Towards optimising characterisation of metal nanoparticles in complex matrices; method selection, comparisons and challenges

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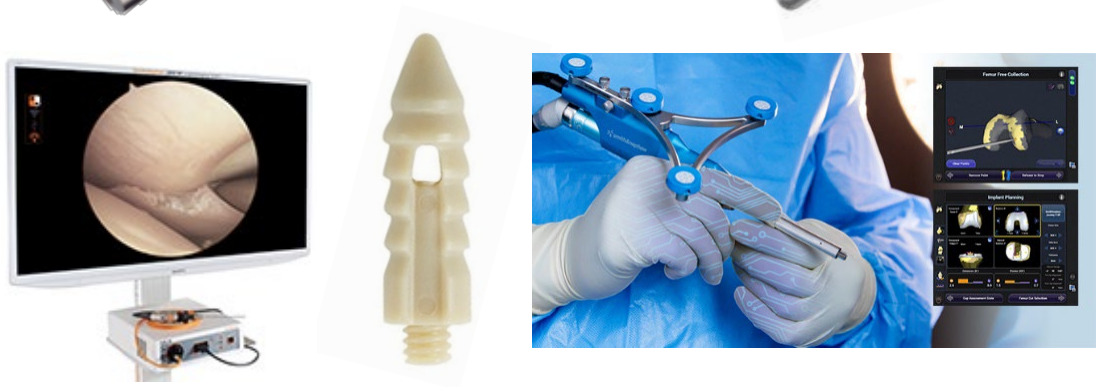
Background and Context; medical devices

Advanced Wound Management
Medium to hi-tech dressings: nano-structured anti-microbial dressings, vacuum dressings, hydro-jet scalpel, ointments, digital patient monitors

Orthopaedics Implants: hips, knees, shoulders, pins, plates, nails

Sports Med Cartilage repair endoscopes, tendon repair products & resorbables, robotic surgery, augmented reality surgery



ENT Plasma tissue ablation

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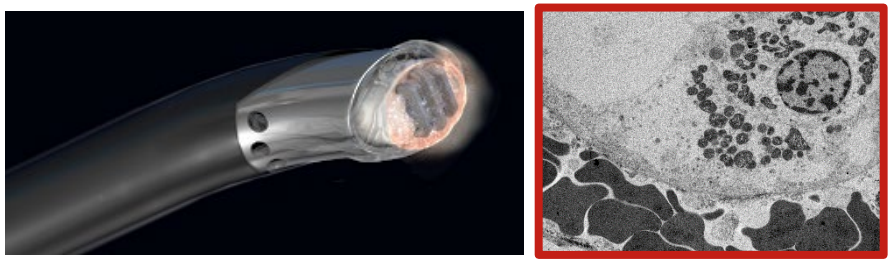
TISSUE! In-vitro cultured cells and ex-vivo tissue

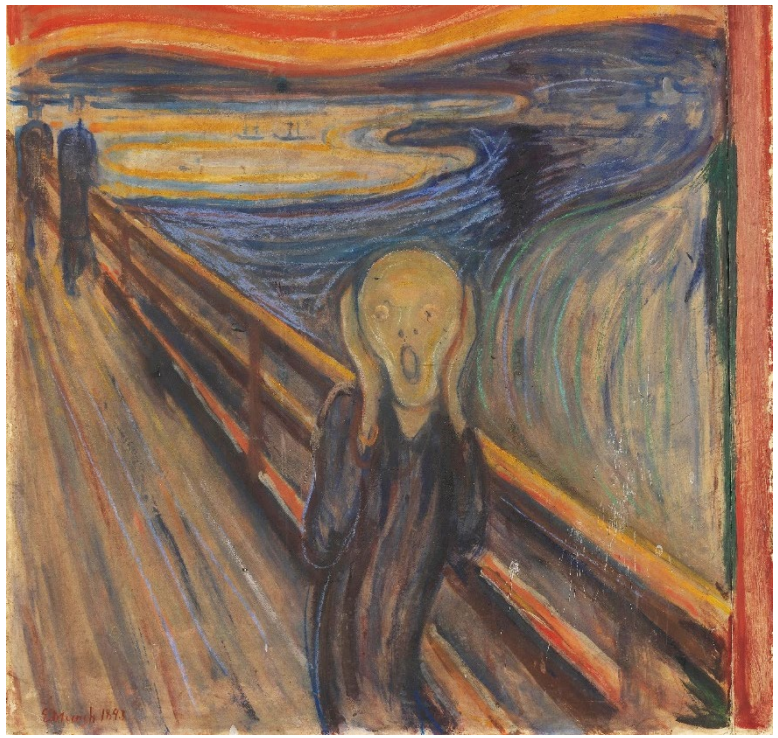


Background and Context; complexity

x(matrices) + y(microstructures) + emerging regs* = analytical complexity

Nanomaterial characterisation in complex matrices =

*Emerging regulations have been helpful;
grateful for guidelines such as ISO10993, part 22



By Edvard Munch - National Gallery of Norway 8 January 2019 (upload date) by Coldcreation, Public Domain, https://commons.wikimedia.org/w/index.php?curid=69541493

Methods: accessibility and selection

$ → $$$ → $$ → $$$

SME → Global Corporate → Medical Devices → Pharmaceuticals

simple methods $ → advanced methods $$$

Inherent methodology uncertainties necessitate multiple methods, increasing budgetary constraints and therefore accessibility

Where to start? complex problem; seek help

Invaluable for:

- Benchmarking methodologies, our own laboratory
- Benchmarking Shared learning and dissemination of expertise/practice across standards institutions, academia and industry
- De-risking product development

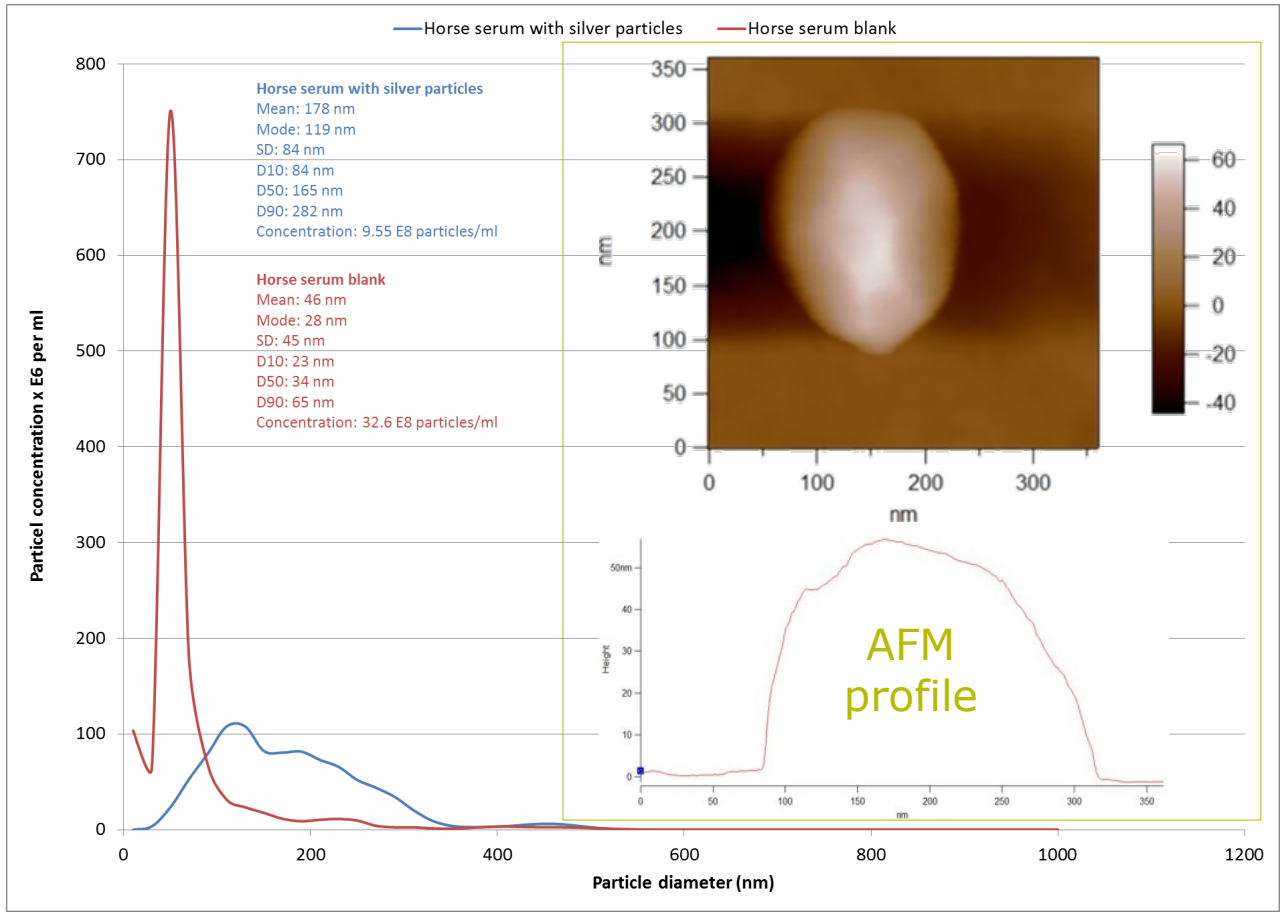
Toughest challenges?

