The detection of HIV using plasmonically active colloidal gold nanoparticles

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Abstract. Localized surface plasmon resonance (LSPR) phenomenon occurs when incident light of specific wavelength excites the free electrons on the gold nanoparticles surface, which then leads to the enhancement of the nanoparticle surface electromagnetic field. The enhanced electromagnetic field has a short decay length and is localized in LSPR as opposed to the surface plasmon resonance (SPR) where the activated surface plasmons propagate. The short electromagnetic field decay length in LSPR means that it is highly sensitive to the refractive index changes near the gold nanoparticles surface rather than the bulk refractive index in SPR. This makes this technique efficient particularly to changes induced by subtle interactions. In this work, LSPR was used to differentiate between samples with HIV and the ones with no HIV. A glass slide was treated with 1% APTES solution in ethanol before depositing a layer of gold nanoparticles. An anti-HIV-gp120 antibody was added as a biorecognition element prior to the addition of the HIV pseudovirus as the analyte. Thereafter the slide was analyzed on an LSPR system using a green LED light. The results showed that when using 50 nm gold nanoparticles, there was a clear distinction between a sample with the HIV pseudovirus and the one without it as shown by the varying light transmission intensities between the negative sample and the sample with the virus. This denotes that LSPR is sensitive enough as a label free detection method for virus detection. This can be used for the development of simple and cost-effective ways of detecting various diseases in developing countries.

1 Introduction

By the end of 2023, globally, there was an estimated 40 million individuals living with Human immunodeficiency virus (HIV), which demonstrates that this infection remains a public health challenge [1] There remains no cure for the HIV infection and according to the World Health Organization (WHO) recommendations all people should get tested and the infected ones are to start treatment as early as they are aware of their HIV status regardless of their CD4 count [2] This therefore has created a need to increase testing platforms, thus reaching as many people as possible irrespective of geographical location and socioeconomic factors. The investigation of photonics-based technologies for HIV diagnostics seeks to provide a sensitive, accurate and simple to use device that can be used at point of care particularly in resource limited settings. Localized surface plasmon resonance (LSPR) is one of the promising photonics-based technologies that can be used for the detection of HIV. The LSPR describes a phenomenon that occurs when the incident light of a specific wavelength excites free electrons on the surface of nanoparticles, which results in the enhancement of the nanoparticle surface

electromagnetic field. The enhanced electromagnetic field has a short, localized decay length, thus making the LSPR highly sensitive to the refractive index changes near the nanoparticles surface [3]. In the LSPR system when the refractive index increases, the wavelength peak of the incident light shifts towards the longer wavelengths (red shift) whereas the decrease of the refractive index causes a blue shift. This shift facilitates the detection of various analytes due to changes in the refractive index [4]. Thereby making this technique efficient particularly to changes induced by subtle interactions.

LSPR biosensing is performed without the use of dyes, thus simplifying the process and reducing costs. Owing to the strong electromagnetic field enhancements, LSPR allows the detection of analytes at low concentration. This type of biosensor can be integrated into microfluidic devices and multiplexed and enabling the simultaneous detection of multiple analytes in portable diagnostic systems. In this work LSPR was used for the detection of Human immunodeficiency virus (HIV) with the aim of developing a laser driven real-time microfluidic, multiplexed device that can be used in resource limited settings. Furthermore, this work aims to contribute towards the UNAIDS 95-95-95 fast track strategy of eradicating HIV/AIDS by 2030. This strategy states that by 2030 95% of people living with HIV (PLHIV) should know their infection status, 95% of those living with the virus should be receiving antiretroviral treatment, and 95% of all those on treatment be virally suppressed [5]The detection of HIV was performed by coating glass slides with gold nanoparticles and functionalizing the slide using anti-HIV-gp120 antibody before the addition of the virus and analyzing on the LSPR system.

