A DNA biosensors-based microfluidic platform for attomolar real-time detection of unamplified SARS-CoV-2 virus

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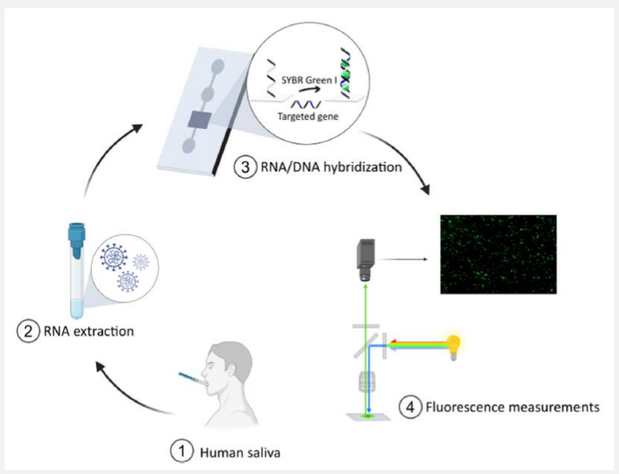
Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a pathogenic coronavirus which emerged in late 2019 and caused the ongoing major pandemic of the coronavirus disease 2019. In addition to the vaccines developed against SARS-CoV-2 social distancing, mass screening of the population and quarantine of the infected people have been efficient in mitigating the pandemic. We herein present the development of a microanalysis platform for SARS-CoV-2 viral charge detection in human saliva samples at attomolar concentrations and which does not require transcription and amplification steps.

Method



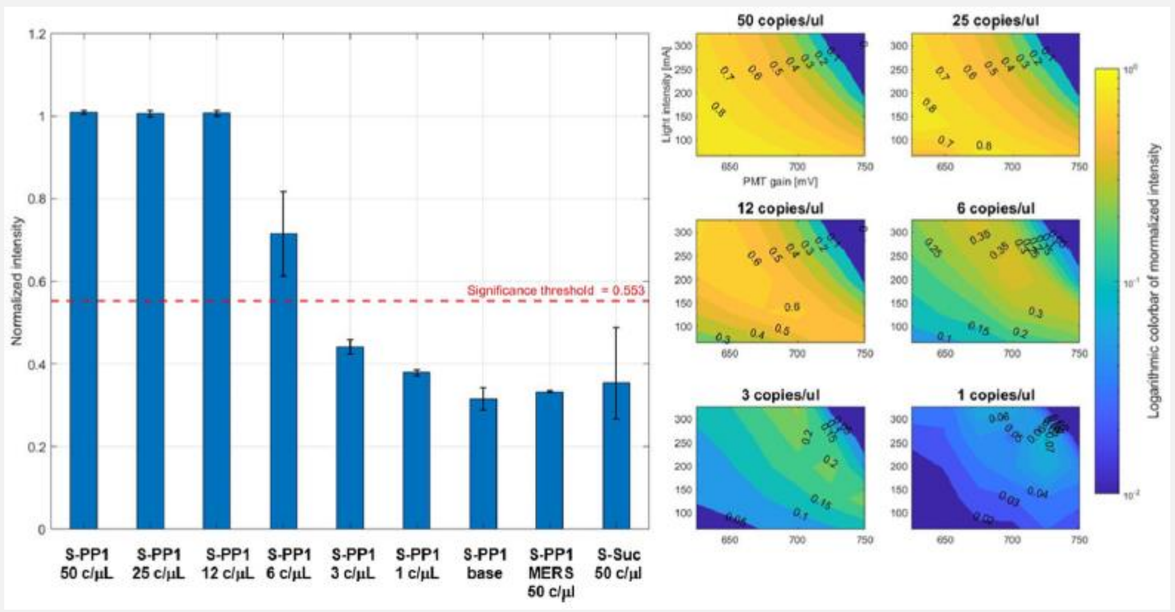
Specific probe was designed and covalently immobilized at the surface of glass slides to fabricate a DNA biosensor, allowing the identification of viral charge by the detection of RNA/DNA hybridization events at the surface of the sensing slides.

