Technical Note
Fluorescent Protein Analysis with the Accuri C6 Flow Cytometer® System

Introduction

Many fluorescent protein (FP) variants can be analyzed using the Accuri C6 Flow Cytometer System. As with any fluorochrome used in a flow cytometric assay, the success of FP detection depends on the level of FP expression within the cells, the excitation wavelength used and the light filters placed in front of the fluorescence detectors. This Technical Note provides guidelines for the C6 user performing FP analysis.

Background

The successful cloning and expression of the green fluorescent protein (GFP), derived from the jellyfish *Aequoria victoria* (Chalfie et al., 1994 and Tsien, 1998), ushered in a new era for fluorescence microscopic studies of living cells and their functional processes. Since the initial use of GFP in gene expression studies, mutagenesis has been used to produce a number of variants (e.g., eGFP, eCFP, eYFP) with varying emission maxima across the blue to yellow wavelengths. In 1999, the gene for a red fluorescent protein (RFP) from the sea anemone *Discosoma striata* was cloned (Matz et al. 1999). Subsequent mutagenesis of this FP has also yielded a wide range of derivatives which have extended the useful spectral emission range into the red region (Baird et al., 2000). Many of these GFP and RFP derivatives have proven useful not only for fluorescence microscopy but flow cytometry as well. The combined use of flow cytometry and FP technologies has been successfully employed in almost every field of biology, including physiology, cell biology, molecular biology, immunology, stem cell research, and microbiology.

References

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W. and Prasher, D. C. (1994). Green fluorescent protein as a marker for gene expression. *Science* 263, 802-805.

Tsien, R. Y. (1998). The green fluorescent protein. *Annu. Rev. Biochem.* 67, 509-544.

Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zaraisky, A. G., Markelov, M. L. and Lukyanov, S. A. (1999). Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat. Biotechnol.* 17, 969-973.

Baird, G. S., Zacharias, D. A. and Tsien, R. Y. (2000). Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc. Nat. Acad. Sci. USA* 97, 11984-11989.

Green FPs. GFP and eGFP (enhanced GFP) will be detected in FL1 when using the standard C6 filter configuration (FL1 = 530/30 BP). However, use of the optional 510/15 BP filter (CP-170) at position FL1 can improve GFP signal detection. For detecting extremely bright GFP signals, the 530/30 BP filter is also available at two attenuation levels: 90% (CP-144) and 99% (CP-142).

Yellow FPs. YFP, eYFP, mCitrine and other YFP derivatives will be detected in FL1 when using the standard C6 filter configuration (FL1 = 530/30 BP).

Simultaneous Green and Yellow FP Detection. GFP and YFP signals can only be separated by using the 510/15 BP filter (CP-170) at position FL1 and the 540/20 BP filter (CP-178) at position FL2, respectively (**Figure 1**). The emission spectra for GFP, YFP and derivatives thereof, overlap considerably. Both of these FPs will be maximally detected in FL1 when using the standard C6 filter configuration (FL1 = 530/30 BP).

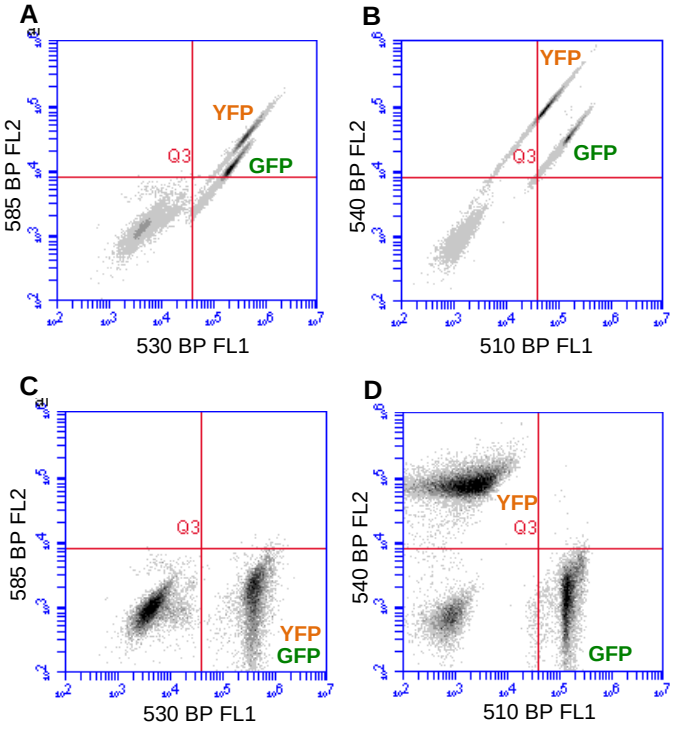

Figure 1. GFP and **YFP** signals are both detected in FL1 using the standard C6 filter configuration (A, C), but can be separated using the 510/15 filter (CP-170) in FL1 and the 540/20 filter (CP-178) in FL2 (B, D). Plots A and B are uncompensated data; plots C and D are compensated.

