Measuring Droplet Volume in Home-Made Microfluidic Devices

To measure droplet volume, purchase some durable, monodisperse polystyrene beads with a hydrophilic coating (e.x. 10-micron carboxylated polystyrene beads from Bangs Labs, product #PC06N-11355. It can be helpful to use fluorescent beads to be sure you can identify them in droplets. Bangs cells these under product # FC06F-10163). Wash and resuspend them in Drop-seq lysis buffer at a concentration of 1000 beads per microliter.

Draw these beads into a syringe with a magnetic mixer (as you would with the standard barcoded beads) and load into a syringe pump.

Load the syringe pump intended for cells with regular PBS. (Since we are co-flowing beads with PBS, we estimate that the concentration of beads in the droplet fluid will be 500 beads per microliter).

Connect all tubing to the appropriate channels in the microfluidic device, and generate droplets.

For a given number of droplets, count the number of beads inside. You should count the beads inside several hundred droplets to make sure that you have a statistically sound estimate.

Divide the total number of beads counted inside droplets by the number of droplets you counted. This is your droplet occupancy.

Here is how to calculate droplet volume:

Droplet volume = (droplet occupancy) / (500 beads per microliter) = # microliters per droplet.