Application Report

High speed ADCs and Amplifiers for Flow Cytometry Applications

Xiaochen Xu, Jacob Freet, Daniel Brock, Jun Shen

ABSTRACT

Flow cytometry is an advanced technology for characterizing suspensions of single cells or particles. It has been used widely in research and clinical applications. Novel semiconductor products have been applied to this biomedical application for miniaturizing flow cytometry instruments without sacrificing performance. [1]

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Trademarks

1 Introduction

As shown in Figure 1-1, Fundamental components of a flow cytometer includes a fluidics system for moving cells, a laser optics system for emitting photons to cells and detecting light scatters from cells, an analog electronics system for converting emitted photons to a voltage pulse, and a digital signal processing/ microcontroller system for correlating the digitized voltage pulses to cell property results. This application note focuses on the analog signal chain electronics, and discusses the design considerations.

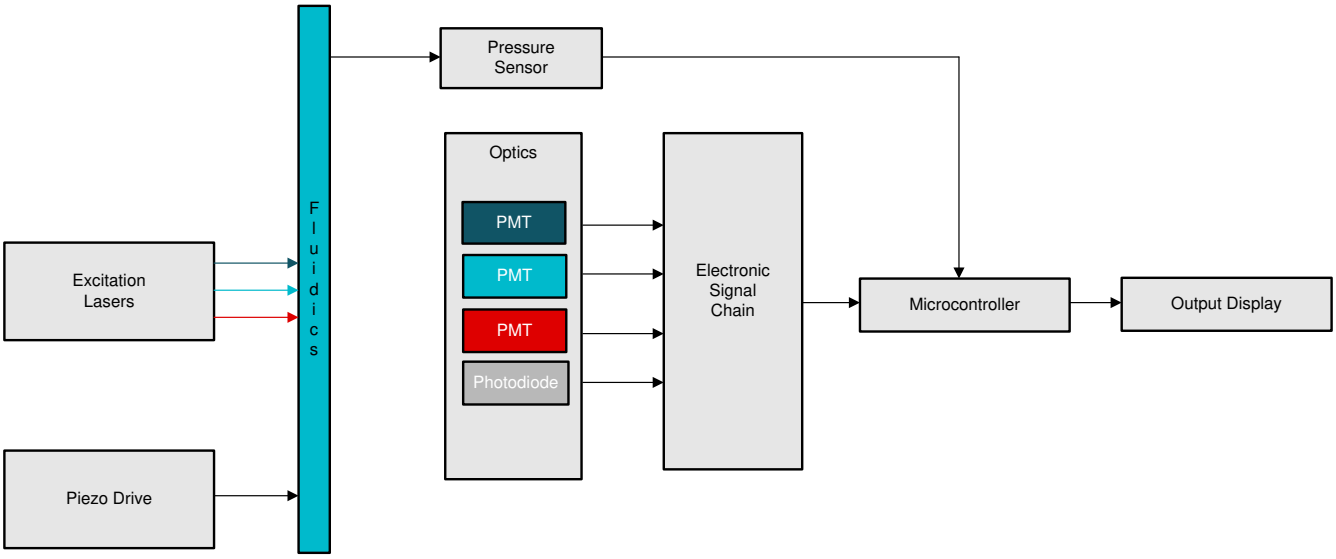


Figure 1-1. System Block Diagram of a flow Cytometer

The analog signal chain of a flow cytometer is shown in Figure 1-2. In order to cover the full range of the fluorescence spectrum, a variety of optical filters and detectors are selected, resulting in a multi-channel signal chain. It is common that a flow cytometer contains over 24 channels of analog signal chain electronics. Different sensors are used for detecting different frequencies and intensities of the scattered signals. As a result, different analog performance is expected in the signal chain. First, review the different photon detectors first; then propose the corresponding amplifier and ADC designs.

