pensabio

A SERVIÇO DA VIDA

CENTRO DE EXCELÊNCIA (CEP)

Suporte e Serviço

1 Engenheiro de Serviços

Cursos e Capacitação

16 Pessoas dedicadas

Assistência Técnica

Treinamento teórico e prático

3 PhD.
2 MSc.

Londres

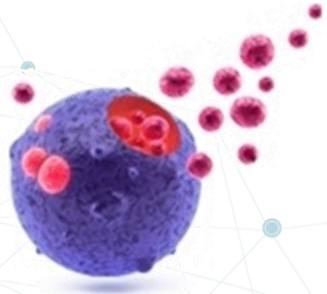
Nova Iorque

Photon

“One photon per second of flux corresponds roughly to the light which an observer in London would see from a candle at the distance of New York”

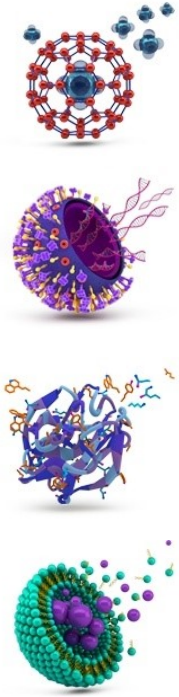
Nanoparticle Tracking Analysis (NTA)

Princípios da técnica e aplicações



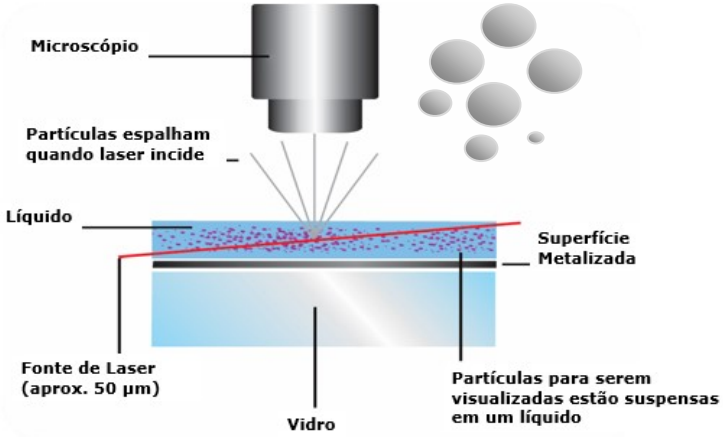
Visualização direta e em tempo real

de particulados entre 10 nm – 1 um*



*Nota: 1-não é uma imagem de alta resolução. 2-Nanopartículas de dióxido de titânio

Análise de partícula por partícula



Modelos de NANOSIGHT

Tamanho e concentração

NANOSIGHT **NS300**

Tamanho das nanopartículas
Concentração (partículas/ml)
Fluorescência (opcional)
Foco automático

Técnica: Nanoparticle Tracking Analysis
Faixa de tamanho: 10 nm – 2000 nm

NANOSIGHT **LM10**

Tamanho das nanopartículas
Concentração (partículas/ml)
Fluorescência (opcional)
Plataforma XY manual

Técnica: Nanoparticle Tracking Analysis
Faixa de tamanho: 10 nm – 2000 nm

- **Faixa de tamanho: 10 nm – 2 µm***

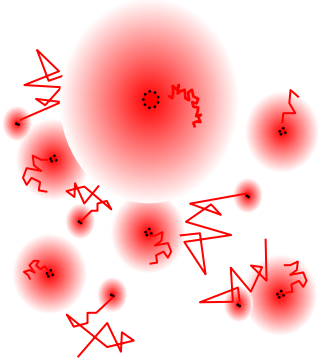
(material dependente)

  - Visualização das nanopartículas em tempo real
  - Análise em suspensão
  - Tamanho e Concentração (partículas/mL)
  - Modo de fluorescência (análise individual de partículas em meios complexos)

O QUE É DETECTADO?

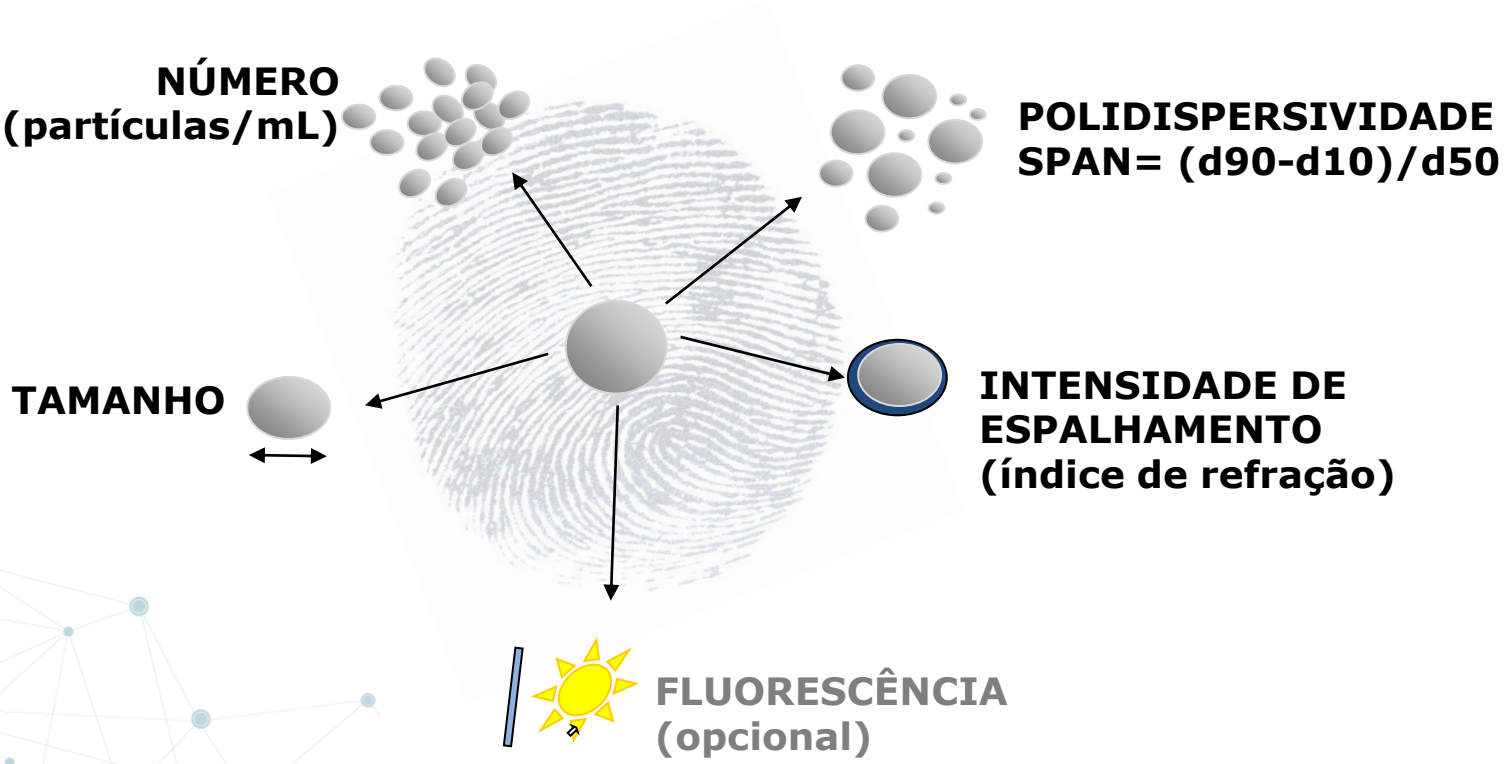
Movimento Browniano

Extracellular vesicles (EVs) purified from serum by ultracentrifugation, sizes 100-500nm



- Nanopartículas se movem
  - *Movimento Browniano*
- Partículas pequenas se movem mais rápido do que partículas maiores
- **Coeficiente de Difusão** pode ser calculado acompanhando e analisando o movimento de cada partícula separadamente, mas simultaneamente

O que o NTA pode oferecer?



