Label-free in vitro modelling of nanoparticle and microorganism dynamics at cell interfaces

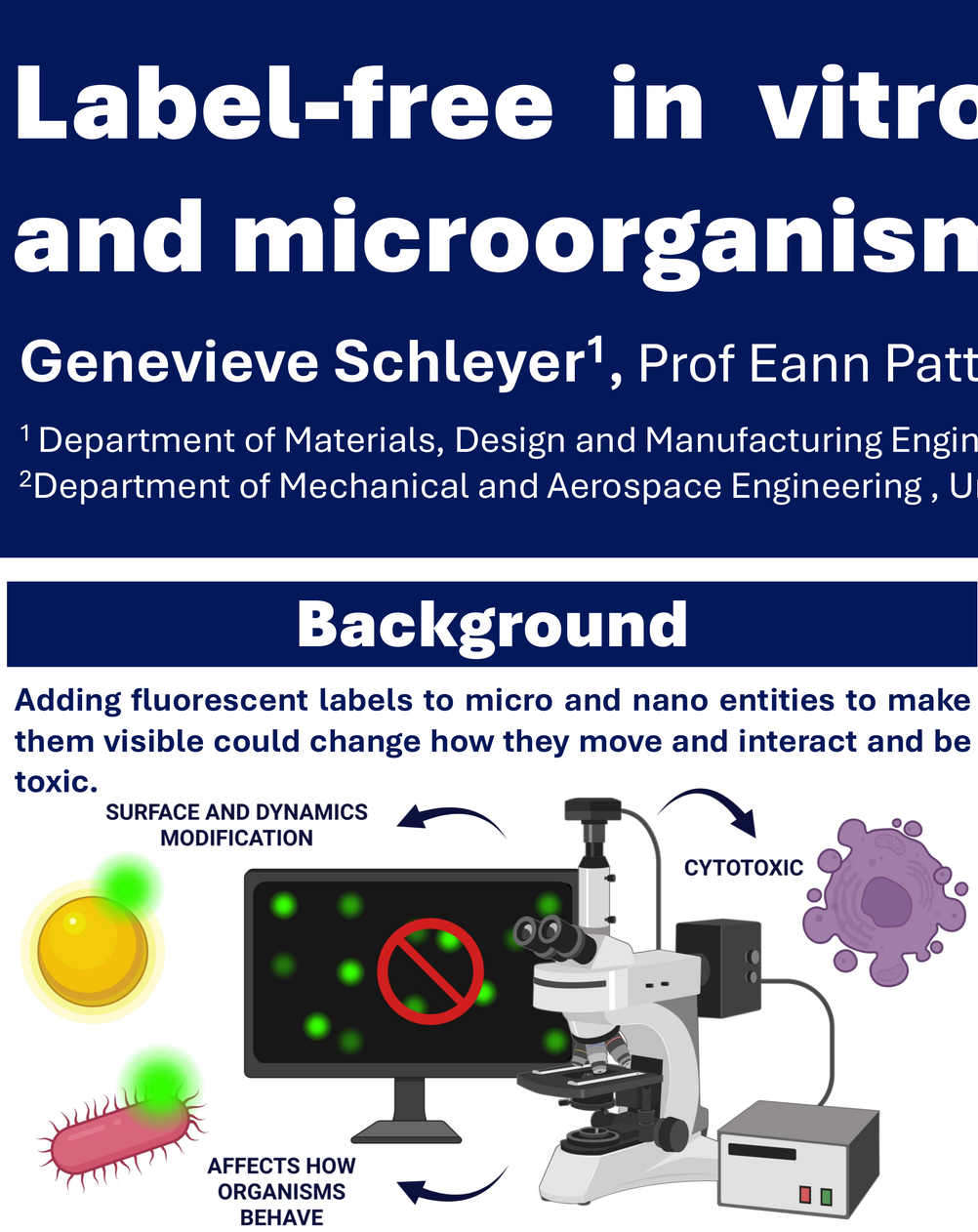
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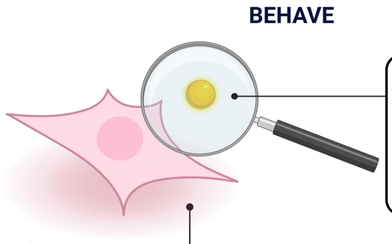
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UKRI Biotechnology and Biological Sciences Research Council

Background

Adding fluorescent labels to micro and nano entities to make them visible could change how they move and interact and be toxic.

