Single-Molecule Characterisation of Ovarian Cancer Biomarkers

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Ovarian Cancer

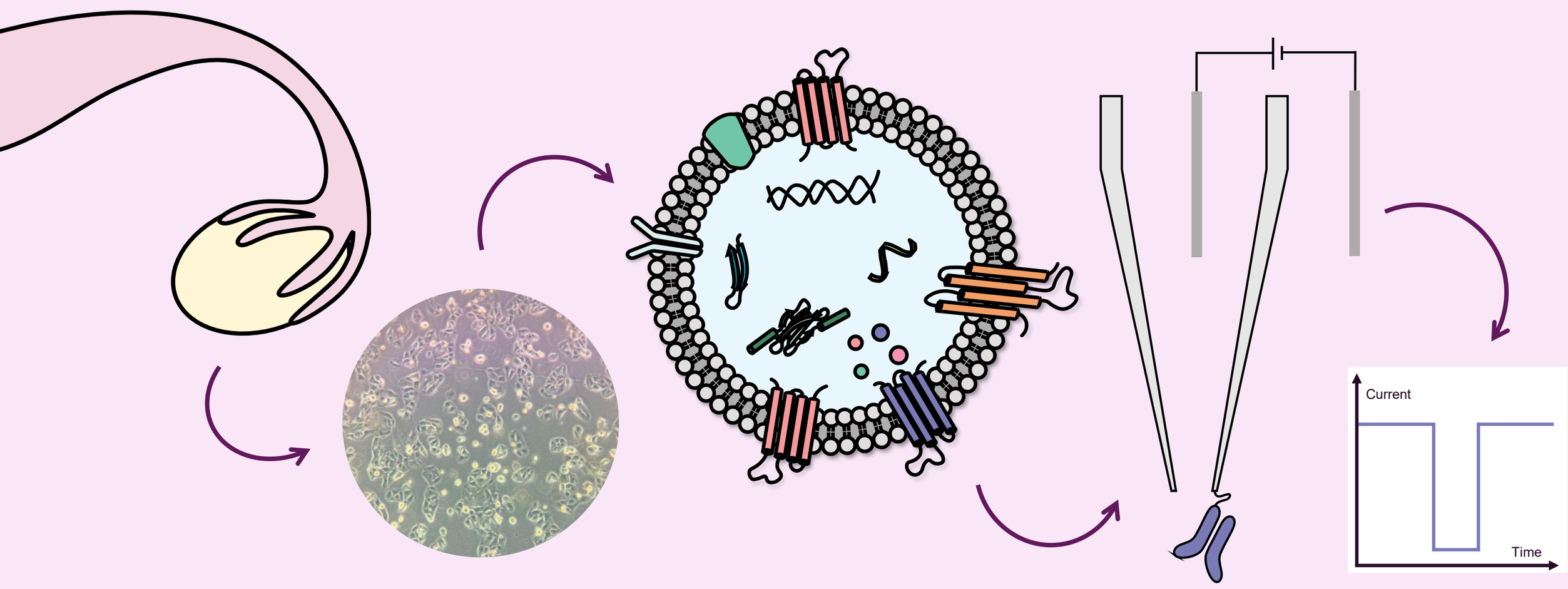
Ovarian cancer (OC) is the 6th most common gynaecological cancer in the UK, with a low five-year prognosis of 40% and recurring in 70% of cases. This is linked to late diagnosis due to non-specific symptoms and ineffective screening methods. **The development of new methods for the early detection of OC is therefore crucial.**

Extracellular Vesicles

Extracellular vesicles (EVs) are nanoscale, lipid bilayer-bound bodies released by all cells which can be isolated from biofluids such as blood. Their characteristics such as content and surface markers tend to match the cell of origin, suggesting disease biomarkers potential. **EVs derived from OC cells have been found to contain specific surface markers**, making them relevant candidates for the development of liquid biopsy diagnostic tests for OC.[1]

Single-Molecule Techniques

Due to high EV polydispersity, single-molecule (SM) techniques are better-suited to EV analysis than bulk methods. SM methods include nanoparticle tracking analysis (NTA), SM fluorescence microscopy, and nanopore sensing (NS). **This work seeks to improve OC diagnoses through SM characterisation of OC-derived EVs surface markers.**



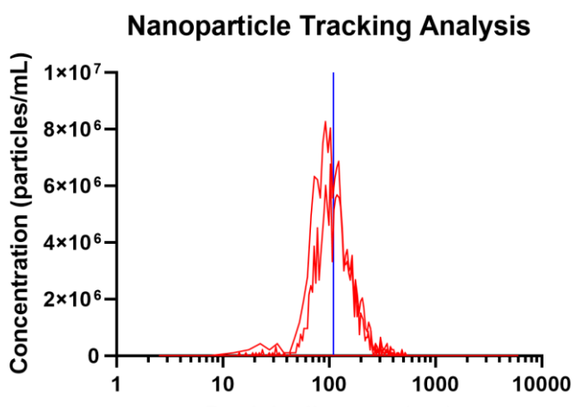
EV Isolation

OC-derived EVs are isolated by size exclusion chromatography (SEC) from the cell media of immortalised OC cell line OVCAR-4, before further characterisation.

