Optical Filter Guidelines for Fluorescent Protein Analysis with the BD Accuri™ C6 Flow Cytometer

Technical Bulletin

Contents

The BD Accuri™ C6 personal flow cytometer can analyze many variants of fluorescent proteins (FPs). As with any fluorochrome used in a flow cytometric assay, the success of FP detection depends on the level of expression within the cells, the excitation wavelength used, and the optical filters placed in front of the fluorescence detectors. This Technical Bulletin provides guidelines for optical filter selection in FP analysis.

Background

The successful cloning and expression of the green fluorescent protein (GFP), derived from the jellyfish *Aequoria victoria*,[1,2] ushered in a new era for studies of living cells and their functional processes. The GFP gene can be introduced into organisms through breeding, injection with a viral vector, or cell transformation, and studied using fluorescence microscopy or flow cytometry. Since the initial use of GFP in gene expression studies, mutagenesis has been used to produce a number of enhanced variants (eg, eGFP, eCFP, eYFP) with varying emission peaks across the blue, cyan, green, and yellow wavelengths.

In 1999, the gene for a red fluorescent protein (RFP) was cloned from the sea anemone *Discosoma striata*.[3] Mutagenesis of this FP has also yielded a wide range of derivatives that have extended the useful spectral emission range into the red region.[4]

The combination 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.

Optical Filters for FP detection on the BD Accuri C6

The BD Accuri C6 flow cytometer can detect many FPs using the standard filter configuration (Table 1).

However, optional filters can increase signal resolution and allow separation of signals that might overlap using the standard configuration. Details on optimally detecting each fluorochrome family can be found in Table 2.

Table 1. FP detection with the standard BD Accuri C6 optical filters.

Detector	Filter	FPs Detected
FL1	533/30	GFP*, YFP*, mCitrine, YPet
FL2	585/40	mOrange, dTomato, DsRed
FL3	670 LP	RFP, mCherry

*Includes enhanced versions

Table 2. Suggested optional filters for optimal detection and separation of FP signals.

Protein	Optimal Filter	Cat. No.	Detector
GFP*	510/15	653184	FL1
YFP*, mCitrine	540/20	653528	FL2
mOrange	565/20	653185	FL2
dTomato, DsRed	585/40	Standard	FL2
RFP, mCherry	610/20	653186	FL2 or FL3

*Includes enhanced versions

Specific filter recommendations for fluorescent protein families

This section contains detailed information about filter performance for each FP family listed in Tables 1 and 2.

Detecting GFP

You can detect GFP and eGFP (enhanced GFP) in FL1 with the standard BD Accuri C6 filter configuration (FL1 = 533/30 BP) (Table 1). However, substituting the optional 510/15 BP filter (Cat. No. 653184) at position FL1 can improve GFP signal detection.

To detect extremely bright GFP signals, the 533/30 BP filter is also available in two optional attenuation levels: 90% (Cat. No. 653173) and 99% (Cat. No. 653172).

Detecting yellow FPs

You can detect YFP, eYFP, mCitrine, and other YFP derivatives in FL1 with the standard BD Accuri C6 filter configuration (FL1 = 533/30 BP).

Detecting green and yellow FPs simultaneously

The emission spectra for GFP, YFP, and their derivatives overlap considerably.

To detect both GFP and YFP signals simultaneously, they must be separated. To do this, use the optional 510/15 BP filter (Cat. No. 653184) at position FL1 and the optional 540/20 BP filter (Cat. No. 653528) at position FL2 (Figure 1).

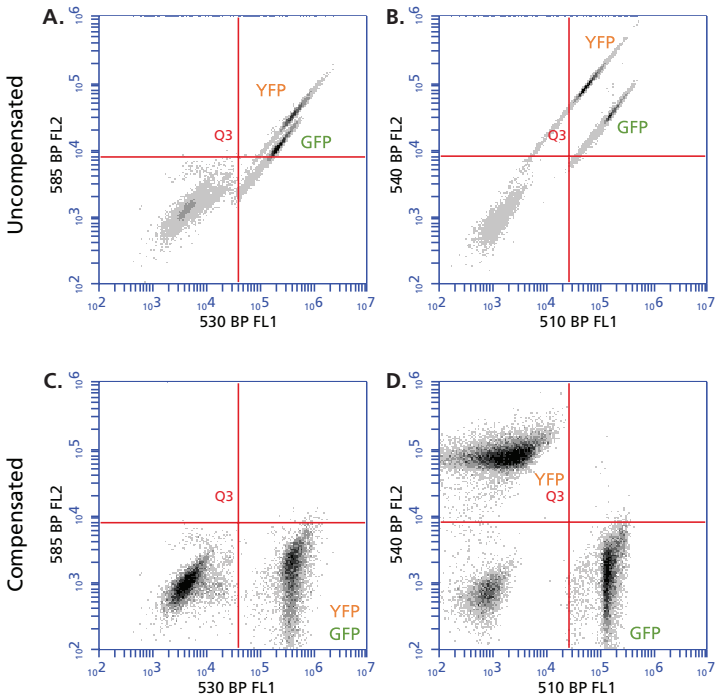

Figure 1. Detection of GFP and YFP.