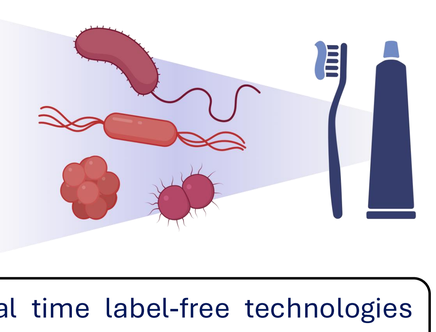




Changes in the surrounding environment, caused by cell activity, or interactions with complex biological fluids, can influence how small particles move.

The development of effective materials at the cellular level requires tools that can capture how nano- to microscale entities interact with complex cell environments...

...and understanding how microorganisms, such as bacteria, approach and colonise host surfaces is critical for advancing next-generation antimicrobial strategies.





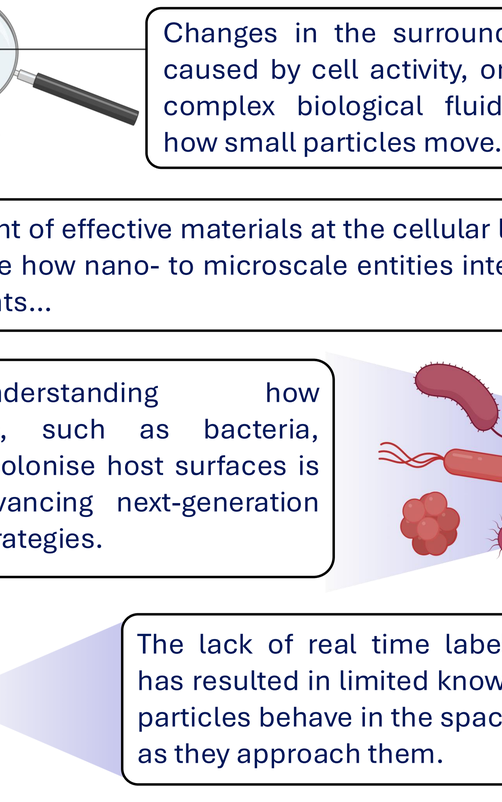
The lack of real time label-free technologies has resulted in limited knowledge of how small particles behave in the space around cells, and as they approach them.

