ANALYSIS & SORTING OF TXRED LABELED BEADS IN A COMBINATORIAL PEPTIDE LIBRARY

OBJECTIVE

The purpose of this experiment was to test the feasibility of using the COPAS™ SELECT instrument for high throughput screening of bead based combinatorial peptide libraries on the basis of fluorescence. In addition, three different types of lasers with different excitation wavelengths were used to determine the ideal wavelength for use with the Texas Red® fluorophore. In order to verify the experiment results, beads were sorted for microscopic inspection.

INTRODUCTION

The COPAS SELECT instrument is able to analyze and sort large objects (120-300 microns) at a high rate (up to 50 objects per second) on the basis of the physical characteristics of size, density and fluorescence signals. In this experiment using the COPAS SELECT instrument, we analyzed and sorted mixed populations of beads with two different fluorescence levels. Non-fluorescent beads were used as a negative control. Once analyzed, the target bead population was dispensed and the results were visually inspected for accuracy.

MATERIALS AND METHODS

The following samples were analyzed and sorted:

Sample A

Contains about 800 mg of Tentagel S NH_2 beads (~ 130 micrometers in diameter). 5% of these beads are labeled with the fluorescent dye Texas Red to only 2.5% of their capacity.

Sample B

Contains about 800 mg of Tentagel S NH_2 beads (same dimensions as above sample). 5% of the beads are labeled with the fluorescent dye Texas Red to an extent of only 0.25% of their capacity.

Additional information

- For Texas Red, the excitation peak is at 595 nm and the emission peak is at 620 nm.
- The following lasers were used to excite the fluorescent dye for detection in the COPAS instrument: argon-krypton laser for 568 nm excitation; argon laser for 514 nm excitation; and a red diode laser for 635 nm excitation. The results from each of the three lasers are shown below.
- The 635 diode laser is also used to generate light-scattering signals for both a length measurement, which is called Time Of Flight (TOF) and an optical density measurement, which is called Extinction (EXT).
- The emission filter used for fluorescence measurements in all three experiments is a narrow bandpass filter at 610 nm with a bandwidth of 20 nm.
- Data was acquired using the COPAS SELECT and analyzed using the WinMDI software.

WORK PERFORMED JUNE 2003 BY:

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RESULTS

Experiment 1: Fluorescence excitation at 568 nm

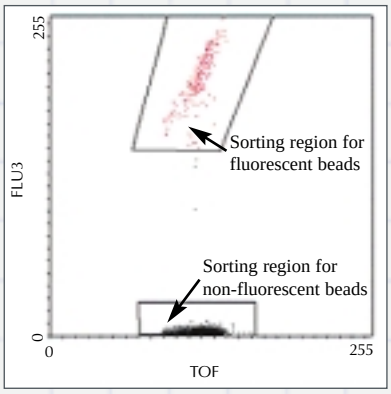

Figure 1. FLU3/TOF for Sample A using an argon-krypton laser (excite at 568 nm). FLU3: Red fluorescence.

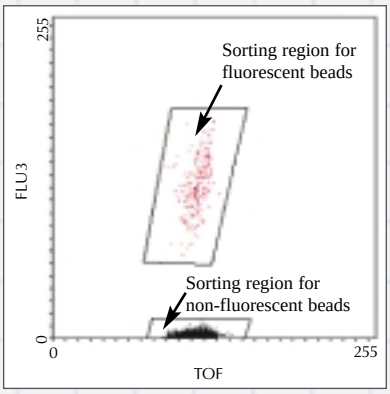

Figure 2. FLU3/TOF for Sample B using an argon-krypton laser (excite at 568 nm). FLU3: Red fluorescence.

