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Terumitsu Azuma
Academia Sinica

Yuen Yung Hui
Academia Sinica

Oliver Y. Chen
Academia Sinica

Yuh-Lin Wang
Academia Sinica

Huan-Cheng Chang (✉ hchang@gate.sinica.edu.tw)
Academia Sinica

Research Article

Keywords: detection, immunoassay, sensitivity, virus

Posted Date: December 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1140710/v1>

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Experiments and Simulations of Thermometric Lateral Flow Immunoassay for Point-of-Care Testing

Terumitsu Azuma^{†,‡}, Yuen Yung Hui[†], Oliver Y. Chen[†], Yuh-Lin Wang^{†,‡}, and Huan-Cheng Chang^{*,†,§,||}

[†]*Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 106, Taiwan*

[‡]*Department of Physics, National Taiwan University, Taipei 106, Taiwan*

[§]*Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 106, Taiwan*

^{||}*Department of Chemistry, National Taiwan Normal University, Taipei 106, Taiwan*

Abstract

Temperature sensing is a promising method of enhancing the detection sensitivity of lateral flow immunoassay for point-of-care testing. A temperature increase of more than 100 °C can be readily achieved by photoexcitation of reporters like gold nanoparticles (GNPs) or colored latex beads (CLBs) on the strips with a laser power below 100 mW. Despite its promise, processes involved in the photothermal detection have not yet been well-characterized. Here, we provide a fundamental understanding of this thermometric assay by combining experiments and simulations using non-fluorescent CLBs as the reporters deposited on nitrocellulose membrane. By measuring the dependence of temperature rises on the number density of membrane-bound CLBs, we determined a 1.5-fold enhancement of the light absorption at 520 nm by the beads (diameter of 0.4 μm). The enhancement, however, was compromised by a 5-fold reduction of the incident laser power due to multiple scattering of the light in this highly

porous medium. The limit of detection was measured to be 1×10^5 particles/mm². In line with previous studies using GNPs as the reporters, the CLB-based thermometric assay provides a 10× higher sensitivity than color visualization, as demonstrated with the immunoassay for nucleocapsid proteins of the SARS-CoV-2 virus.

Introduction

The COVID-19 epidemic has emerged as a major public health concern since its first identification in Wuhan, China, on December 2019. Over the past 2 years, tremendous efforts have been made on a global level to fight the SARS-CoV-2 virus.¹ Alongside disease research and vaccine development, immunodiagnosics has been extensively carried out to understand and decipher the disease states of patients. Although various types of detection tools have been available,² there is still an urgent need for rapid, quantitative, and sensitive immunodiagnostic platforms in the combat against COVID-19.³ These technologies are fundamental to the development of better methods to prevent and control the epidemic, whose spread is exponentially growing and intrinsically unpredictable.⁴

Enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA) are two commonly used immunodiagnostic tools.⁵ Both methods are based on specific antigen-antibody reactions. While ELISA has been serving as the gold standard in the field for decades, the assay is time-consuming and costly. Particularly, it requires secondary antibodies linked with enzymes (such as horseradish peroxidase and alkaline phosphatase) as reporters in the individual assays and needs significant expertise to carry out the tests. LFIA, in contrast, is fast, low-cost, and scalable. It can rapidly diagnose a sample containing antigens or antibodies of interest by capillary flow across a membrane in typically 15 min.⁶ The principle of LFIA (or paper chromatography at that time) was first proposed by Yalow and Berson in 1959.⁷ The technique was originally developed to study the interaction between insulin and insulin-binding

antibody on a paraffin paper by radioactive detection. After 6 decades of development, the paper is now replaced by cellulose membrane and the scope of laboratory diagnosis has been expanded into multiplex formats to measure the immunoreactions of various samples in trace quantities. LFIA first appeared in commercial use as a general-purpose pregnancy test in 1988 and has become one of the most promising methods for point-of-care testing (POCT),⁸ including one-step home diagnosis of COVID-19 today.

