

Single Cell ICP-MS: Exposure, Dose and Response of Fresh Water Phytoplankton to Gold NPs

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HUMAN HEALTH • ENVIRONMENTAL HEALTH

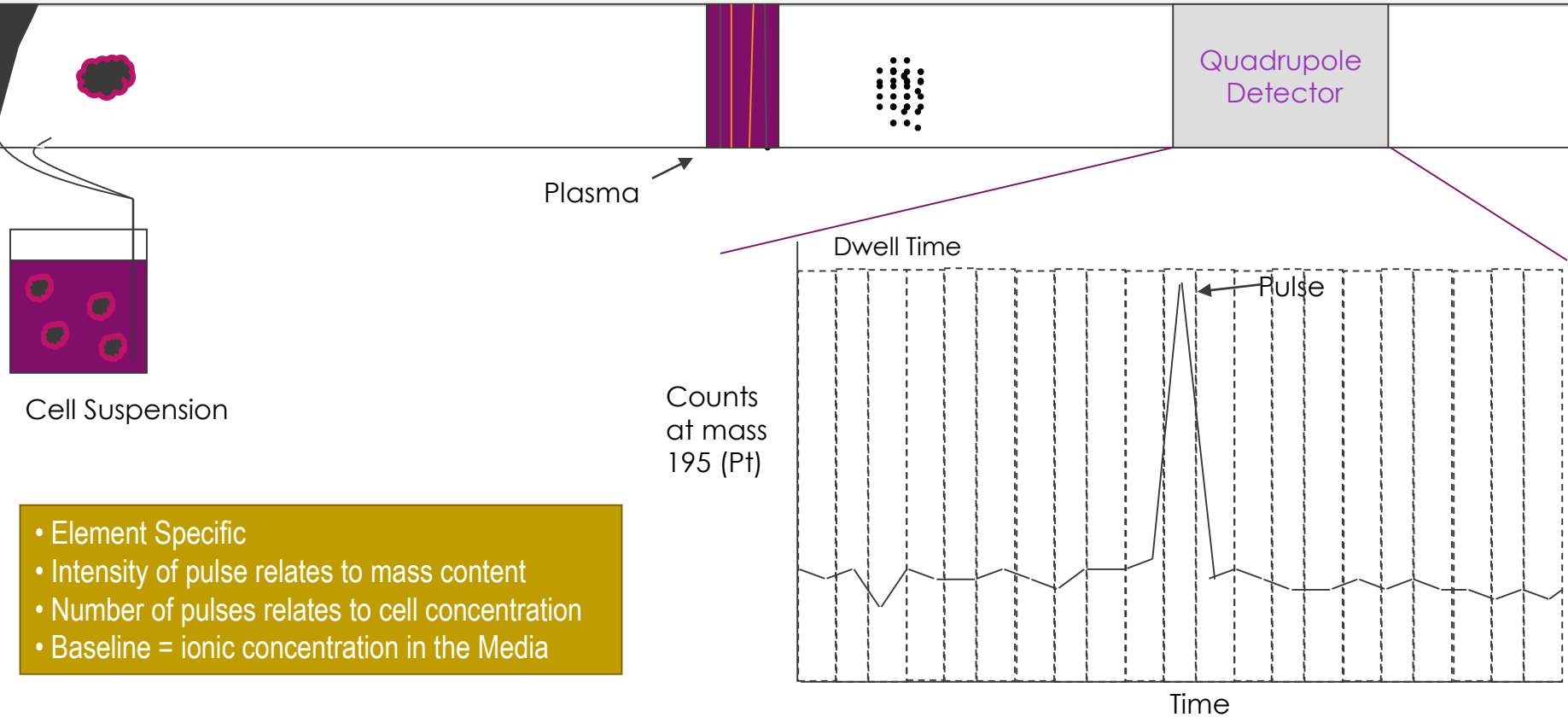
- Introduction to Single Cell (SC)-ICP-MS Analysis
 - Concept of Single Cell (SC)-ICP-MS
 - Considerations to proper analysis in Single Cell
 - Syngistix™ Single Cell Application Module

- Algae Cells Experiment
 - Uptake of NPs into algae cells
 - Uptake of ionic metals into algae cells