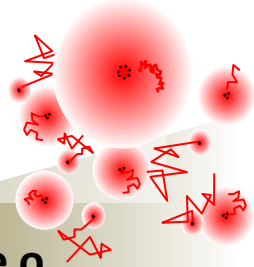
01 Tamanho:
Stokes-Einstein

Como calcular tamanho?

$$d(H) = \frac{K_B\, T}{3\, \pi\, \eta\, D}$$

K_B = Constante de Boltzmann
η= Viscosidade
T = Temperatura

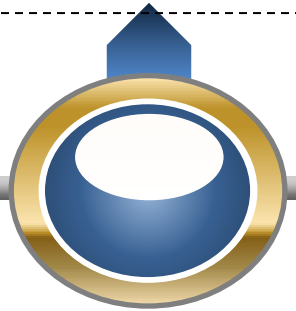
1. ***Obtém* o Coeficiente de Difusão**
2. **Mede o *Movimento Browniano* em tempo real**
3.



Limites de Detecção do NTA

Tamanho

Concentração



MÍNIMO: 10 – 40 nm

- ✓ Tipo de material
- ✓ Comprimento de onda e potência da iluminação
- ✓ Sensibilidade da câmera

MÁXIMO: 1000 – 2000 nm

- ✓ Movimento Browniano
- ✓ Viscosidade do solvente

MÍNIMA: Aprox 10^6 partículas/ mL

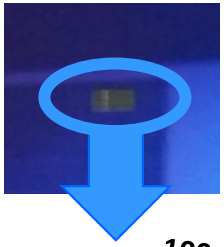
- ✓ Requer maior duração da análise

MÁXIMA: Aprox 10^9 partículas / mL

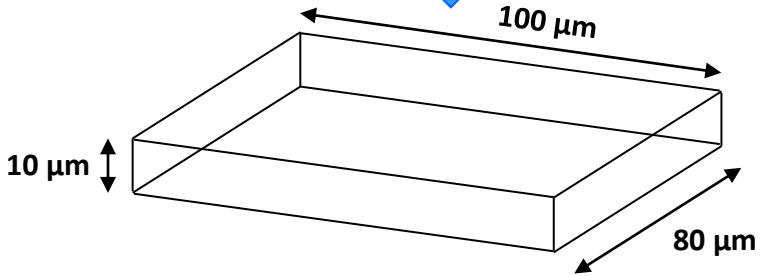
- ✓ Dificuldade de distinção das partículas ao seu redor

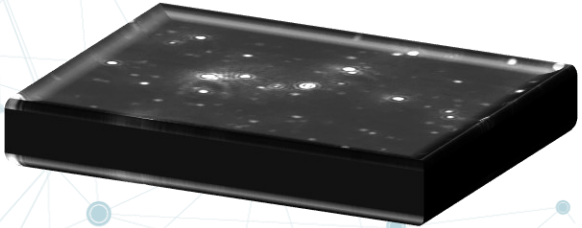
02 Concentração:

Contar partículas em um volume conhecido



Módulo do laser
(parte superior)

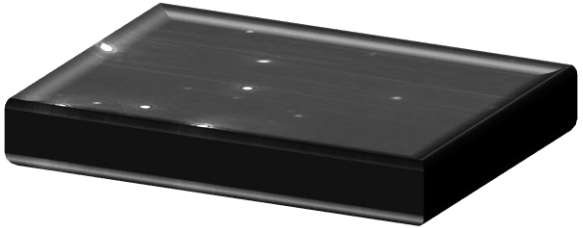




10^9 Partículas/mL

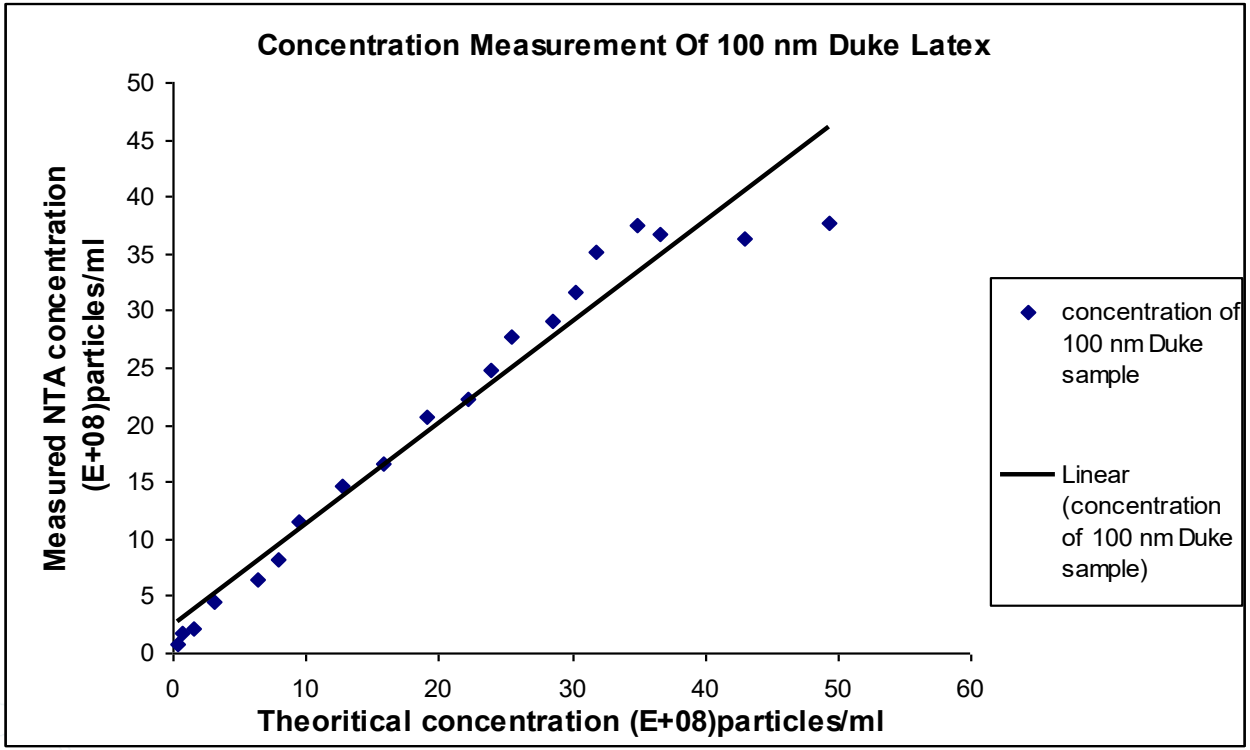
Partículas/mL

Número absoluto de concentração



10^6 Partículas/mL

Linearidade da Análise de Concentração

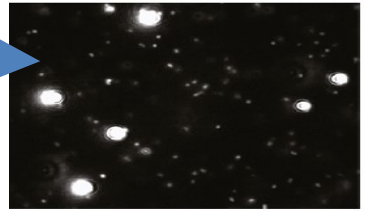


Os resultados teóricos e experimentais são muito próximos nas faixas de 10e6 até 10e9 partículas por mL

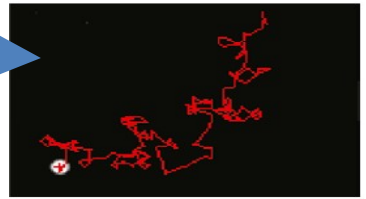
Como funciona?

- A técnica NTA possui capacidade única de promover a caracterização de tamanho baseada em número de forma rápida, sem preparo prévio da amostra (apenas diluição, se necessário).