Colloidal gold nanoparticles (GNPs) are the most widely used reporters in LFIA because the assay can be conducted colorimetrically by eye without the need of an instrument.^{9,10} The technique takes advantage of the fact that GNP has an exceptionally strong absorption in the optical region, known as surface plasmon resonance (SPR),¹¹ caused by the interaction between light and electrons on the surface of GNPs. For spherical GNPs of 40 nm in diameter (i.e. particles commonly used in LFIA),¹² the SPR band has a molar extinction coefficient of $8.42 \times 10^9 \text{ M}^{-1}\text{cm}^{-1}$ or an absorption cross section of about $3 \times 10^{-11} \text{ cm}^2/\text{particle}$ at 530 nm.^{13,14} The limit of detection (LOD) of these particles in the test zone of a LFIA strip made of nitrocellulose (NC) membrane was reported to be 3.78×10^6 particles/mm², measured by using a photo scanner for the color intensity in the blue channel.¹⁵ Although this color readout approach is direct and low-cost, the sensitivity is relatively low, with a LOD in the range of 1 – 10 ng/mL for the analyte concentration.

In view of this deficiency, various techniques have been developed to enhance the sensitivity as well as the quantification capability of the GNP-based LFIA. These techniques include surface-enhanced Raman scattering (SERS), photothermal detection (PTD), and photoacoustic (PA) detection.¹⁶ Among them, PTD is most practical and has readily achieved a 10× improvement in detection sensitivity.¹⁷⁻²⁰ A PTD system typically consists of a continuous-wave laser as the energy source to heat up the reporters, which absorb visible light and then emit infrared photons. An infrared camera measures the temperature rises of the

reporters trapped in control and test zones of the strips. Since the amount of the heat generated is linearly proportional to the amount of the trapped particles, this thermometric method is well suited for quantitative analysis.

The objective of this work is to provide a fundamental understanding of this so-called thermometric lateral flow immunoassay (TLFIA) using non-fluorescent CLBs (diameter of 0.4 μm) as the reporters and a green laser (wavelength of 520 nm) as the heat source. The reasons to choose CLBs in this study are twofold. First, the applicability of PTD to CLBs in LFIA is not yet known. Second, CLBs outperform GNPs in terms of multiplexing capability because organic dyes of different colors can be uniformly incorporated into the polystyrene matrix.²¹ For fluorescent polystyrene particle of 40 nm in diameter, each of them can contain more than 300 dye equivalents.²² Gaigalas et al.²³ have reported an absorption cross section of $\sigma \approx 2 \times 10^{-8} \text{ cm}^2/\text{particle}$ for 2.5- μm polystyrene spheres doped with green fluorescent dyes. Assuming a constant number density of dye molecules in particles of different sizes, scaling this value linearly with their volume gives $\sigma \approx 8 \times 10^{-11} \text{ cm}^2/\text{particle}$ for the 0.4- μm CLBs. This number is 3 $\times$ as large as that of 40-nm GNPs and is expected to further increase for CLBs containing non-fluorescent dyes with a concentration of up to 20%.²⁴

Here, we provide a systematic investigation on the laser-induced temperature changes as a function of the number density of CLBs on NC membrane, which is a highly light scattering medium. Both experiments and simulations are performed to assess important parameters such as effective laser powers used in the excitation as well as effective absorption cross sections of the membrane-bound CLBs involved in the PTD. Understanding in depth these light absorption processes, together with the heat transfer behaviors of these reporters on the LFIA strips, is anticipated to facilitate the establishment of a better and higher-sensitivity POCT platform. The utility of TLFIA is finally demonstrated with an immunoassay for nucleocapsid proteins of the SARS-CoV-2 virus.

Results and discussion

Turbidity. Prior to the experiments and simulations, knowing the structure of the NC membrane is crucial. SEM serves well the purpose. **Fig. 1** presents two SEM images of a typical NC membrane used in this experiment. As seen, the NC membrane has a highly fibrous and porous architecture, with a pore size of about 5 – 15 μm in diameter. Based on these images, a NC membrane model was constructed in **Fig. 2** with the pores represented by regularly arranged empty cylinders to simplify the numerical simulations with COMSOL, which is a computer software that can analyze a wide range of physical phenomena including structural mechanics and heat conduction (convection/diffusion).²⁵

As revealed by the SEM images shown in **Fig. 1**, NC membrane is a highly porous medium and thus can scatter light strongly. A significant intensity attenuation can occur when light passes through it. Turbidity (τ) is a useful parameter to describe the light scattering property of a matrix, defined as²⁶

$$\tau = -\frac{\ln(I/I_0)}{d} = -\frac{\ln[(I_0 - I_s)/I_0]}{d}, \quad (1)$$

where I_0 is the incident light intensity, I is the transmitted light intensity, I_s is the intensity of light scattered by the NC membrane, and d is the membrane thickness. We measured the turbidity by use of the 520-nm laser and found $\tau = 1.6 \times 10^2 \text{ cm}^{-1}$ for the dry NC membrane after properly taking into account the reflection loss of light from the polystyrene backing. The high turbidity suggests that most of the light scattered by the NC fibers in the membrane will have directions differing from that of the incoming beam. Multiple scattering occurs, resulting in a longer path of the light through the sample. In the presence of absorbing particles like CLBs in the matrix, the scattering leads to multiple absorption of light by the same particles, which in effect increases their absorption cross sections.²⁷

