

READY TO USE DAILY QC FLUOROSPHERES

This application is being filed on January 10, 2024, as a PCT International Patent Application and claims the benefit of and priority to U.S. Provisional Patent
5 Application Serial No. 63/479,305, filed on January 10, 2023, the entire disclosure of which is incorporated by reference in its entirety.

INTRODUCTION

Biological laboratory instruments automatically measure analytes and compositions. Using automated instruments to perform blood chemistry and cell analysis increases
10 laboratory efficiency and the test accuracy. As laboratories became more dependent on these instruments, and as the instruments became more sensitive at measuring samples at lower and lower concentrations, it became necessary to calibrate the instruments frequently to assure that the data produced was both accurate and precise.

One such instrument is a flow cytometer. In flow cytometry, single cells in solution
15 flow through a beam of laser light resulting in light scattered in the forward and the side directions. The scattered light is then collected by photodetectors, analyzed and the cells are counted according to the analyzed characteristics. In addition to measuring scattered light, flow cytometers can also measure fluorescence, e.g., fluorescence labeled antibodies or markers. Flow cytometers have become more sensitive and
20 capable of measuring more parameters as the technology has matured. With this technological maturity has come complexity such that a flow cytometer is now able to measure the intensity of multiple fluorescent stains, particle size, and structure as measured by scattering angle at substantially the same time.

To ensure the quality of patient results, flow cytometers require frequent, multiple
25 calibrations and quality control prior to analyzing and reporting patient results. Such calibrations can be time consuming, adding cost and reducing the useful daily working hours of the instrument in the lab.

Currently, flow cytometers equipped with both an infrared ("IR") laser (e.g., 808 nm) and additional lasers (e.g., less than 800 nm) require the use of two different quality
30 control kits or processes (e.g., two different sets of fluorospheres) before the performance of all lasers in the flow cytometer is verified. For example, a Beckman Coulter CytoFLEX Platform flow cytometer equipped with an IR (808 nm) laser requires the use of two different single peak quality control ("QC") fluorospheres to perform quality control on the instrument: CytoFLEX Ready to Use Daily QC

Fluorospheres (PN C65719) are used to evaluate the performance of the five non IR lasers (UV, Violet, Blue, Yellow, and Red) while the CytoFLEX Daily IR QC Fluorospheres (PN C06147) are used to evaluate the performance of the IR laser on the instrument.

- 5 Quality control fluorospheres designed for non-IR lasers, like the CytoFLEX Ready to Use QC Fluorospheres, are not excited by the IR laser and are therefore not suitable to qualify IR channels on a flow cytometer with an IR laser. In contrast, quality control fluorospheres designed for IR lasers, like the CytoFLEX IR QC Fluorospheres, contain dye that is excited by the IR laser and cannot be used to qualify UV, Violet, Blue,
10 Yellow-Green, or Red lasers on a flow cytometer.

Accordingly, a user of a flow cytometer with an IR laser must currently use two different quality control fluorospheres to confirm the flow cytometer is calibrated and ready for samples. The need to use two different quality control fluorospheres, one for non-IR lasers and one for an IR laser, is time consuming and burdensome for the user.

- 15 The present invention eliminates the need for two different sets of quality control fluorospheres when performing quality control on a flow cytometer equipped with an IR laser by providing one set of QC fluorospheres that can be used for quality control. Simply, the present invention provides a single set of fluorospheres, rather than two different sets of fluorospheres, for quality control of all lasers on a flow cytometer,
20 thereby eliminating the need to quality control a flow cytometer twice with two different sets of fluorospheres.

An embodiment of the present compositions, methods, and kits will contain fluorospheres composed of at least two dyes incorporated in the polystyrene beads that can be excited by all lasers (UV, Violet, Blue, Yellow, Red, and IR) in a flow
25 cytometer. The present invention includes a complete daily quality control fluorosphere solution for a flow cytometer. Fluorosphere suspensions, methods of use, and kits of the present invention can be used to evaluate alignment of all lasers, calculate laser delay, and evaluate fluidic stability on a flow cytometer.

- 30 An embodiment of the present invention will have specifications of less than 5 μ s difference in delay settings, less than 20% percent difference in target gain settings, and less than 5% percent difference in target median fluorescence intensity. In an embodiment of the present invention, the robust coefficient of variation ("rCV") must be less than 6% in target detector channels. In an embodiment, the target detector channel will either be detector 3 or detector 4 for UV, Violet, Blue, Yellow-Green, and

Red channels, and detector 1 or detector 2 for IR channels. These channels are selected because the dyes are brightest in these regions with low rCVs.

Currently, non-IR fluorospheres, are sold in a 1-peak, 3-peak, 4-peak, 6-peak, 8-peak, and 9-peak formats for molecules of equivalent soluble fluorochrome ("MESF")

- 5 calibration to determine detector sensitivity, but these fluorospheres do not contain IR dye and are not suitable for quality control of an IR laser in a flow cytometer. In order to meet the needs of users who desire flow cytometry sensitivity assessment as part of their QC process, an alternative embodiment of the present invention includes QC Fluorospheres in a multipeak format (Peak 7, Peak 4, Peak 2) and assigned molecules
- 10 of equivalent soluble fluorochrome ("MESF") values. This embodiment is used to calculate the MESF sensitivity of the instrument. This embodiment is an optional quality control composition, method, and kit for determining sensitivity and can be used in conjunction with single peak embodiments.

BRIEF SUMMARY OF THE INVENTION

- 15 The compositions, methods, and kits of the present invention offer significant advantages over the currently available fluorospheres used for quality control of a flow cytometer having both IR and non-IR lasers. The compositions, methods, and kits of the present invention eliminate the need to use two different quality control fluorospheres, and perform two different quality controls, by combining dyes that are
- 20 excited by both non-IR and IR lasers into a single fluorosphere. Accordingly, a single suspension of the present invention has fluorophores comprising both IR and non-IR excitable dyes and therefore eliminates the need to quality control IR lasers with one set of quality control fluorospheres and quality control non-IR lasers with a second set of quality control fluorospheres.

25 **Compositions for quality control of a flow cytometer.**

- An embodiment of the present invention is a suspension for quality control of a flow cytometer comprising fluorospheres, wherein the suspension of fluorospheres include individual fluorospheres having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one fluorescence emission between 355
- 30 nm and 800 nm when excited at a wavelength of less than 800 nm, at least one surfactant, and at least one stabilizer or preservative. The individual fluorospheres are encapsulated with at least one dye having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one dye having at least one

fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm.

In an embodiment, the at least one dye having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser is selected from the group consisting of: Aqua Green, Jade Green, Cy Green, Indo cyanine green (ICG), Cy7, or Cy7.5, IR dye 800CW, or any combination thereof. In an embodiment, the at least one dye having an infrared fluorescence emission greater than 800 nm is excited with an infrared laser at a wavelength of 808 nm.

In an embodiment, the at least one dye having at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm is selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof. In certain embodiments, the individual fluorospheres are encapsulated with at least seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited at a wavelength of less than 800 nm selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof. In certain embodiments, the individual fluorospheres are encapsulated with seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited by five or six lasers having the following wavelengths: 355 nm, 375 nm, 405 nm, 488 nm, 561 nm, and 638 nm. In certain embodiments, the individual fluorospheres are encapsulated with eight dyes having eight fluorescence emissions when excited by six or seven lasers having the following wavelengths: 355 nm (UV), 375 nm, 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared).

The fluorospheres of the present invention may be polystyrene beads. In an embodiment, the fluorospheres have a diameter of between 2.5 μm and 6.5 μm , between 2.8 μm and 3.4 μm , or are about 3.0 μm .

In certain embodiments, the fluorospheres in the suspension have a concentration between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL, a concentration between 0.9×10^6 fluorospheres/mL and 1.1×10^6 fluorospheres/mL, or concentration of about 1.0×10^6 fluorospheres/mL.

In an embodiment of the present invention, the at least one surfactant is selected from the group consisting of: an ionic surfactant, a non-ionic surfactant, Sodium Dodecyl Sulfate (SDS), NP-40s, Ecosurf EH-9, Ecosurf SA-9, Ecosurf Tween 20, Triton X-11, or any combination thereof. In certain embodiments, the at least one surfactant is at a concentration between 0.01% and 1% based on the total volume of the suspension. In certain embodiments, the at least one surfactant is EcoSurf EH-9 at a concentration is 0.05% based on the total volume of the suspension.

In an embodiment of the present invention, the at least one stabilizer is selected from the group consisting of: a stabilized reducing agent, a stabilized thiol containing compound, (S)-2-Aminobutane-1,4-dithiol hydrochloride, dithiothreitol (DTT), Tris(2-carboxyethyl)phosphine (TCEP) or any combination thereof. In an embodiment of the present invention, the at least one preservative is selected from the group consisting of: sodium azide, thimerosal, or any combination thereof

Methods for quality control of a flow cytometer.

An embodiment of the present invention includes a method for quality controlling a flow cytometer using a single peak comprising: (a) loading a quality control suspension of the present invention, (b) evaluating at least one of the following: (i) evaluating power of at least one laser in the flow cytometer based on a single peak analysis, (ii) evaluating EPS of at least one laser in the flow cytometer based on a single peak analysis, (iii) evaluating laser delay of at least one laser in the flow cytometer based on a single peak analysis, (iv) evaluating gain of at least one laser in the flow cytometer based on a single peak analysis, (v) evaluating rCV of at least one laser in the flow cytometer based on a single peak analysis, and (c) determining whether the flow cytometer passes or fails quality control based on the evaluations in step (b).

In an embodiment, the evaluating in step (b) includes evaluating each of steps (b)(i) through (b)(v). In an embodiment, the evaluation in step (b) is performed on an infrared laser and at least one laser with a wavelength of less than 800 nm. In certain embodiments, the evaluation in step (b) is performed on seven lasers including the following wavelengths: 355 nm (UV), 375 nm (UV), 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared). In certain embodiments, the 355nm (UV) laser, 405 nm (Violet) laser, 488 nm (Blue) laser, 561 nm (Yellow-Green) laser, and 638 nm (Red) laser are evaluated on selected detector channel 3 or detector channel 4 of the flow cytometer, and the 808 nm (Infrared) laser is evaluated on selected detector channel 1 or detector channel 2 of the flow cytometer.

In certain embodiments, the method includes generating a quality control report following step (c). In additional embodiments, the method for quality controlling a flow cytometer is performed at least once per day. In certain embodiments, the method for quality controlling a flow cytometer is performed before using the flow cytometer to
5 analyze samples.

An alternative embodiment of the present invention includes a method for quality controlling a flow cytometer using multiple peaks comprising: (a) loading a quality control suspension of the present invention into the flow cytometer to perform a quality control analysis based on more than one peak, (b) adjusting the brightest peak to a
10 target median fluorescence intensity, (c) reading the median fluorescence intensity and rCV for each of the peaks, (d) unloading the quality control suspension from the flow cytometer following step (c), (e) loading polystyrene beads without encapsulated fluorescence into the flow cytometer, (f) reading the median fluorescence intensity for the polystyrene beads without encapsulated fluorescence, and (g) calculating a
15 sensitivity and background. In certain embodiments, the multiple peaks include three peaks comprising a bright peak, a mild peak, and a dim peak.

In an embodiment, the target median fluorescence intensity for step (f) is between 500,000 and 4×10^6 . In certain embodiments, the sensitivity and background comprise MESF sensitivity, Quantum efficiency, or Background.

20 In certain embodiments, step (a) loading a quality control suspension of the present invention into the flow cytometer to perform a quality control analysis based on more than one peak is concurrent with step (e) loading polystyrene beads without fluorescence into the flow cytometer. In certain embodiments, a method further includes generating a quality control report following step (k).

25 **Kits for quality control of a flow cytometer.**

An embodiment of the present invention includes a kit for performing a method of the present invention, the kit comprising a suspension of the present invention, at least one vial to hold the suspension, and instructions for using the kit. In certain embodiments, a
30 kit may further comprise a second vial to hold a suspension of the present invention. In certain embodiments, the vials are each 10mls.

In certain embodiments, a kit will include a second vial containing a suspension of polystyrene beads with no dye. In certain embodiments, the diameter of the polystyrene beads with no dye in a second vial is about 1 μm . In certain embodiments, the

concentration of the polystyrene beads in the second vial is between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1** shows an embodiment of a presently disclosed method for quality controlling a flow cytometer with a suspension of the present invention using a single peak.
- Figure 2** shows an alternative embodiment of a presently disclosed method for quality controlling a flow cytometer with a suspension of the present invention using multiple peaks.
- Figure 3** shows an analysis of dye leak over time from fluorospheres with a single dye in order to understand dye leak and its impact on rCVs and singlet percentage. Specially, Figure 3 shows the gating strategy for each dye. Figure 3A shows the gating strategy for fluorospheres with UV dye. Figure 3B shows the gating strategy for fluorophores with Light Yellow dye. Figure 3C shows the gating strategy for fluorophores with Yellow dye. Figure 3D shows the gating strategy for fluorospheres with Nile Red dye. Figure 3E shows the gating strategy for fluorospheres with Purple dye. Figure 3F shows the gating strategy for fluorospheres with Blue dye. Figure 3G shows the gating strategy for fluorospheres with Sky Blue dye. Figure 3H shows the gating strategy for fluorospheres with Aqua Green dye (IR excitable dye). Figure 3I shows the gating strategy for fluorospheres with CyGreen dye (IR excitable dye). Figure 3J shows the mean diameter (um) for each of the single dyes.
- Figure 4** shows an analysis of dye leak over time (Day 0, Day 3, Day 16, Day 22, and Day 24) from fluorospheres with a single dye. Specifically, Figure 4 shows the median fluorescence intensity ("MdFl") % difference on emitted channels (Figures 4A, 4C, 4E) at Day 0, Day 3, Day 16, Day 22, and Day 24, and the MdFl for all the spectrum (Figures 4B, 4D, 4F) at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4A shows the MdFl % difference for fluorospheres with UV on emitted channels and Figure 4B shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4C shows the MdFl % difference for fluorospheres with Light Yellow dye on emitted channels and Figure 4D shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4E shows the MdFl % difference for fluorospheres with Yellow dye on emitted channels and Figure 4F shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4G shows the MdFl % difference

for fluorospheres with Nile Red dye on emitted channels and Figure 4H shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4I shows the MdFl % difference for fluorospheres with Purple dye on emitted channels and Figure 4J shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4K shows the MdFl % difference for fluorospheres with Blue dye on emitted channels and Figure 4L shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4M shows the MdFl % difference for fluorospheres with Sky Blue dye on emitted channels and Figure 4N shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4O shows the MdFl % difference for fluorospheres with Jade Green dye (IR excited) on emitted channels and Figure 4P shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4Q shows the MdFl % difference for fluorospheres with CyGreen dye (IR excited) on emitted channels and Figure 4R shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24. Figure 4S shows the MdFl % difference for fluorospheres with Aqua Green dye (IR excited) on emitted channels and Figure 4T shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

Figure 5 shows the analysis of dye leak from fluorospheres of the present invention based upon dilution at three different volumes (0.5ml, 1.0ml, and 2.0ml) to a concentration of 1×10^6 beads/sample using two different buffers Ecosurf EH-9 (CAS: 64366-70-7) and Ecosurf SA-9 (CAS:68937-66-6). Figure 5A-5C are based upon Table 10 herein and disclose the gate analysis for fluorospheres of the present invention (Set 7.2) diluted to 0.5ml with Ecosurf EH-9 four days after dilution (Figure 5A), diluted to 1.0ml with Ecosurf EH-9 four days after dilution (Figure 5B), and diluted to 2.5mls with Ecosurf EH-9 four days after dilution (Figure 5C). Figures 5D-5F shows the MdFl % difference for the samples analyzed in Figures 5A-5C, respectively. Figures 5G-5I show the MdFl % difference for fluorospheres of the present invention diluted to 0.5ml with Ecosurf SA-9 (Figure 5G), diluted to 1.0ml with Ecosurf SA-9 (Figure 5H), and diluted to 2.5ml with Ecosurf SA-9 (Figure 5I). Figures 5J-5K shows MdFl over time (Day 0, Day 4, Day 7, Day 18, Day 20, Day 25, Day 27) for fluorospheres of the present invention diluted to 0.5 ml in Ecosurf EH-9 (Figure 5J) and diluted to 2.5ml in Ecosurf EH-9 (Figure 5K). Figures 5L-5M show MdFl over time (Day 0, Day 4, Day 7,

Day 18, Day 20, Day 25, Day 27 (only for Ecosurf EH-9)) for fluorospheres of the present invention diluted to 2.5 ml in Ecosurf EH-9 (Figure 5L) and diluted to 2.5ml in Ecosurf SA-9 (Figure 5M).

