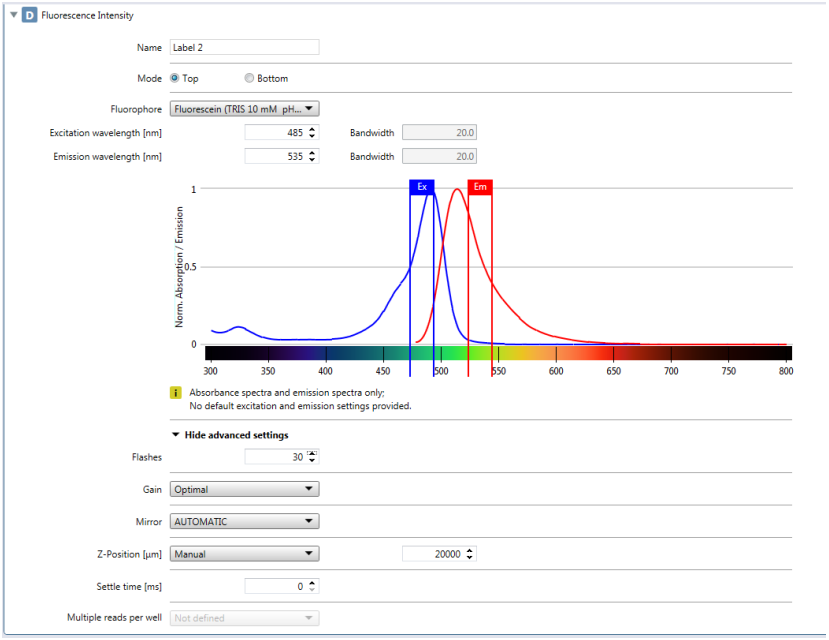


    - distance from border of read regions

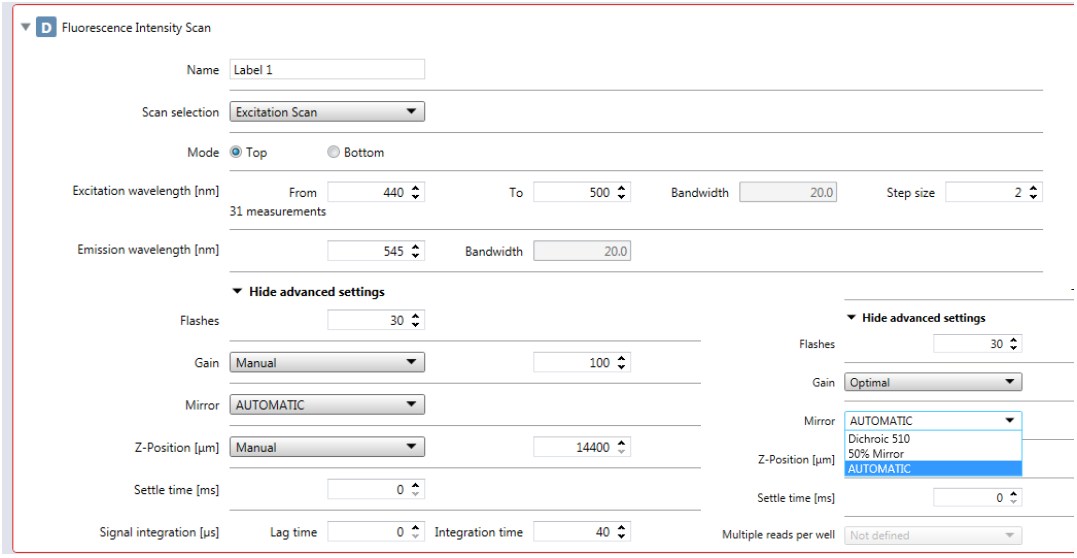


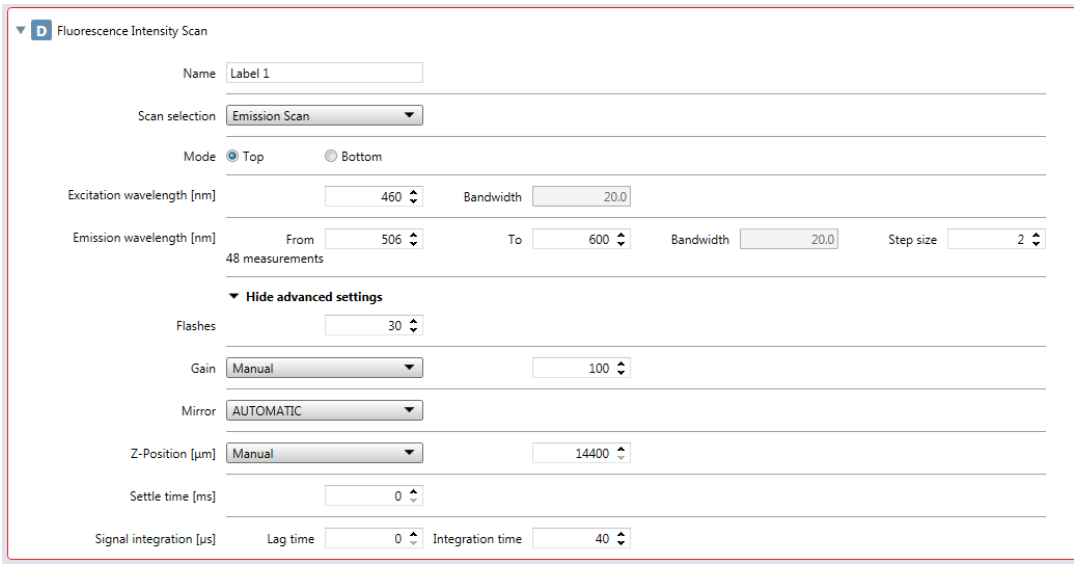
49. **Absorbance scan** over a range of wavelengths
