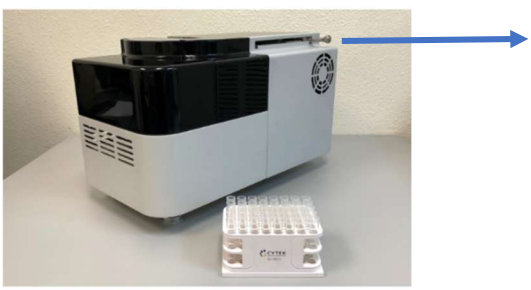
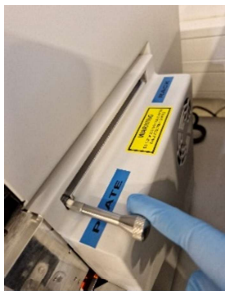
Loader Operation Protocol

1. Loader Overview

The Automated Sample Loader (ASL) is an automated loading system that mixes samples and delivers racks and plates to the cytometer for acquisition. The ASL resuspends samples using an orbital shaker. It supports a range of 96-well plates (96 U-Bottom, 96 V-Bottom, 96 Flat-Bottom, 96 Deep) and a 40-tube Tube Rack. The manual handle is located on the right-hand side of the loader and it is a mechanical level used to make the instrument work in Plate mode, Tube mode or Tube Rack mode. The lever can be moved when the unit is switched on or off.

Important: If you switch from tube to plate acquisition, make sure there is no tube on the SIP (Sample Injection Port) and that the sample line is in the instrument. If a tube is on the SIP or the sample line is outside the SIP, the system will be damaged when you pull the lever to plate mode (towards you).





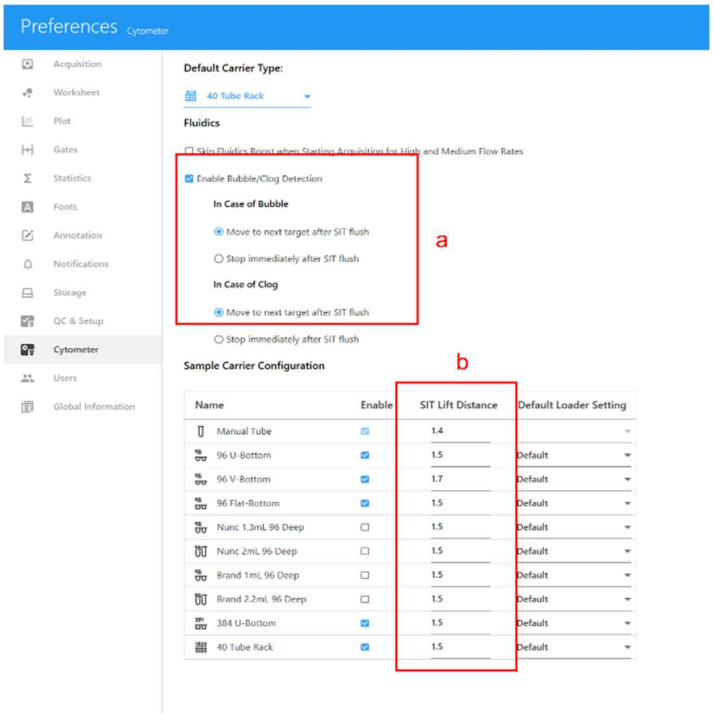
2. Loader Start-up

a) Turn on the cytometer and computer (if not already switched on). Turn on the power to the Loader by pressing the switch on the back of the unit.
b) Launch the SpectroFlo software and log in using the appropriate username. Once you have logged in to the software, the cytometer initialization procedure begins. When the loader is connected to the software, the status light in the bottom right-hand corner of the screen displays a green tick. A red cross appears if the loader is not powered up or connected to the software.
c) Load a tube of DI water and wait for the self-diagnosis of the unit and loader to be completed. NB: If a shutdown or Clean Flow Cell in rack tube has been performed before your arrival, once the SpectroFlo software has been opened, the rack will be automatically moved and you will have access to the SIP to load the water tube.

