MAY 06, 2020
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BIOSENSORS FOR PANDEMICS

PHOTONIC PLATFORM FOR DETECTION OF SIGNIFICANT LOW AMOUNT OF DNA

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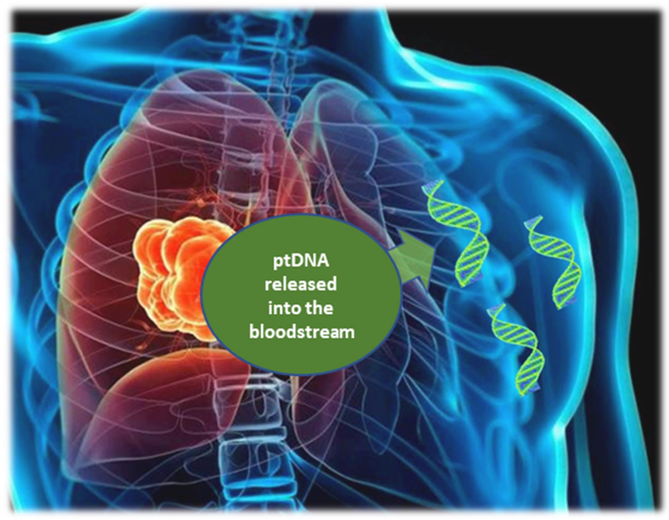
IFAE

Introduction

Liquid biopsy has the potential to revolutionize the future of cancer diagnostics and disease management. The biomarkers for cancer present in blood are very diluted (~ pg/µL) and difficult to measure. Droplet digital PCR (dPCR), real time PCR and Next Generation Sequencing are techniques that can measure minimum concentrations of biomarkers such as free circulating DNA fragments relevant to cancer diagnostics. The adoption of these techniques has been held back by complicated workflow, specific reagents, costly equipment and slow read out times.

Optics and photonics technologies, and high-sensitivity molecular diagnostic tests, already play key roles in the practice of healthcare. These technologies are essential for developing advanced tools for observing and measuring symptoms and treating patients with less invasive, more cost-effective methods.



Photonic platform design

Photonic platform setup

Our approach is based on light sensors sensitive to single photons and with excellent time resolution. The setup consists of the following components:

- a LED operated in pulse mode (pulse width ~20-30 ns)
- a set of short pass filter to eliminate all photons with $\lambda > 500$ nm
- a 10 mm light path quartz cuvette containing the sample with the wavelength shifter
- short pass filters to eliminate all photons with $\lambda < 500$ or 550 nm
- 2 different Photomultiplier (PMT) sensitive to single photons (MAGIC PMT or El PMT)

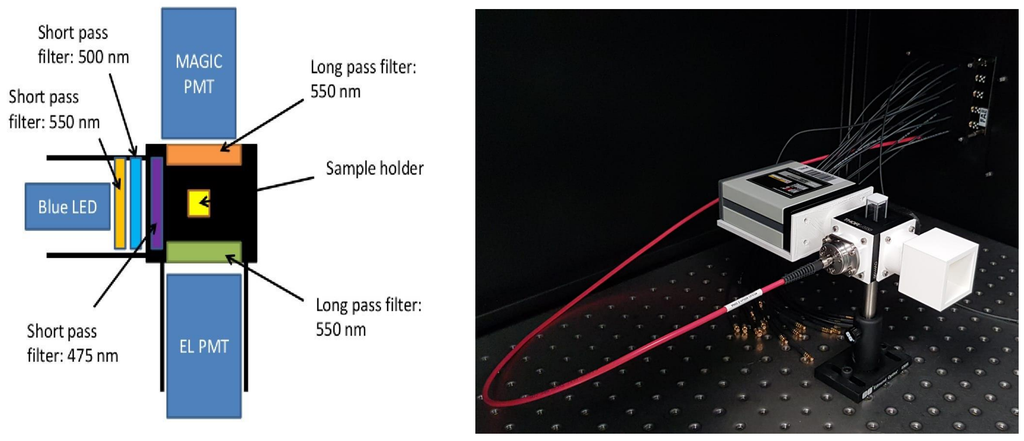


FIG 1. Diagram of the setup for detecting free DNA (Left) and a picture of these setup (Right)

LED election for DNA detection

The blue LED used for the excitation of the sample has the principal emission around the 470 nm, but also have a tiny emission at around the 625 nm following official data sheet (FIG 2).

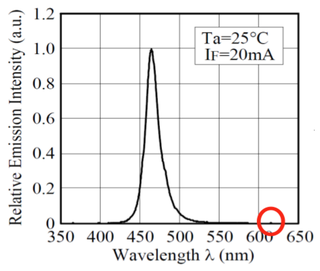


FIG 2. Emission spectrum of the Blue LED

Fluorescence dye for DNA detection

Nucleic Acid Staining Solution Midori Green was used for detecting DNA. This stain emits green fluorescence when bound to DNA or RNA. It has two secondary fluorescence excitation peaks and one strong excitation peak centered around 490 nm. The fluorescence emission is centered at ~530 nm. (FIG 3).