Effective absorption cross section. In the inset of **Fig. 3**, we show an optical image of red latex beads deposited on the NC membrane. The red spot was prepared by dropping 0.5 μL of the concentrated CLB solution (1.05×10^{12} particles/mL) onto the substrate to mimic the presence of these particles in the control or test zones of LFIA strips. As seen, the CLBs formed a coffer-ring-like structure with a diameter of 3.0 mm. We measured the temperature changes of the sample using the infrared camera equipped in the TLFIA reader to examine whether or not the radiation energy absorbed by CLBs could be rapidly transferred to the NC membrane and released as heat. **Fig. 4** shows a schematic diagram of the experimental setup. Considering that the laser beam had a diameter of only 0.6 mm, which is smaller than the CLB spot size, we moved the sample mounted on the motorized translation stage to allow the laser beam to come across the center of the spot. Presented in **Fig. 3** is a typical result of the thermometric measurement for the red latex beads exposed to the 520-nm laser with an output power of 10 mW. A temperature rise of 40 $^{\circ}\text{C}$ could be readily achieved, with an intensity variation of $\sim 10\%$ within the 3-mm band profile. Given this spot size and the NC membrane thickness of 0.1 mm, we obtained a mean number density of 7.5×10^{11} particles/ cm^3 for the CLBs uniformly distributed in the membrane.

To elucidate the photothermal process observed above, one needs to understand first how light is absorbed by CLBs in the highly scattering medium. We referred to the modified Beer–Lambert law, which was developed as a basis for near-infrared spectroscopy of biological tissues.^{28,29} The law treats the illuminated tissue, which is highly scattering also, as being optically homogeneous as

$$-\ln(I/I_0) = \mu_a L + G, \quad (2)$$

where μ_a is the absorption coefficient of tissue and L is the total mean path length of detected photons, and G is a geometry-dependent factor representing the intensity loss caused by light scattering. Given $\mu_a = \sigma N$ in our case, Eq. (2) becomes

$$-\ln(I/I_0) = \sigma NL + G = \sigma_{eff} Nd + G, \quad (3)$$

where N is the number density of absorbing particles in the membrane, and σ and $\sigma_{eff} \equiv \sigma L/d$ are the absorption cross section and effective absorption cross section of the membrane-bound particles, respectively. Note that in this equation, although the membrane-bound CLBs themselves also contribute to multiscattering, the effect is small compared with that caused by the medium alone, particularly at the low number density regions. To simplify subsequent analysis, we assume that the intensity loss due to the light scattering by CLBs in the NC matrix is negligible, i.e. $G = \tau d$, and write

$$-\ln(I/I_0) = (\sigma_{eff} N + \tau) d. \quad (4)$$

Prior to our measurements for σ_{eff} , we first determined σ by acquiring the extinction spectra of red and black latex beads in water after 1000 $\times$ dilution of their stock solutions. As shown in **Fig. 5**, a large background associated with Mie scattering of the sub-micron particles is clearly visible in the individual spectra. Although both the beads showed significant absorption at 520 nm, their absorption cross sections could not be precisely determined due to the lack of knowledge of the corresponding scattering cross sections. To circumvent this problem, we measured the absorbance of CLBs dispersed in a glycerol/water mixture (glycerol:water = 9:1, v/v) to reduce the light scattering effect.³⁰ The mixture is known to have a refractive index of $n = 1.47$ at 520 nm, close to $n = 1.59$ of polystyrene. Although there remains a refractive index mismatch, we corrected the effect by Mie scattering calculations for undyed polystyrene microspheres of the same size in the mixture. **Fig. 5** displays the absorption spectra of both the beads in 90% glycerol/water as well as their scattering-resulted

extinctions predicted by the calculations, from which we estimated an absorption cross section of $\sigma = 5.2 \times 10^{-10} \text{ cm}^2/\text{particle}$ and $\sigma = 2.7 \times 10^{-10} \text{ cm}^2/\text{particle}$ for the red and black latex beads, respectively, at 520 nm.

