accuri CYTOMETERS

Expanding fluorescence detection options with the Accuri® C6 Flow Cytometer® System

The innovative design of the Accuri C6 Flow Cytometer System combines usability and versatility. With Accuri's new Selectable Lasers Module, the numerous fluorophore combinations that the C6 can detect are further expanded through the use of alternative optical filters and adjustable detector-laser associations. Here we demonstrate the increased versatility by using alternative filters and selectable lasers to optimize the detection of specific fluorophore and fluorescent protein combinations.

Ease of use

The Accuri C6 Flow Cytometer is an affordable, easy-to-use benchtop instrument that makes flow cytometry accessible to individual research labs. Its innovative design includes standardized detectors that eliminate the need for voltage and gain adjustments, making flow cytometry manageable for the novice user. The simplicity of the system does not hinder its appeal to the more advanced user, who will appreciate the flexibility of the C6. The instrument's internal design allows the user to easily access and exchange optical filters, facilitating optimal detection of diverse combinations of fluorophores and fluorescent proteins. In addition, the recently released Selectable Lasers Module allows the user to quickly adjust detector-laser associations through the software interface, further expanding possible fluorophore combinations and applications.

Fluorescent protein detection

The successful cloning and expression of fluorescent proteins (FPs) such as GFP, YFP and RFP has allowed scientists to tag proteins and track their expression dynamically, greatly advancing many areas of research in the biological sciences[1–4]. Many FPs can be detected using the standard optical configuration of the C6, and optional filters are available that can increase signal resolution and distinguish signals that overlap when the standard configuration is used (**Table 1**).

Optimizing signal separation

GFP and YFP are both detected in FL1 using the standard filter configuration. Simultaneous detection of these FPs is possible on the C6 with an alternative filter combination. Replacing the standard 530/30 band-pass (BP) filter in FL1 with a 510/15 BP filter and exchanging the standard 585/40 BP filter in FL2 for a 540/20 BP filter results in detection of distinct signals from GFP (maximum-emission wavelength, or Em-max, 507 nm) and YFP (Em-max = 529 nm) in FL1 and FL2, respectively (compare **Fig. 1a**,**b**).

Optimizing signal detection

Signal detection of red fluorescent proteins such as mCherry (Em-max = 610 nm) can be enhanced by exchanging the standard 670 long-pass (LP) filter in FL3 with a 610/20 BP filter (compare **Fig. 1c**,**d**).

Selectable lasers module

Accuri's new Selectable Lasers Module further increases the combinations of fluorophores that can be used with the C6. Selectable

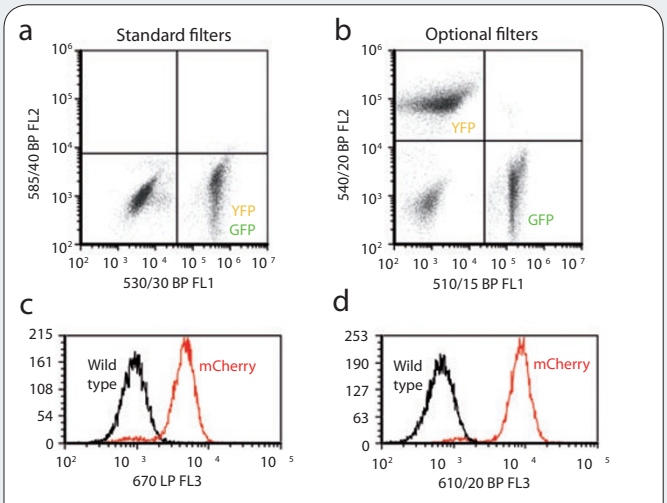

Figure 1 | Optional filters. (**a**,**b**) Data from CHO cells transfected with the YFP mCitrine mixed with GFP-labeled cells. The two labels are indistinguishable using standard filters (**a**) but are clearly resolved using the indicated optional filters (**b**). (**c**,**d**) mCherry-expressing CHO cells (red) are better resolved from wild-type cells (black) using the optional 610/20 BP filter (**d**) instead of the standard 670 LP filter (**c**).

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APPLICATION NOTES

Table 1 | C6 optical configurations

3-blue 1-red[a]		
Detector	**Filter options**	**Fluorophore[c]**
FL1	530/30 BP (standard)	FITC, GFP, YFP, CFSE
	510/15 BP	GFP
FL2	585/40 BP (standard)	PE, PI, PE–Texas red
	540/20 BP	YFP
FL3	670 LP (standard)	PerCP, PerCP-Cy5.5, PE-Cy5, PE-Cy7, PI, 7-AAD
	610/20 BP	RFP, PI, PE–Texas red
FL4	675/25 BP (standard)	APC, PE-Cy5, AF-647