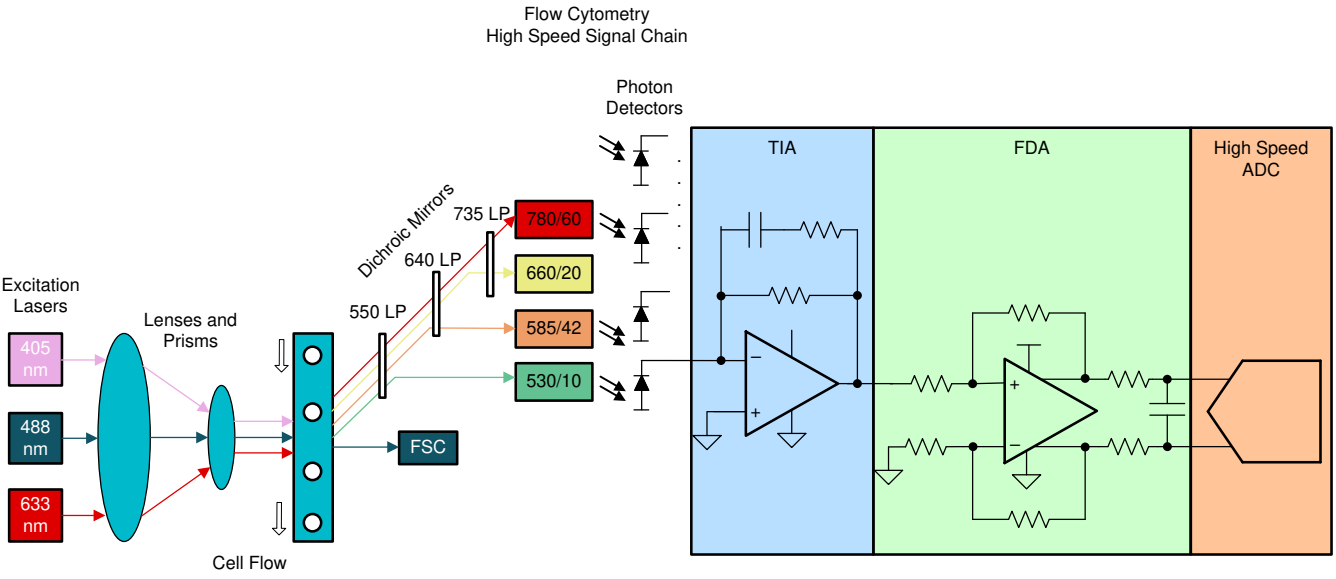


Figure 1-2. Analog Signal Chain of a Flow Cytometer

2 Photon Detectors in Flow Cytometers

Photmultiplier tubes(PMTs), Silicon photomulitplers (SiPMs), photo diodes(PDs), and avalanche photodiodes(APDs) are commonly used sensor technologies in flow cytometry. PMT with high intrinsic optical gain usually has high sensitivity and low noise, which makes PMT suitable for detecting low light or low scattering signals. Photo diodes don't have the intrinsic gain and are suitable for detecting the bright forward light and side light scatter from the transmitting laser wavelength. Compared to PD, APD' sensitivity sits between the PMT and PD. SiPMs are the latest photon detector, which has comparable sensitivity and is more compact and cost effective. The sensitivity of these detectors also depends on the wavelengths see [3]. In order to cover the wide fluorescence spectrum of scattered signals from cells, multiple sensor technologies are likely used in a flow cytometer system for performance and cost optimization. Table 2-1 shows the key specifications of these sensors.

Table 2-1. Photon detector Comparison

Type	PMT	APD	PD	Silicon PM
Part Number	R9220	AD500	H7422-50	S13720-1325CS/PS
Dark Current	10 nA	0.3 nA	0.5 nA	0.5 µA
Peak current	100 µA	0.25 mA	2 µA	2 mA
I/V R at 2Vpp	20 KΩ	80 KΩ	1 MΩ	1 KΩ
Peak λ	450 nm	700 nm	800 nm	660 nm

3 Analog Signal Chain in Flow Cytometers

A typical circuit configuration between a optical sensor and an ADC is shown in Figure 3-1. Due to the peak current differences, different resistances of R7 are selected in the transimpedance amplifier OPA858 in Figure 3-1. More design guidelines can be found in [4,5] and TIDU535. In flow cytometer, the received scattering signal from a photon detector is typically a unipolar pulse. The fully differential amplifier, THS4541, converts the unipolar pulse signal to differential outputs biased at the ADC common mode voltage [5]. The first stage OPA858 or OPA818 with FET inputs achieves low current noise, suitable for MΩ feedback resistors. Bipolar operational amplifier OPA855 and LMH6629 also can be used when low value feedback resistor is selected. THS4541 generates the 2Vpp differential outputs with a ADC common mode voltage of 0.75 V. TINA simulation was carried out. The time domain waveforms (Figure 3-2), output noise, and SNR are summarized in Figure 3-2 and Table 3-1.

