

Novel Detection Methods for Protein-Protein Interactions.

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Objective

- To develop a label free, real time technique to quantify protein interactions with good sensitivity and specificity using imaging ellipsometry.

Materials & Methods

- The initial aim is to study the interactions of Sigma class GST proteins from *Fasciola Hepatica*, an economically important zoonotic parasite.[1]
- Imaging Ellipsometry offers the combined power of ellipsometry for sub-nanometer thickness determination and microscopy for high resolution small area imaging.
- Linear polarised light is turned elliptical on interaction with a sample material (Fig1).

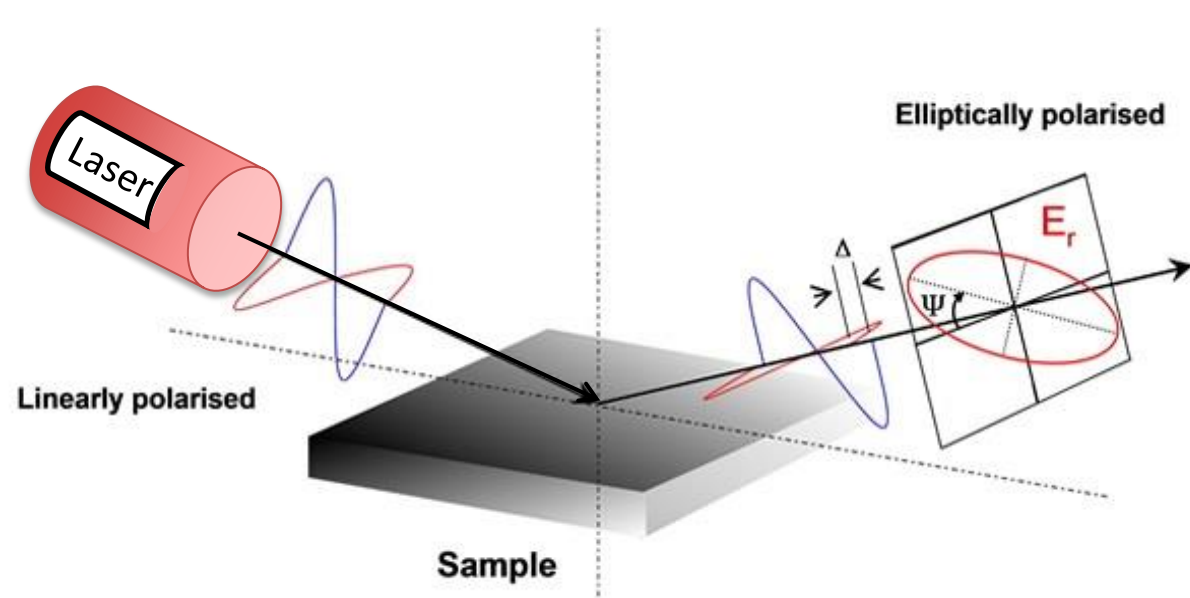


Fig 1 Schematic showing the principal of ellipsometry

- The shift in dielectric properties as a result of proteins interacting can be detected by changes in the amplitude ratio (Ψ) and the phase difference (Δ), seen in fig 1 above.
- The change in Ψ and Δ can be used as a proxy for film thickness. And hence monitor the real time process of interaction.