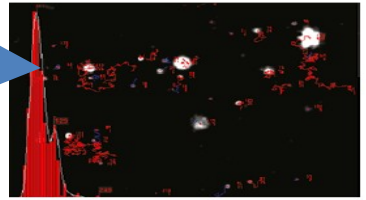
1.Laser ilumina as nanopartículas que se tornam um ponto de luz micrométrico
2.Câmera grava um vídeo do Movimento Browniano das partículas em suspensão

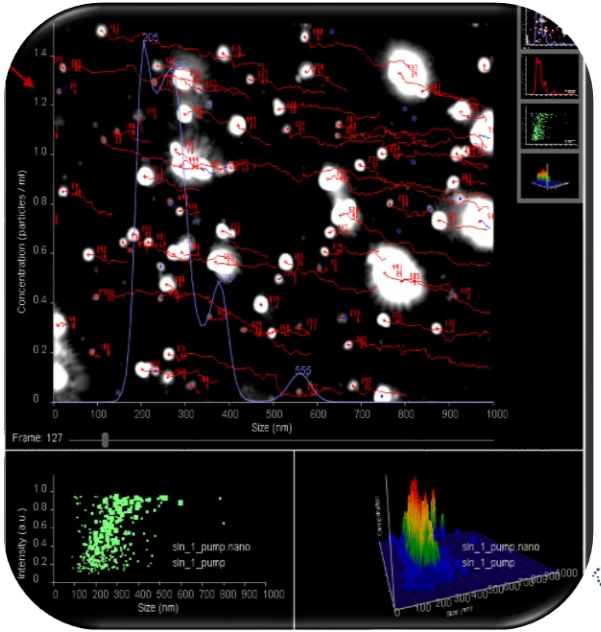


3.Software processa o vídeo para capturar o coeficiente de difusão da amostra
4. Acompanha todas as partículas uma-a-uma e simultaneamente



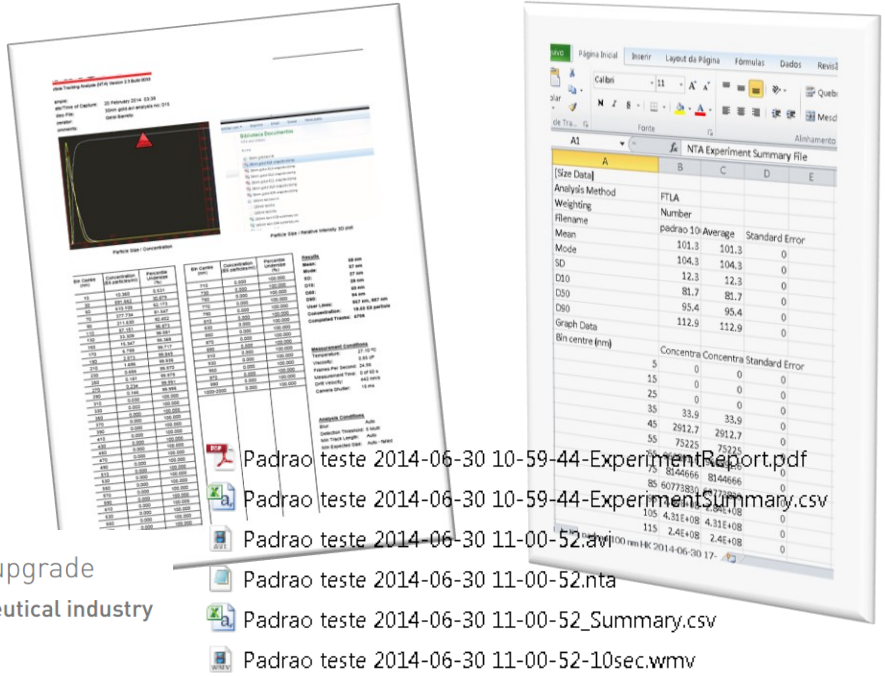
5.Cálculo do D_H pela equação de Stokes-Einstein
6.Gráfico de distribuição de tamanho
7. Valores de concentração (partículas/mL)



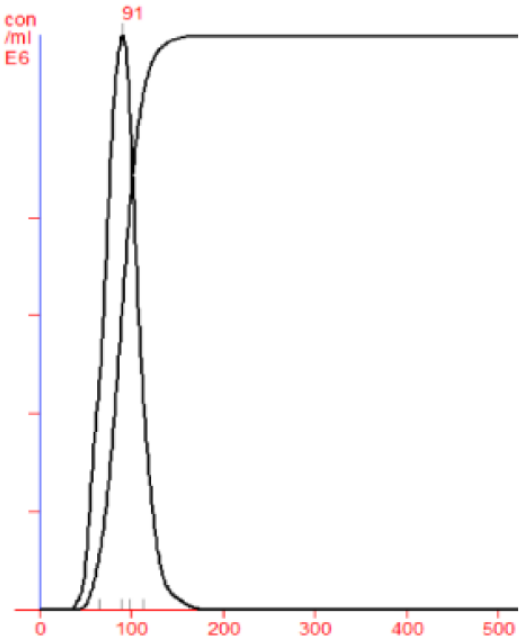


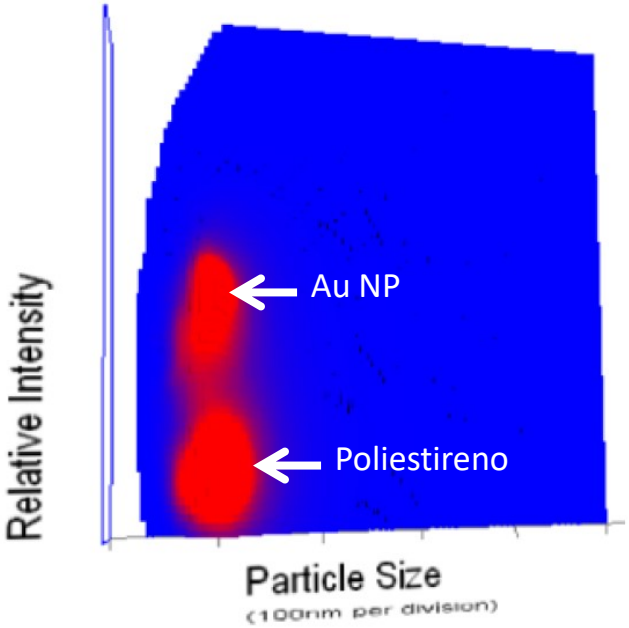
RELATÓRIO

- Exportação de forma rápida e prática de dados em arquivos de Excel, vídeo e relatório em pdf de uma única medida ou de várias
  - *PDF Report*
  - *Summary*
  - *Experiment Summary*
  - *AllTracks*
  - *Particle Data*
  - *Microsoft WMV*
  - *Microsoft WMV 10 sec*



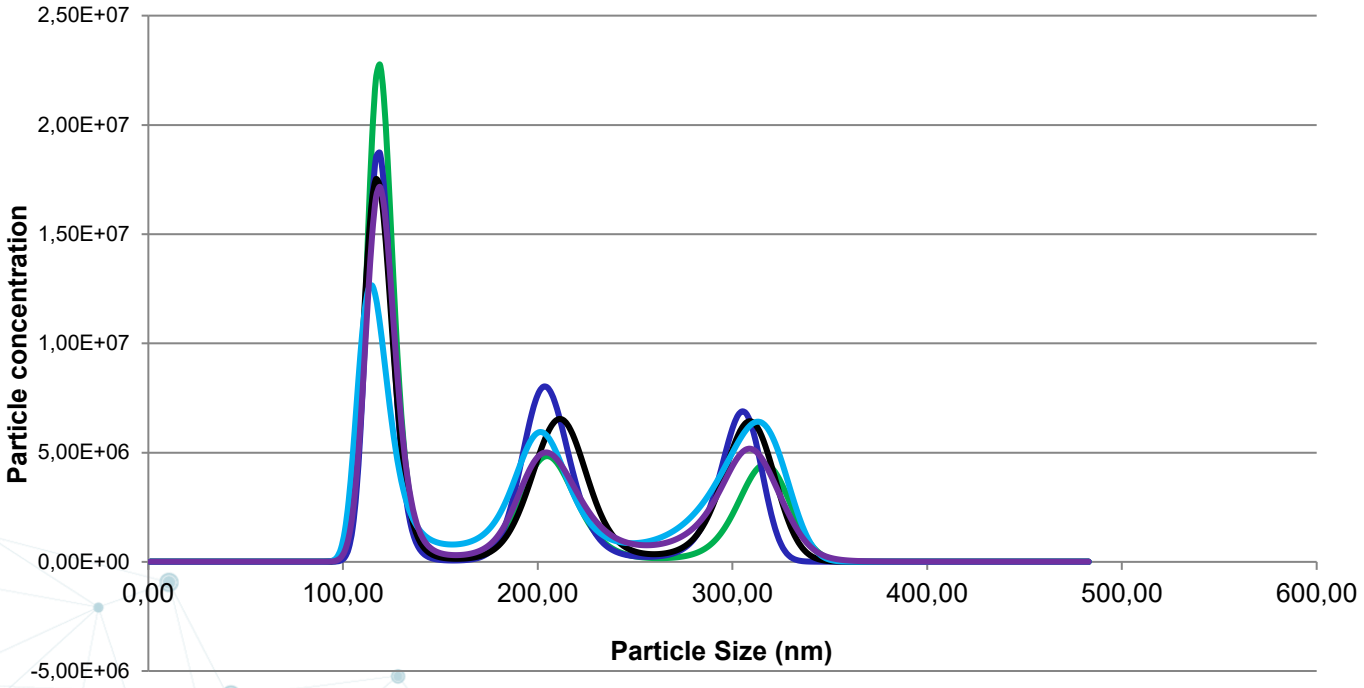
Separando Populações de Tamanhos Similares?



