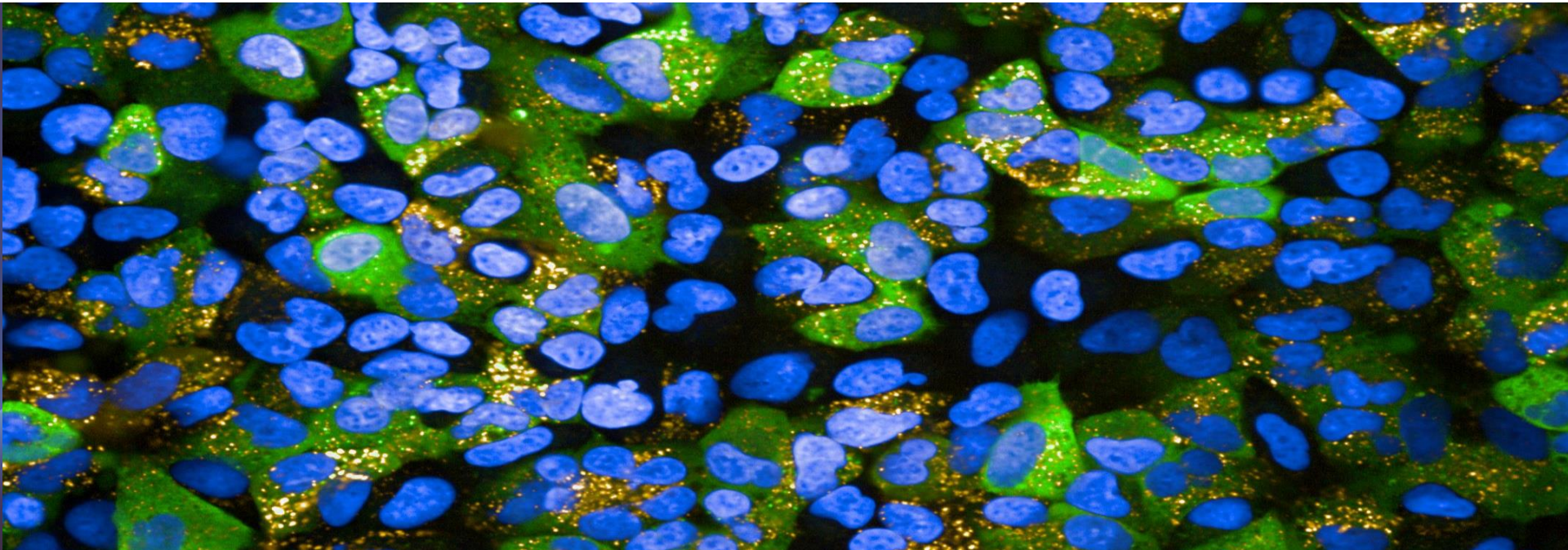
PerkinElmer 单颗粒、单细胞ICP-MS分析在环境和生物分析中的应用

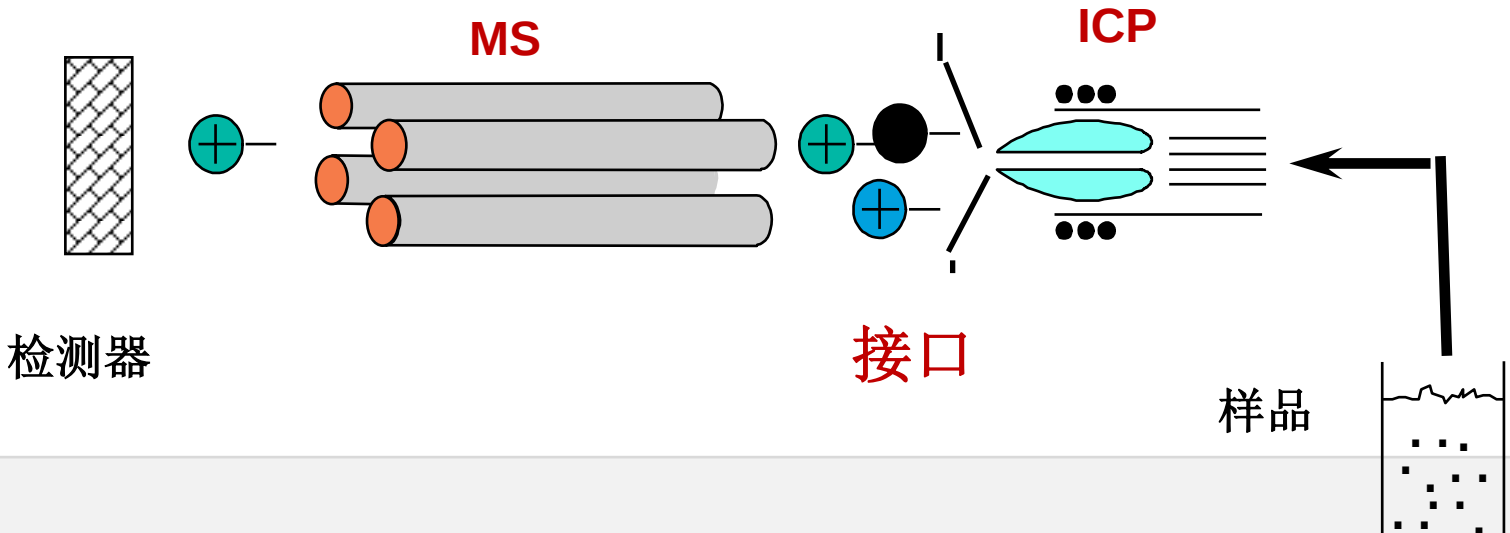
- 朱敏，min.zhu@perkinelmer.com,15301601129

- 14th, August, 2018

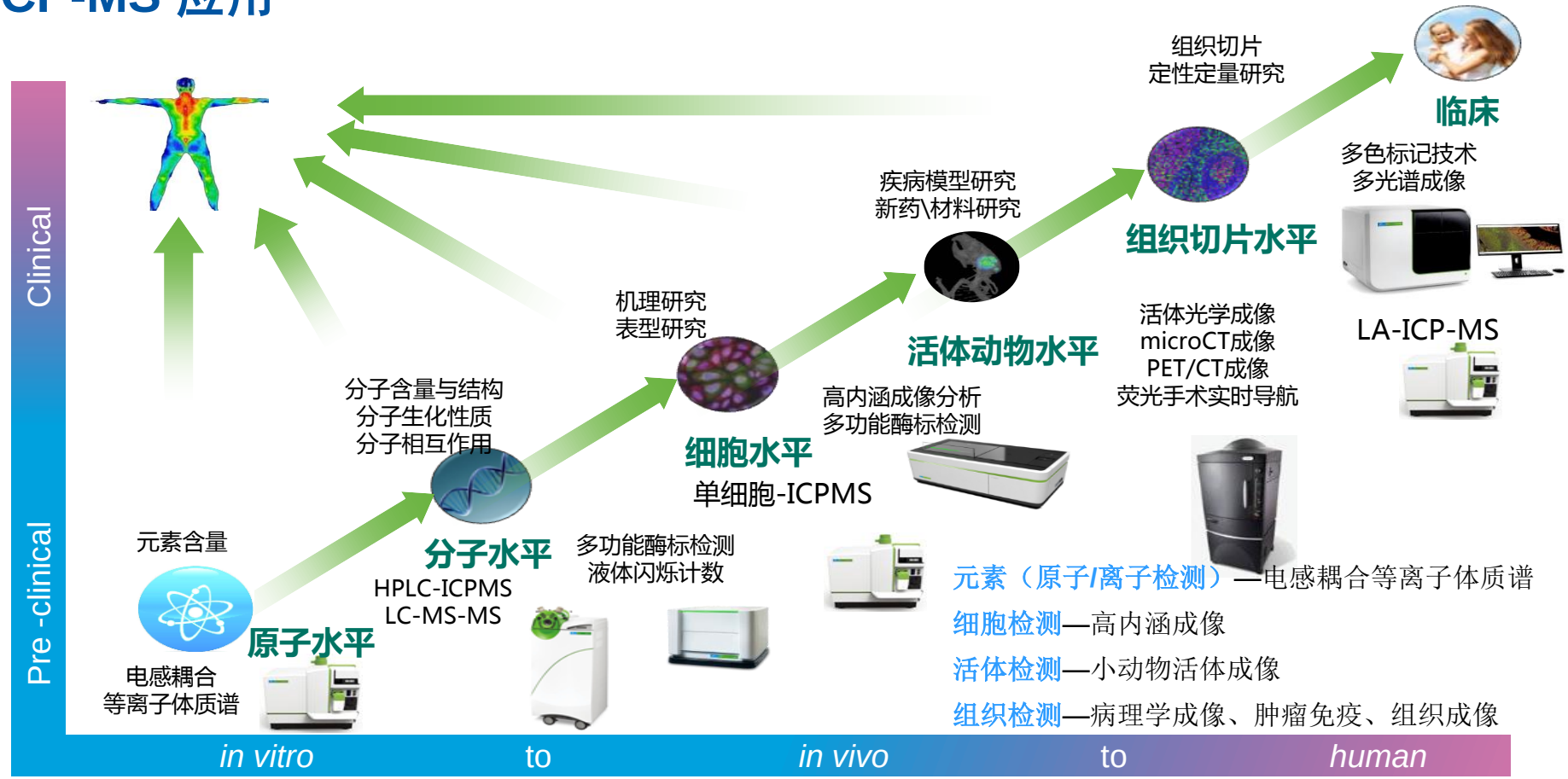


ICP-MS技术

- 电感耦合等离子体质谱（ICP-MS，Inductively Coupled Plasma Mass Spectrometry）是以独特的**接口**技术将电感耦合等离子体（ICP）的高温电离特性与四极杆质量分析器（MS）的快速灵敏扫描的优点相结合而形成一种**元素、同位素分析和单颗粒、单细胞分析**技术。