- Conclusion

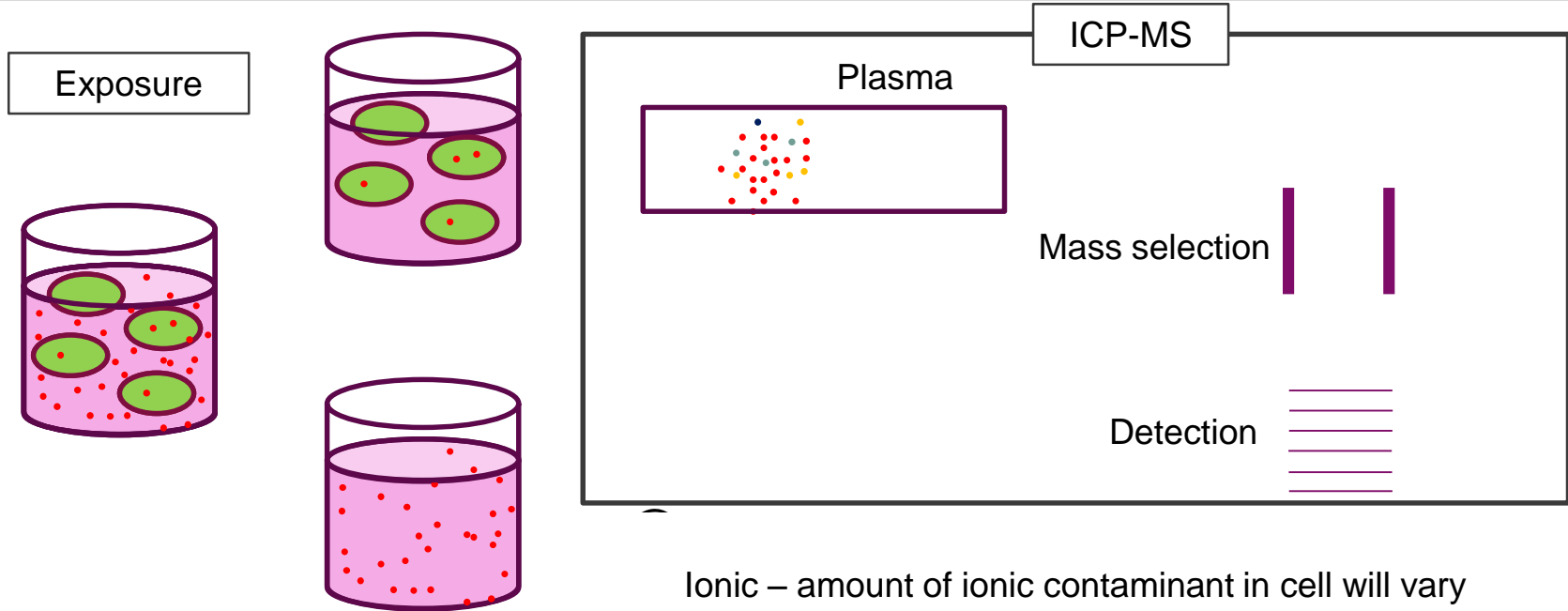


Concept of Single Cell ICP-MS Analysis



- Element Specific
- Intensity of pulse relates to mass content
- Number of pulses relates to cell concentration
- Baseline = ionic concentration in the Media

Exposure and Single cell ICP-MS Analysis



Ionic – amount of ionic contaminant in cell will vary

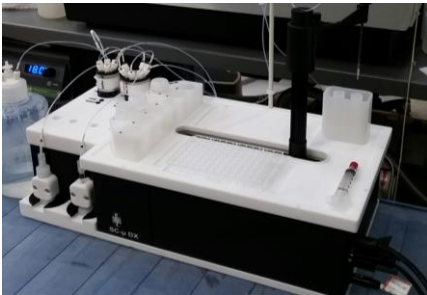
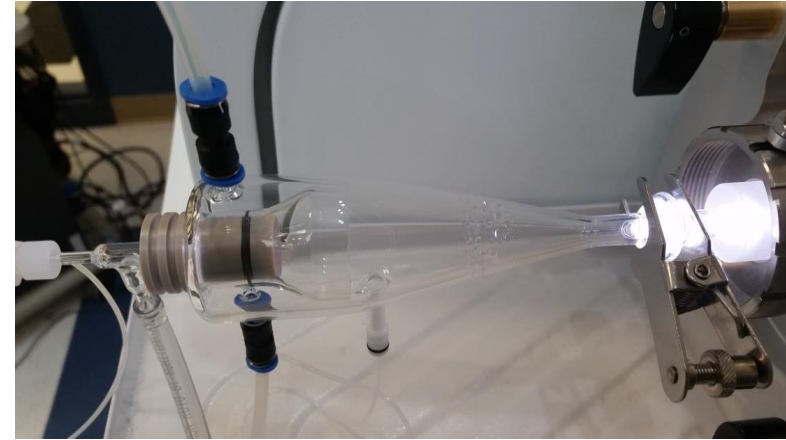
$I \neq II \neq III.$