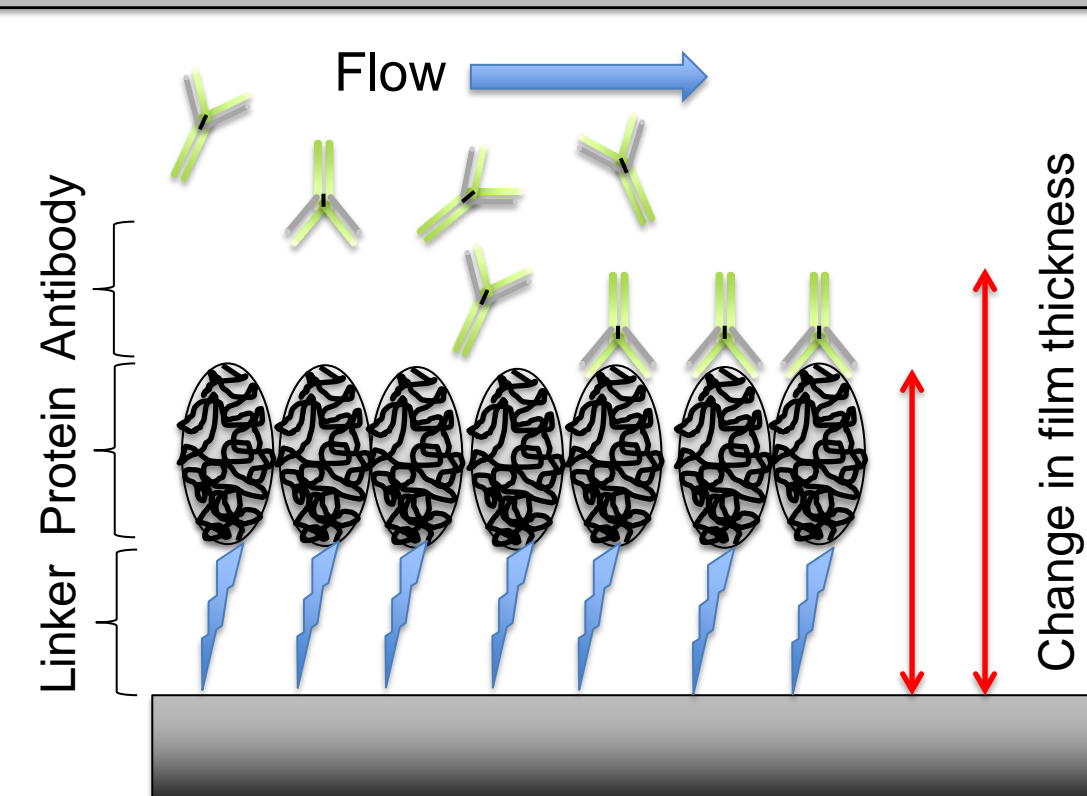


Fig 2 Schematic of the Protein array.

