DNA biosensor combining single-wavelength colorimetry and digital Lock-in Amplifier within a Smartphone

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Supporting information

Digital LIA algorithm

The algorithm of digital LIA can be found in **Fig.S1**. In SBLIA, the raw waveform, which is received by audio jack, is first digitally mixed with reference signal. Then, it is passed to a digital low pass filter (LPF) which is realized by an averaging filter. The low pass filter controls the integration time (τ) of the LIA. τ can be adjusted during experiment through the UI. Increasing τ will increase the number of data point being averaged. This action narrows LIA pass band and consequently reduce the noises.

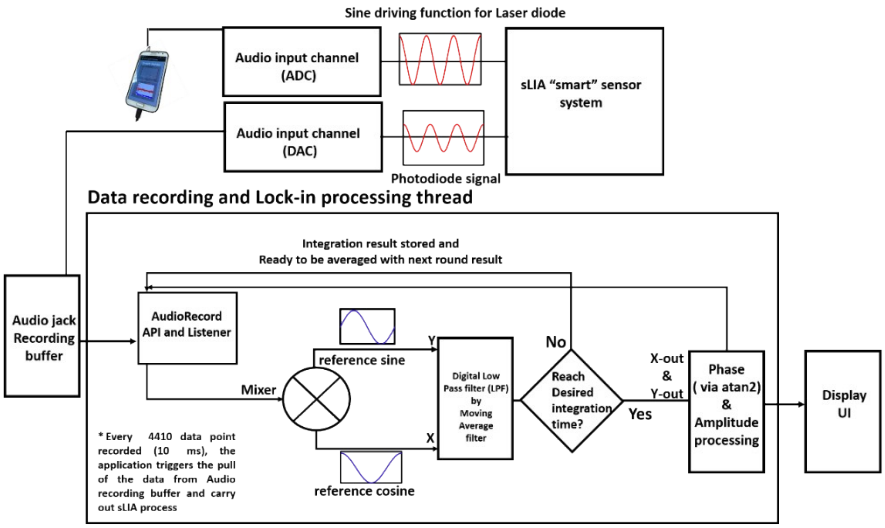

Fig.S1 the SBLIA software algorithm

SBLIA detection under difference background light condition

Herein, we demonstrate the claimed SBLIA system's advantage that it can work under different background light source condition without the need for a calibration or an enclosure to separate the system from the environment.

For this purpose, we have carried our SBLIA detection under two different settings that mimic the point-of-care environment. First, during SBLIA detection, the ceiling light is switched on and off to evaluate the effect of change in the average intensity of the background light. Such senario is common to point-of-care environment. Secondly, we have also investigated how SBLIA can exclude a specific 120 Hz fluctuation, which is a common source of noise in optical measurement. In this case, an external laser, which act as our noise source, is modulated in 120 Hz by a function generator. The external laser diode then irradiates on photodiode detector of SBLIA system during the sensogram measurement process.

As shown in **Fig.S2 (a)** and **(b)**, raw waveform data from the audio jack, as well as the amplitude sensogram, are monitored during a SBLIA detection to evaluate the effect of background lighting condition (f=2.5 kHz, V_{audio}=40 mV and τ =60 ms). **Fig.S2 (a)** shows the audio jack raw waveform data when SBLIA detection is carried out under different background conditions. The figure demonstrates that raw waveform data remain unaffected when ceiling light are switched on and off (Black Trace for ceiling light off, and blue trace for ceiling light on). Also, when the 120 Hz noise source irradiates on the photodiode, we see that only the AC part of the noise is detected by SBLIA. The DC level of the waveform is zero for all case of measurement. This is due to the fact that there are low pass filters on both SBLIA system and within the audio jack to exclude the signal below ~20 Hz. Therefore, SBLIA in not affected by difference in the average background light intensity. This allows the SBLIA to be used without calibration to the ambient light intensity[1]. Apart from the capacity to avoid noise from DC level change, the SBLIA also excludes AC noises that are not on the reference frequency (in this case, 2.5 kHz). As result, even though we can observe the 120 Hz slow modulation mixed with our 2.5 kHz source signal in the waveform (**Fig.S2 (a)** red trace), the noise is entirely excluded in the final amplitude sensogram as shown in the **Fig.S2 (b)**. In the figure, we have observed that SBLIA sensogram remains at a constant intensity whether ceiling light is off or on (blue area) or when the external source is introduced (red area).

AuNP synthesis

The gold nanoparticle is prepared using citrate mediate reduction. 100 mL aqueous solution of $HAuCl_4$ (41 mg, 1.0 mM) was heated to reflux and stirred vigorously.