NP – will be an integer of a single NP depending on number of NPs/cell

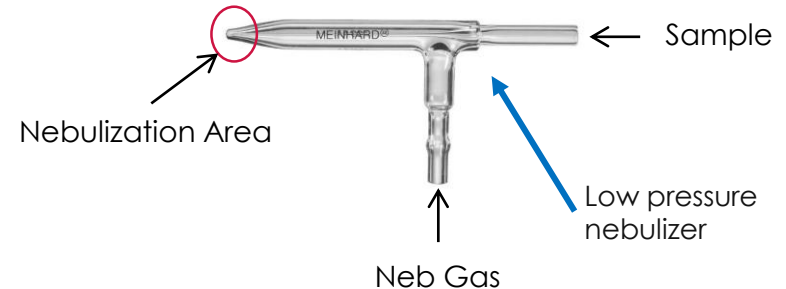
$II = 3I, III = 2I$

Hardware needed for Single Cell – ICP-MS

- Nebulizer – Should ensure the nebulization of cell suspensions while maintaining integrity of the cell.
- Spray Chamber – Should ensure the delivery of cells to the plasma while maintaining accurate transport efficiency.
- Auto Sampler – Should ensure sample mixing as cells tend to settle in vials and offer temperature control to maintain cell viability

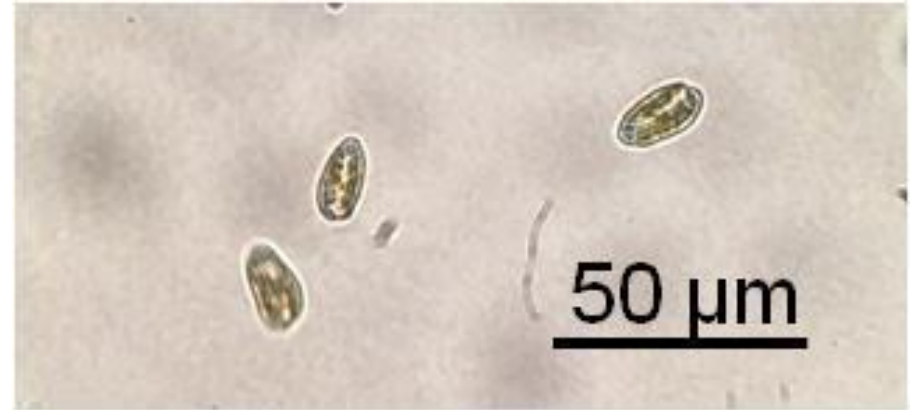


Syringe driven autosampler



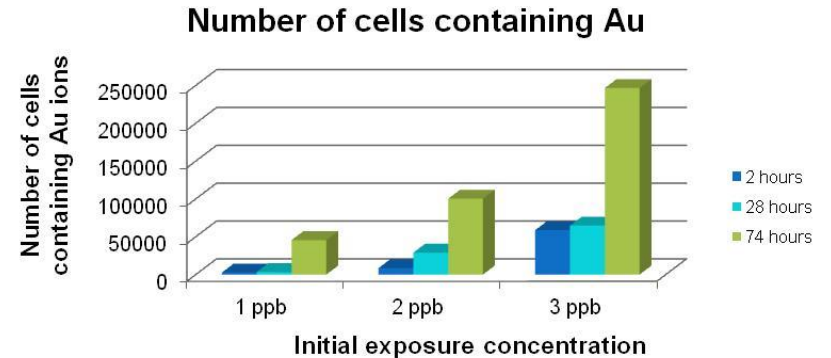
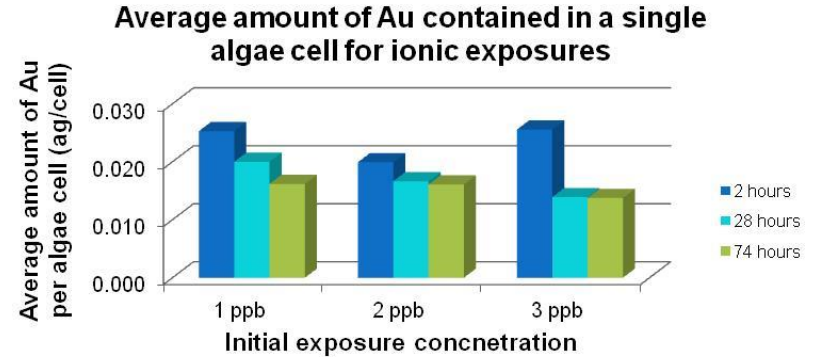
Algae experiment: Exposure Conditions