2 Methodology

2.1 Localized surface plasmon resonance system

The work presented here was performed using an LSPR system as described in Mcoyi et al 2024 [6] The system consisted of a 520 nm light emitting diode (LED) as a light source (Thorlabs, PL201, Pout < 1mW). The light was directed to the XYZ stage, where a glass sample slide was placed for detection. A focusing lens was used to couple the light into the optical fiber, which guided the light to the spectrometer connected to the computer for spectra acquisition.

2.2 Preparation of a sample slide and samples

Glass slides were washed by sonication in acetone, absolute ethanol, and ultrapure water. After drying with nitrogen gas, the slides were then treated with oxygen plasma (hydroxylation). Glass slides were then silane treated by incubation in 1% APTES (3-Aminopropyltriethoxysilane) (Sigma-Aldrich) for 24hr at room temperature. After incubation, the slides were washed with water and dried. Multiple wells were created on the glass slide for different samples (which are gold nanoparticles (AuNPs), gold nanoparticles conjugated to the anti-HIV gp120 antibody (Abcam, ab21179) (AuNPs-Ab) and the conjugate with the virus (AuNPs-Ab-virus). In the first well, 250µl of 60 nm (Cytodiagnostics, Canada) nanoparticles were added, in wells 2 and 3, 250 µl of the AuNPs-Ab conjugate was added (conjugation process outlined in section 2.3) and allowed stabilize by letting them stand for one hour at room temperature. In the third well, 100µl of the HIV pseudovirus was added and incubated for 1 hour at 4°C. Pseudovirus production is summarized in section 2.4.

2.3 Conjugation of the antibody to the nanoparticles

Similar ratios of N-hydroxysuccinimide (NHS) (Sigma-Aldrich, 56480), 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) (Sigma-Aldrich, E7750) and polyethylene glycol (SH-PEG-COOH) (Sigma-Aldrich, 757845) were mixed in ultrapure water and shaken for an hour. Thereafter, the anti-HIV-1 gp120 antibody (Ab) was added to the solution and further mixed for 2 hours on an orbital shaker before filtering and washing twice with 1X phosphate-buffered saline (PBS) in a 50 kDA filter. Fifteen microlitres of the activated antibody were eluted by inverting the filter and spinning it down. Subsequently, 12µL of the activated antibody was mixed with 200µL of 1:2 diluted 50 nm gold nanoparticles and incubated for an hour to form a AuNPs-Ab bioconjugate. This was then characterized by UV-Vis absorption spectroscopy.

2.4 Production of the HIV pseudovirus

The HIV pseudovirus was produced by transfecting 293T/17 cells using a transfection reagent as described by Lugongolo et al [7]. Briefly, cells were seeded in a T75 flask with 4µg of HIV-1 Plasmid and 8µg of env HIV-1 plasmid backbone. The supernatant containing the pseudovirus was harvested after 48 hours and filtered with a 0.45µm filtration system and stored in cryovials at 80°C.

3 Results

To optimize and characterize the LSPR system, a simulation approach was used to analyze the impact of gold nanoparticle size on the LSPR peak shift. It is documented that the optical response of gold nanoparticles differs from small to large nanoparticles. In small nanoparticles, the response is mainly dominated by absorption, while in larger nanoparticles, the optical outcome is dominated by scattering [8]. Theoretical modeling of gold nanoparticles is possible with Mie theory, which is adapted in Maxwell's equation for spherical particles accommodate optical outcomes and has been successfully used to model optical extinction. Figure 1A shows the simulated effect of nanoparticle size with corresponding LSPR peak shifts and how peaks shift with varying nanoparticle sizes. The LSPR peak shifts increase as nanoparticle size increases.

