



CYTEK
TRANSCEND THE CONVENTIONAL

Aurora

Operation Protocol

Reference Controls
(for Library)

FCF-Berg

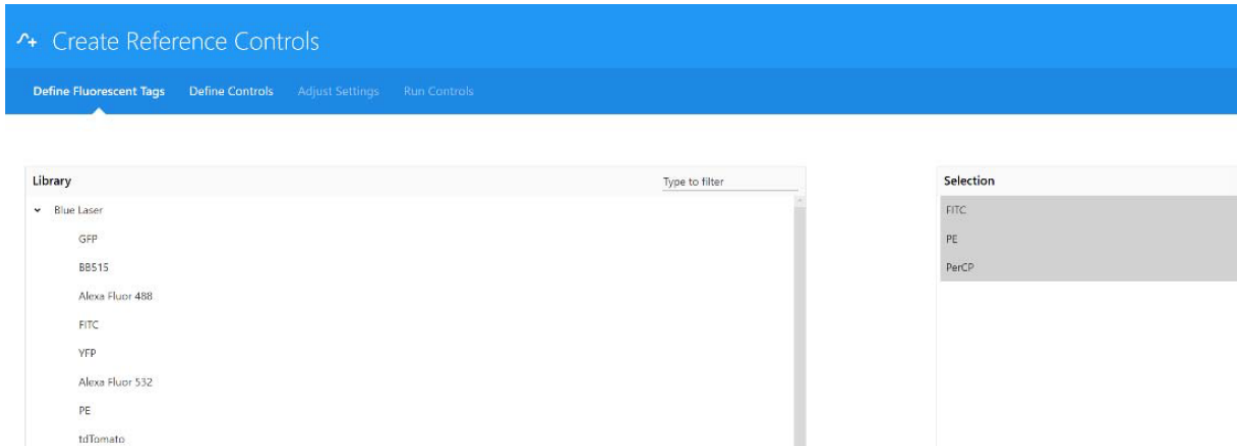
Tuebingen University
Department of Internal Medicine II

Reference Controls

- 1) Run Daily QC
- 2) QC & Setup modul > Reference Controls > New Reference Controls > wizard opens:

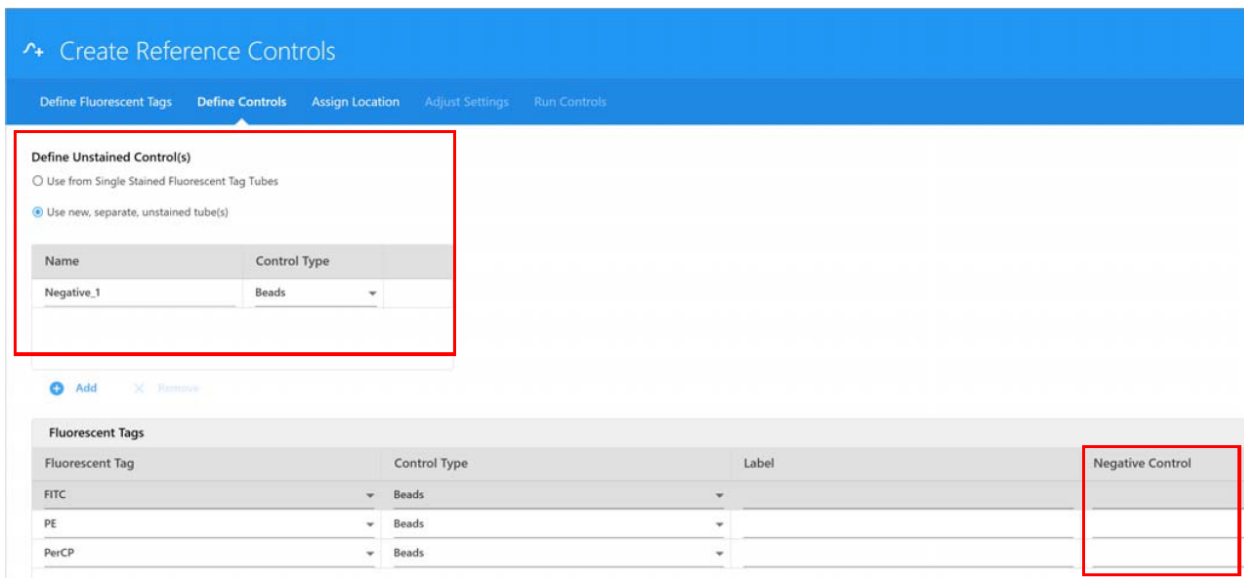


> search and select **fluorescent tags** (by dragging and dropping, double-click or Add button)