ICP-MS 应用



元素（原子/离子检测）—电感耦合等离子体质谱

细胞检测—高内涵成像

活体检测—小动物活体成像

组织检测—病理学成像、肿瘤免疫、组织成像

纳米科技与生活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Nanotechnology in our lives
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NANO PRODUCTS
NANO SAFETY
SOCIAL & ETHICAL
REGULATION
THE NANO DEBATE
NANO NEWS
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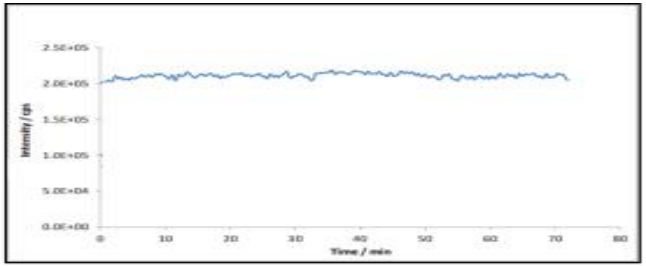
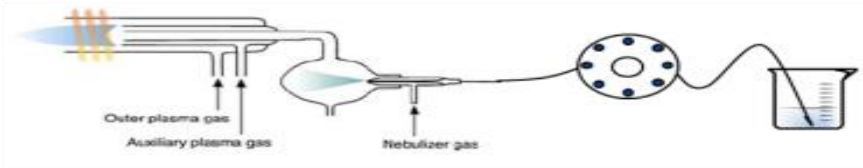
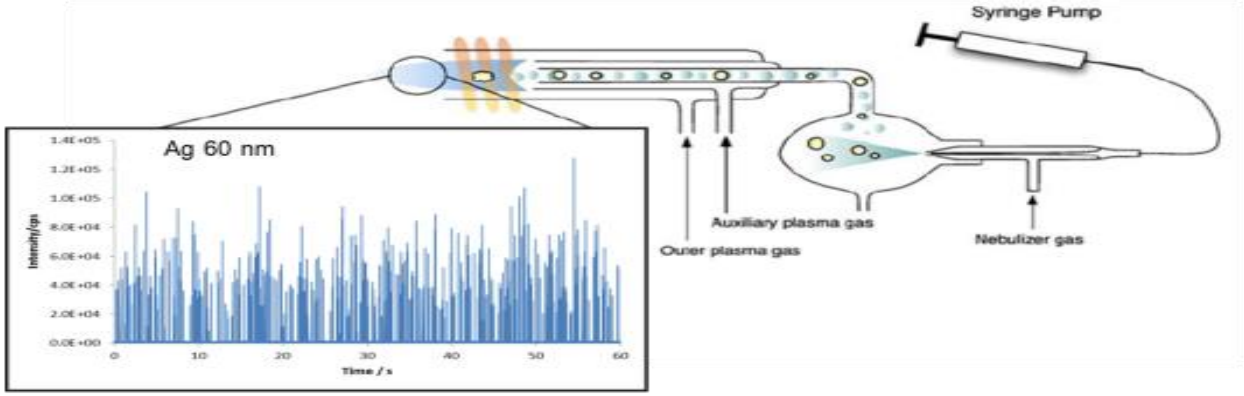
Click on the things in the picture to find out about nano in our lives.

常规ICP-MS测定与SP-ICP-MS单颗粒测定的区别



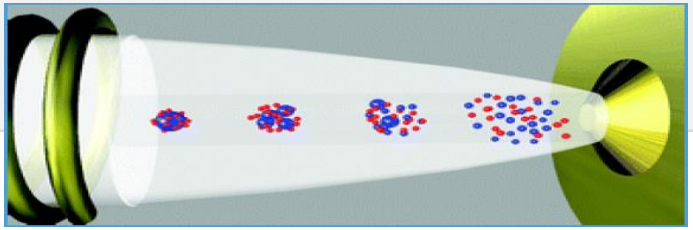
连续稳定的信号（溶液）





单独的信号（纳米粒子）

SP-ICP-MS 原理



- 只有当纳米颗粒产生的“离子云”经过检测器时才会检测到信号，其他信号为背景信号.
- 分析信号强度与离子云中离子数量呈正比.
- 每个“离子云”检测到的离子数量与颗粒的质量成正比.
- 颗粒的质量与粒径成正比.