Early Results

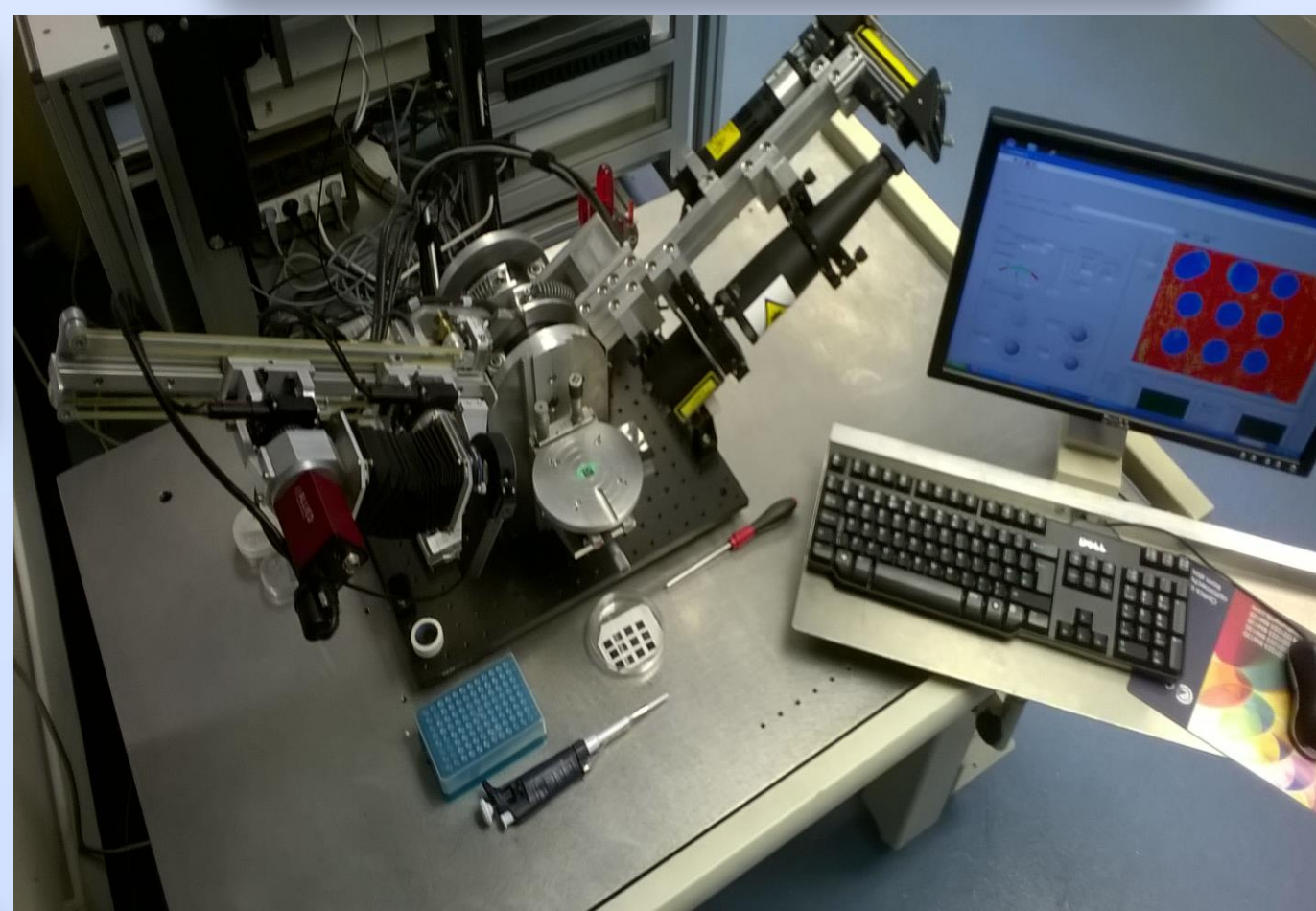


Fig 3 The imaging ellipsometer in use

- Previous studies here at Aberystwyth University have provided a proof of principle.
- The ellipsometer is now undergoing detailed characterisation.
- Lateral resolution is $11\mu\text{m}$, vertical (thickness) resolution should be under 1nm . [2]
- Trials to develop micro arraying techniques are underway. Robotic pipetting and piezo electric printing are being evaluated.