- Figure 6** shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 6A-6D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel U3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 6A shows the rCV analysis of Set 7.2. Figure 6B shows the rCV analysis of set 10.1 diluted in sodium dodecyl sulfate (“SDS”) to 1×10^6 fluorospheres per ml. Figure 6C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 6D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.
- Figure 7** shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 7A-7D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel U3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 7A shows the rCV analysis of Set 7.2. Figure 7B shows the rCV analysis of set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 7C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 7D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.
- Figure 8** shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 8A-8D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel B3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 8A shows the rCV analysis of Set 7.2. Figure 8B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 8C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 8D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 9 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 9A-9D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel Y3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 9A shows the rCV analysis of Set 7.2. Figure 9B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 9C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 9D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 10 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 10A-10D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel R3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 10A shows the rCV analysis of Set 7.2. Figure 10B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 10C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 10D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 11 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 11A-11D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel IR1A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 11A shows the rCV analysis of Set 7.2. Figure 11B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 11C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 11D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 12 shows a comparison of the MdFl for channels IR1A (Figures 12A-12C) and IR2A (Figures 12D-12F) for the same samples evaluated in Figure 11 and Figure 13. Specifically, Figure 12A shows the MdFl analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 12B shows the MdFl analysis of Set 10.2 diluted and

resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 12C shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide. Figure 12D shows the MdFl analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 12E shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 12F shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 13 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 13A-13D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel IR2A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 13A shows the rCV analysis of Set 7.2. Figure 13B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 13C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 13D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 14 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 14A-14D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel IR3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 14A shows the rCV analysis of Set 7.2. Figure 14B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 14C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 14D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 15 shows an MdFl analysis (channel IR1A and IR2A) of Set 10.1 and Set 10.2 based upon light exposure of 1500 lux or 3500lux at 0 Hours, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, and 6 hours. Figure 15A shows the MdFl analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml with a light exposure of 3500lux (channel IR1A). Figure 15B shows the MdFl analysis of Set 10.1 washed in SDS and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide with a light

exposure of 3500lux (channel IR1A). Figure 15C shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide (channel IR1A). Figure 15D shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9 and 0.02% sodium azide with a light exposure of 1500lux (channel IR1A). Figure 15E shows the MdFl analysis of Set 10.2 washed with SDS and resuspended in 0.1% ethanolamine, 0.05% Ecosurf EH-9 and 0.02% sodium azide with a light exposure of 1500lux (channel IR1A). Figure 15F shows the MdFl analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml with a light exposure of 1500lux (channel IR2A). Figure 15G shows the MdFl analysis of Set 10.1 washed in SDS and resuspended in SDS with 0.05% Ecosurf EH-9, and 0.02% sodium azide with a light exposure of 1500lux (channel IR2A). Figure 15H shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide with a light exposure of 1500lux (channel IR2A). Figure 15I shows the MdFl analysis of Set 10.2 diluted in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide with a light exposure of 1500lux (channel IR2A). Figure 15J shows the MdFl analysis of Set 10.2 washed in SDS and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide with a light exposure of 1500lux (channel IR2A).

Figure 16 shows the fluorosphere preparation process for an embodiment of the present invention, specifically, Set 11 and Set 12. Fluorosphere polymerization (300) is followed by the addition of non-IR dyes (302). IR dyes are added next (304) followed by dialysis (306), washing (308), and resuspension (310).

Figure 17 shows the evaluation of fluorospheres of the present invention containing either Aqua Green dye or Jade Green dye as the IR excited dye. Both sets of fluorospheres were prepared as shown in Figure 16. The MdFl analysis is for Peak 2 (Figures 17A-17F), Peak 4 (Figures 17G-17L), and Peak 7 (Figures 17M-17R). Figure 17A shows the MdFl analysis (Peak 2) of fluorospheres with Aqua Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17B shows the MdFl analysis (Peak 2) of fluorospheres with Jade Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17C shows the MdFl analysis (Peak 2) of fluorospheres with Aqua Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17D shows the MdFl analysis (Peak 2) of fluorospheres with Jade Green dye that were

incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17E shows the MdFl analysis (Peak 2) of fluorospheres with Aqua Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17F shows the MdFl analysis (Peak 2) of fluorospheres with Jade Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17G shows the MdFl analysis (Peak 4) of fluorospheres with Aqua Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17H shows the MdFl analysis (Peak 4) of fluorospheres with Jade Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17I shows the MdFl analysis (Peak 4) of fluorospheres with Aqua Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17J shows the MdFl analysis (Peak 4) of fluorospheres with Jade Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17K shows the MdFl analysis (Peak 4) of fluorospheres with Aqua Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17L shows the MdFl analysis (Peak 4) of fluorospheres with Jade Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17M shows the MdFl analysis (Peak 7) of fluorospheres with Aqua Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17N shows the MdFl analysis (Peak 7) of fluorospheres with Jade Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17O shows the MdFl analysis (Peak 7) of fluorospheres with Aqua Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17P shows the MdFl analysis (Peak 7) of fluorospheres with Jade Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17Q shows the MdFl analysis (Peak 7) of fluorospheres with Aqua Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17R shows the MdFl analysis (Peak 7) of fluorospheres with Jade Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17S and Figure 17T shows the differences at Peak 2, Peak 4, and Peak 7 between fluorospheres with Aqua Green dye (Figure 17S) and fluorospheres with Jade Green dye (Figure 17T) at channel IR1A. Figure 17U and Figure 17V show the differences at Peak 2, Peak 4, and Peak 7 between fluorospheres with Aqua Green dye (Figure 17S) and fluorospheres with Jade Green dye (Figure 17T) at channels U3A, V3A, B3A, Y3A, and R3A.

DETAILED DESCRIPTION

While the concepts of the present disclosure are illustrated and described in detail in the figures and descriptions herein, results in the figures and their description are to be considered as examples and not restrictive in character; it being understood that only
5 the illustrative embodiments are shown and described and that all changes and modifications that come within the spirit of the disclosure are desired to be protected. Unless defined otherwise, the scientific and technology nomenclatures have the same meaning as commonly understood by a person in the ordinary skill in the art pertaining to this disclosure.

- 10 It will be understood by one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the compositions, methods, and kits described herein are readily apparent from the description of the disclosure contained herein in view of information known to the ordinarily skilled artisan, and may be made without departing from the scope of the disclosure or any embodiment thereof.
- 15 Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, preferred methods and materials are now described.

Definitions.

- As used herein, “g” represents gram; “L” represents liter; “mg” represents “milligram
20 (10⁻³ gram);” “mL” or “cc” represents milliliter (10⁻³ liter). One “μL” equals to one microliter (10⁻⁶ liter). The unit of temperature used herein is degree Celsius (°C).

- The term “about” is used in conjunction with numeric values to include normal variations in measurements as expected by persons skilled in the art, and is understood to have the same meaning as “approximately” and to cover a typical margin of error,
25 such as ±15%, ±10%, ±5%, ±1%, ±0.5%, or even ±0.1% of the stated value. Whether or not modified by the term “about,” the claims include equivalents to the quantities.

- It should be noted that, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise. For example, reference to “a method” includes having two or more
30 methods that are either the same or different from each other. It should also be noted that the term “or” is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

In the interest of brevity and conciseness, any ranges of values set forth in this specification contemplate all values within the range and are to be construed as support for claims reciting any sub-ranges having endpoints which are real number values within the specified range in question. By way of a hypothetical illustrative example, a disclosure in this specification of a range of from 1 to 5 shall be considered to support claims to any of the following ranges: 1-5; 1-4; 1-3; 1-2; 2-5; 2-4; 2-3; 3-5; 3-4; and 4-5.

The term “substantially” or “about” is utilized herein to represent the inherent degree of uncertainty that can be attributed to any quantitative comparison, value, measurement, or other representation. The term “substantially” or “about” is also utilized herein to represent the degree by which a quantitative representation can vary from a stated reference without resulting in a change in the basic function of the subject matter at issue.

The term “comprise,” “comprises,” and “comprising” as used herein, specify the presence of the stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof.

The term “quality control” or “quality controlling” as used herein, specify the use of disclosed compositions, methods, and kits to standardize a flow cytometer in order to ensure the reliability and accuracy of data collected by the flow cytometer.

General Description.

The present invention includes a suspension for quality control of a flow cytometer comprising fluorospheres, wherein the suspension of fluorospheres include individual fluorospheres having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm, at least one surfactant, and at least one stabilizer or preservative.

The individual fluorospheres are encapsulated with at least one dye having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one dye having at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm.

Embodiments of the present invention have at least two dyes (one IR excitable dye and one non-IR excitable dye) but may have more than two dyes. Fluorospheres of the present invention may between 2 and 15 dyes, between 2 and 13 dyes, between 2 and

11 dyes, between 2 and 9 dyes, between 2 and 8 dyes, or between 2 and 7 dyes.

Fluorospheres of the present invention may have greater than 2 dyes, greater than 3 dyes, greater than 4 dyes, greater than 5 dyes, greater than 6 dyes, greater than 7 dyes, or greater than 8 dyes. Fluorospheres of the present invention may have less than 15
5 dyes, less than 13 dyes, less than 11 dyes, less than 9 dyes, less than 7 dyes, less than 5 dyes, or less than 3 dyes.

In an embodiment, the at least one dye having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser is selected from the group consisting of: Aqua Green, Jade Green, Cy Green, Indo cyanine green (ICG), Cy7, or Cy7.5, IR
10 dye 800CW, or any combination thereof. Embodiments can include any commercially available dye that is excitable by IR lasers. In certain embodiments, fluorospheres contain more than 1 IR excitable dye, more than 2 IR excitable dyes, or more than 3 IR excitable dyes. In an embodiment, the at least one dye having an infrared fluorescence emission greater than 800 nm is excited with an infrared laser at a wavelength of 808
15 nm. In certain embodiments, the at least one dye having an infrared fluorescence emission is excited with an IR laser having any suitable wavelength.

In an embodiment, the at least one dye having at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm is selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum
20 dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof. Embodiments can include any commercially available dye that is excitable at a wavelength of less than 800 nm. In certain embodiments, fluorospheres have between 1 and 12 non-IR excitable dyes (e.g., dyes excitable at less than 800 nm with a fluorescence emission of
25 between 355 nm and 800 nm), between 1 and 10 non-IR excitable dyes, between 1 and 8 non-IR excitable dyes, between 1 and 6 non-IR excitable dyes, between 1 and 4 non-IR excitable dyes, between 1-3 non-IR excitable dyes. In certain embodiments, fluorospheres have greater than 2 non-IR excitable dyes, greater than 4 non-IR excitable dyes, greater than 6 non-IR excitable dyes, greater than 8 non-IR excitable
30 dyes, or greater than 10 non-IR excitable dyes. In certain embodiments, fluorospheres have less than 12 non-IR excitable dyes, less than 10 non-IR excitable dyes, less than 8 non-IR excitable dyes, less than 6 non-IR excitable dyes, less than 4 non-IR excitable dyes, or less than 2 non-IR excitable dyes.

In certain embodiments, the individual fluorospheres are encapsulated with at least seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited at a wavelength of less than 800 nm selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof. In certain embodiments, the individual fluorospheres are encapsulated with seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited by six lasers having the following wavelengths: 355 nm, 405 nm, 488 nm, 561 nm, and 638 nm. While specific laser wavelengths are mentioned, any commercially available laser with a non-IR wavelength is contemplated by the present invention. In certain embodiments, the individual fluorospheres are encapsulated with eight dyes having eight fluorescence emissions when excited by seven lasers having the following wavelengths: 355 nm (UV), 375 nm (UV), 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared). In certain embodiments, the fluorospheres may be excited by at least 2 lasers (one IR and one non-IR). In embodiments, the fluorospheres are excited by at least 3 lasers, at least 4 lasers, at least 5 lasers, at least 6 lasers, at least 7 lasers, or at least 8 lasers. In embodiments, the fluorospheres are excited by between 2 and 12 lasers, between 2 and 10 lasers, between 2 and 8 lasers, between 2 and 6 lasers, or between 2 and 4 lasers.

The fluorospheres of the present invention may be polystyrene beads. The present invention is not limited to polystyrene beads and contemplates the use of fluorospheres made of any material that is suitable for application of more than one dye and use with a flow cytometer. In an embodiment, the fluorospheres have a diameter of between 2.5 μm and 6.5 μm , between 2.8 μm and 3.4 μm , or are about 3.0 μm . In embodiments, the fluorospheres have a diameter of less than 10 μm , less than 8.0 μm , less than 6.5 μm , less than 5.0 μm , less than 4.0 μm , less than 3.5 μm , or less than 3.0 μm . In embodiments, the fluorospheres have a diameter of greater than 1.0 μm , greater than 2.0 μm , greater than 2.5 μm , greater than 2.8 μm , greater than 3.0 μm , greater than 4.0 μm , or greater than 4.0 μm . In embodiments, the fluorospheres within a suspension have different diameters and are not all the same diameters. In embodiments, the fluorophores within a suspension are substantially the same diameter.