50. **Fluorescence intensity** at fixed wavelengths. Tip: choose fluorophore from the list

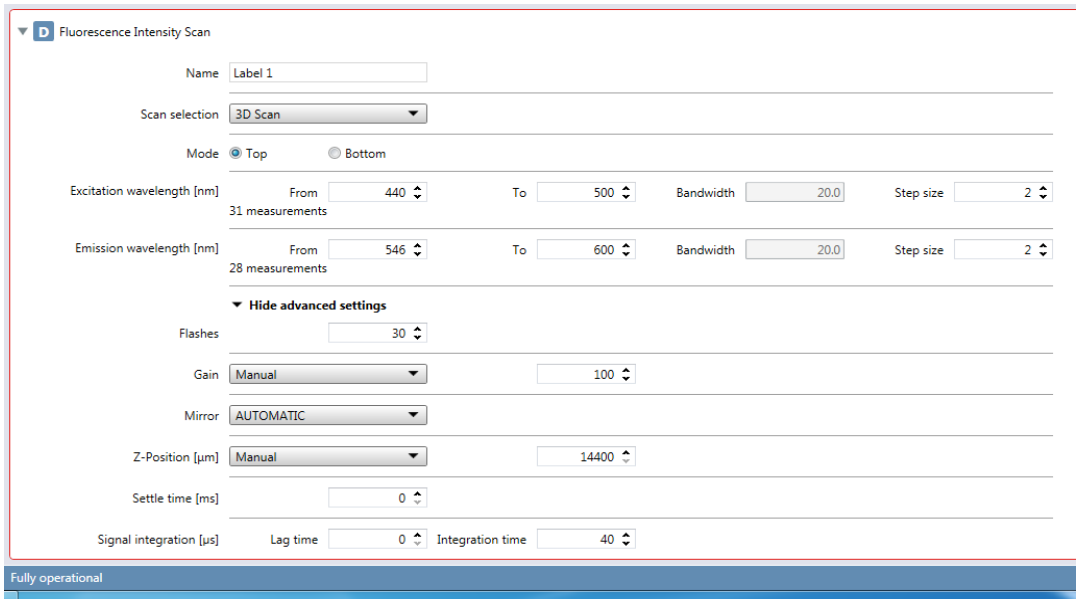




51. **Fluorescence intensity scan**, with either fixed or variable (range) excitation and emission