2-blue 2-red[b]		
Detector	**Filter options**	**Fluorophore[c]**
FL1	530/30 BP	FITC, GFP, YFP, CFSE
	510/15 BP	GFP
FL2	585/40 BP	PE, PI, PE–Texas red
	540/20 BP	YFP
FL3	780/60 BP	APC-Cy7
FL4	675/25 BP	APC, PE-Cy5, AF-647

4-blue[b]		
Detector	**Filter options**	**Fluorophore[c]**
FL1	530/30 BP	FITC, GFP, YFP, CFSE
	510/15 BP	GFP
FL2	585/40 BP	PE, PI, PE–Texas red
	540/20 BP	YFP
FL3	780/60 BP	PE-Cy7
	610/20 BP	RFP, PI, PE–Texas red
	630/30 BP	PE–Texas red, PI
FL4	675/25 BP	PE-Cy5, PerCP-Cy5.5

Blue and red text denotes filters for detecting signals excited by the blue laser and red laser, respectively. [a]C6 standard configuration is 3-blue 1-red with 530/30 BP filter in FL1, 585/40 BP filter in FL2, 670 LP filter in FL3 and 675/25 BP filter in FL4. [b]Available with the Selectable Lasers Module. [c]Examples of common fluorophores, not a complete list.

Lasers gives users the option to adjust the laser-detector associations from the standard '3-blue 1-red' configuration (FL1, FL2 and FL3 detecting signals excited by the blue laser, and FL4 detecting those from the red laser) to alternative configurations of '2-blue 2-red' (FL1 and FL2 detecting signals excited by the blue laser, and FL3 and FL4 detecting those from the red laser) or '4-blue' (FL1–FL4 detecting signals from the blue laser).

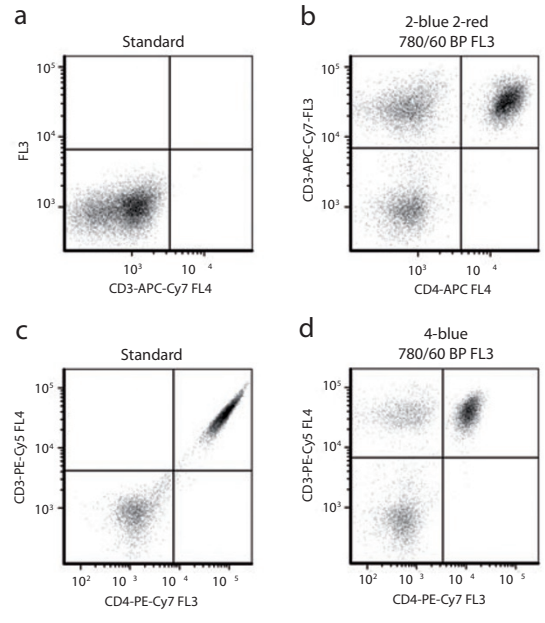

Figure 2 | Selectable lasers. (**a**,**b**) Lymphocytes from human peripheral blood stained with αCD3-APC-Cy7 are not detected using the standard filter configuration (**a**) but are easily detected and resolved from αCD4-APC–stained cells in the 2-blue 2-red configuration with a 780/60 BP filter in FL3 (**b**). (**c**,**d**) Lymphocytes from human peripheral blood stained with αCD3-PE-Cy5 and αCD4-PE-Cy7 have overlapping signals in the standard configuration (**c**) but are easily distinguished in the 4-blue configuration with a 780/60 BP filter in FL3 (**d**).

By combining these configurations with optional filters included in the module, the user can optimize the laser-detector associations for a wide variety of desired fluorophore combinations. For example, the standard configuration of the C6 is not conducive to APC-Cy7 detection, as its emission (Em-max = 780 nm) is not in the range of the associated (FL4) detector (675/25 BP; **Fig. 2a**). Swapping the standard FL4 filter with a 780/60 BP filter allows detection of APC-Cy7. Users can also distinguish signals from APC and APC-Cy7, when the two are used in combination, by operating in the 2-blue 2-red mode and exchanging the standard 670 LP filter in FL3 with the 780/60 BP filter (**Fig. 2b**). Another fluorophore combination that can be optimized using Selectable Lasers is PE-Cy5 and PE-Cy7. The signals from these fluorophores overlap in FL3 under the standard configuration (**Fig. 2c**) but are easily separated when the 4-blue configuration is used with a 780/60 BP filter in FL3 (**Fig. 2d**).

Summary

Optional filters and the Selectable Lasers Module are inexpensive tools to easily expand the versatility of the Accuri C6 Flow Cytometer.

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2. Matz, M.V. *et al.* Fluorescent proteins from nonbioluminescent *Anthozoa* species. *Nat. Biotechnol.* **17**, 969–973 (1999).
3. Baird, G.S. *et al.* Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc. Natl. Acad.* Sci. *USA* **97**, 11984–11989 (2000).
4. Campbell, R.E. *et al.* A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. USA* **99**, 7877–7882 (2002).