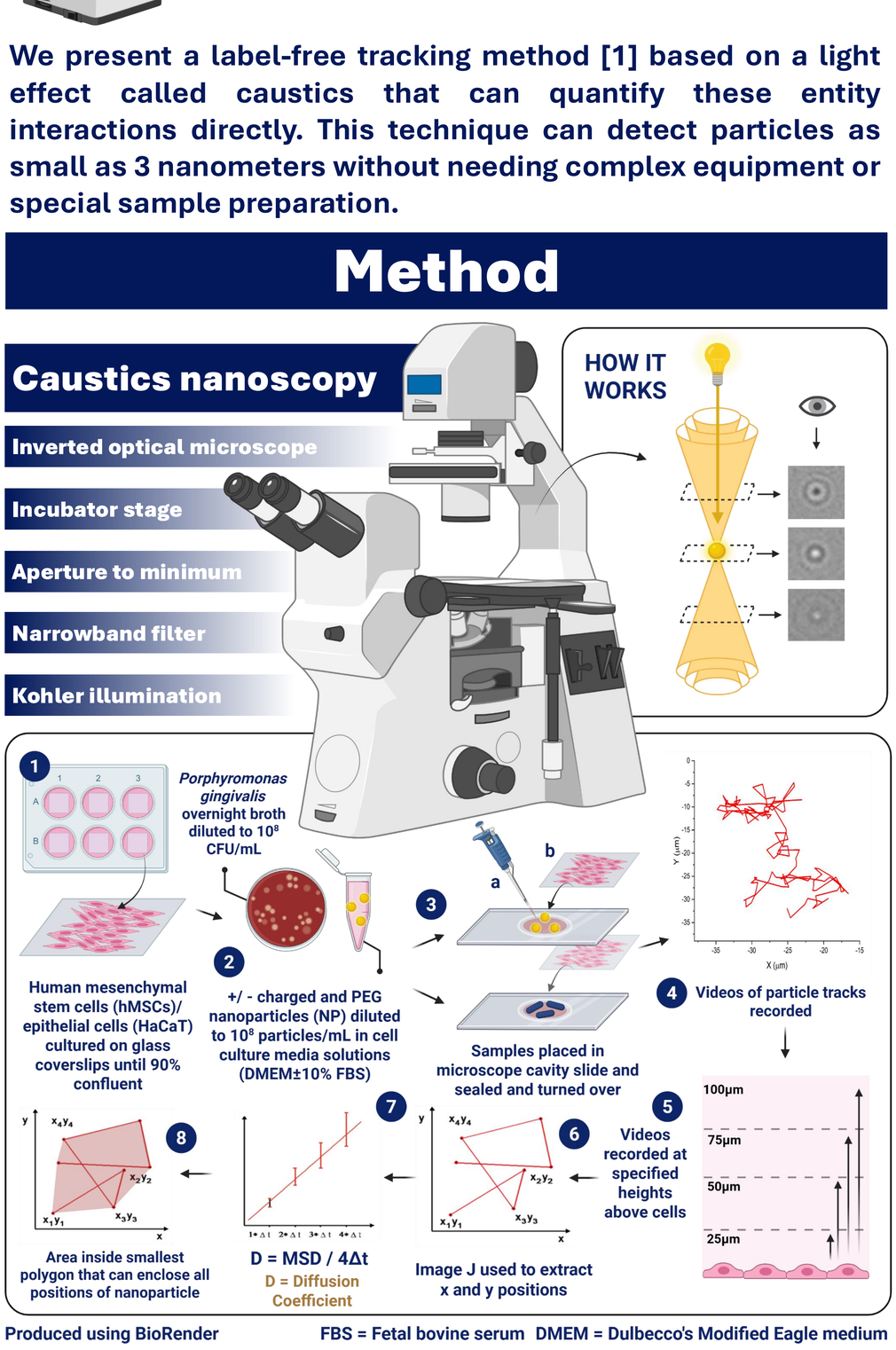
We present a label-free tracking method [1] based on a light effect called caustics that can quantify these entity interactions directly. This technique can detect particles as small as 3 nanometers without needing complex equipment or special sample preparation.

Method

Caustics nanoscopy

- Inverted optical microscope
- Incubator stage
- Aperture to minimum
- Narrowband filter
- Kohler illumination





Produced using BioRender

FBS = Fetal bovine serum DMEM = Dulbecco's Modified Eagle medium

Results

1 NP visualisation

NP = Nanoparticle

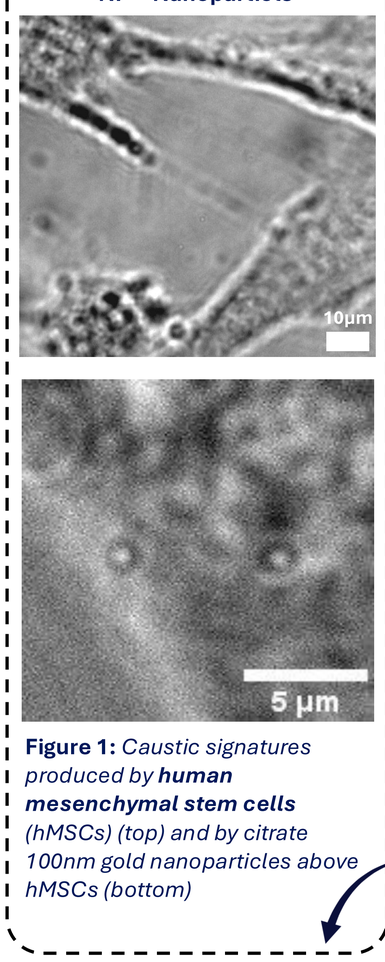


Figure 1: *Caustic signatures produced by **human mesenchymal stem cells** (hMSCs) (top) and by citrate 100nm gold nanoparticles above hMSCs (bottom)*