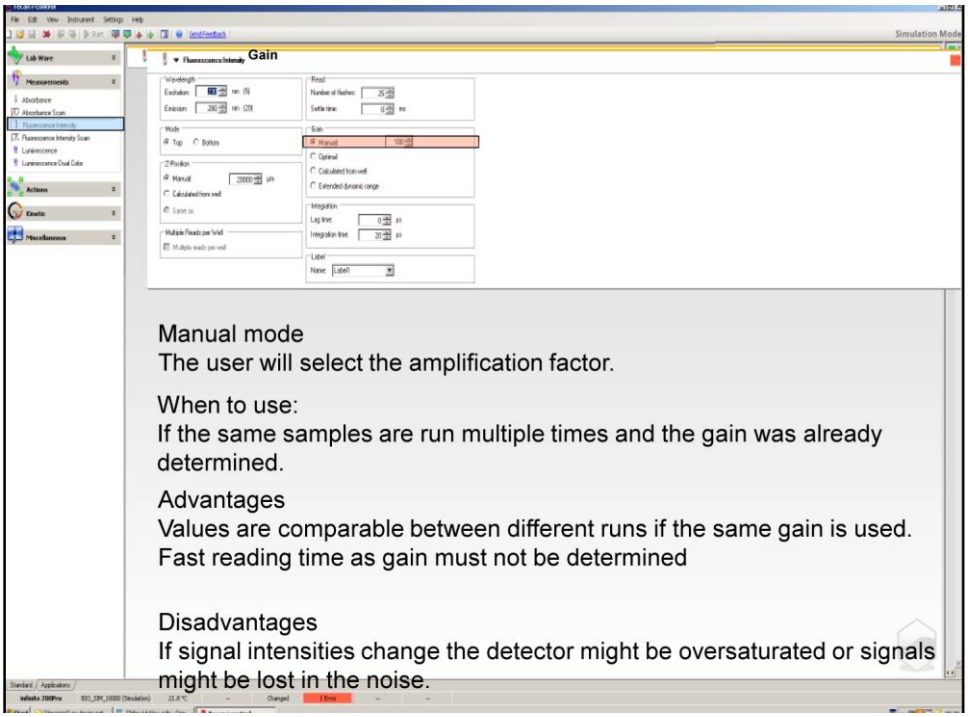




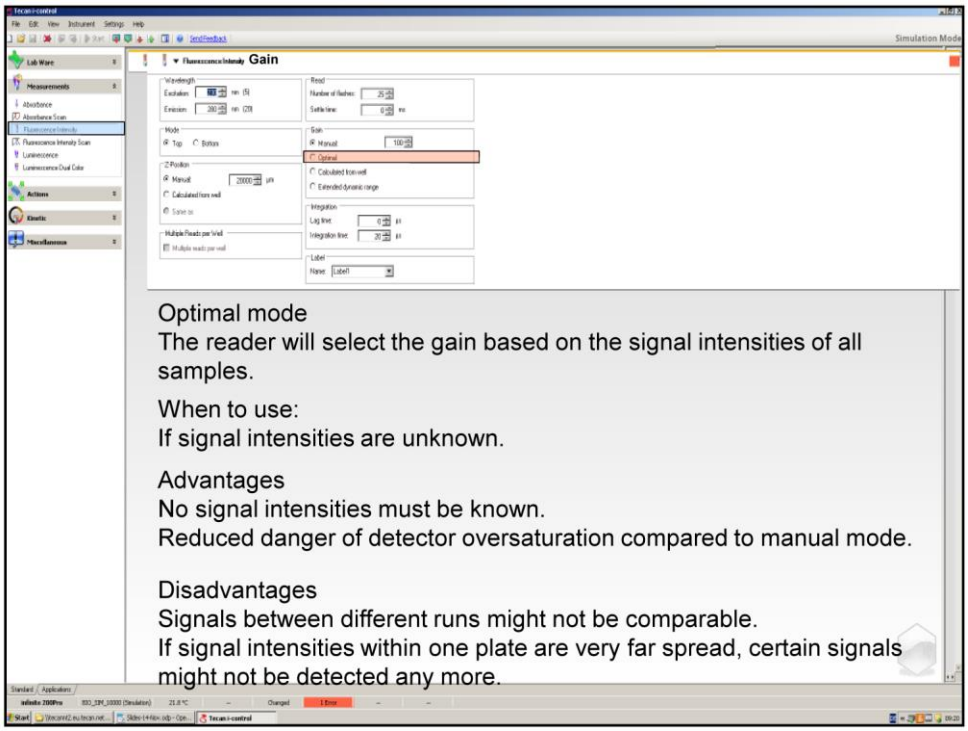
Options :

- **Bandwidth:** can be be selected in a flexible way, thanks to monochromator
- **Step size:** increment in nm of the steps to vary the excitation/emission band in a fluorescence intensity scan. The measurement of each step is performed at a controllable **bandwidth**
- **Number of flashes:** for optimal performance use the default number of flashes indicated in the instrument
- **Mirror** (refers to dichroic mirrors in fluorescence): in general keep on AUTOMATIC
- **Gain** (RFU Relative fluorescence unit):Gain values should be between 60 and 255