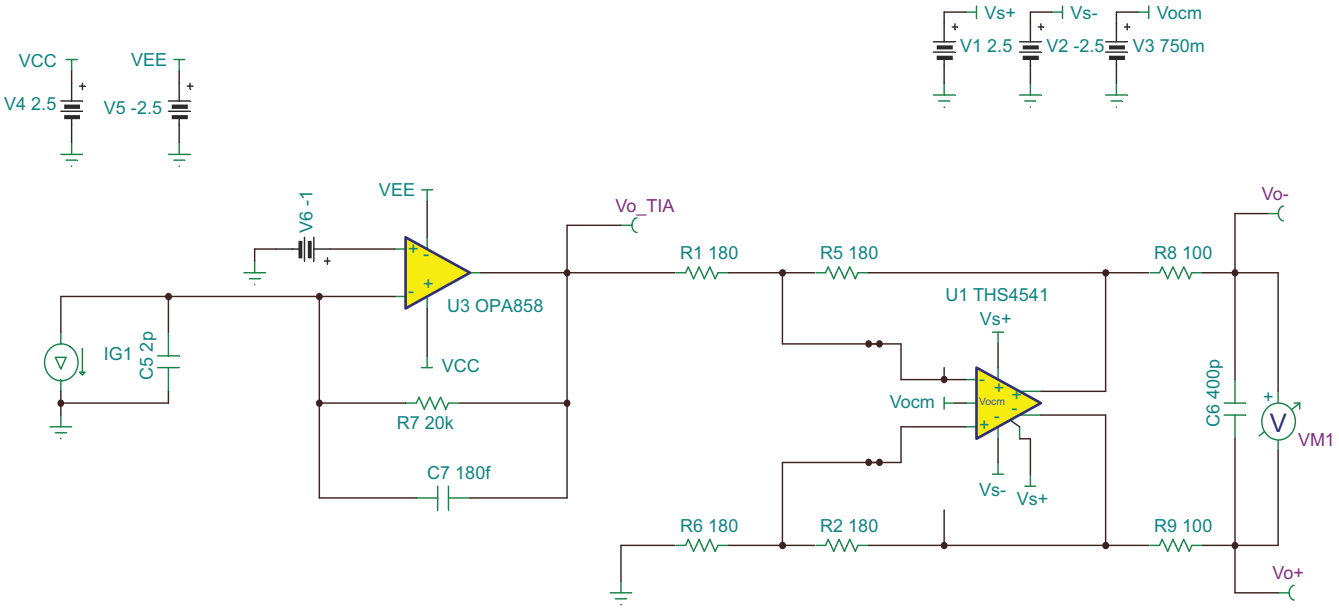


Figure 3-1. Transimpedance amplifier and Fully Differential Amplifier for photon detectors

