

# Monkeypox Virus (MPXV) Neutralizing Antibodies Diagnosis with Spectral-Phase Surface Plasmon Resonance 3D Imaging Biosensor

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**Abstract** – In this paper, we present the detection of Monkeypox virus (MPXV) neutralizing antibodies with a spectral-phase SPR 3D imaging biosensor. To the best of our knowledge, this is the first demonstration of SPR imaging for MPXV neutralizing antibodies detection in the literature. The imaging biosensor provides 3-dimensional molecular binding information in the x-, y- and time dimensions, enables real-time multiplex molecular bindings detection. In the optical design, a plasmonic sensing surface is placed in between a pair of polarizers with perpendicular orientation to block light transmission. At plasmonic resonance, an additional phase value is introduced for p-polarized light, while s-polarized light remains unchanged. This rotates the polarization ellipse and enables light transmission at specific plasmonic resonance wavelengths, generating the spectral-phase SPR image. In refractive index (RI) measurements, the sensor RI sensitivity was calculated to be 1191.2 Hue units/RIU. The biosensor was further applied to detect MPXV neutralizing antibodies with a 4 x 4 gold biosensor array immobilized with specific MPXV proteins and bovine serum albumin (BSA) as control. The limit of detection (LOD) was found to be 0.27 ng/μL with detection time of less than 16 mins. This value is significantly lower than the neutralizing antibodies level (3.0 ng/μL) stimulated by MPXV vaccine reported in previous study.

**Keywords:** Monkeypox virus; MPXV; Neutralizing antibodies; SPR Imaging; Biosensor; Spectral-phase

## 1. Introduction

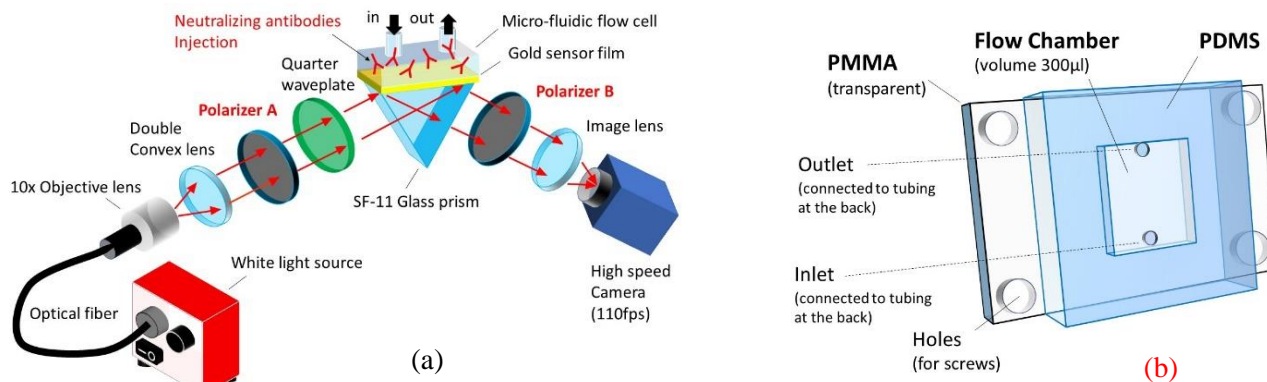
According to World Health Organization (WHO), an outbreak of Monkeypox happened in May 2022 and rapidly spread across Africa countries, Europe, the Americas and Southeast Asia. Monkeypox cases have been reported in over 120 countries worldwide. Between January 2022 to August 2024, cumulative infection cases reached one million and 220 deaths was reported. The Monkeypox virus (MPXV) belongs to the orthopoxvirus genus within the poxviridae family of vaccinia virus (VACV), variola virus (VARV) and cowpox virus (CPXV) [1]. The diagnosis of MPXV neutralizing antibodies in patients contributes not only to vaccination development and efficacy evaluation, but also to governmental public health planning during outbreak. Current detection methods include Plaque-Reduction Neutralization Test (PRNT), Focus Reduction Neutralization Test (FRNT) and Enzyme-Linked Immunosorbent Assay (ELISA) [2]. However, PRNT and FRNT suffer from long detection time of 3-5 days and 1-2 days, respectively, while ELISA involves complex labeling and sandwich detection process [2]. Furthermore, trained staffs and centralized laboratory facilities are required, which are often lacking in Monkeypox outbreak areas, such as the Democratic Republic of the Congo and other Africa countries.