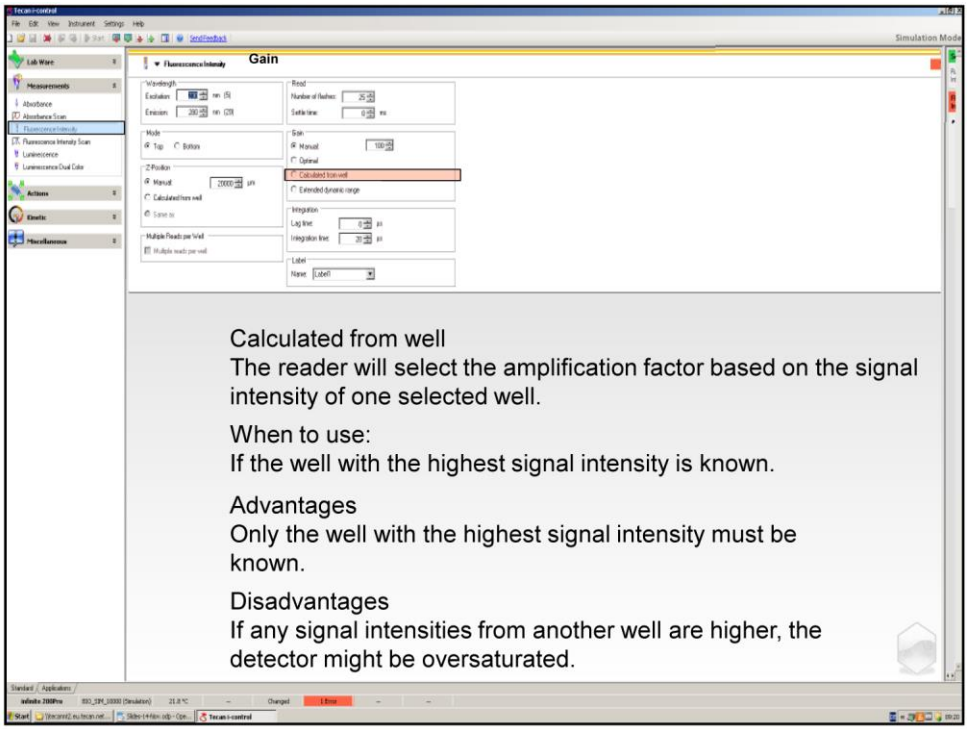
Manual mode



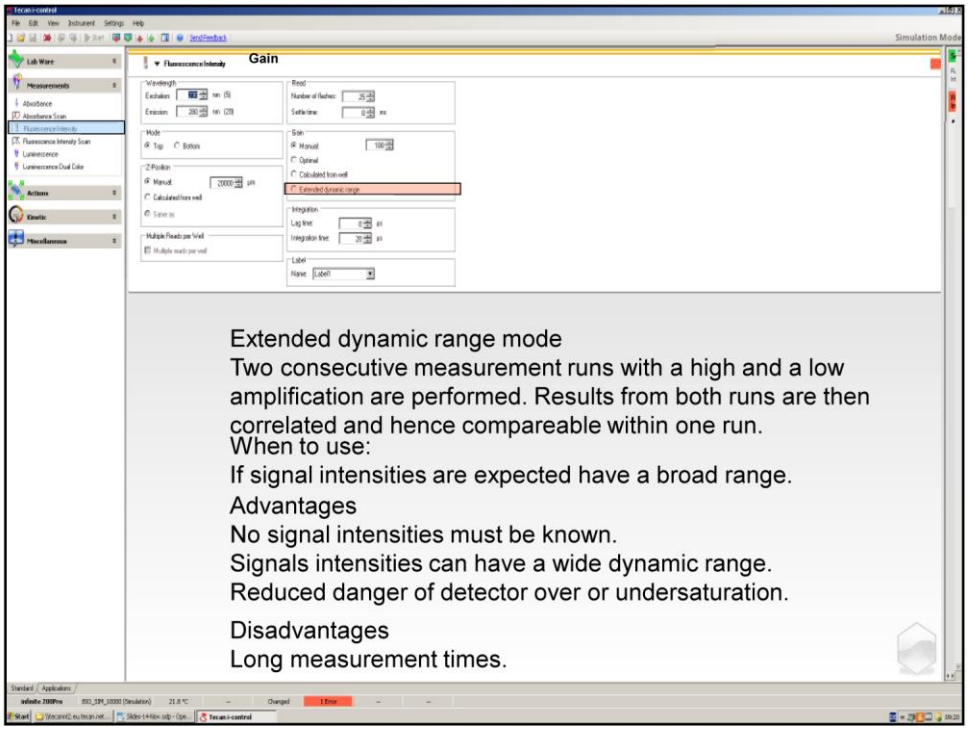
Optimal mode



Calculated from well



Extended dynamic range mode



- **Z-position** (adapts the focus of the detector to the filling volume, and hence optimizes the sensitivity).

Calculate from well: One well can be selected from which the Z-position is calculated. The well should contain one of the fluorophores used in the assay! Note: In general sample volumes within one plates should be kept constant. If volumes are different within one plate, it is best to calculate the position from a well that represents an average filling.

