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Helium Ion Microscopy of Platelet-Tumour Cell and Platelet-Nanoparticle Interactions

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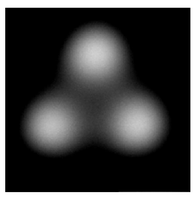
Introduction

Helium ion microscopy has demonstrated promising capabilities for imaging untreated biological samples. There are many examples that act as proofs of concept, however the images generally serve as demonstration of the technique or to aid descriptions of systems [1-3]. In this project I set out to see if this kind of imaging can give insight into the mechanisms of platelet-tumour cell interactions and platelet-nanoparticle interactions.

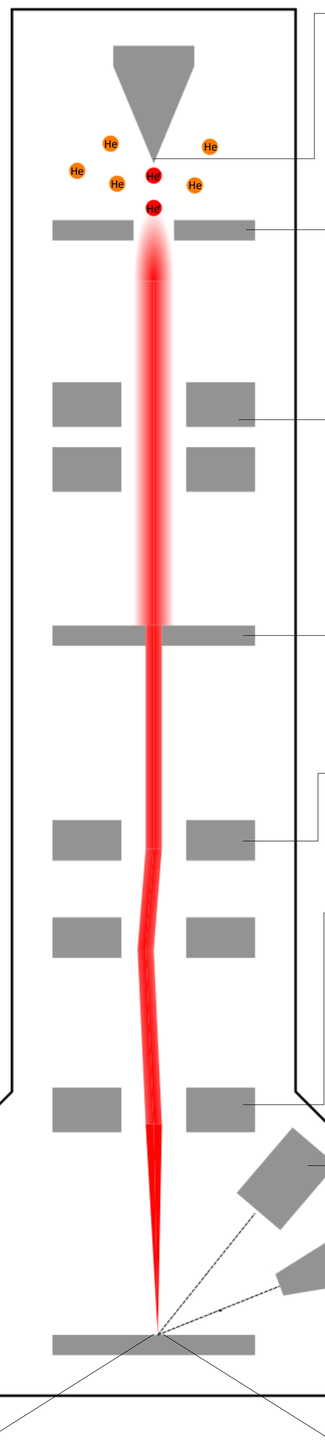
The Helium Ion Microscope

Developed in 2006, the helium ion microscope (HIM) is a charged particle microscope, much like the scanning electron microscope (SEM) in which a helium ion beam is accelerated towards a sample to create an image . It offers many advantages over SEM that allows the imaging biological sample without complicated sample preparation. When detecting secondary electrons, it is highly surface sensitive allowing the characterisation of nanoscale surface details [4].



Gas Field Ion Source Gun

Helium gas is passed over an atomically sharp tip cooled to 80K. A high voltage is applied creating a strong electric field that ionises the helium atoms. The image above shows the three atom configuration at the tip of the source gun , called a trimer [5].



Extractor

The extractor diode is negatively biased with respect to the source to accelerate the helium ions down the column of the microscope creating a beam.

Condenser and Alignment

The beam passes through a condenser lens to make the beam parallel then through lenses to align the beam with the optical column

Aperture

This limits the beam current and determines the final probe size. A larger apertures increase beam current but reduce depth of field.

Stigmatic and Scanning Lenses

Quadrupole lenses ensure the beam focuses uniformly. Scanning coils deflected the beam across the surface of the sample.

Objective Lens

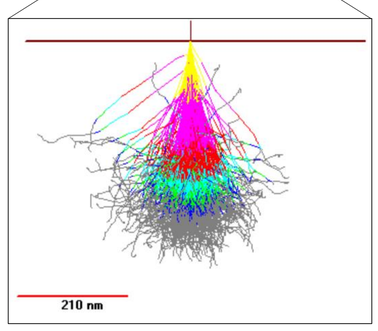
The beam is focussed to a point. The size of the point is the probe size.

Everhart-Thornley Detector

Secondary electrons are emitted and detected to form an image. This method of detection is very surface sensitive making it good for surface characterisation.