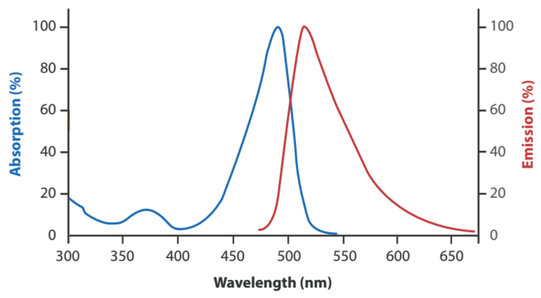


FIG 3. Absorption/Emission Spectrum Midori Green

Tests

Optimization of detection system

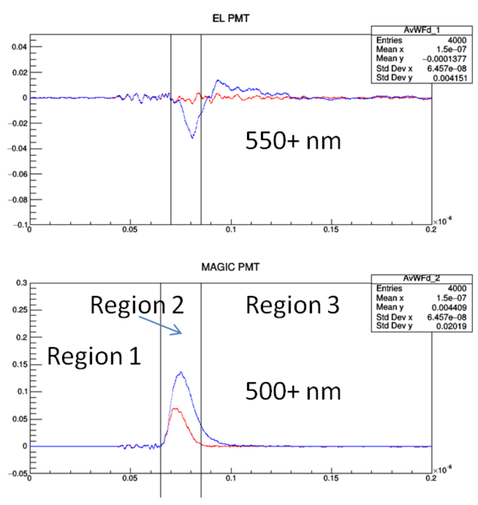


FIG 4. Average waveforms of both PMT for signal and background.

Two different samples and two different Photomultiplier were used, measured and compared :

1. Red: pure doubled strained DNA (dsDNA)
2. Blue: dsDNA + 1/10000 Midori Green DNA stain

550+ nm (EL PMT):
- Ratio region 1 (Signal to background): 1.00341
- Region 2: 13.5768
- Region 3: -21.9035 (problematic due to overshoot)

500+ nm (MAGIC PMT):
- Region 1: 1.78613 (both integrals close to 0)
- Region 2: 2.28086
- Region 3: 27.5077

DNA measurements

Comparison between pure Midori Green DNA stain sample and Midori Green DNA stain with dsDNA samples:

- EL PMT for tests were used. Unfortunately, MAGIC PMT which provided best separation in first tests did not work. Applying machine learning methods for statistical classification were used
- Different concentration of dsDNA were measured. Even the smallest dsDNA concentration 0.1 pg/µL show difference to background sample. In comparison to usual fluorometer (CYTATION 5) the signal of 62.5 pg/µL dsDNA was lost.

However, these results very preliminary and more measurements needed to confirm the results and understand systematic

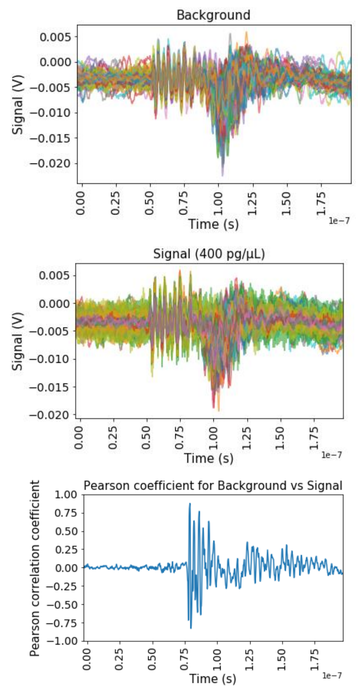


FIG 5. (Top): background, (Middle): signal, (Bottom): Pearson coefficient showing where both differ

In summary, our technology could be a promising tool for cancer detection. The principle of the our photonic detection system is based on exposing a sample of wavelength shifting material coupled to free DNA to light of certain wavelength and detecting afterwards the wavelength shifted light and provides information about the amount of free DNA in the sample. Current photodiodes devices have a low internal gain and a relatively large dark current, leading to the fact that at least few thousand photons are needed for detection. The developed setup is using PMT for the detection of the light. This kind of sensor has a high internal gain and allows therefore the detection of single photons. Furthermore, PMTs allow to measure the arrival time of the different photons with high precision which allow to detect significant lower amounts of free DNA (at picomolar range).

Moreover, the detection of low amount nucleic acid without amplification or sequencing opens the market for detection of another diseases such as: (I) Viral diseases (COVID-19..) by viral RNA or (II) Nosocomial infections by pathogenic bacterial DNA.

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ACKNOWLEDGEMENTS:

ATTRACT

This work was supported by the European Union's Horizon 2020 program ATTRACT, project PHIL
https://attract-eu.com/selected-projects/photonic-system-for-liquid-biopsy-phil/

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