Values on the Huck plate reader

Z-position on the Huck Tecan plate reader		
Plate: Number of wells	***Volume (µl)***	***z-position (µm)***
96	300	22781
	250	21844
	200	21374
	150	20723
	100	19912
	75	19412
	50	19163
384	30-100	23300
11536	3-10	22775
• Values not checked for the Spark M10		

- **Settle time [ms]**: duration of measurement
- **Signal integration:** lag time and integration time[ms]

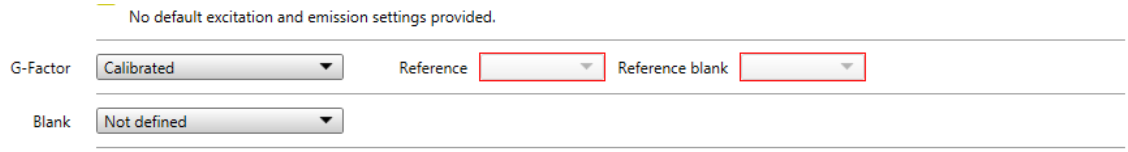
52. **Fluorescence polarization**

Fluorescence polarization measures rotational immobility of a fluorescently labeled compound due to its environment. Fluorescence polarization is defined by the following equation:

$$P = \frac{(I_{||} - I_{\perp})}{(I_{||} + I_{\perp})}$$

Where P equals polarization, $I_{||}$ equals the emission intensity of the polarized light parallel to the plane of excitation and $I_{\perp}$ equals the emission intensity of the polarized light perpendicular to the plane of excitation. FP is suitable for binding

studies, because tumbling of molecules may be dramatically reduced after binding to a much larger site, resulting in high polarization values.



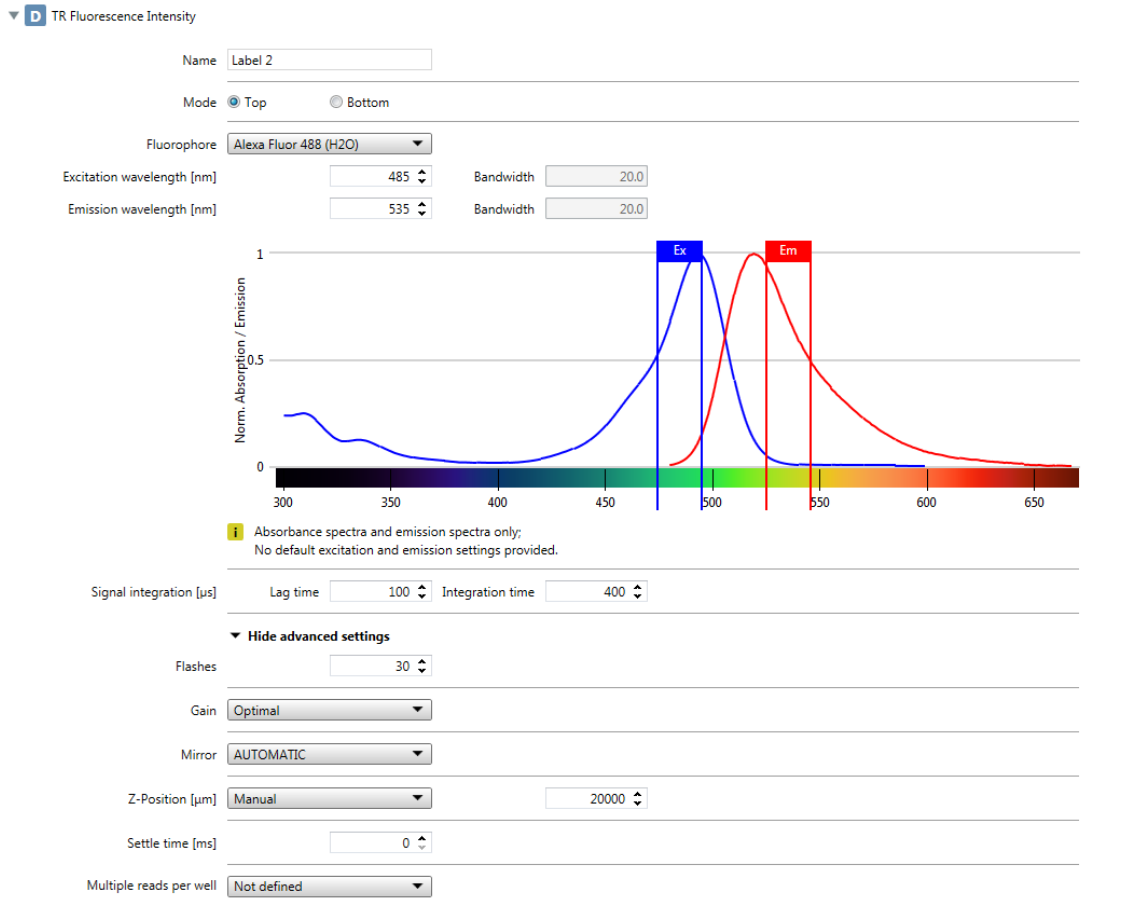
The G-factor or grating factor is an instrumental preference of the emission optics for the horizontal orientation to the vertical orientation. It can be measured by moving the excitation polarizer to the horizontal orientation and comparing the intensities when the emission polarizer is vertically and horizontally polarized respectively. G is wavelength dependent and requires at least one well containing fluorophore (reference) used in the assay and one well containing the buffer solution without fluorophore (reference blank). Once the G-factor was calculated for a certain fluorophore (assay type) it can be set manually for all further measurements using the same fluorophore.