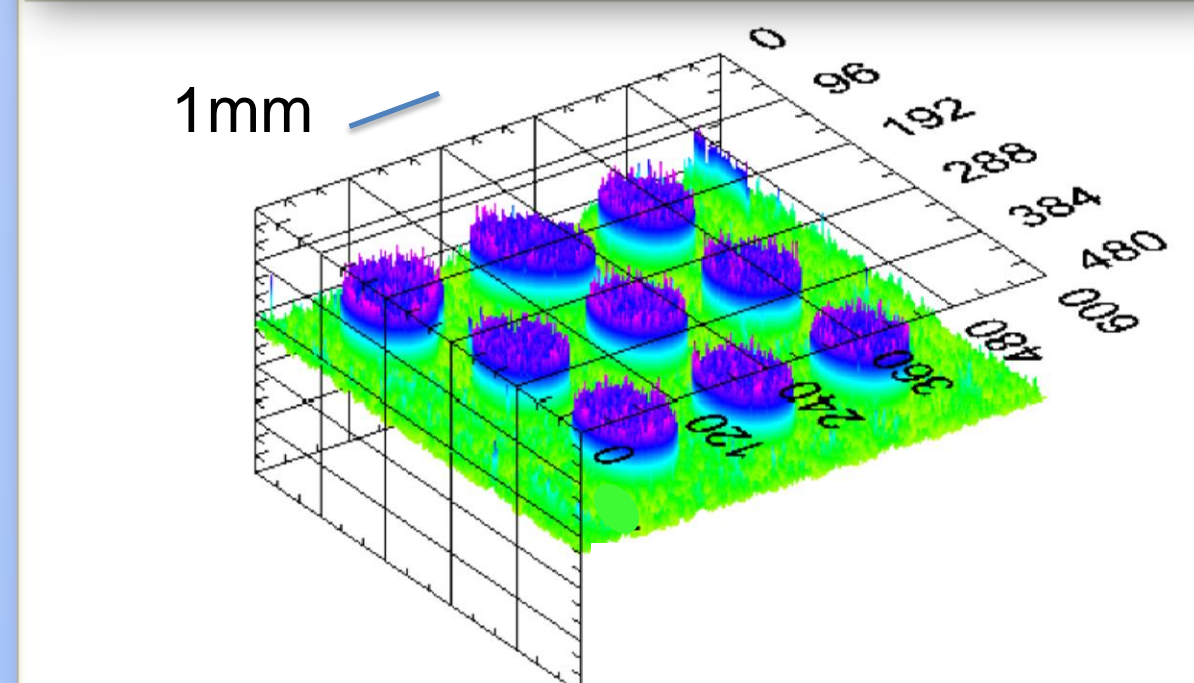
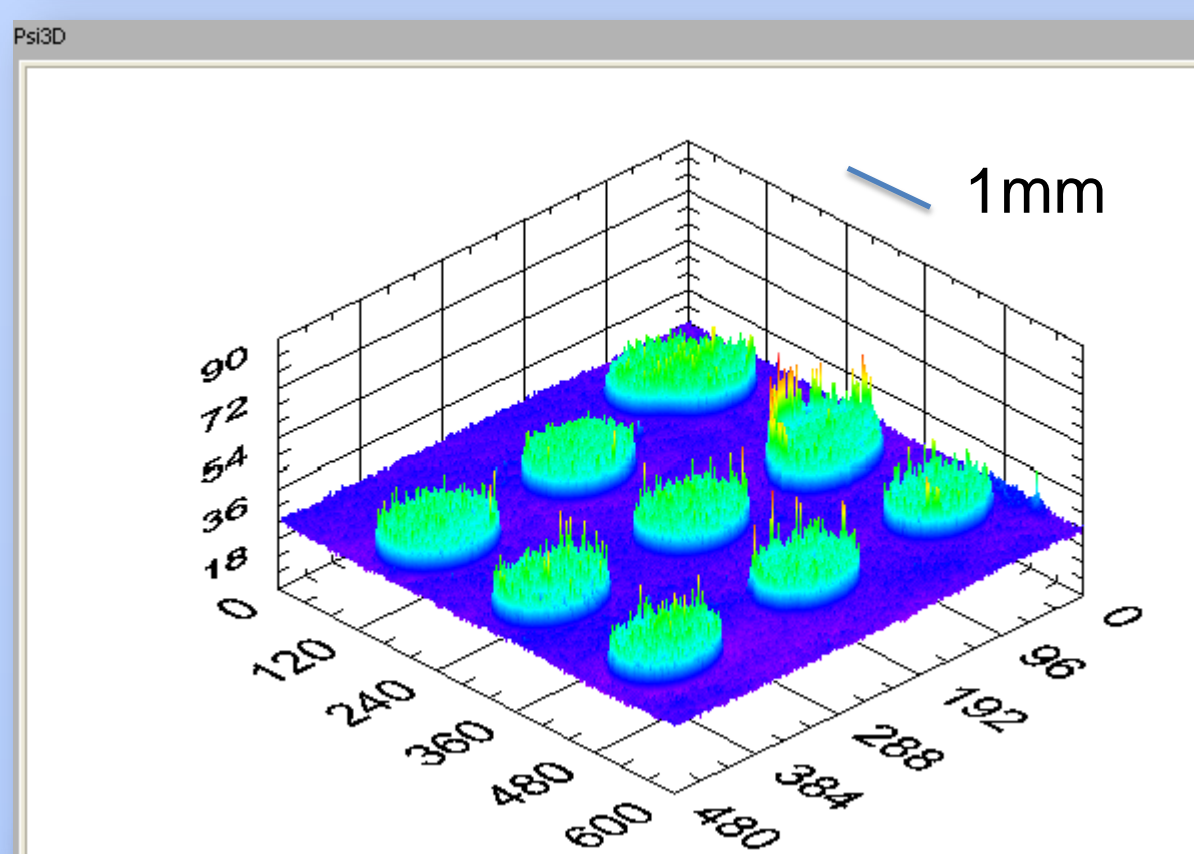


Fig 4 Two examples of Bovine serum albumin on a silicon substrate evaluated using imaging ellipsometry

Potential Impact

- Diagnostics:** Sera from animals (or humans) can be passed across an array of known proteins and rapidly evaluated using this process as a disease detection method (Fig 5).
- Drug Development:** Small molecules can be used as ligands to identify potential chemotherapeutics for target proteins.
- Protein function analysis:** Transient protein interactions can be studied in real time and label free – significant advantage over current protein interaction technologies (Fig 6).
- Antibody characterization:** Imaging ellipsometry will allow the study of cross-reactivity and specificity of antibodies for multiple applications.