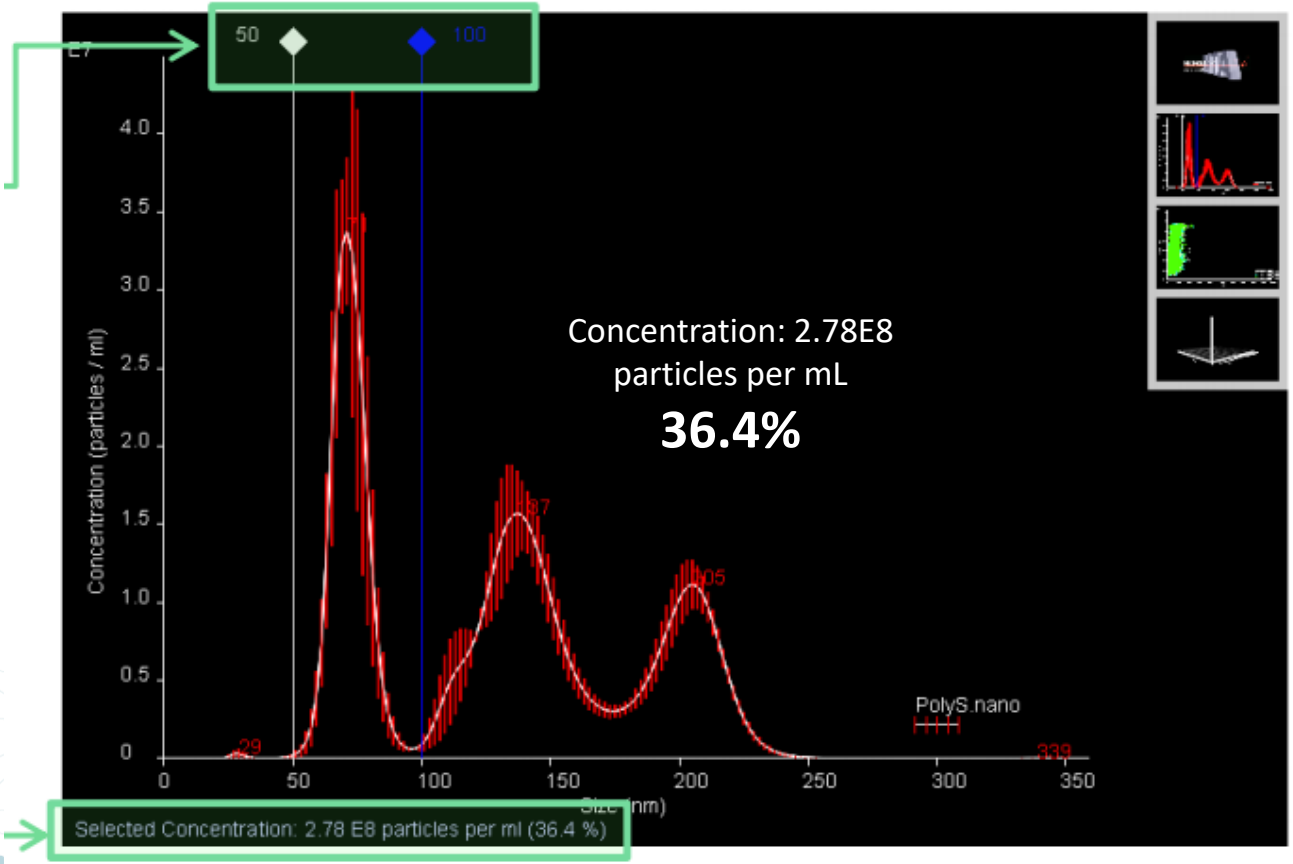


Mistura de Poliestireno (92nm) e Nanopartícula de ouro (90nm)

Resolução de pico a pico

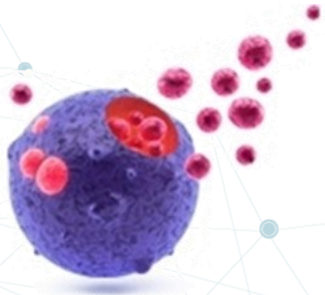


Resolução de pico a pico

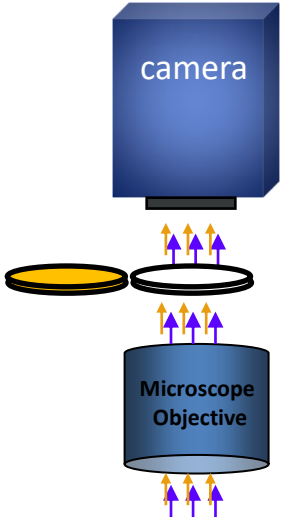


Nanoparticle Tracking Analysis (NTA)

Fluorescência (opcional)



Fluorescent Mode available



Choice of clear or fluorescent filter:

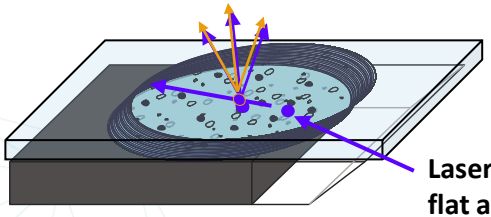
With clear filter, all light transmitted
-scatter is dominant and captured by CCD

With long pass filter, scattered light blocked
-fluorescence is captured by CCD

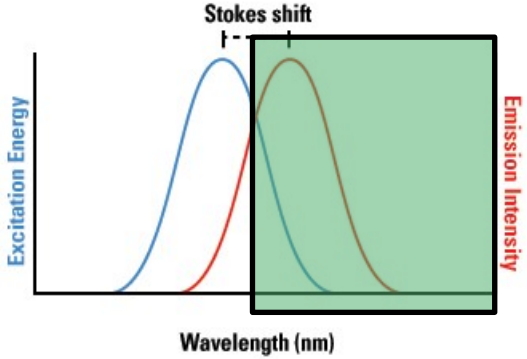
All light collected by objective...

- ALL particles scatter the laser beam.

-Only particles with fluorescence capabilities or label will also fluoresce.



Laser beam illuminates optical flat and transmits through the sample

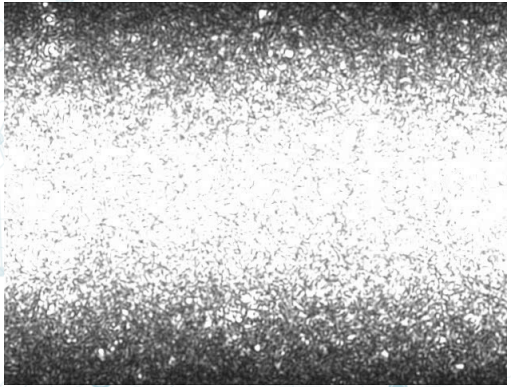


Modo de Fluorescência

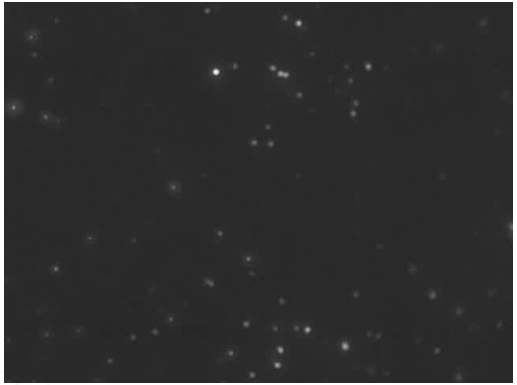
- Permite a medição de partículas específicas
- Partículas fluorescentes de 100nm em 100% FBS
- Amostra mantida em temperatura constante de 37 ºC
- Viscosidade da amostra: 1,33 cP

