Nano-plasmonic acceleration of nucleic acid amplification for pathogen detection

An automated system that couples microfluidics with plasmonic hot electron injection to accelerate colorimetric detection of DNA and RNA amplification is shown to achieve 95% detection accuracy in human saliva samples. This technique uses different amplification assays for pathogen identification and can differentiate between viral variants and subtypes.

This is a summary of:
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The mission

The lengthy protocols involved with assays based on nucleic acid detection and replication through nucleic acid probes (amplification assays) make it difficult to rapidly detect pathogens at the point of need, let alone automate the process from the sample preparation to the result[1]. This restriction hinders decision-making regarding managing the spread of viral respiratory infections, which — owing to their rising incidence — increasingly affect the global populace and economy. Such limitations were particularly highlighted in the early stages of the COVID-19 pandemic when there was a worldwide effort to develop and use diagnostic tests. Currently, antigen tests are the tests of choice for point-of-need settings owing to their simple and fast operation. However, antigen tests have lower sensitivity than polymerase chain reaction (PCR) tests[2], hampering their applicability for informing healthcare decision-making at the onset of infection.

The solution

We propose a technique — QolorEX — for obtaining a label-free quantitative colorimetric readout at single nucleotide resolution, which can obtain a result within minutes. This approach integrates phenol red-based reverse transcription loop-mediated isothermal amplification (RT-LAMP) and rolling circle amplification (RCA) colorimetric assays[3] with miniaturized plasmonic nanosurface microfluidics to achieve plasmonic hot-spot catalysis[4]. To streamline the amplification reaction, we developed a microfluidic cartridge that uses tunable angle-dependent microfluidic actuation to integrate the full cycle of sample collection, lysis, addition of assay reagents, nucleic acid amplification, and detection (Fig. 1a). To reduce user-introduced errors, we developed an illumination-coupled imaging box with automated actuators to handle, heat, and image the microfluidic cartridge without requiring input from the user. This automation allows the user to tap a button on a mobile phone application to begin the sequential operation of the microfluidic cartridge. The colorimetric readout is automatically detected by the complementary metal-oxide-semiconductor (CMOS) camera integrated with the imaging box. A machine-learning algorithm then analyses the readout and establishes positive or negative results; the result is then sent to the user's mobile phone.

We demonstrated that the rapid colorimetric readout obtained with QolorEX strongly depends on the injection of light-excited 'hot' electrons from the surface of self-assembled plasmonic nanoparticles into the mixture of sample and assay reagents in the sensing chamber. These hot electrons accelerate the nucleophilic reaction in the polymerization step of the amplification, which in turn results in the label-free pH-dependent colour transformation of phenol red from fuchsia to yellow (Fig. 1b). We found that plasmonic surfaces with 400 nm diameter nanoparticles offered higher electromagnetic-field enhancement and associated plasmonic hot-spot catalysis effects than plasmonic surfaces with other nanoparticle sizes, resulting in a 9-fold acceleration in the amplification reaction rate on average.

We validated the QolorEX system using various respiratory viruses and bacteria. We also demonstrated that QolorEX can differentiate between SARS-CoV-2 (sub)variants at the level of single nucleotide polymorphism. Additionally, QolorEX has a detection limit of 5 RNA copies per microlitre, which enables an accurate diagnosis from the onset of infection when viral loads are low. We also tested 33 SARS-CoV-2 positive and 15 negative human saliva samples. For these samples, QolorEX achieved a sample-to-answer time of 13 minutes with 95% accuracy, which is comparable to the gold standard quantitative PCR test. The limited user involvement, high accuracy, sensitivity, automation, and rapidity of QolorEX mean that this technique could address the need for PCR-grade tests in remote settings.

Future directions

QolorEX is truly a versatile platform. Although we tested it with colorimetric LAMP and RCA assays, it could potentially work with any colorimetric nucleic acid amplification assay. Moreover, QolorEX can detect any nucleic acid target and is not limited to only viruses and bacteria. In the future, QolorEX could be integrated with different amplification assays or used to investigate new applications such as environmental studies, food testing, and the discovery and efficacy assessment of drugs.

Currently, QolorEX requires clear sample media to enable colorimetric signal detection. Thus, further development is needed to allow the incorporation and testing of opaque media such as whole blood and stool samples. Additionally, the current approach uses pH-sensitive dyes for the colorimetric readout, which limits the pH range of samples that can be tested.