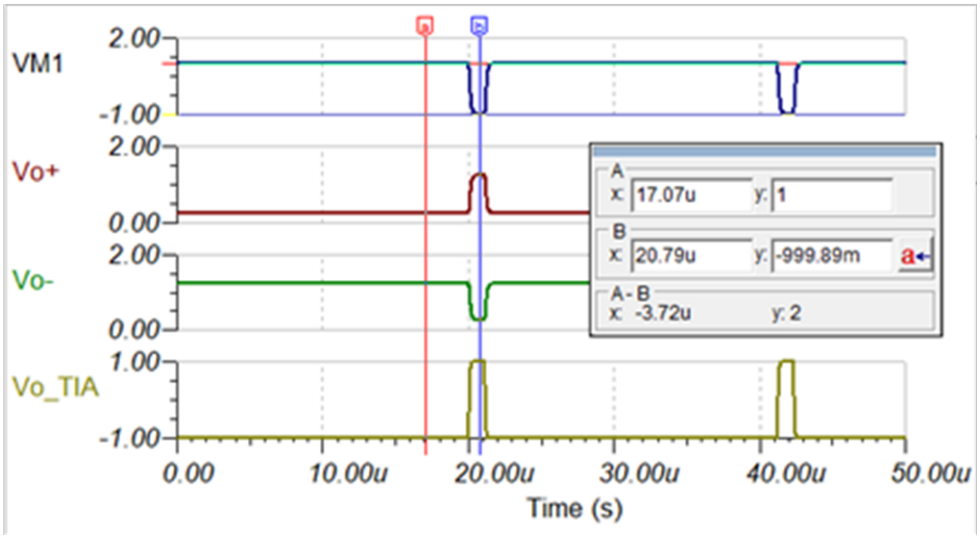


Figure 3-2. Time domain waveforms

Table 3-1 shows that the transimpedance amplifier designed for SiPM peak current could ideally reach about 95 dBFS signal to noise ratio (SNR). [2] reported that a stained cell can be 10,000 times brighter than an unstained cell which gives a SNR requirement of 80 dBFS. Thus a system would be optimized between 80 dBFS and 95 dBFS SNR. Other than SNR, the ADC sampling rate, power consumption, and cost are also important aspects in the flow cytometer system design. Certainly a high speed ADC with 95 dBFS SNR would achieve the best performance while it may significantly impact the system cost-effectiveness.

Table 3-1. SNRs for different photon detector with TIAs outputs

	Photodiode	APD	PMT	SiPM
Feedback R7	1 MΩ	80 KΩ	20 K	1 KΩ
Output Noise	230 µVRMS	66 µVRMS	35 µVRMS	13 µVRMS
SNR at 2Vpp	70 dBFS	81 dBFS	86 dBFS	95 dBFS

Scattering signals vary depending on the cell properties. Flow cytometer needs to study the time domain waveform, including waveform shape, peak, frequency and so on. The scattered signals are commonly visualized as a Gaussian shape pulse with a frequency from 100s KHz to several MHz. [2] concluded the relationship between the peak detection error and No. of samples of each pulse, which is listed in Table 3-2. For example, in order to reach the peak detection error of 0.1%, about 120 samples are needed to cover the whole pulse. If the pulse was 1 µs long, a system would require an ADC to have a sample rate of 120 MSPS. Certainly it is very challenging to have an ADC to meet both sample rate of >120 MSPS and >95 dBFS SNR at a reasonable cost and power consumption. System designers have to create innovative methods to reach both goals.

Table 3-2. ADC Over Sample Rate vs. Peak Detection Error

Peak Detection Error %	No. of Samples Per Pulse
0.1	120
0.15	96
0.5	48
1.0	40
2.0	24

Ideally, we hope to reach these SNR and sample rates goals simultaneously since the scattering signal from a cell at a certain location is captured by one photon detector channel only once. When it is limited by the ADC specification, a duplicate ADC channel for the fully differential amplifier may be considered as shown in Figure 3-3.