- Cell line: ***Cryptomonas Ovata***
- NPs: 60 nm Au NIST
 - ~ 57 nm diameter
 - ~ 1770 attograms / NP
- 200,000 cells / mL
 - 12:12 light dark cycle
 - Temperature 20 °C
 - 77 hour exposure
 - Samples taken at 2, 29, 53 and 77 hours
- Initial exposure concentrations
 - NPs: 200,000 part. / mL and 600,000 part / mL
 - Ionic: 1, 2, and 3 ppb



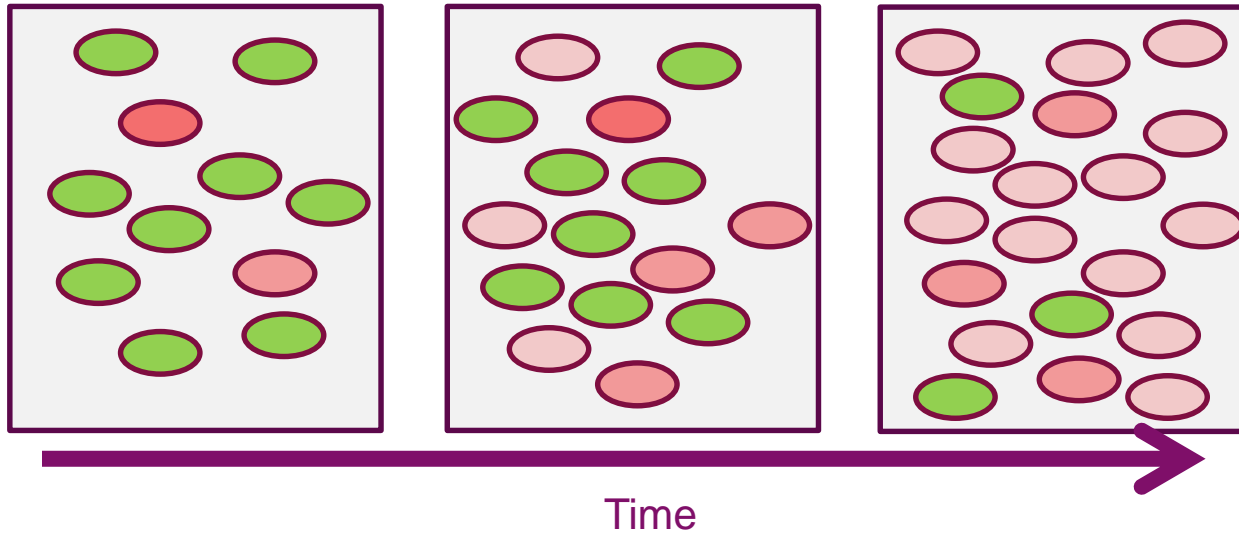
Au ionic uptake into cells

- For ionic exposures, there is not direct correlation between exposure and amount of Au per cell
- Over time, mass of Au per cell drops
- Amount of cells containing Au metal increases



Au ionic uptake into cells

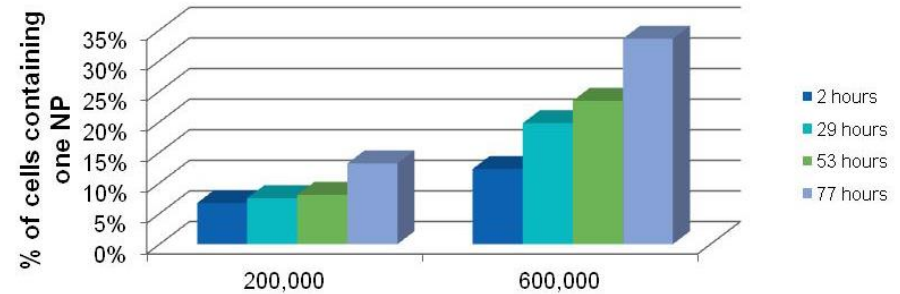
- Increase in number of algal cells
- Increase in number of cells containing metal
- Equilibration and dilution and through population over time cell population