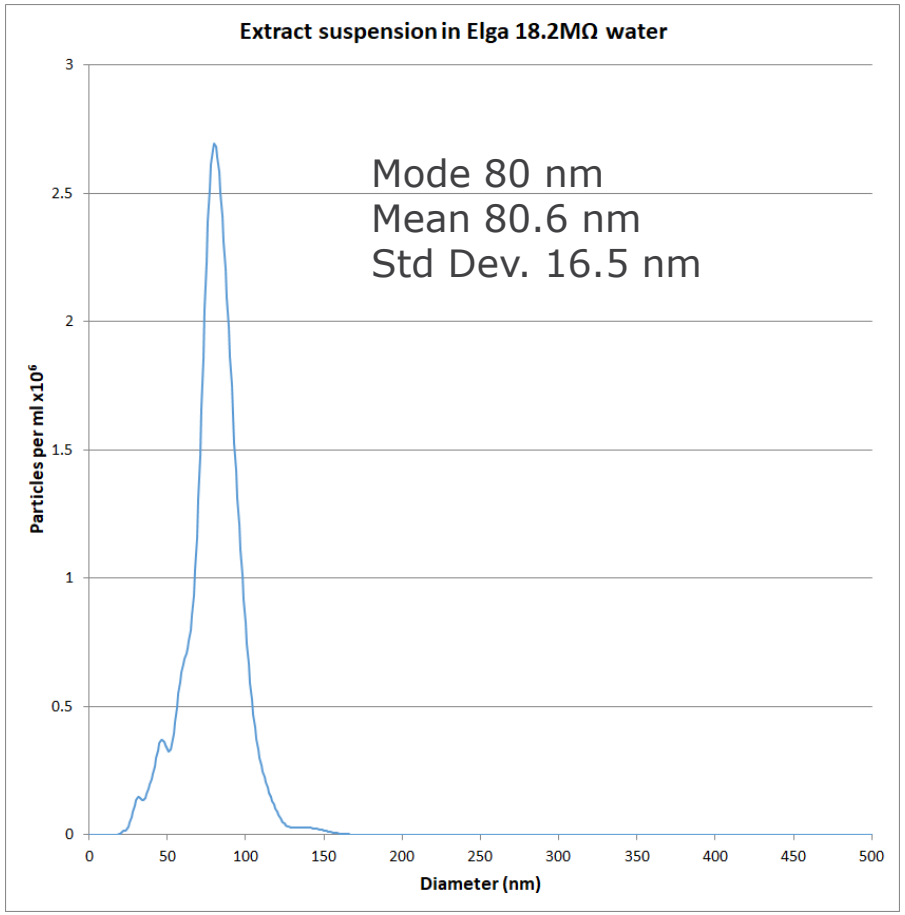
- Characterisation sub 100nm, but more so sub 30nm
- Characterisation in complex matrices
- Understanding fate of nanomaterials in biological systems (us!)
  → environmental fate

Characterisation sub 100nm and 'simple' matrices; Example system 1, Ag NP's of ca. 80nm diameter

'Simple' media – in-vitro dressing extracts by multiple techniques:

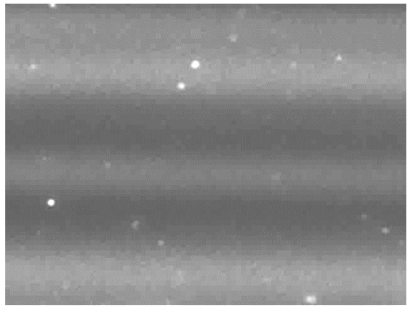
PTA (NanoSight) sample extracted into horse serum; sera contained (eicosahedral) pestivirus (BVD) as well as protein aggregates, convoluting acquired data

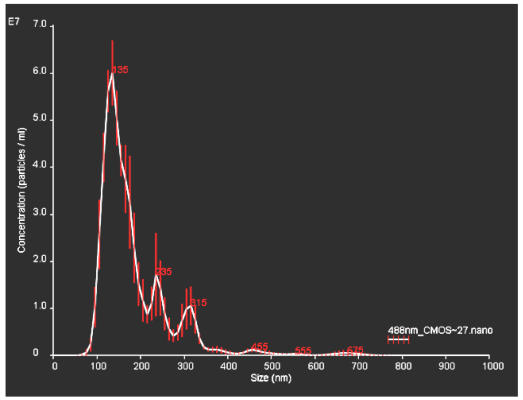




Characterisation sub 100nm and ‘simple’ matrices; Example system 2, Ag NP’s of ca. 34nm diameter