GFP and YFP signals are both detected in FL1 with the standard BD Accuri C6 filter configuration (A, C), but can be separated by using the 510/15 filter (Cat. No. 653184) in FL1 and the 540/20 filter (Cat. No. 653528) in FL2 (B, D). ***A, B.*** *Uncompensated data.* ***C, D.*** *Compensated data.*

Detecting mOrange, dTomato, and DsRed

You can detect the strongest signals from mOrange, dTomato, and DsRed in FL2 with the standard BD Accuri C6 filter configuration (FL2 = 585/40 BP). However, they will also emit significant signal in FL3 (670 LP) (Figure 2A). For this reason, avoid using these FPs simultaneously with RFP or mCherry.

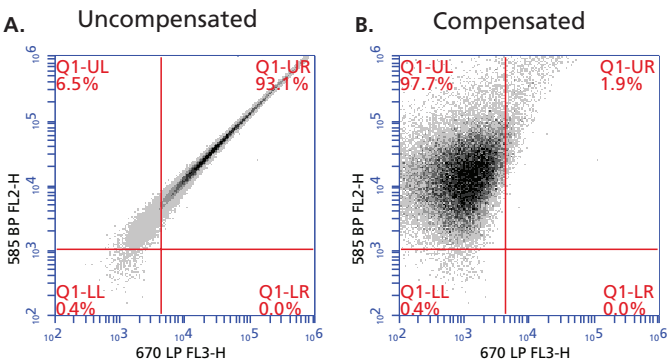

Figure 2. Detection of DsRed in FL2 and FL3.

THP-1 cells labeled with DsRed-conjugated antibody show the strongest signal in FL2 (585/40 BP) of the BD Accuri C6 flow cytometer. However, they also show significant signal in FL3 (670 LP). ***A.*** *Uncompensated data.* ***B.*** *Compensated data (FL3 – FL2 = 83%).*

Detecting mCherry, RFP, and other red FPs

You can detect strong signals from mCherry, RFP, and other red FPs in FL3 with the standard BD Accuri C6 filter configuration (FL3 = 670 LP). However, substituting the optional 610/20 BP filter (Cat. No. 653186) in FL3 can improve resolution from background (Figure 3).

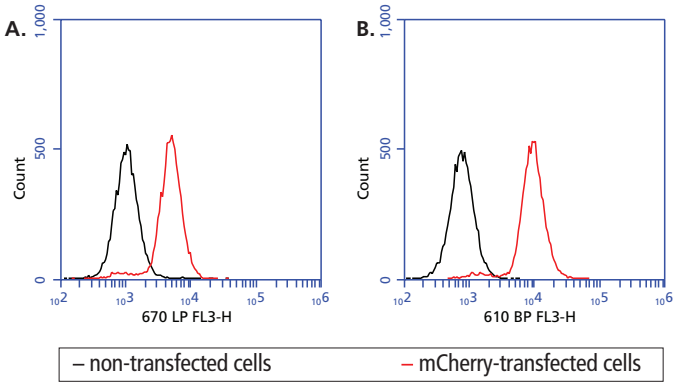

Figure 3. Detection of mCherry.

A. *mCherry expression in stably-transfected CHO cells (red line) can be discriminated from controls (black line) in FL3 with the standard BD Accuri C6 filter configuration (FL3 = 670 LP).*
B. *Discrimination is improved by using the optional 610/20 BP filter (Cat. No. 653186).*

In addition, mCherry, RFP, and other red FPs will also emit significant signal in FL2 (585/40 BP, Figure 4). Therefore care must be taken when using these simultaneously with fluorescent proteins such as dTomato, DsRed or mOrange.

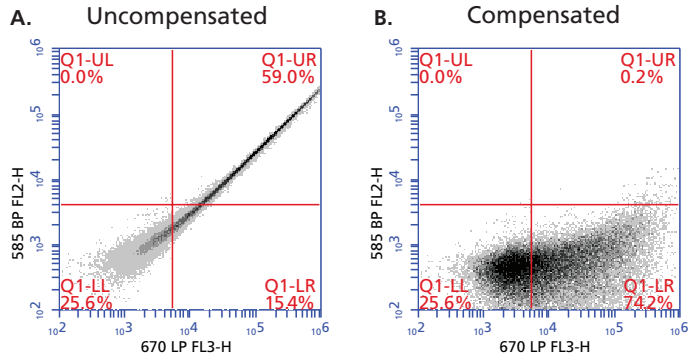

Figure 4. Detection of mCherry in FL2 and FL3.

293T cells transfected with mCherry show the strongest signal in FL3 (670 LP) but also emit significant signal in FL2 (585/40 BP).
A. *Uncompensated data.* ***B.*** *Compensated data (FL2 – FL3 = 25%).*

References

1. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. *Science.* 1994;263:802-805.
2. Tsien RY. The green fluorescent protein. *Annu Rev Biochem.* 1998;67:509-544.
3. Matz MV, Fradkov AF, Labas YA, et al. Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat Biotechnol.* 1999;17:969-973.
4. Baird GS, Zacharias DA, Tsien RY. Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc Nat Acad Sci USA.* 2000;97:11984-11989.

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