Setup procedure for guava easyCyte™ with a Single Laser

Important note:

The guava instruments are based on microcapillary flow. This technology offers some advantages for general flow cytometry but the instruments are different from traditional flow cytometry and the performance of LEGENDplex™ is also different. The Forward and Side Scatter profile of the beads look different and the guava instrument may have a lower resolution in the Forward Scatter channel. While the guava easyCyte™ may not be the best instrument for LEGENDplex™ in this regards, following these instructions will help in getting the best data possible. For comparison of data generated in different experiments machine settings should remain the same. Analysis of the data should also remain as similar as possible with the LEGENDplex™ software.

1. **Start up the Instrument**

   Perform instrument startup and verification check following the manufacturer's recommendations.

2. **Select the Application from the main Menu:**

   Several applications, which are called assays, are provided in the guavaSoft package. This guide is written based in ExpressPlus and ExpressPro. The ExpressPlus Assay is for performing one-, two-, and three-color assays. ExpressPro is for performing assays with up to six colors, or where time, area, and/or width parameters are necessary. InCyte can also be used to collect six colors, time, area, and/or width parameters. When using instruments with two lasers there is no need to do compensations. Instructions for ExpressPro are also provided.

   2.1 Select ExpressPlus from the dropdown menu (Figure 1)

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Figure 1.
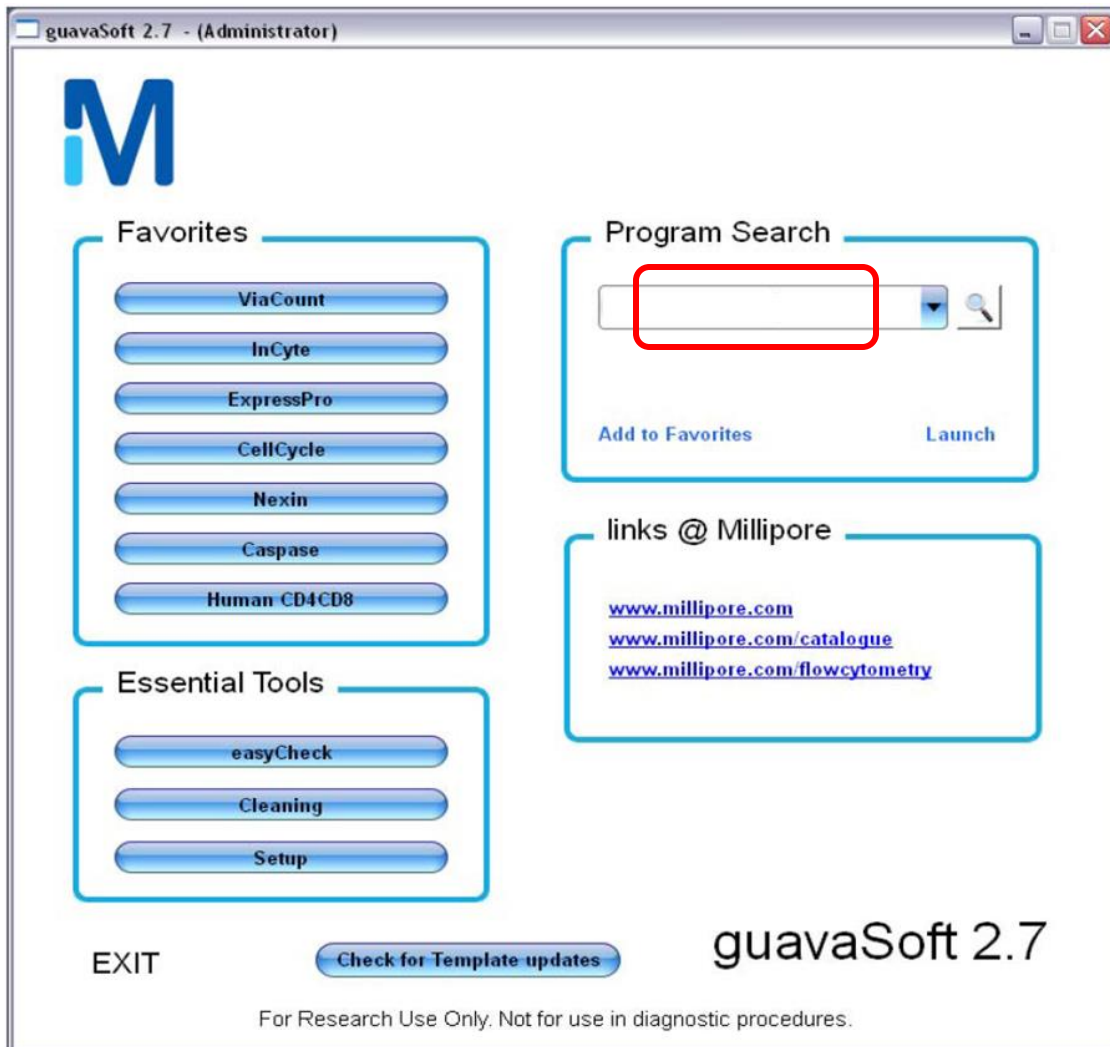
2.2 Create a Worklist following the guava EasyCyte™ User's manual. Follow the software instructions if prompted to put required tubes/solutions for instrument tray check. If you created a Worklist already, click Start Worklist.

