Label-free ratiometric monitoring of interferon gamma dynamics with spectrally filtered Si photodiode pairs

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Abstract: We report a label-free ratiometric interferon gamma (IFN-γ) sensor based on spectrally filtered Si photodiode (PD) pairs anchored with aptamer probes. Our sensor can rapidly detect two-color fluorescence change resulting from the aptamer-IFN-γ binding events. © 2021 The Author(s)
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Miniaturized, cost-effective cytokine sensing devices are essential for health monitoring and critical care of patients with acute immune responses [1]. Yet, most electrochemical cytokine sensors suffer from signal drifting, device-to-device variation, and require costly research-grade potentiostats [2]. On the other hand, most optical cytokine sensors are limited by bulky optical readout and costly labeling steps [3]. To this end, here we present an on-chip optical ratiometric aptasensing device to monitor the dynamics of an essential immune response biomarker, IFN-γ, in a label-free manner. Our device is formed by a pair of spectrally filtered Si photodiodes (PD) that are surface functionalized by aptamer probes, which can rapidly detect two-color fluorescence change resulting from the aptamer-IFN-γ binding events at pM levels. Combined with its chip-scale construct and high specificity, our cytokine sensor may have possible use in point-of-care (POC) and therapeutic screening applications.

To achieve ratiometric aptasensing of IFN-γ in a label-free manner, we chose to sequentially surface functionalize our spectrally-filtered PD pair (see details below) with biotinylated bovine serum albumin (BSA), Alexa Fluor 555 (AF555) conjugated streptavidin, and biotinylated Cy5-labeled DNA aptamer probe designed to specifically bind to IFN-γ. This aptamer probe will self-hybridize itself when no IFN-γ binds to it, pulling Cy5 close to AF555 to form a Förster resonance energy transfer (FRET) pair (with AF555 [Cy5] being the donor [acceptor]). When IFN-γ binds to the aptamer probe, the structural change of the aptamer will separate Cy5 and AF555, lessening the FRET effect (i.e. leading to an increase [decrease] of the AF555 [Cy5] emission signals under 550/15 nm excitation) (Fig. 1a). On the device side, we first fabricated a pair of spectrally filtered PDs based on Si p-i-n diodes routed in a 2-by-1 cross-bar structure as we reported before [4]. Briefly, Si PDs were built from the plasma-enhanced chemical vapor deposition based amorphous Si layers, and contacted by indium tin oxide (ITO) and Cr/Au layers at their p- and n-terminals, respectively. We then sequentially patterned two distinct photoresist-based spectral filters on two PD pixels (thermally crosslinked for 2 hrs, dry etched by photoresist-based sacrificial layers, and passivated by SU8 layers), allowing them to selectively detect AF555 and Cy5 signals (i.e. AF555 and Cy5 pixels), respectively (Fig. 1b). This spectrally-filtered PD pair was next wire-bonded onto a printed circuit board, and packaged in a polydimethylsiloxane (PDMS) based microfluidic channel (Fig. 1c). The latter allowed us to functionalize the device by sequentially introducing 1mg/ml biotinylated BSA in deionized water for 1 hr, 0.2 mg/ml AF555-labeled streptavidin in 1 × phosphate buffered saline (PBS) for 10 mins, and 1 μM biotinylated Cy5-laybeled aptamers in 1 × PBS for 2 hrs in the dark at room temperature.

