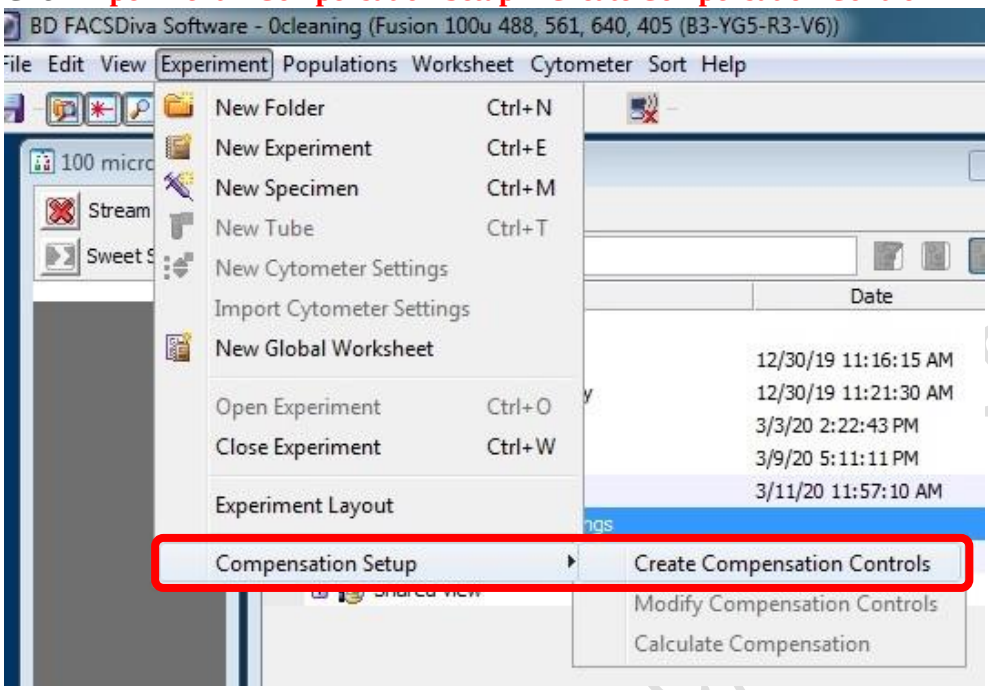
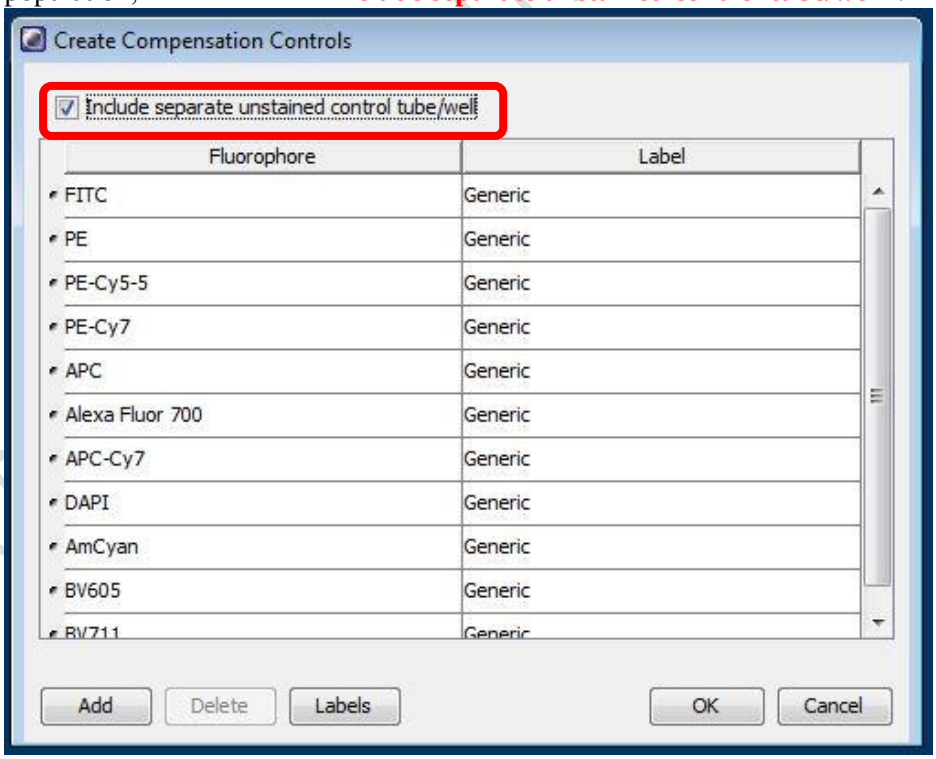
BD FACSDiva Compensation Protocol