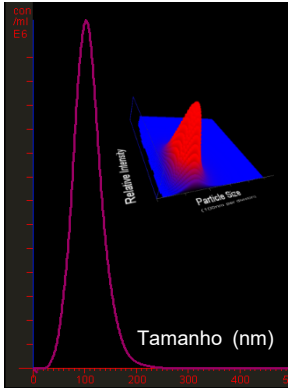
Available laser sources (nm)	Standard filter provided (nm)
Violet 405	430 long pass
Blue 488	500 long pass
Green 532	565 long pass
Red 638	650 long pass

Modo de luz espalhada



Modo de fluorescência



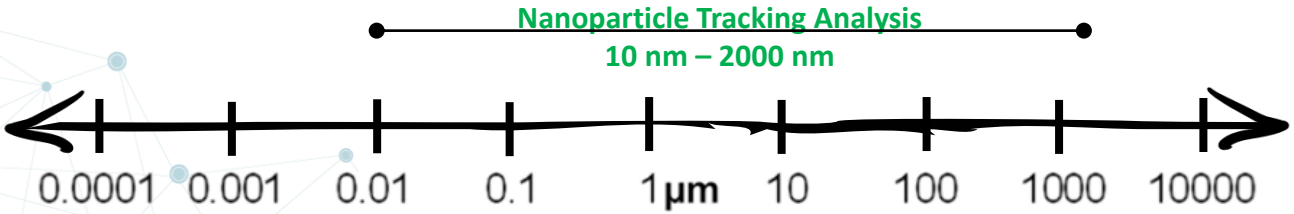


Moda: 103 nm
Concentração: 9.5 x10^8 partículas/mL

Seringa de fluxo

Normas relacionadas

NTA e nanotoxicidade

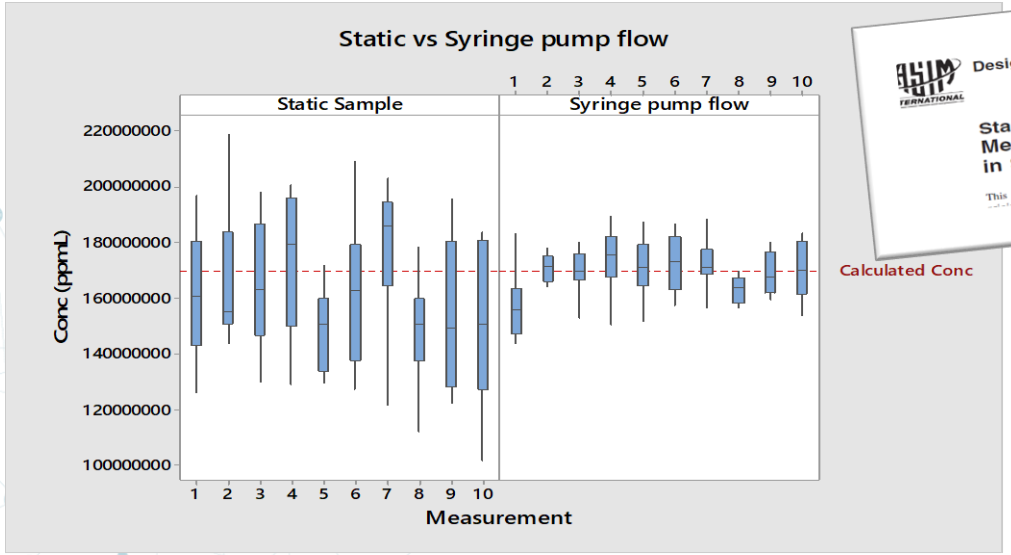


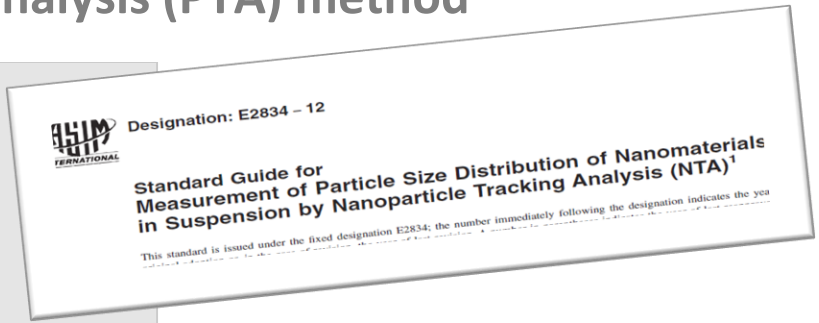
Normas relacionadas

- Mais de **11.600** resultados do NanoSight no Google Acadêmico (08/09/2020).

› **ASTM E2834-12 Guide on NTA technique**

› **ISO 19430 standard: Particle tracking analysis (PTA) method**





Designation: E2834 – 12

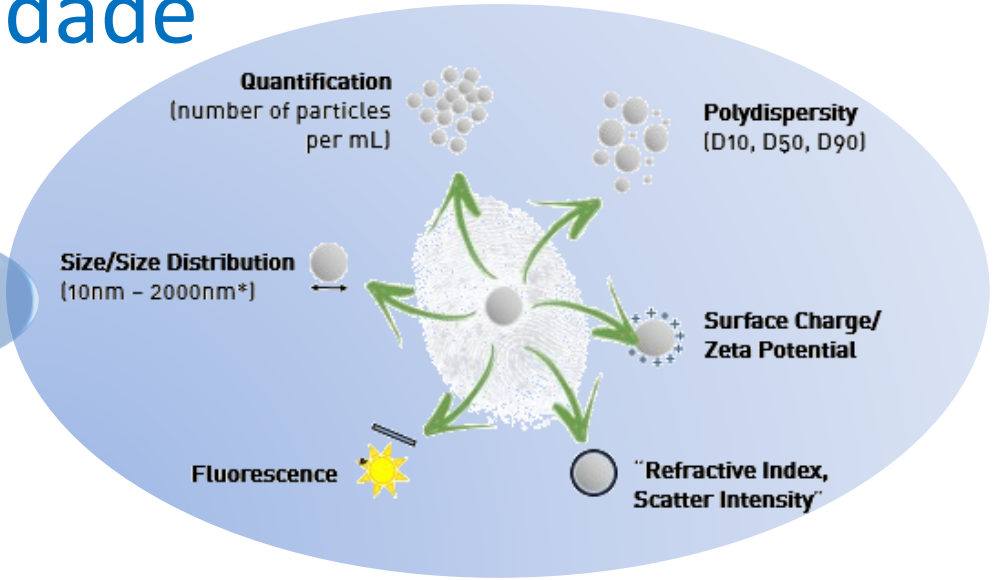
Standard Guide for
Measurement of Particle Size Distribution of Nanomaterials
in Suspension by Nanoparticle Tracking Analysis (NTA)[1]

This standard is issued under the fixed designation E2834; the number immediately following the designation indicates the yea

Estudos de nanotoxicidade

Métodos Biofísicos

NTA

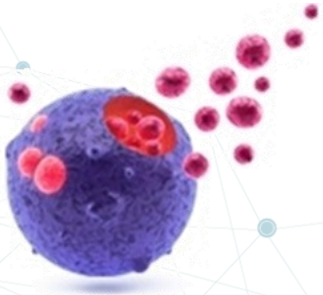


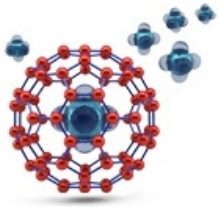
Necessita de testes realizados em fluidos biologicamente relevantes

- ❑ Composição química
- ❑ Concentração
- ❑ Tamanho
- ❑ Distribuição de Tamanho baseada em número/Polidispersão
- ❑ Área de superfície
- ❑ Morfologia
- ❑ Carga de superfície
- ❑ Estado de agregação
- ❑ Recobrimento
- ❑ Partículas rastreáveis
- ❑ Estabilidade/comportamento ao longo do tempo

Aplicações

Biológicas & Não-biológicas

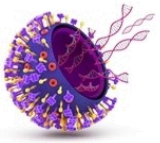




PRINCIPAIS APLICAÇÕES DO NTA

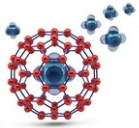
Bio applications

- **Vesículas Extracelulares** (Exossomos e microvesículas)
- **Drug delivery**
- **Agregação de Proteínas**
- Vírus e VLP
- Nanotoxicologia