3. Setting-up an experiment with the loader

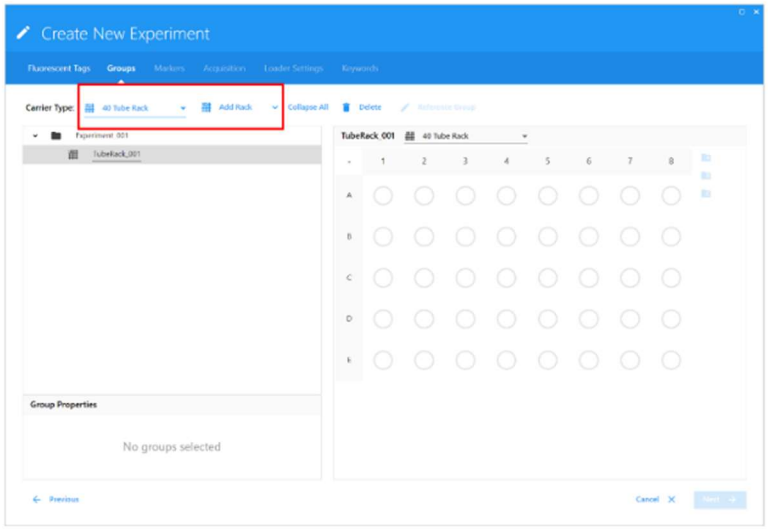
1) In the Preferences Cytometer menu:
   - Configure bubble/plug detection preferences as shown in the following figure (**a**).
   - If you are using a plate type for the first time, set the SIT (Sample Injection Tube) lift distance (**b**). To do this, place water in position A1 on a test plate and check that you have the correct flow rate for the corresponding flow rate (see table below). If the measured flow rate is too low or if you get a "bubble detection" message, that means the sample line is too high, so decrease the SIT lift distance value. If you get a flow rate equal to 0, that means the sample line is stuck at the bottom of the well, so you'll need to increase the SIT lift distance value.

Flow rate	µl/minute
Low	15
Medium	30
High	60 (tubes) 100 (plaques)



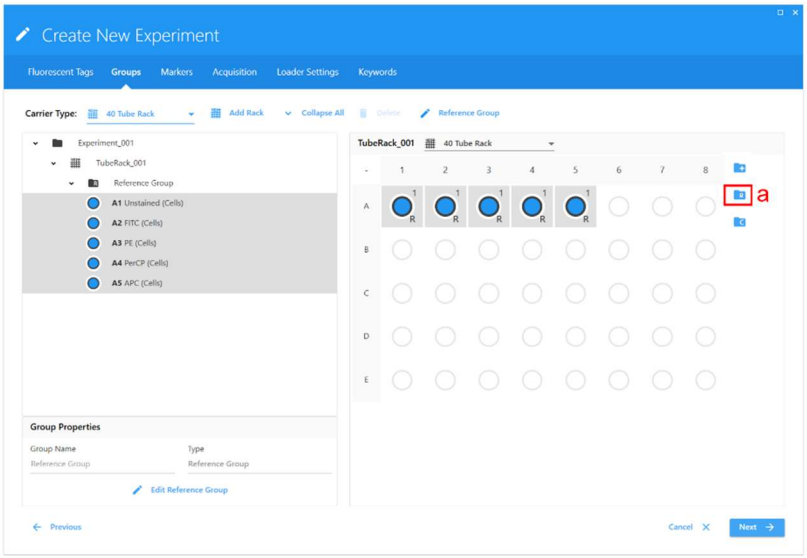
2) In the **Group** tab of the experiment create/edit module:

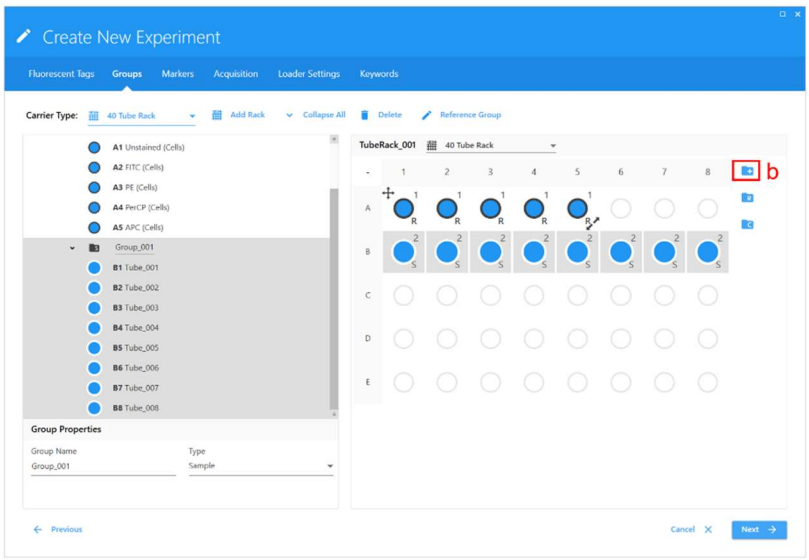
- Select the Carrier type and add it by clicking on Add Rack/Plaque. A tube/plate plan appears on the right.