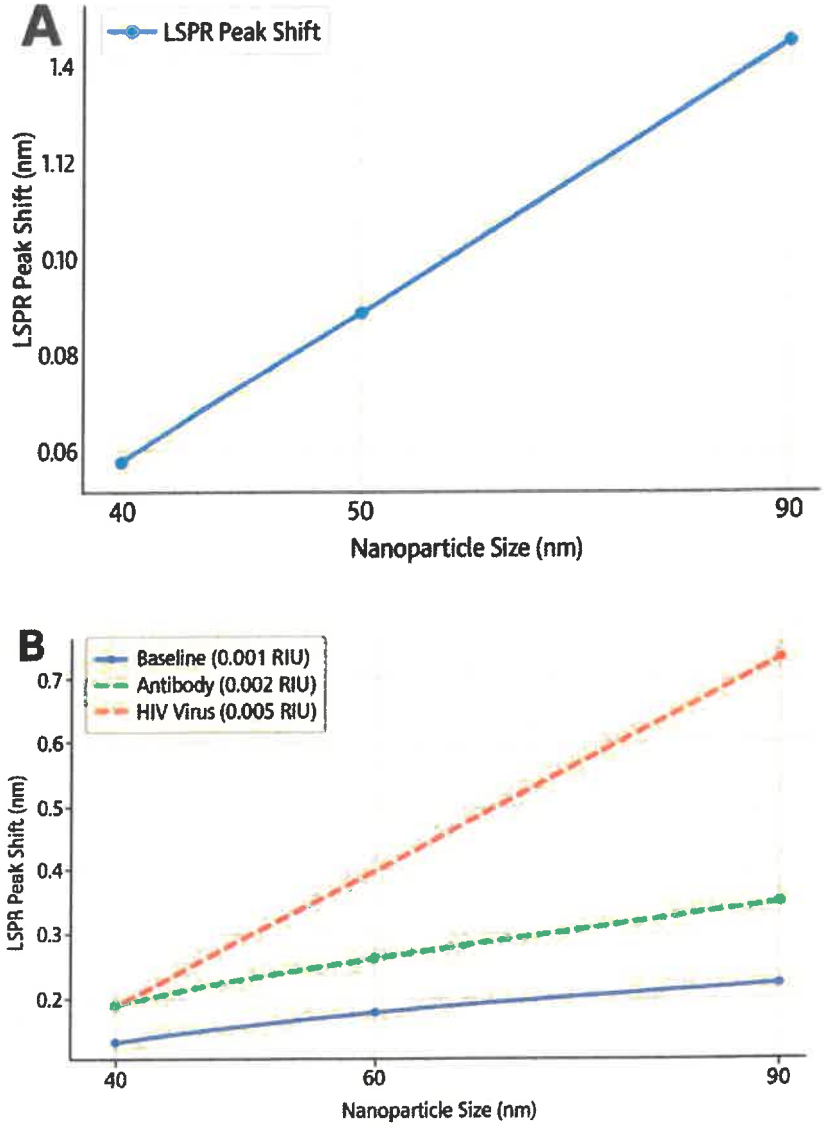

Figure 1: Simulated effect of nanoparticles showing (A) different nanoparticle sizes and their LSPR peak shifts. (B) Simulated effect of nanoparticles with varying sizes (blue line), nanoparticles conjugated to antibody (green line) and nanoparticles conjugated to the antibody and the virus (red line) and their LSPR peak shifts.

The shifts are distinctively visible for all sizes of gold nanoparticles, with 40 nm exhibiting the smallest peak shifts, and 90 nm exhibiting the highest peak shifts. An increase in nanoparticle sizes correlates with higher LSPR wavelength peaks as larger nanoparticles absorb at higher wavelengths. The LSPR wavelength shift can be further induced by refractive index changes around the nanoparticle surface as shown in figure 1B. In this instance, the impact of refractive index as small as 0.001 was observed for antibody binding to the gold nanoparticles, and 0.003 following the addition of the virus to the nanoparticles-antibody conjugate. This analysis suggests that the bigger sized gold nanoparticles are more sensitive to subtle refractive index changes. The results exhibit a true relationship between LSPR peak wavelength shifts and changes in refractive indices, which tends to follow a linear trend [3, 9]. The simulation data was used to guide the selection of the gold nanoparticles sizes for the experimental work. The data suggests that 90 nm gold nanoparticles are ideal for analyzing and detecting HIV through refractive-index sensing. However, the LSPR extinction in bigger sized nanoparticles is predominantly due to scattering rather than absorption exhibited by small sized nanoparticles. The transition between absorption to scattering occurs around a diameter of 70 nm. The experiment focused on absorption, and after fixing the peak absorption wavelength for transmitted intensity to analyze the binding of HIV to specific antibody, the experimental work was performed using 50 nm sized gold nanoparticles. The 50 nm nanoparticles used in the study were first diluted from 1:1 to 1:5 dilution to determine the best dilution that would be used for the experiments. At higher concentrations, nanoparticles can form aggregates, which tend to alter LSPR properties thus leading to a broader LSPR peak which reduces sensitivity. As such it is recommended to dilute the nanoparticles to achieve minimal intraparticle interaction [10]. Figure 2 shows UV-Vis spectra of 50 nm gold nanoparticles at different dilutions. The absorption peak of the 50 nm gold nanoparticles is at 525 ± 5 nm. The dilution of the gold nanoparticles in distilled water resulted in a 1 nm wavelength peak shift, which aligns with literature [11]. The proceeding dilutions did not result in a further refractive index change, which suggests that the gold nanoparticles were stable and that low concentrations did not induce refractive index changes.