信号强度

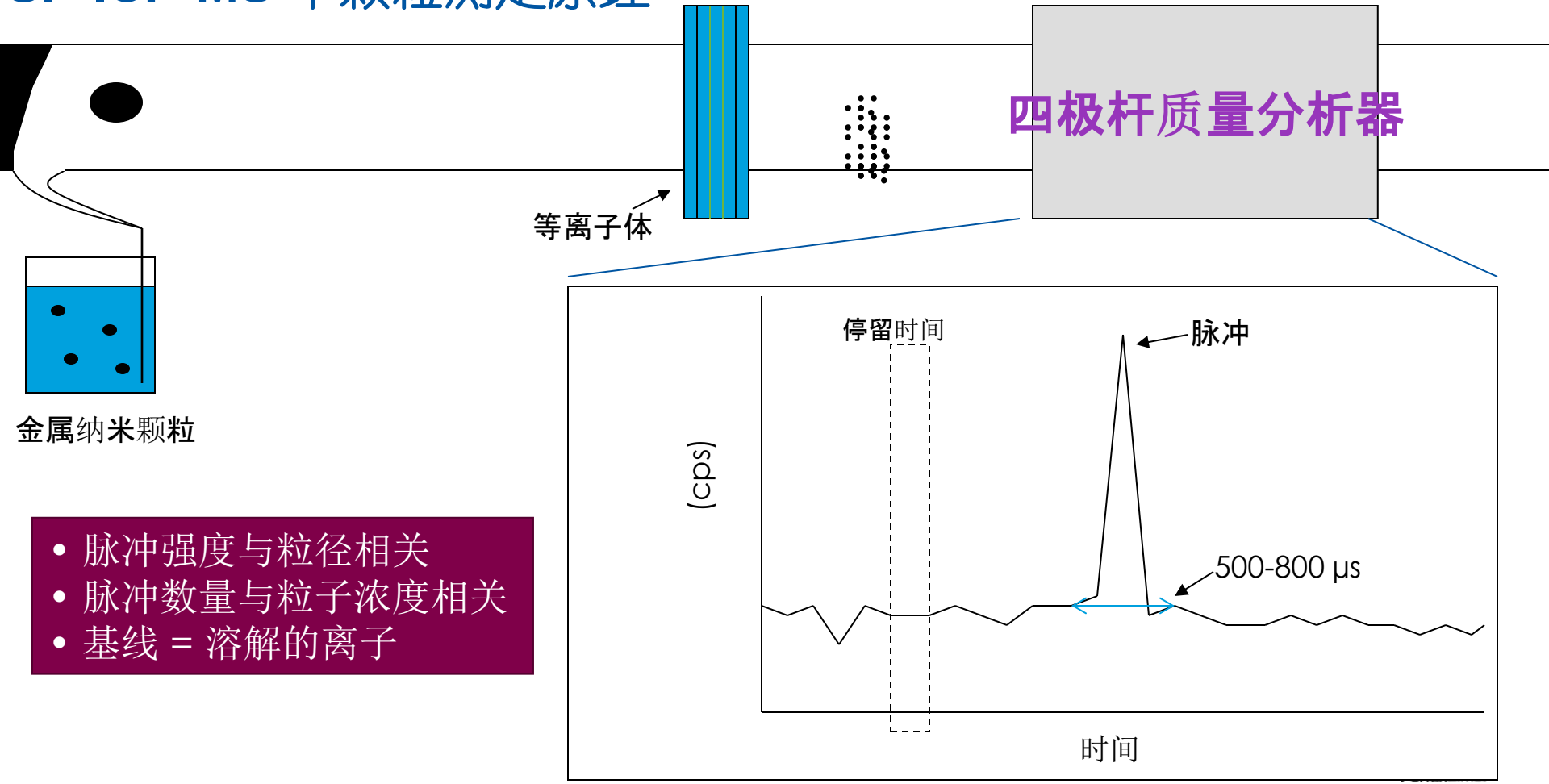
↓

颗粒质量

↓

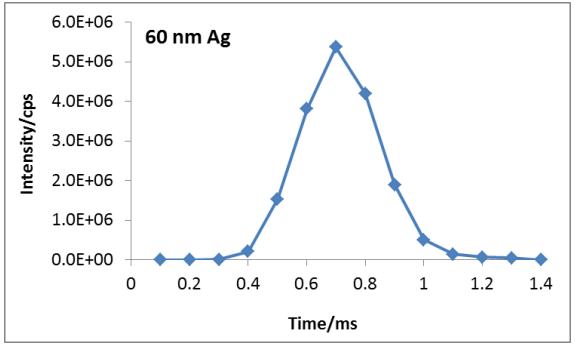
颗粒粒径

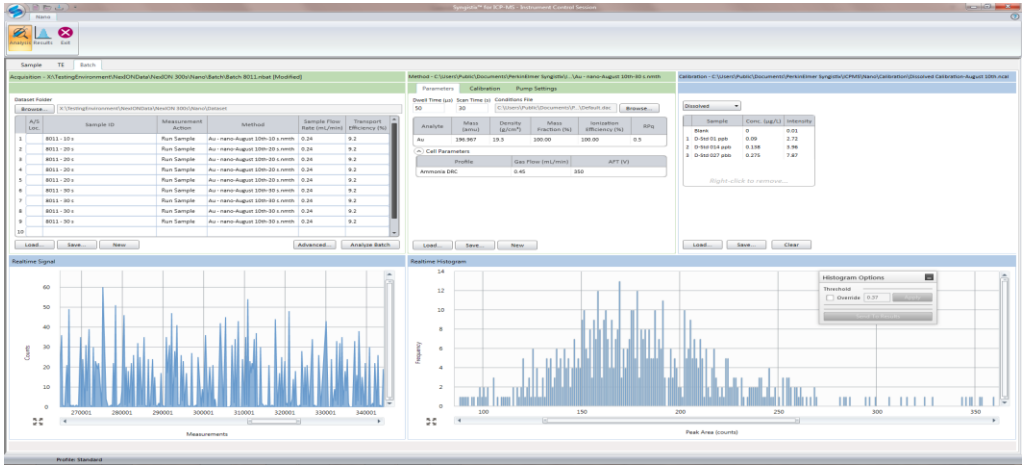
SP-ICP-MS 单颗粒测定原理

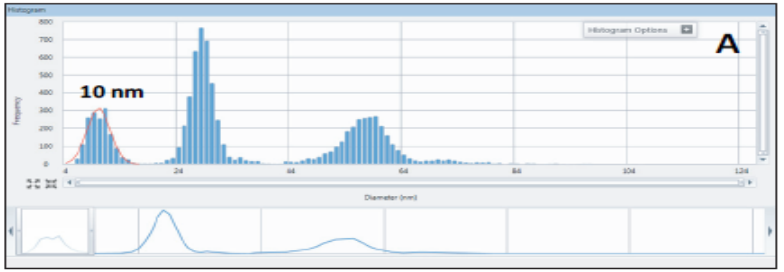


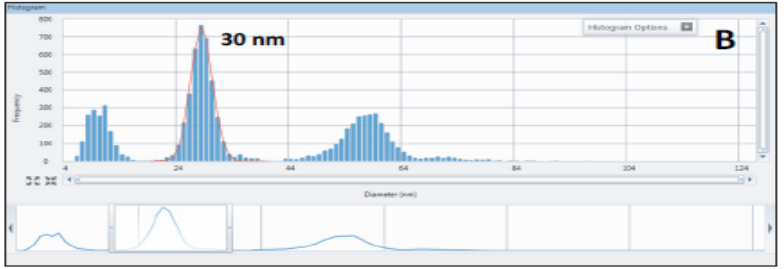
- 脉冲强度与粒径相关
- 脉冲数量与粒子浓度相关
- 基线 = 溶解的离子

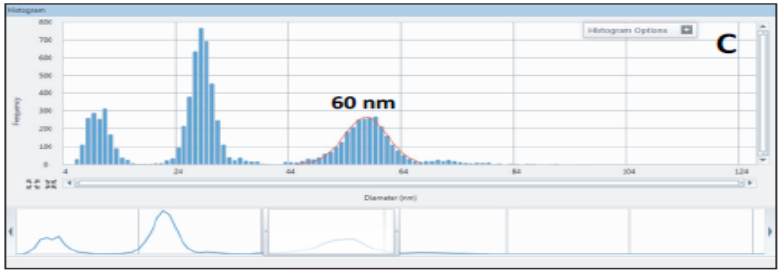
纳米颗粒的粒径和数量


60 nm Ag
Intensity/cps
6.0E+06
5.0E+06
4.0E+06
3.0E+06
2.0E+06
1.0E+06
0.0E+00
0
0.2
0.4
0.6
0.8
1
1.2
1.4
Time/ms




A
10 nm


B
30 nm

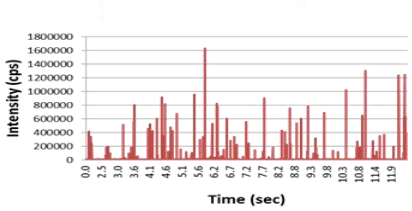

C
60 nm

sp-ICP-MS 环境纳米颗粒应用研究

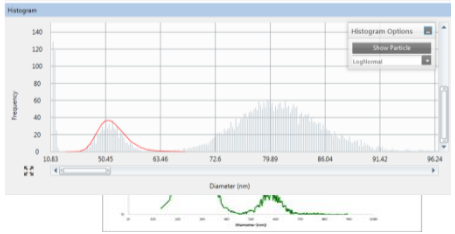
Sampling

SP-ICP-MS Analysis

Time Resolved Data



Size distribution



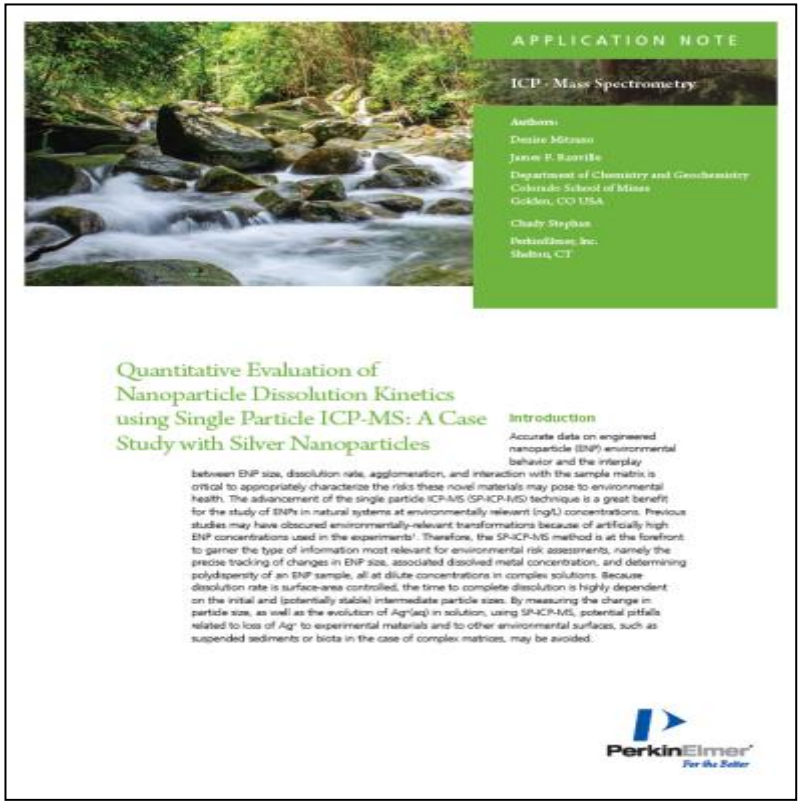
Nanoparticles in Drinking water

Effectiveness of Three Water Treatment Plants Removing TiO_2 Particles and Dissolved Ti.

Plant	Pre/ Post Treatment	Most Frequent Size (nm)	Particle Concentration (particles/mL)	Dissolved Concentration (µg/L)
1	Pre Post	170 < MDL	432,000 < MDL	17.9 1.21
2	Pre Post	156 < MDL	451,000 < MDL	11.7 1.17
3	Pre Post	153 76	425,000 17,237	10.6 < MDL

Sample	Au			Ag			TiO_2		
	Most Freq Size (nm)	Part Conc. Spike Recovery	Diss Conc. Spike Recovery	Most Freq Size (nm)	Part Conc. Spike Recovery	Diss Conc. Spike Recovery	Most Freq Size (nm)	Part Conc. Spike Recovery	Diss Conc. Spike Recovery
1	98	97%	80%	98	97%	80%	102	9%	84%
2	97	88%	84%	97	88%	84%	87	6%	88%
3	101	94%	89%	101	94%	89%	87	6%	112%

Tracking Nanoparticles Dissolution/Aggregation



APPLICATION NOTE

ICP - Mass Spectrometry

Authors:
Denise Mitrano
James F. Ranville
Department of Chemistry and Geochemistry
Colorado School of Mines
Golden, CO USA

Chady Stephan
PerkinElmer, Inc.
Shelton, CT

Quantitative Evaluation of Nanoparticle Dissolution Kinetics using Single Particle ICP-MS: A Case Study with Silver Nanoparticles

Introduction

Accurate data on engineered nanoparticle (ENP) environmental behavior and the interplay between ENP size, dissolution rate, agglomeration, and interaction with the sample matrix is critical to appropriately characterize the risks these novel materials may pose to environmental health. The advancement of the single particle ICP-MS (SP-ICP-MS) technique is a great benefit for the study of ENPs in natural systems at environmentally relevant (ng/L) concentrations. Previous studies may have obscured environmentally-relevant transformations because of artificially high ENP concentrations used in the experiments[1]. Therefore, the SP-ICP-MS method is at the forefront to garner the type of information most relevant for environmental risk assessments, namely the precise tracking of changes in ENP size, associated dissolved metal concentration, and determining polydispersity of an ENP sample, all at dilute concentrations in complex solutions. Because dissolution rate is surface-area controlled, the time to complete dissolution is highly dependent on the initial and (potentially stable) intermediate particle sizes. By measuring the change in particle size, as well as the evolution of $Ag^{+}_{(aq)}$ in solution, using SP-ICP-MS, potential pitfalls related to loss of Ag^{+} to experimental materials and to other environmental surfaces, such as suspended sediments or biota in the case of complex matrices, may be avoided.

