PhD Project Proposal

PhD Project Title	**Lectin arrays for electrochemical detection of dysplastic cell binding**
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Second supervisor (if applicable)	Prof. Rebecca Fitzgerald, Department of Oncology

Project Outline	Glycans show potential as biomarkers, considering their abundance on cell surfaces, their reported alterations in the early stages of cancer development and their binding to plant-derived lectins (carbohydrate binding proteins). Glycans are complex carbohydrates attached on cell surface proteins via glycosylation. Altered glycosylation has been known to occur either at the onset and/or during tumour progression. Identifying these changes at early disease stages may aid in accurately identifying at-risk individuals for bespoke medical advice.[1] Here, we aim to incorporate this strategy as a viable tool for ex vivo diagnosis of dysplasia, showing proof of principle using Barrett's Oesophagus (BO) biopsies.[2] Preliminary work in the Fitzgerald group has identified a number of potential lectin biomarkers capable of discriminating between cells from Barrett's tissue relative to normal squamous tissue. Lectin electrochemical biosensors have shown detection limits down to the fM-aM levels, however successful clinical translation is yet to be achieved.[3] Glycosylation patterns on cells may heterogeneous and expression levels may vary, therefore an array approach, where multiple lectins are immobilised in distinct areas of a device, will be preferred.[4] The array will be optimised to include lectins that will bind to normal tissue, BO tissue and other tissues from the gastrointestinal tract. A pattern or fingerprint will emerge, able to efficiently detect dysplasia with high confidence. The array will be optimised to identify control biopsy areas similar to the positive control on a pregnancy test. Owens and co-workers have focused on developing electrochemical sensing devices that operate in complex samples.[5] A device array will be fabricated in a Wheatstone bridge format that will allow reference based substraction of non-specific binding, enhancing the confidence in the results obtained. This has been used previously by Owens and colleagues to detect biomarkers from 10s of circulating tumour cells.[6]
Project plan	1. A matrix of lectin biomarkers will be identified that will give highest confidence in identifying dysplastic cells, while discarding non-dysplastic cells. 2. Devices will be designed to incorporate sensing arrays with a Wheatstone bridge configuration. Inkjet printing will be investigated for fabrication 3. Lectins will be immobilised and electrochemical impedance spectroscopy will be used to show glycan binding and then cell binding, validated with immunofluorescence staining.

	4. Additive manufacturing will be used to produce 3D printed housings for sample introduction, binding and washing 5. Devices will be tested with cell lines and then with normal tissues or BO biopsies. Protocols will be optimised for enhancing cell capture of dissociated cells while reducing non-specific binding 6. Cells from the non-endoscopic Cytosponge will be tested with the device.
Main methods to be used	Device fabrication (NanoScience), design, photolithography, inkjet printing Device characterisation; Electrical Impedance Sensing Device housing and fluidics; 3D printing Surface biofunctionalisation and characterisation; Microscopy – AFM and confocal Biochemical assay development; optimisation of binding, SPR Cell culture; growth of cell lines, handling of biopsies and opportunity to work on Cytosponge samples depending on progress The expertise is up and running in the Owens laboratory and samples are readily available from the Fitzgerald lab.
Key References	1. Neves, A. *et al.* Detection of early neoplasia in Barrett's esophagus using lectin-based near-infrared imaging: an ex vivo study on human tissue. *Endoscopy* **50**, 618–625 (2018). 2. Peters, Y. *et al.* Barrett oesophagus. *Nat Rev Dis Primers* **5**, 35 (2019). 3. Chen, L. *et al.* Organic Electrochemical Transistors for the Detection of Cell Surface Glycans. *ACS Appl. Mater. Interfaces* **10**, 18470–18477 (2018). 4. Dang, K., Zhang, W., Jiang, S., Lin, X. & Qian, A. Application of Lectin Microarrays for Biomarker Discovery. *ChemistryOpen* **9**, 285–300 (2020). 5. Curto, V. F. *et al.* Organic transistor platform with integrated microfluidics for in-line multi-parametric in vitro cell monitoring. **3**, 17028 (2017). 6. Braendlein, M. *et al.* Lactate Detection in Tumor Cell Cultures Using Organic Transistor Circuits. *Advanced Materials* 1605744 (2017) doi:10.1002/adma.201605744.