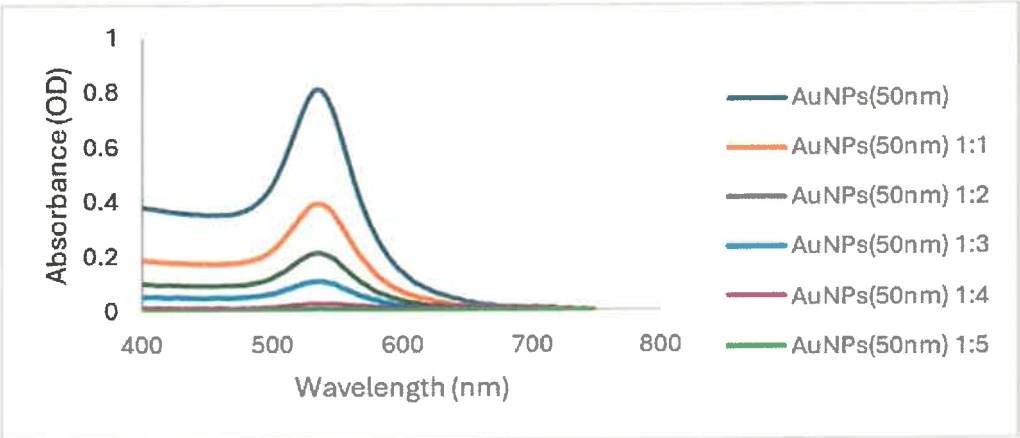

Figure 2: The UV-Vis spectra of gold nanoparticles (50nm) at different dilutions.

The nanoparticles at 1:2 dilution were used in this work, and they were conjugated with the antibody and the virus was also added to the conjugate. The UV-Vis results in figure 3 exhibit a small peak near 280 nm, typical of protein presence in the antibody sample and sample with virus. The sample with the virus had a notable peak at 280 nm due to high protein levels from the antibody and the virus. AuNPs display a strong plasmon resonance peak around 535 nm, which slightly shifts and decreases in intensity when conjugated with antibody and virus to 538 nm, indicating successful binding events. These spectral changes confirm that the LSPR of AuNPs is sensitive to surface modifications, making this technique effective for detecting biomolecular interactions. The wavelength shift and reduction in the peak intensity after the addition of the antibody the virus is typical of nanoparticles when molecules bind to their surface or when their local environment changes [12].