In certain embodiments, the fluorospheres in the suspension have a concentration between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL, a concentration between 0.9×10^6 fluorospheres/mL and 1.1×10^6 fluorospheres/mL, or concentration

of about 1.0×10^6 fluorospheres/mL. In embodiments, the fluorospheres in a suspension have a concentration of less than 2.0×10^6 fluorospheres/mL, less than 1.5×10^6 fluorospheres/mL, or less than 1.0×10^6 fluorospheres/mL. In embodiments, the fluorospheres in a suspension have a concentration of greater than 0.3×10^6 fluorospheres/mL, greater than 0.8×10^6 fluorospheres/mL, greater than 1.0×10^6 fluorospheres/mL, greater than 1.1×10^6 fluorospheres/mL, or greater than 1.5×10^6 fluorospheres/mL. The above concentrations of fluorospheres refers to the concentration of fluorospheres when the fluorospheres are used for quality control of a flow cytometer. Higher or lower concentrations are contemplated for storage and shipping of the fluorospheres.

In an embodiment of the present invention, the at least one surfactant is selected from the group consisting of: an ionic surfactant, a non-ionic surfactant, Sodium Dodecyl Sulfate (SDS), NP-40s, Ecosurf EH-9, Ecosurf SA-9, Ecosurf Tween 20, Triton X-11, or any combination thereof. The present invention contemplates the use of any commercially available surfactant. In certain embodiments, the at least one surfactant is at a concentration between 0.01% and 1% based on the total volume of the suspension, at a concentration of between 0.05% and 0.9 % based on the total volume of the suspension, between 0.1% and 0.8% based on the total volume of the suspension, or between 0.3% and 0.6% based on the total volume of the suspension. In embodiments, the at least one surfactant is at a concentration of less than 1.0% based on the total volume of the suspension, less than 0.8% based on the total volume of the suspension, or less than 0.6% based on the total volume of the suspension. In certain embodiments, the at least one surfactant is at a concentration of greater than 0.01% based on the total volume of the suspension, greater than 0.1% based on the total volume of the suspension, greater than 0.3% based on the total volume of the suspension, or greater than 0.5% based on the total volume of the suspension. In certain embodiments, the at least one surfactant is EcoSurf EH-9 at a concentration is 0.05% based on the total volume of the suspension. The above concentration of surfactant refers to the concentration of surfactant when the fluorosphere suspension is used for quality control of a flow cytometer. Higher or lower concentrations are contemplated for storage and shipping.

In embodiments, there is at least 2 surfactants, at least 3 surfactants, at least 4 surfactants, or at least 5 surfactants in a suspension. In embodiments, there are less than

5 surfactants, less than 4 surfactants, less than 3 surfactants, or less than 2 surfactants in a suspension.

In an embodiment of the present invention, the at least one stabilizer is selected from the group consisting of: a stabilized reducing agent, a stabilized thiol containing
5 compound, (S)-2-Aminobutane-1,4-dithiol hydrochloride, dithiothreitol (DTT), Tris(2-carboxyethyl)phosphine (TCEP) or any combination thereof. The present invention contemplates the use of any commercially available stabilizer.

In embodiments, there is at least 2 stabilizers, at least 3 stabilizers, at least 4 stabilizers, or at least 5 stabilizers in a suspension. In embodiments, there are less than 5
10 stabilizers, less than 4 stabilizers, less than 3 stabilizers, or less than 2 stabilizers in a suspension.

In an embodiment of the present invention, the at least one preservative is selected from the group consisting of: sodium azide, thimerosal, or any combination thereof. The present invention contemplates the use of any commercially available preservative.

15 In embodiments, there is at least 2 preservatives, at least 3 preservatives, at least 4 preservatives, or at least 5 preservatives in a suspension. In embodiments, there are less than 5 preservatives, less than 4 preservatives, less than 3 preservatives, or less than 2 preservatives in a suspension.

An embodiment of the present invention includes a method for quality controlling a
20 flow cytometer using a single peak comprising: (a) loading a quality control suspension of the present invention, (b) evaluating at least one of the following: (i) evaluating power of at least one laser in the flow cytometer based on a single peak analysis, (ii) evaluating EPS of at least one laser in the flow cytometer based on a single peak analysis, (iii) evaluating laser delay of at least one laser in the flow cytometer based on
25 a single peak analysis, (iv) evaluating gain of at least one laser in the flow cytometer based on a single peak analysis, (v) evaluating rCV of at least one laser in the flow cytometer based on a single peak analysis, and (c) determining whether the flow cytometer passes or fails quality control based on the at least one evaluation in step (b).

In an embodiment, the evaluating in step (b) includes evaluating each of steps (b)(i)
30 through (b)(v) or any combination thereof.

In an embodiment, the evaluation in step (b) is performed on an infrared laser and at least one laser with a wavelength of less than 800 nm. In embodiments, the evaluation in step (b) is performed on less than all the lasers in the flow cytometer, and can include evaluation of any combination of lasers in the flow cytometer. In certain embodiments,

the evaluation in step (b) is performed on seven lasers having the following wavelengths: 355 nm (UV), 375 nm (UV), 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared). In certain embodiments, the 355nm (UV) laser, 405 nm (Violet) laser, 488 nm (Blue) laser, 561 nm (Yellow-Green) laser, and 638 nm (Red) laser are evaluated on detector channel 3 or detector channel 4 of the flow cytometer, and the 808 nm (Infrared) laser is evaluated on detector channel 1 or detector channel 2 of the flow cytometer. In other embodiments, the lasers evaluated in step (b) are evaluated on additional detector channels.

In certain embodiments, the method includes generating a quality control report following step (c). The quality control report may include a pass or fail analysis of each evaluated laser in the flow cytometer. It may also include specific measurements for each evaluated laser in the flow cytometer. In additional embodiments, the method for quality controlling a flow cytometer is performed at least once per day. In certain embodiments, the method for quality controlling a flow cytometer is performed before using the flow cytometer.

An alternative embodiment of the present invention includes a method for quality controlling a flow cytometer using multiple peaks, rather than a single peak, comprising: (a) loading a quality control suspension of the present invention into the flow cytometer to perform a quality control analysis based on more than one peak, (b) adjusting the brightest peak to a target median fluorescence intensity, (c) reading the median fluorescence intensity and rCV for each of the peaks, (d) unloading the quality control suspension from the flow cytometer following step (c), (e) loading polystyrene beads without fluorescence (e.g., no dye) into the flow cytometer (or loading a mixture of polystyrene beads with and without fluorescence), (f) reading the median fluorescence intensity for the polystyrene beads without fluorescence (e.g., no dye) and (g) calculating a sensitivity and background. In certain embodiments, the multiple peaks include three peaks comprising a bright peak, a mild peak, and a dim peak. In an embodiment, the multiple peaks include more than 1 peak, more than 2 peaks, more than 3 peaks, more than 4 peaks, more than 5 peaks, or more than 6 peaks. In an embodiment, the at multiple peaks includes more than 1 peak but less than 7 peaks, less than 6 peaks, less than 5 peaks, or less than 4 peaks.

In an embodiment, the target median fluorescence intensity for step (f) is between 500,000 and 4×10^6 . In an embodiment, the target median fluorescence intensity is greater than 500,000, greater than 1×10^6 , greater than 2×10^6 , or greater than 4×10^6 . In

an embodiment, the target median fluorescence intensity is less than 5×10^6 , less than 4×10^6 , less than 3×10^6 , or less than 2×10^6 . In certain embodiments, the sensitivity and background comprise MESF sensitivity, Quantum efficiency, or Background.

In certain embodiments, step (a) loading a quality control suspension of the present invention into the flow cytometer to perform a quality control analysis based on more than one peak is concurrent with step (e) loading polystyrene beads without fluorescence into the flow cytometer. In certain embodiments, a method further includes generating a quality control report following step (k). In embodiments, the quality control report may include a pass or fail analysis of each evaluated laser in the flow cytometer. It may also include specific measurements for each evaluated laser in the flow cytometer.

An embodiment of the present invention includes a kit for performing a method of the present invention, the kit comprising a suspension of the present invention, at least one vial to hold the suspension, and instructions for using the kit. In certain embodiments, a kit may further comprise a second vial to hold a suspension of the present invention. In certain embodiments, the vials are each 10mls. In embodiments, a kit may include more than 1 vial, more than 2 vials, more than 3 vials, more than 4 vials, or more than 5 vials. In embodiments, a kit may include less than 5 vials, less than 4 vials, less than 3 vials, or less than 2 vials. The vials may be of appropriate size and the vials within the kit may be of different sizes.

In certain embodiments, a kit will include at least a second vial (but may include additional vials) containing a suspension of fluorospheres with no dye, e.g., polystyrene beads with no dye. In certain embodiments, the diameter of the polystyrene beads with no dye in a second vial is about $1 \mu\text{m}$ but can also be of different diameter or a mix of diameters. In certain embodiments, the concentration of the polystyrene beads in the second vial is between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL, a concentration between 0.9×10^6 fluorospheres/mL and 1.1×10^6 fluorospheres/mL, or concentration of about 1.0×10^6 fluorospheres/mL. In embodiments, the polystyrene beads in the second vial have a concentration of less than 2.0×10^6 fluorospheres/mL, less than 1.5×10^6 fluorospheres/mL, or less than 1.0×10^6 fluorospheres/mL. In embodiments, the polystyrene beads in the second vial have a concentration of greater than 0.3×10^6 fluorospheres/mL, greater than 0.8×10^6 fluorospheres/mL, greater than 1.0×10^6 fluorospheres/mL, greater than 1.1×10^6 fluorospheres/mL, or greater than 1.5×10^6 fluorospheres/mL. The above concentrations of polystyrene beads with no dye

refers to the concentration of polystyrene beads when the polystyrene beads are used for quality control of a flow cytometer. Higher or lower concentrations are contemplated for storage and shipping of the fluorospheres.

Having now described the present disclosure in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for
5 purposes of illustration only and are not intended to be limiting of the disclosure.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the disclosure, and are not intended to limit the scope of what the
10 inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, molecular weight is average molecular weight, temperature is in degrees Celsius, and pressure is at or
15 near atmospheric.

Example 1

An embodiment of the presently disclosed method for quality controlling a flow cytometer using a single peak is shown in Figure 1. Referring to Figure 1, which depicts a flow chart for steps to quality control a flow cytometer, a user of a flow
20 cytometer selects a quality control bead target file on the flow cytometer (100). A user then loads a suspension for quality control of a flow cytometer of the present invention designed for single peak analysis into the flow cytometer (102). The user then starts the quality control process (104) and brings bead fluorescence to target value. The quality control process does one or more of the following: evaluates the laser power for one or
25 more lasers in the flow cytometer (106), evaluates the EPS for one or more lasers in the flow cytometer (108), evaluates laser delay for one or more lasers in the flow cytometer (110), evaluates the gain for one or more lasers in the flow cytometer (112), and/or evaluates rCV for one or more lasers in the flow cytometer (114). If any evaluation step fails for any laser, then the quality control fails (120) and the user is notified by a
30 quality control report (118). If each evaluation step passes, then the quality control passes (116) and a user is notified by a quality control report (118). Following the generation of the quality control report (pass or fail) (118), the quality control process

ends (122) and a user unloads the quality control suspension and cleans the flow cytometer (124).

Example 2

An embodiment of the presently disclosed method for quality controlling a flow cytometer using a single peak followed by using multiple peaks is shown in Figure 2. Referring to Figure 2, steps 200 through 224 are the same as shown in Figure 1 and represent the steps for quality controlling a flow cytometer using a single peak. Following step 216, a user has the ability to perform an additional quality control analysis using multiple peaks, which begins by the user selecting an enhanced quality control analysis (226). An enhanced quality control analysis can be performed daily, weekly, monthly, a specific number of days, or never (226). If the timing for performing an enhanced quality control analysis has not occurred yet, e.g., it has only been 1 day since the last enhance quality control analysis and the timing is set for every 3 days (228), then the flow cytometer does not perform the enhanced quality control analysis and generates a quality control report (218). If it is time to perform an enhance quality control analysis (228) then the user can decide whether to perform an enhanced quality control analysis (230). If the user elects to move forward with an enhanced quality control analysis then the user unloads the single peak suspension (232), selects an enhanced quality control target file (234), and loads an enhanced multiple peak suspension of the present invention into the flow cytometer (236). The user then selects an enhanced quality control analysis (238), adjusts the brightest peak to target MdfI (240), and the flow cytometer reads the MdfI and rCV for the Bright, Middle, and Dim peaks in an embodiment using a 3-peak analysis (242). The user then unloads the enhanced multiple peak suspension of the present invention (244) and loads blank fluorospheres (beads with no dye) (246) before selecting continuation of the enhanced quality control analysis (248). The MdfI is read for the blank fluorospheres (250), followed by calculation of MESF sensitivity for specific channels 252, and generation of an enhanced quality control report (254), and the termination of the enhanced quality control analysis (256).

Example 3

To better understand the dye leak from fluorospheres, thereby providing a better understanding of how best to combine and use non-IR and IR dyes in a single fluorosphere, single dye fluorospheres were evaluated for dye leak, rCV, and singlets

percentage over time. Fluorospheres with the following single dyes were evaluated:
UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, Aqua Green, and Cy
Green. All fluorospheres were prepared by and ordered from Spherotech, Inc.
(<https://www.spherotech.com/>), 27845 Irma Lee Circle, Unit 101, Lake Forest, IL
5 60045.

Each single dye fluorosphere suspension was diluted to 1×10^6 fluorospheres/mL in
0.05% Ecosurf EH-9, available from Sigma Aldrich (CAS: 64366-70-7). Once diluted,
samples were stored in FACS tubes, covered with foil to protect them from the light,
and stored at 4 °C. The dilution day is Day 0. Prior to measurement, samples were
10 vortexed for 5 seconds and acquired for 60 seconds with a threshold of FSC Height at
600,000. Each single dye suspension was acquired and assessed for rCV, singlet
percentage, and MdFl difference in comparison to Day 0. An rCV of less than 5% was
considered passing. Singlet events divided by total fluorosphere events (% singlets) of
greater than 85% was considered passing. MdFl minus MdFl at Day 0 divided by MdFl
15 at Day 0 equals the % MdFl difference.

Table 1 represents the preparation of each single dye fluorosphere suspension
evaluated.

Table 1: Preparation of Single Bead Peaks.

	Weight % Solids (w/v)	Number of Microspheres/mL	Mean diameter (μ m)	Amount from stock sln for 1×10^6 /mL (μ L)
UV	1	2.08E+09	2.06	0.48
Light Yellow	1	1.81E+09	2.16	0.55
Yellow	1	2.27E+09	2.00	0.44
Nile Red	1	2.49E+09	1.94	0.40
Purple	1	2.38E+09	1.97	0.42
Blue	1	5.06E+08	3.30	1.97
Sky Blue	0.2	5.10E+08	1.93	1.96
Aqua Green	0.014	1.00E+07	3.00	100.00
Cy Green	0.014	1.00E+07	3.00	100.00

20 The gating strategy is disclosed in Figure 3 for each of the single dye fluorosphere
suspensions. The percentage of singlets at Day 0 and Day 3 is disclosed in Table 2 and
Table 3, respectively. The Day 3 control, which represents samples freshly diluted on
Day 3, is disclosed in Table 4,

Table 2: Percentage of Singlets for Single Dye Fluorospheres at Day 0.