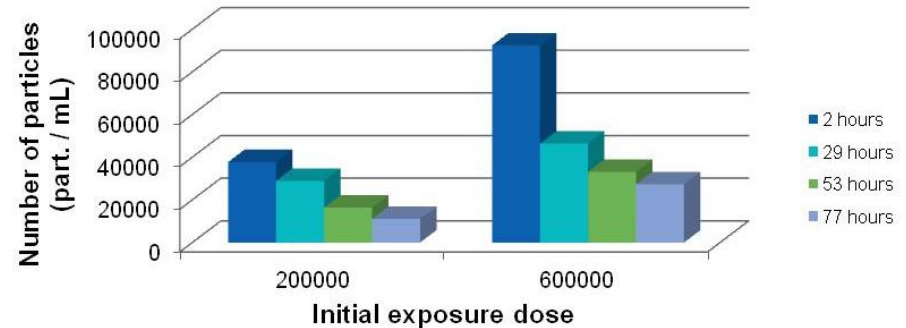
Au nanoparticle uptake over time

- There is an increase of the % of cells containing one NP with increased concentration
- Increases are also seen for all exposures over time
- Decrease in the number of NP particles per cell over time

% of algal cells containing one NP



Change in exposure over time



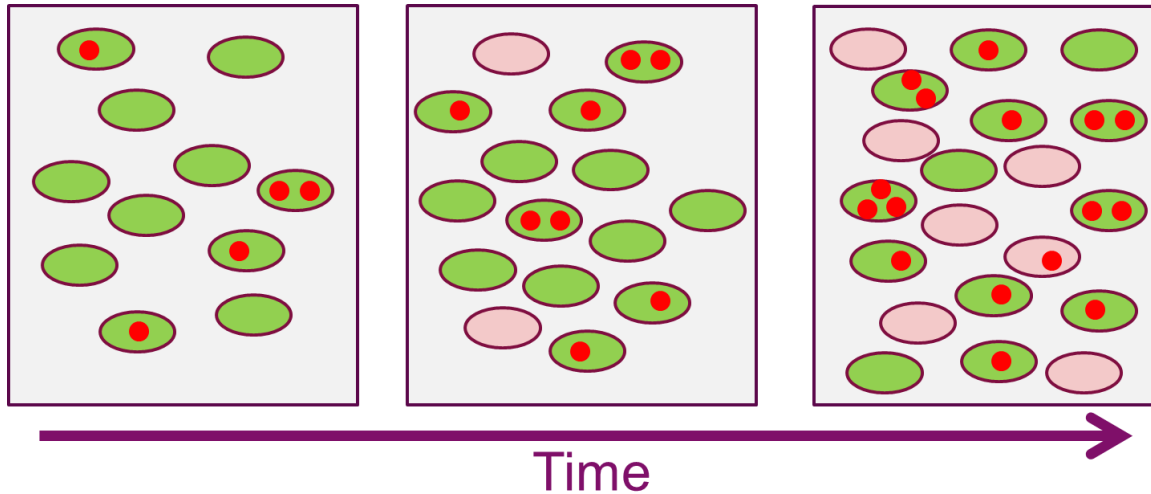
NP uptake over time

- The main peak at around 1700 ag is due to single NPs per cell (labelled 1P/1C)
- The number of cells containing 1P increases with time
- Over time, the presence of two (around 3400 ag) and three (around 5100 ag) particles per cell increases (Marked as 2p/1c and 3p/1c)
- Dashed black line marks the initial mass of NPs in cells
- There is a decrease in the amount of Au/cell over time suggesting possible dissolution of Au in the cell followed by depuration.