For CLBs, one would expect their absorption cross sections to be unchanged after deposition on the NC membrane since the dye molecules responsible for light absorption are incorporated into the interior of the polystyrene spheres. In contrast, the SPR bands of GNPs may markedly be altered after deposition as the particles can easily form aggregates on the NC membrane (Supplementary **Fig. S1**). The difference renders CLBs more appealing than GNPs in the present study. Despite this distinct advantage, issues associated with multiscattering of light within the highly porous membrane are still a concern. The multiscattering can not only give rise to an enhancement of light absorption as discussed earlier, but also result in an attenuation of the excitation light intensity. To experimentally measure σ_{eff} in Eq. (4), we used the TLFIA reader to investigate how the temperature rises varied with the CLB concentrations of the droplets deposited on the strips. This PTD approach serves well for the purpose because it has been previously demonstrated that the photothermal spectra obtained for absorbing molecules or nanoparticles dispersed in scattering medium corresponds to the absorption component of the sample's extinction.³¹⁻³³

Our experiment started with a temperature measurement for a sample spot prepared with concentrated CLB solution (4% w/w) and excited by a 520-nm laser at a power of 10 mW. The power used in this measurement was kept low to avoid photodamage of the dye molecules embedded in the microspheres. **Fig. 6a** shows temporal evolutions of the measured temperature rises as a function of the excitation time for both red and black latex beads. The temperature rise reached its steady state in 30 s and no noticeable photodamage of the particles was found within the temperature measurement time of 120 s. We recorded the concentration-dependent temperature changes of both CLBs deposited on the strips within this time window.

As shown by the data in **Fig. 6b**, the temperature rise increased nearly linearly with the CLB number density at small N but was gradually saturated at $N = 4 \times 10^{11}$ particles/cm³. To interpret the experimental findings, we consider that the magnitude of the temperature change is in linear proportion to the amount of the energy absorbed by the samples as $\Delta T \propto I_a = I_0 - I_s - I$, where I_a is the intensity of the light absorbed by CLBs. According to Eqs. (1) and (4), the temperature change can be written as

$$\Delta T = CI_a = C(I_0 - I_s - I) = CI_0 \exp(-\tau d) [1 - \exp(-\sigma_{eff} Nd)], \quad (5)$$

where the parameter C is a function of laser power, the heat capacity of NC membrane, sample volume, and the rate of energy transfer from the heated particle to its environment. By fitting the experimental data in **Fig. 6b** with Eq. (5) and keeping the value of C the same in these two cases, we obtained $\sigma_{eff} = 7.5 \pm 0.7 \times 10^{-10}$ cm²/particle for the red CLBs and $\sigma_{eff} = 4.7 \pm 0.5 \times 10^{-10}$ cm²/particle for the black CLBs at 520 nm. Notably, both the values are about 1.5-fold as large as that estimated by direct absorption of the beads dispersed in the glycerol-water mixture.

Effective excitation laser intensity. An understanding of the PTD process is not complete if its heat transfer dynamics is not known. To deduce such information, we conducted numerical simulations with COMSOL and considered the thermal diffusion in three dimensions.²⁵ In the simulations, we assumed that the heat generated by laser irradiation was rapidly transferred from CLBs to the NC membrane and the presence of these particles in the membrane did not affect much the heat transfer dynamics. Following Eq. (5), we write the heat generation as

$$Q = \frac{I_a}{d} = \frac{I_0 \exp(-\tau d)}{d} [1 - \exp(-\sigma_{eff} Nd)] = \frac{I_{eff}}{d} [1 - \exp(-\sigma_{eff} Nd)], \quad (6)$$

where $I_{eff} \equiv I_0 \exp(-\tau d)$ is the effective laser intensity. For the 520-nm laser used in this study, it has a specified beam diameter of 0.6 mm ($1/e^2$), corresponding to a full width at half

maximum (FWHM) of 0.35 mm. Given a laser power of 10 mW and $\exp(-\tau d) = 0.20$, we calculated the laser intensities to be $I_0 = 10 \text{ W/cm}^2$ and $I_{eff} = 2.0 \text{ W/cm}^2$, which suggests an attenuation of the incident laser power by 80% due to the strong light scattering by the membrane. Interestingly, the loss is compensated by a gain (σ_{eff}/σ) of the optical path length of the light in the medium.