Day 0	Singlets	Beads population	Singlets%
UV	927	1956	47%
Light Yellow	120	1000	12%
Yellow	5940	12440	48%
Nile Red	2513	4162	60%
Purple	1465	2534	58%
Blue	20482	22521	91%
Sky Blue	637	2000	32%
Aqua Green	8602	10253	84%
Cy Green	9613	10960	88%

Table 3: Percentage of Singlets for Single Dye Fluorospheres at Day 3.

Day 3	Singlets	Beads population	Singlets%
UV	193	684	47%
Light Yellow	136	474	29%
Yellow	5159	10350	50%
Nile Red	2824	5615	50%
Purple	447	1346	33%
Blue	4228	5715	74%
Sky Blue	146	553	26%
Jade Green	8978	10206	88%
Aqua Green	3619	5722	63%
CyGreen	8708	10184	86%

- 5 **Table 4:** Percentage of Singlets for Single Dye Fluorospheres that were Freshly Diluted on Day 3.

Day 3 Ctr	Singlets	Beads population	Singlets%
UV	1042	2212	47%
Light Yellow	140	772	18%
Yellow	3338	7233	46%
Nile Red	2638	3928	67%
Purple	1025	1578	65%
Blue	24246	26581	91%
Sky Blue	1356	4483	30%

Aqua Green	9051	11016	82%
CyGreen	9809	11009	89%

An rCV analysis of each single dye fluorosphere suspension was conducted at Day 0 (Table 5), Day 3 (Table 6), Day 16 (Table 7), Day 22 (Table 8), and Day 24 (Table 9) at the following channels: U3-A, U4-A, V3-A, V4-A, B3-A, B4-A, Y3-A, Y4-A, R3-A,

5 R4-A, IR1-A, IR2-A, and IR3-A. A CytoFlex LX flow cytometer from Beckman

Coulter was used for all readings.

Table 5: rCV Analysis of Single Dye Fluorospheres at Day 0.

Day 0	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
UV	4.7	4.55	3.41	3.38	27.77	32.2	279.18	227.41	294.19	538.03	18.78	65.52	-222.45
Light Yellow	4.89	5.38	1.99	2.09	8.05	8.49	157.27	102.13	181.02	563.72	18.54	61.48	-153.77
Yellow	95.89	28.67	2.4	2.34	2.4	2.37	68.76	84.92	575.58	12733.89	24.43	80.28	-262.99
Nile Red	31.74	27.57	14.89	10.13	2.86	2.92	3.06	4.35	9.84	12.29	20.13	67.46	-238.57
Purple	192.11	129.14	60.27	63.65	18.04	2.51	3.68	3.94	4.45	4.51	21.08	73.96	-242.17
Blue	32.87	22.19	11.79	12.27	8.02	7.55	11.93	6.09	5.98	5.85	21.04	46.72	-263.82
Sky Blue	62.27	46.66	26.99	30.82	13.75	21.3	19.08	4.42	3.5	2.72	14.57	16.06	48.19
Aqua Green	17.33	12.57	4.95	4.61	3.97	3.97	6.78	5.97	5.49	6.55	9.87	9.64	9.81
Cy Green	49.88	34.42	13.54	13.66	7.36	6.28	9.42	5.16	2.84	2.42	2.9	2.84	3.73

Table 6: rCV Analysis of Single Dye Fluorospheres at Day 3.

Day 3	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
UV	4.89	4.74	3.96	4.77	27.07	35	422.25	192.36	695.08	559.5	19.98	71.33	-248.26
Light Yellow	3.45	3.56	1.71	1.64	7.34	8.63	129.93	120.02	321.47	658.64	15.57	45.1	-196.06
Yellow	94.05	28.59	2.38	2.25	2.44	2.42	69.98	81.43	803.24	33457.34	17.98	57.52	-245.7
Nile Red	27.51	22.64	11.37	7.58	2.11	2.15	2.78	3.21	7.25	9.9	18.64	62.11	-291.7
Purple	116.57	92.67	53.53	69.18	18.58	2.36	3.55	3.8	4.6	4.69	16.35	53.04	-247.65
Blue	30.95	21.43	11.64	11.87	7.79	7.57	11.26	6.02	6.22	6.06	19.92	44.41	-271.55
Sky Blue	62.06	50.67	26.79	26.67	14.22	22	21.45	4.97	5.46	4.44	13.5	13.75	58.56
*Jade Green	33.21	19.31	10.58	10.23	6.73	5.42	5.85	7.33	9.79	8.07	2.76	2.79	3.03
Aqua Green	17.26	12.75	4.96	4.76	4.01	4.02	6.85	5.8	5.52	6.52	9.64	9.42	9.62
CyGreen	51.36	33.62	13.44	14.24	7.09	6.24	9.36	5.01	3.02	2.5	2.52	2.57	3.25

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Table 7: rCV Analysis of Single Dye Fluorospheres at Day 16.

Day 16	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
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UV	5.03	5.13	4.09	4.87	29.87	37.45	426.37	170.69	788.87	512.91	18.76	51.88	-273.13
Light Yellow	4.08	4	1.67	1.77	7.04	8.45	127.58	115.78	361.59	-2937.46	15.96	50.53	-488.13
Yellow	86.75	27.24	2.15	2.05	2.7	2.6	68.35	81.03	636.36	-4155.91	15.61	47.55	-505.59
Nile Red	25	20.61	11.11	7.63	2.94	2.88	2.31	2.59	6.58	8.59	18.07	52.43	-403.93
Purple	102.14	79.57	58.73	57.12	19.42	3.43	3.34	3.36	5.65	5.61	17.03	53.68	-514.54
Blue	29.04	23	12.28	12.27	8.51	7.73	11.19	6.14	6.28	6.16	19.37	37.92	-307.15
Sky Blue	40.31	50.06	27.41	31.12	9.14	17.87	24.07	4.73	5.2	2.58	14.83	17.1	58.17
*Jade Green	29.79	18.23	10.63	10.31	6.72	6	6.1	7.42	10.08	8.37	2.65	2.67	3
Aqua Green	16.32	12.28	4.94	4.96	4.18	4.19	5.85	5.5	5.57	6.59	9.16	8.97	9.05
CyGreen	47.73	32.79	13.57	13.59	7.12	6.16	9.07	4.66	2.94	2.44	1.84	1.85	2.73

Table 8: rCV Analysis of Single Dye Fluorospheres at Day 22.

Day 22	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
UV	2.52	2.79	3.78	3.82	38.91	61.48	122.19	199.59	374.25	-458.28	27.1	103.27	-1245.38
Light Yellow	2.56	3.31	3.18	2.61	33.35	62.22	479.33	2032.42	451.64	462.89	21.14	73.97	-262.08
Yellow	142.86	52.19	2.48	2.28	2.55	2.38	122.36	154.59	1439.31	2724.81	22.14	97.78	-447.71
Nile Red	26.42	23.66	22.65	7.85	1.67	1.58	2.36	1.95	8.11	8.72	26.71	119.23	856.36
Purple	202.26	147.84	69	108.89	35.75	9.75	20.8	18.74	18.08	20.64	37.56	57.2	-134.27
Blue	35.35	21.71	12.3	12.78	7.79	7.22	11.12	5.44	5.51	5.39	21.39	43.21	-452.23
Sky Blue	239.32	179.59	66.52	75.06	48.15	4.42	5.62	6.42	5.7	6.65	33.21	135.41	1934348
*Jade Green	31.38	18.8	10.21	9.95	6.76	5.94	6.65	7.73	9.51	7.94	3.91	3.99	4.08
Aqua Green	17.92	12.9	5.27	4.5	3.61	3.54	5.67	5.26	5.5	6.37	10.59	10.46	10.43
CyGreen	50.96	33.89	14.07	13.97	7.17	6.02	9.01	4.54	2.98	2.67	5.41	5.58	5.76

Table 9: rCV Analysis of Single Dye Fluorospheres at Day 24.

Day 24	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
UV	3.65	3.94	5.14	5.56	45.91	56.67	8187.25	213.76	353.06	687.06	25.58	115.66	-172.94
Light Yellow	48.42	48.57	86.46	86.96	72.22	73.83	475.67	557.11	732.14	367240	66.18	279.48	-913.27
Yellow	139.6	51.42	6.07	5.87	2.65	2.54	119.15	141.39	919.44	5460.23	59.68	365.51	-319.87
Nile Red	44.25	38.9	20.28	12.81	2.61	2.5	2.3	2.51	8.83	10.77	54.14	301.35	-343.75
Purple	168.26	129.94	109.68	115.65	43.48	5.15	3.27	4.83	7.7	7.87	75.76	503.55	-343.12

Day 24	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
Blue	8497.15	43855.3	3270.85	420.3	19.81	18.32	64.03	54.83	133.17	133.31	509.98	435.49	507.85
Sky Blue	152.24	128.59	77.48	85.09	43.8	6.26	7.43	7.58	9.65	9.99	80.29	224.65	1442.38
*Jade Green	32.92	19.07	11.45	11.03	6.47	5.86	6.28	7.46	9.44	7.81	31.34	30.62	31.12
Aqua Green	39	32.47	28.82	28.16	4.12	3.99	6.18	5.39	5.98	7.39	57.39	56.18	58.01
CyGreen	77.9	63.67	38.99	38.87	6.82	5.87	8.82	4.41	3.56	3.26	54.01	53.38	54.43

rCVs failed for fluorospheres with Aqua Green at Day 0. rCVs for fluorospheres with Cy Green failed at Day 24. rCVs for fluorospheres with Jade Green failed at Day 20.

The MdFl calculation and MdFl % difference was also evaluated and disclosed in

- 5 Figure 4. More specifically, Figure 4A shows the MdFl % difference for fluorospheres with UV on emitted channels and Figure 4B shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

Figure 4C shows the MdFl % difference for fluorospheres with Light Yellow dye on emitted channels and Figure 4D shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

- 10 Figure 4E shows the MdFl % difference for fluorospheres with Yellow dye on emitted channels and Figure 4F shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

Figure 4G shows the MdFl % difference for fluorospheres with Nile Red dye on

- 15 emitted channels and Figure 4H shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

Figure 4I shows the MdFl % difference for fluorospheres with Purple dye on emitted channels and Figure 4J shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

- 20 Figure 4K shows the MdFl % difference for fluorospheres with Blue dye on emitted channels and Figure 4L shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

Figure 4M shows the MdFl % difference for fluorospheres with Sky Blue dye on emitted channels and Figure 4N shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

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Figure 4O shows the MdFl % difference for fluorospheres with Jade Green dye (IR excited) on emitted channels and Figure 4P shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

Figure 4Q shows the MdFl % difference for fluorospheres with CyGreen dye (IR excited) on emitted channels and Figure 4R shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

Figure 4S shows the MdFl % difference for fluorospheres with Aqua Green dye (IR excited) on emitted channels and Figure 4T shows the MdFl for the same fluorospheres across the spectrum at Day 0, Day 3, Day 16, Day 22, and Day 24.

The dyes became dimmer after Day 22. Cy Green was dimmer in the IR laser, followed by Cy Green. Jade Greed provided the brightest MdFl. There was dye leak over time for Jade Green fluorospheres.

Example 4

The stability of fluorospheres of the present invention were evaluated following dilution to different volumes using different buffers. Fluorospheres of the present invention (Set 7.2) having 8 dyes were prepared for evaluation. The fluorospheres included UV dye, Light Yellow dye, Yellow dye, Nile Red dye, Purple dye, Blue dye, Sky Blue dye, and Jade Green. The Jade Green was added to the fluorospheres after the other dyes were added. The fluorospheres were washed with 0.01% NP-40 and suspended in a buffer of 0.01% NP-40.

Fluorospheres were diluted to a concentration of 1×10^6 fluorospheres/sample to a total volume of either 0.5 ml, 1.0 ml, or 2.5 ml using either Ecosurf EH-9 (CAS: 64366-70-7) and Ecosurf SA-9 (CAS:68937-66-6). Samples were freshly diluted with 0.05% Ecosurf EH-9 to 1.0 ml for use as a control. Samples were vortexed for 5 seconds and then acquired for 60 seconds. Each fluorosphere suspension was acquired and assessed for rCV, singlet percentage, and MdFl difference in comparison to Day 0. An rCV of less than 5% was considered passing. Singlet events divided by total fluorosphere events (% singlets) of greater than 85% was considered passing. MdFl minus MdFl at Day 0 divided by MdFl at Day 0 equals the % MdFl difference. A CytoFlex LX flow cytometer from Beckman Coulter was used for all readings.

Fluorospheres (Set 7.2) were diluted to 0.5 ml, 1.0 ml, and 2.5 ml final volume with Ecosurf EH-9 at Day 0. Fluorospheres (Set 7.2) were also diluted 0.5 ml, 1.0 ml, and 2.5 ml final volume with Ecosurf SA-9 at Day 0. The analysis of percent singlets was performed at Day 0, Day 4, Day 7, Day 18, Day 20, Day 25, and Day 27 as disclosed in

Table 10 and Table 11. Gate analysis of the Day 4 samples diluted with Ecosurf EH-9 is shown in Figure 5.

Table 10: Percent Singlet Analysis for Each of the Six Samples at Day 0, Day 4, and Day 7.

	Beads Events	Singlet Events	Singlets
Set 7.2 p.7 0.5mL EH-9	16315	14262	87%
Set 7.2 p.7 1mL EH-9	8077	7293	90%
Set 7.2 p.7 2.5mL EH-9	3157	2953	94%
Set 7.2 p.7 0.5mL SA-9	15088	14127	94%
Set 7.2 p.7 1mL SA-9	7632	7034	92%
Set 7.2 p.7 2.5mL SA-9	14898	13941	94%
Set 7.2 p.7 0.5mL SA-9 Day 4	15058	13669	91%
Set 7.2 p.7 1mL SA-9 Day 4	6809	6238	92%
Set 7.2 p.7 2.5mL SA-9 Day 4	2953	2762	94%
Set 7.2 p.7 0.5mL EH9 Day 4	3188	2014	63%
Set 7.2 p.7 1mL EH9 Day 4	1670	1051	63%
Set 7.2 p.7 2.5mL EH9 Day 4	616	456	74%
Set 7.2 p.7 0.5mL EH-9 Day 7	2180	1380	63%
Set 7.2 p.7 1mL EH-9 Day 7	1245	723	58%
Set 7.2 p.7 2.5mL EH-9 Day 7	278	194	70%
Set 7.2 p.7 0.5mL SA-9 Day 7	14515	13294	92%
Set 7.2 p.7 1mL SA-9 Day 7	7067	6588	93%
Set 7.2 p.7 2.5mL SA-9 Day 7	3429	3040	89%

5

Table 11: Percent Singlet Analysis for Each of the Six Samples at Day 18, Day 20, Day 25, and Day 27.