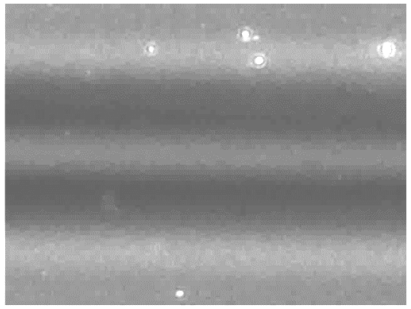
Control - CSWF Filtered

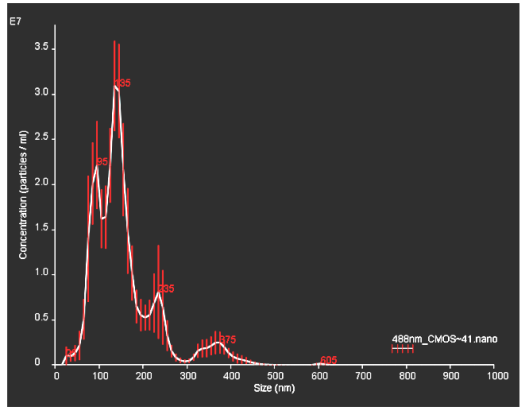




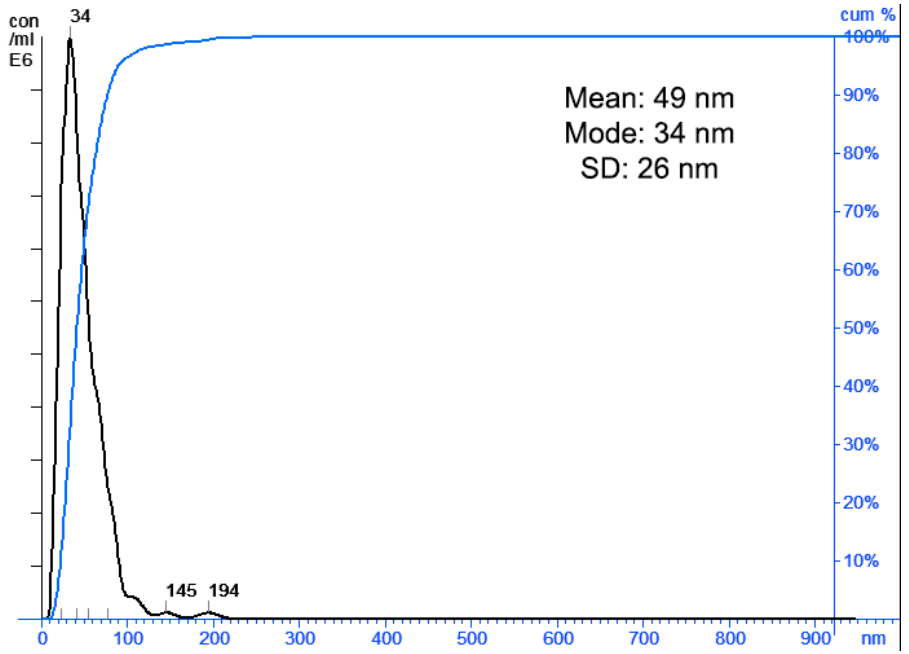
Polydispersed system with a broad particle size, ranging from approximately 60-700 nm.
Average modal particle size: 128.1 nm.

Dressing CSWF Filtered



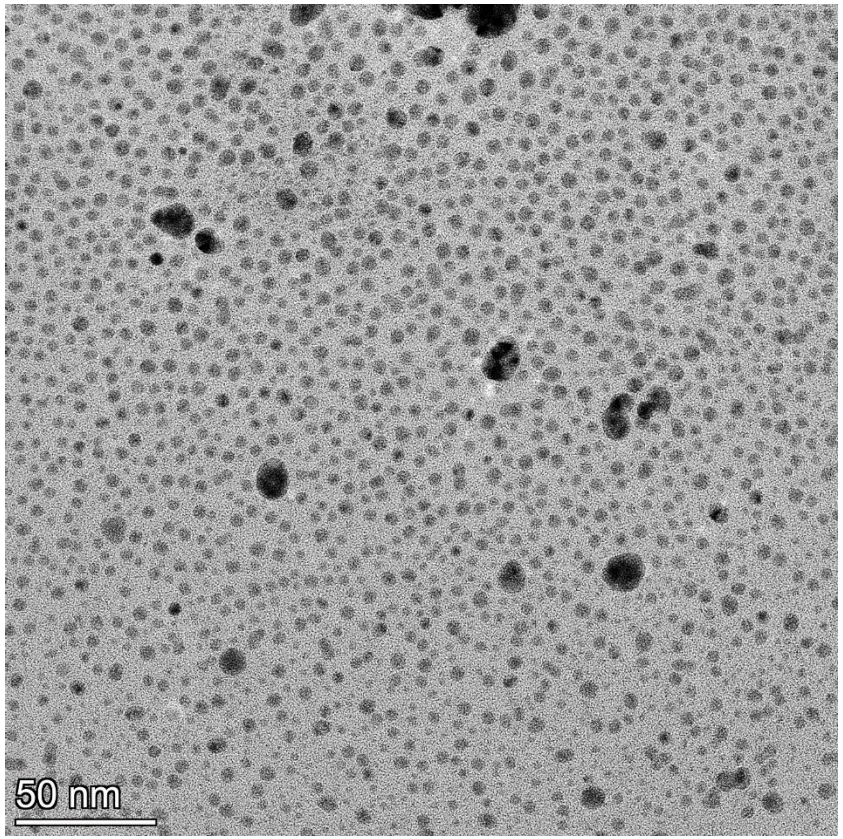


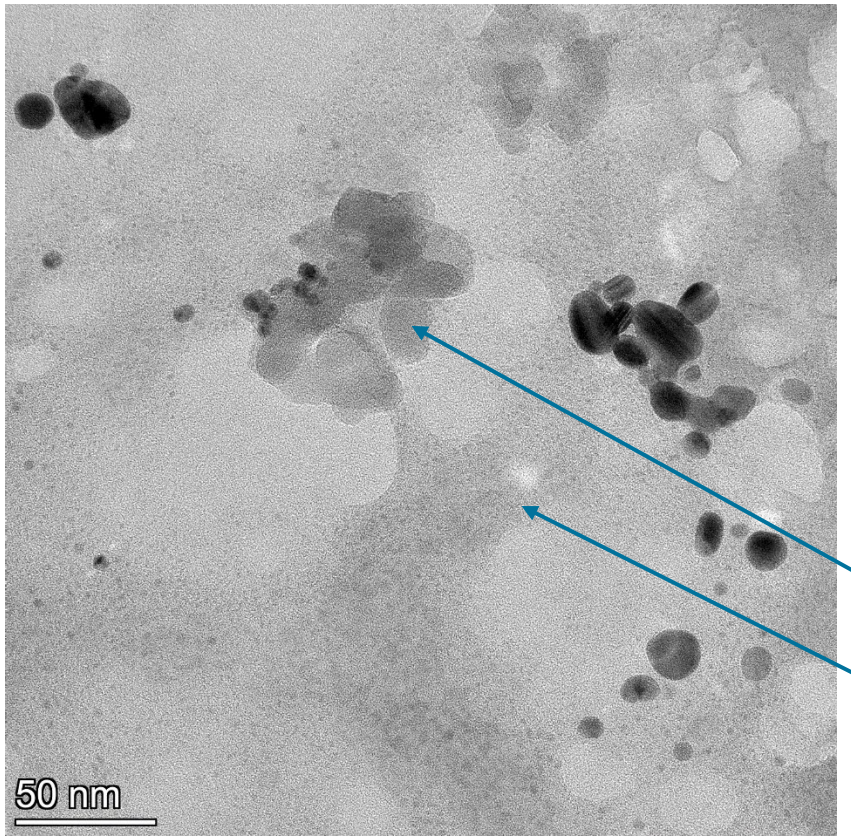
Polydispersed system with a broad particle size, ranging from approximately 30-600 nm.
Average modal particle size: 118.3 nm.



Characterisation sub 30nm and ‘simple’ matrices; Example system 3, Ag NP’s sub 30nm

Simple extraction medium, samples extracted into 5% Aq. EtOH (similar osmolarity and hydrophilicity as wound fluid, but without the proteins and other ‘bio-debris’)





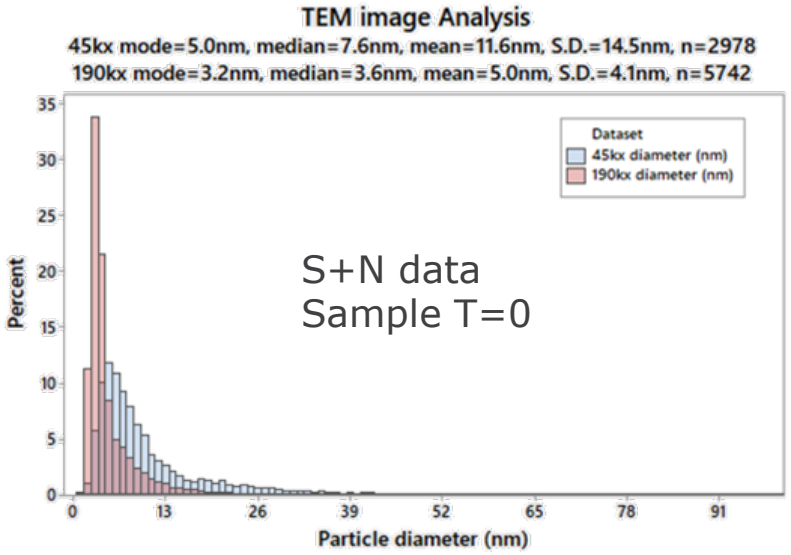
Co-extracted materials; simple matrix??