A portion of Sample A was run on the COPAS SELECT to verify that the instrument can distinguish between fluorescent and non-fluorescent bead populations. Beads were analyzed based on the fluorescence parameter FLU3, where FLU3 represents the red emission (from 600 to 620 nm) and the size parameter, TOF. As shown in Figure 1, two distinct populations with different fluorescence properties (shown along the FLU3 axis) were detected. Each dot on the dot plot is one single event. A sorting region was drawn to collect either fluorescent or non-fluorescent beads. 200 beads from the non-fluorescent group and 50 beads from the fluorescent group were collected and visual inspection under a fluorescent microscope confirmed that all sorted beads had the expected fluorescence properties, giving a 100% sorting accuracy. The same experiment was performed with a portion of beads from Sample B with the same results (Figure 2). The average fluorescence intensity for the fluorescent and non-fluorescent beads of both samples is shown in Table 1.

	Fluorescent beads	Non-fluorescent beads	Ratio
Sample A	206.4 ± 20.0 (n = 208)	2.4 ± 1.5 (n = 4102)	86.0
Sample B	122.7 ± 17.4 (n = 241)	2.3 ± 1.4 (n = 5328)	53.3

Table 1. *Average intensity (mean ± standard deviation) of red fluorescence generated by excitation at 568 nm with an argon-krypton laser. Ratio: average fluorescence intensity of fluorescent beads / average fluorescence intensity of non-fluorescent beads.*

Experiment 2: Fluorescence excitation at 514 nm

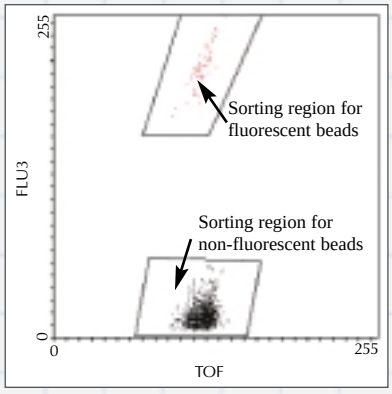

Figure 3. FLU3/TOF for Sample A using argon laser (excitation at 514 nm).

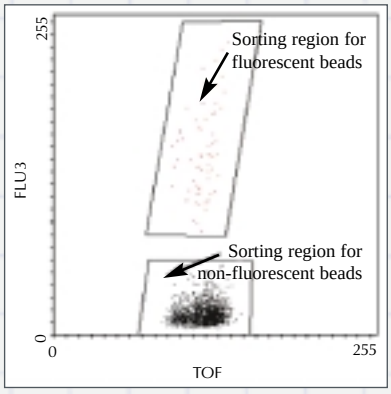

Figure 4. FLU3/TOF for Sample B using argon laser (excitation at 514 nm).

As in Experiment 1, a portion of Sample A and a portion of Sample B were run (independently) on the COPAS instrument. Similar to the results in Experiment 1, the dot plot Figures from Experiment 2 show that two distinct populations of beads with different red fluorescence intensities were detected in both samples. However, notice in Table 2, the ratio between the intensity of fluorescence from the fluorescent beads and the non-fluorescent beads is comparatively lower than the ratio shown in Table 1, suggesting that the 568 nm laser is a better excitation source for detection of the Texas Red fluorescence.

200 beads from the non-fluorescent group and 50 beads from the fluorescent group were collected using the sorter for both Sample A and B. Visual inspections under a fluorescent microscope confirmed that all sorted beads had the expected fluorescence properties, giving a 100% sorting accuracy.

	Fluorescent beads	Non-fluorescent beads	Ratio
Sample A	215.6 ± 15.1 (n = 61)	17.5 ± 8.6 (n = 1149)	12.3
Sample B	147.1 ± 35.3 (n = 64)	15.8 ± 6.6 (n = 1637)	9.3

Table 2. *Average intensity (mean ± standard deviation) of red fluorescence generated by excitation at 514 nm with an argon laser. Ratio: average fluorescence intensity of fluorescent beads / average fluorescence intensity of non-fluorescent beads.*

Experiment 3: Fluorescence excitation at 635nm

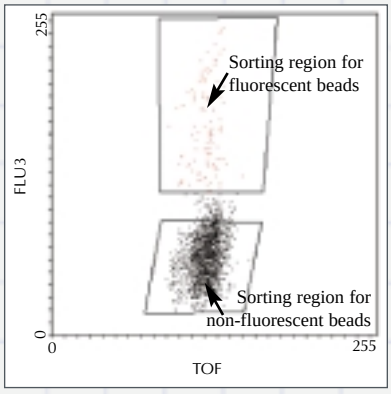

Figure 5. FLU3/TOF for Sample A using red diode laser (excitation at 635 nm).

