

Optimization of Bead Loading for MAGPIX®

MAGPIX® Chamber Capacity

Luminex Flow Cytometry vs. MAGPIX®

In a Luminex flow cytometer*, beads are injected single file through a cuvette and are counted and classified as they pass by a laser. When the bead count for all regions reaches the desired value, the system stops acquisition and moves on to the next well. In the MAGPIX® system, the entire Sample Load Volume is injected into a chamber where the beads are imaged. The image is then processed by the software, and the data is presented to the user.

MAGPIX Bead Loading

The MAGPIX chamber has an upper end capacity of ~60,000 - 65,000 beads. Since MAGPIX loads all the beads in the Sample Load Volume into the chamber, the total number of beads in the Sample Load Volume is limited by this threshold. As a general rule, the end user should target a final count of 1,250 beads per region in the Sample Load Volume. (See sample load examples on page 3.)

At lower bead concentrations, the Total Classified Events increase linearly with the number of beads in the Sample Load Volume. As the bead concentration loaded into the chamber increases, the image processing algorithms start to throw away beads that are interfering with each other, and the Total Classified Events do not increase as fast as the concentration. As the bead concentration approaches the chamber capacity, the Total Classified Events stop increasing with increased bead concentration. If the chamber is overloaded, the Total Classified Events will decrease.

Figure 1: Flow Cytometry vs. Fluorescent Imaging

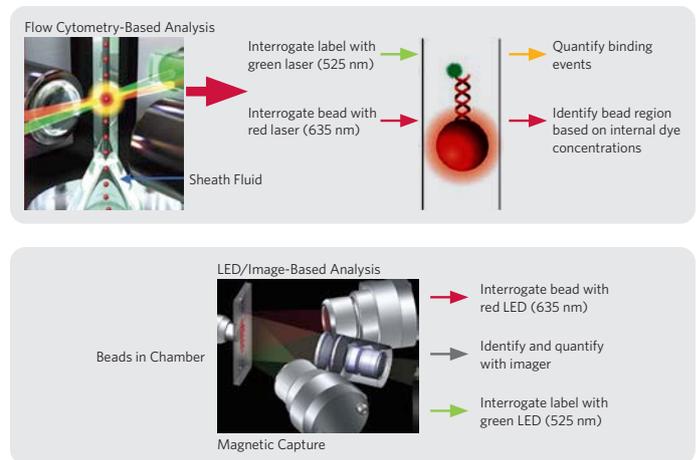
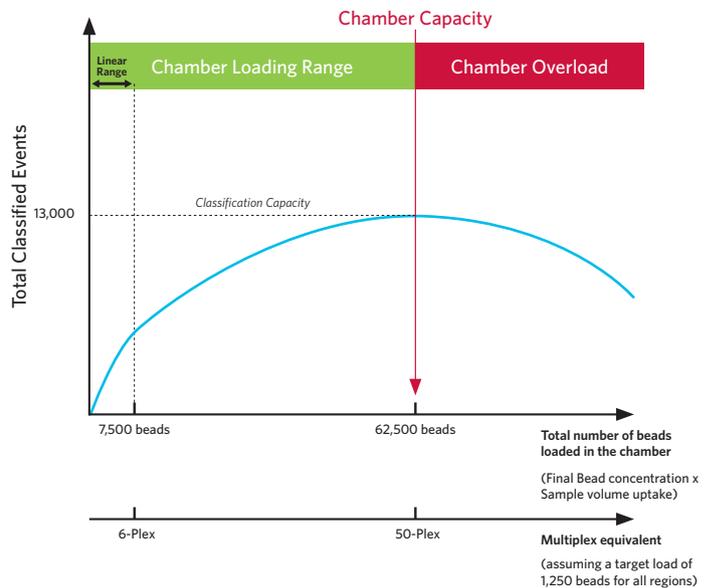


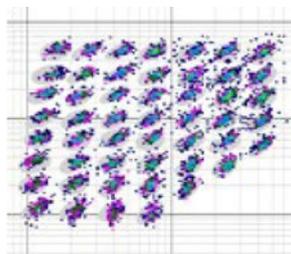
Figure 2: MAGPIX® Chamber Loading Capacity