Non-bio applications

- **Polímeros e coloides**
- Ink jet inks e pigmentos
- Nanopartículas Magnéticas
- Quantum dots (Qdots)
- Nanobolhas
- Cerâmica
- Aditivos de combustível
- Nanotubos de carbono
- Cosméticos
- Alimentos



NATURE PROTOCOLS

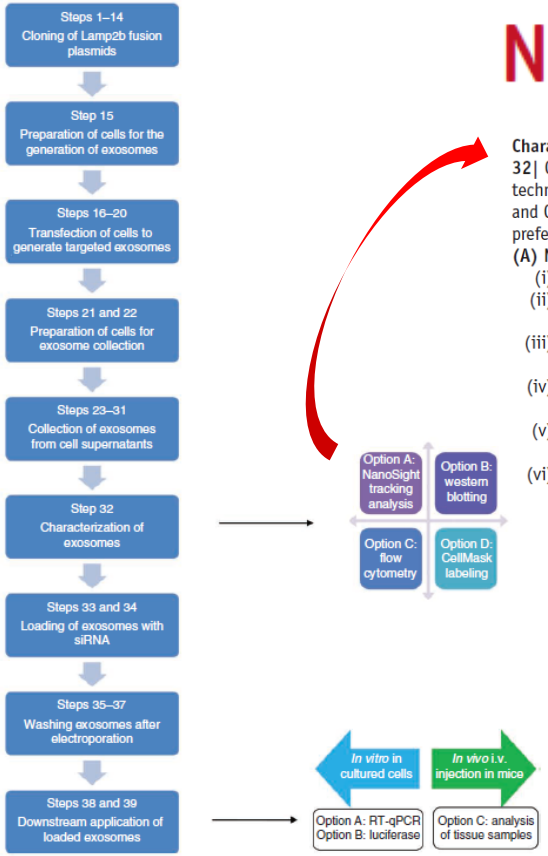


Figure 1 | Flowchart depicting the nine stages for exosome-mediated delivery of siRNA *in vitro* and *in vivo*.

Characterization of exosomes

32| Characterization of exosomes is essential for ascertaining their presence and quality. Perform the characterization techniques described below (nanoparticle tracking analysis, option A; western blotting, option B; flow cytometry, option C; and CellMask labeling, option D). This is commonly done after Step 34 onward (exosome loading), as exosomes should preferentially be loaded (Step 33) on the day of collection.

(A) Nanoparticle tracking analysis ● TIMING 0.5 h

(i) Verify size measurements using NIST-traceable polystyrene microspheres.

(ii) Dilute exosomes in PBS immediately before use. Typically, an initial dilution of 1:100 is made. If necessary, adjust the exosome suspension to achieve an exosome count of between 2×10^8 per ml and 1.0×10^9 per ml.

(iii) Load the diluted sample into the sample chamber and adjust the camera focus if necessary. The exosomes should appear as sharp dots of light.

(iv) Analyze the sample, using the NTA's *script control* facility application, to record five 20-s videos with a sample advance, followed by a 5-s delay between each recording.

(v) Visually check that the five size profiles on the screen are in concordance. Repeat the measurement if the profiles are not in close agreement.

(vi) Analyze the videos in *batch process* mode and export the results to Microsoft Excel (see ANTICIPATED RESULTS).

(A) Nanoparticle tracking analysis ● TIMING 0.5 h

(B) Western blotting ● TIMING 6 h

(C) Flow cytometry ● TIMING 4 h

(D) CellMask plasma membrane stain ● TIMING 24 h

Fonte: http://www.nature.com/nprot/journal/v7/n12/full/nprot.2012.131.html

Chapter 22

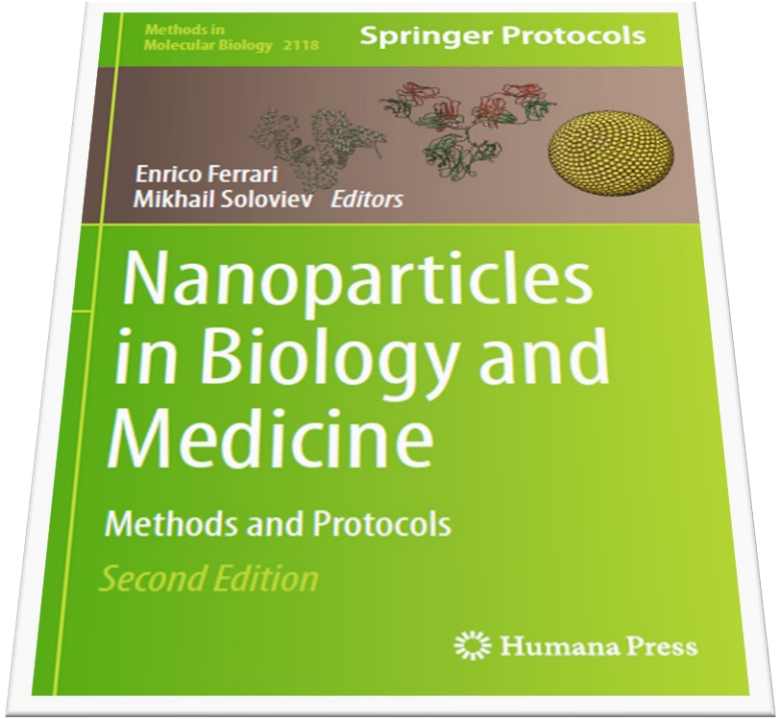
Nanoparticle Tracking Analysis for Multiparameter Characterization and Counting of Nanoparticle Suspensions

Duncan Griffiths, Pauline Carnell-Morris, and Matthew Wright

Abstract

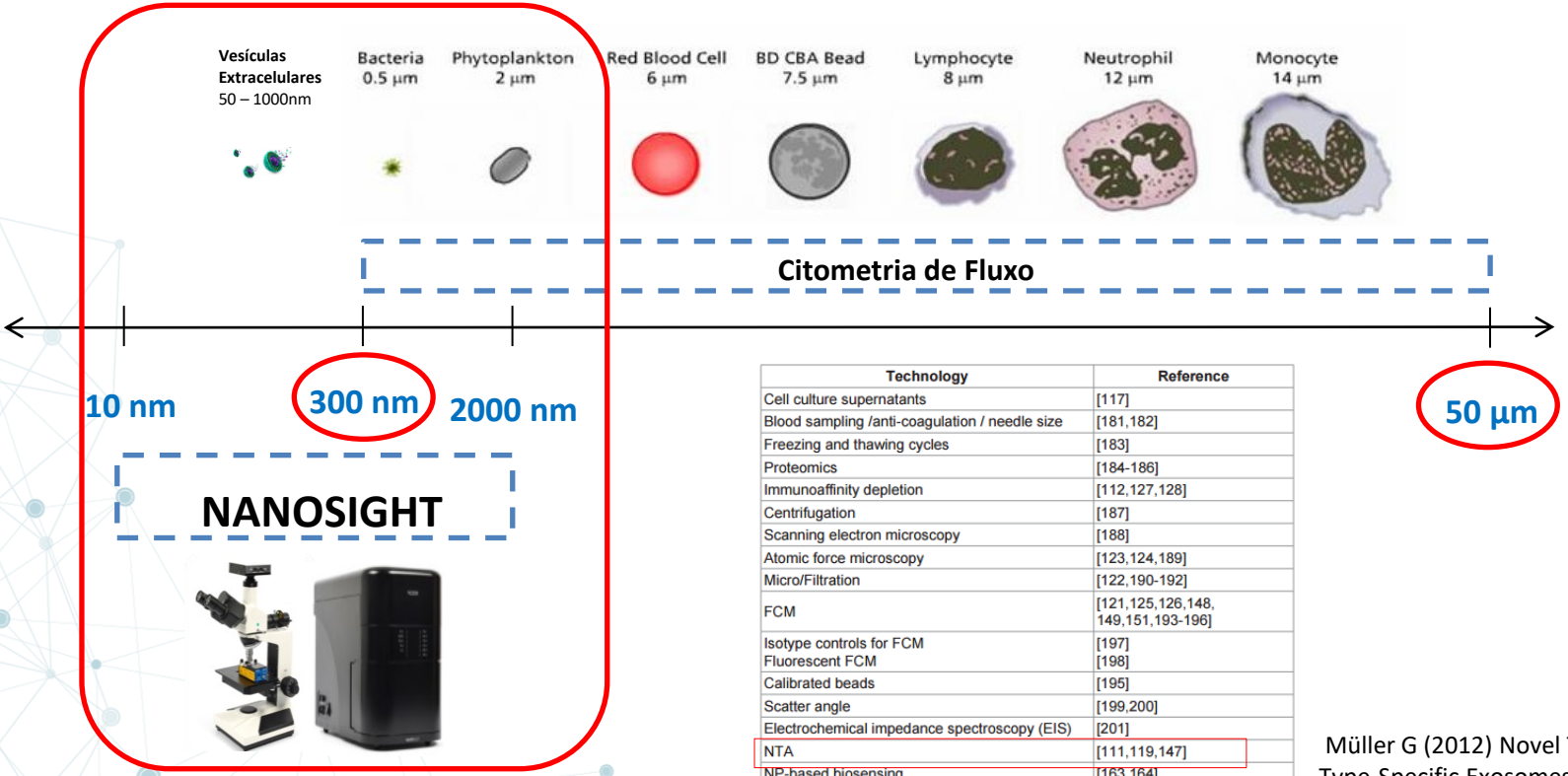
Nanoparticle tracking analysis (NTA) provides direct and real time visualization, sizing and counting of particulate materials between 10 nm and 1 μm in liquid suspension. The technique works on a particle by particle basis, relating the degree of movement under Brownian motion to the sphere equivalent hydrodynamic diameter particle size, allowing for high-resolution particle size distributions to be obtained within minutes. NTA has been used in studying protein complexes and protein aggregates, protein nanoparticles, metal nanoparticles, silica nanoparticles, viruses, cellular vesicles and exosomes to name just a few. Here we describe application of NTA to the analysis of model nanospheres of ~100 nm in liquid suspension, the size being representative of the middle of the NTA working range. The technique described can be adapted for use with nearly all particulate materials with sizes between approximately 10 nm and 1 μm, with appropriate adjustments to instrument settings.