	Beads Events	Singlet Events	Singlets
0.5mL EH9 Day 18	1830	1170	64%
1mL EH9 Day 18	586	358	61%
2.5mL EH9 Day 18	232	180	78%
0.5mL SA9 Day 18	14739	13714	93%
1mL SA9 Day 18	7188	6404	89%
2.5mL SA9 Day 18	3277	2878	88%
0.5mL EH9 Day 20	770	481	62%
1mL EH9 Day 20	166	125	75%
2.5mL EH9 Day 20	175	122	70%
0.5mL SA9 Day 20	9836	9136	93%
1mL SA9 Day 20	5961	5568	93%
2.5mL SA9 Day 20	3446	3209	93%
0.5mL EH9 Day 25	1206	773	64%
1mL EH9 Day 25	353	236	67%
2.5mL EH9 Day 25	158	118	75%
0.5mL SA9 Day 25	7267	6755	93%

	Beads Events	Singlet Events	Singlets
1mL SA9 Day 25	6558	6117	93%
2.5mL SA9 Day 25	3662	3437	94%
0.5mL EH9 Day 27	1363	963	71%
1mL EH9 Day 27	517	340	66%
2.5mL EH9 Day 27	77	55	71%
0.5mL SA9 Day 27	9896	9167	93%
1mL SA9 Day 27	4636	4291	93%

- Fluorospheres (Set 7.2) were diluted to 0.5 ml, 1.0 ml, and 2.5 ml final volume with Ecosurf EH-9 at Day 0. Fluorospheres (Set 7.2) were also diluted 0.5 ml, 1.0 ml, and 2.5 ml final volume with Ecosurf SA-9 at Day 0. An analysis of rCVs was performed
- 5 on each sample at Day 0, Day 4, Day 7, Day 18, Day 20, Day 25, and Day 27 for each of the following channels: U3-A, U4-A, V3-A, V4-A, B3-A, B4-A, Y3-A, Y4-A, R3-A, R4-A, IR1-A, IR2-A, and IR3-A.

Table 12: rCV Analysis for Each of the Six Samples at Day 0, Day 4, and Day 7.

	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
0.5mL EH-9	3.98	4	1.83	1.82	1.46	1.58	2.47	2.48	3.39	3.34	2.63	2.56	3.71
1mL EH-9	3.98	3.96	1.87	1.84	1.51	1.6	2.42	2.44	3.45	3.44	4.61	4.39	5.33
2.5mL EH-9	3.92	3.87	1.84	1.88	1.55	1.65	2.36	2.34	3.19	3.22	4.19	4	4.85
0.5mL SA-9	4.1	4.16	1.82	1.8	1.55	1.65	2.31	2.31	3.17	3.16	9.62	9.26	9.74
1mL SA-9	4.28	4.34	1.88	1.84	1.6	1.7	2.42	2.4	3.28	3.31	10.33	9.75	10.62
2.5mL SA-9	3.93	3.95	1.84	1.81	1.55	1.63	2.28	2.31	3.19	3.18	5.57	5.23	6.16
0.5mL SA-9 Day 4	4.16	4.22	1.96	1.94	1.51	1.6	2.77	2.77	2.88	2.88	2.53	2.48	3.69
1mL SA-9 Day 4	4.14	4.21	1.91	1.9	1.46	1.57	2.74	2.73	2.81	2.81	2.75	2.57	3.74
2.5mL SA-9 Day 4	4	3.98	1.88	1.88	1.42	1.5	2.72	2.74	2.76	2.82	2.69	2.56	3.83
0.5mL EH9 Day 4	3.89	3.83	2.01	2.02	1.42	1.49	2.63	2.62	2.69	2.63	2.36	2.27	3.45
1mL EH9 Day 4	3.53	3.58	1.95	1.95	1.49	1.59	2.63	2.64	2.69	2.71	2.17	2.24	3.5
2.5mL EH9 Day 4	4.01	4.02	2.12	2.09	1.27	1.42	2.82	2.72	2.73	2.73	2.38	2.08	3.28
0.5mL EH-9 Day 7	3.79	3.75	2.06	1.99	1.43	1.55	2.62	2.75	2.79	2.87	3.46	3.69	4.45
1mL EH-9 Day 7	3.97	4.03	2.05	1.99	1.59	1.69	2.59	2.67	2.69	2.68	2.82	2.5	3.61
2.5mL EH-9 Day 7	3.91	4.04	2.12	1.94	1.72	1.63	2.49	2.47	2.78	2.68	2.62	2.98	4.38
0.5mL SA-9 Day 7	4.15	4.2	1.94	1.94	1.46	1.55	2.79	2.82	2.91	2.89	2.55	2.56	3.55
1mL SA-9 Day 7	4.08	4.12	1.94	1.95	1.41	1.52	2.74	2.7	2.84	2.82	2.74	2.73	3.65
2.5mL SA-9 Day 7	4.37	4.52	1.96	1.91	1.54	1.63	2.73	2.77	2.9	2.91	2.33	2.41	3.43

Table 13: rCV Analysis for Each of the Six Samples at Day 18, Day 20, Day 25, and Day 27.

	U3-A	U4-A	V3-A	V4-A	B3-A	B4-A	Y3-A	Y4-A	R3-A	R4-A	IR1-A	IR2-A	IR3-A
0.5mL EH9 Day 18	3.74	3.78	2.09	2.16	2	2.07	2.18	2.29	3.34	3.42	2.31	2.27	3.09
1mL EH9 Day 18	3.73	3.82	1.98	1.89	2.17	2.23	2.09	2.15	3.33	3.31	2.66	2.66	3.4
2.5mL EH9 Day 18	3.85	3.96	2.19	2.32	1.99	1.98	2.47	2.3	3.29	3.23	3.26	3.02	3.55
0.5mL SA9 Day 18	3.94	3.97	1.82	1.78	1.91	1.95	2.17	2.21	3.16	3.18	2.23	2.18	3.19
1mL SA9 Day 18	4.09	4.13	1.96	1.89	1.98	2	2.36	2.39	3.35	3.36	5.37	5.59	5.84
2.5mL SA9 Day 18	4.17	4.15	1.96	1.91	1.97	1.97	2.33	2.35	3.34	3.36	5.53	5.65	5.97
0.5mL EH9 Day 20	4.01	4.04	2.38	2.31	2.17	2.23	2.3	2.26	3.04	3.12	3.6	3.44	4.48
1mL EH9 Day 20	3.74	4.13	2.41	2.06	2.59	2.63	2.57	2.49	3.09	2.95	5.45	4.48	6.07
2.5mL EH9 Day 20	3.39	3.37	1.89	1.84	2.14	2.27	2.27	2.18	2.67	2.8	3.33	3.17	4.08
0.5mL SA9 Day 20	4.07	4.11	2.18	2.14	2.08	2.14	2.56	2.6	3.36	3.35	3.83	3.63	4.48
1mL SA9 Day 20	4.01	4.03	2.22	2.13	2.2	2.24	2.62	2.61	3.47	3.44	3.84	3.71	4.6
2.5mL SA9 Day 20	4.14	4.28	2.16	2.11	2.15	2.19	2.59	2.66	3.39	3.39	3.78	3.46	4.45
0.5mL EH9 Day 25	3.77	3.95	2.11	2.11	2	2.1	2.42	2.34	2.96	2.97	2.78	2.8	3.51
1mL EH9 Day 25	3.27	3.33	2.15	2.08	1.72	1.78	2.33	2.41	2.71	2.76	2.6	2.78	3.41
2.5mL EH9 Day 25	4.32	4.64	2.08	2.11	1.98	2.15	2.21	2.55	2.78	2.84	2.62	2.75	3.46
0.5mL SA9 Day 25	3.85	3.94	2.03	1.98	1.87	1.91	2.32	2.3	2.98	2.95	2.51	2.38	3.35
1mL SA9 Day 25	3.9	3.99	2.07	2.06	1.8	1.81	2.26	2.33	2.96	2.96	5.21	5.43	5.68
2.5mL SA9 Day 25	3.87	3.96	2.06	2	1.98	2.01	2.2	2.2	3.1	3.13	2.63	2.52	3.58
0.5mL EH9 Day 27	3.9	3.95	2	1.94	1.91	2	2.06	2.18	3.07	3.03	3.53	3.29	4.23
1mL EH9 Day 27	3.39	3.47	2.02	2.01	1.79	1.94	2.25	2.25	3.68	3.38	2.99	2.92	3.83
2.5mL EH9 Day 27	3.72	4.03	1.88	1.73	2.39	2.19	2.2	2.21	3.28	3.2	3.14	2.94	3.32
0.5mL SA9 Day 27	3.87	3.9	1.94	1.94	1.97	1.99	2.26	2.26	3.29	3.33	2.75	2.5	3.59
1mL SA9 Day 27	3.75	3.89	1.96	1.93	2.05	2.07	2.16	2.15	3.15	3.21	2.98	2.76	3.67

- 5 The MdFl calculation and MdFl % difference was also evaluated and disclosed in Figures 5D-5M. More specifically, Figures 5D-5F show the MdFl % difference for fluorospheres of the present invention diluted to 0.5ml with Ecosurf EH-9 (Figure 5D), diluted to 1.0ml with Ecosurf EH-9 (Figure 5E), and diluted to 2.5ml with Ecosurf EH-

9 (Figure 5F). Figures 5G-5I show the MdFl % difference for fluorospheres of the present invention diluted to 0.5ml with Ecosurf SA-9 (Figure 5G), diluted to 1.0ml with Ecosurf SA-9 (Figure 5H), and diluted to 2.5ml with Ecosurf SA-9 (Figure 5I). Figures 5J-5K shows MdFl over time (Day 0, Day 4, Day 7, Day 18, Day 20, Day 25, Day 27) for fluorospheres of the present invention diluted to 0.5 ml in Ecosurf EH-9 (Figure 5J) and diluted to 2.5ml in Ecosurf EH-9 (Figure 5K). Figures 5L-5M show MdFl over time (Day 0, Day 4, Day 7, Day 18, Day 20, Day 25, Day 27 (only for Ecosurf EH-9)) for fluorospheres of the present invention diluted to 2.5 ml in Ecosurf EH-9 (Figure 5L) and diluted to 2.5ml in Ecosurf SA-9 (Figure 5M)

10 **Example 5**

The stability of fluorospheres of the present invention were evaluated following exposure to light. Fluorospheres of the present invention (Set 10) having 8 dyes were prepared for evaluation. The fluorospheres included UV dye, Light Yellow dye, Yellow dye, Nile Red dye, Purple dye, Blue dye, Sky Blue dye, and Jade Green. Jade Green was added to the fluorospheres at the same time as the other dyes. The fluorospheres were washed with 0.01% NP-40. One set of fluorospheres of the present invention (Set 10.1) were diluted to 1×10^6 fluorospheres/mL in SDS (Set 10.1 SDS Diluted) following the wash with 0.01% NP-40. A second set of fluorospheres (Set 10.1) were resuspended in 0.05% Ecosurf EH-9 and 0.02% sodium azide following a wash with the same buffer (Set 10.1 Wash + Resuspended in Ecosurf+SA). A third set of fluorospheres (Set 10.2) were diluted and then resuspended in a buffer of 0.05% Ecosurf EH-9 and 0.02% sodium azide (Set 10.2 SDS Diluted Ecosurf+SA). A fourth set of fluorospheres (Set 10.2) were diluted and resuspended in a buffer of 0.1% ethanolamine, 0.05% Ecosurf EH-9 and 0.02% sodium azide (Set 10.2 SDS Diluted ETA+Ecosurf+SA). All fluorosphere suspensions were diluted or resuspended to a working concentration of 1×10^6 fluorospheres/mL.

All samples were vortexed for 5 seconds prior to acquisition. Sample were exposed to 1500lux for different time periods: 0 minutes (control), 15 minutes, 30 minutes, 1 hours, 2 hours, 4 hours, and 6 hours. Regular light exposure in a lab is presumed to be 400-800lux. Threshold of the FSC height was 100,000. The fluorospheres were acquired and assessed for rCV, singlet percentage, and MdFl difference in comparison to Day 0.

Figures 6-14 show the rCV analysis of each sample.

Figure 6 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 6A-6D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel U3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 6A shows the rCV analysis of Set 7.2. Figure 6B shows the rCV analysis of set 10.1 diluted in sodium dodecyl sulfate ("SDS") to 1×10^6 fluorospheres per ml. Figure 6C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 6D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 7 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 7A-7D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel U3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 7A shows the rCV analysis of Set 7.2. Figure 7B shows the rCV analysis of set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 7C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 7D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 8 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 8A-8D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel B3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 8A shows the rCV analysis of Set 7.2. Figure 8B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 8C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 8D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 9 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 9A-9D show rCV analysis of fluorospheres of the present invention (Set

7.2 compared to Set 10.1 and Set 10.2) at channel Y3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 9A shows the rCV analysis of Set 7.2. Figure 9B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 9C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 9D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 10 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 10A-10D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel R3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 10A shows the rCV analysis of Set 7.2. Figure 10B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 10C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 10D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 11 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 11A-11D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel IR1A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 11A shows the rCV analysis of Set 7.2. Figure 11B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 11C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 11D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 12 shows a comparison of the MdFl for channels IR1A (Figures 12A-12C) and IR2A (Figures 12D-12F) for the same samples evaluated in Figure 11 and Figure 13. Specifically, Figure 12A shows the MdFl analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 12B shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 12C shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide. Figure 12D shows the

MdFl analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 12E shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 12F shows the MdFl analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 13 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 13A-13D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel IR2A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 13A shows the rCV analysis of Set 7.2. Figure 13B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 13C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 13D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Figure 14 shows the analysis of light exposure (1500lux at different time points) to fluorospheres of the present invention based upon an analysis of rCV in comparison to Day 0. Figures 14A-14D show rCV analysis of fluorospheres of the present invention (Set 7.2 compared to Set 10.1 and Set 10.2) at channel IR3A at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours. Specifically, Figure 14A shows the rCV analysis of Set 7.2. Figure 14B shows the rCV analysis of Set 10.1 diluted in SDS to 1×10^6 fluorospheres per ml. Figure 14C shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.05% Ecosurf EH-9 and 0.02% sodium azide. Figure 14D shows the rCV analysis of Set 10.2 diluted and resuspended in SDS with 0.1% ethanolamine, 0.05% Ecosurf EH-9, and 0.02% sodium azide.

Table 14 shows the rCV analysis of each sample over time after exposure to 3500lux. The rCV analysis was conducted at the following channels: U3-A, V3-A, B3-A, Y3-A, IR1-A, and IR2-A.