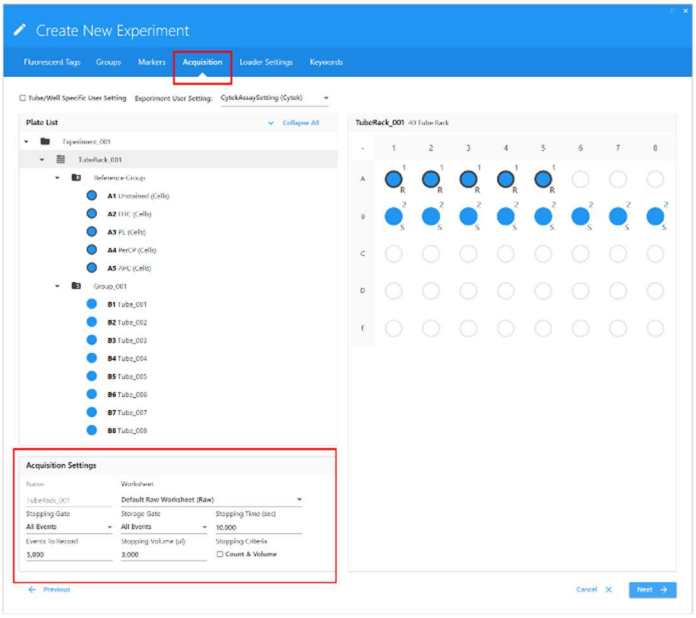
- On the rack/plate plan, select the wells for reference group acquisition by clicking on the "**R**" icon on the right (**a**) and the wells for sample group acquisition by clicking on the "**+**" icon on the right (**b**). The tubes in the reference group will automatically be placed in increasing order of excitation wavelength of the corresponding fluorochromes, and

fluorochromes excited by the same laser in increasing order of emission peak wavelength. If this order doesn't match your plate layout, you can change the position of the wells by dragging them on the plate view.





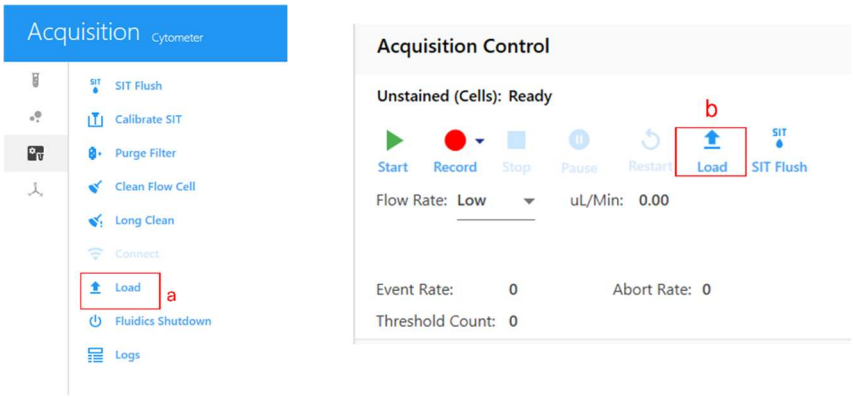
3) <u>In the **Acquisition** tab of the experiment create/edit module:</u>
Configure the experiment parameters. These include the worksheet and stopping acquisition criteria. You can apply the same parameters to the whole experiment (reference group and sample group) or configure specific parameters for different groups as well as for different tubes within a group.
<u>Important</u>: In Stopping Volume always put 60µl less than what you have in the tubes or plates, because at the beginning of acquisition there is a boost which causes 36µl loss plus a small extra volume to be lost for Record Delay Time (see next paragraph).