To examine the wavelength selectivity of the PD pair (before packaging steps), we measured the transmittance spectra of spectral filters (coated on a dummy glass slide) and the photocurrents of each spectrally-filtered PD pixel at 3 wavelength windows (provided by a fluorescence microscope): 550/15 nm (260 mW), 575/25 nm (310 mW), and 640 nm (231 mW). In the former (Fig. 2a), we observed that: 1) both filters can effectively reject the 550/15 nm light we will use to excite AF555, 2) the filter applied to the AF555 pixel can effectively reject Cy5 emission near 666 nm, whereas the filter applied to the Cy5 pixel can effectively reject AF555 emission near 580 nm. In the latter (Fig. 2b), we found that the *I-V* curves measured in both pixels show clear wavelength selectivity that matches the transmittance spectra of their spectral filters. For instance, the AF555 [Cy5] pixel showed maximum photocurrent, I_{ph} (defined in [4]), under 575/25 nm [640/30 nm] illumination. Notably, the rejection ratios of the AF555 pixel ($I_{ph\_575nm}/I_{ph\_550nm}$ and $I_{ph\_575nm}/I_{ph\_640nm}$ at $V_{bias} = -5$ V) and the Cy5 pixel ($I_{ph\_640nm}/I_{ph\_550nm}$ and $I_{ph\_640nm}/I_{ph\_575nm}$ at $V_{bias} = -5$ V) were (49, 45) and (37, 35), respectively, suggesting good wavelength selectivity of the spectrally-filtered PD pair (Fig. 2c).

To conduct the ratiometric aptasensing experiments, we built an off-board multiplexing circuit to alternately bias the AF555 and Cy5 pixels at $V_{bias} = -5$ V as we reported before [4], with the photocurrent of each pixel being pre-amplified into a voltage readout, V_{out} (filtered by a 100 Hz low-pass filter and a 50/60 Hz noise eliminator). To alleviate the photo-bleaching effect, we illuminated the PD pair with 550/15 nm light pulses that have t_{on}/t_{off} = 18/162 s (i.e. 3-min period); when the light pulse was on, the multiplexing circuit was synchronized to sample the V_{out} of each pixel

for 3 s at 2000 S/s, with the last 2.5 s of data reaching to the steady state and being averaged as the pixel signal, V_{sig}. After the aforementioned surface functionalization steps, we kept the device in 1 × PBS, and conducted a control experiment by sequentially introducing 0.1 pM – 1 nM streptavidin in 1 × PBS at a 15-min interval to the PD pair via the PDMS channel (Fig. 3a). As expected, we did not observe significant change of V_{sig} from both AF555 and Cy 5 pixels since streptavidin does not bind to the aptamer. In contrast, we then kept the device in 1 × PBS again, and sequentially introduced 0.1 pM – 1 nM IFN-γ in 1 × PBS at a 15-min interval to the PD pair (Fig. 3b). This time, we observed that: 1) the V_{sig} values of the AF555 [Cy5] pixel increased [decreased] when IFN-γ concentration increased from 0.1 pM to 10 pM, likely due to an increased number of aptamer-IFN-γ binding events; 2) the V_{sig} values did not change further when 100 pM and 1 nM IFN-γ was applied, likely because the aptamers on PD pixels have been saturated by the IFN-γ bound to them; and 3) the V_{sig} values at each IFN-γ concentration rapidly reached a steady state in about 6 min in both pixels. Consequently, our spectrally-filtered PD pair ratiometrically monitored the IFN-γ dynamics at pM levels (see normalized $V_{sig\_AF555}/V_{sig\_Cy5}$ in Fig. 3c) in a label-free manner, suggesting its promise towards POC applications that require timely immune response monitoring.

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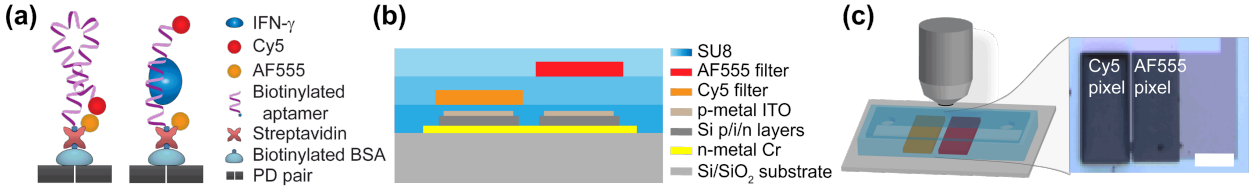

Figure 1. Experimental setup. (a) Illustration of on-chip ratiometric aptasensing of IFN-γ with a PD pair. (b) Illustration of the spectrally-filtered PD pair in a cross-sectional view. (c) Illustration of the fabricated PD pair packaged in a microfluidic channel. Scale bar, 100 μm.