In this paper, we propose a spectral-phase surface plasmon resonance (SPR) 3D imaging biosensor for rapid MPXV neutralizing antibodies diagnosis. The biosensor provides rapid detection results in 16 mins without requiring any labeling process. Furthermore, it is a potential point-of-care diagnostic tool, which contributes to patient care in developing countries experiencing Monkeypox outbreaks. In the optical design, the plasmonic sensor head is placed between two crossed polarizers for light blocking. At plasmonic resonance wavelength, a differential phase response is introduced between p- and s- polarized light [3], which rotates the polarization ellipse and allows light at resonance wavelengths [4] to pass through, resulting in a spectral-phase plasmonic color image. In addition, the excitation beam is expanded to cover a 2D gold sensor spots array (4 x 4 elements). As video data are captured over the sensor surface, the biosensor can provide three dimensional (3D) molecular binding information in the x-, y- and time dimensions, which enables real-time acquisition of multiplex molecular binding curves for Monkeypox virus protein-neutralizing antibody interactions. Compared to the spectra-phase SPR imaging works we previously reported in [5, 6], this paper contributes to the first demonstration of MPXV neutralizing antibody detection using SPR imaging in the literature [7], while Bovine Serum Albumin (BSA) and COVID-19 antibody detection were reported in [5] and [6] respectively.

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## 2. Material and Methods

The experimental optical setup was constructed as described in Fig. 1a. A fiber coupled halogen lamp was used as the broadband light source, which covers the spectral range of 350-1800nm (Thorlab, model:OSL2). Subsequently, a telescope optical system consisting of a 10x objective lens (Olympus, model:10x Olympus plan achromat objective) and a double convex achromatic lens (Edmund Optics, model:50mm dia. x 75mm) expanded the beam to approximately 30mm in diameter. A 4 x 4 gold sensor array was sputtered onto an SF-18 equilateral glass prism for plasmonic biosensing. It is integrated with a Polydimethylsiloxane (PDMS) based microfluidic flow cell for sample injection. The plasmonic sensing element was placed between polarizer A and B with near perpendicular polarization angles to block light transmission. However, at plasmonic resonance, additional light phase difference was introduced between the p- and s- polarized light, that rotated the polarization ellipse and allowed light to pass through. As plasmonic excitation is a wavelength dependent process, a plasmonic spectral profile was encoded into the spectral-phase SPR image. Finally, a high-speed CMOS camera (Basler, model: acA1440-220uc) was used to capture sequence of plasmonic images up to 113 fps (corresponding to a temporal resolution of 0.0088s), which were further analyzed using a custom-built MATLAB program. Spectral-phase SPR imaging enabled two dimensional (2D) detection. At video format, the sequence of SPR images can provide 3D multiplexed molecular bindings curves in the time domain. Fig. 1b shows structure of the flow cell, where a flow chamber was patterned at the center of a Polydimethylsiloxane (PDMS) layer. The size of the chamber was approximately 1.5 x 1.5 cm<sup>2</sup> and with the fluidic volume of ~300μL. During detection, a 5ml syringe was connected to the flow cell through tubing for static sample injection.



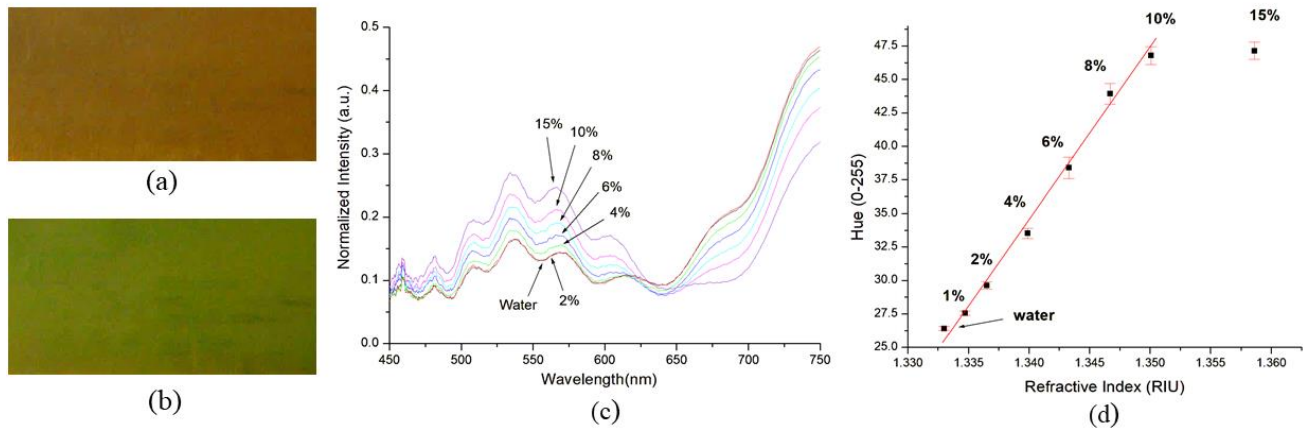
**Fig. 1.** (a) Optical scheme of the Spectral-Phase SPR 3D imaging biosensor (b) Diagram of flow-cell