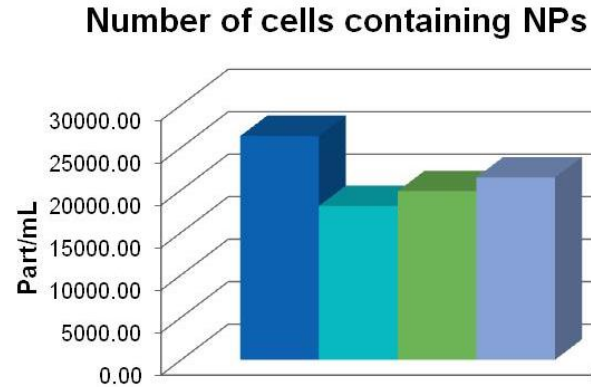
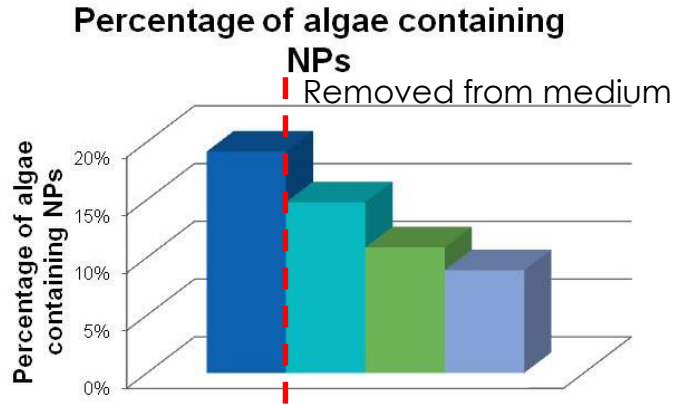


Au NP uptake into cells

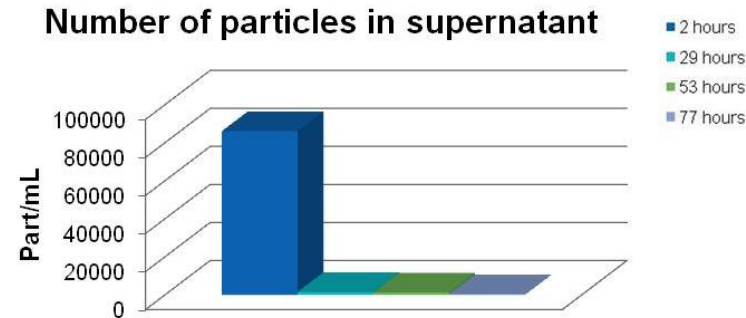
- Increase in number of algal cells
- Increase in number of cells containing NP, decrease in % of cells containing NPs
- Dissolution of NP within cells. Dilution by division
- Increase in number of cells containing more than one NP



What happens if the cells are removed from the exposure media after 24 hours?



Exposure:
600,000 Au 60 nm



- The % of cells containing NPs decreases over time → increase in total number of cells
- The absolute number of cells containing NPs does not alter much
- Measuring the supernatant shows that there are no NPs returning to the water column from the cells.

Conclusions

- Single cell ICP-MS is an emerging technology allowing users to Monitor metal content within individual cells for:
 - Intrinsic metal content
 - Uptake of ionic contaminants
 - Uptake of nanoparticulate contaminants
- SC-ICP-MS handles lower cell numbers compared to conventional methods and uses minimal to no sample preparation
- Single Cell ICP-MS was used to measure the uptake of ionic and nanoparticulate gold into fresh water algae (*Cryptomonas ovata*) focusing on:
 - Uptake of ionic metal vs. time
 - Uptake of metal nanoparticles vs. time
 - Quantification of uptake as number of particles / cell (or biological entity)
 - Correlation of nanoparticle concentration with cell uptake rate
 - Insight into algae uptake during life cycle

10th – 12th January 2017

spICP-MS: DATA ANALYSIS WORKSHOP

RIKILT Wageningen University & Research
The Netherlands



www.nanofase.eu



empir.npl.co.uk/innanopart



www.nanodefine.eu

Day1 (10th Jan): Lectures
Day2 (11th Jan): Computer exercises
Day3 (12th Jan): Hands-on-training (*optional*)

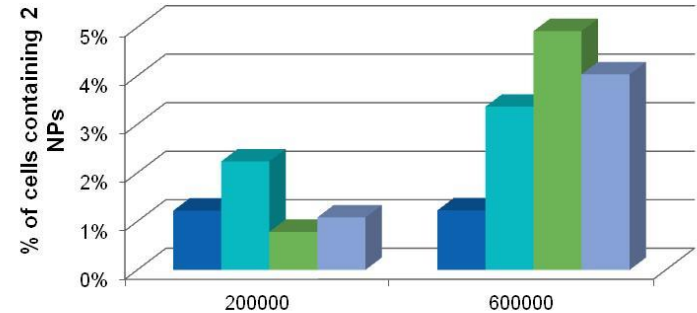
Please register until 09.12.2016 under
[http://www.wur.nl/
en/activity/spICP-MS-data-analysis-workshop.htm](http://www.wur.nl/en/activity/spICP-MS-data-analysis-workshop.htm)