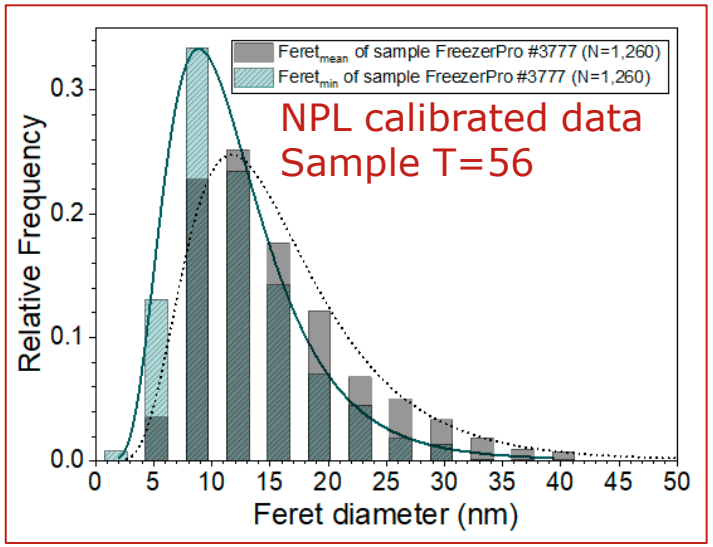
fumed silica

sodium-polyacrylate

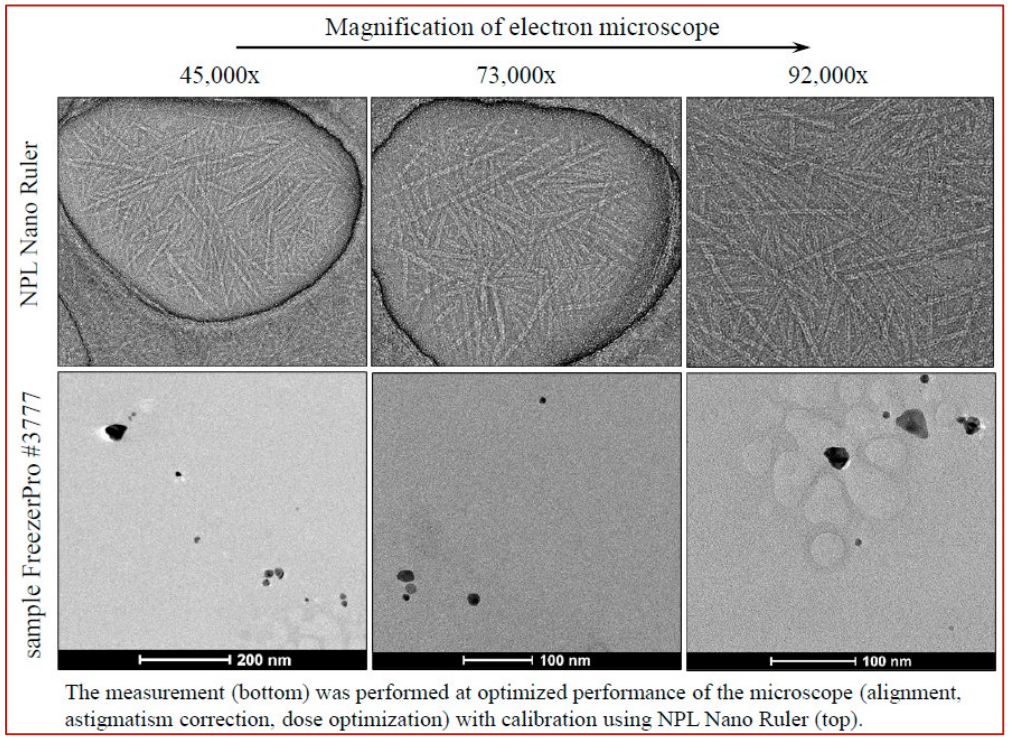
TEM, 200 mesh Cu with amorphous carbon support films, field-width 300 nm

Characterisation sub 30nm and 'simple' matrices; Example system 3, Ag NP's sub 30nm

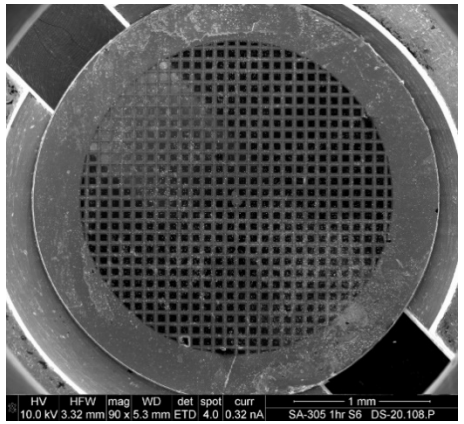


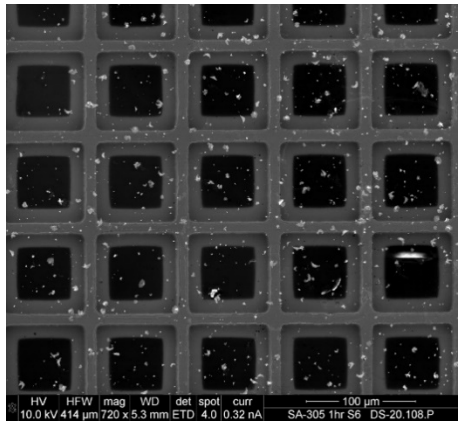


- 2µL deposited on grid = 190 millionths of total sample volume
- if homogenous across grid, each square carries only $4x10^{-9}$ % of total sample!



The measurement (bottom) was performed at optimized performance of the microscope (alignment, astigmatism correction, dose optimization) with calibration using NPL Nano Ruler (top).



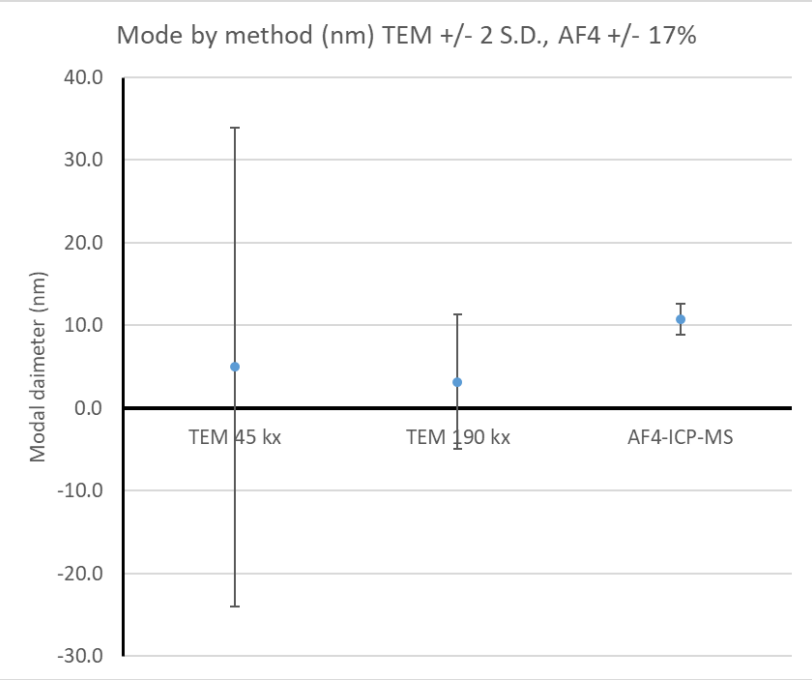


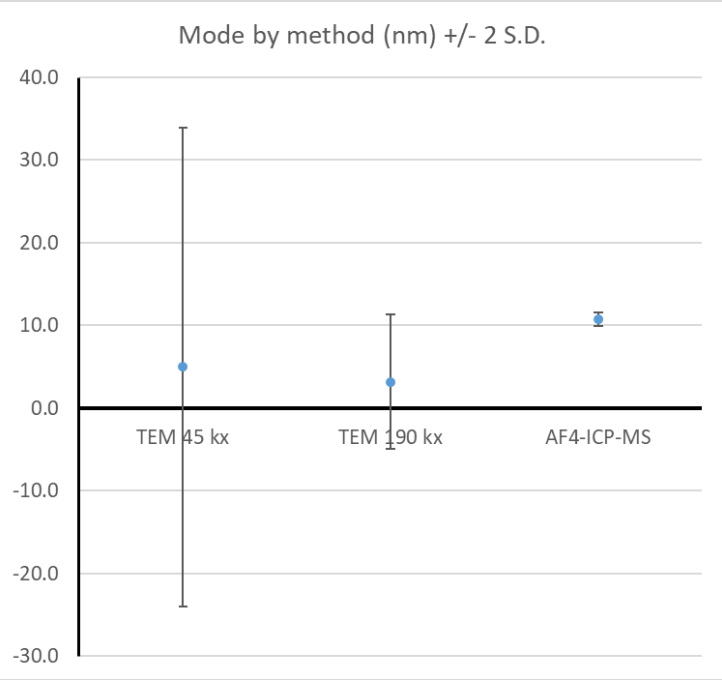
Characterisation sub 30nm and 'simple' matrices; Example system 3, Ag NP's sub 30nm