Table 14: rCV analysis of Fluorosphere Samples Over Time Following Exposure to 3500Lux.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Set 10.1 SDS Diluted by SphT ctr	3.55	1.48	1.49	2.62	3.02	4.33	4.94
Set 10.1 SDS Diluted by SphT 15 min	3.37	1.43	1.48	2.58	2.94	4.32	4.88
Set 10.1 SDS Diluted by SphT 30min	3.71	1.44	1.33	2.8	3.21	4.3	4.91
Set 10.1 SDS Diluted by SphT 1h	3.56	1.46	1.39	2.65	3.03	4.23	4.78

Set 10.1 SDS Diluted by SphT 2h	3.69	1.42	1.38	2.66	3.07	4.27	4.77
Set 10.1 SDS Diluted by SphT 4h	3.56	1.57	1.52	2.72	3.02	4.18	4.68
Set 10.1 SDS Diluted by SphT 6h	3.55	1.52	1.56	2.67	2.93	4.52	4.96
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 ctr	3.5	1.41	1.59	2.61	2.89	4.36	4.93
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 15 min	3.65	1.48	1.51	2.53	2.94	4.3	4.9
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 30min	3.45	1.45	1.54	2.57	2.88	4.38	4.93
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 1h	3.67	1.42	1.55	2.71	3.07	4.5	5.02
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 2h	3.73	1.46	1.52	2.72	2.99	4.38	4.92
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 4h	3.63	1.42	1.48	2.6	2.86	4.22	4.81
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 6h	3.46	1.48	1.59	2.67	3.15	6.75	6.54
Set 10.2 SDS Diluted EcoSurf+SA ctr	3.55	1.41	1.5	2.51	2.87	4.49	5.08
Set 10.2 SDS Diluted EcoSurf+SA 15 min	3.59	1.43	1.5	2.56	2.88	4.39	4.99
Set 10.2 SDS Diluted EcoSurf+SA 30 min	3.56	1.44	1.44	2.51	2.84	4.31	4.91
Set 10.2 SDS Diluted EcoSurf+SA 1h	3.52	1.42	1.48	2.52	2.78	4.3	4.89
Set 10.2 SDS Diluted EcoSurf+SA 2h	3.44	1.39	1.52	2.46	2.69	4.15	4.71
Set 10.2 SDS Diluted EcoSurf+SA 4h	3.42	1.41	1.51	2.5	2.73	4.26	4.84
Set 10.2 SDS Diluted EcoSurf+SA 6h	3.45	1.39	1.5	2.51	2.67	4.32	4.76
Set 10.2 SDS Diluted ETA+EcoSurf+SA ctr	3.6	1.54	1.53	2.57	2.84	4.44	4.89
Set 10.2 SDS Diluted ETA+EcoSurf+SA 15 min	3.47	1.45	1.49	2.46	2.74	4.41	4.92
Set 10.2 SDS Diluted ETA+EcoSurf+SA 30 min	3.52	1.44	1.51	2.45	2.75	4.12	4.7
Set 10.2 SDS Diluted ETA+EcoSurf+SA 1 h	3.54	1.42	1.45	2.53	2.74	4.15	4.69
Set 10.2 SDS Diluted ETA+EcoSurf+SA 2h	3.4	1.47	1.48	2.48	2.72	4.08	4.64
Set 10.2 SDS Diluted ETA+EcoSurf+SA 4h	3.55	1.42	1.49	2.55	2.61	4.1	4.61
Set 10.2 SDS Diluted ETA+EcoSurf+SA 6h	3.58	1.43	1.48	2.54	2.71	4.08	4.59
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 1ctr	3.37	1.43	1.47	2.46	2.61	3.87	4.37
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 15 min	3.51	1.53	1.58	2.51	2.8	4.25	4.8
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 30 min	3.55	1.54	1.58	2.65	2.86	4.32	4.9
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 1h	3.57	1.5	1.52	2.63	2.78	4.16	4.67
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 2h	3.31	1.49	1.69	2.46	2.58	4.31	4.79

Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 4h	3.4	1.52	1.66	2.64	2.95	4.53	4.94
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 6h	3.59	1.7	1.52	2.63	4.88	11.33	10.37

Table 15 shows the % singlet analysis of each sample over time after exposure to 3500lux.

Table 15: % Singlet Analysis of Fluorosphere Samples Over Time Following Exposure to 3500Lux.

5

TubeName	Population	Singles Events	Singlets %
Set 10.1 SDS Diluted by SphT ctr	4215	3775	90%
Set 10.1 SDS Diluted by SphT 15 min	9158	8245	90%
Set 10.1 SDS Diluted by SphT 30min	9459	8153	86%
Set 10.1 SDS Diluted by SphT 1h	9549	8310	87%
Set 10.1 SDS Diluted by SphT 2h	9482	8365	88%
Set 10.1 SDS Diluted by SphT 4h	9056	7894	87%
Set 10.1 SDS Diluted by SphT 6h	9678	8541	88%
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 ctr	1917	1687	88%
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 15 min	6274	5534	88%
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 30min	4876	4269	88%
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 1h	5594	4833	86%
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 2h	4732	3955	84%
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 4h	4016	3484	87%
Set 10.1 SDS Wash+Resuspended in EcoSurf+SA r1 6h	6637	5663	85%
Set 10.2 SDS Diluted EcoSurf+SA ctr	6859	5818	85%
Set 10.2 SDS Diluted EcoSurf+SA 15 min	8900	7716	87%
Set 10.2 SDS Diluted EcoSurf+SA 30 min	7042	6052	86%
Set 10.2 SDS Diluted EcoSurf+SA 1h	9685	8309	86%
Set 10.2 SDS Diluted EcoSurf+SA 2h	8169	7097	87%
Set 10.2 SDS Diluted EcoSurf+SA 4h	7779	6780	87%
Set 10.2 SDS Diluted EcoSurf+SA 6h	9390	8020	85%
Set 10.2 SDS Diluted ETA+EcoSurf+SA ctr	3657	3230	88%
Set 10.2 SDS Diluted ETA+EcoSurf+SA 15 min	9425	8414	89%
Set 10.2 SDS Diluted ETA+EcoSurf+SA 30 min	8632	7609	88%
Set 10.2 SDS Diluted ETA+EcoSurf+SA 1 h	6998	6232	89%
Set 10.2 SDS Diluted ETA+EcoSurf+SA 2h	6073	5418	89%
Set 10.2 SDS Diluted ETA+EcoSurf+SA 4h	5887	5217	89%
Set 10.2 SDS Diluted ETA+EcoSurf+SA 6h	9501	8353	88%
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 1ctr	941	803	85%

Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 15 min	5032	4492	89%
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 30 min	6743	6037	90%
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 1h	7482	6671	89%
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 2h	6720	5959	89%
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 4h	5496	4880	89%
Set 10.2 SDS Washed+Resuspended in ETA+EcoSurf+SA pH:7 r3 6h	8115	7195	89%

Example 6

To investigate the best potential IR dye for use with fluorospheres of the present invention, fluorospheres of the present invention having either Jade Green, Aqua Green, or Cy Green were evaluated. Fluorospheres of the present invention (Set 11) having 8 dyes were prepared for evaluation. The fluorospheres included UV dye, Light Yellow dye, Yellow dye, Nile Red dye, Purple dye, Blue dye, Sky Blue dye, and either Aqua Green or Cy Green. In addition, fluorospheres of the present invention (Set 12) having 8 dyes were prepared for evaluation. The fluorospheres included UV dye, Light Yellow dye, Yellow dye, Nile Red dye, Purple dye, Blue dye, Sky Blue dye, and Jade Green. All three Sets (Set 11 with Aqua Green, Set 11 with Cy Green, and Set 12 with Jade Green) were prepared as shown in Figure 16.

Fluorospheres with Cy Green (Set 11 with Cy Green) did not perform as well as fluorospheres with Aqua Green (Set 11 with Aqua Green) or Jade Green (Set 12 with Jade Green). Accordingly, only analysis for fluorospheres with Aqua Green or Jade Green are included.

An MdFl analysis was performed on the samples. Figure 17 shows the evaluation of fluorospheres of the present invention containing either Aqua Green dye or Jade Green dye as the IR excited dye. The MdFl analysis is for Peak 2 (Figures 17A-17F), Peak 4 (Figures 17G-17L), and Peak 7 (Figures 17M-17R).

Figure 17A shows the MdFl analysis (Peak 2) of fluorospheres with Aqua Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.

Figure 17B shows the MdFl analysis (Peak 2) of fluorospheres with Jade Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.

Figure 17C shows the MdFl analysis (Peak 2) of fluorospheres with Aqua Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.

- Figure 17D shows the MdFl analysis (Peak 2) of fluorospheres with Jade Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- Figure 17E shows the MdFl analysis (Peak 2) of fluorospheres with Aqua Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- 5 Figure 17F shows the MdFl analysis (Peak 2) of fluorospheres with Jade Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- Figure 17G shows the MdFl analysis (Peak 4) of fluorospheres with Aqua Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- Figure 17H shows the MdFl analysis (Peak 4) of fluorospheres with Jade Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- 10 Figure 17I shows the MdFl analysis (Peak 4) of fluorospheres with Aqua Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- Figure 17J shows the MdFl analysis (Peak 4) of fluorospheres with Jade Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure
- 15 17K shows the MdFl analysis (Peak 4) of fluorospheres with Aqua Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17L shows the MdFl analysis (Peak 4) of fluorospheres with Jade Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- Figure 17M shows the MdFl analysis (Peak 7) of fluorospheres with Aqua Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- 20 Figure 17N shows the MdFl analysis (Peak 7) of fluorospheres with Jade Green dye that were incubated at 22°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- Figure 17O shows the MdFl analysis (Peak 7) of fluorospheres with Aqua Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- 25 Figure 17P shows the MdFl analysis (Peak 7) of fluorospheres with Jade Green dye that were incubated at 32°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17Q shows the MdFl analysis (Peak 7) of fluorospheres with Aqua Green dye that were incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18. Figure 17R shows the MdFl analysis (Peak 7) of fluorospheres with Jade Green dye that were
- 30 incubated at 50°C at Day 0, Day 5, Day 7, Day 11, Day 14, and Day 18.
- Figure 17S and Figure 17T shows the differences at Peak 2, Peak 4, and Peak 7 between fluorospheres with Aqua Green dye (Figure 17S) and fluorospheres with Jade Green dye (Figure 17T) at channel IR1A. Figure 17U and Figure 17V show the differences at Peak 2, Peak 4, and Peak 7 between fluorospheres with Aqua Green dye

(Figure 17S) and fluorospheres with Jade Green dye (Figure 17T) at channels U3A, V3A, B3A, Y3A, and R3A.

An evaluation of the MdFl % difference for each sample as function of time (Day 0, Day 5, Day 7, Day 11, Day 14, and Day 22) combined with temperature (22°C, 32°C, and 50°C) was also conducted and is presented in Tables 16-18 below.

Table 16A: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (22°C), across Several Channels for Peak 2.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P2 Day 5 22C	2%	0%	0%	1%	-3%	4%	3%
Jade P2 Day 7 22C	2%	0%	1%	0%	-4%	-2%	-2%
Jade P2 Day 11 22C	2%	0%	2%	-1%	-4%	-7%	-7%
Jade P2 Day 14 22C	1%	0%	2%	0%	-5%	-12%	-12%
Jade P2 Day 18 22C	1%	-1%	2%	0%	-4%	-15%	-15%
Aqua P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P2 Day 5 22C	-1%	0%	0%	0%	0%	-5%	-7%
Aqua P2 Day 7 22C	0%	1%	1%	1%	1%	-10%	-10%
Aqua P2 Day 11 22C	0%	0%	0%	-1%	-1%	-12%	-13%
Aqua P2 Day 14 22C	-1%	0%	1%	0%	0%	-14%	-15%
Aqua P2 Day 18 22C	-1%	3%	3%	0%	-2%	-15%	-17%
Jade P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 22C	1%	-1%	1%	0%	0%	-2%	-1%
Jade MIX Day 7 22C	0%	-1%	1%	-1%	-2%	-5%	-5%
Jade MIX Day 11 22C	-1%	-1%	1%	-1%	-3%	-7%	-7%
Jade MIX Day 14 22C	0%	-1%	2%	-3%	-3%	-9%	-9%
Jade MIX Day 18 22C	0%	2%	4%	-4%	-6%	-9%	-10%
Aqua P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P2 Day 5 22C	-1%	0%	0%	0%	0%	-5%	-7%
Aqua P2 Day 7 22C	0%	1%	1%	1%	1%	-10%	-10%
Aqua P2 Day 11 22C	0%	0%	0%	-1%	-1%	-12%	-13%
Aqua P2 Day 14 22C	-1%	0%	1%	0%	0%	-14%	-15%
Aqua P2 Day 18 22C	-1%	3%	3%	0%	-2%	-15%	-17%

Table 16B: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (22°C), across Several Channels for Peak 4.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P4 Day 5 22C	0%	0%	0%	0%	0%	-3%	-3%
Jade P4 Day 7 22C	1%	0%	0%	0%	0%	-7%	-7%
Jade P4 Day 11 22C	0%	-1%	-1%	-1%	-1%	-7%	-7%
Jade P4 Day 14 22C	-1%	-2%	-1%	-1%	-1%	-9%	-9%
Jade P4 Day 18 22C	-1%	-1%	0%	-1%	-1%	-10%	-10%
Aqua P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P4 Day 5 22C	0%	0%	0%	0%	0%	-3%	-3%
Aqua P4 Day 7 22C	0%	-1%	-1%	0%	-1%	-6%	-6%
Aqua P4 Day 11 22C	1%	0%	0%	0%	-1%	-9%	-9%
Aqua P4 Day 14 22C	0%	0%	0%	-1%	-1%	-11%	-11%
Aqua P4 Day 18 22C	-1%	0%	0%	-1%	-2%	-10%	-10%
TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 22C	-1%	-1%	-1%	-1%	-1%	-2%	-2%
Jade MIX Day 7 22C	-1%	-1%	-1%	-1%	-1%	-5%	-5%
Jade MIX Day 11 22C	-1%	-1%	-1%	-1%	-1%	-6%	-6%
Jade MIX Day 14 22C	0%	-1%	-1%	-2%	-2%	-9%	-8%
Jade MIX Day 18 22C	0%	-1%	-1%	-4%	-3%	-9%	-9%
Aqua P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 22C	-1%	-1%	0%	-1%	-2%	-5%	-5%
Aqua MIX Day 7 22C	1%	0%	0%	0%	-2%	-10%	-10%
Aqua MIX Day 11 22C	1%	0%	0%	0%	-1%	-11%	-11%
Aqua MIX Day 14 22C	2%	1%	1%	0%	-1%	-11%	-10%
Aqua MIX Day 18 22C	0%	0%	0%	-1%	-2%	-13%	-13%

