

# Multicolor Detection of CD45 on Human Monocytes with Quantum Dots in a High-Throughput Format



QUANTUM DOT

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## Abstract

Multicolor quantum dot probes emitting from 525 to 705 nm can be effectively excited with a single 488 nm laser, resulting in signals equal to the best organic dyes, but that are clearly discriminated with simple color filters. Using a plate based 4-color laser scanning system with a single 488 or 405 nm laser for excitation, we have detected the binding of quantum dot conjugates to CD45 on human monocytes. The colors were easily discriminated with little or no spill-over in the detector for adjacent colors, allowing very clean discrimination of up to four individually labelled cells in a mixed population in a single well, as well as clear detection and quantitation of relative signals on cells labelled with multiple quantum dots. The lack of color overlap in these materials enables the detection of multiple cellular parameters in a high-throughput cell-analysis system, increasing the information density of a screen.

## Introduction

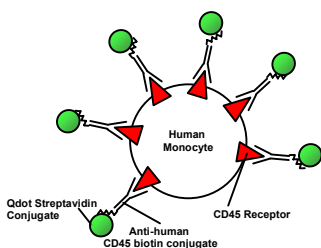
Acumen Explorer™ combines the object recognition capability of image-based systems with the high read speeds of traditional bulk readers. At the heart of the system is a proprietary, non-confocal optic system which permits focus-free, area-based scanning, collecting up to 4 channels of data in a single scan. It also allows scanning of 96, 384 and 1536 well plates in the same time. To prevent problems of variable cell number, patchy stimulation and edge effects, Acumen Explorer allows the researcher to define the scan area within each well. Options include scanning the whole well area, center well or user-defined strips.

Qdot® Streptavidin Conjugates are a class of fluorescent labelling reagents based on state-of-the-art quantum dot technology possessing the unique and highly desirable characteristics of Qdot semiconductor nanocrystal particles. Qdot Conjugates provide a superior platform for a plethora of applications in biological research and diagnostic assays due to their unique optical properties. The narrow emission spectra of these Qdot Conjugates allow for simple multiplexing in immunohistochemical experiments. The level of discrimination allows simple acquisition of multicolor images using the Qdot Streptavidin Conjugates range. The polymer shell is directly coupled to streptavidin to create the new Qdot Streptavidin Conjugate family of fluorescent bio-labels. Quantum dots have been used as conjugates of Streptavidin and IgG to label a number of biological targets<sup>1-4</sup>.

By using antibodies directed against CD45 receptors expressed on human monocytes, we have devised a model system to demonstrate the multiplexing capability of using multicolor quantum dot probes emitting from 525 to 705 nm on the laser scanning cytometer, the Acumen Explorer.

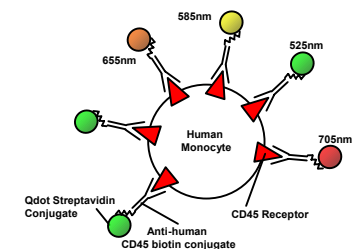
This multiplexing technique could potentially be applied to many assay applications, including cellular screening for several disease states, in which changes in cell receptor densities can act as therapeutic markers and for the monitoring of changes in cellular receptor numbers in *ex vivo* stimulation experiments.

## 1 Monocytes labelled individually with Qdot Conjugate



Schematic representation of a human monocyte labelled with Qdot Streptavidin Conjugate.

## 4 Monocytes labelled with ratios of 4 Qdot Conjugates

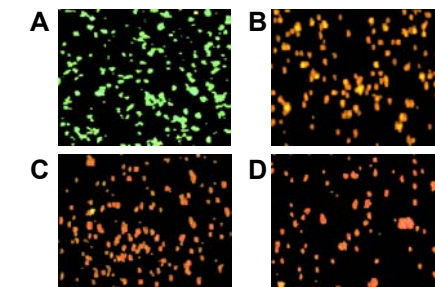


Schematic representation of a human monocyte labelled with a 3 times ratio 525 nm Qdot Streptavidin Conjugate on a single cell.

## 6 Assay Protocol

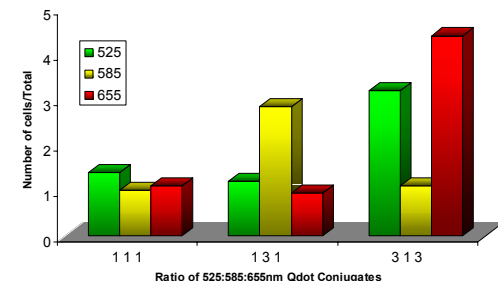
- Human monocytes were isolated from "buffy coat" using standard isolation procedures and separation techniques (Histopaque gradients) as described in the literature.
- Monocytes were washed 3X with sterile PBS pH 7.4 buffer (37°C) and resuspended at 10<sup>6</sup>/mL. Cells were fixed by the addition of 4% paraformaldehyde (equal volumes).
- Cells were washed 3X with PBS and resuspended (10<sup>6</sup>/mL) in serum free RPMI medium.
- Cells were incubated overnight with mouse anti-human CD45 biotin conjugate at room temperature at 1/50 dilution. Following incubation, the monocytes were washed 5X with PBS. Qdot Streptavidin Conjugates were added at a final concentration of 20 nM and incubated overnight.
- Cells were washed 3X with PBS and scanned on the Acumen Explorer

## 2 Well view of monocytes individually labelled with single Qdot Conjugates



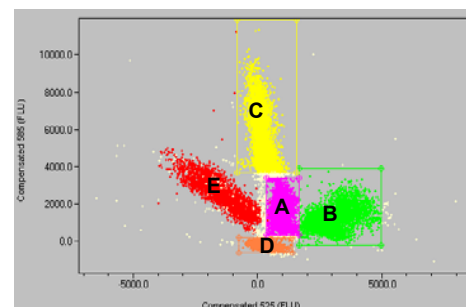
Monocytes labelled with: A. 525 nm, B. 585 nm, C. 655 nm and D. 705 nm, Qdot Conjugates.

## 3 Mixed population of individually labelled monocytes



Identification of different ratios of individually labelled monocytes with 3 different Qdot Conjugates in a single well.

## 5 Monocytes labelled with different ratios of 4 different Qdot Conjugates on a single cell



Scatter chart showing easy discrimination of monocytes labelled with 5 different ratios of Qdot Conjugates. The ratios are described in the corresponding table table.

Population	Qdot Conjugate Ratios			
	525nm	585nm	655nm	705nm
A	1	1	1	1
B	3	1	1	1
C	1	3	1	1
D	1	1	3	1
E	1	1	1	3

## Conclusion

In this study, we have demonstrated that by using human monocytes as a model system, we can easily detect multiple quantum dot conjugates in a mixture of individually labelled cells. We further showed that we could identify distinct populations of single human monocytes labelled with different ratios of Qdot Conjugates. This was achieved by using the unique optical properties of quantum dots, in conjunction with the Acumen Explorer. The lack of color overlap in these materials enables the detection of multiple cellular parameters in a high-throughput cell-analysis system, increasing the possible information density of a screen.

## References

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