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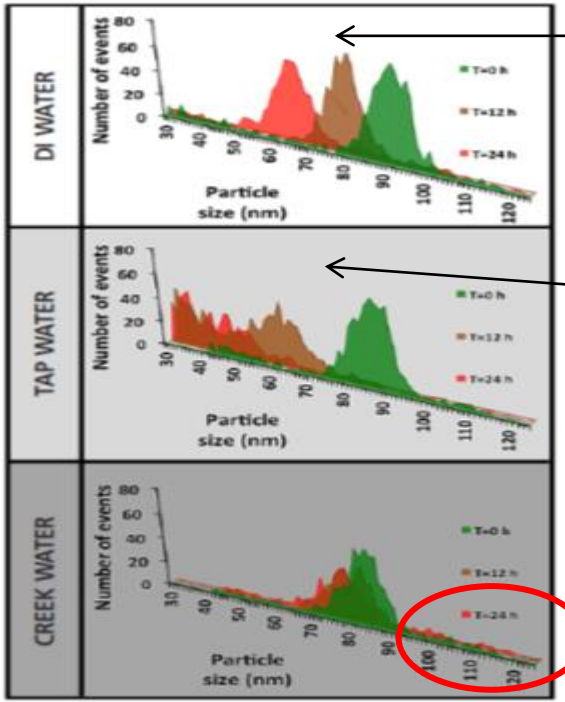
For the Better



Dissolution

Accelerated Dissolution

Agglomeration

Figure 2. Particle size distribution of Ag ENP suspended in various water chemistries (DI, tap, and creek waters) over 24 h. Evidence of decreasing particle diameter with time through particle oxidation and dissolution in some samples (e.g. DI and tap waters) with less change in particle size observed in other samples, (e.g. creek water).

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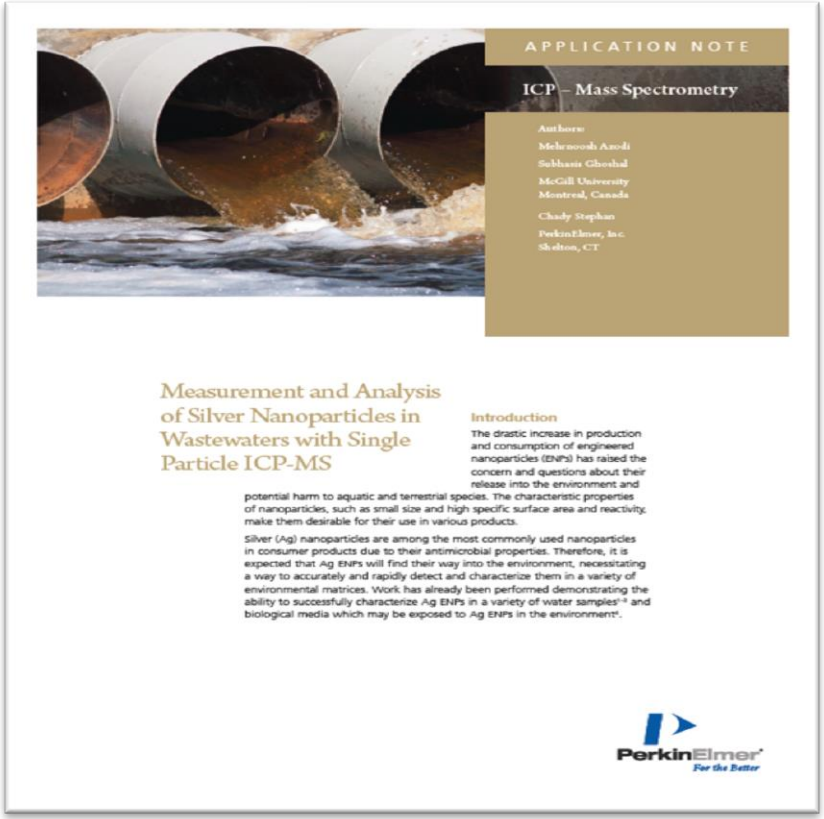
Silver Nanoparticles in Wastewaters



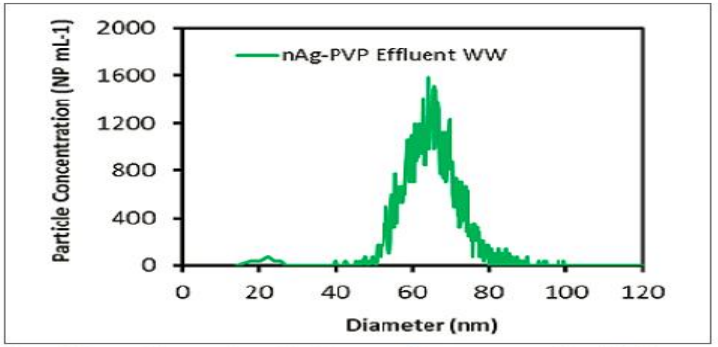


Figure 2. Measured Ag particle size distribution in effluent wastewater diluted 1000 times.

Table 2. Results from Analysis of Wastewater Samples Spiked with Ag NPs.

Sample	Mean Particle Size (nm)	Spiked Particle Conc. (particles/mL)	Measured Particle Conc. (particles/mL)
Effluent Wastewater	66.3±0.2	50,000	54,691±1185
Mixed Liquor Wastewater	63.7±0.4	50,000	53,123±1216

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Bioavailability of Nanoparticles in Tomato Plants

APPLICATION NOTE

ICP – Mass Spectrometry

Authors:
Yinglee Dan[1,2], Weilan Zhang[2], Xingmao Ma[2], Hongjun Shi[3,4], Chady Stephan[4]

[1]Department of Chemistry, Missouri University of Science and Technology
[2]Center for Single Nanoparticle, Single Cell, and Single Molecule Monitoring (CSNM), Missouri University of Science and Technology
[3]Department of Civil and Environmental Engineering, Southern Illinois University
[4]PerkinElmer, Inc.

Gold Nanoparticle Uptake by Tomato Plants Characterized by Single Particle ICP-MS

Introduction

With the increasing use of engineered nanoparticles (ENPs) in a variety of products and processes, there is concern about the release of ENPs into and impact on the environment. One aspect of the environmental impact of ENPs that must be explored is their uptake by plants, as ENPs can make their way to plants via migration through water and/or soil. If ENPs end up in food crops, this is a potential pathway to human exposure.

The challenge arises in how to measure ENPs in plant materials and, more specifically, in sample preparation. To our knowledge, current sample preparation techniques have limited capability to conserve the concentration and characteristics of nanoparticles (NPs) once they enter plant tissues, as they mainly depend on acid digestion. These limitations can be avoided by careful choice of the ENP extraction procedure and performing the analysis with single particle ICP-MS (SP-ICP-MS), the combination of which will preserve the particle size information, allow for rapid analysis of a large number of samples, and yield results on the particle size, concentration, and size distribution.

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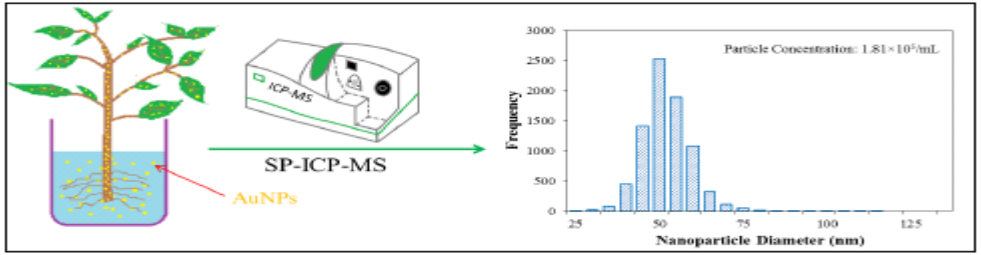

Table 1. NexION 300/350D Instrumental and Analytical Parameters.