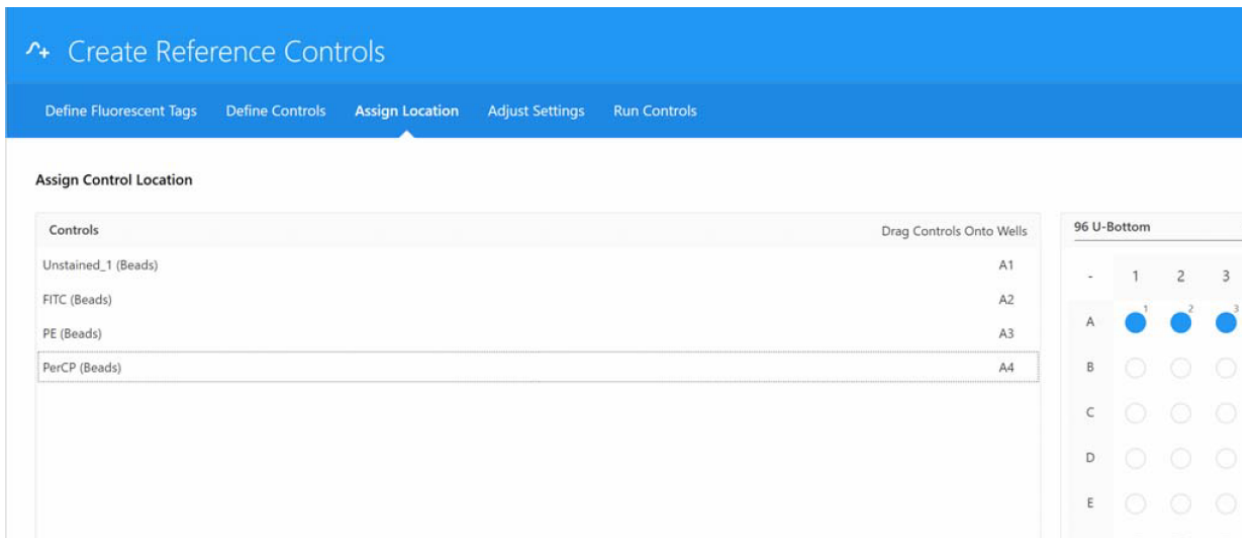


> **Next**

- 3) Define **unstained control(s)**
Choose either single-stained fluorescent tags, or new, separate, unstained for the control tubes.



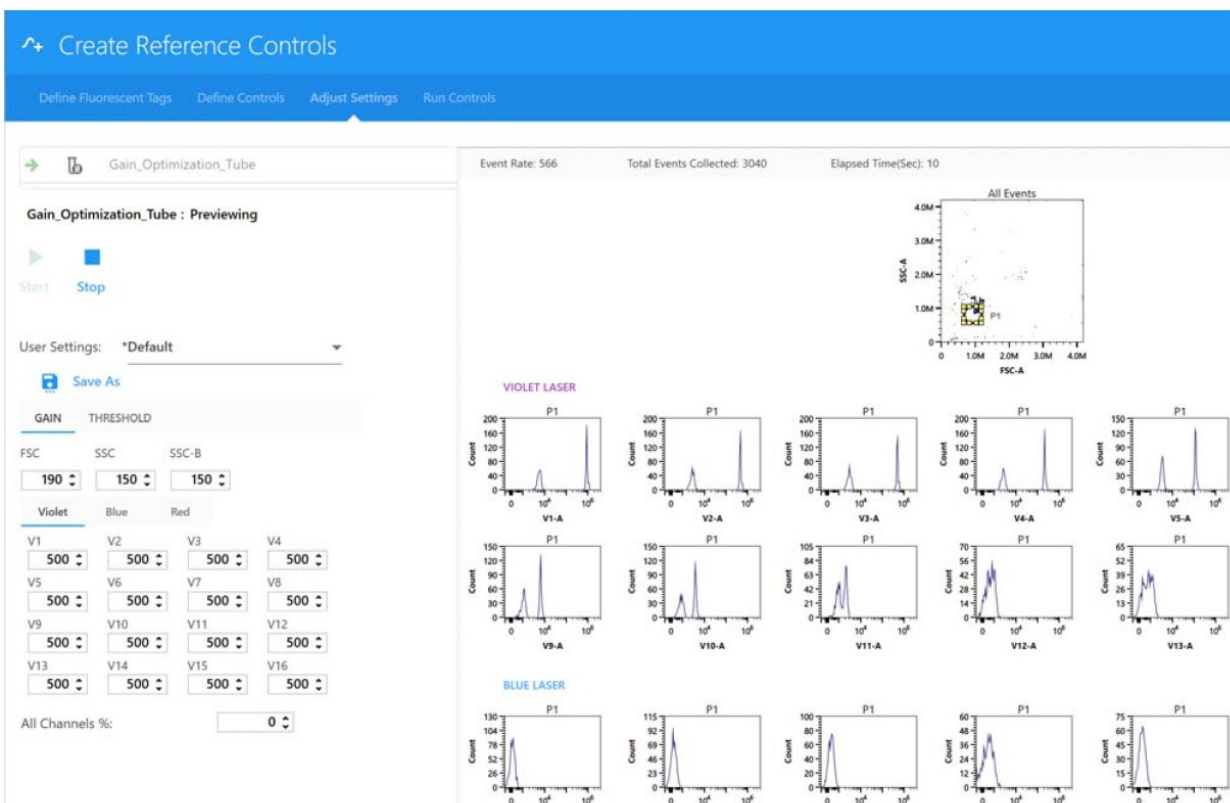
- 4) Define the **control type (beads or cells)** for each fluorescent tag in the table
- 5) (Optional) enter labels associated with the fluorescent tag for identification and tracking
- 6) Click **Next**
- 7) Load control sample(s)