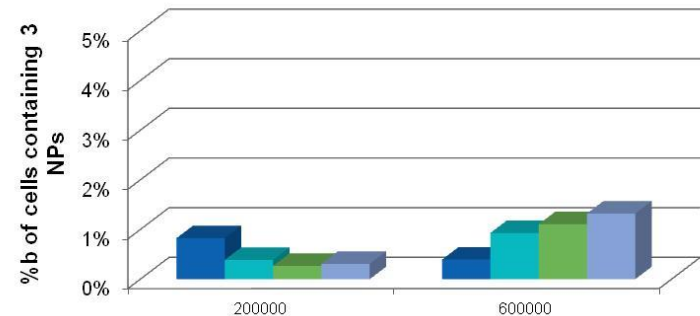
Cells containing multiple NPs

- Multiples of NPs per cell can be measured easily over time.
- Multiple particles per cell are seen only at higher concentration due to the amount of available NPs per cell

2 NP/Cell



3 NP/Cell



Single Cell Application Module

The screenshot displays the 'Single Cell' application window within the 'SynGota™ for ICP-MS - Instrument Control Session'. The interface is divided into several functional areas:

- Method Parameters:** A table defining the analysis method with columns for Dissolved Std, Particle Std, and Pump Settings.
- Calibration Parameters:** A table showing calibration data for Au (μg) and Au (nm).
- Acquisition Parameters:** Controls for Start Pump, Sample Flow Rate, and various analysis options.
- Advanced options:** A section containing an 'Advanced...' button for further configuration.
- Real-Time Signal:** A plot showing 'Counts' versus 'Measurements' over time.
- Real-Time Distribution Histogram:** A plot showing 'Frequency' versus 'Peak Area (counts)'. A 'Histogram Options' dialog is open, showing a 'Threshold' of 1.36.
- Calibration Graph:** A plot of 'Intensity (counts)' versus 'Mass (μg)' for Au 196.967 - Particle, showing a linear relationship.

Single Cell Application Module

File Information

Method Parameters

Dissolved Calibration

Ionic Calibration

Adjustable bin Size

Adjustable Integration Window

Scrolling List of Results

Results Table

Sample	Analyte	Most Freq. Mass (ag)	Mean Mass (ag)	No. of Peaks	Mean Inten. (counts)	Cell Conc. (parts/mL)	Diss. Inten. (counts)	Diss. Conc. (ppb)	Bin Size (ag)	Start (ag)	End (ag)
Algae - 800K - 21 hours-3	Au 196.967	1648	1762	79	187.82	12834	0.01	-0.029	100		
Algae - 800K - 21 hours-9-1	Au 196.967	1648	1774	69	189.17	10423	0.01	-0.03	100		
Algae - 800K - 21 hours-9-2	Au 196.967	1648	1781	66	189.59	9970	0.01	-0.03	100		
Algae - 800K - 21 hours-5-1	Au 196.967	1648	1813	96	196.46	14502	0.01	-0.034	100		
Algae - 800K - 21 hours-5-2	Au 196.967	1648	1748	156	187.04	25685	0.01	-0.033	100		
Algae - 800K - 21 hours-5-3	Au 196.967	1648	1714	80	182.85	13385	0.01	-0.033	100		
Algae - 800K - 21 hours-5-4	Au 196.967	1648	1767	70	188.76	10574	0.01	-0.033	100		
Algae - 800K - 21 hours-2	Au 196.967	1648	1593	152	171.57	22961	0.01	-0.03	100		
Algae - 800K - 21 hours-2-1	Au 196.967	1648	1678	122	178.93	13429	0.01	-0.031	100		
Algae - 800K - 21 hours-2-2	Au 196.967	906	1248	122	207.45	18818	0.01	-0.031	100		
Algae - 800K - 21 hours-3	Au 196.967	1648	1833	231	195.85	14894	0.01	-0.032	100		
Algae - 800K - 21 hours-9-1	Au 196.967	906	1150	196	207.39	29607	0.01	-0.032	100		
Algae - 800K - 21 hours-9-2	Au 196.967	906	1164	163	209.77	24622	0.01	-0.032	100		
Algae - 800K - 21 hours-9-3	Au 196.967	1006	1392	163	216.23	29093	0.01	-0.032	100		
Algae - 1 gpb- 21 hours-1	Au 196.967	148	250	53	24.43	8006	0.06	0.015	100		
Algae - 1 gpb- 21 hours-1-1	Au 196.967	248	266	39	24.08	5891	0.05	0.013	100		
Algae - 1 gpb- 21 hours-2-1	Au 196.967	48	186	29	16.66	4381	0.05	0.012	100		
Algae - 1 gpb- 21 hours-2	Au 196.967	48	436	8	42.5	1209	0.05	0.01	100		
Algae - 1 gpb- 21 hours-2-1	Au 196.967	148	315	3	30.33	453	0.05	0.01	100		