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EXPERT OPINION

"This work presents a comprehensive package combining microfluidics, electronics, and nanoplasmonic transduction mechanisms with a robust in-lab evaluation of the device using several clinically relevant viruses and bacteria. The authors use the bioassay study to successfully distinguish between bacterial and viral infection within minutes, which is an application that is both important and extremely difficult to achieve." **Nikhil Bhalla, Ulster University, Belfast, UK.**

FIGURE

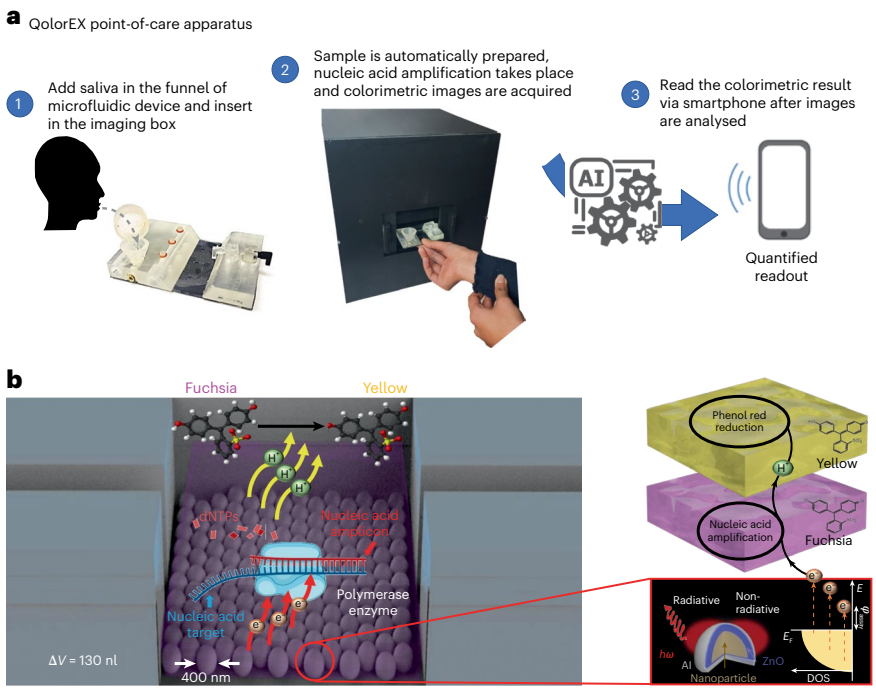

Fig. 1 | QolorEX operation and hot electron injection. a, A schematic of the operation of QolorEX. The user spits saliva into the collection funnel and puts the microfluidic cartridge inside the imaging box. The results are then automatically relayed through a smartphone app. **b**, Upon light excitation, the plasmonic nanomaterial accelerates the amplification assay owing to the excess electrons at the reaction interface. The increased amplification rate increases the rate of production of protons, which decrease the pH of the medium, causing the phenol red to rapidly change colour from fuchsia to yellow in the presence of the target pathogen (shown in the inset). dNTP, deoxyribonucleotide triphosphate; DOS, density of states; E, energy; E_F, Fermi energy; ϕ_{assay}, oxidation reaction initiation energy; $\hbar\omega$, photon energy; ΔV, sensing chamber volume. © 2023, AbdElFatah, T. et al.

BEHIND THE PAPER

In early 2020, we discussed the idea of plasmonic enhanced colorimetric read-out and the integration with microfluidics. It was during lockdown when we first came across RT-LAMP assays and had the idea to use a plasmonic enhanced colorimetric readout to facilitate COVID-19 diagnosis. From the beginning, we achieved faster detection times and lower detection limits than the original work[3]! Yet, we did not know what the source of this improvement was. For six months, we scoured the literature across various disciplines. One day, T.AF. stumbled upon a study of the nucleophilic reaction that occurs during DNA amplification[5]. Just the previous day, we had discussed the idea of electron injection from the surface of the plasmonic materials under light excitation. We wondered whether injected electrons could accelerate the amplification reaction. Confirming this hypothesis required many experimental validations. Yet, we shall never forget that feeling when everything clicked together. **T.AF., M.J. and S.M.**

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FROM THE EDITOR

"The work by AbdelFatah et al. presents a benchtop package combining nanoplasmonic transduction, microfluidics, and a colourimetric signal readout for smartphones with a quantitative output and is a step towards the development of an automated modular molecular diagnostic platform. Furthermore, the study demonstrates the robust distinction between clinically relevant pathogenic viruses and bacteria infection within minutes, opening up opportunities for deploying and adopting such technologies in resource-limited settings." **Raghavendra Palankar, Associate Editor, *Nature Nanotechnology*.**