Later on, 10 mL of tri-sodium citrate (114 mg, 38.8 mM) solution was quickly added to this solution. The solution was then heated for 10 min and was allowed to cool to room

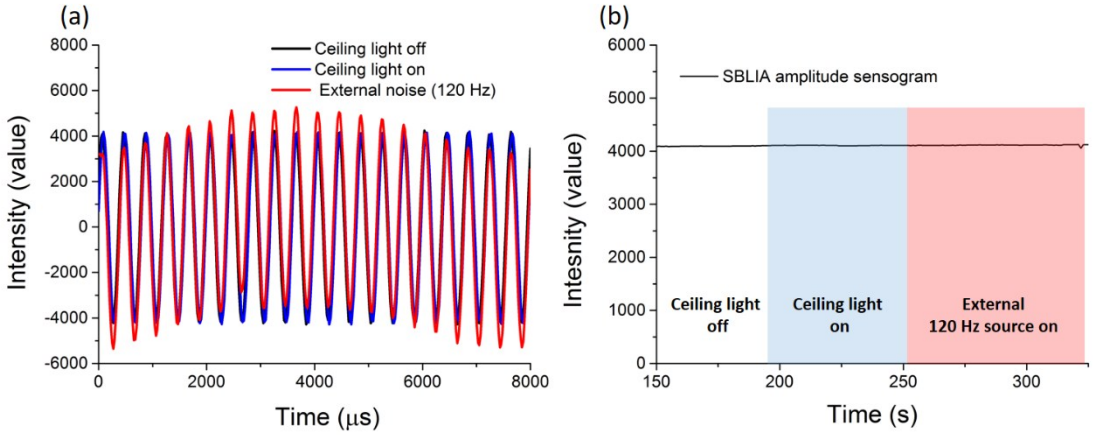

Fig.S2 (a) raw waveform data from audio jack under different background light condition (b) SBLIA amplitude sensorgram under different background condition.

temperature while being rigorously stirred. Before AuNP solution is finally stored at 4 degree, some filtering is necessary. The average diameter of AuNPs was found to be around 13 nm, as determined with a particle size analyzer.

Sample preparation for target DNA detection

The procedure for target DNA detection is comprised of three steps: DNA hybridization, AuNP-DNA incubation and then the colorimetry measurement.

Step1 (DNA hybridization):

6.4 μL of 10 μM probe ssDNA is incubated with a designated amount of 10 μM target DNA in a 1 ml vial. 6.4 uL of probe ssDNA correspond to 200 nM in the final detection process. Meanwhile, 0, 0.8, 1.6, 3.2, 4.8 uL of 10 μM target DNA will correspond to 0, 20, 40, 80, 120 nM of target DNA in the final detection. To facilitate optimized hybridization condition, the sample is then added with 3 μL of 2 M NaCl and filled with TBE buffer to 27 μL. Finally, incubate for 5 minutes.

Step2 (AuNP-DNA incubation):

Add 250 μL of AuNP and 150 μL of de-ionized water to the sample in step 1. Incubate 10 minutes before colorimetry measurement.

**Trouble shooting: If the color change may not as prominent, adjust the salt concentration in step1 to optimize colorimetry result. This will enhance the color contrast between samples with different target DNA.*

Converting SBLIA-AuNP colorimetry measurement data into absorbance unit

(AU) for comparison

Fig.S3 shows a typical result of SBLIA-AuNP colorimetry sensogram. In order to compare important system properties, e.g NSR and LOD, between SBLIA and UV-Visible spectrometer, the sensing result must be weighted on the same measurement units. In contrast to to UV-Visible spectrometer, SBLIA provides intensity data in terms of digital values. Therefore, a conversion is needed.

Under present context, absorbance reference level is defined by the AuNP solution that contains only 200 nM probe DNA and zero target DNA (This will be defined as "blank sample" hereafter). Therefore, the data from the SBLIA-AuNP measurement can be converted to AU by definition of absorbance:

$$A_c = -\,log\left(\frac{I_c}{I_{c=0}}\right) (eq.S1)$$

Where I_c is the SBLIA intensity as a function of target DNA concentration C in AuNP. $I_{c=0}$ is the SBLIA intensity when blank sample is placed in the cuvette.

In case of UV-Visible spectrometer, our UV-Visible absorbance data uses empty air transmission as the reference level (i.e, 100% transmission). In such case, when AuNP solution contains target DNA with C concentration, the UV-Visible absorbance data can be defined as:

$$A_c^{UV-Vis} = -\log\left(\frac{I_c}{I_{air}}\right) (eq.S2)$$

As to the blank sample, we have:

$$A_{c=0}^{UV-Vis} = -\log\left(\frac{I_c = 0}{I_{air}}\right) (eq.S3)$$

We can obtain absorbance, in terms of blank sample as reference, simply by subtracting **(eq.S6)** with **(eq.S7)**. In this way, we have:

$$A_c = -\log\left(\frac{I_c}{I_{c=0}}\right) = A_c^{UV-Vis} - A_{c=0}^{UV-Vis} \quad \textbf{(eq.S4)}$$

Using **(eq.S1)** and **(eq.S4)**, results from both system can be evaluated on the same basis.