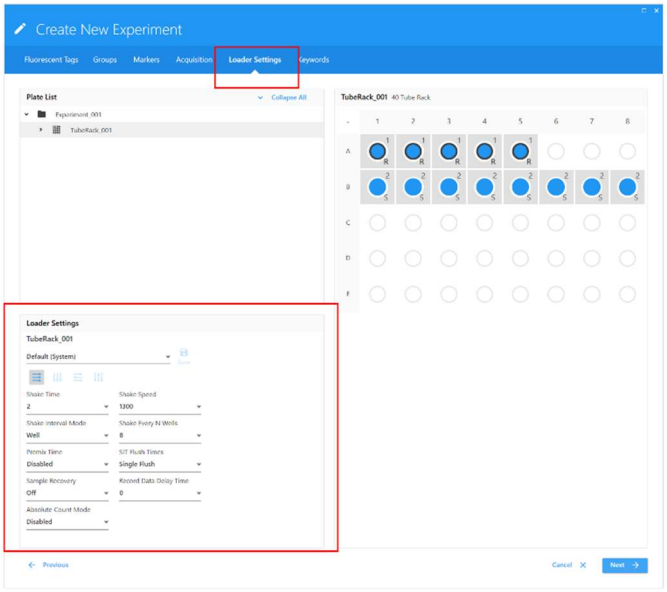


4) In the **Loader Settings** tab of the experiment create/edit module:
Configure the loader's operating parameters. These include shaking configuration, SIT Flush Times, Sample Recovery and Record Data Delay Time.
Important:

- If you're doing plate-based acquisition, pay attention to Shake Speed, because depending on the volume you have in your wells and their depth, an unsuitable Shake Speed could cause your samples to overflow. If you are using a particular type of plate for the first time, we recommend you carry out a test with water.
- Since the analyzer's sheath liquid is milli-Q water, always select OFF for Sample Recovery.

5) Once you have set up your experiment, you can position your plate or tube rack with your samples and click on Load.
Important: Each time you place a plate or rack tube on its support (including for Shutdown or Clean Flow Cell), remember to perform a new Load so that the support is in the correct XY position. You can access the Load either in the Cytometer tab (**a**) (when an experiment is not open, e.g. before performing the Shutdown or Clean Flow Cell) or in the Acquisition Control (**b**) (when an experiment is open).



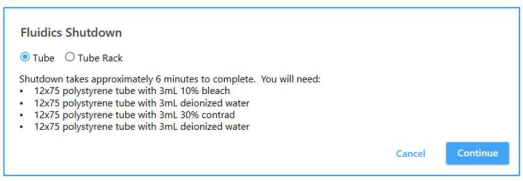


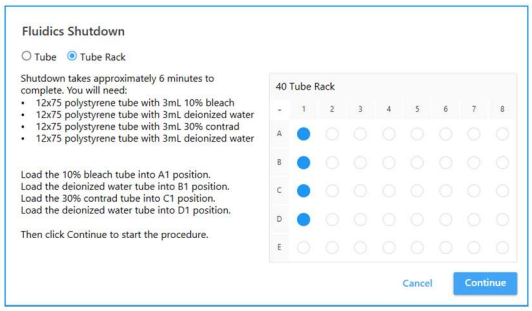
Paramètre	Description
Selected Settings (Paramètres sélectionnés)	Trois paramètres de chargeur sont disponibles : Default (Défaut), High Throughput (Haut débit) et Low Carryover (Contamination croisée faible). Vous pouvez créer vos propres paramètres personnalisés.
Acquisition order (Ordre d'acquisition)	Sélectionnez l'ordre dans lequel vous souhaitez analyser la plaque. Les puits sont acquis par : • rangée de gauche à droite (A1-A12, B1-B12, etc.) • colonne de haut en bas (1A-1H, 2A-2H, etc.) • rangée de gauche à droite, puis de droite à gauche (A1-A12, B12-B1, C1-C12, etc.) • colonne de haut en bas, puis de bas en haut (1A-1H, 2H-2A, etc.)
Shake Time (Durée d'agitation)	Sélectionnez la durée (en secondes) d'agitation de la plaque/du portoir de tubes. Vous pouvez aussi désactiver la durée d'agitation.
Shake Speed (Vitesse d'agitation)	Sélectionnez la vitesse de l'agitateur orbital (en tr/min).
Shake Interval Mode (Mode d'intervalle d'agitation)	Sélectionnez l'intervalle d'agitation : tous les N puits/tubes ou après un délai déterminé. Vous pouvez aussi désactiver l'agitation.
Shake Every N Wells, or Shake Interval (Agiter tous les N puits, ou intervalle d'agitation)	Sélectionnez la fréquence (nombre de puits/tubes ou durée en secondes) d'agitation de la plaque/du portoir de tubes.
Premix Time (Durée de prémélange)	Sélectionnez la durée (en secondes) de l'agitation de la plaque/du portoir de tubes avant l'acquisition du premier tube/puits.
SIT Flush Times (Nombre de rinçage de la SIT)	Un rinçage de la SIT est effectué sur le poste de lavage après chaque acquisition. Choisissez Single Flush (Rinçage simple), Double Flush (Rinçage double) ou Disabled (Désactivé) si vous ne souhaitez pas effectuer de rinçage de la SIT.
Sample Recovery (Récupération des échantillons)	Permet de redéposer dans le puits tout échantillon restant dans la SIT une fois l'acquisition terminée.
Record Data Delay Time (Délai de temporisation d'enregistrement des données)	Sélectionnez la durée de prévisualisation des données d'un puits/tube, en secondes, avant le début de l'enregistrement après avoir cliqué sur Record (Enregistrer).