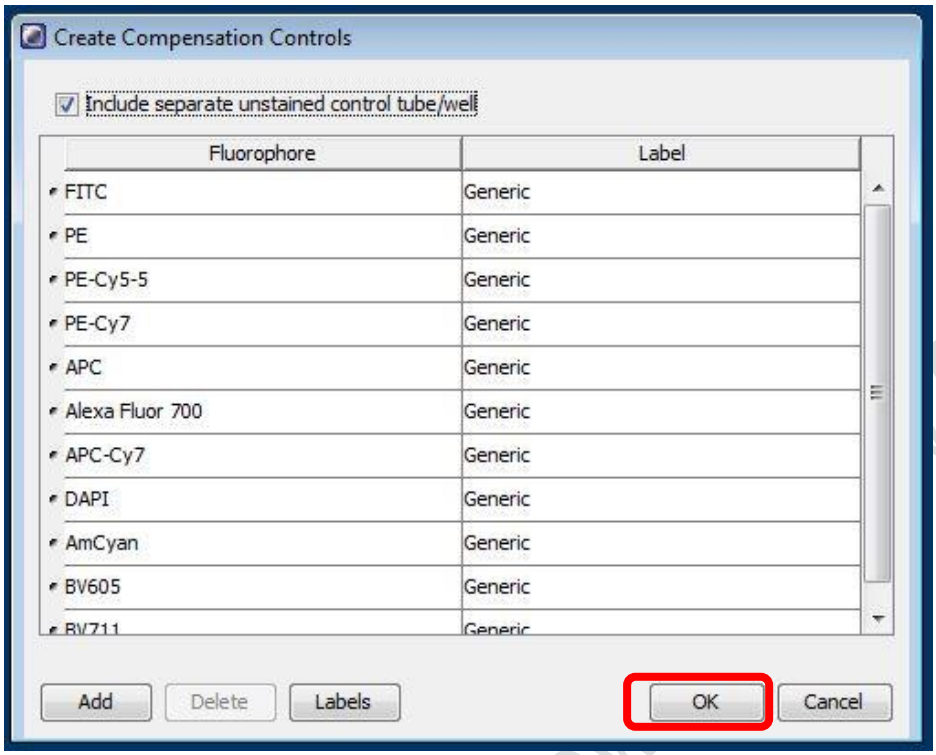
1. Click **Experiment > Compensation Setup > Create Compensation Control**



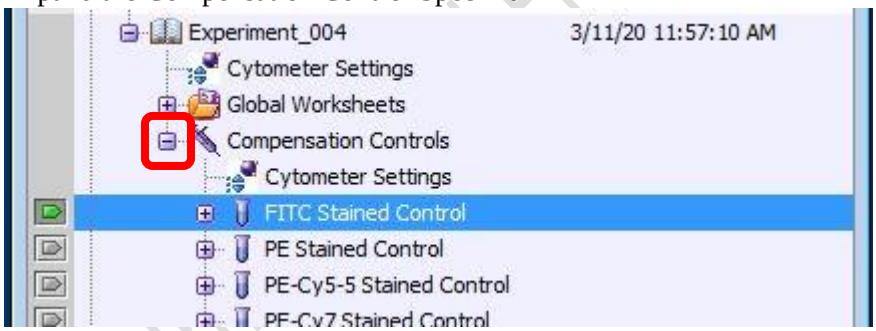
2. If any one of your single stain controls is known to be 100% positive, i.e. no negative population, check the box "**Include separate unstained control tube/well**".