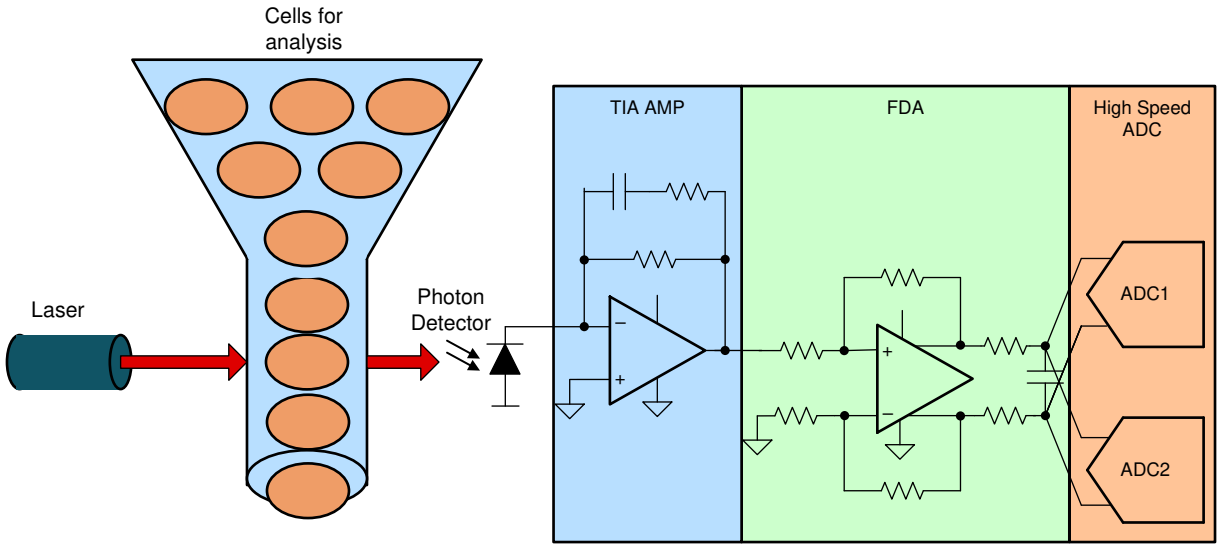
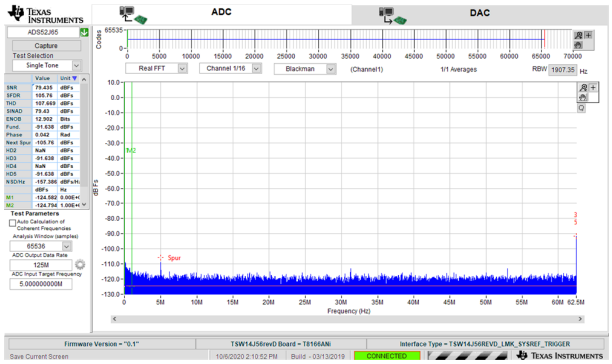


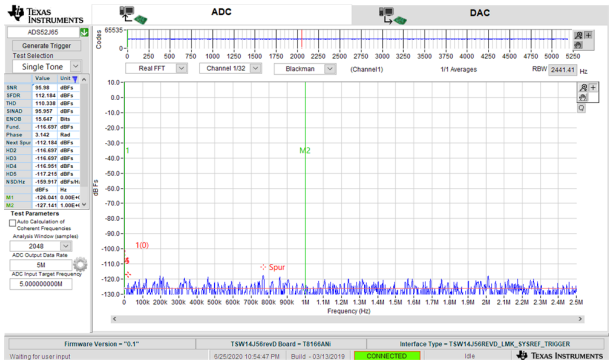
Figure 3-3. Analog Signal Chain with a duplicate ADC channel

For example, one ADC channel is optimized for SNR for detecting stained cells from unstained ones; and the other ADC channel is optimized for high sample rate for achieving high accuracy peak detection. Either two different ADCs could be used for different optimization goals; or the same high speed ADC with digital processor could be programmed differently to achieve 80 dBFS SNR at 125 MSPS and >95 dBFS SNR at 5 MSPS. The measurement results, by using digital decimation, are shown in Figure 3-4, Figure 3-5 and Figure 3-6, Figure 3-7. By using the same ADC, the hardware design and software design are more straightforward, instead of handling different ADCs with different analog specifications and digital features. The system software can superpose two ADC outputs to realize both accurate peak detection with 8 ns sampling interval and weak scattering detection with a dynamic range of >95 dBFS. In addition, the programmable decimation filter enables system designers to optimize SNR after matching the filter response to the scattered signal bandwidth.