3. Select an instrument settings option.

3.1 Click Settings (Figure 2)

Note: To define, edit and allocate gates any time during the procedure please refer to the guava easyCyte™ User's guide.
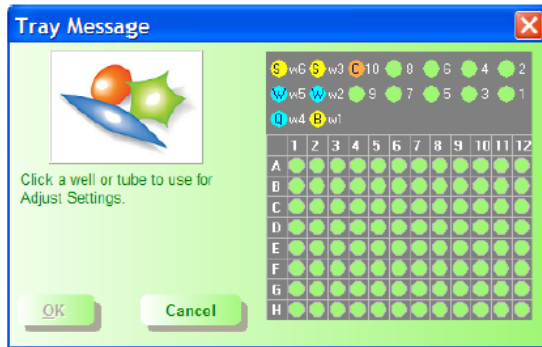
Figure 2.
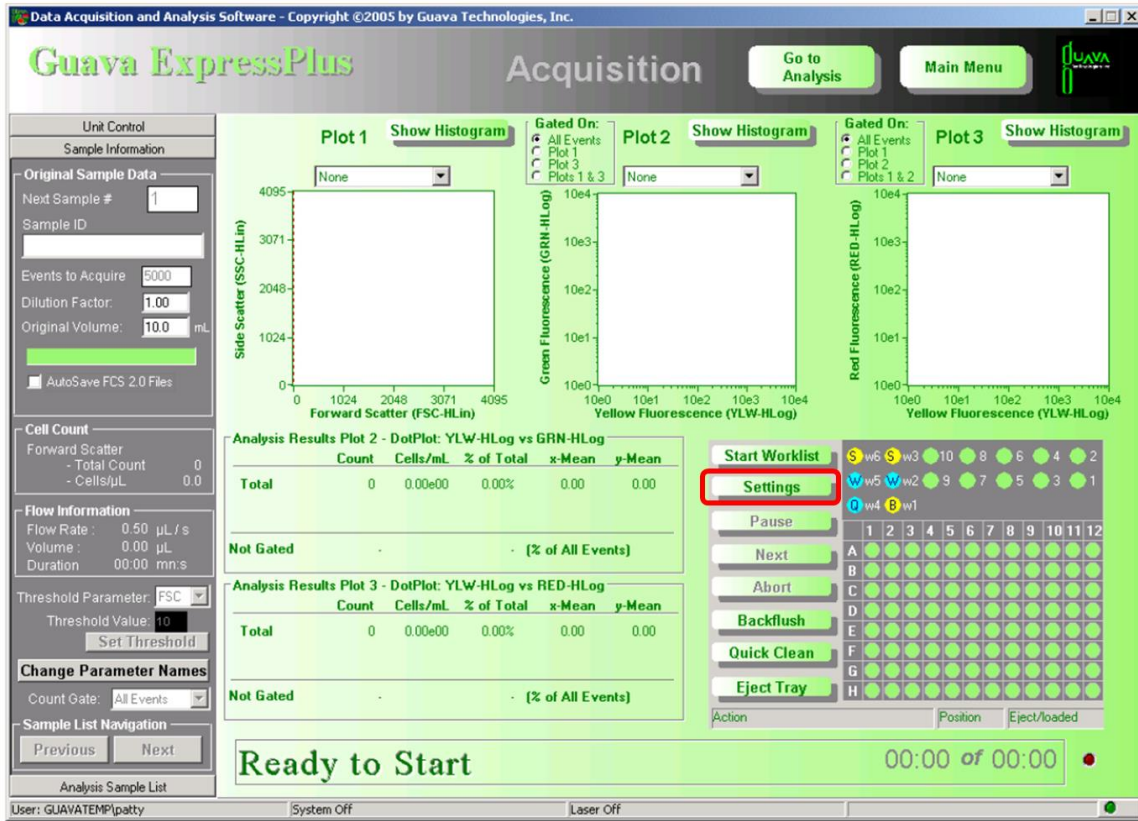
3.2 A window will pop up (Figure 3). To adjust instrument settings, click **Adjust Settings**. If you performed this step in a previous LEGENDPlex™ assay, retrieve the settings by clicking **Retrieve Settings**. Select a setting file and click **Open**.