Table 16C: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (22°C), across Several Channels for Peak 7.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P7 Day 5 22C	0%	0%	0%	0%	0%	0%	0%
Jade P7 Day 7 22C	1%	1%	1%	1%	1%	0%	0%
Jade P7 Day 11 22C	0%	0%	0%	1%	0%	1%	1%
Jade P7 Day 14 22C	1%	1%	1%	1%	1%	2%	2%
Jade P7 Day 18 22C	0%	-1%	0%	-1%	-1%	2%	2%
Aqua P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P7 Day 5 22C	-2%	-1%	0%	-1%	0%	0%	0%
Aqua P7 Day 7 22C	-2%	-1%	-1%	-1%	-1%	-1%	-2%
Aqua P7 Day 11 22C	-1%	-1%	-1%	-1%	-1%	-1%	-1%
Aqua P7 Day 14 22C	-3%	-1%	-1%	-1%	-1%	1%	1%
Aqua P7 Day 18 22C	-3%	-2%	-1%	-2%	-2%	-2%	-2%
TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 22C	0%	0%	0%	0%	0%	1%	2%
Jade MIX Day 7 22C	0%	0%	0%	0%	0%	1%	1%
Jade MIX Day 11 22C	-1%	0%	0%	0%	-1%	2%	2%
Jade MIX Day 14 22C	0%	0%	0%	-1%	-1%	2%	2%
Jade MIX Day 18 22C	0%	0%	0%	-3%	-2%	3%	3%
Aqua P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 22C	-3%	-1%	-1%	-1%	-1%	-1%	-1%
Aqua MIX Day 7 22C	-1%	0%	0%	0%	-1%	-1%	-1%
Aqua MIX Day 11 22C	0%	0%	0%	0%	0%	0%	-1%
Aqua MIX Day 14 22C	0%	0%	0%	0%	0%	1%	1%
Aqua MIX Day 18 22C	-2%	-1%	0%	-1%	-1%	1%	1%

Table 17A: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (32°C), across Several Channels for Peak 2.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P2 Day 5 32C	-3%	-4%	-1%	-7%	-7%	-18%	-20%
Jade P2 Day 7 32C	-5%	-5%	-2%	-7%	-8%	-22%	-24%
Jade P2 Day 11 32C	-6%	-6%	-1%	-8%	-8%	-25%	-27%
Jade P2 Day 14 32C	-7%	-6%	0%	-8%	-9%	-29%	-31%
Jade P2 Day 18 32C	-7%	-6%	0%	-6%	-9%	-29%	-31%
Aqua P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P2 Day 5 32C	-3%	-3%	-1%	-3%	-3%	-21%	-24%
Aqua P2 Day 7 32C	-1%	-2%	0%	-2%	-3%	-25%	-27%
Aqua P2 Day 11 32C	-2%	-2%	0%	-2%	-4%	-29%	-31%
Aqua P2 Day 14 32C	-4%	-2%	0%	-2%	-4%	-31%	-34%
Aqua P2 Day 18 32C	-1%	0%	1%	1%	-1%	-18%	-19%

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 32C	-5%	-5%	-1%	-5%	7%	-20%	-21%
Jade MIX Day 7 32C	-6%	-6%	0%	-6%	2%	-22%	-23%
Jade MIX Day 11 32C	-6%	-6%	0%	-5%	1%	-25%	-26%
Jade MIX Day 14 32C	-6%	-6%	-1%	-6%	-1%	-25%	-27%
Jade MIX Day 18 32C	-7%	-7%	-1%	-7%	-1%	-26%	-29%
Aqua P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 32C	-6%	-4%	-2%	-3%	-2%	-20%	-21%
Aqua MIX Day 7 32C	-3%	-3%	-1%	-3%	-3%	-24%	-26%
Aqua MIX Day 11 32C	-4%	-3%	0%	-2%	-3%	-28%	-29%
Aqua MIX Day 14 32C	-4%	-3%	-1%	-3%	-4%	-28%	-30%
Aqua MIX Day 18 32C	-1%	0%	1%	2%	-1%	-17%	-18%

Table 17B: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (32°C), across Several Channels for Peak 4.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P4 Day 5 32C	-2%	-3%	-1%	-5%	-2%	-20%	-20%
Jade P4 Day 7 32C	-3%	-3%	-1%	-3%	-2%	-24%	-24%
Jade P4 Day 11 32C	-3%	-3%	-1%	-4%	-2%	-26%	-26%
Jade P4 Day 14 32C	-4%	-4%	-1%	-4%	-2%	-27%	-27%
Jade P4 Day 18 32C	-4%	-3%	-1%	-4%	-2%	-29%	-29%
Aqua P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P4 Day 5 32C	-1%	-1%	0%	-2%	-1%	-15%	-15%
Aqua P4 Day 7 32C	-1%	-1%	-1%	-1%	-2%	-17%	-17%
Aqua P4 Day 11 32C	0%	-1%	-1%	-1%	-2%	-20%	-20%
Aqua P4 Day 14 32C	-1%	-1%	0%	0%	-2%	-21%	-20%
Aqua P4 Day 18 32C	0%	0%	0%	2%	1%	-13%	-12%

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 32C	-3%	-3%	-1%	-3%	-2%	-20%	-20%
Jade MIX Day 7 32C	-3%	-3%	-1%	-4%	-2%	-22%	-22%
Jade MIX Day 11 32C	-3%	-3%	-1%	-3%	-2%	-24%	-24%
Jade MIX Day 14 32C	-4%	-4%	-1%	-4%	-3%	-25%	-25%
Jade MIX Day 18 32C	-4%	-5%	-2%	-5%	-3%	-27%	-27%
Aqua P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 32C	-3%	-2%	-1%	-2%	-3%	-17%	-17%
Aqua MIX Day 7 32C	0%	-1%	0%	-1%	-3%	-21%	-21%
Aqua MIX Day 11 32C	0%	-1%	0%	-1%	-2%	-23%	-23%
Aqua MIX Day 14 32C	0%	-1%	0%	-1%	-2%	-23%	-23%
Aqua MIX Day 18 32C	0%	0%	1%	1%	1%	-14%	-14%

Table 17C: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (32°C), across Several Channels for Peak 7.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P7 Day 5 32C	0%	0%	0%	0%	0%	0%	0%
Jade P7 Day 7 32C	0%	0%	1%	1%	0%	1%	0%
Jade P7 Day 11 32C	1%	0%	1%	1%	0%	1%	1%
Jade P7 Day 14 32C	1%	1%	1%	2%	1%	1%	1%
Jade P7 Day 18 32C	-1%	-1%	0%	-1%	-1%	3%	2%
Aqua P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P7 Day 5 32C	-3%	-1%	0%	-1%	-1%	-2%	-2%
Aqua P7 Day 7 32C	-1%	-1%	-1%	-1%	-1%	-2%	-2%
Aqua P7 Day 11 32C	-1%	-1%	0%	0%	-1%	-1%	-1%
Aqua P7 Day 14 32C	-3%	-2%	-1%	-1%	-1%	-1%	-2%
Aqua P7 Day 18 32C	-4%	-2%	-2%	0%	-1%	-1%	-2%

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 32C	-1%	0%	0%	0%	0%	0%	0%
Jade MIX Day 7 32C	0%	0%	0%	0%	0%	1%	1%
Jade MIX Day 11 32C	0%	0%	0%	0%	0%	2%	2%
Jade MIX Day 14 32C	0%	0%	0%	0%	0%	2%	2%
Jade MIX Day 18 32C	0%	-1%	-1%	0%	-1%	2%	1%
Aqua P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 32C	-4%	-2%	-1%	-2%	-2%	-3%	-3%
Aqua MIX Day 7 32C	-1%	-1%	-1%	-1%	-1%	-2%	-3%
Aqua MIX Day 11 32C	-1%	-1%	0%	-1%	-1%	-1%	-1%
Aqua MIX Day 14 32C	-2%	-1%	-1%	-1%	-1%	0%	0%
Aqua MIX Day 18 32C	-1%	0%	0%	1%	1%	1%	1%

Table 18A: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (50°C), across Several Channels for Peak 2.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P2 Day 5 50C	-13%	-13%	-3%	-11%	-12%	-33%	-37%
Jade P2 Day 7 50C	-14%	-13%	-3%	-10%	-13%	-37%	-41%
Jade P2 Day 11 50C	-15%	-14%	-2%	-11%	-13%	-41%	-45%
Jade P2 Day 14 50C	-16%	-15%	-3%	-11%	-15%	-44%	-47%
Jade P2 Day 18 50C	-17%	-15%	-3%	-10%	-15%	-46%	-50%
Aqua P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P2 Day 5 50C	-6%	-6%	-1%	-3%	-6%	-37%	-40%
Aqua P2 Day 7 50C	-7%	-7%	-2%	-4%	-9%	-41%	-45%
Aqua P2 Day 11 50C	-8%	-8%	-2%	-4%	-10%	-44%	-49%
Aqua P2 Day 14 50C	-9%	-8%	-2%	-4%	-10%	-45%	-50%
Aqua P2 Day 18 50C	-10%	-9%	-2%	-6%	-11%	-47%	-52%
TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 50C	-13%	-12%	-2%	-10%	-11%	-34%	-37%
Jade MIX Day 7 50C	-16%	-14%	-3%	-10%	-12%	-35%	-40%
Jade MIX Day 11 50C	-16%	-14%	-2%	-10%	-13%	-40%	-43%
Jade MIX Day 14 50C	-15%	-14%	-2%	-9%	-13%	-41%	-44%
Jade MIX Day 18 50C	-16%	-15%	-3%	-11%	-13%	-42%	-46%
Aqua P2 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 50C	-9%	-8%	-2%	-5%	-9%	-35%	-38%
Aqua MIX Day 7 50C	-7%	-8%	-2%	-4%	-9%	-39%	-41%
Aqua MIX Day 11 50C	-8%	-8%	-3%	-5%	-11%	-40%	-44%
Aqua MIX Day 14 50C	-9%	-7%	-1%	-3%	-10%	-43%	-47%
Aqua MIX Day 18 50C	-9%	-7%	-1%	-4%	-11%	-45%	-48%

Table 18B: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (50°C), across Several Channels for Peak 4.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P4 Day 5 50C	-6%	-7%	-2%	-5%	-4%	-33%	-33%
Jade P4 Day 7 50C	-6%	-8%	-1%	-6%	-5%	-36%	-37%
Jade P4 Day 11 50C	-7%	-9%	-2%	-5%	-5%	-40%	-40%
Jade P4 Day 14 50C	-9%	-9%	-2%	-5%	-5%	-41%	-41%
Jade P4 Day 18 50C	-9%	-10%	-2%	-5%	-7%	-44%	-44%
Aqua P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P4 Day 5 50C	-2%	-2%	0%	-1%	-2%	-24%	-24%
Aqua P4 Day 7 50C	-2%	-3%	-1%	-1%	-4%	-28%	-28%
Aqua P4 Day 11 50C	-3%	-4%	-1%	-2%	-5%	-31%	-31%
Aqua P4 Day 14 50C	-4%	-4%	-1%	-1%	-5%	-32%	-32%
Aqua P4 Day 18 50C	-4%	-4%	0%	-1%	-6%	-35%	-34%
TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 50C	-7%	-7%	-2%	-6%	-5%	-34%	-34%
Jade MIX Day 7 50C	-9%	-10%	-3%	-7%	-7%	-36%	-37%
Jade MIX Day 11 50C	-9%	-9%	-2%	-6%	-7%	-39%	-40%
Jade MIX Day 14 50C	-8%	-10%	-2%	-6%	-6%	-41%	-41%
Jade MIX Day 18 50C	-9%	-11%	-3%	-6%	-7%	-43%	-43%
Aqua P4 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 50C	-3%	-3%	-1%	-2%	-4%	-29%	-29%
Aqua MIX Day 7 50C	-2%	-3%	0%	-2%	-5%	-31%	-31%
Aqua MIX Day 11 50C	-3%	-4%	-1%	-2%	-6%	-34%	-34%
Aqua MIX Day 14 50C	-3%	-3%	0%	-1%	-5%	-35%	-35%
Aqua MIX Day 18 50C	-4%	-3%	0%	-1%	-6%	-37%	-37%

Table 18C: MdFl % Difference for Fluorospheres having either Aqua Green or Jade Green as a Function of Time (Day 0, Day 5, Day 7, Day 11, Day 14, Day 18) and Temperature (50°C), across Several Channels for Peak 7.

TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade P7 Day 5 50C	-2%	-2%	0%	0%	-1%	1%	1%
Jade P7 Day 7 50C	-2%	-2%	1%	1%	0%	3%	2%
Jade P7 Day 11 50C	0%	-2%	2%	2%	0%	5%	4%
Jade P7 Day 14 50C	0%	-2%	2%	3%	1%	6%	4%
Jade P7 Day 18 50C	-2%	-3%	2%	2%	0%	9%	8%
Aqua P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua P7 Day 5 50C	-3%	-3%	0%	0%	-1%	-2%	-3%
Aqua P7 Day 7 50C	-3%	-4%	-1%	-1%	-3%	-1%	-2%
Aqua P7 Day 11 50C	-4%	-4%	0%	0%	-4%	1%	0%
Aqua P7 Day 14 50C	-5%	-4%	0%	0%	-4%	1%	0%
Aqua P7 Day 18 50C	-5%	-5%	0%	0%	-5%	4%	3%
TubeName	U3-A	V3-A	B3-A	Y3-A	R3-A	IR1-A	IR2-A
Jade P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Jade MIX Day 5 50C	-2%	-2%	0%	0%	-1%	2%	1%
Jade MIX Day 7 50C	-3%	-4%	-1%	0%	-2%	3%	2%
Jade MIX Day 11 50C	-3%	-3%	1%	1%	-1%	6%	5%
Jade MIX Day 14 50C	-2%	-3%	1%	2%	0%	7%	6%
Jade MIX Day 18 50C	-2%	-4%	1%	2%	0%	10%	9%
Aqua P7 Ctr	0%	0%	0%	0%	0%	0%	0%
Aqua MIX Day 5 50C	-4%	-3%	-1%	-1%	-3%	-2%	-3%
Aqua MIX Day 7 50C	-2%	-3%	0%	0%	-3%	-1%	-1%
Aqua MIX Day 11 50C	-3%	-4%	-1%	-1%	-4%	2%	1%
Aqua MIX Day 14 50C	-3%	-4%	0%	1%	-3%	2%	1%
Aqua MIX Day 18 50C	-4%	-4%	1%	1%	-4%	4%	3%

NUMBERED CLAUSES

1. A suspension for quality control of a flow cytometer comprising:
fluorospheres, wherein the suspension of fluorospheres include individual fluorospheres having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm;
at least one surfactant; and
at least one stabilizer or preservative.

2. The suspension of clause 1, wherein the individual fluorospheres are encapsulated with at least one dye having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one dye having at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm.

3. The suspension of clause 2, wherein the at least one dye having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser is selected from the group consisting of: Aqua Green, Jade Green, Cy Green, Indo cyanine green (ICG), Cy7, or Cy7.5, IR dye 800CW, or any combination thereof.

4. The suspension of clause 3, wherein the at least one dye having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser is Aqua Green.

5. The suspension of any one of clauses 2-4, wherein the at least one dye having an infrared fluorescence emission greater than 800 nm is excited with an infrared laser at a wavelength of 808 nm.