OVCAR-4 tissue culture → OVCAR-4 EV isolation by SEC → EV characterisation by SM techniques

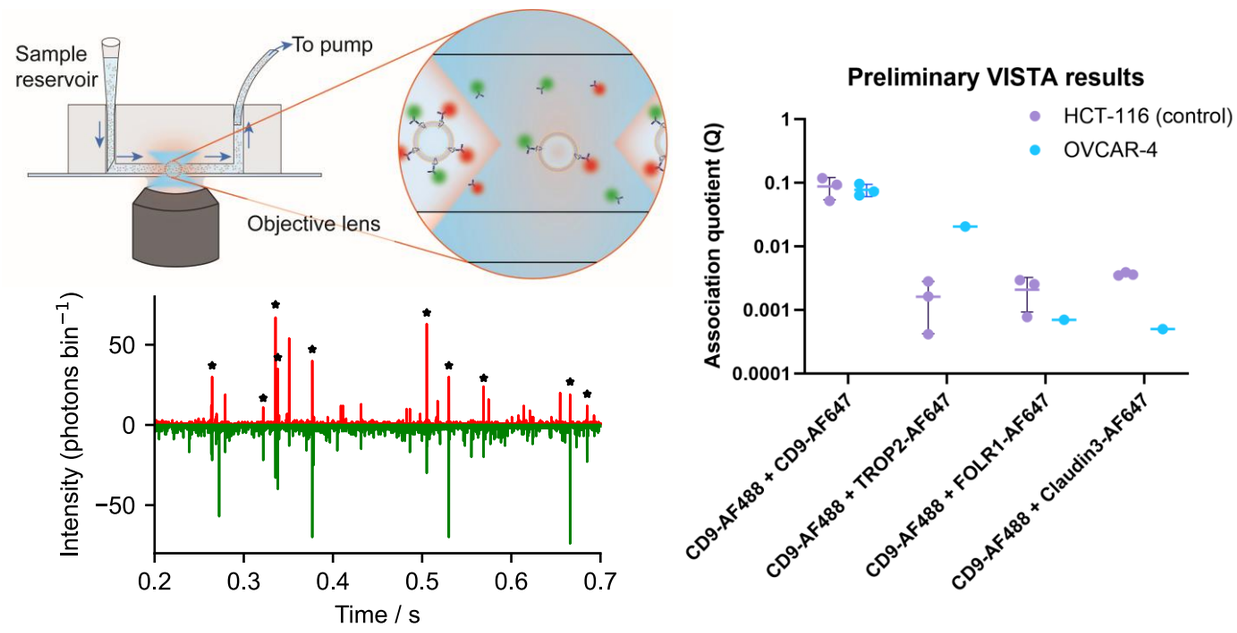
Nanoparticle Tracking Analysis

NTA measures the hydrodynamic diameter of particles and their sample concentration. However, **a key limitation of NTA is its lack of specificity to EVs**, meaning that other particles present in the sample such as lipoproteins may also be detected.



Single-Molecule Confocal Microscopy

Vesicle **I**maging by **S**ingle-molecule **T**wo-colour coincidence detection **A**nalysis (**VISTA**) is a fluorescence microscopy technique where EVs are detected in flow by two target antibodies labelled with orthogonal fluorophores. EVs containing both markers of interest will yield coincident events in both wavelengths.[2] In this work, common EV surface markers such as the tetraspanin CD9 are targeted to **confirm the presence of EVs**, and potential OC surface markers TROP-2, FOLR-1, and Claudin-3 are investigated.