Thank you

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The background of the slide is a 3D rendering of a molecular structure, likely a carbon nanotube or a similar nanoscale material. It features a hexagonal lattice of atoms, with several large, textured spheres (possibly representing molecules or nanoparticles) scattered throughout. The color palette is primarily blue and green, with a bright yellow rectangular box containing the company name.

NANOLYTICA™

Thank you

For questions please contact Chady Stephan or Ruth Merrifield:

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Single Cell ICP-MS

- A lateral application to Single Particle ICP-MS allowing the **measurement of metal content in individual cells**
- A technique that allows the **differentiation between ionic concentration (M^+) present in the culture media and ionic concentration present in individual cells** without any **prior separation**
- It is **element specific** and could potentially provide us with **precise cell counting** if the transport efficiency is well established
- Capable of quantifying the **metal content or particles by individual cell**
- Capable of tracking the **uptake of metals or particles** by individual cells
- Allows the analysis of cell suspension at **low cell concentrations**



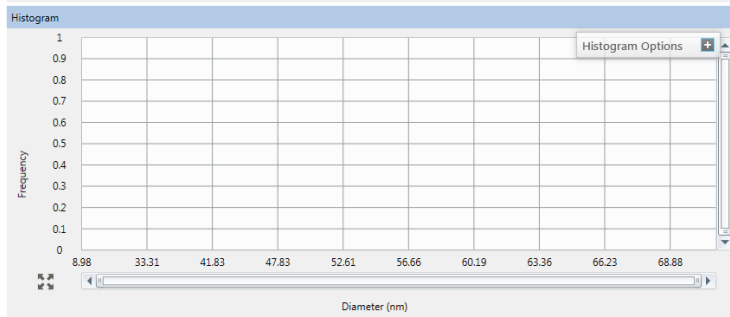
What data can be acquired with SC-ICP-MS

- **Measurable within cells**
 - Intrinsic metals
 - Uptake of NPs
 - Uptake of ionic metal
- **Exposure**
 - Amount of metal in culture/suspension media
 - Amount of NPs in culture/suspension media
- **Dose**
 - Number of cells containing a metal of interest
 - % of cells containing metal
 - Amount of metal per cell
 - Number of NPs/Cell

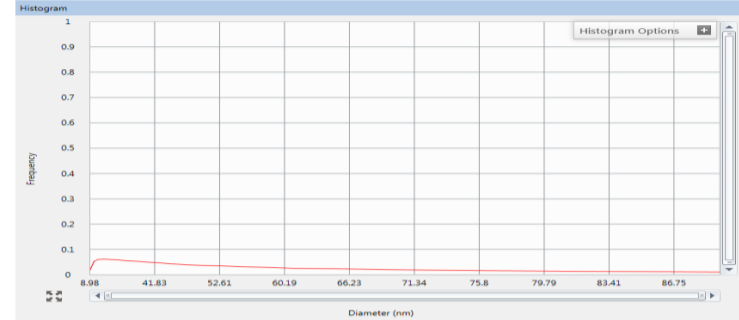
Conditions



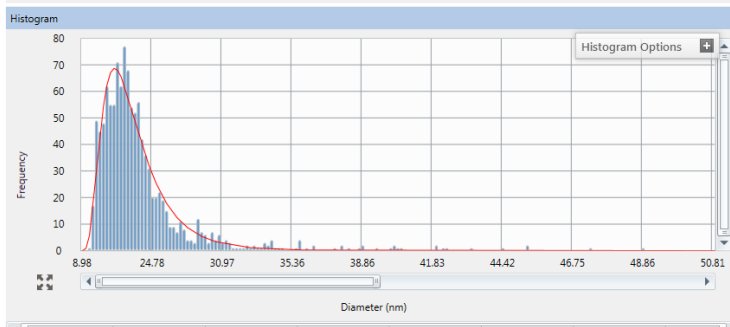
A: Cell control



B: Ionic control - 1 ppb Au



C: Cells + ionic (1 ppb Au)



D: Cells + Au NPs (60 nm NIST)