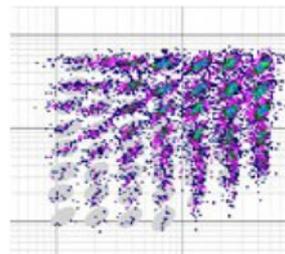
The MAGPIX system pushes the entire Sample Load Volume into the chamber. Thus, the images can have too many beads in them to give accurate results. The image-processing algorithms favor the brighter beads over the dimmer beads. In higher plex assays, the lower intensity regions will have fewer counts than the high intensity regions. As the chamber becomes overloaded, the user will see the DotPlot start to drop in the lowest CL1 and CL2 regions first. Figure 3-A shows a 50-plex DotPlot with 1,250 beads per region in the Sample Load Volume; Figure 3-B shows the same DotPlot with 3,750 beads per region in the Sample Load Volume (*three times the number suggested*).

Figure 3: MAGPIX® 50-Plex DotPlots

A: 1,250 beads per region



B: 3,750 beads per region



In general, if all the beads in your mix are at the same concentration, then the ratio of the region with the highest counts to the region with the lowest counts should be approximately 5 or less ($\text{Max Count} / \text{Min Count} \leq 5$). If the ratio is higher than 5, it is likely that the chamber is overloaded.

The user is able to control the number of beads in the sample load by adjusting the initial bead concentration, the final resuspension volume, and the Sample Load Volume picked up from the well and loaded into the chamber (*See Figure 4*). Care must be taken to minimize bead losses during assay wash steps, as this will also impact the final bead load.

When troubleshooting overloaded DotPlots, the first parameter to change is the Sample Load Volume. The Sample Load Volume is set to 50 μL by default but can be varied from 20-200 μL . By decreasing the Sample Load Volume, you decrease the number of beads loaded into the chamber. Similarly, if you experience lower than desired bead counts in all the regions, then the Sample Load Volume can be increased to increase the number of beads loaded into the chamber. If after setting the Sample Load Volume to the minimum value of 20 μL , there are still too many beads in the chamber, then the resuspension volume in the last step of the assay can be increased to decrease the bead concentration. If the resuspension volume cannot be changed, then the initial bead concentration can be adjusted.

Sample load examples:

Example 1:

2,500 beads per region for a 25-Plex assay = 62,500 beads

Final resuspension volume of 100 μL

Sample Load Volume set to 50 μL = $\frac{1}{2}$ of 62,500 beads = 31,250 beads (1,250 per region)

Example 2:

5,000 beads per region for a 25-Plex assay = 125,000 beads

Final resuspension volume of 100 μL

Sample Load Volume set to 25 μL = $\frac{1}{4}$ of 125,000 beads = 31,250 beads (1,250 per region)