Bandwidth	For the monochromator, select the bandwidth for excitation and emission if supported by the connected instrument.
G-Factor	Select **Calibrated** for automatic calibration of the G-factor by the instrument. Select a reference identifier and the reference blank identifier used for blanking according to the plate layout as defined in the Plate strip. Select **Manual**, if the measurement is carried out with a G-factor value manually defined by the user or with a calibrated value already available for the selected wavelength combination. If no calibrated G-factor is available, the default value of 1 will be displayed and marked as **Uncalibrated G-Factor**. Otherwise, a calibrated value will be displayed and marked as **Calibrated G-Factor**. Both the uncalibrated and the calibrated G-factors can be manually changed by the user. Use the **Reset** button to recall the original calibrated value.

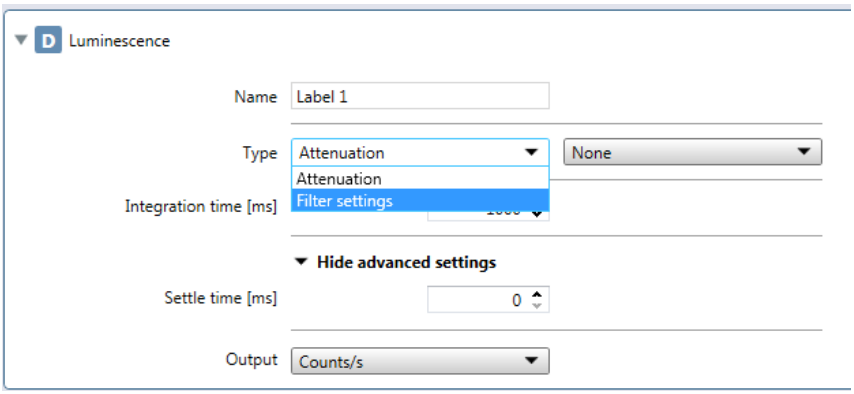
53. **TR fluorescence intensity**

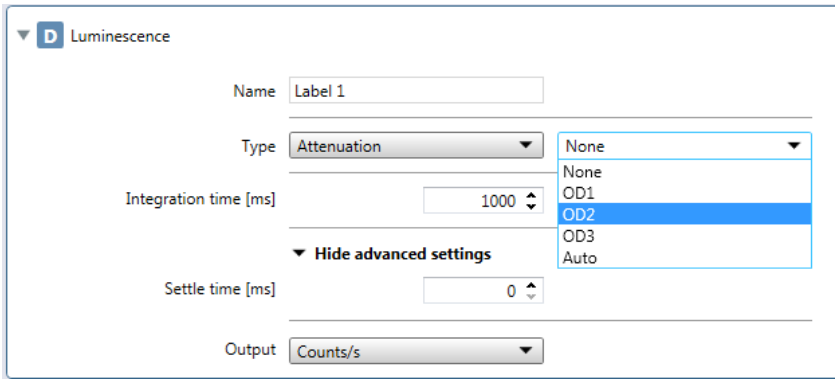
Time-resolved fluorescence applications apply fluorescent acceptors with a long-lived fluorescence signal (e.g. lanthanides like Europium and Terbium). Consequently, the short-lived unspecific background fluorescence signal can be excluded by using a time delay between the excitation and

signal integration thus maximizing the signal to background ratio.