To test the validity of our model, we first compared the experimental data with the simulations conducted under the steady-state condition, $\partial T/\partial t = 0$. **Fig. 7** shows the measured and calculated temperature rises as a function of the number density of CLBs on the NC membrane. With the particle number density varying over 3 orders of magnitude, good agreement was achieved between experiments and simulations using $\tau = 1.6 \times 10^2 \text{ cm}^{-1}$ and $\sigma_{eff} = 7.5 \times 10^{-10} \text{ cm}^2/\text{particle}$ (red) and $\sigma_{eff} = 4.7 \times 10^{-10} \text{ cm}^2/\text{particle}$ (black), despite that offsets in the y-axis at the zero CLB number density were found in both measurements (inset in **Fig. 6b**). These offsets appeared due to laser heating of the air-dried NC membrane with deionized water as the sample only (Supplementary **Fig. S2**). We further compared the spatial temperature profiles of the heated areas between measurements and calculations using the same input parameters. As shown in **Fig. 8** for red latex beads only, satisfactory agreements in both peak height and width were reached between these two sets of data over a wide number density range. The remaining discrepancies are attributed to the structural inhomogeneity of the NC membrane, the approximation that $G = \tau d$, as well as the variation of τ , whose value depends on the sample wetness.

In **Fig. 9**, we also compare the measured temporal temperature changes with simulations for membrane-bound red latex beads using Eq. (6) with the input parameters of $N = 7.5 \times 10^{11} \text{ particles/cm}^3$, $I_{eff} = 2.0 \text{ W/cm}^2$, and $\sigma_{eff} = 7.5 \times 10^{-10} \text{ cm}^2/\text{particle}$ over different time periods. The simulations predicted a less steep temperature rise than that observed in experiment. We ascribed the discrepancies to the fibrous structure of the NC membrane, where highly localized

heating could occur in these highly inhomogeneous filaments. Noticeably, the experimental result indicated that the temperature rise could rapidly reach 80% of its steady-state value within 1 s of the excitation. However, only about 20% of the incident laser power was useful for the heating.

The ability to perform quantitative analysis is an essential feature of TLFIA. We demonstrated the ability by fitting the experimental data with a linear line over the low number density region of CLBs (inset in **Fig. 6b**). The good fit indicates that the technique is well suitable for quantitative purpose. For experiments conducted with a laser power of 10 mW, we obtained a slope of 3.8×10^{-10} K·cm³/particle and 2.7×10^{-10} K·cm³/particle for red and black latex beads, respectively. Based on the fact that the highest temperature resolution of the infrared camera is 0.1 °C, it suggests an ultimate sensitivity of about 3×10^4 particles/mm² for both particles on the 0.1-mm-thick NC membrane. With the use of a 40-mW laser, the sensitivity is expected to be enhanced to 1×10^4 particles/mm², comparable to that of fluorescent nanodiamonds (diameter of 100 nm) reported in our previous work using a magnetic modulation technique to achieve background-free detection.³⁴ However, in practice, we found that the NC membrane itself was also heated up by 4 °C when excited by the high-power laser for only 3 s (Supplementary **Fig. S2**). This effect, together with the fluctuations (~1 °C over 10 min) of room temperature in our lab,³⁵ deteriorated the sensitivity of this temperature sensing to LOD $\approx 1 \times 10^5$ particles/mm² for both CLBs.

CLB-based COVID-19 testing. After laying a solid foundation for the TLFIA platform, we applied it for quantitative diagnostics of the nucleocapsid proteins (NPs) of SARS-CoV-2 using commercially available COVID-19 antigen test strips. Specifically, the strips employed red latex beads (0.4 μm in diameter) to mark the test lines and black latex beads (0.4 μm in diameter) to mark the control lines. Shown in **Fig. 10a** is the result of a representative measurement for

the temperature profiles of both control and test zones of the COVID-19 antigen test strip after the assay. The strip was irradiated with a 520-nm laser at a power of 40 mW to enhance the signal-to-noise ratio. As expected, both the CLBs could absorb the green light, leading to temperature rises. Comparing these two temperature rises with the data presented in **Fig. 6**, we estimated that the amounts of black and red latex beads captured on the control and test zones of the strip were 1.3×10^7 particles/mm² and 8.6×10^5 particles/mm², respectively, for the assay conducted at the NP concentration of 1.5 ng/mL.