Flood Gun

The sample is exposed to a low energy electron beam between scans to negate charge build up. This allows non-conducting samples to be imaged without coating.



Ion Interactions and Contrast

The ions are scattered as the enter the sample forming an interaction volume from which secondary electrons are emitted. This causes the signal detected to be dependent on the angle of surface. This creates topographical contrast and allows shape to be interpreted [6].

Image of IONiSE Monte Carlo simulations of 30KeV He beam on carbon target with n=500. This estimates a penetration depth of 500nm and SE yield of 1.4.

Acknowledgements

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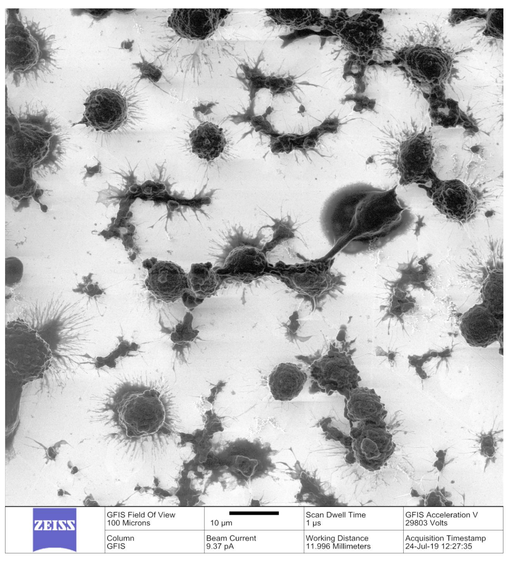
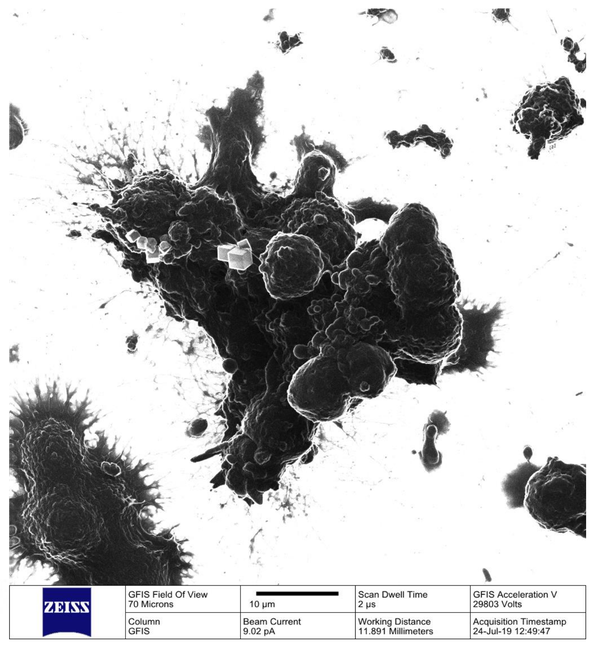
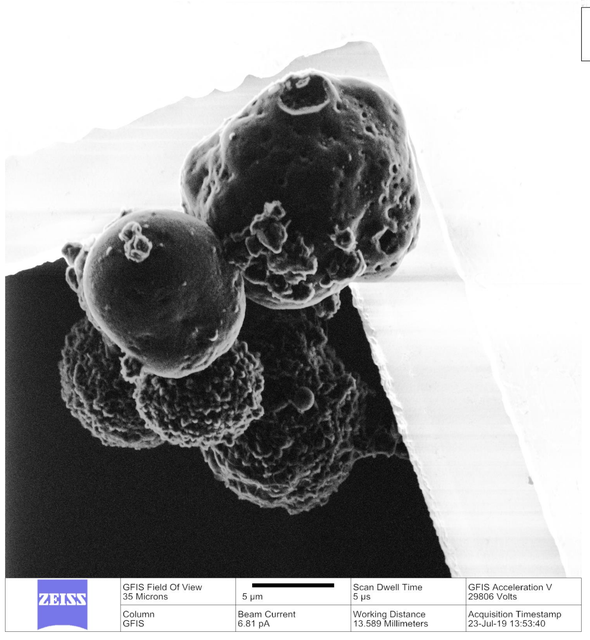
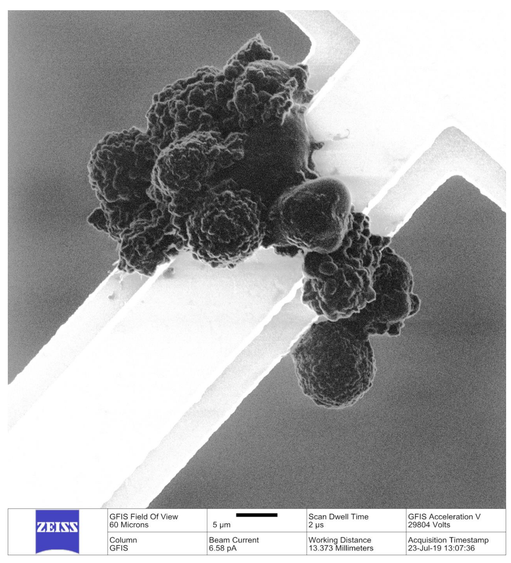
Platelet-Tumour Cell Interactions