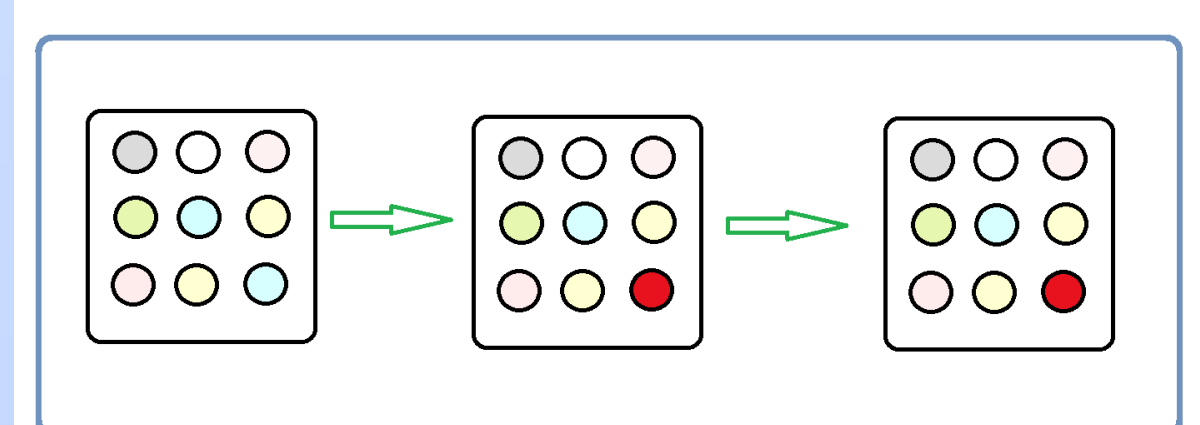


Fig 5 A mixed Protein array showing disease diagnosis (antigen antibody)

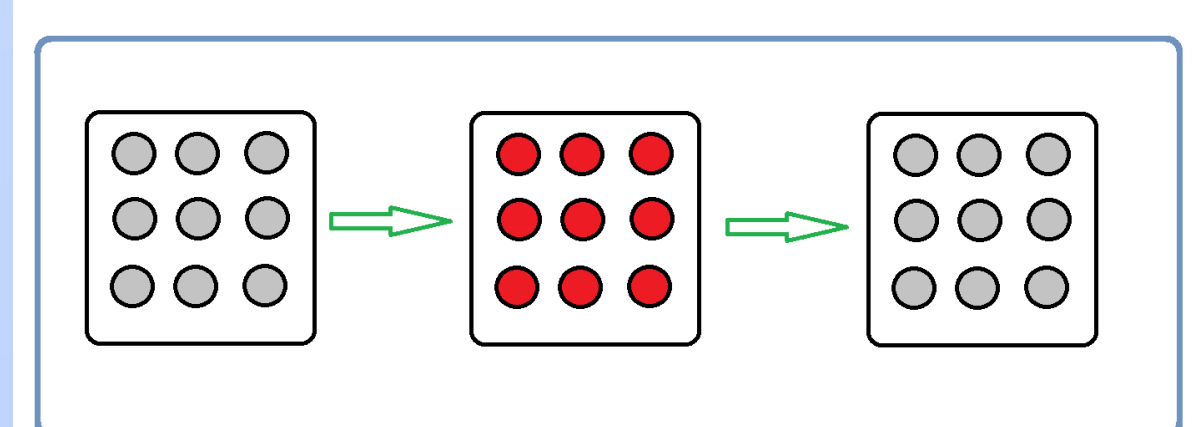


Fig 6 Protein array showing a transient interaction.

Conclusion

- Proteins are the functional unit of the genome and thus, the investigation of protein-protein interactions is of significant interest.
- Observing these interactions label free and in real time offers opportunities to quickly detect multiple analytes and monitor complex interaction processes – significant advances over current protein interaction technologies.

References:

- Morpew et al "In vitro biomarker discovery in the parasitic flatworm *Fasciola Hepatica* for monitoring chemotherapeutic treatment." *Open Proteomics* (2014) 85-95
- Z Wang & G.Jin "A Label free multisensing immunosensor based on imaging ellipsometry" *Anal.Chem* 2003 75, 6119-6123