Questions of statistical quality:

Practicable limit to number of particles evaluated using direct visualisation methods, (TEM, AFM)

Bulk methods such as sp-ICP-MS or AF4-ICP-MS offer potential for analysing larger volumes of sample, PTA and DCS also improve statistics, but have matrix convolution and sensitivity issues

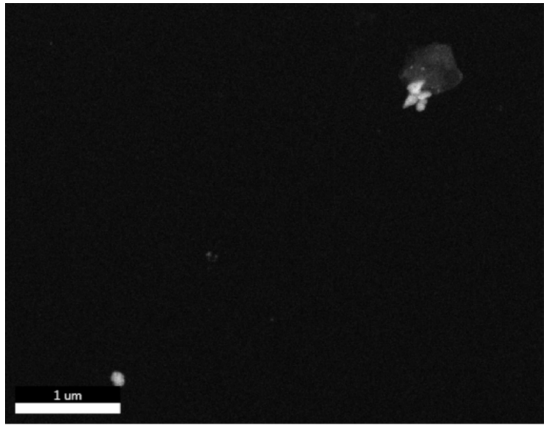


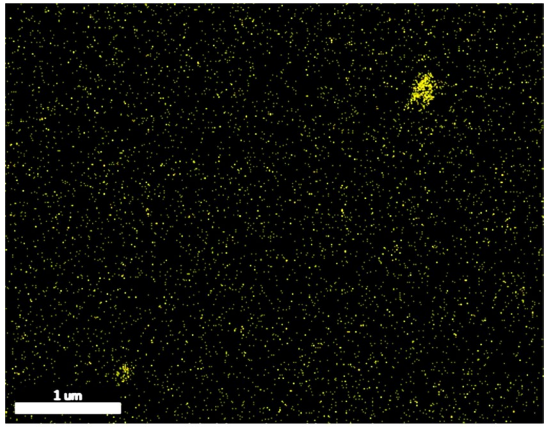


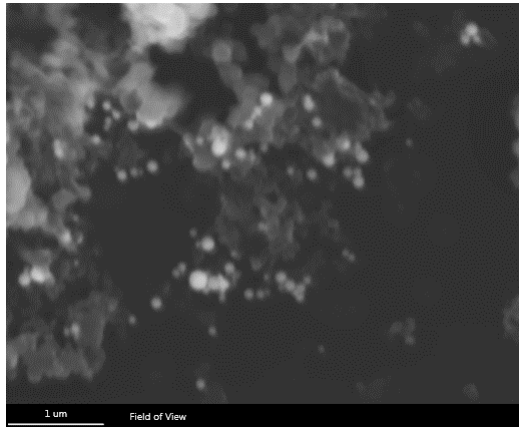
Sample ID	Total Ag*, mg kg^{-1}	Modal particle size, nm	Ag particle number concentration (in the main size fraction), kg^{-1}
Extracts 1 hour			
Blank	Below LOQ	n/a	n/a
Extract 1	15.23	11	2.29×10^{15}
Extract 2	15.07	10	2.51×10^{15}
Extract 3	12.84	11	1.76×10^{15}
Extract Pooled	19.78	10	4.05×10^{15}

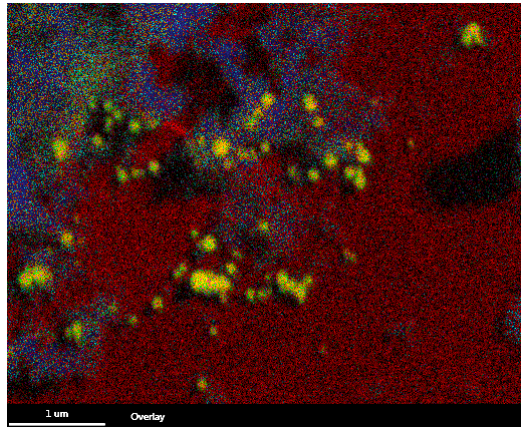
Characterisation sub 100nm; chemical speciation

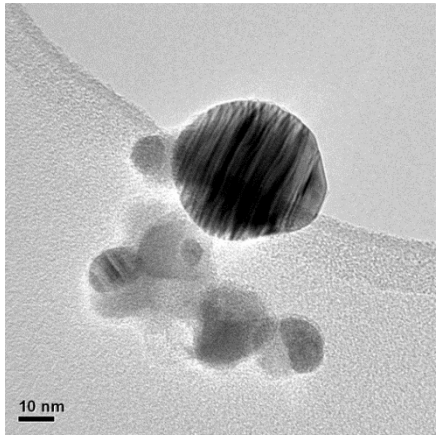
TEM grid mounted samples (minimising e-beam/sample interaction volume); FEG-SEM Energy Dispersive X-ray microanalysis