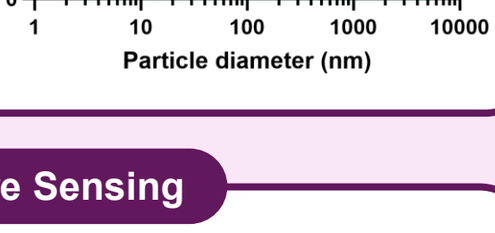


Nanopore Sensing

NS is a SM technique where an analyte is driven through a pore by an applied potential difference and characterised by the observed drop in current. NS platforms can be functionalised to detect specific elements such as EV surface markers and can be adapted into portable devices, making this a **highly versatile detection method with point-of-care applications for OC.**[3]

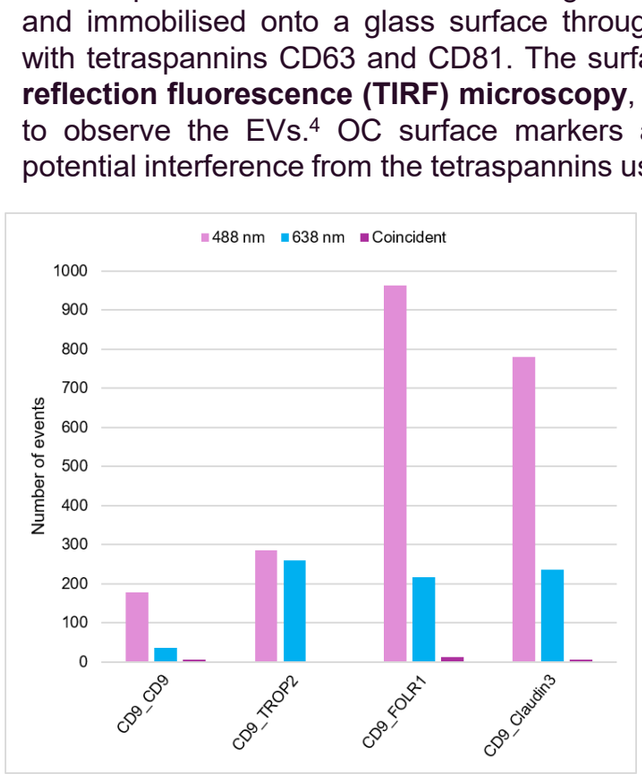
Nanopore fabrication and characterisation → Nanopore functionalisation → EV detection by NS

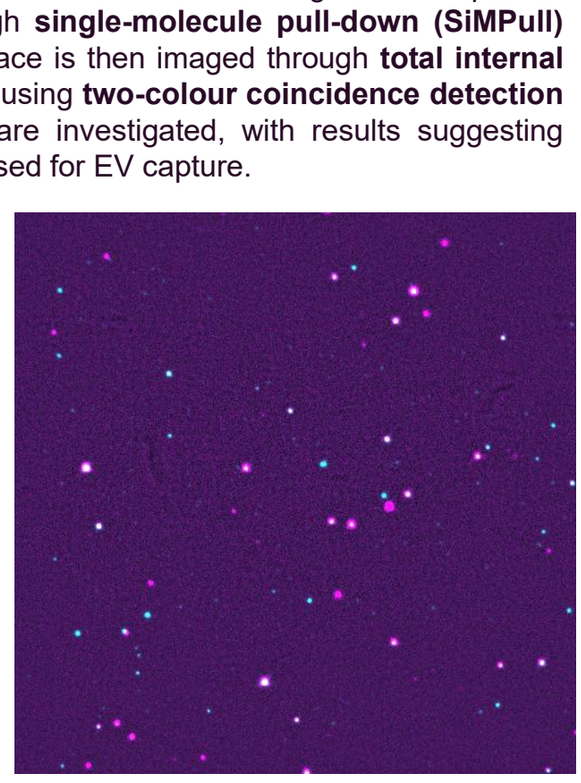
This work focuses on the set up of a NS platform to detect OC-derived EVs. The fabrication and aperture characterisation of quartz nanopores has been optimised to yield nanopores of **aperture diameters ranging from 105 to 255 nm**.



Single-Molecule Pull-Down

EV samples are incubated with two target antibodies labelled orthogonal fluorophores and immobilised onto a glass surface through **single-molecule pull-down (SiMPull)** with tetraspannins CD63 and CD81. The surface is then imaged through **total internal reflection fluorescence (TIRF) microscopy**, using **two-colour coincidence detection** to observe the EVs.[4] OC surface markers are investigated, with results suggesting potential interference from the tetraspannins used for EV capture.





Future Work

Future work will investigate EVs derived from further OC cell lines, as well as healthy fallopian controls, using a variety of protein markers, before moving on to characterising patient-derived EVs. Nanopore functionalisation will also be investigated to achieve detection of OC-derived EV surface markers, and to adapt this method for accessible point-of-care applications in diagnostics.

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References

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