Utilising a Multiplexed Gold Nanoparticle-Based Sensor for Antibody and Antigen Detection

Philip H. James-Pemberton, Aaron Westlake, Mark W. Helliwell, Shivali Kohli, Rouslan V. Olkhov, Benjamin M. Farrar and Andrew M. Shaw

University of Exeter
Attomarker

1. The Aim

Using a multiplexed sensor, based on a gold nanoparticle array, to quantify SARS-CoV-2 antibodies in serum and SARS-CoV-2 antigens in saliva.

2. Background

- The multiplexed array technology has already been used for profiling of monoclonal antibodies to *Yersinia Pestis* [1] and the characterisation of whole serum containing BSA antibodies [2]. Therefore it was decided that preliminary testing would be performed using printed SARS-CoV-2 antigens. This established that the array could be used for antibody profiling against multiple antigens simultaneously [3].
- Printed antibody arrays using the multiplexed array technology have previously demonstrated an ability to perform quantitative testing for analytes [4], therefore an investigation to determine the capability of a multiplexed antibody array in detecting SARS-CoV-2 antigens was conducted.

3. Equipment

- The Light Scattering Array Reader (LiScAR) detects changes in brightness of gold nanoparticles when the refractive index around them is changing.
- Gold nanoparticle arrays are manufactured in-house and functionalised with both reference and target-specific biological surfaces.
- The difference in brightness change between reference and target-specific surfaces is turned into a signal using the attached PC (see Fig 1-3).
- The biophotonic platform used for this work has been used for previously published studies and described in detail [2].

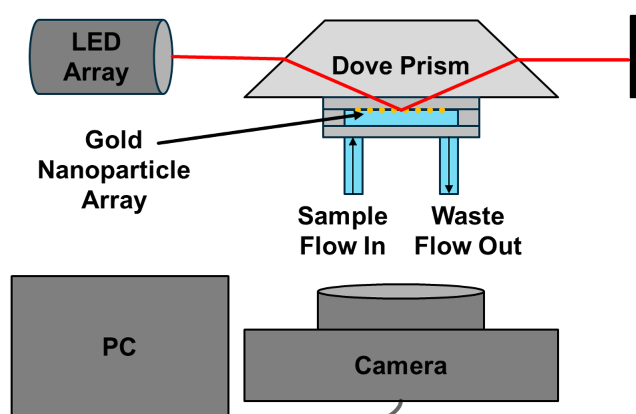

Fig. 1. The internal configuration of the Light Scattering Array Reader (LiScAR)

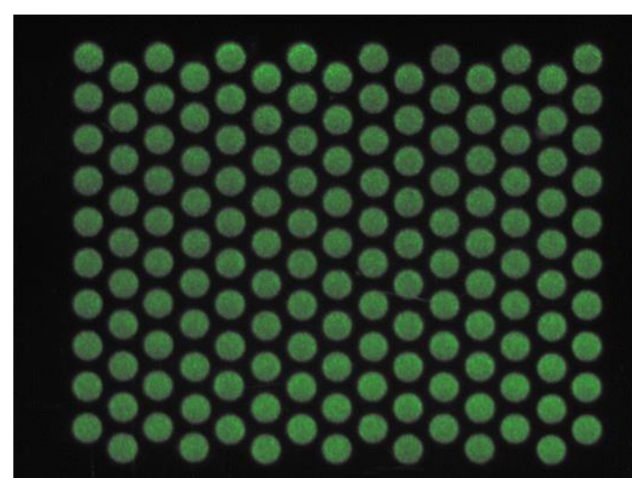

Fig. 2. The view of a gold nanoparticle array when mounted in the LiScAR device.

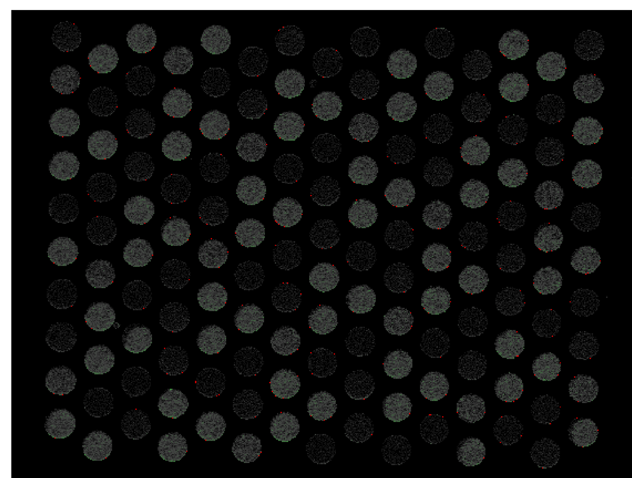

Fig. 3. A difference picture showing the response of aCRP detection spots during an assay