2 Presence of cells influences NP dynamics

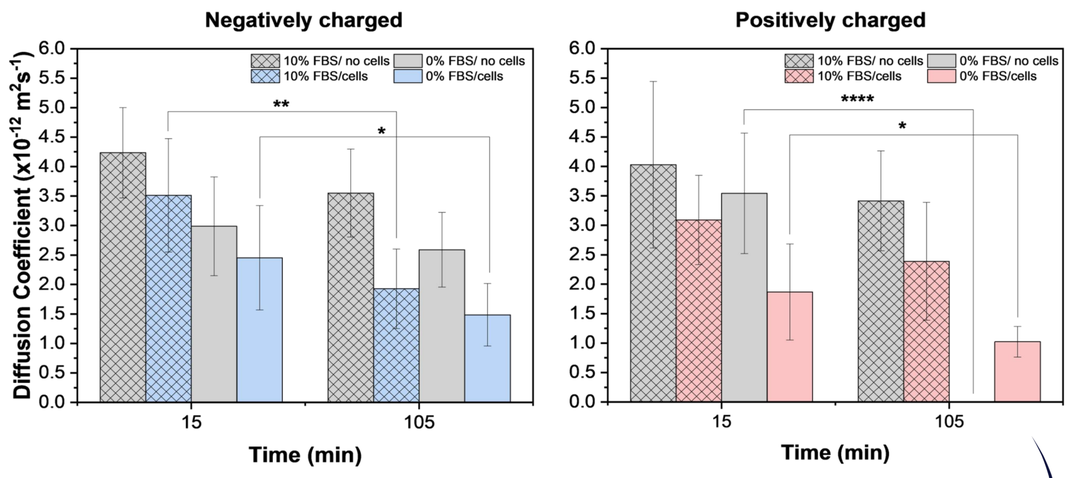


Figure 2: Diffusion Coefficient of *gold nanoparticles with a diameter of 100nm in DMEM (Dulbecco's modified Eagle medium) with 10% FBS (Fetal bovine serum) and no FBS both **in the presence (colour) and absence (grey) of cells** as a function of time after exposure to cell/control surface, tracked 50µm above the cell layer surface. (* p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)*

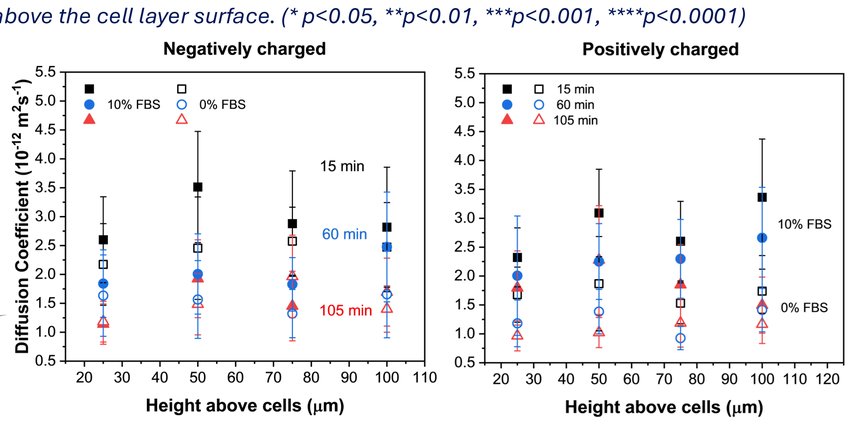


Figure 3: *Diffusion Coefficient of gold nanoparticles with a diameter of 100nm. Several factors influence nanoparticle dynamics including **charge and environmental conditions.***

3 Cell type influences dynamics

Negatively charged NPs, DMEM + 10% FBS

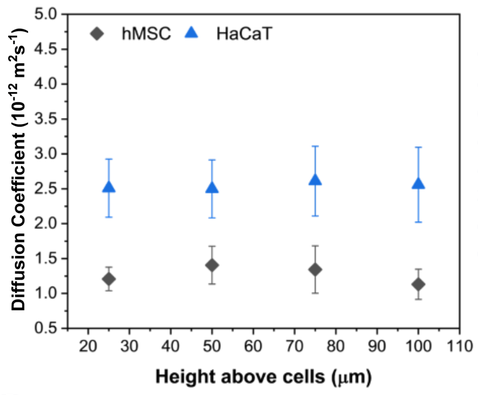


Figure 4: *Diffusion coefficient of negatively charged (citrate) 100nm gold nanoparticles in DMEM + 10% FBS as a function of height above **two different cell layers.***