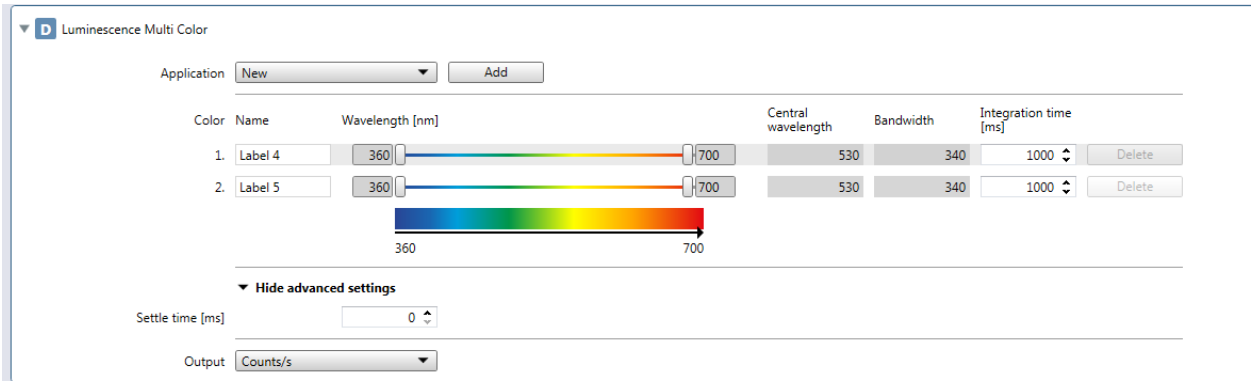


54. **Luminescence**

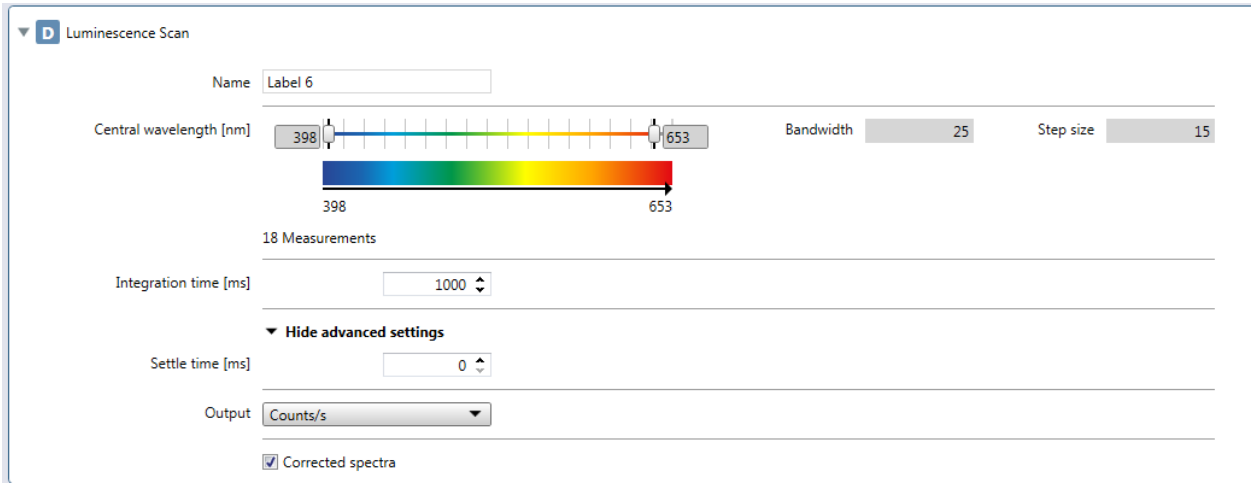




55. **Luminescence multi color**

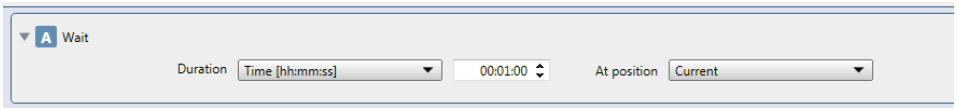


56. **Luminescence scan**

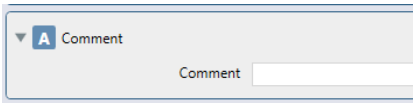


57. **Action:**

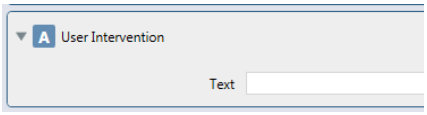
- Wait



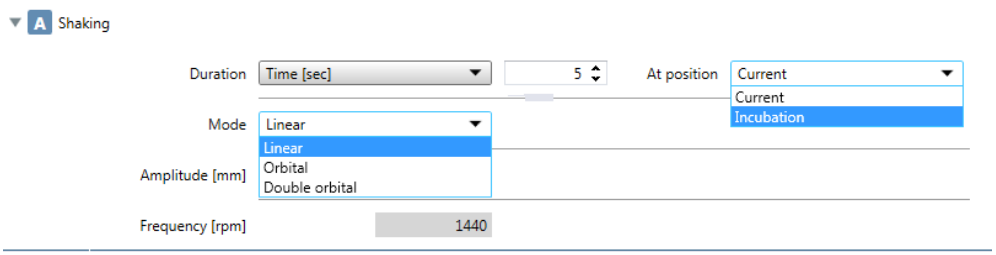
- Comment



- User intervention



- Shaking

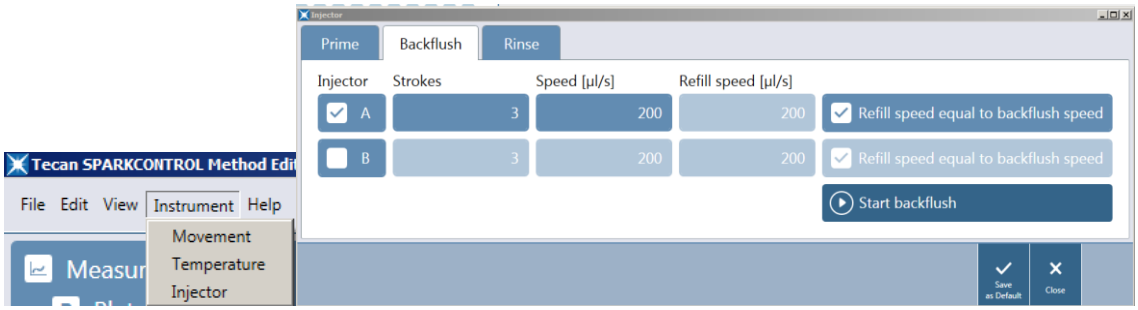


- Condition

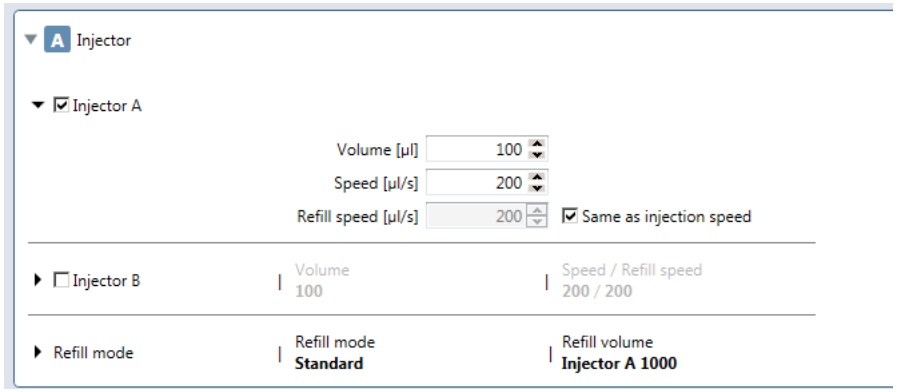
- Injector

To prepare the injector select in the upper horizontal menu bar Instrument > Injector