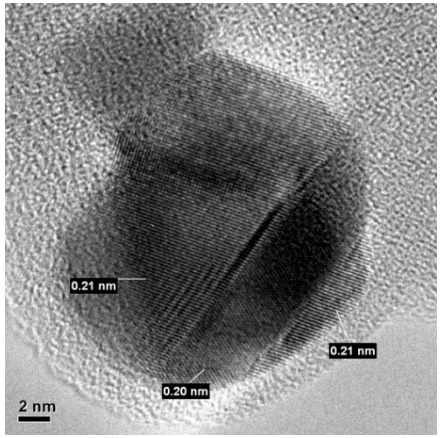


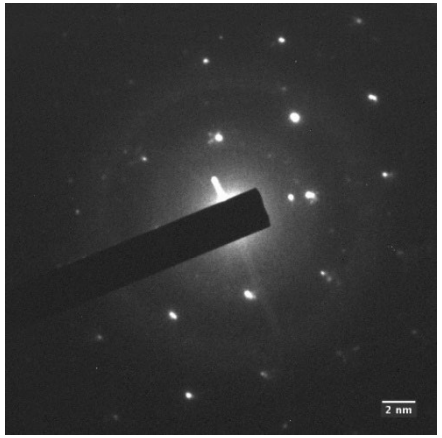








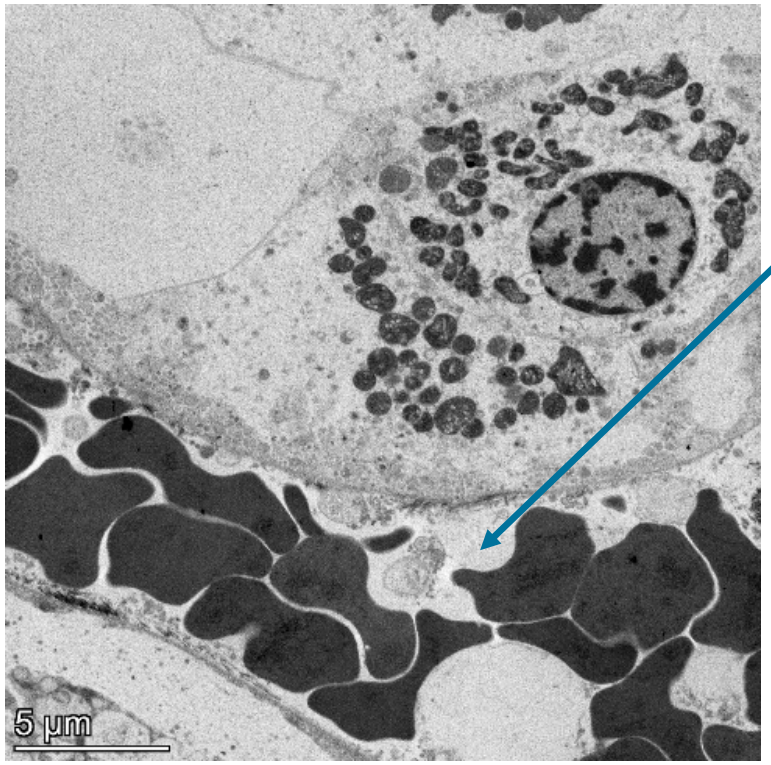




Measure d-spacing (nm)	Reference Pattern d-spacing (nm)	Alternate phase
0.233	0.235 / Ag (111)	
0.206	0.204 / Ag (200)	
0.146	0.145 / Ag (220)	
0.121 (faint ring)	0.123 / Ag (311)	0.121 / AgO (222) 0.118 / Ag_2O (004)

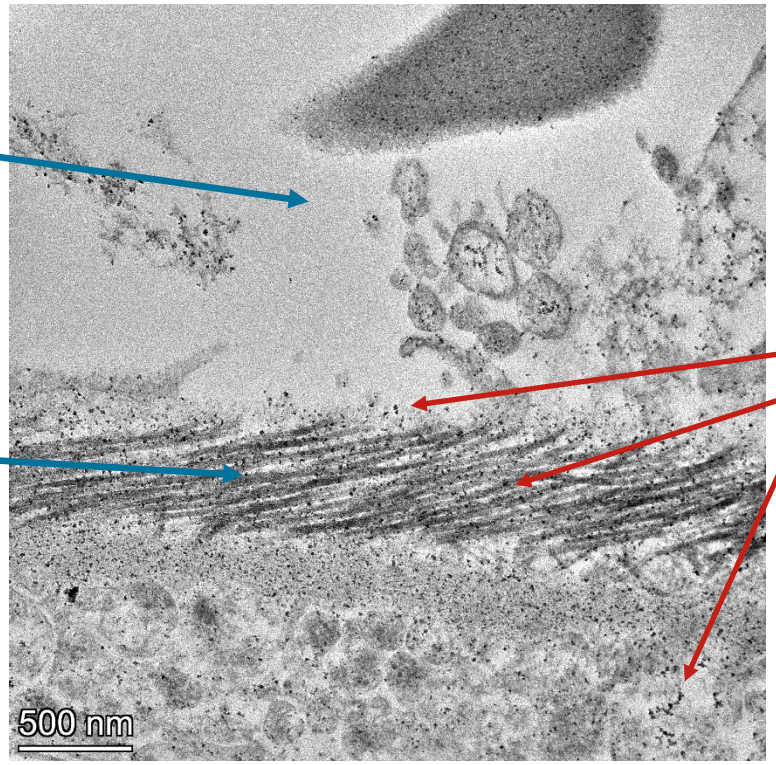
Single particle and AF ICP-MS provide chemical speciation as well as size data from larger volumes, but with cut-off sizes ca. 30nm and sub-5 nm respectively

Characterisation in complex matrices/biology



micro-vessel lumen

micro-vessel wall; collagen fibrils



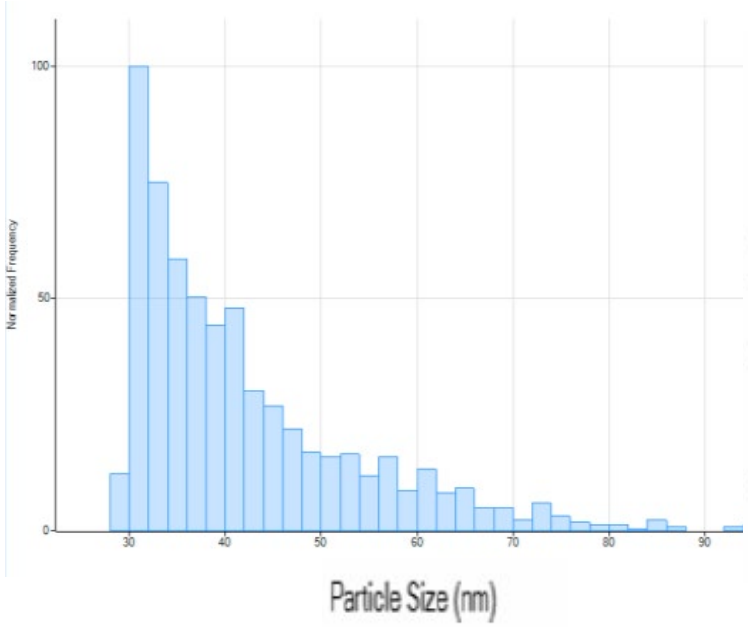
Intriguing electron-dense particles

TEM or other high vacuum direct visualisation methods:

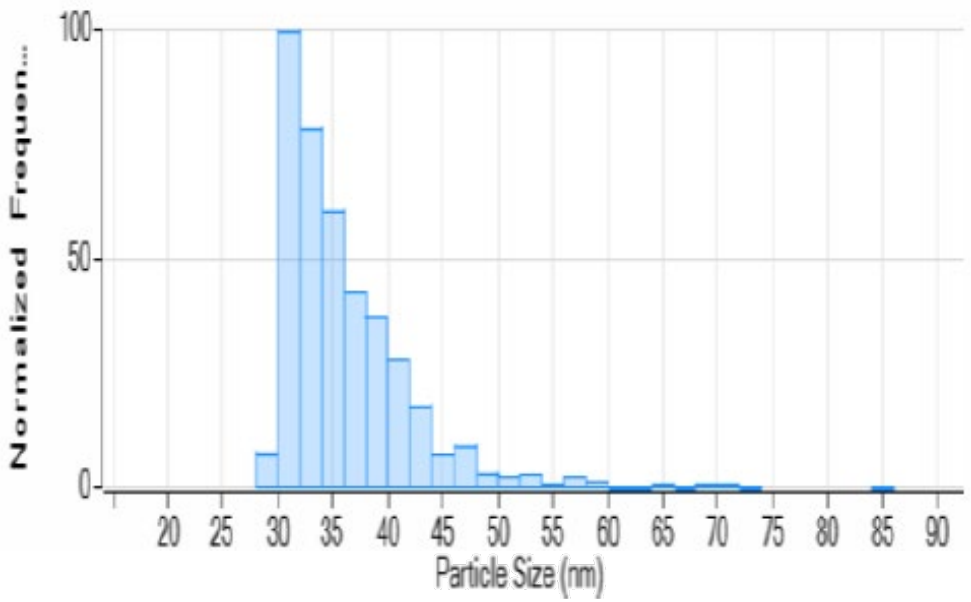
- Lengthy multiple fixation, post-fixation, rinsing, dehydration, resin-embedding and staining, counter-staining steps prior to imaging, maximising chances of analyte loss or modification
- Staining artefacts, beam-sensitivity (thermal distortion artefacts), stage/sample drift

Understanding fate of nanomaterials in biological systems (mammals/us!)