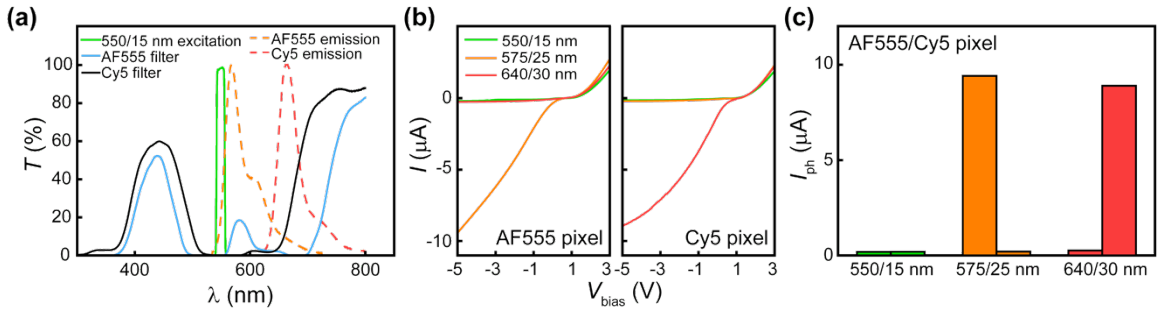

Figure 2. Optical characterizations of the PD pair. (a) Filter design, including the measured transmittance spectra of AF555 and Cy5 filters, the 550/15 nm excitation spectrum, and the emission spectra of AF555 and Cy5. (b) *I-V* curves of the PD pair at 3 wavelength windows. (c) Wavelength selectivity of the PD pair (left columns: AF555 pixel; right columns: Cy 5 pixel) based on the photocurrent (I_{ph}) measured at $V_{bias} = -5$ V in Fig. 2b.

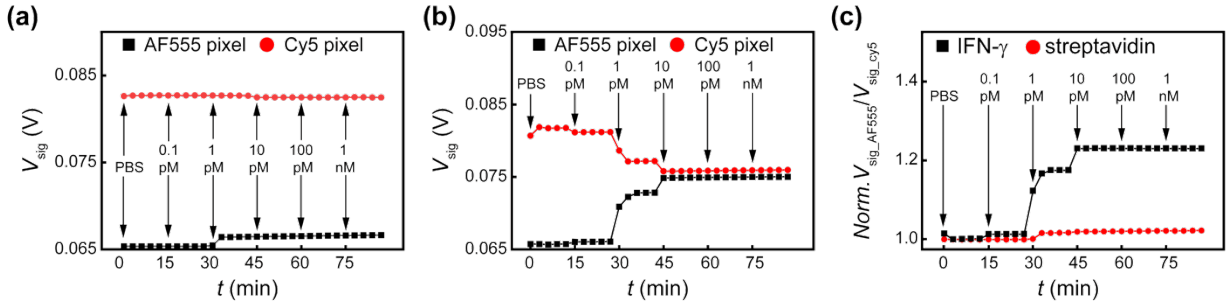

Figure 3. Ratiometric aptasensing of IFN-γ using the PD pair. (a) Measured V_{sig}-trace with 0.1 pM – 1 nM streptavidin being introduced to the PD pair. (b) Measured V_{sig}-trace with 0.1 pM – 1 nM IFN-γ being introduced to the PD pair. (c) Ratiometric readout trace of the PD pair (normalized $V_{sig\_AF555}/V_{sig\_Cy5}$). Vertical solid lines mark the time point when PBS, streptavidin, or IFN-γ was introduced to the PD pair.

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