8) > click **Start** to preview sample data > adjust scale and (optional) adjust **CytekAssaySettings** (threshold, gain settings)
→ to click **Restart** helps to refresh the preview

9) Once gain settings have been confirmed, unstained and reference controls are ready for **acquisition**.

NOTE: Dim markers may not separate from the negative population regardless of how much the gain is increased.



10) Preview the data for the remaining controls by clicking **Stop**, then:

- Tube mode: Loading the next control onto the SIP and clicking Start.
- Plate mode: Selecting the next control well and clicking Start.

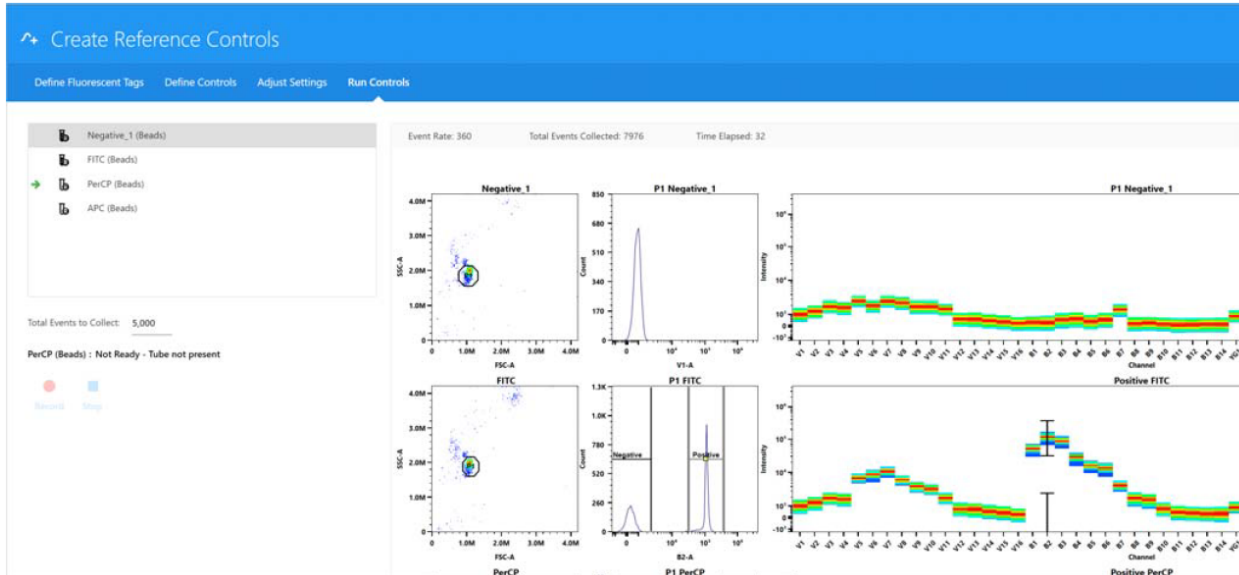
11) Select **Next** when you are satisfied with the gain settings. Proceed to running controls.

12) If you are running in Tube mode, place a tube of the appropriate single-stained particles on the SIP.

13) 14 Click **Record** to begin acquiring.

Make sure to follow the order listed in the left-hand panel. When running in plate mode, you can select to record a single well/control or the entire plate.

NOTE: During acquisition the spectra plot for each fluorescent control is displayed. The plots show all the channels across all lasers in the x-axis vs mean fluorescence intensity (MFI) of the fluorescent tag.



14) During acquisition, obtain spectral information by moving the polygon gate on the FSC-A vs SSC-A plot to include the population of interest.

Hold down the **Ctrl** key while adjusting the gate to move the polygon gates for all the scatter plots at once. The gated population appears in the histogram, which is set approximately to the peak emission channel of the fluorescent tag to be acquired. The emission spectrum of the population is displayed in the spectrum plot.

Adjust the positive gate on the histogram. The software automatically displays the emission spectrum of the positive particle in the spectrum plot. SpectroFlo software sets the default gate near or on the peak emission channel. The gate can be selected manually. It is best to set the gate on the brightest emission, as this can make distinguishing the positive and negative populations easier and provides better visualization of the spectrum.

NOTE: Unmixing results are unaffected by the position of the interval gate in the spectrum plot.

Readjust the positive and/or negative gate on the histogram, if necessary.

15) Continue recording each control.

16) Select Save to save the reference controls.