4 Protein corona affects dynamics

NPs in DMEM + 10% FBS, HaCaT cell layer

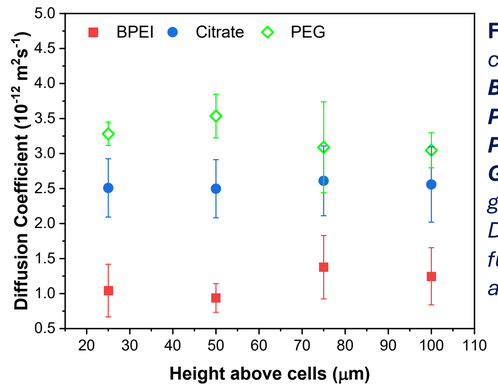


Figure 5: *Diffusion coefficient of **citrate, BPEI (Branched Polyethylenimine) and PEG (Polyethylene Glycol) coated** 100nm gold nanoparticles in DMEM + 10% FBS as a function of height above a HaCaT cell layer.*

6 Bacteria visualisation

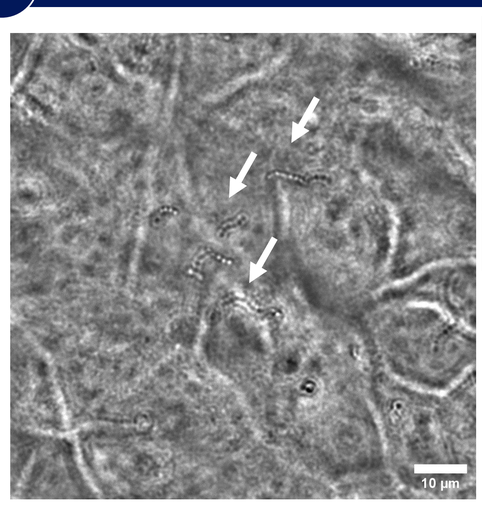


Figure 6: *Streptococcus sanguinis (white arrows) on the surface of human epithelial cells (HaCaT) using caustics nanoscopy*

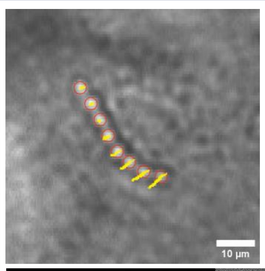


Figure 7: *Tracks of individual bacterium (Streptococcus sanguinis) at cell surface*

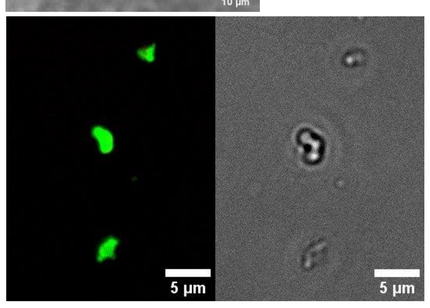


Figure 8: *Porphyromonas gingivalis fluorescent LIVE/DEAD stain vs Caustics on glass slide with no human cells present*

5 Convex hull analysis

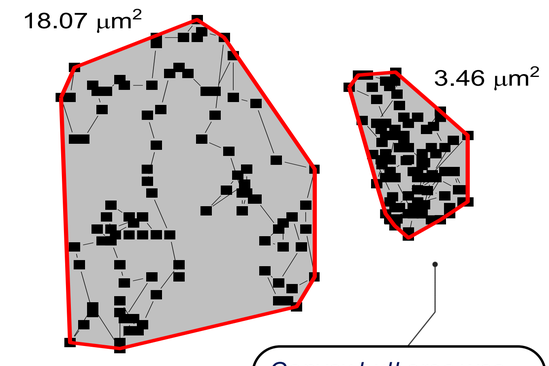


Figure 9: *convex hull areas of negatively charged nanoparticles in DMEM with 10% FBS 15 min after exposure to cells.*

Convex hull area was not uniform when measured over a fixed period of measurement for all particles and media conditions suggesting environment heterogeneity.

Conclusions

- Developed a **cost-effective** technology platform that enables **label-free, real-time** tracking of nanoscale entities and extraction of quantitative dynamic information in multi-component biological systems
- **Comprehensive model** of nanoparticle diffusion above a cell layer has been developed
- Provided a robust tracking technique and **fundamental models** that can be applied to ***multiple*** scenarios and aid in the design *in vitro* models

REFERENCES

[1] Patterson, E.A. and Whelan, M.P. (2008). Optical Signatures of Small Nanoparticles in a Conventional Microscope. *Small*, 4(10), pp.1703–1706.

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