Sp-ICP-MS Ag NP's in blood



Sp-ICP-MS Ag NP's in liver

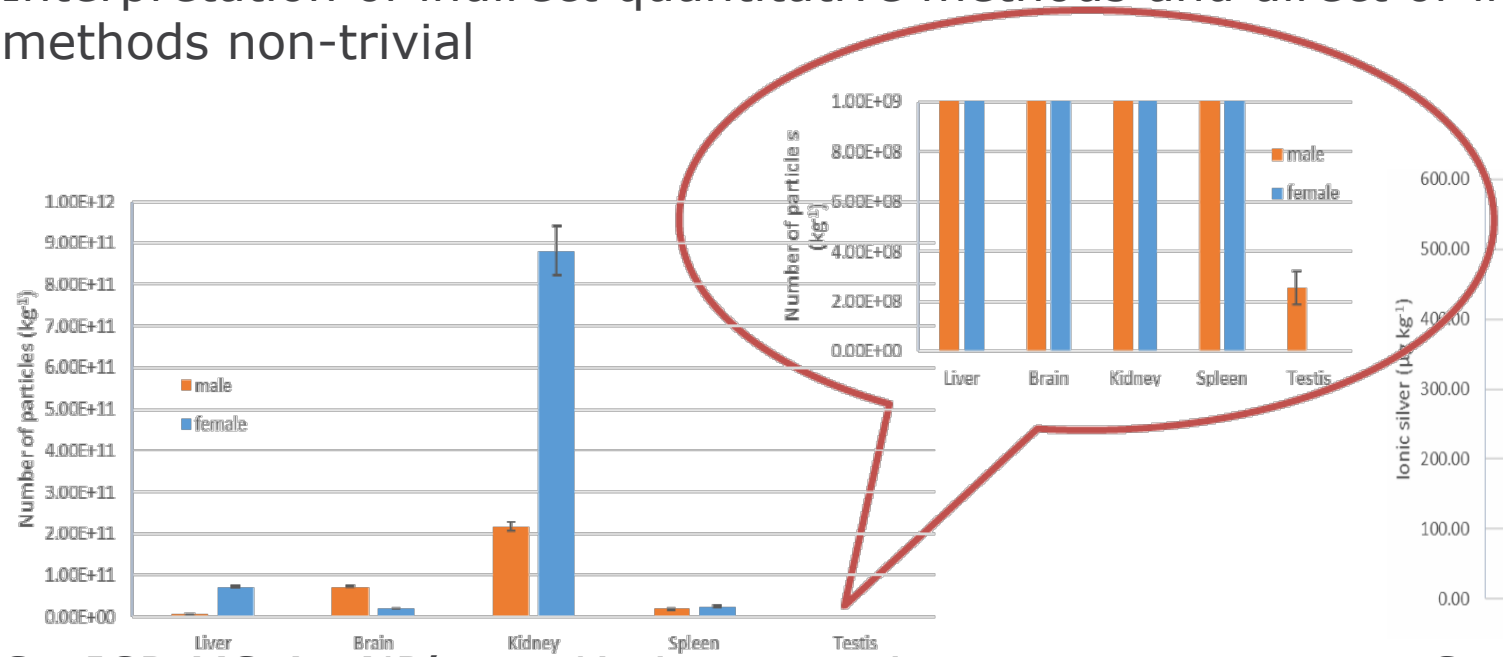


Sp-ICP-MS of digested body fluids/tissue provides number quantification, *ionic quantification, size distribution and speciation, but with <30nm cut-off*

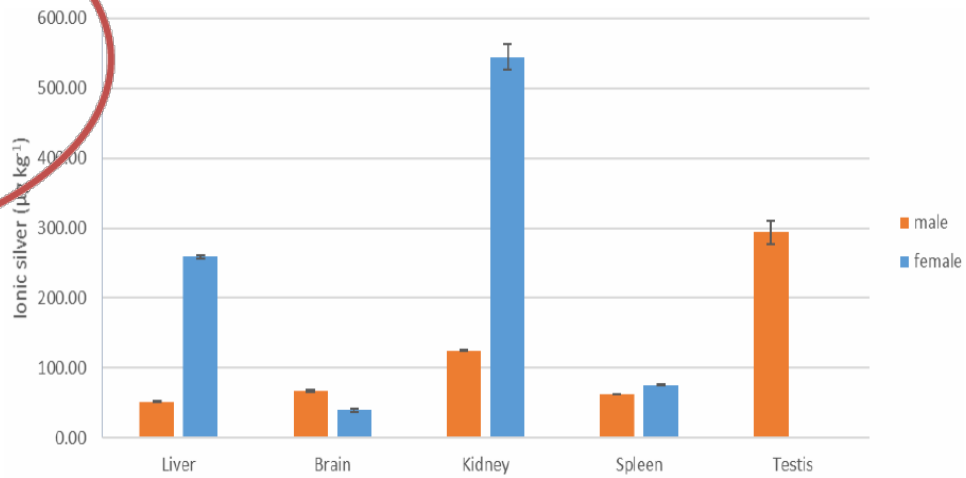
AF4-ICP-MS also provides number quantification and mean diameters, with cut-off at ca. <3nm, but not (directly) ionic concentration nor size distribution data

Understanding fate of nanomaterials in biological systems (mammals/us!)

Interpretation of indirect quantitative methods and direct or indirect qualitative visualisation methods non-trivial