Figure 3.

3.3 Once Adjust Settings is selected, a dialog box appears prompting you to select the sample for adjusting settings (Figure 4). Click to select the well/tube used to adjust settings, then click **OK**.

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Figure 4.

  3.4 The Adjust Settings screen appears (Figure 5), allowing you to adjust the threshold, the PMT voltages, and optimize the display of the data.

4. Set up PMT Voltages and Compensation

The Setup Beads 3: Raw Beads are used to set up the PMT voltage of the Classification Channel RED and channel GRN. The Setup Beads 2: PE Beads are used to set up the PMT voltage of the reporter channel YLW. All three setup beads are needed for setting up Compensation. Follow the instructions below for setting up the PMT voltages and compensation:

 4.1 **Sonicate the Raw Beads for 1 minute in a bath sonicator to resuspend and reduce aggregates. If you do not have access to a sonicator vortex the vial for at least 2 minutes.**

 4.2 Transfer 400µL of the Raw Beads to a fresh FACS tube.

 4.3 Make sure the events rate is within the recommended range of the machine (usually the recommended flow rate is medium; the events/µL should be less than or equal to 500).

 4.4 **Carefully adjust the gain and voltage for FSC and SSC so that both beads populations are visible and easy to gate. Adjust the FSC threshold to exclude unwanted events (Figure 5).**

 4.5 Adjust the GRN voltage so that the signal for the majority of beads is between 10^0 and 10^1 (Figure 5).

 4.6 Adjust the voltage for YLW in the fluorescent dot plots so that the signal for majority of beads is between 10^0 and 10^1 (Figure 5).

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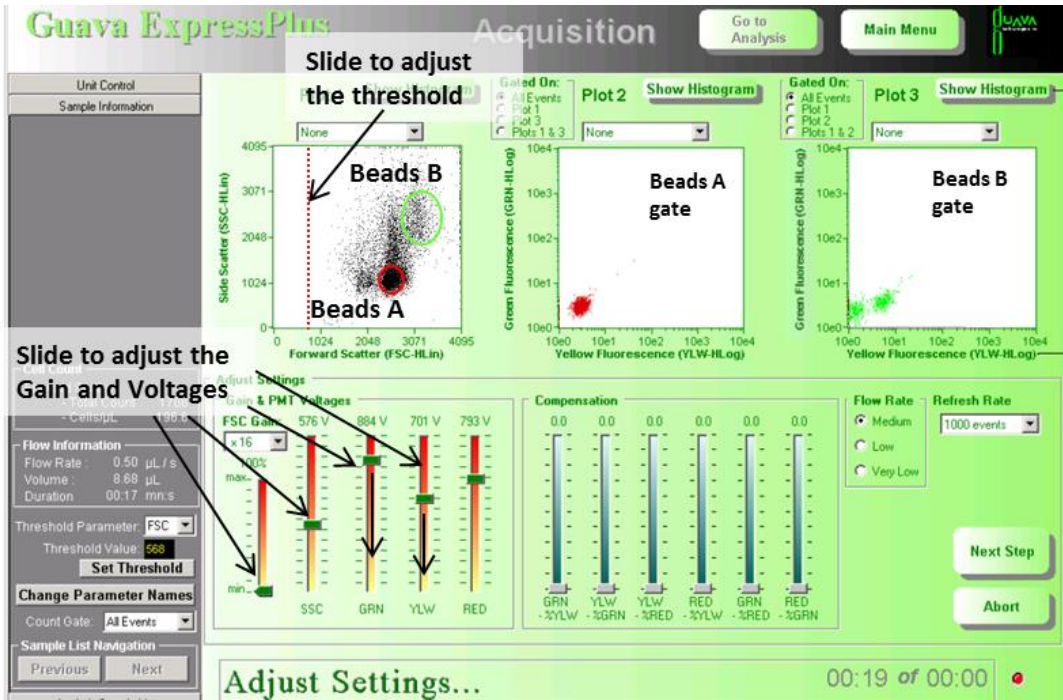
Figure 5.