125 MSPS, SNR = 80 dBFS

Figure 3-4. ADS52J65 SNR improvement with on-chip digital decimation processor

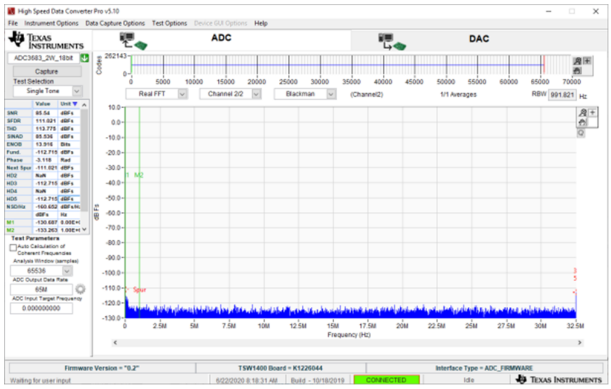


125 MSPS, SNR = 96 dBFS w/ 25x decimation

Figure 3-5. ADS52J65 SNR improvement with on-chip digital decimation processor

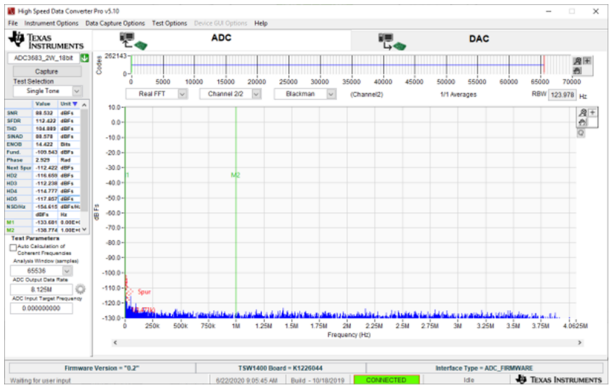
Considering most flow cytometer systems require more than 8 channels, the low power 16-bit 8-CH 125 MSPS ADS52J65 with 9×9 mm package enables system designers to achieve compact design easily. Its JEDS204B interface achieves >10 Gbps and is capable of compressing all ADC data on single differential pair , which significantly reduces the PCB design complexity. The ADS52J65 equips with comprehensive digital features: the programmable digital filter can be used to smooth pulse shape; the offset correction feature can compensate for system DC components due to multiple gain stages or the detector dark current; the I/Q demodulation feature can act as an approximate envelop detector, instead of complex Hilbert transform, when the pulse frequency is known or can be estimated.

TI also offers 2-channel 18-bit 65 MSPS ADC3683 and 4-channel 16-bit 100 MSPS ADS5263 with LVDS outputs. They are suitable for cost-effective FPGAs without JES204B interface. The same digital decimation feature exists to improve ADC SNR significantly. When photodiode is used and the SNR requirement on ADC is much lower, 14-bit ADCs with a SNR of 75 dBFS are suitable for cost-effective flow cytometers. 8-channel 14-bit 80 MSPS ADS5294 and 16-channel 14-bit 65 MSPS ADS52J90 are suitable candidates for photodiode based signal chains.



65 MSPS, SNR = 85.5 dBFS

Figure 3-6. ADC3683 SNR improvement with on-chip digital decimation processor



65 MSPS, SNR = 88.5 dBFS, w/ 4x Decimation

Figure 3-7. ADC3683 SNR improvement with on-chip digital decimation processor

4 Summary

In summary, the TI transimpedance amplifiers OPA858, OPA818, OPA855, OPA320, fully differential amplifiers THS4541, THS4551, and high speed high resolution ADCs (ADS52J65, ADC3683, ADS5263, ADS5294, ADS52J90) complete the high speed signal chain for flow cytometer. These devices enable system designers to achieve low power, high accurate flow cytometer systems in a portable form factor without compromising analog performance.

5 References

1. Cossarizza, A., Chang, H.-D., Radbruch, A., Akdis, M., Andr ¨a, I., Annunziato, F., Bacher, P. et al., Guidelines for the use of flow cytometry and cell sorting in immunological studies. Eur. J. Immunol. 2017. 47: 1584–1797 https ://onlinelibrary.wiley.com/doi/10.1002/eji.201646632
2. Snow, Christopher “Kit. "Flow cytometer electronics." Cytometry Part A: The Journal of the International Society for Analytical Cytology 57.2 (2004): 63-69.
3. https://hub.hamamatsu.com/sp/hc/resources/FC_Webinar.pdf\
4. Designing Photodiode Amplifier Circuits with OPA128
5. Samir Cherian, Maximizing the dynamic range of analog front ends having a transimpedance amplifier https://www.ti.com/lit/an/slyt773/slyt773.pdf

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