4. Assay Design

- A sandwich assay is performed on the sensor surface.
- This method makes use of the antibody-antigen interaction, either by functionalising the sensor with antigen, as shown in Fig 4, or with antibodies.
- The steps of the process are outlined below and shown in Fig 4, with Fig 5 giving an example as to the PC readout obtained from the sensor during the assay:

1) The empty sensor surface is ready to be used – in this case functionalised with SARS-CoV-2 Spike protein (S).
2) The sample is injected and anti-Spike IgG binds to the immobilised antigen on the surface.
3) A detection step consisting of another antibody, specific to human IgG, is used to enhance the signal and improve specificity.
4) The captured sample antibody and detection antibody are washed away, allowing the sensor to be reused.

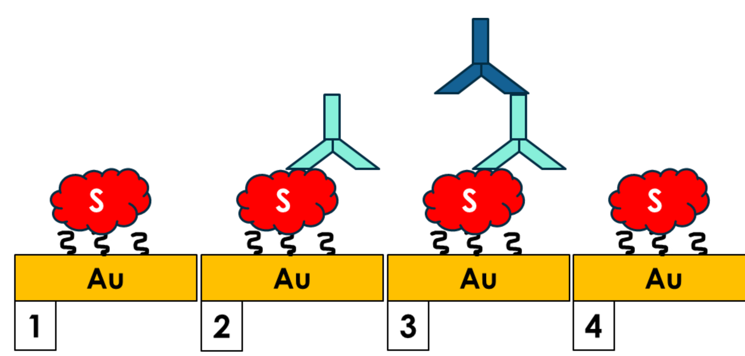

Fig. 4. Step-by-step process of a sandwich assay to detect anti-Spike antibodies

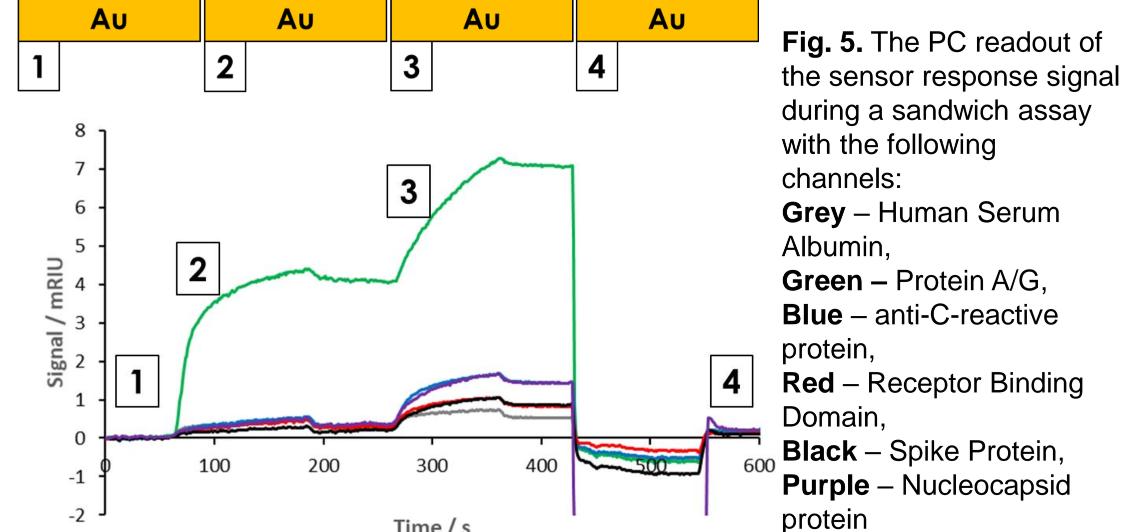

Fig. 5. The PC readout of the sensor response signal during a sandwich assay with the following channels:
Grey – Human Serum Albumin,
Green – Protein A/G,
Blue – anti-C-reactive protein,
Red – Receptor Binding Domain,
Black – Spike Protein,
Purple – Nucleocapsid protein

5. SARS-CoV-2 Antibody Detection

- A commercially sourced set of 400 samples (200 positive and 200 negative) were tested using the printed array described in Fig 5 and the total antibody (IgG + IgA + IgM) responses were recorded as the sandwich assay seen in Fig 4.
- The assay design used a detection mixture to measure all antibody responses as a combined signal, allowing the assay length to be kept to ten minutes.