In the inset of **Fig. 10b**, we show a photograph of the strips after assay over a wide NP concentration range of 0 – 100 ng/mL. The LOD of color visualization with the naked eye was about 1.5 ng/mL. In contrast, TLFIA was able to detect NPs in the sample solution with a concentration as low as ~0.1 ng/mL, which is lower than that of the visual inspection by one order of magnitude (**Fig. 10b**). It has been well documented in literature that each SARS-CoV-2 virion contains 35 – 40 viral RNA-protein (vRNP) complexes and within the individual vRNP, about 800 nt of the genomic RNA are wrapped around 12 copies of NP.^{36,37} Given a molecular weight of ~100 kDa for the NP dimer,³⁸ the LOD of ~0.1 ng/mL suggests a detection limit of $\sim 3 \times 10^6$ virions/mL for the SARS-CoV-2 virus. It should be emphasized here that the LOD of TLFIA so determined for the NP of SARS-CoV-2 is primarily limited by the large variations between test strips as well as the non-specific binding among capture antibodies, BSA, and detection antibodies, rather than the intrinsic sensitivity of TLFIA. A 10-fold reduction of the LOD is possible if better manufactured strips and antibody pairs are available. These features, together with further technological improvements, will make TLFIA useful as a rapid and sensitive tool to quantify the levels of SARS-CoV-2 infection and other diseases among patients in clinics and hospitals.³⁹

Conclusion

We have systematically conducted experiments with a home-built TLFIA reader and performed simulations with COMSOL for photothermal measurements of CLBs deposited on NC membrane strips. Our results show a 1.5-fold enhancement of the light absorption by the membrane-bound beads, along with a 5-fold diminution of the incident laser power, due to multiple light scattering of the highly porous medium. We validated the quantification capability of this method with concentration-dependent temperature measurements for the NC-bound CLBs and also a testing for the nucleocapsid proteins of SARS-CoV-2 virus, showing that the CLB-based TLFIA is able to offer a 10× higher sensitivity than color visualization with the naked eye. The present study provides a solid basis for the implementation of TLFIA as a quantitative tool for immunodiagnosics with high promises to achieve a detection sensitivity and a measurement accuracy comparable to those of ELISA. We expect that continuous improvement and process optimization of the technology will open up new avenues and perspectives of LFIA, enabling its practical applications to address a wide range of key issues in the life sciences.

Finally, we note that the theoretical modeling for the multiscattering of light by NC membrane as presented in this work is general and applicable to fluorescence-based LFIA as well. The assay employs reporters like fluorescent polystyrene beads, quantum dots, and fluorescent nanodiamonds, etc.,^{34,40,41} whose detection sensitivity depends on the amount of light absorbed by the particles, similar to that of PTD.

Methods

Chemicals and Materials. Undyed polystyrene microspheres were obtained from Sigma-Aldrich, red and black latex beads were from Fisher Scientific, gold colloid was from BBI Solutions, NC membrane was from Millipore, bovine serum albumin (BSA), phosphate-buffered saline (PBS), and all other chemicals were from MilliporeSigma and used without

further purification. Both SARS-CoV-2 NPs and COVID-19 antigen test strips were obtained from BF Biotech, Taiwan.

Sample preparation. Two types of CLBs were used in this study: red and black latex beads with specified diameters of 0.41 μm and 0.42 μm , respectively. They were deposited on NC membrane strips, each of which was 78 mm long and 4 mm wide with a polystyrene backing of 100 μm in thickness.⁴² To prepare the samples for PTD, droplets (0.5 μL /each) of CLB suspensions after serial dilution of the stock solution (4%, w/w) were added at the centers of the NC membrane strips to form spots. The strips were then air-dried and placed in plastic cassettes prior to measurements.

Instrumentation. The home-built TLFIA reader consisted of a continuous-wave 520 nm laser (OBIS, Coherent) as the excitation source, a sample holder for the plastic cassette, an Arduino UNO board to control a stepper motor mounted on a linear translation stage (SEMC1D-50, SF Technology), and an infrared camera (SC325, FLIR) to detect thermal radiation. A program written in LabVIEW 2020 (Laboratory Virtual Instrumentation Engineering Workbench) scanned the sample across the laser spot and analyzed the data collected by the camera. **Fig. 4** shows an instrument layout of the TLFIA system.

Scanning electron microscopy (SEM). Structures of the NC membrane (FF120HP Plus, Millipore) were interrogated with a scanning electron microscope (Phenom ProX, Phenom-World) that acquired the images. Elemental mapping was conducted with energy-dispersive X-ray spectroscopy (EDS) featured by the instrument.