Estimation of Limit of detection (LOD)

Noise of the SBLIA system is obtained using the sensogram as shown in inset of **Fig.S3** (and in **Fig.3)**. Standard deviation of the sensogram during a sufficient long time period (>100τ) determines the corresponding noise level. Note that there exists certain signal spike that are much greater than system noises (As indicated by the red arrow in the inset of **Fig.S3**. These spikes are due to sudden and instantaneous drops in audio output channel (due to instability of smartphone), which can be easily excluded with outlier's rule as they are generally much greater than 3 times the standard deviation. For all noise estimation, outlier are excluded beforehand. For UV-

Visible spectrometer, the noise is estimated by averaging 4 consecutive measurement on 650 nm. For both SBLIA and UV-Visible system, the noise are all measured the same integration time. In case of the measurements shown in **Fig.4**, SBLIA gives an averaged noise around 3.7×10^{-4} AU and UV-Vis gives an averaged noise around 1.59×10^{-3} AU and.

In context of sensor application, LOD is estimated by taking the 3 times standard deviation on the calibration curve shown in **Fig. 4(d).** Therefore, LOD, in terms of AU signal intensity, for SBLIA-AuNP DNA sensing is around 0.0011 AU and 0.00477 AU for UV-Visible spectrometer. Using the calibration curve shown in the **Fig.4(d)** (AU=0.00144*C for SBLIA and AU=0.0011*C for UV-Visible spectrometer), we obtained the LOD for SBLIA-AuNP DNA sensing as 0.77 nM while it is 4.27nM for UV-Vis spectrometer.

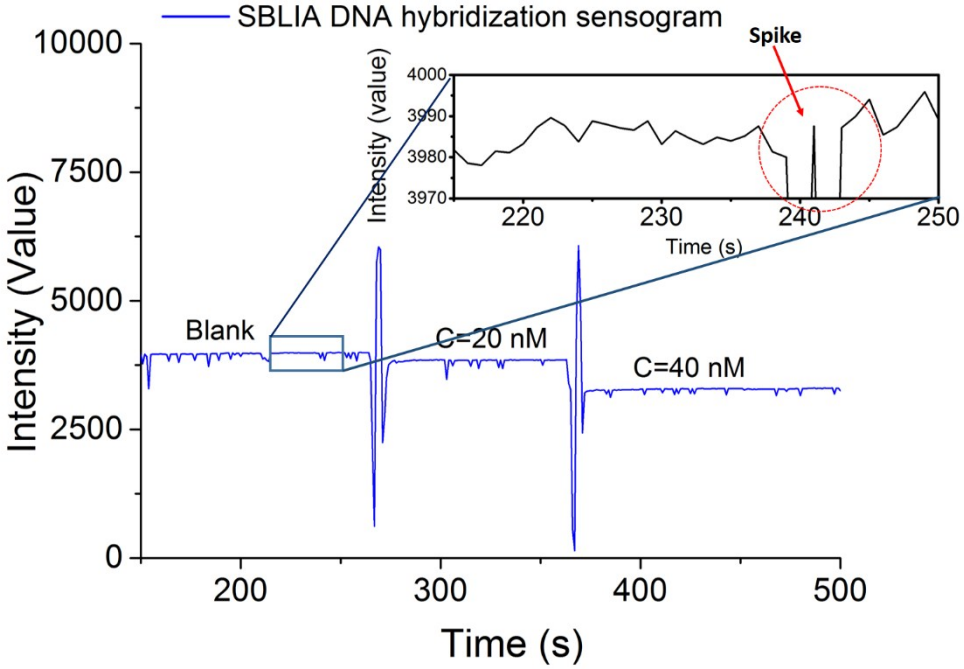

Fig.S3 typical DNA sensorgram of sLIA "smart" biosenspr

Phase sensogram of SBLIA system

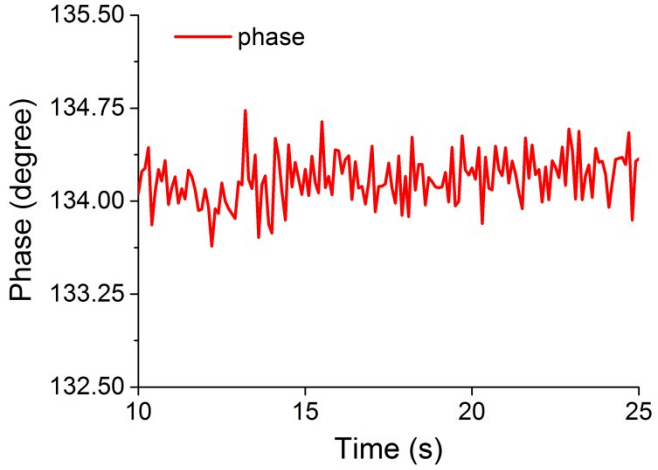

Fig.S4 phase signal extracted with SBLIA