MPXV L1R neutralizing antibodies (40889-T62, 1μg/μL), L1R protein (40889-V07E, 0.25μg/μL) and control Bovine Serum Albumin (BSA, 810651) protein samples were purchased from Sino Biological and Sigma-Aldrich respectively. In the biosensing experiment, MPXV proteins (0.25 μg/μL) and control BSA proteins (2.94 μg/μL) were immobilized onto the 4 x 4 gold sensor array using the following surface chemistry procedures. Initially, all gold sensor spots were incubated in an 11-Mercatoundecanoic acid solution (10.8mM) for 28 hours to produce a self-assembled monolayer (SAM). Subsequently, mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (0.4M) and N-Hydroxysuccinimide (NHS) (1M) solution were applied to activate the sensor surfaces. It was followed by a protein immobilization process of 3 hours. Finally, the sensor surface was rinsed with phosphate-buffered saline (PBS) buffer before performing MPXV neutralizing antibodies detection.

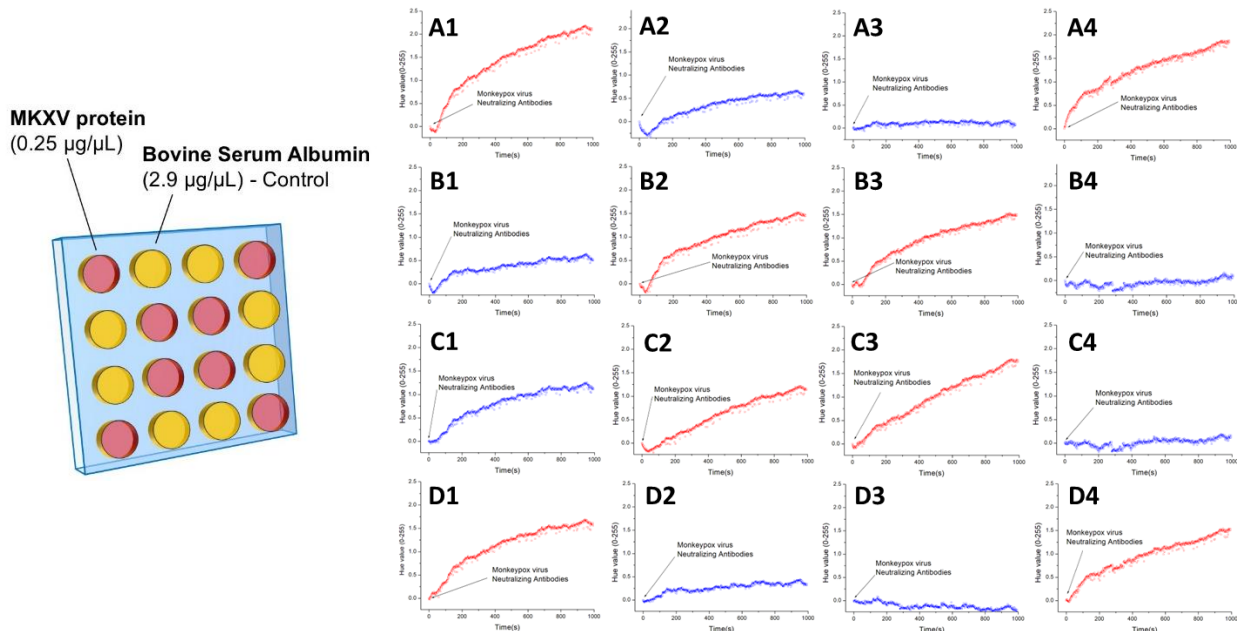
## 3. Results and Discussion

Performance of the spectral-phase 3D SPR Imaging sensor was first characterized with NaCl solutions ranged from 0% to 15%, which corresponding to refractive index values between 1.3330 to 1.3585 RIU [8]. Fig. 2a and 2b are the experimental spectral-phase SPR images of water (0%, 1.3330 RIU) and 15% NaCl solution (1.3585 RIU) respectively. A distinct green shift is observed with increasing refractive index values. In addition, a fiber-based spectrometer (Ocean Insight, model: Ocean HR high-resolution spectrometer) was used to analyze the visible range spectral profiles. Fig. 2c presents the plasmonic excitation spectra of 0% (water), 2%, 4%, 6%, 8%, 10% and 15% NaCl solutions. The spectra exhibit a significant intensity increase within the green (500-570 nm) and yellow (570-590 nm) spectral ranges, while the red spectral region (620-750 nm) is suppressed. The