4.7   Adjust the RED settings so that the signal for all beads is between 10^1 and 5 x 10^3 (Figure 6).

Figure 6.
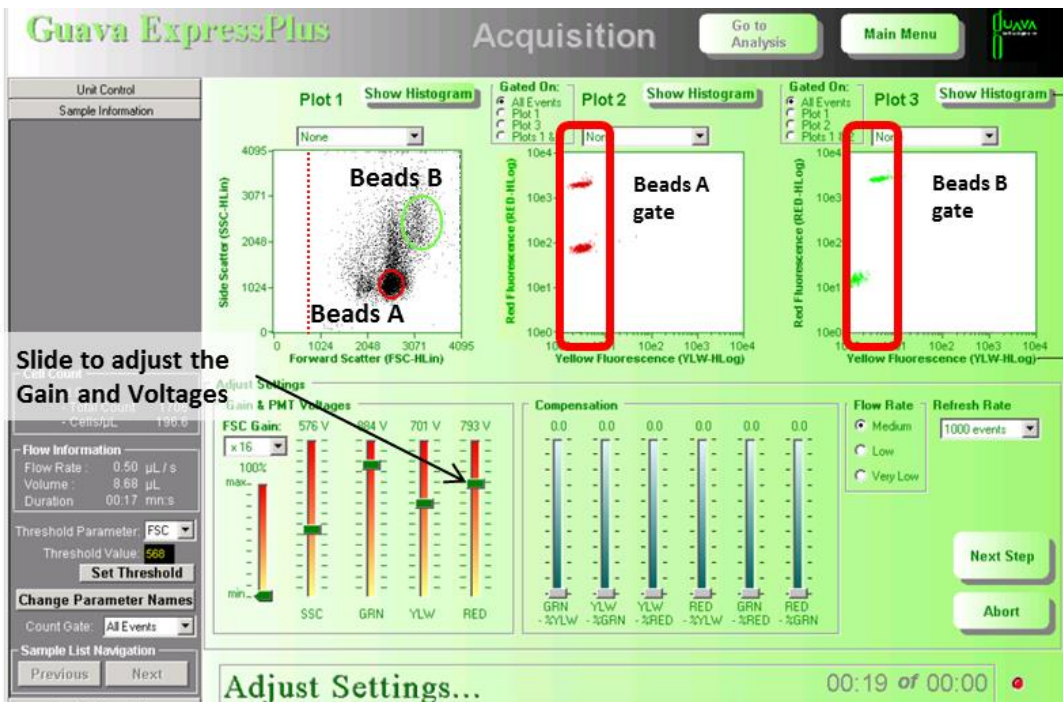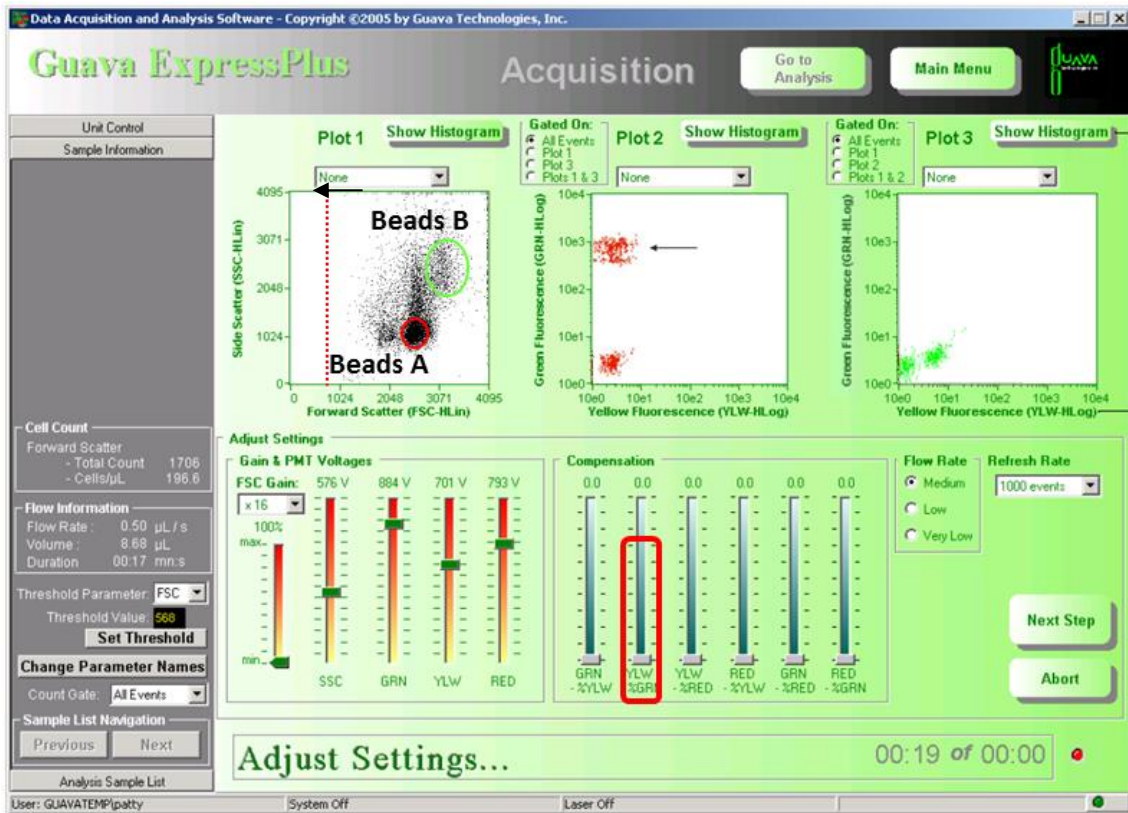
4.8   Sonicate for 1 minute or vortex the vial of the Setup Beads 1: FITC Beads for 2 minutes to resuspend the beads and minimize aggregates.