Parameter	Value
Nebulizer	Concentric (glass)
Nebulizer Flow	1.08 L/min
Spray Chamber	Baffled Cyclonic (glass)
ICP RF Power	1600 W
Analyte	Au
Mass	197 amu
Dwell Time	0.1 ms
Settling Time	0 ms
Sampling Time	100 sec
Number of Data Points Acquired	1 million per sample
Au Density	19.3 g/cm^3

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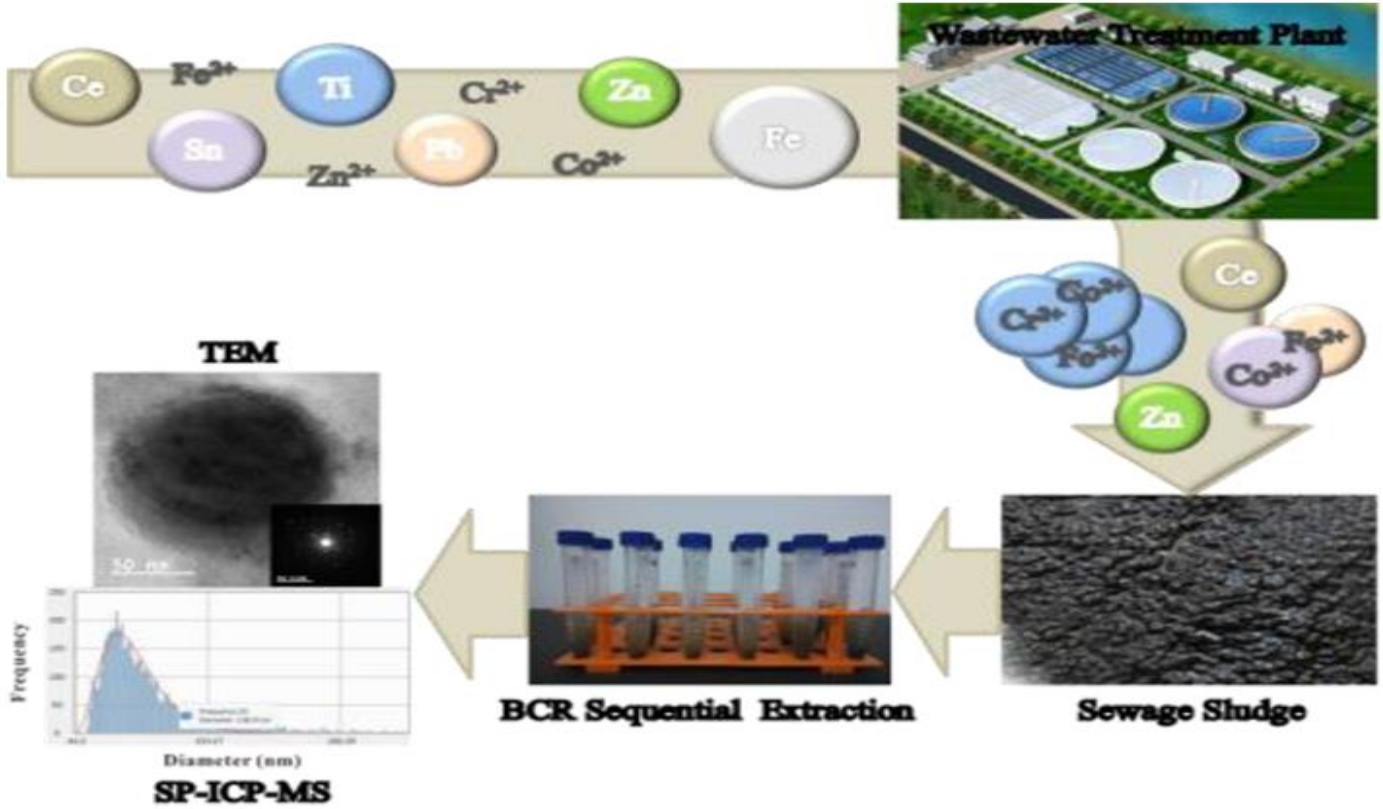
Metal containing Nanoparticles in Sludges

華東師範大學 EAST CHINA NORMAL UNIVERSITY
Environmental Science & Technology
Environmental Risk Implications of Metals in Sludges from Waste Water Treatment Plants: The Discovery of Vast Stores of Metal-Containing Nanoparticles
Feiyun Tou,[†] Yi Yang,[‡,⊥] Jingnan Feng,[†] Zuoshun Niu,[†] Hui Pan,[†] Yukun Qin,[†] Xingpan Guo,[†] Xiangzhou Meng,[§] Min Liu,[†] and Michael F. Hochella[‖,⊥]

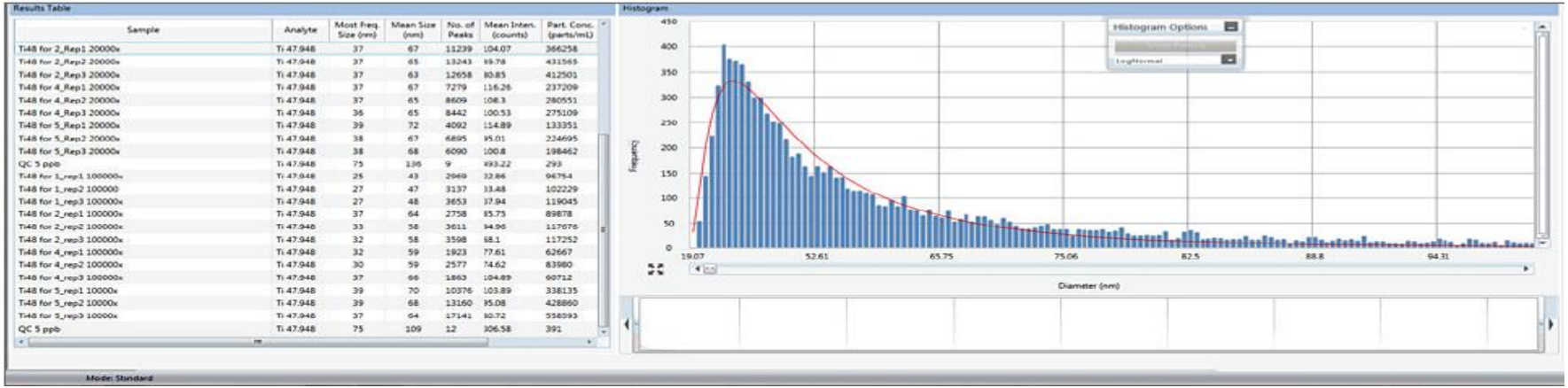


DOI: 10.1021/acs.est.6b05931

Nanoparticles in Sunscreens

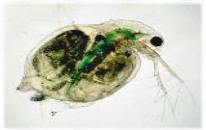
Sunscreen	SPF	TiO_2 Content (%)
1	60+	4.9
2	50	6
3	45	0
4	50	6
5	45+	5.1

Sunscreen	Dilution Factor	Most Frequent Size (nm)	Particle Size Distribution (nm)	Particle Concentration (particles/mL)
1	100,000	32	24 – 58	102,229
2	100,000	34	24 – 64	117,252
3*	100,000	-	-	-
4	100,000	33	24 – 61	63,000
5	20,000	42	28 – 67	198,462



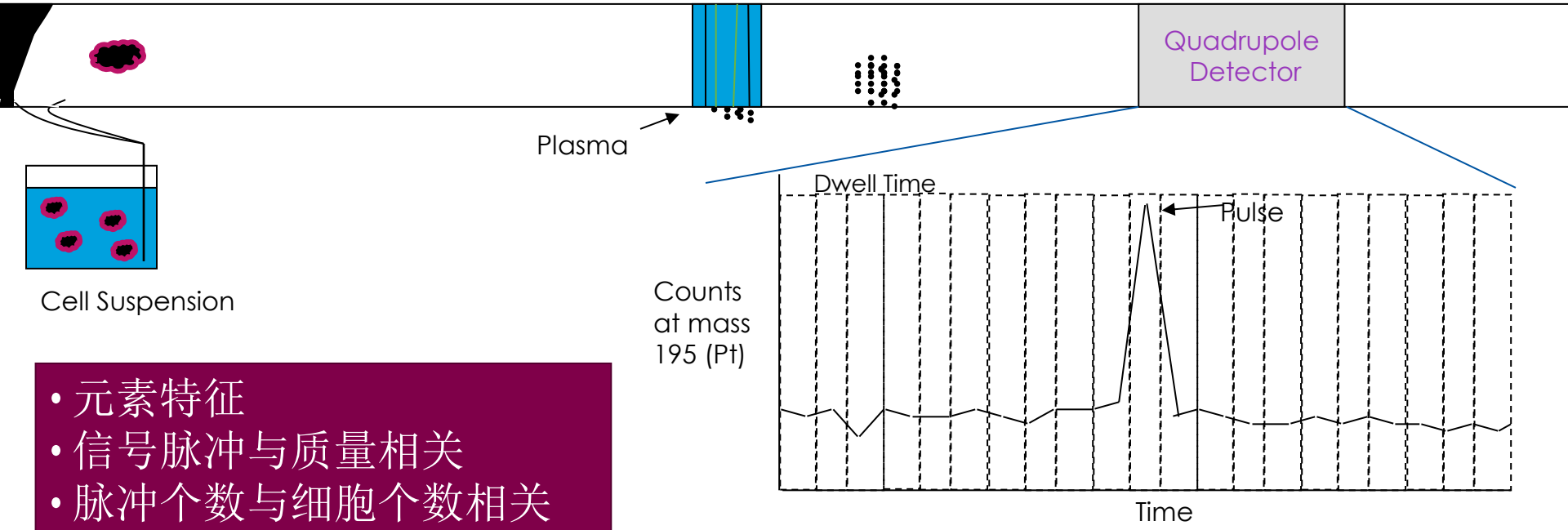
样品稀释20000倍

Nanoparticles in Biological Samples

Urine 	1/10 dilution in 0.5% NH_4OH + 0.1% Triton
Blood 	1/20 dilution in 0.5% NH_4OH + 0.1% Triton
Tissues 	24-hour digestion with Tetramethylammonium Hydroxide (TMAH -20% w/w) followed by centrifugation
Organisms 	24-hour digestion with Tetramethylammonium Hydroxide (TMAH - 20% w/w) followed by centrifugation