Figure 4: Bead Count Optimization

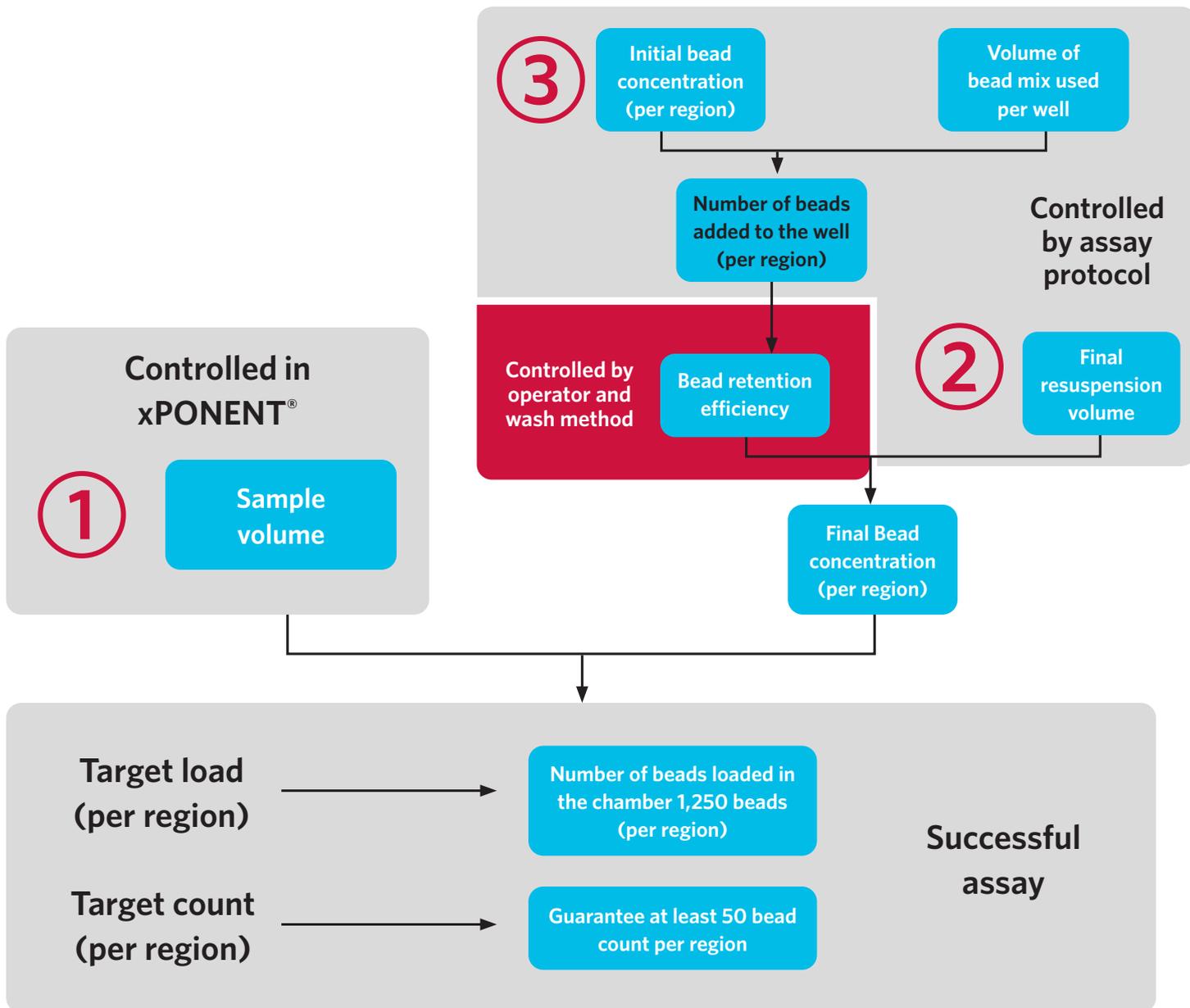
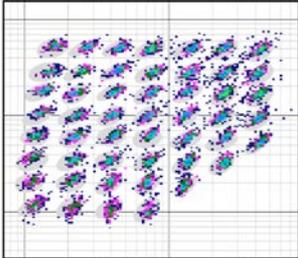
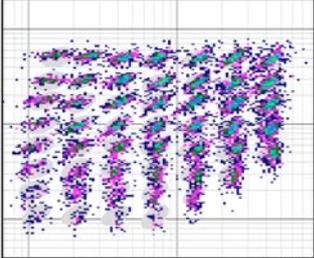
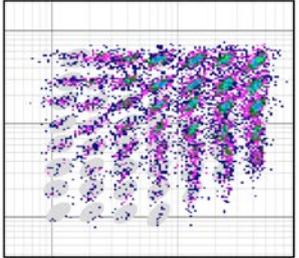
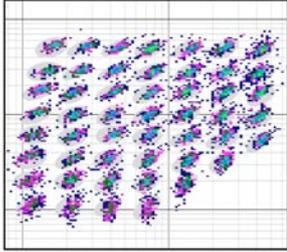
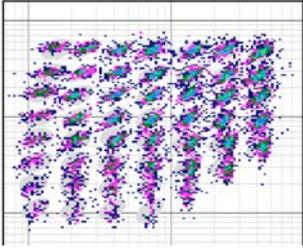
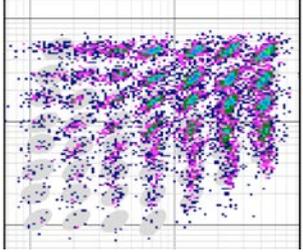


Figure 4: Bead Count Optimization

	Target Load	Overload	Overload
Final Bead Concentration: 2,500 Beads/ Region/100 MI			
Sample Volume	50 µl	100 µl	100 µl
Number of Beads Loaded (Per Region)	1,250	2,500	3,750
Total Number of Beads Loaded (For a 50-Plex)	62,500	125,000	187,500
	Target Load	Overload	Overload
Final Bead Concentration: 5,000 Beads/ Region/100 MI			
Sample Volume	25 µl	50 µl	75 µl
Number of Beads Loaded (Per Region)	1,250	2,500	3,750

If bead concentration is uncertain:

1. Create protocol and batch using an estimate concentration and the guidance table to define "Sample Volume":

Example:

- The Assay protocol does not specify the initial bead concentration, so it is estimated at 2,000 beads per region per 100 μ L.
- The final resuspension volume is specified by the protocol at 150 μ L.
- The guidance table suggests a "Sample Volume" between 75 μ L to 100 μ L to reach the target load of 1,250 beads per region. Assuming there maybe some bead loss during the assay preparation process, the sample volume of 100 μ L is chosen.