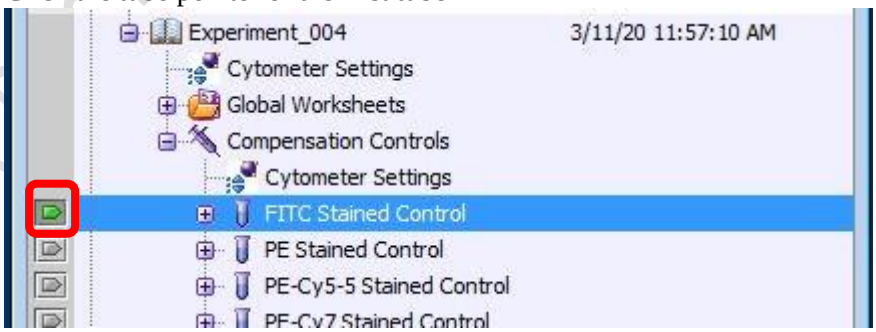
3. Click **OK**



4. Expand the Compensation Control Specimen



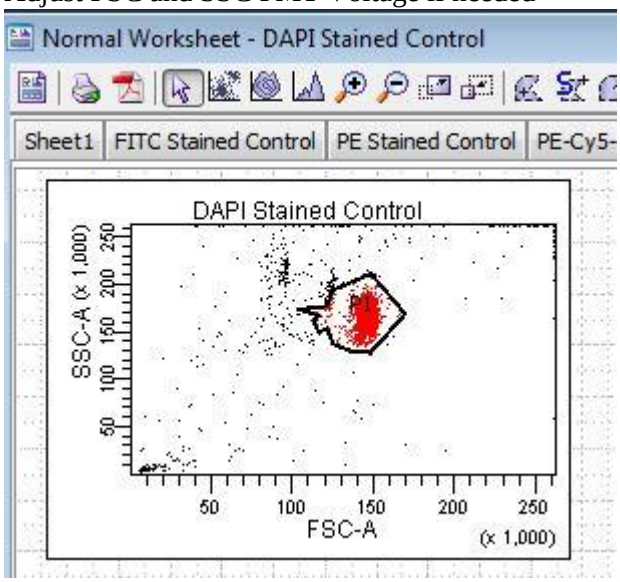
5. Click the tube pointer of the first tube



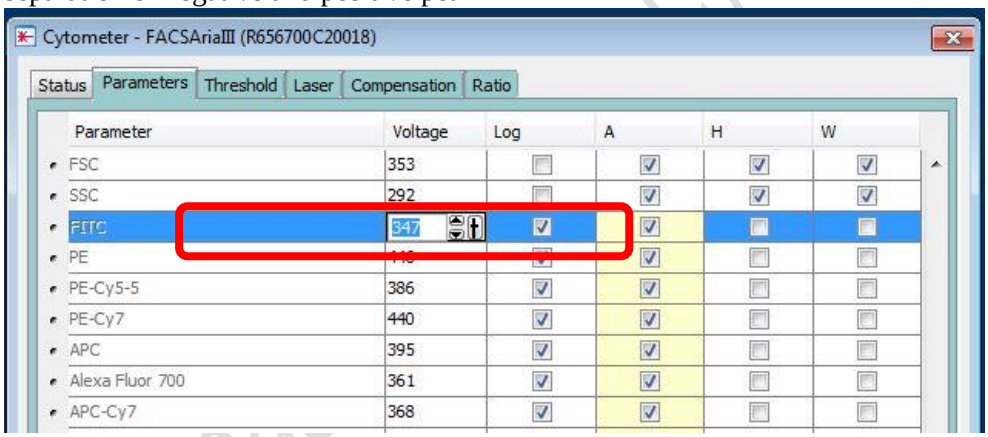
6. Load the single stained controls according to the tube label, i.e. run FITC single stain when the tube pointer is pointing at "FITC Stained Control"

7. Go to Acquisition Dashboard, Click **Acquire Data or Load in AriaSORP or Fusion**.

8. Go to Normal Worksheet, move the P1 gate to include major cluster.
Adjust FSC and SSC PMT Voltage if needed

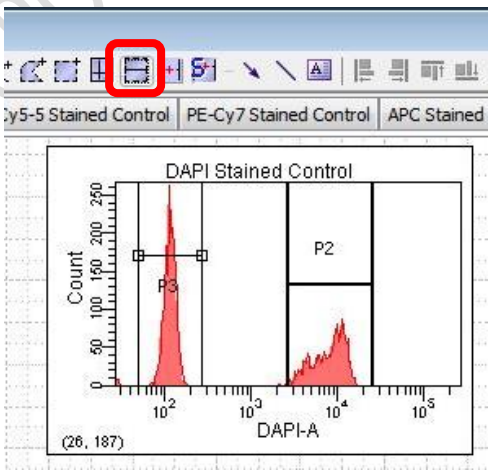


9. Go to Cytometer window, Fine tune the corresponding fluorescence PMT voltage to have best separation of negative and positive peak