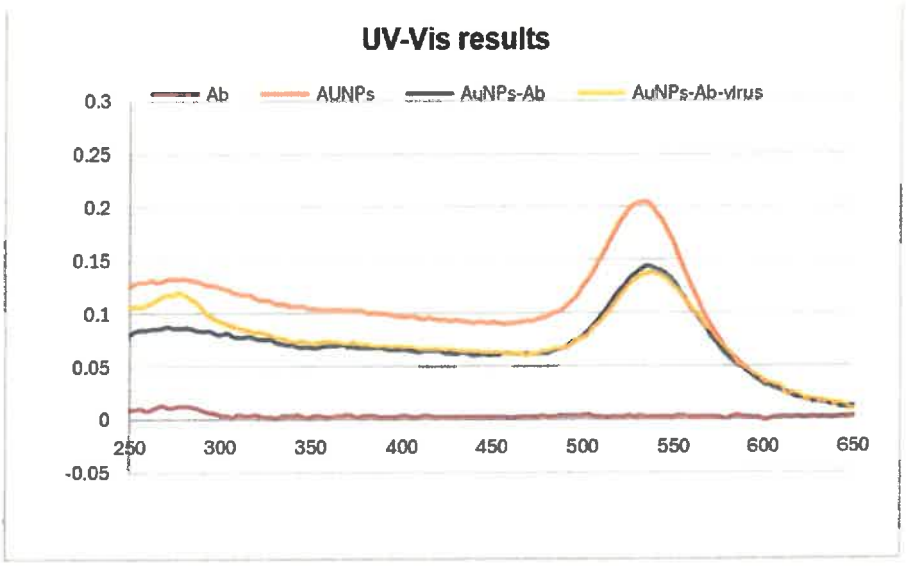

Figure 3: The UV-Vis spectra of gold nanoparticles (535 nm), antibody conjugated with the antibody (538 nm), and the virus bound to the conjugate (538 nm).

Herein, LSPR analysis was performed using a 520 nm LED light. To distinguish between samples with HIV and the ones without HIV, transmittance intensity was measured. As shown in figure 4, AuNPs exhibit the highest transmittance intensity of 36000, followed by the AuNPs+Ab conjugate at 32000, while the sample containing the virus showed a transmittance intensity of 27000.

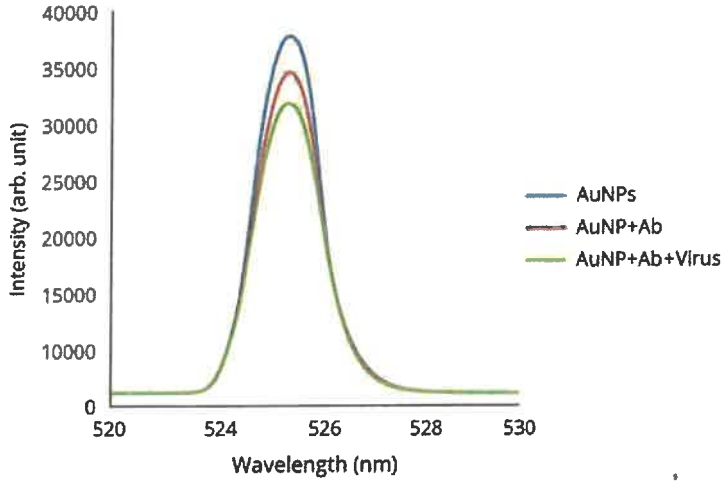

Figure 4: The transmittance intensity of gold nanoparticles, gold nanoparticle-antibody conjugate as well as the sample in which the virus was bound to the conjugate.

The results demonstrated that as different substances bind to the surface of the slide, the amount of transmitted light decreases. The variation in transmitted light levels across different samples is attributed to changes in refractive index. This further confirms that the virus was successfully bound to the antibody, which is specific to the virus used in this study. The notable differences in the transmitted intensity levels among the three samples

confirm the sensitivity of the LSPR to refractive index changes, as also demonstrated in figure 1B. The control sample containing nanoparticles only is expected to have a lower refractive index compared to the other two samples, while the sample, with the virus would exhibit the lowest transmittance intensity.

4 Conclusion

These preliminary findings confirmed the sensitivity of LSPR for refractive index changes as demonstrated by the notable differences in transmittance intensity levels of the different samples. Further work is still required to investigate how variations in nanoparticles geometry affect plasmonic behaviour and determine the refractive index sensitivity observed here. In addition, the specificity, the limit of detection of the LSPR technique in detecting HIV needs to be investigated.

5 Acknowledgements

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