Platelets are a component of blood that form aggregates in response to injuries in blood vessels to prevent bleeding. It is known that platelets interact with tumour cells and can aid metastasis in a process called platelet cloaking [7]. The exact mechanism of the interaction however is not known. We conducted experiments in which prostate cancer were exposed to platelets to study the interaction. There were compared to control samples of healthy prostate cells and samples in which aggregation was prohibited. The samples were fixed and then dehydrated before imaging using HIM.





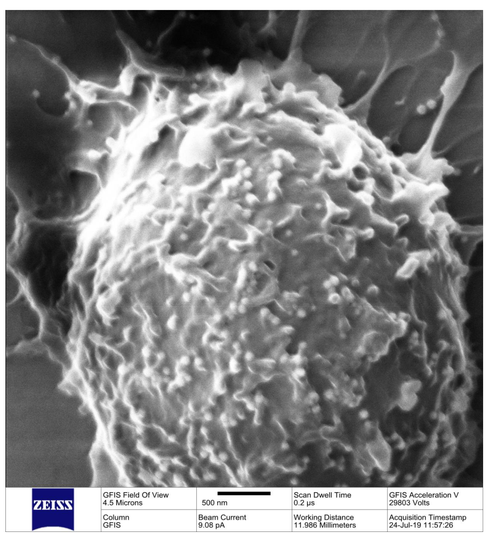




Upper left and right: Platelets exposed to prostate cancer cells on a substrate. Platelets can be seen forming a coating around the cancer cells and aggregates can be seen spreading across the substrate. Lower left and right: platelets exposed to tumour cells in solution then drop cast onto copper transmission electron microscopy grids. Individual platelets can be seen attaching to the cells.

Platelet-Nanoparticles Interactions

Nanoparticles are being investigated as a method for targeted drug delivery. It has been found however that certain sizes of nanoparticles can interact with platelets and cause aggregation. We conducted experiments in which platelets were exposed to gold nanoparticles of 60nm and 70nm in size. The 60nm particles had been found to induce platelet aggregation. By imaging with HIM we found evidence the interaction is due to nanoparticles binding to the surface of the platelets.



Platelet exposed to 60nm gold nanoparticles coated with PEI. The nanoparticles can be seen on the surface of the platelet and on the pseudopodia extending on the substrate.

Conclusions

Imaging of platelet-tumour cell interactions showed platelets attaching to the cancer cells then forming aggregates to coat the cells. The experiments carried out on a substrate show these aggregates spreading out, however this feature is lost during the preparation process of the samples in solution. Imaging of the platelet-nanoparticle systems showed evidence that the interaction is caused by nanoparticle attaching to the surface of the platelet.

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