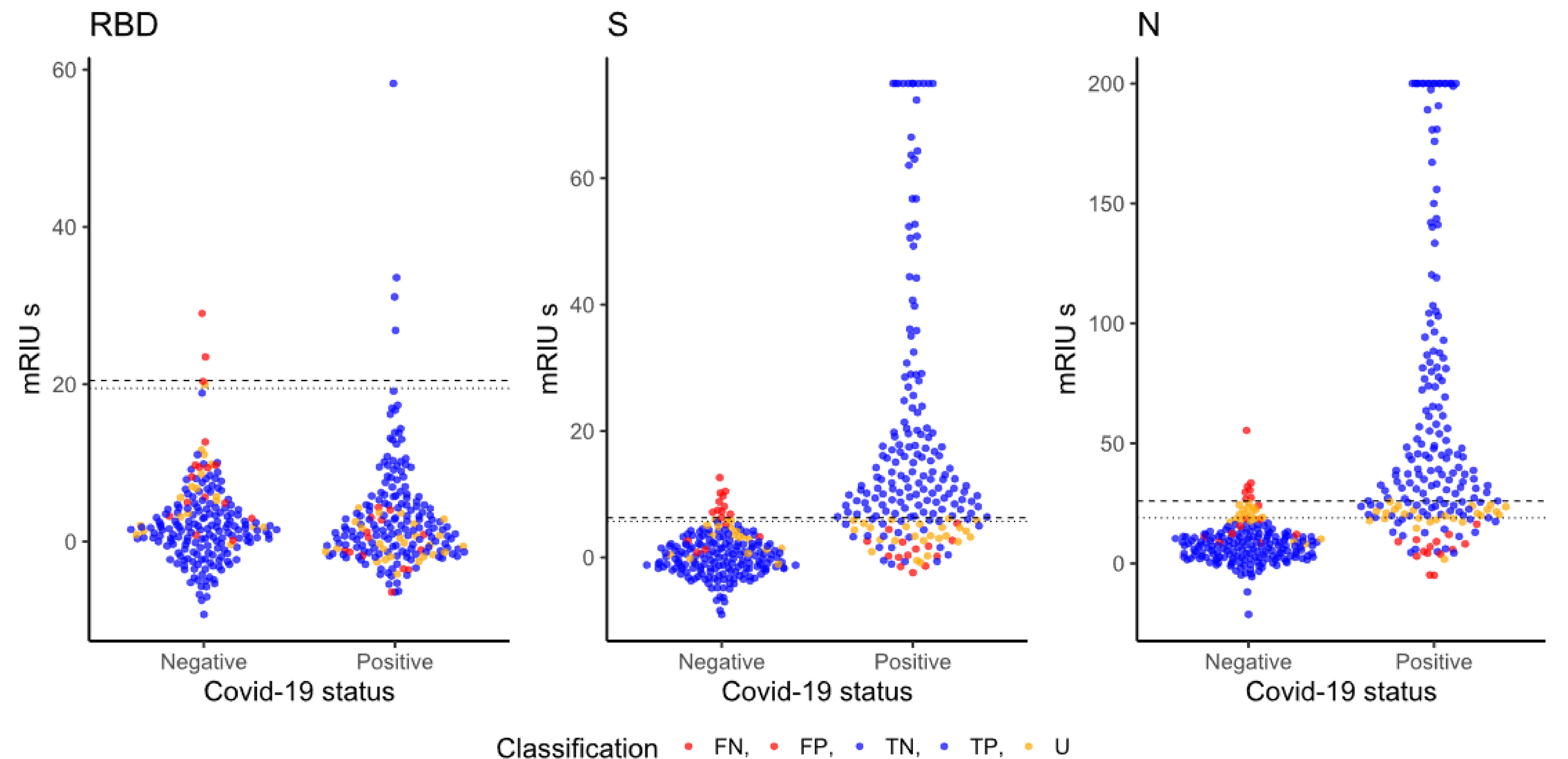

Fig. 6. Beeswarm plots showing the distribution of responses of the 200 negative and 200 positive samples to the total antibody test on three separate antigen channels.
The colours of the dots denote the overall classification of the sample, with blue indicating agreement with supplied SARS-CoV-2 antibody status, red indicating disagreement, and orange indicating an unclassified sample.
The dashed line denotes the positivity threshold and the dotted line the unclassified threshold.
The logic used to classify samples: a sample status is set to positive if any of the three antigen channels returned a positive result.

- Thresholds for each channel were independently determined by maximising the Youden Index [5]. This maximises an index *J*, where *J = sensitivity + specificity – 1*. An unclassified region was also used to take into account the error in measurement.
- Using these calculated thresholds for each channel to classify each sample, a sensitivity of 92% (95%CI 87-96%) and specificity of 91% (95%CI 86-95%) were calculated for the total antibody test.
- This demonstrates the assay capability in correctly classifying real SARS-CoV-2 samples.