2. Before clicking on "Run Batch" from the "Plate Layout" on the Batches page, select the "Single Step" feature.

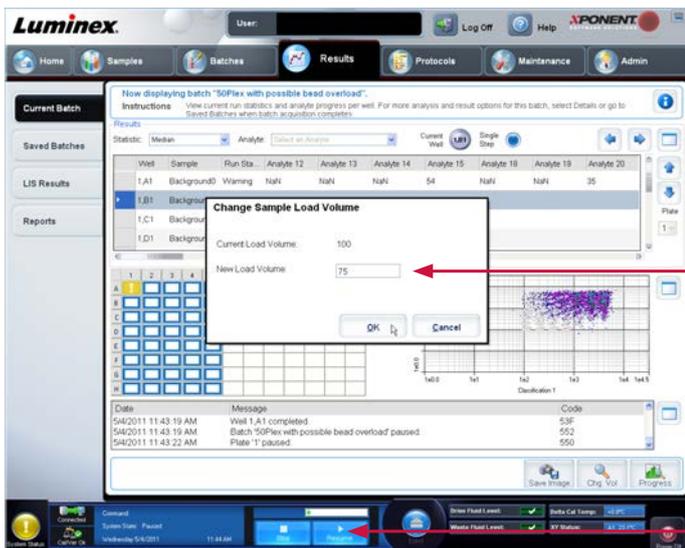
3. Start the batch.



4. xPONENT® will switch to the "Result" page, and show the "Current Batch" tab. The "Single Step" feature shows as selected.

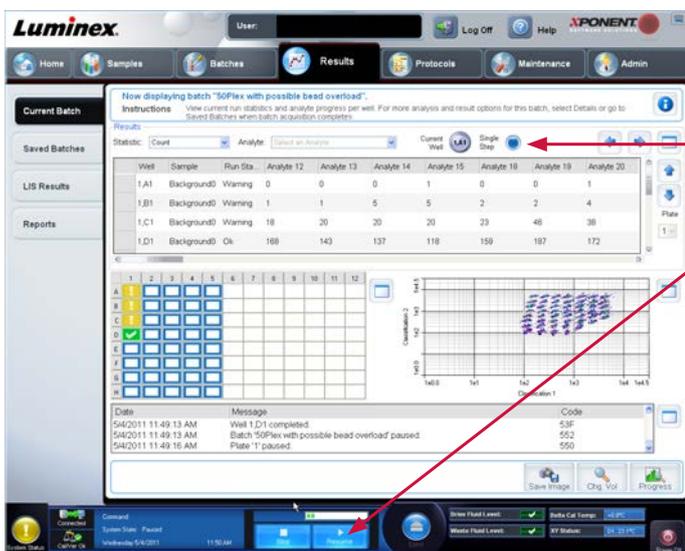
5. After the first well, the system pauses automatically.

If the "Sample Load Volume" has to be changed (based on a warning for low counts, a bad DotPlot, and/or a ratio between the highest count and the lowest count above 5), click on the "Chg. Vol" icon.



6. Specify a new Sample Load Volume (allowed range 20 to 200 μ L). The change is logged into the "Current Batch" display. **Caution:** The change is implemented for the current batch only. The protocol used to create the batch remains unchanged.

7. Resume the batch.



8. After next well, the system pauses automatically again.

9. If needed, repeat steps 5-8 until bead loading is optimized.

10. Once the bead counts are optimized, and the DotPlot is satisfactory, unselect the "Single Step" feature and resume the acquisition of all remaining wells without automatic pause implemented.

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For more information, please visit www.luminexcorp.com/MAGPIX

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HEADQUARTERS

UNITED STATES

+1.512.219.8020

info@luminexcorp.com

EUROPE

+31.73.800.1900

europa@luminexcorp.com

CANADA

+1.416.593.4323

info@luminexcorp.com

CHINA

+86.21.8036.9888

info@luminexcorp.com

JAPAN

+81.3.5545.7440

info@luminexcorp.com

FL137353