Optical extinction spectroscopy. Extinction spectra of undyed polystyrene microspheres and CLBs in aqueous solution were obtained in 10-mm cuvettes using a standard UV-Vis spectrophotometer (U-3310, Hitachi). Turbidity of the NC membrane was measured in an attenuation mode with the detector positioned at an angle that was 180° relative to the incident light beam. A continuous-wave 520-nm laser (OBIS, Coherent) with an output power of 1 mW served as the light source and a photodiode (SM1PD1B, Thorlabs) with an active area of 10 × 10 mm² behind a Ø9 mm clear aperture detected the signals. Neutral density filters were applied to attenuate the laser intensity to avoid saturation of the detector.

Immunoassays. The levels of SARS-CoV-2 NPs were measured by using the TLFIA reader, along with commercially available COVID-19 antigen test strips. To carry out the assays, we first prepared the NP solutions in PBS containing ~1% BSA after serial dilution with the same buffer, followed by dipping the strips vertically in the sample solutions (100 µL/each) for 15 min to complete the capillary flow. The air-dried strips were finally placed in plastic cassettes for TLFIA measurements. All the experiments were repeated in triplicate.

Mie scattering calculations. Scattering cross sections of undyed polystyrene microspheres with diameters of 0.1, 0.6, and 1.1 µm were calculated based on the Mie theory. We employed a MATLAB program, similar to that developed by Matzler,⁴³ and inputted the refractive indexes (*n*) of water and polystyrene listed in **Table 1** to perform the calculations. The calculated values were then compared with experimental measurements using a standard UV-Vis spectrophotometer (Supplementary **Fig. S3**).⁴⁴

Heat transfer calculations. The simulations were conducted with COMSOL Multiphysics (Comsol AB).⁴⁵ We selected blocks and cylinders from the “Geometry” list of the program to

construct a model for the strip. For the sake of simplicity, the NC membrane and polystyrene backing of the strip were represented by two rectangular blocks with the same dimensions of 10 mm (length) $\times$ 4 mm (width) $\times$ 100 μ m (thickness), and the pores were represented by empty cylinders with dimensions of 15 μ m (diameter) $\times$ 100 μ m (height) and separated by 25 μ m from each other (**Fig. 2a**). To reduce the computational load without losing much the precision, the porous region was confined to be 10 mm (length) $\times$ 0.84 mm (width) $\times$ 100 μ m (thickness) only at the center of the membrane. Additionally, the cylinders at the boundary of the laser-irradiated region (i.e. the circled area in **Fig. 2b**) were removed to facilitate the calculations. Overall, the model consisted of a total of 13200 vertical cylinders to delineate the high porosity of the NC membrane.

To simulate the heat transfer processes, we selected the interface “Heat Transfer in Solids or Fluids” of the COMSOL program and used the following thermal conduction equation to map out the temperature changes of the system both temporally and spatially:

$$\rho C_p(\partial T/\partial t) = \nabla \cdot (k \nabla T) + Q, \quad (7)$$

where ρ is the material density (kg/m³), C_p is the heat capacity [J/(kg·K)], k is the thermal conductivity [W/(m·K)], T is the temperature (K), and Q is the amount of heat generated (W/m³). **Table 1** lists the values of ρ , C_p , and k for four materials that play key roles in the simulations.⁴⁶⁻⁴⁹ To run the model, we first defined the boundary conditions by entering the temperature 293.15 K at the four faces of the walls lying in the x - z and y - z planes (denoted in **Fig. 2**) and then computed both the time-independent and time-dependent temperature profiles for the NC membrane with polystyrene backing.

Data Availability

All the data are available on request from the corresponding author (H.C.C.).

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Acknowledgements

This work was supported by Academia Sinica and the Ministry of Science and Technology, Taiwan, with Grant No. 110-2113-M-001-046 -MY3. We thank Fang-Zhen Liao and Yi-Xiu Tang for experimental assistance as well as Ching-Ya Cheng and Chia-Lung Hsieh for Mie scattering calculations.

Author Contributions

T.A. and Y.Y.H. performed the experiments, implemented the simulations, analyzed the data, and wrote the manuscript, O.Y.C. performed the experiments, Y.L.W. edited the manuscript, and H.C.C. analyzed the data and wrote the manuscript. All authors reviewed the manuscript.

Competing Interests

The authors declare no competing financial interests.

Additional Information

Supplementary information. SEM images of GNPs on NC membrane (Fig. S1), temporal temperature changes of heated CLBs on NC membrane (Fig. S2), Mie scattering calculations for undyed polystyrene microparticles (Fig. S3).

Correspondence and requests for materials should be addressed to Huan-Cheng Chang. (E-mail: hchang@gate.sinica.edu.tw)

Table 1. Parameters used in the heat transfer calculations.