4.9   Transfer 200 μL of the FITC Beads to a fresh FACS tube. Add 200 μL of Raw Beads and mix well (This will generate FITC-positive and FITC-negative populations of beads and is needed for proper compensation).

4.10   Run the mixed FITC and Raw Beads.

4.11   On the GRN vs YLW dot plot (Figure 7), the beads will display as FITC- negative and FITC-positive population (indicated by an arrow). Note: FITC beads are only of small size, falling in the Beads A gate.
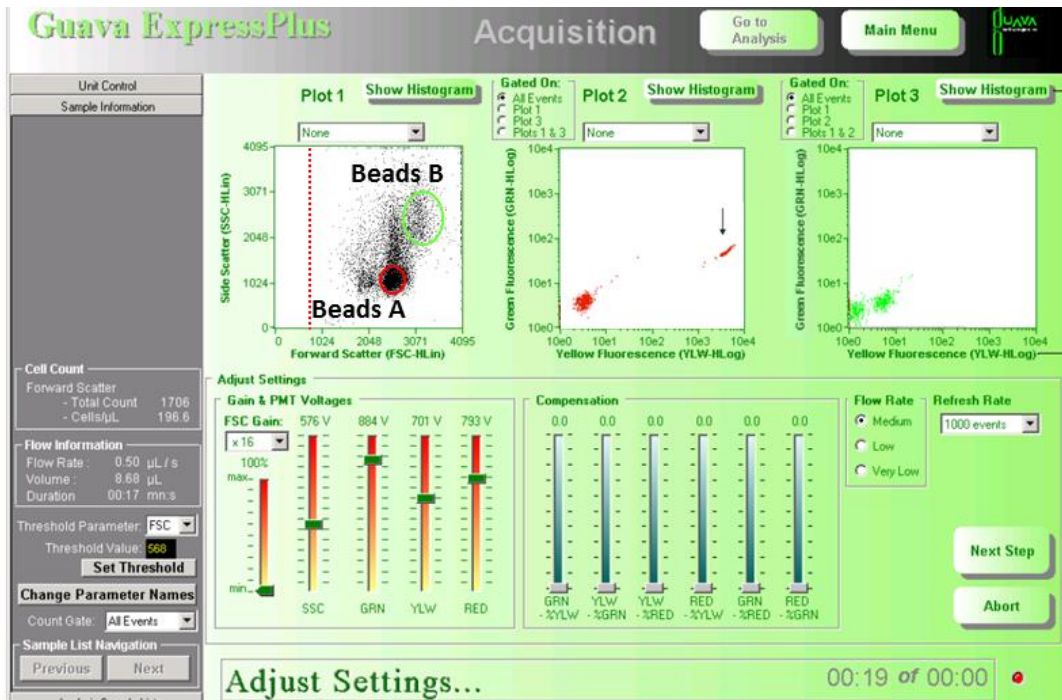
Figure 7.
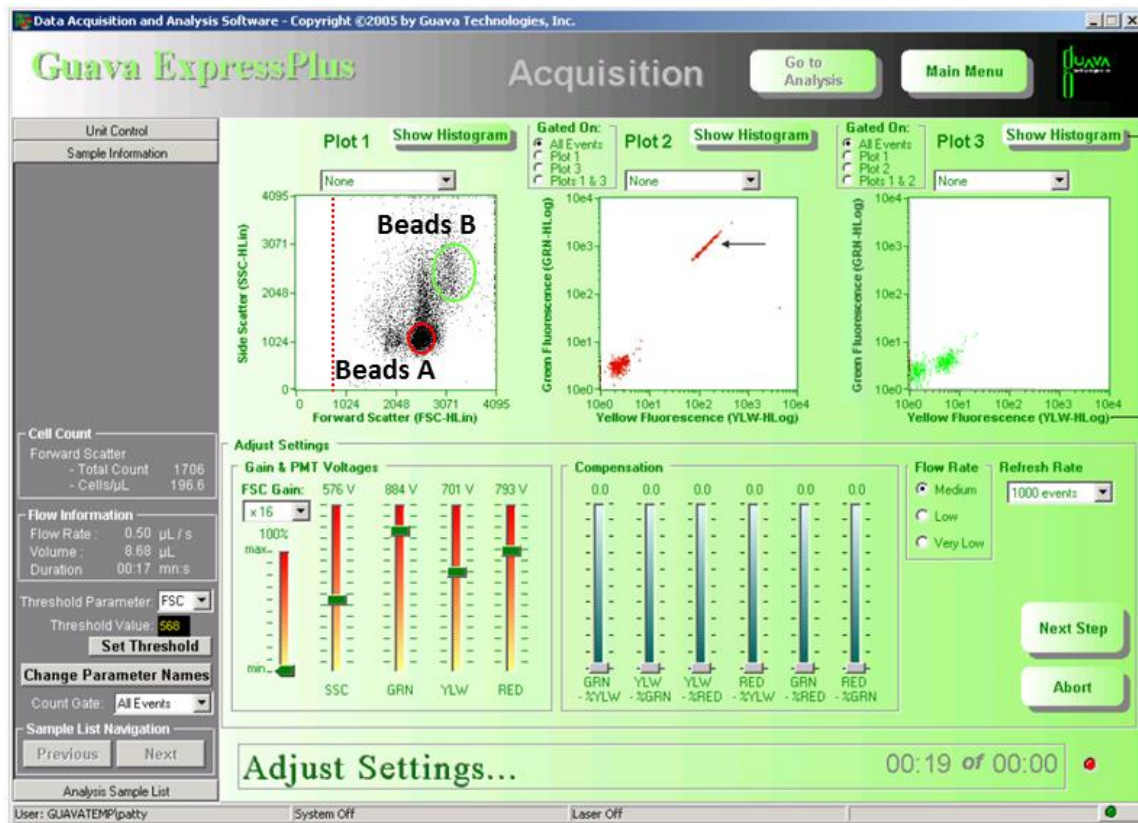
4.12   Adjust the YLW-%GRN compensation setting so that FITC negative and FITC positive populations have similar mean YLW Fluorescence intensity (Figure 8).

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Figure 8.