Key words NTA, Nanoparticle tracking analysis, Nanoparticles, Sizing, Counting, Visualization, NanoSight, Light scattering



OPÇÕES PARA ANÁLISE DE TAMANHO:

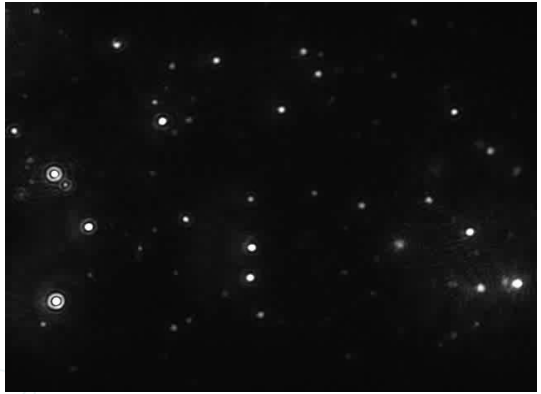
Vesículas Extracelulares

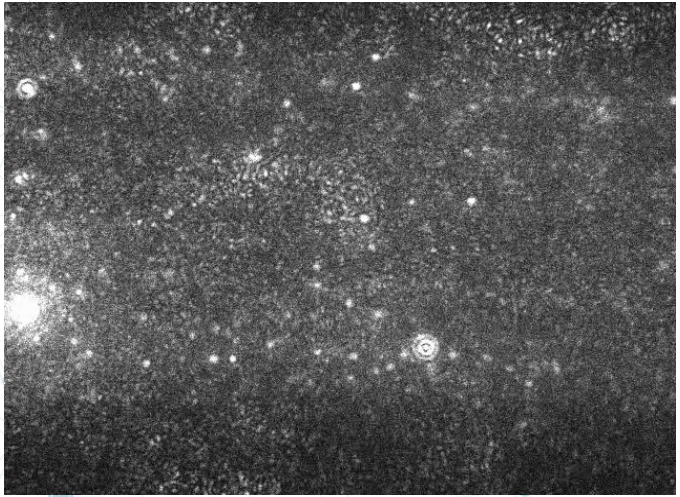


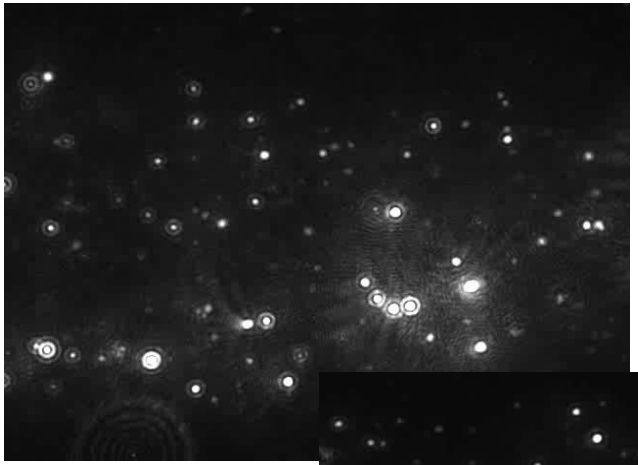
Technology	Reference
Cell culture supernatants	[117]
Blood sampling /anti-coagulation / needle size	[181,182]
Freezing and thawing cycles	[183]
Proteomics	[184-186]
Immunoaffinity depletion	[112,127,128]
Centrifugation	[187]
Scanning electron microscopy	[188]
Atomic force microscopy	[123,124,189]
Micro/Filtration	[122,190-192]
FCM	[121,125,126,148, 149,151,193-196]
Isotype controls for FCM Fluorescent FCM	[197] [198]
Calibrated beads	[195]
Scatter angle	[199,200]
Electrochemical impedance spectroscopy (EIS)	[201]
NTA	[111,119,147]
NP-based biosensing	[163,164]
Microfluidic isolation	[202]
Standardization	[203]

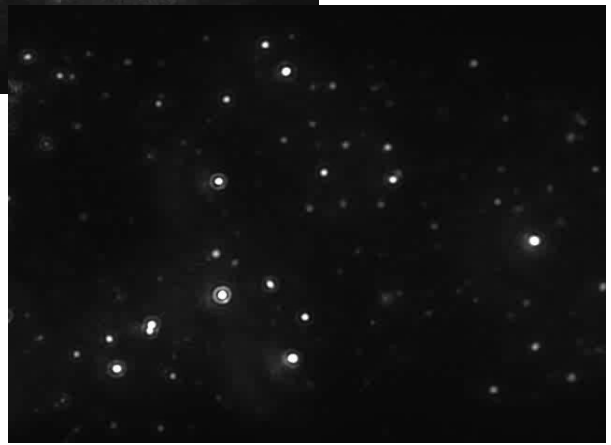
Müller G (2012) Novel Tools for the Study of Cell Type-Specific Exosomes and Microvesicles. J Bioanal Biomed 4: 046-060.