A new windows opens in which the rinse, prime and backflush volumes and speeds can be set



Then add the injector function to the program if necessary

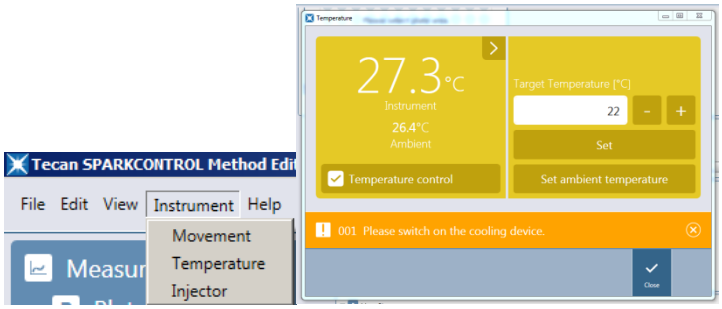


- Move plate

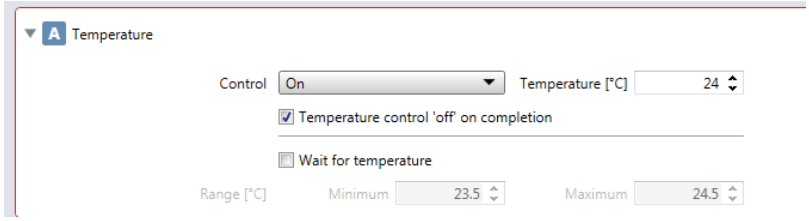
- Temperature

Through upper bar menu > Instrument > temperature

Set desired target temperature. Operation range 18-42°C Note: set cooling about 5°C lower than desired temperature, but do not set the measurement temperature below 18°C .



Possibility to define target temperature to reach before starting measurements in “Action”



Kinetic loop