6. SARS-CoV-2 Antigen Detection

- An array to detect multiple SARS-CoV-2 antigens was printed, with polyclonal antibodies to N protein, and two different polyclonal antibodies to S protein (referred to as channel 1 and channel 2 in Fig 7). Reference spots were coated with recombinant HSA and two calibration channels of protein A/G and anti-C-reactive protein were also printed. The arrays were blocked with milk before use.
- To calculate a Limit of Detection (LoD), saliva samples were made using 'blank' pooled saliva collected pre-pandemic and diluted five-fold in dilution buffer. Nucleocapsid and Spike antigens were added to make a series of known concentrations.
- The data seen in Fig 7 were collected simultaneously on all three anti-antigen channels, with each sample being recorded using a sandwich assay configuration as seen in Fig 8.
- The LoD was determined for all three proteins, with the N protein detection being the most sensitive with a value of 0.96 nM. This corresponds to a calculated viral concentration of 6×10^8 viral particles/mL. This limit was not improved by combining the channels due to the noise level in the two anti-Spike channels.

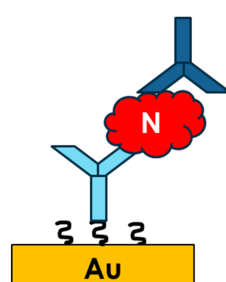

Fig. 8. The sandwich assay to detect nucleocapsid protein with an immobilised antibody, sample binding, and then a detection antibody flowed across the array.

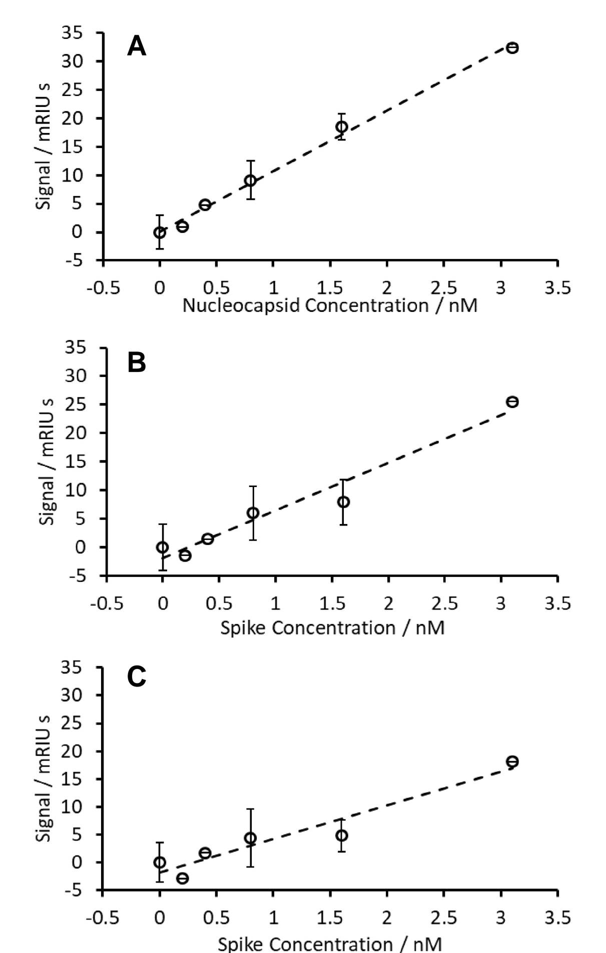

Fig. 7. Plots showing the sensor response to samples of N and S at 0.0, 0.2, 0.4, 0.8, 1.6, 3.1 nM. **A)** Shows anti-Nucleocapsid channel data, **B)** shows anti-Spike channel 1 data, and **C)** shows anti-Spike channel 2 data.

7. References

1. Read, T., et al., *Kinetic epitope mapping of monoclonal antibodies raised against the Yersinia pestis virulence factor LcrV.* Biosensors and Bioelectronics, 2015. **65**: p. 47-53.
2. Olkhov, R.V., J.D. Fowke, and A.M. Shaw, *Whole serum BSA antibody screening using a label-free biophotonic nanoparticle array.* Analytical Biochemistry, 2009. **385**(2): p. 234-241.
3. Shaw, A.M., et al., *Real-world evaluation of a novel technology for quantitative simultaneous antibody detection against multiple SARS-CoV-2 antigens in a cohort of patients presenting with COVID-19 syndrome.* Analyst, 2020.
4. James-Pemberton, P., et al., *Accuracy and precision analysis for a biophotonic assay of C-reactive protein.* Analyst, 2020. **145**(7): p. 2751-2757.
5. Youden, W.J., *Index for rating diagnostic tests.* Cancer, 1950. **3**(1): p. 32-35.