Sp-ICP-MS Ag NP's per Kg in organ tissue

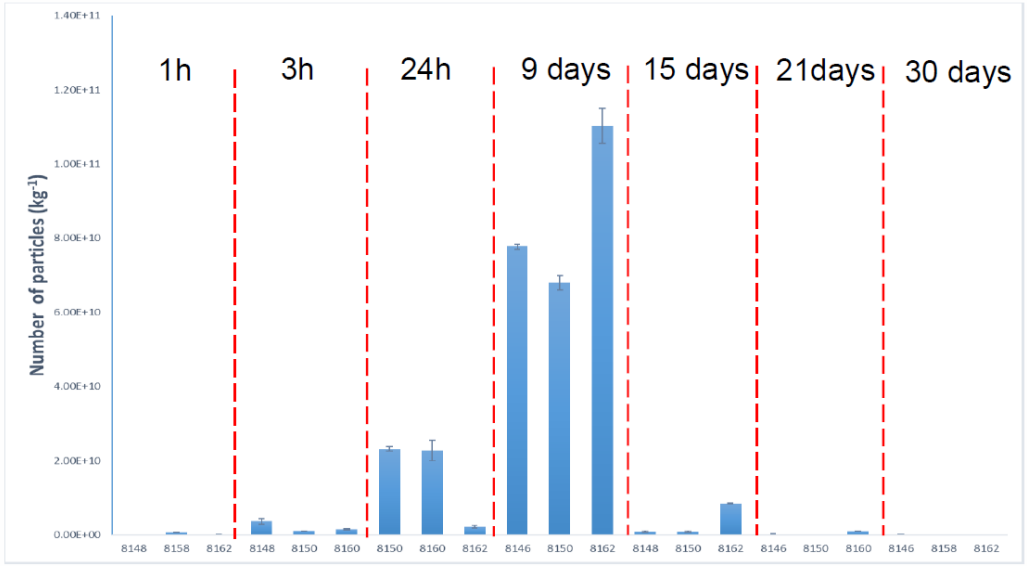


Sp-ICP-MS ionic µg/Kg Ag in blood

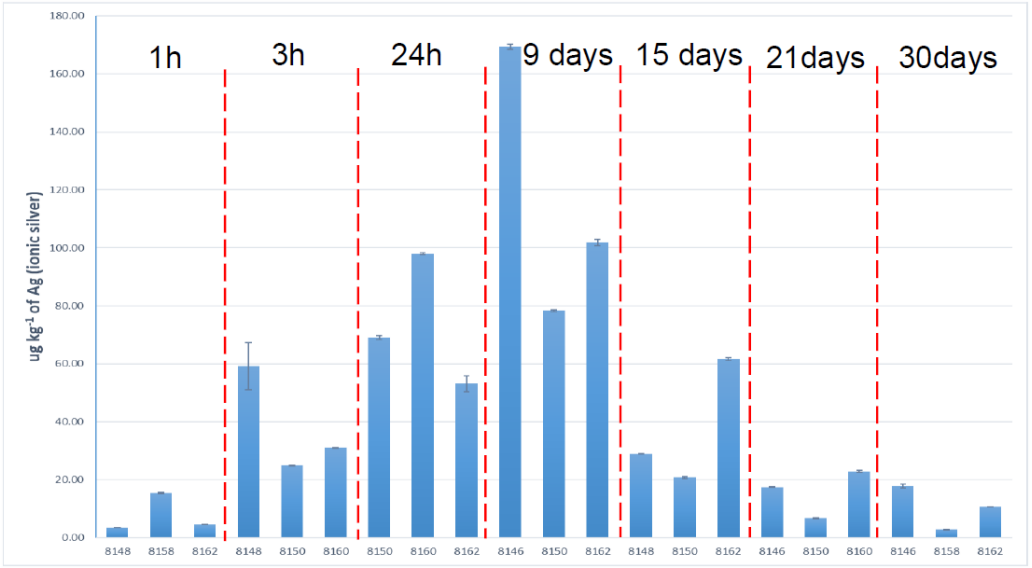
Sp-ICP-MS of digested organ tissue capable of quantifying Ag NP and ionic Ag levels by organ type (experimental data obtained from wound skin, kidney, liver, spleen, brain, testes and blood)

AF4-ICP-MS also likely to be able to quantify Ag NP levels by organ type, (experimental data successfully obtained for silica from same organs and blood)

Understanding fate of nanomaterials in biological systems (mammals/us!)



Sp-ICP-MS Ag NP's per Kg in blood



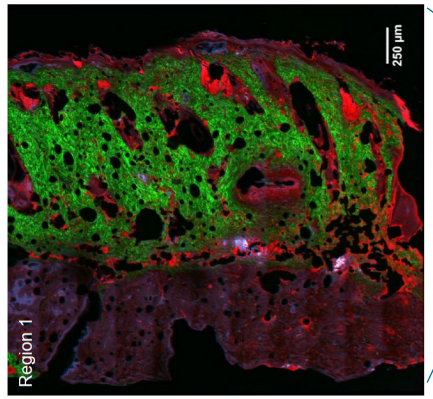
Sp-ICP-MS Ionic µg/Kg Ag in blood

Inter-comparison/interpretation of indirect quantitative bulk methods and direct or indirect qualitative visualisation methods is non-trivial

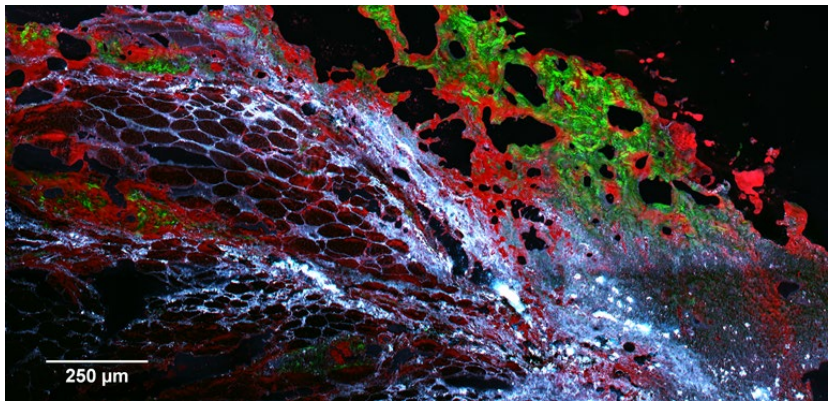
Understanding fate of nanomaterials in biological systems (mammals/us!)

Stimulated Raman Scattering (qualitative)

LA-ToF-SIMS (quant)



controls



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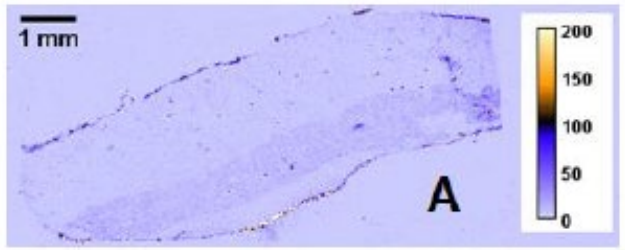
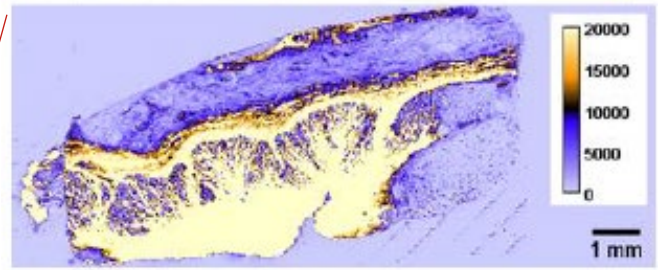
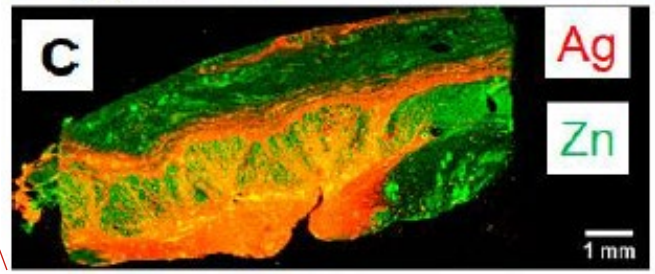






SRS and LA-ToF-SIMS from 40µm cryo-sectioned hydrated tissue

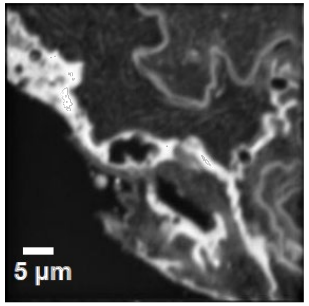
Red: CH_2stretching @ 2850 cm^{-1}

Green: Collagen (Second Harmonic Generation)

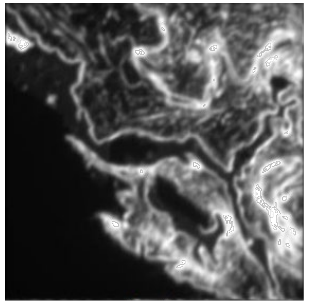
Cyan hot: possible Ag NP (Off-resonance@ 2770 cm^{-1})

SRS off-resonance from photothermal lensing effects – not definitively Ag

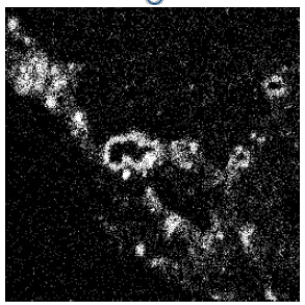
3D Nano-SIMS (quant?)



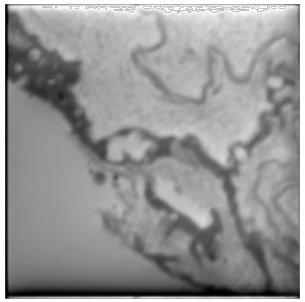
^{16}O



$^{12}C^{14}N$



^{107}Ag



Secondary electron

Nano-SIMS 80nm section
epoxy resin embedded
low signal for Ag
Ag washed away (prep)?

Conclusions, (or perhaps a wish list?)

Accessibility to appropriate methodologies

Need for policy-makers to recognise and support industry and regulators via subsidised access to well funded national core facilities with regard to demands of nanomaterial characterisation

1nm – 100nm uncertainties

Can mitigate complexity by employing multiple methods, but remains analytically challenging, particularly below 30nm, i.e. 1/3rd of the regulatory 1-100nm range

Appropriate inter-method comparison & interpretation

Strategies to avoid comparing 'apples with pears', remains scope for comparative studies

Guidance and practical regulation

More guidance documents are emerging across regulatory territories, each is welcome and so far guidance has been pragmatic. Encourage metrology community, notified bodies and industry to keep exchanging information, best practice

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