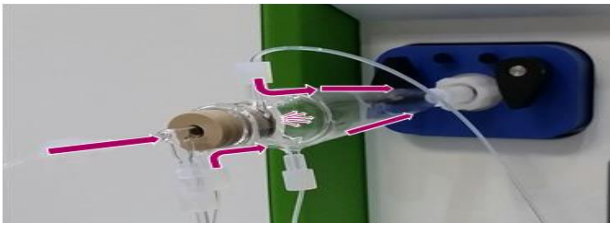
- Evans et al. Extraction and Analysis of Silver and Gold Nanoparticles from Biological Tissues Using Single Particle Inductively Coupled Plasma Mass Spectrometry, Environ. Sci. Technol. 2013, 47, 14315−14323

Single Cell ICP-MS 工作原理



- 元素特征
- 信号脉冲与质量相关
- 脉冲个数与细胞个数相关
- 基线为悬浮浓度

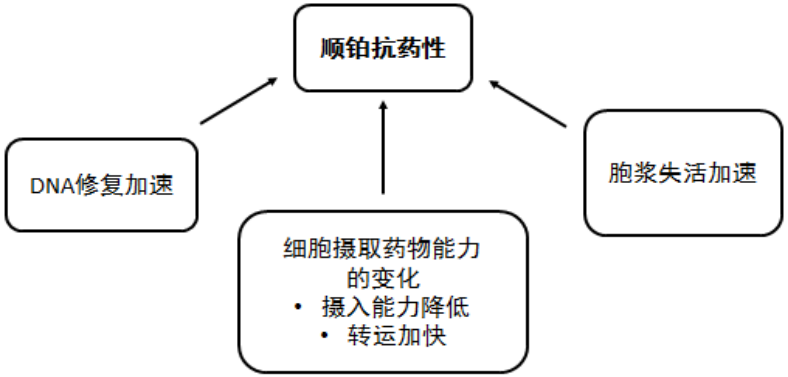
Single Cell ICP-MS 应用研究

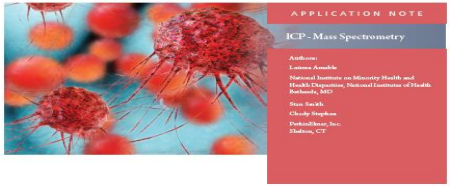


- 癌症研究
- 环境毒理学
- 生物技术和细胞科学
- 药物设计
- 。 。 。

基于单细胞-ICP-MS 的卵巢癌细胞对顺铂吸收研究新方法

- 一般病人对于顺铂初始治疗响应良好，但存在复发问题且对铂系药物产生抗药性
- 抗药性可能途径





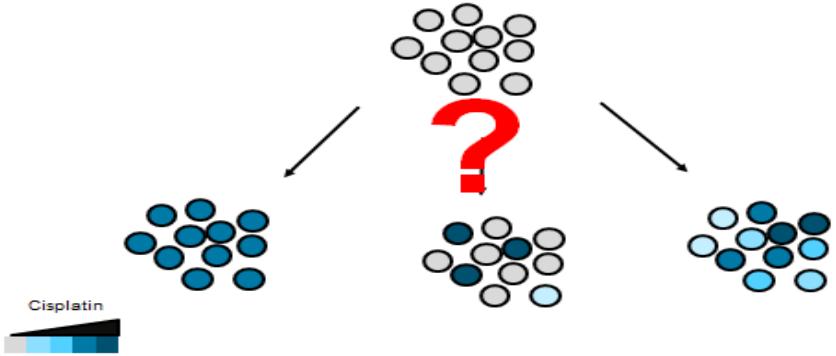
New Research Evaluating Cisplatin Uptake in Ovarian Cancer Cells by Single Cell ICP-MS

Introduction

Cisplatin, carboplatin, and oxaliplatin are the most widely used of platinum-based cancer chemotherapy drugs in the Western world. Cisplatin's effectiveness is due to its ability to bind to the DNA, resulting in DNA-platinum (Pt) adducts, which bend the DNA. The cells must then repair the DNA damage, otherwise DNA replication is blocked resulting in cell death[1].

Many cancers are initially sensitive to platinum-based treatment, but patients frequently relapse with tumors displaying resistance to further cisplatin therapy[2]. Cisplatin drug resistance is due to three major molecular mechanisms: increased DNA repair, increased cytosolic inactivation, and altered cellular accumulation. Decreased cellular uptake or increased cellular export of cisplatin constitutes the mechanisms involved in altered cellular accumulation[3].

- 传统AA，ICP，MAS技术只能测定吸收平均值，无法区分细胞间差异
- 顺铂以及其他药物的吸收存在特异性，即吸收/不吸收；吸收多与少的差异



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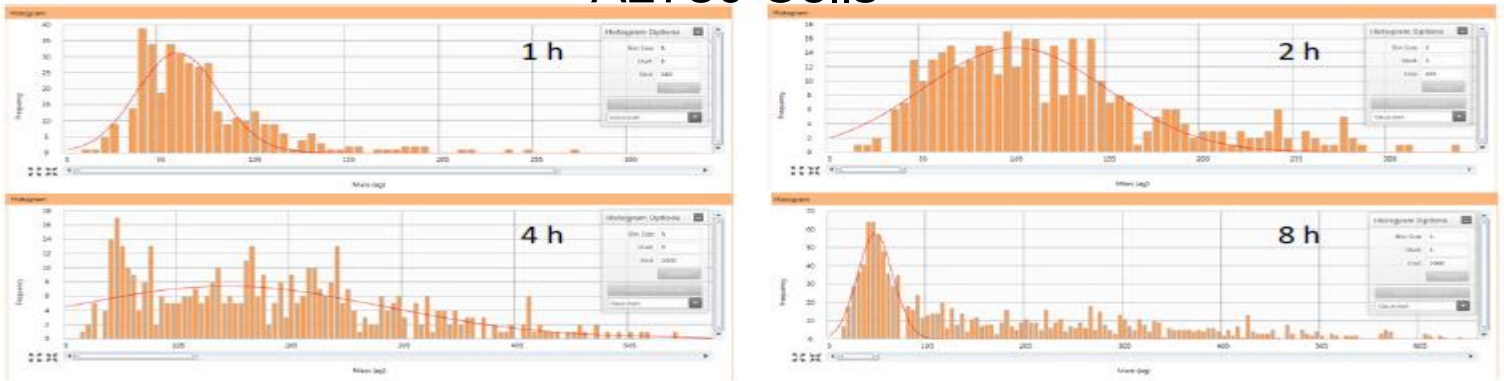
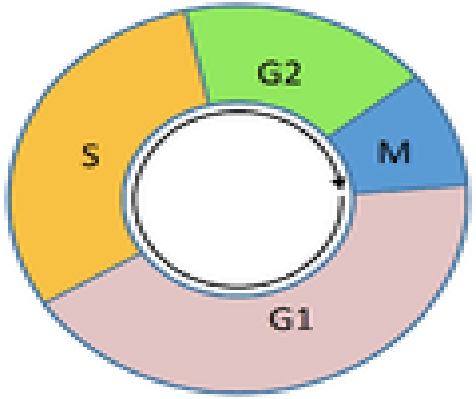
卵巢癌细胞2种不同细胞株随时间的吸收对比

A2780 Cells



A2780/CP70 Cells

细胞周期与血清饥饿处理



Cell division.wmv

- ✓ 推理顺铂吸收率分布不同可能与细胞周期有关（或细胞内DNA含量有关？）
- ✓ 细胞周期的四个阶段
  - G1 phase- First growth phase
  - S phases- DNA synthesis phase
  - G2 phase- Second growth phase
  - Mitosis (M) phase- Cells divide