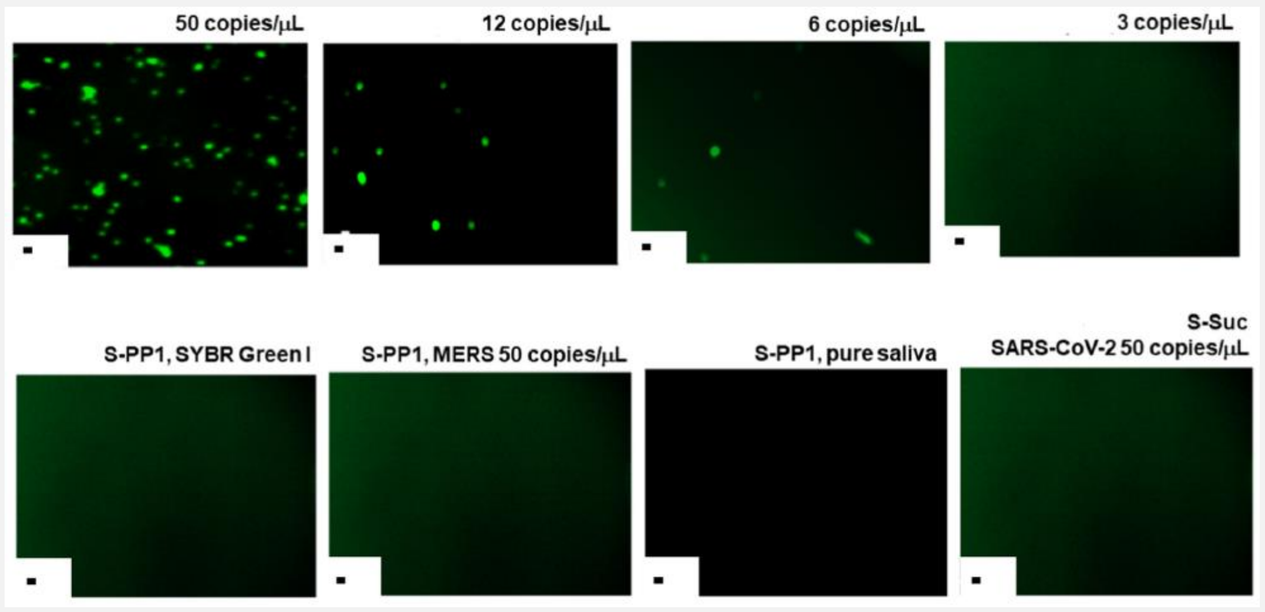
Saliva samples (20 µL) containing decreasing concentrations of SARS-CoV-2 virus were added to the surface of functionalized slides, with SYBR Green I.

A baseline experiment was performed on the slides, in the presence of the fluorescent dye only. Control samples included the use of slides without DNA probe immobilized with SARS-CoV-2 containing saliva and functionalized slides with MERS containing saliva.

Results

- In the absence of immobilized DNA probe, the readout values were close to the baseline, giving evidence for the absence of non-specific light emission.
- The specificity of the sensing slides for SARS-CoV-2 RNA detection was evidenced by the absence of positive readout after incubation with MERS containing saliva.
- At the baseline measurement, a margin of 50% of the base value was added to set the safety margin.
- All concentrations above 6 copies per µL led to readout values satisfying the safety margin and were thus reliably detected.





Further validation of the sensing slides was performed by immunofluorescence detection of SARS-CoV-2 RNA duplexed with the immobilized probe with saliva samples at different viral concentrations. The fluorescent emission was dependent on the viral load concentration and decreased over the studied range in accordance with the diminution of binding events.

Conclusions

Our system enabled the detection of SARS-CoV-2 virus from unamplified samples with a limit of detection of 6 copies per µL (10 aM), in 15 min and with high specificity.

The approach used and the platform design hold the potential to be easily adapted, in a future development, to the detection of other viruses by simple modification of the immobilized probe sequence.

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