4.13 Sonicate for 1 minute or vortex the vial of the Setup Beads 2: PE Beads for 2 minutes to resuspend the beads and minimize aggregates.

4.14 Transfer 200 µL of the PE Beads to a fresh FACS tube. Add 200 µL of Raw Beads and mix well (This will generate PE-positive and PE-negative populations of beads and is needed for proper compensation.

4.15 Run the mixed PE and Raw Beads.

4.16 On the GRN vs YLW dot plot (Figure 9), the beads will display as PE- negative and PE-positive population (indicated by an arrow). Note: PE beads are only of small size, falling in the Beads A gate.

4.17 Adjust the YLW settings so that median fluorescent intensity of the PE beads falls within the lot-specific range labeled on the PE Beads vial.
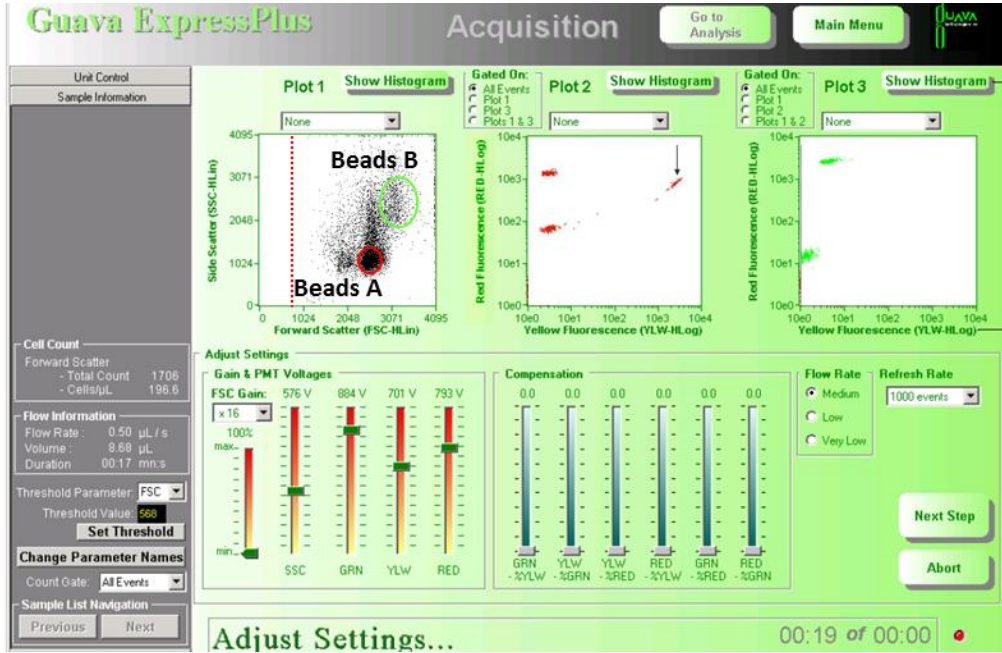
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Figure 9.