- ✓ 血清饥饿处理使得细胞失去生长因子，细胞生长停留在G1阶段
- ✓ 进一步实验验证

NIH National Institute on Minority Health and Health Disparities

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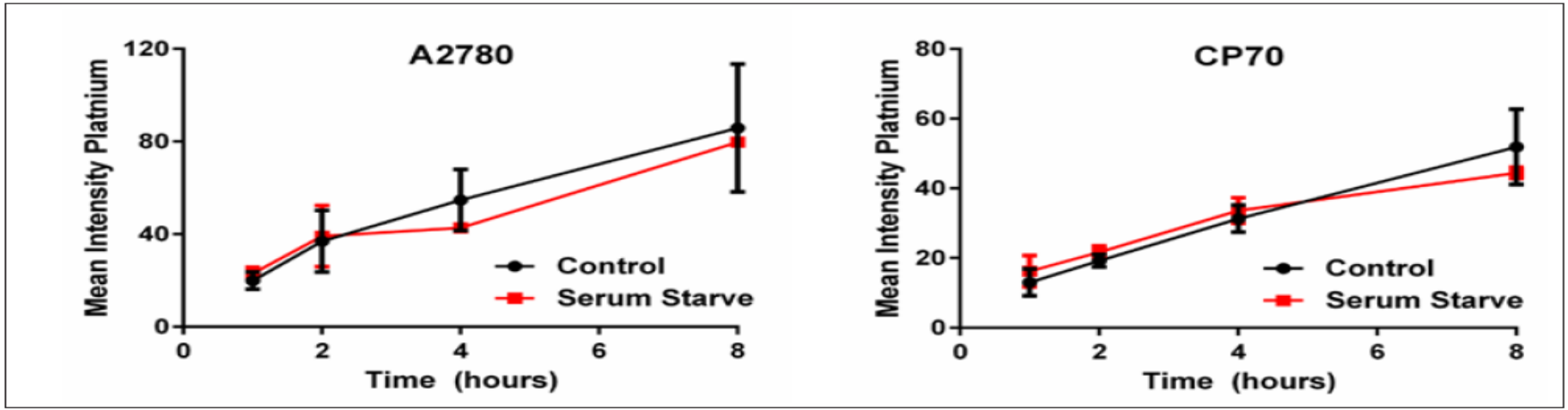
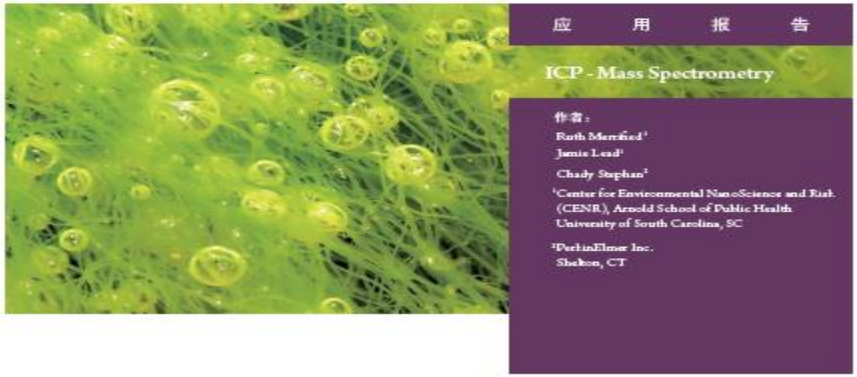
顺铂吸收与血清饥饿处理相关性



血清饥饿对A2780与A2780/CP70顺铂吸收影响不显著

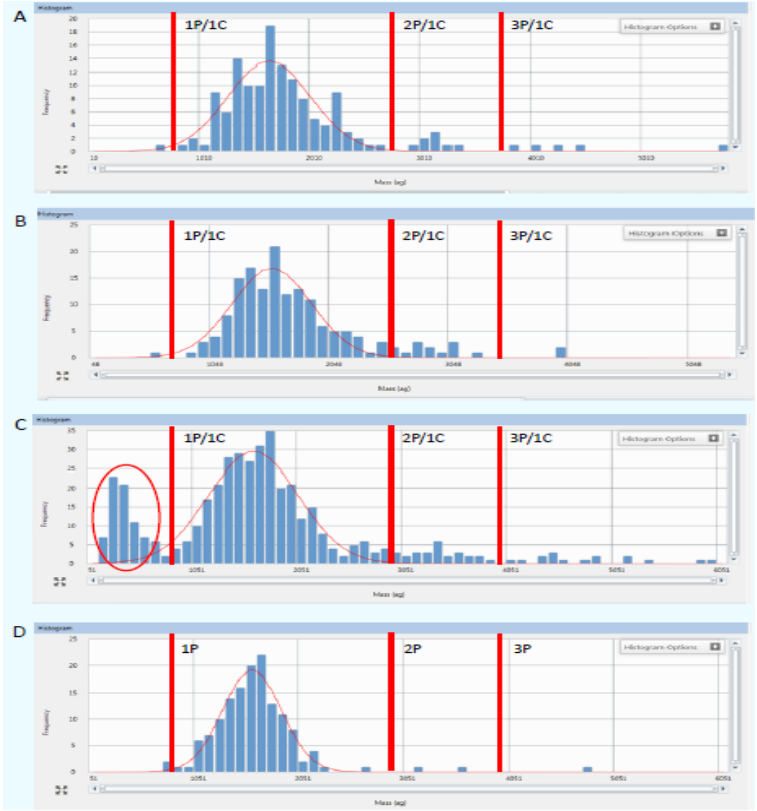
淡水藻类对金纳米颗粒和金离子的摄入行为



利用单细胞 ICP-MS 监测淡水藻类对金纳米颗粒和金离子的摄入行为

引言

对于人类健康和环境安全来说，监测单细胞对于金属离子和纳米颗粒（NPs）的摄入都是非常重要的[1-3]。目前，利用 ICP-MS 对于细胞内金属含量的常规测定方法为：通过离心或过滤将细胞从其天然培养介质中分离出来，再用新鲜介质进行清洗，然后用酸消解后上机检测[4]。采用这种方法可以得到一定数量细胞中金属的总量，而无法获得单个细胞的相关数据。单个细胞内金属的含量只能通过假定所有细胞内含有的金属颗粒或离子浓度相同，通过计算获得。而通过透射电子显微镜（TEM）[5]，扫描电子显微镜（SEM）[6]或荧光示踪法[7]的辅助表征，证明利用这种方法获得的单细胞数据并不准确。如果利用上述显微方法对细胞摄入纳米颗粒进行表征，又存在耗时长，人为误差大的缺点。而且，TEM 和 SEM 法只能定性，也容易由于纳米颗粒标示物化学性质不稳定而导致假阳性结果。



PerkinElmer

单个细菌中铁含量的测定

应　用　文　章

电感耦合等离子体质谱仪

作者：
Mariko Ikehata
Jennifer Woolcock
Michael E. P. Murphy
不列颠哥伦比亚大学生命科学中心微生物与免疫学系，加拿大不列颠哥伦比亚省温哥华市
Ruth Merrifield
Chady Stephan
PerkinElmer, Inc.,
加拿大安大略省伍德布里奇镇

利用 SC-ICP-MS 法测定单个细菌细胞中的铁含量

引言

铁是细菌细胞内部进行各种生物过程所必须的金属辅助因子。通常，铁作为一种可抑制细菌生长的营养元素，细胞中的总铁含量限额取决于细胞的生长状态和代谢需要。细菌已进化出复杂的系统来调节细胞中铁含量。[1] 细胞内多余的可溶性铁是有毒的，这是由于过量的可溶性铁产生会损伤细胞组分的活性氧，这意味着必须严格控制细胞中的铁含量。然而，目前我们尚无法确定单个细胞内及整个细胞群内铁含量的调节情况。在确定细胞生长条件和应激反应的影响时，包括因使用抗生素产生的应激反应，在近乎实时地测定细菌细胞中的铁含量可提供关于细菌中铁耐受限值的信息。有趣的是，某些具有杀菌作用的粘土矿物和粘土提取物中的可溶性铁含量很高，这可能会破坏细菌细胞内的铁平衡，导致细菌死亡。[2] 此外，监测单个细胞内的铁含量还可了解细胞中铁的分布情况，从而确定细胞群的同质性。

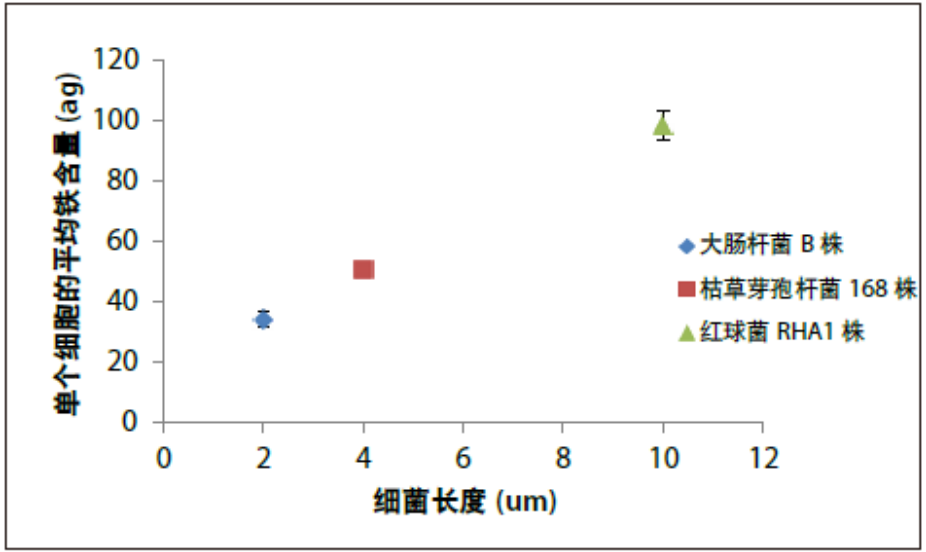
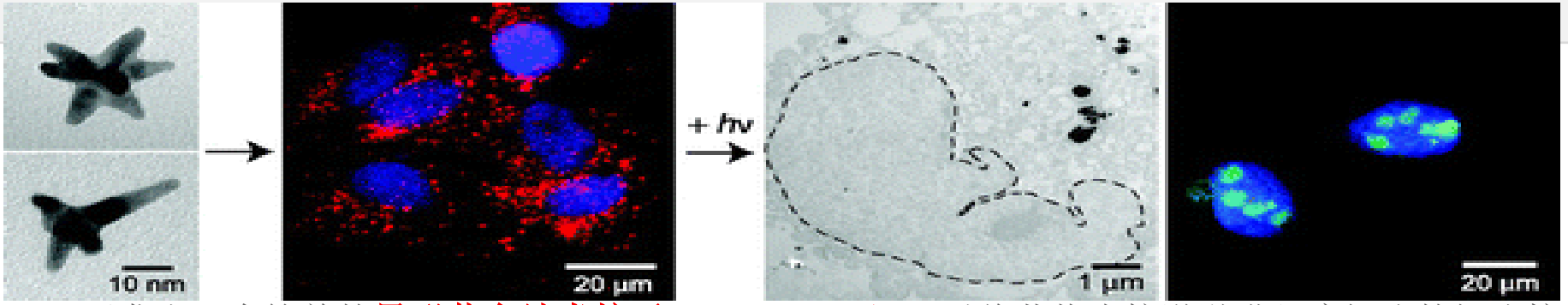