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mOrange, dTomato, dsRed. These FPs will have the strongest signals in FL2 with the standard C6 filter configuration (FL2 = 585/40 BP), but will also have significant signal in FL3 (670 LP) (Figure 2). For this reason, these FPs are not ideal for simultaneous use with RFP or mCherry.

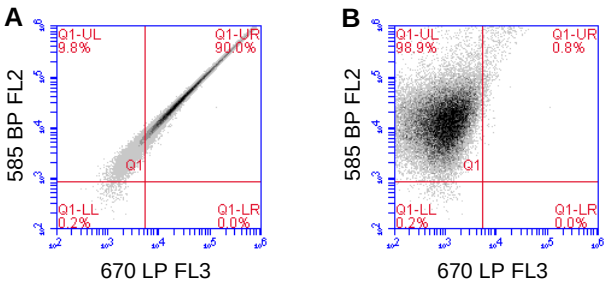

Figure 2. THP-1 cells labeled with dsRed-conjugated antibody show strongest signal in FL2 (585/40 BP) of the C6, but also show significant signal in FL3 (670 LP). Uncompensated data (A), Compensated data (B, FL3 – FL2 = 83%)

mCherry, RFP and other Red FPs. Use of the optional 610/20 BP filter (CP-174) in FL3 improves resolution of these FPs from background (Figure 3 A, B). These FPs emit strong signals detected in FL3 with the standard C6 filter configuration (FL3 = 670 LP), but will also have significant signal in FL2 (585/40 BP, Figure 4).

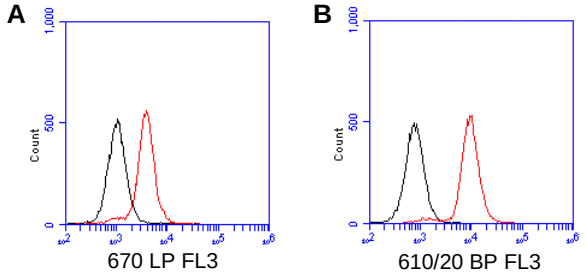

Figure 3. Detection of **mCherry** expression in stably-transfected CHO cells is improved by use of the optional 610/20 filter, CP-174 (B) as compared to the standard Accuri C6 670 LP filter (A). Black line = non-transfected cells. Red line = mCherry transfected cells.

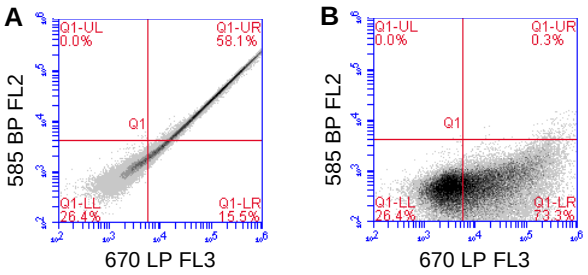

Figure 4. 293T cells transfected with mCherry show strongest signal on FL3 (670 LP) but also have a significant signal in FL2 (585/40 BP). Uncompensated data (A), Compensated data (B, FL2 – FL3 = 25%)

Suggested Filter Configurations

Many FPs can be detected using the standard filter configuration of the C6 (Table 1), but optional filters are available that may increase signal resolution and allow separation of signals which might overlap using the standard configuration (Table 2).

Standard Filters

Detector	Filter	FP Detected
FL1	530/30	GFP*, YFP*, mCitrine, YPet
FL2	585/40	mOrange, dTomato, dsRed
FL3	670 LP	RFP, mCherry

Table 1. FP detection with the standard Accuri C6 light filters. *Includes enhanced versions of both FPs.

Optional Filters

FP	Optimal Filter	Part Number	Detector
GFP*	510/15	CP-170	FL1
YFP*, mCitrine	540/20	CP-178	FL2
mOrange	565/20	CP-172	FL2
dTomato, dsRed	585/40	Standard	FL2
RFP, mCherry	610/20	CP-174	FL2 or FL3

Table 2. Suggested filters for optimal detection and separation of FP signals. *Includes enhanced versions of both FPs.

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