91 spectral-phase SPR images were then quantified by extracting Hue component of the HSV color space, where the Hue value  
 92 represents the dominate wavelength of color [9]. Fig. 2d illustrates the Hue values as a function of refractive index. It  
 93 demonstrates a linear relationship within 1.3330-1.3501 RIU. Furthermore, slope of the sensor response curve yields a sensor  
 94 sensitivity of 1191.2 Hue units/RIU.



95 **Fig. 2.** Spectral-phase SPR images (a) water (b) 15% NaCl solution (c) Reflection spectra for different concentrations of NaCl  
 96 solutions ranged from 0% (water) to 15% (d) Sensor response curve for different reflective index samples.



97 **Fig. 3.** Distribution map of the specific Monkeypox virus protein and control Bovine Serum Albumin protein molecules on the  
 98 biosensor array spots. A1-D4 shows 16 molecular binding curves recorded for all sensor spots in parallel.

99 The spectral-phase imaging biosensor was further applied for the detection of MPXV neutralizing antibodies. Fig 3 shows the  
 100 distribution map of MKXV protein (red sensor spots) and the control bovine serum albumin (BSA) protein (yellow sensor spots)  
 101 in the biosensor array. The functionalized sensor surface was initially kept at phosphate buffer saline (PBS), followed by the  
 102 injection of MPXV neutralizing antibodies ( $13 \text{ ng}/\mu\text{L}$ ) to facilitate MPXV protein–antibody bindings. Fig.3 presents the  
 103 molecular binding curves recorded for all 16 sensor spots. On average, the injection of  $13 \text{ ng}/\mu\text{L}$  MPXV antibody produced a  
 104 sensor response of 1.6 Hue units. In Fig 3, the discernible signals observed at sensor spots A2, B1, C1 and D2 are attributed to  
 105 non-specific bindings, potentially resulting from physical adsorption of MPXV proteins. The non-specific sensor spots can serve  
 106 as control sensor sites. The final sensor response for MPXV antibody was subtracted with the average non-specific sensor  
 107 responses (0.33 Hue units). In addition, the sensor measurement stability was determined to be 0.033 Hue units based on 500  
 108 measurements in PBS buffer. According to the literature [10], the biomolecular detection sensitive can be estimated to be  $0.27$   
 109  $\text{ng}/\mu\text{L}$  using equation (1). This value is significantly lower than the previously reported neutralizing antibodies level ( $3.0 \text{ ng}/\mu\text{L}$ )  
 110 induced by MPXV vaccine [11].

$$\text{Sensor biosensing Resolution} = \frac{\text{Concentration of biomolecules}}{\text{Sensor response}} \times \text{Sensor measurement stability} \quad (1)$$

Furthermore, the observed binding kinetics difference between A1 and C3 sensor spots may cause by spatial non-uniformity of MPXV antibody molecules within the flow cell (1.5 x 1.5 cm<sup>2</sup>). Such variations could be minimized by using a syringe pump to maintain a continuous flow during detection. In addition, different concentrations of MPXV neutralizing antibodies ranged from 0 ng/μL (PBS buffer), 1.56 ng/μL, 3.125 ng/μL, 6.25 ng/μL to 12.5 ng/μL were sequentially injected onto the MPXV protein functionalized surface. Each antibodies sample was incubated for 45mins within the detection chamber to facilitate MPXV protein-antibody binding interactions. After subtracting the PBS baseline, the normalized sensor responses were plotted in Fig. S1 (Appendix). The results demonstrate a linear relationship between the sensor signals and MPXV neutralizing antibodies concentrations. Slope of the sensor response curve also provides the detection sensitivity of the biosensor. It is determined to be 8.45 ng/μL /Hue units.

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#### Conflicts of interest

The authors have nothing to disclose.

#### Data availability statement

Data underlying the results presented in this paper can be obtained from the author upon reasonable request.

#### Author contribution statement

Wong CL. contributes to the conception of works, experimental design, data analysis and manuscript preparation.

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