4. Loader Shutdown

Systematically check the MRI reservation website to see if another slot is reserved after yours. Leave the cytometer on standby or switch it off, depending on reservations.

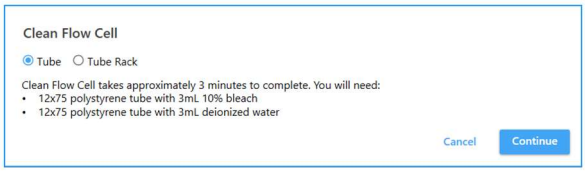
1) <u>If there is no slot reserved after you, so you are the last user for the schedule of the day, shutdown the system:</u>
   - In the Cytometer menu from Acquisition module, select Fluidics Shutdown and follow instructions. You can choose whether to carry out the procedure manually or with a tube rack:

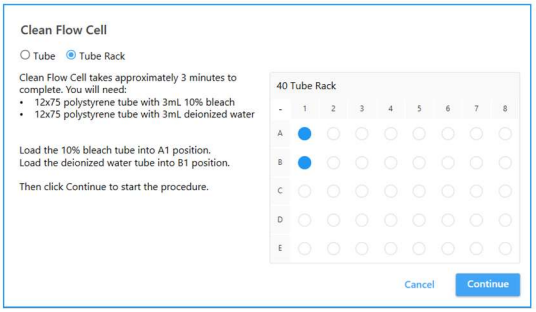




   - Allow the shutdown procedure to complete, then click Done
   - Make sure the SIT is submerged in the DI water at the end of the procedure.
   - Turn off the cytometer and the sample loader.
   - Exit SpectroFlo software.
   - Log out of your MRI session (logout icon on desktop).
   - Leave the computer on.

2) <u>If there is a slot reserved after you:</u>
   In the Cytometer menu from Acquisition module, select Clean FlowCell and follow instructions. You can choose whether to carry out the procedure manually or with a tube rack:





- After completing Clean Flow Cell, leave the tube of DI water on the SIP.
- Turn off the Sample Loader
- Exit SpectroFlo software.
- Log out of your MRI session (logout icon on desktop).
- Leave the computer on.

Do not forget:

1) If you switch from tube to plate acquisition, **make sure there is no tube on the SIP** (Sample Injection Port) and that the sample line is in the instrument. If a tube is on the SIP or the sample line is outside the SIP, the system will be damaged when you pull the lever to plate mode (towards you).
2) If you are using a plate type for the first time, set the SIT lift distance and **do a test with water** to adapt the shake speed to the volume you have in the wells.
3) In Acquisition Settings, always set **Stopping Volume to 60µl less** than what you have in the tubes/plates, and always select **OFF for Sample Recovery**.
4) Each time you place a plate or tube rack on its support (including for Shutdown or Clean Flow Cell), remember to **perform a Load** so that the support is in the correct XY position.