4.18 Adjust the GRN-%YLW compensation setting so that PE negative and PE positive populations have similar mean GRN fluorescence intensity.
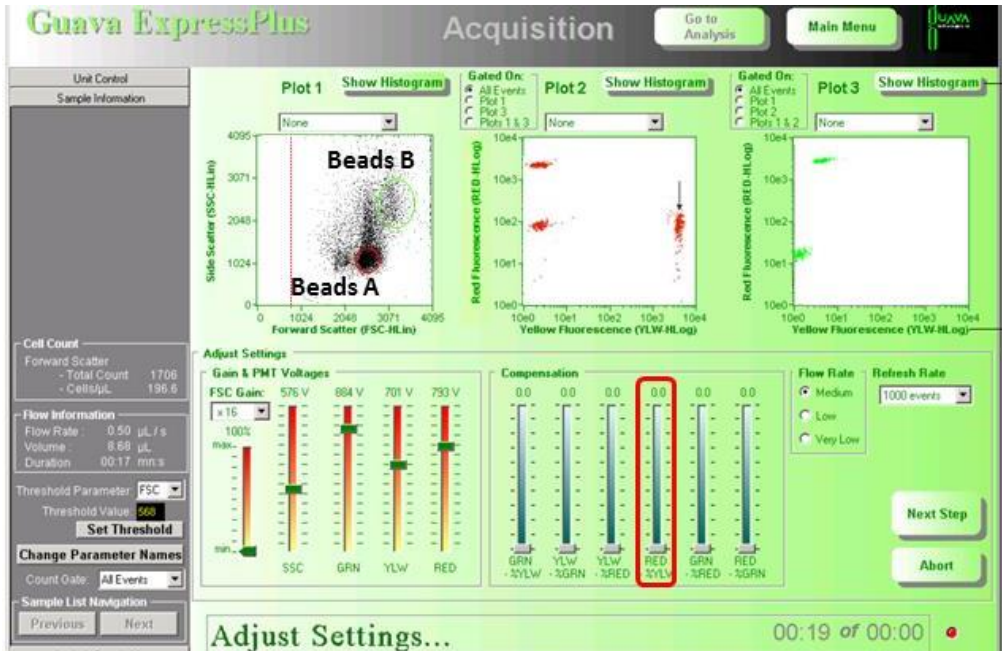
Figure 10.
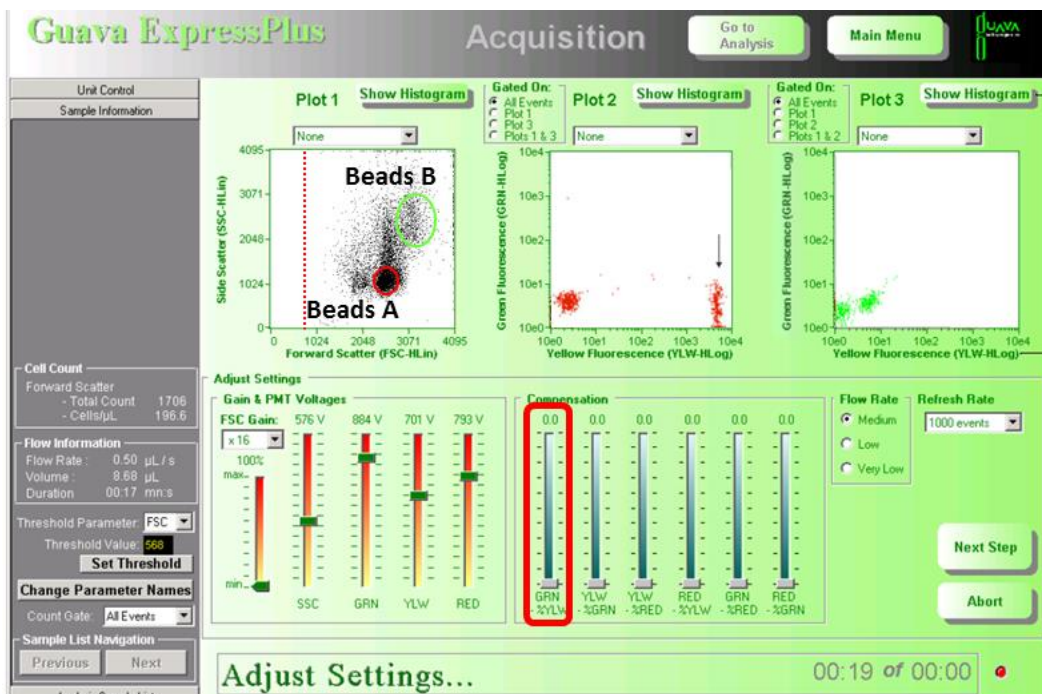
4.19   On the RED vs YLW dot plot, the mixed beads now display as PE negative and PE positive populations (Figure 11, indicated by an arrow).

Figure 11.

4.20   Adjust the RED-%YLW compensation setting so that the RED-low population and the PE positive population have similar median RED signal.

Figure 12.

4.21 The flow cytometer is now compensated and ready for sample analysis.

Additional recommendations when collecting and analyzing your samples:

1) **Collect 2500 beads in either bead gate A or B, or about 300 beads per analyte. Do not collect just the 5,000 events total that is set as default in ExpressPro or ExpressPlus. Do not collect more than 10,000 beads as this could cause problems during data analysis.**

2) **When analyzing the samples with the LEGENDPlex™ Data Analysis Software do manual gating to select the beads, do not use the autogate function. Save the settings and try to use the same gating for subsequent experiments.**