10. Move the interval gate (P2) to include the positive peak

11. Use **interval gate** to gate out negative peak (P3) if there is no separate unstained control.



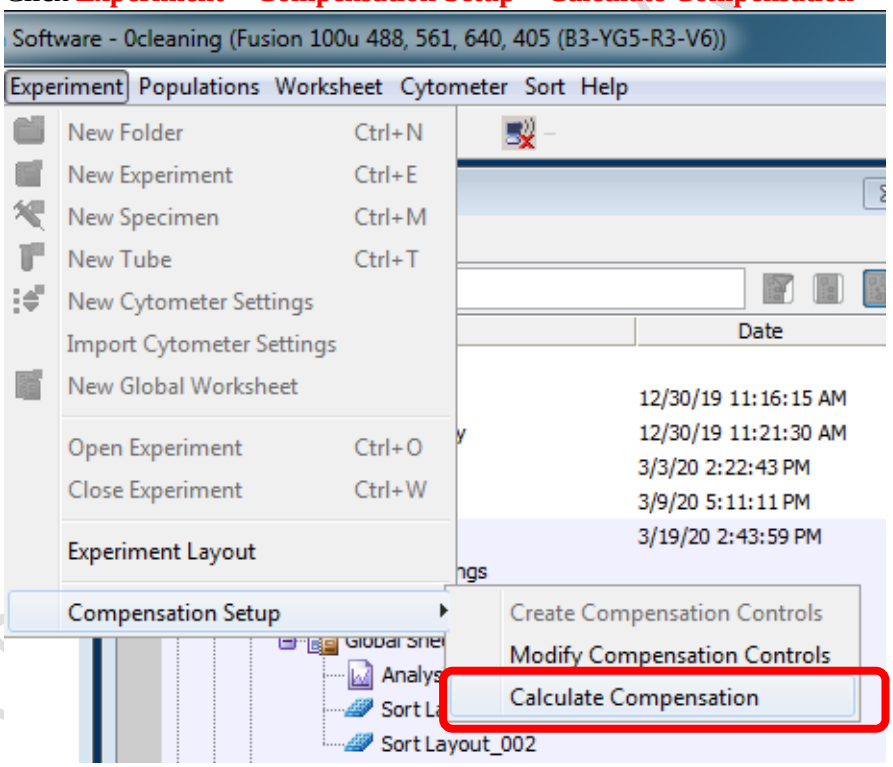
12. Go to Acquisition Dashboard, Click **Stop Acquiring or Unload in AriaSORP or Fusion.**

    *DO NOT Record Data at this point

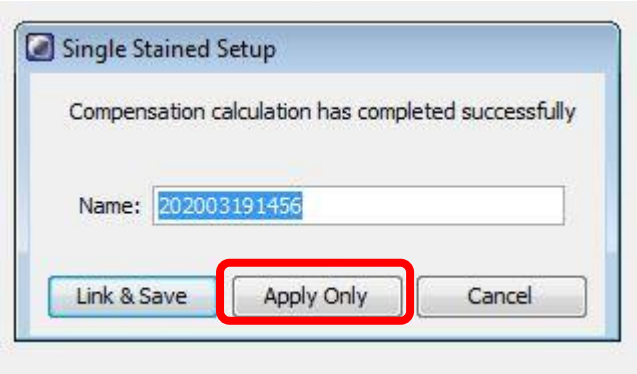
13. Repeat step 5 - 12 with all the single stained controls.

14. After optimising the PMT voltage of ALL the fluorescence channel, load each single stained control and click **Record Data** for ALL single stain controls

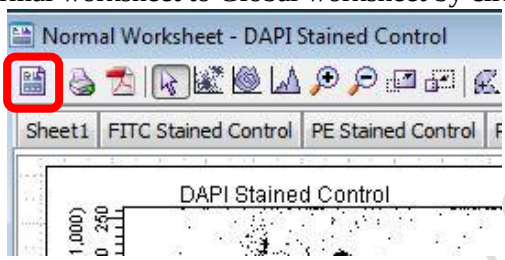
15. Click **Experiment > Compensation Setup > Calculate Compensation**



16. Click Link and Save for the most stringent practice, i.e. cannot adjust PMT voltage anymore **OR** Click **Apply Only** (recommended) for some flexibility on PMT voltage adjustment of your samples.



17. Switch Normal worksheet to Global worksheet by clicking the first icon on the left

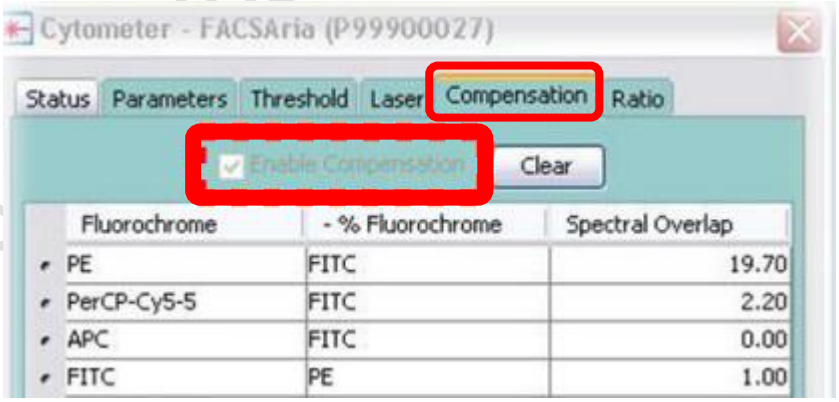


18. Create a **new specimen**

19. Expend the new specimen and **click the tube pointer** of Tube_001

20. Run a sample that is fully stained

21. Visualise the compensated data by go to **Cytometer > Compensation > Check the box of Enable Compensation**



22. Adjust the compensation value if needed