6. The suspension of any one of clauses 2-5, wherein the at least one dye having at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm is selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof.

7. The suspension of any one of clauses 2-6, wherein the individual fluorospheres are encapsulated with at least seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited at a wavelength of less than 800 nm selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof.

8. The suspension of any one of clauses 2-7, wherein individual fluorospheres are encapsulated with seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited by five lasers having the following wavelengths: 355 nm, 405 nm, 488 nm, 561 nm, and 638 nm.

9. The suspension of any one of clauses 2-8, wherein the individual fluorospheres are encapsulated with eight dyes having eight fluorescence emissions when excited by six lasers having the following wavelengths: 355 nm (UV), 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared).

10. The suspension of any one of clauses 1-9, wherein the fluorospheres are polystyrene beads.

11. The suspension of any one of clauses 1-10, wherein the fluorospheres have a diameter of between 2.5 μm and 6.5 μm .

12. The suspension of any one of clauses 1-11, wherein the fluorospheres have a diameter between 2.8 μm and 3.4 μm .

13. The suspension of any one of clauses 1-12, wherein the fluorospheres have a diameter of about 3.0 μm .

14. The suspension of any one of clauses 1-13, wherein the fluorospheres in the suspension have a concentration between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL.

15. The suspension of any one of clauses 1-14, wherein the fluorospheres in the suspension have a concentration between 0.9×10^6 fluorospheres/mL and 1.1×10^6 fluorospheres/mL.

16. The suspension of any one of clauses 1-15, wherein the fluorospheres in the suspension have a concentration of about 1.0×10^6 fluorospheres/mL.

17. The suspension of any one of clause 1-16, wherein the at least one surfactant is selected from the group consisting of: an ionic surfactant, a non-ionic surfactant, Sodium Dodecyl Sulfate (SDS), NP-40s, Ecosurf EH-9, Ecosurf SA-9, Ecosurf Tween 20, Triton X-11, or any combination thereof.

18. The suspension of any one of clauses 1-17, wherein the at least one surfactant is at a concentration between 0.01% and 1% based on the total volume of the suspension.

19. The suspension of any one of clauses 1-18, wherein the at least one surfactant is EcoSurf EH-9 is at a concentration is 0.05% based on the total volume of the suspension.

20. The suspension of any one of clauses 1-19, wherein the at least one stabilizer is selected from the group consisting of: a stabilized reducing agent, a stabilized thiol containing compound, (S)-2-Aminobutane-1,4-dithiol hydrochloride, dithiothreitol (DTT), Tris(2-carboxyethyl)phosphine (TCEP) or any combination thereof.

21. The suspension of any one of clauses 1-20, wherein the at least one preservative is selected from the group consisting of: sodium azide, thimerosal, or any combination thereof.

22. A method for quality controlling a flow cytometer using a single peak comprising:

(a) loading a quality control suspension of any one of clauses 1-21 into a flow cytometer;

(b) evaluating at least one of the following:

(i) evaluating power of at least one laser in the flow cytometer based on a single peak analysis;

(ii) evaluating EPS of at least one laser in the flow cytometer based on a single peak analysis;

- (iii) evaluating laser delay of at least one laser in the flow cytometer based on a single peak analysis;
- (iv) evaluating gain of at least one laser in the flow cytometer based on a single peak analysis;
- (v) evaluating rCV of at least one laser in the flow cytometer based on a single peak analysis; and
- (c) determining whether the flow cytometer passes or fails quality control based on the evaluations in step (b).

23. The method of clause 22, wherein the evaluating in step (b) includes evaluating each of steps (b)(i) through (b)(v).

24. The method of any one of clauses 22-23, wherein the evaluation in step (b) is performed on an infrared laser and at least one laser with a wavelength of less than 800 nm.

25. The method of any one of clauses 22-24, wherein the evaluation in step (b) is performed on seven lasers having the following wavelengths: 355 nm (UV), 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared).

26. The method of clause 25, wherein the 355 (UV) laser, 405 nm (Violet) laser, 488 nm (Blue) laser, 561 nm (Yellow-Green) laser, and 638 nm (Red) laser are evaluated on detector channel 3 or detector channel 4 of the flow cytometer, and wherein the 808 nm (Infrared) laser is evaluated on detector channel 1 or detector channel 2 of the flow cytometer.

27. The method of any one of clauses 22-26, further comprising: generating a quality control report following step (c).

28. The method of any one of clauses 22-27, wherein the method for quality controlling a flow cytometer is performed at least once per day.

29. The method of any one of clauses 22-27, wherein the method for quality controlling a flow cytometer is performed before using the flow cytometer.

30. A method for quality controlling a flow cytometer using multiple peaks comprising:

- (a) loading a quality control suspension of any one of clauses 1-21 into the flow cytometer to perform a quality control analysis based on more than one peak,
- (b) adjusting the brightest peak to a target median fluorescence intensity;
- (c) reading the median fluorescence intensity and rCV for each of the peaks;
- (d) unloading the quality control suspension from the flow cytometer following step (c);
- (e) loading polystyrene beads without fluorescence into the flow cytometer;
- (f) reading the median fluorescence intensity for the polystyrene beads without fluorescence; and
- (g) calculating a sensitivity and background.

31. The method of clause 30, wherein there are three peaks, a bright peak, a mild peak, and a dim peak.

32. The method of any one of clauses 30-31, wherein the target median fluorescence intensity for step (f) is between 500,000 and 4×10^6 .

33. The method of any one of clauses 30-32, wherein the sensitivity and background comprise MESF sensitivity, Quantum efficiency, or Background.

34. The method of any one of clauses 30-33, wherein step (a) is concurrent with step (e).

35. The method of any one of clauses 30-34, further comprising: generating a quality control report following step (k).

36. A kit for performing the method according to any one of clauses 22-35, the kit comprising:

- a suspension of any one of clauses 1-21;
- at least one vial to hold the suspension; and
- instructions for using the kit.

37. The kit of clause 36, further comprising a second vial to hold the suspension.

38. The kit of clause 37, wherein the two vials are each 10mls.

39. The kit of clause 36, further comprising a second vial containing a suspension of polystyrene beads with no dye.

40. The kit of clause 39, wherein the diameter of the polystyrene beads in the second vial is about 1 μm .

41. The kit of any one of clauses 39-40, wherein the concentration of the polystyrene beads in the second vial is between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL.

42. A suspension for quality control of a flow cytometer comprising:
fluorospheres encapsulated with at least one dye having a fluorescence emission, wherein the suspension of fluorospheres include individual fluorospheres having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm;
at least one surfactant; and
at least one stabilizer or preservative.

43. The suspension of clause 42, wherein the suspension of fluorospheres having a fluorescence emission greater than 800 nm when excited with an infrared laser are encapsulated with at least one dye selected from the group consisting of: Aqua Green, Jade Green, Cy Green, Indo cyanine green (ICG), Cy7, or Cy7.5, IR dye 800CW, or any combination thereof.

44. The suspension of clause 42 or 43, wherein the suspension of fluorospheres having a fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm are encapsulated with at least one dye selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof.

45. The suspension of any one of clauses 42-44, wherein the individual fluorospheres are encapsulated with at least seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited at a wavelength of less than 800 nm selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof.

46. The suspension of any one of clauses 42-45, wherein the individual fluorospheres are encapsulated with eight dyes having eight fluorescence emissions when excited by six lasers having the following wavelengths: 355 nm (UV), 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared).

47. The suspension of any one of clauses 42-46, wherein the fluorospheres have a diameter of between 2.5 μm and 6.5 μm .

48. The suspension of any one of clauses 42-47, wherein the fluorospheres in the suspension have a concentration between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL.

49. The suspension of any one of clauses 42-48, wherein the at least one surfactant is selected from the group consisting of: an ionic surfactant, a non-ionic surfactant, Sodium Dodecyl Sulfate (SDS), NP-40s, Ecosurf EH-9, Ecosurf SA-9, Ecosurf Tween 20, Triton X-11, or any combination thereof and the at least one stabilizer is selected from the group consisting of: a stabilized reducing agent, a stabilized thiol containing compound, (S)-2-Aminobutane-1,4-dithiol hydrochloride, dithiothreitol (DTT), Tris(2-carboxyethyl)phosphine (TCEP) or any combination thereof and the at least one preservative is selected from the group consisting of: sodium azide, thimerosal, or any combination thereof.

50. The suspension of any one of clauses 42-49, wherein the at least one surfactant is at a concentration between 0.01% and 1% based on the total volume of the suspension.

51. A method for quality controlling a flow cytometer using a single peak comprising:

- (a) loading a quality control suspension of any one of clauses 42-50 into a flow cytometer;
- (b) evaluating at least one of the following:
 - (i) evaluating power of at least one laser in the flow cytometer based on a single peak analysis;
 - (ii) evaluating EPS of at least one laser in the flow cytometer based on a single peak analysis;
 - (iii) evaluating laser delay of at least one laser in the flow cytometer based on a single peak analysis;
 - (iv) evaluating gain of at least one laser in the flow cytometer based on a single peak analysis;
 - (v) evaluating rCV of at least one laser in the flow cytometer based on a single peak analysis; and
- (c) determining whether the flow cytometer passes or fails quality control based on the evaluations in step (b).

52. The method of clause 51, wherein the evaluating in step (b) includes evaluating each of steps (b)(i) through (b)(v).

53. A method for quality controlling a flow cytometer using multiple peaks comprising:
- (a) loading a quality control suspension of any one of clauses 42-49 into the flow cytometer to perform a quality control analysis based on more than one peak,
 - (b) adjusting the brightest peak to a target median fluorescence intensity;
 - (c) reading the median fluorescence intensity and rCV for each of the peaks;
 - (d) unloading the quality control suspension from the flow cytometer following step (c);
 - (e) loading polystyrene beads without fluorescence into the flow cytometer;
 - (f) reading the median fluorescence intensity for the polystyrene beads without fluorescence; and
 - (g) calculating a sensitivity and background.

54. The method of clause 53, wherein there are three peaks, a bright peak, a mild peak, and a dim peak.

55. The method of any one of clauses 53-54, wherein the target median fluorescence intensity for step (f) is between 500,000 and 4×10^6 .

56. The method of any one of clauses 53-55, wherein the sensitivity and background comprise MESF sensitivity, Quantum efficiency, or Background.

CLAIMS

What is claimed is:

1. A suspension for quality control of a flow cytometer comprising:
fluorospheres encapsulated with at least one dye having a fluorescence emission, wherein the suspension of fluorospheres include individual fluorospheres having an infrared fluorescence emission greater than 800 nm when excited with an infrared laser and at least one fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm;
at least one surfactant; and
at least one stabilizer or preservative.
2. The suspension of claim 1, wherein the suspension of fluorospheres having a fluorescence emission greater than 800 nm when excited with an infrared laser are encapsulated with at least one dye selected from the group consisting of: Aqua Green, Jade Green, Cy Green, Indo cyanine green (ICG), Cy7, or Cy7.5, IR dye 800CW, or any combination thereof.
3. The suspension of claim 1 or 2, wherein the suspension of fluorospheres having a fluorescence emission between 355 nm and 800 nm when excited at a wavelength of less than 800 nm are encapsulated with at least one dye selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof.
4. The suspension of any one of claims 1-3, wherein the individual fluorospheres are encapsulated with at least seven dyes having seven fluorescence emissions between 355 nm and 800 nm when excited at a wavelength of less than 800 nm selected from the group consisting of: a small organic dye, a phycobiliprotein, quantum dots, a polymer dye, a fluorescent protein, a tandem dye, UV, Light Yellow, Yellow, Nile Red, Purple, Blue, Sky Blue, or any combination thereof.

5. The suspension of any one of claims 1-4, wherein the individual fluorospheres are encapsulated with eight dyes having eight fluorescence emissions when excited by six lasers having the following wavelengths: 355 nm (UV), 405 nm (Violet), 488 nm (Blue), 561 nm (Yellow-Green), 638 nm (Red), and 808 nm (Infrared).
6. The suspension of any one of claims 1-5, wherein the fluorospheres have a diameter of between 2.5 μm and 6.5 μm .
7. The suspension of any one of claims 1-6, wherein the fluorospheres in the suspension have a concentration between 0.4×10^6 fluorospheres/mL and 1.5×10^6 fluorospheres/mL.
8. The suspension of any one of claims 1-7, wherein the at least one surfactant is selected from the group consisting of: an ionic surfactant, a non-ionic surfactant, Sodium Dodecyl Sulfate (SDS), NP-40s, Ecosurf EH-9, Ecosurf SA-9, Ecosurf Tween 20, Triton X-11, or any combination thereof and the at least one stabilizer is selected from the group consisting of: a stabilized reducing agent, a stabilized thiol containing compound, (S)-2-Aminobutane-1,4-dithiol hydrochloride, dithiothreitol (DTT), Tris(2-carboxyethyl)phosphine (TCEP) or any combination thereof and the at least one preservative is selected from the group consisting of: sodium azide, thimerosal, or any combination thereof.
9. The suspension of any one of claims 1-8, wherein the at least one surfactant is at a concentration between 0.01% and 1% based on the total volume of the suspension.
10. A method for quality controlling a flow cytometer using a single peak comprising:
- (a) loading a quality control suspension of any one of claims 1-9 into a flow cytometer;
 - (b) evaluating at least one of the following:
 - (i) evaluating power of at least one laser in the flow cytometer based on a single peak analysis;
 - (ii) evaluating EPS of at least one laser in the flow cytometer based on a single peak analysis;
 - (iii) evaluating laser delay of at least one laser in the flow cytometer based on a single peak analysis;

- (iv) evaluating gain of at least one laser in the flow cytometer based on a single peak analysis;
- (v) evaluating rCV of at least one laser in the flow cytometer based on a single peak analysis; and
- (c) determining whether the flow cytometer passes or fails quality control based on the evaluations in step (b).

11. The method of claim 10, wherein the evaluating in step (b) includes evaluating each of steps (b)(i) through (b)(v).

12. A method for quality controlling a flow cytometer using multiple peaks comprising:
- (a) loading a quality control suspension of any one of claims 1-9 into the flow cytometer to perform a quality control analysis based on more than one peak,
 - (b) adjusting the brightest peak to a target median fluorescence intensity;
 - (c) reading the median fluorescence intensity and rCV for each of the peaks;
 - (d) unloading the quality control suspension from the flow cytometer following step (c);
 - (e) loading polystyrene beads without fluorescence into the flow cytometer;
 - (f) reading the median fluorescence intensity for the polystyrene beads without fluorescence; and
 - (g) calculating a sensitivity and background.

13. The method of claim 12, wherein there are three peaks, a bright peak, a mild peak, and a dim peak.

14. The method of any one of claims 12-13, wherein the target median fluorescence intensity for step (f) is between 500,000 and 4×10^6 .

15. The method of any one of claims 12-14, wherein the sensitivity and background comprise MESF sensitivity, Quantum efficiency, or Background.