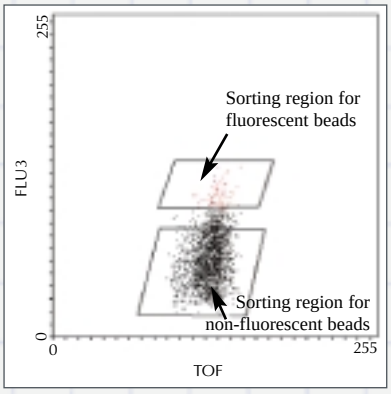

Figure 6. FLU3/TOF for Sample B using red diode laser (excitation at 635 nm).

Samples were prepared as in previous experiments. Similar to Experiments 1 and 2, beads were analyzed based on FLU3 and TOF. Notice that in Figures 5 and 6 however, the two populations for the fluorescent and non-fluorescent beads do not have clear demarcations as those shown in Figures 1-4, suggesting that the 635 nm diode laser is a poor source for exciting the Texas Red fluorophore. This is also reflected in the much reduced ratio as shown in Table 3.

Nevertheless, the beads can still be separated based on their fluorescence properties using the 635 nm laser. For Sample A, 200 beads from the non-fluorescent group and 50 beads from the fluorescent group were collected. Visual inspection confirmed that all sorted beads had the expected fluorescence properties, giving a 100% sorting accuracy. For Sample B, 200 beads from the non-fluorescent group were collected and again visual inspection confirmed that all sorted beads had the expected fluorescence properties. 20 beads were collected from the fluorescent group. However, only 17 beads were found to be fluorescent, giving a sorting accuracy of 85%.

	Fluorescent beads	Non-fluorescent beads	Ratio
Sample A	165.4 ± 36.4 (n = 106)	60.4 ± 16.3 (n = 1587)	2.74
Sample B	112.5 ± 12.3 (n = 61)	56.7 ± 17.0 (n = 1566)	1.98

Table 3. *Average intensity (mean ± standard deviation) of red fluorescence generated by excitation at 635 nm with a red diode laser. Ratio: average fluorescence intensity of fluorescent beads / average fluorescence intensity of non-fluorescent beads. Based on the visual inspection result, the sorting and analysis region for fluorescent beads of sample B may contain a small fraction of non-fluorescent beads.*

Conclusion

These three experiments demonstrate that the COPAS SELECT may be used to separate fluorescent and non-fluorescent beads from a mixed population. Either the 568 nm argon-krypton or the 514 nm argon laser can be used as the excitation source resulting in 100% purity of the selected bead type. However, as shown in the above results and also as was expected knowing the fluorescence property of Texas Red, the 568 nm laser is superior compared to the 514 nm laser. Given that the emission peak for Texas Red is at 620 nm, the signal/noise ratio may be further enhanced by substituting the 635 nm diode laser with a 670 nm diode laser.

About Union Biometrica, Inc.

Union Biometrica, Inc. is the pioneer in the development and manufacture of high throughput systems for the screening of "large" Particles such as large cells and cell clusters, beads used in combinatorial chemistry libraries, small seeds, and for viable small model organisms including *C. elegans*, *Drosophila*, and zebrafish. An alternative to manual sorting (under a microscope), COPAS™ systems sort and dispense objects (sized 40 – 1,500 microns) based on size and fluorescence parameters. Automating this process offers increased speed, sensitivity, quantification, and repeatability of experiments, thereby facilitating some experiments that previously were not even feasible.

More information about Union Biometrica, Inc. may be found at http://www.unionbio.com or contact us at +(617) 591-1211 or sales@unionbio.com.

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