Materials	ρ [kg/m ³]	C_p [J/(kg·K)]	k [W/(m·K)]
Nitrocellulose	1.4×10^3	1.5×10^3	0.2
Polystyrene	1050	1100	0.12
Water	993	4182	0.606
Air	1.2	1005	0.0262

Fig. 1 SEM images of NC membrane. SEM analysis of the structure of NC membrane with a magnification of 570 $\times$ (left) and 6600 $\times$ (right). Scale bars: 100 μm (left) and 10 μm (right).

Fig. 2 Photothermal model of laser-irradiated CLBs on NC membrane. **a** General and **b** top views of the photothermal model used in COMSOL simulations for the laser-irradiated NC membrane with polystyrene backing. Dimensions of the NC membrane are 10 mm (length) $\times$ 4 mm (width) $\times$ 0.1 mm (depth) with a porous region of 10 mm (length) $\times$ 0.84 mm (width) $\times$ 0.1 mm (depth). The pores holding air are 15 μm in diameter and separated by 25 μm from each other. The black circle denotes the laser-irradiated region with a beam diameter (FWHM) of 0.35 mm.

Fig. 3 Temperature profile of a laser-irradiated CLB spot on NC membrane. The measurement, conducted with a TLFIA reader, is insensitive to room lighting due to the detection of thermal radiation. The corresponding optical image of the spot made of red latex beads is shown in the inset.

Fig. 4 Instrument layout of the TLFIA reader. The excitation laser is unfocussed and has a beam diameter of 0.6 mm with an adjustable output power of 0 – 40 mW. The instrument is portable, having dimensions of 32 cm (length) × 20 cm (width) × 12 cm (height).

Fig. 5 Extinction spectra of CLBs in solution. The red and black latex beads were suspended in water and 90% glycerol/water for the measurements. Concentrations of the both beads are 0.004%. Dashed curves represent scattering-resulted extinctions predicted by Mie calculations for undyed polystyrene microspheres (diameters of 0.41 μm and 0.42 μm) in 90% glycerol/water.

Fig. 6 Photothermal detection of CLBs on NC membrane. a Temporal temperature changes of laser-irradiated red and black latex beads on NC membrane strips. Inset: enlarged view of the time evolutions over 0 – 3 s. **b** Variations of temperature changes with the number densities of laser-irradiated red and black latex beads on NC membrane strips. The effective absorption cross sections of both particles are obtained by fitting the experimental data to Eq. (5) in text using the same value of C in both fittings. Inset: enlarged view of data in the low number density region. Solid curves are best fits of the experimental data with the linear function, $y = Ax + B$, where A and B are constants.

Fig. 7 Comparison between experiments and simulations. The comparison is made for the temperature changes of both red and black latex beads on NC membrane strips as functions of their number densities upon laser irradiation. Inset: enlarged view of data in the low number density region.

Fig. 8 Comparison between simulations and experiments. The comparison is made for the temperature profiles of laser-irradiated red latex beads on NC membrane as a function of the number density of the beads: **a** experiments and **b** simulations. Parameters used in the simulations are $N = 3.7 \times 10^9 - 7.5 \times 10^{11}$ particles/cm³, $I_{eff} = 2.0$ W/cm², and $\sigma_{eff} = 7.5 \times 10^{-10}$ cm²/particle.

Fig. 9 Comparison between simulations and experiments. The comparison is made for the temporal temperature changes of laser-irradiated red latex beads on NC membrane. Inset: enlarged view of the time evolutions over 0 – 3 s. Parameters used in the simulations are $N = 7.5 \times 10^{11}$ particles/cm³, $I_{eff} = 2.0$ W/cm², and $\sigma_{eff} = 7.5 \times 10^{-10}$ cm²/particle.

Fig. 10 COVID-19 testing with TLFIA. **a** Temperature profile of CLBs captured on a COVID-19 antigen test strip for SARS-CoV-2 NP, obtained with the TLFIA reader. The assay is conducted at the NP concentration of 1.5 ng/mL. Integrated areas of the two peaks denoted by “Control (C)” and “Test (T)” are calculated to obtain the T/C ratio. **b** Comparative LFIA for SARS-CoV-2 NPs by thermometric detection and color visualization (inset) with COVID-19 antigen test strips. The solid curve is a best fit of the experimental data to a hyperbola function. The black arrow indicates the LOD of visual inspection and the dashed line indicates the LOD of TLFIA, estimated from the standard deviation of the blank experiment with a confidence level of 99% (or $3\times$ standard deviation).

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