图 4. 以单个细菌细胞长度对应测得的单个细胞平均铁含量作图。从图上可看出，细胞长度与测得的单个细胞内铁含量之间呈正相关，其中长度最长的细菌（红球菌 RHA1 株），其细胞内铁含量最高，而长度最短的细菌（大肠杆菌 B 株）中，其细胞内铁含量最低。

ACS Nano：纳米颗粒能直接载药入癌细胞核

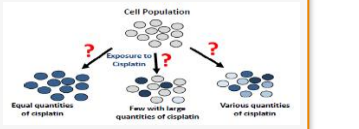


开发出一个简单的**星形黄金纳米粒子（ nanostar）**可以将药物直接递送进入癌细胞的细胞核中。相关研究论文发表在*ACS Nano*杂志上。论文主要作者是Teri W. Odom博士。首次发表报告称他们制造了一个简单的纳米粒子可以针对癌症细胞的细胞核，他们利用电子显微镜观察药物纳米颗粒进入细胞核能极大地改变癌细胞的细胞核的形状，他们注意到光滑、椭圆形的细胞核会变得凹凸不平，有深的褶皱。他们发现细胞核的形状变化与癌细胞的死亡和数量下降有关。

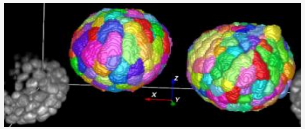
- **nanostar**纳米粒子约25纳米宽、黄金材料制造。纳米颗粒的形状具有较大的表面积，使其能够携带高负荷浓缩的药物分子。在这项研究中，研究人员利用一种称为AS1411的单链DNA适体。每个nanostar可以携带约1000股的适体药物，附着在其表面。
- **nanostars**的另一个优点是生物相容性很好，这是普通纳米颗粒不具备的特点。该新技术也可以在外科手术中使用的，一旦肿瘤切除，外科医生可以再使用黄金**nanostars**以消除癌细胞周围组织中杂散剩余的肿瘤细胞。（生物谷：Bioon）

纳米药物设计

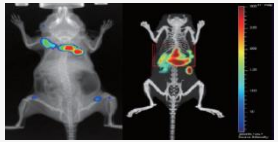
分子机制



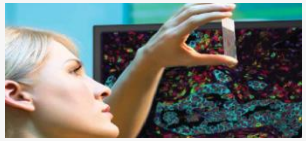
细胞分析

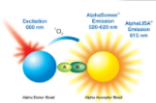


活体成像



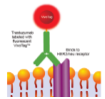
定量病理





Alpha 检测试剂

HCS专用微孔板

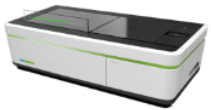


活体成像试剂

Opal 多标试剂

多模式检测系统
EnVision

单细胞ICP-MS
NexIon2000



高内涵成像分析系统
Operetta CLS

小动物活体成像
Spectrum CT

Micro CT 成像系统
Quantum GX2

小动物活体
PET/CT G8

多光谱定量病理系统
Vectra Polaris

PerkinElmer

定量分析金纳米颗粒在癌细胞中的吸收

APPLICATION NOTE

ICP - Mass Spectrometry

Authors:

Stefan Wilhelm

Stephenson School of Biomedical Engineering
University of Oklahoma
Norman, Oklahoma

Ryan C. Bensen, Naga Rama Kothapalli, Anthony W. G. Burgett

Chemistry and Biochemistry
University of Oklahoma
Norman, Oklahoma

Ruth Merrifield, Chady Stephan

PerkinElmer, Inc.
Woodbridge, Canada

Quantification of Gold Nanoparticle Uptake into Cancer Cells using Single Cell ICP-MS

Introduction

Cancer is a disease that is characterized by the uncontrolled growth and spread of abnormal cells. According to the American Cancer Society, cancer is the second most common cause of death in the US.[1] Current treatments for various cancers include surgery, radiation, immunotherapy, and chemotherapy. Although these conventional therapies may improve patients' overall survival and quality of life, they also have several limitations. For example, in conventional cancer chemotherapy, small-molecule-based cancer therapeutics distribute non-specifically throughout the entire human body. The consequence is that these drugs do not only kill cancer cells but also destroy healthy cells in the body causing severe side effects for cancer patients,[2] leading to a need for new therapies that can target diseased cells.

Nanoparticles (NPs) have been of significant interest over the last two decades as they offer attractive benefits for drug delivery to overcome limitations in conventional chemotherapy.[3] Nanoparticles can be engineered to carry both drugs and imaging probes to simultaneously detect and treat cancer. They may also be designed to specifically target diseased tissues and cells in the body. A number of nanoparticle-based cancer therapeutics have been approved for clinical use and/or are currently under development.[4,5] Advantages that rationally engineered nanoparticles may offer over conventional small-molecule drugs include: (i) prolonged circulation time in the body; (ii) reduction of nonspecific cellular uptake and undesirable off-target and side effects; and (iii) improvement in cellular interactions through specific cancer cell targeting moieties.

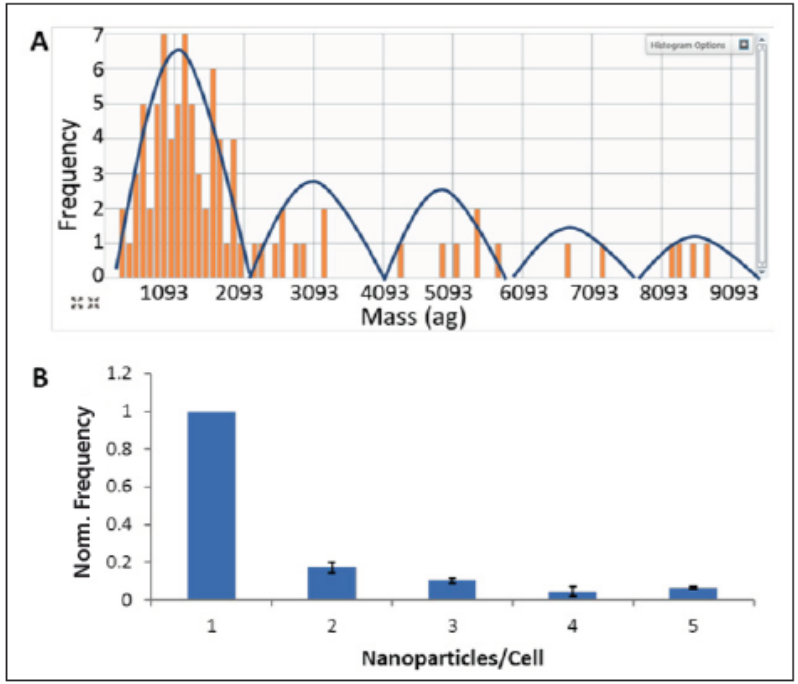


Figure 5. SC-ICP-MS analysis of human bladder cancer cells (T24) incubated with PEGylated 50-nm (gold core size) AuNPs for 4 h, using a PerkinElmer NexION system. (A) Histogram of gold mass distribution for individual T24 cells – blue curves highlight populations of cells containing one or more AuNPs per cell. (B) Normalized frequency distribution histogram of cells containing one or more AuNPs (norm. to number of cells containing one AuNP; n=3) – bars indicate average values with standard deviations.

单颗粒、单细胞 SP-ICP-MS分析技术

- PerkinElmer 公司极力倡导的单颗粒、单细胞ICP-MS(single particle-ICPMS)技术被公认为一种定性和定量测定含有特定元素的低浓度的单颗粒最有前途的方法。
- 单颗粒目标可以是纳米粒子，也可以是PM2.5/PM10等大气粉尘颗粒物，或者合成的超细颗粒物及包合物等，更可以是生命科学研究前沿的细胞，藻类，病毒等活体小颗粒型目标物。
- 相对传统的元素监测方法，SP-ICP-MS技术可快速有效并提供更多的信息：它能够测定颗粒尺寸分布、颗粒个数，颗粒内部元素的浓度、颗粒外部溶解出来的元素浓度等。而且，它能够区分含有不同元素的特定粒子。

谢　　谢！