Exemplos de Exossomos e Microvesículas com Concentração e Pureza diferentes



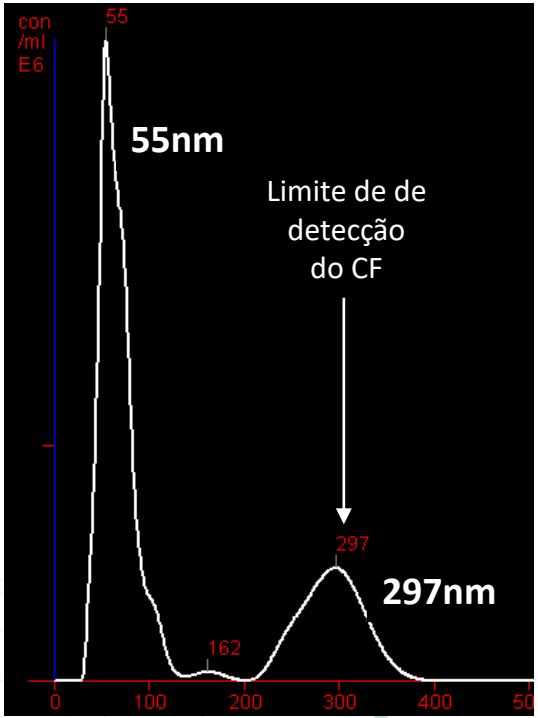




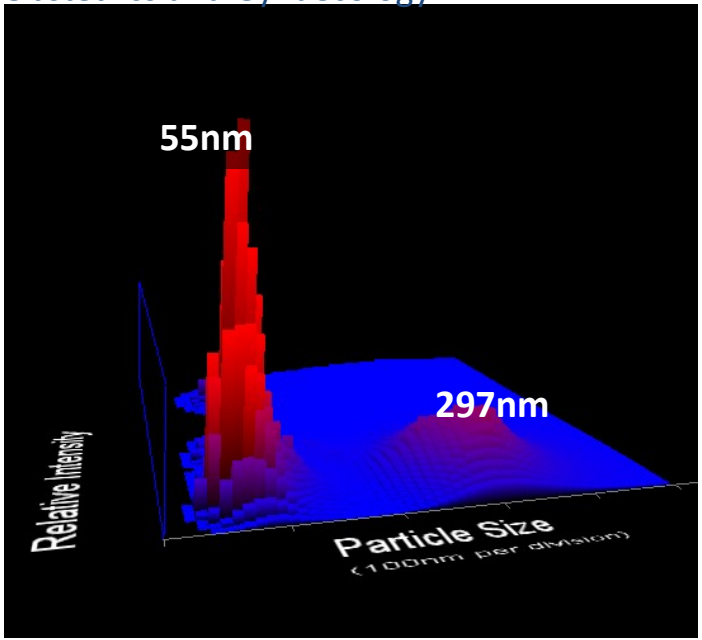


Nanovesículas no plasma

→ indicador de pré-eclâmpsia?



Chris Gardiner; Nuffield Department of Obstetrics and Gynaecology



Contents lists available at SciVerse ScienceDirect

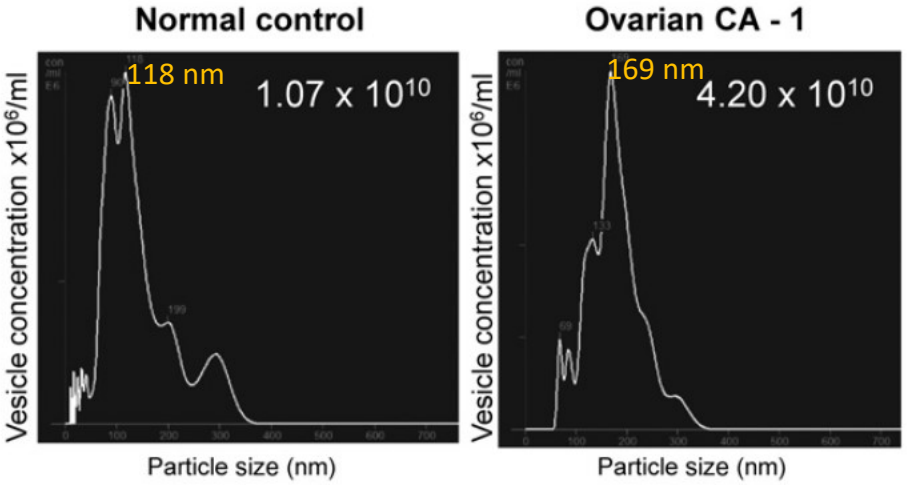
Analytical Biochemistry

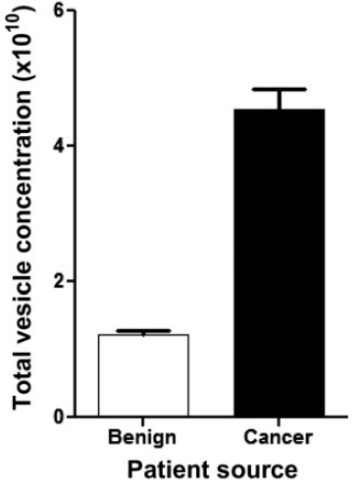
journal homepage: www.elsevier.com/locate/yabio

Nanoparticle analysis of circulating cell-derived vesicles in ovarian cancer patients

Cicek Gercel-Taylor [a], Safinur Atay [b], Richard H. Tullis [c], Mehmet Kesimer [d], Douglas D. Taylor [a,*]

Analytical Biochemistry 428 (2012) 44–53





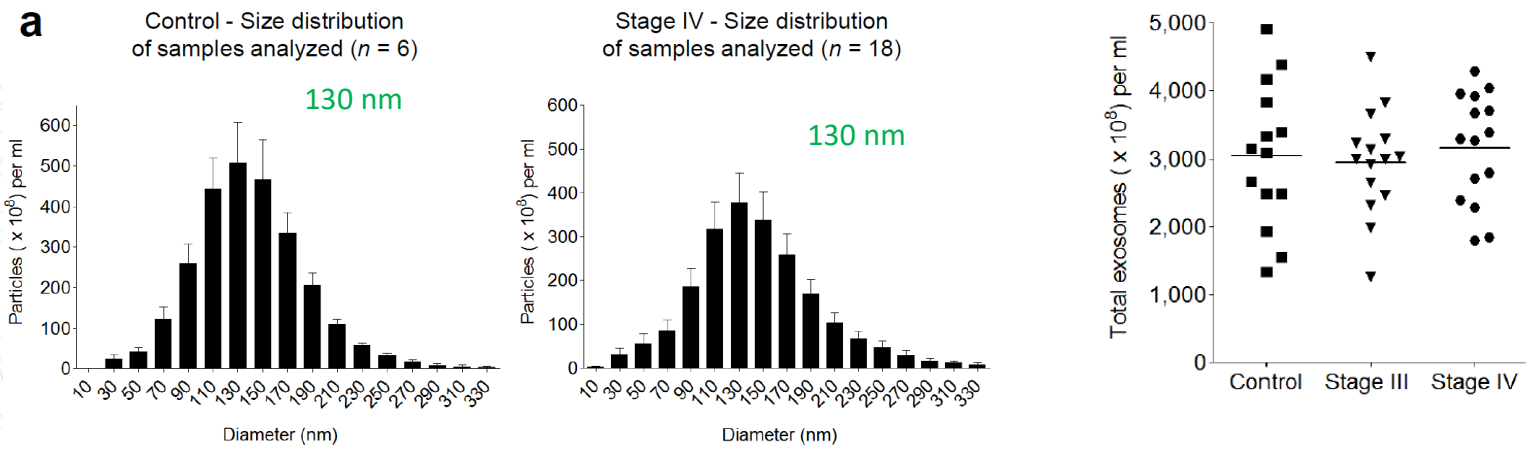
› **Aumentou 4x** o número de exossomas de células com câncer

nature medicine

ARTICLES

Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET

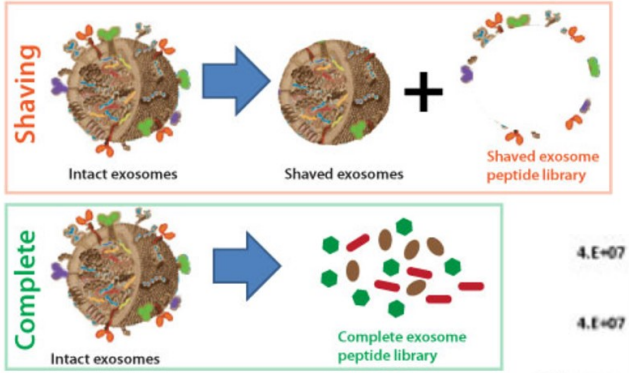
Héctor Peinado[1], Maša Alečković[2], Simon Lavotshkin[3], Irina Matei[1], Bruno Costa-Silva[1,4], Gema Moreno-Bueno[5], Marta Hergueta-Redondo[5], Caitlin Williams[1], Guillermo García-Santos[1], Cyrus M Ghajar[6], Ayuko Nitadori-Hoshino[1], Caitlin Hoffman[7], Karen Badal[1], Benjamin A Garcia[2], Margaret K Callahan[8], Jianda Yuan[9], Vilma R Martins[4], Johan Skog[10], Rosandra N Kaplan[11], Mary S Brady[12], Jedd D Wolchok[8,9,13], Paul B Chapman[8,13], Yibin Kang[2,14,15], Jacqueline Bromberg[8,13] & David Lyden[1,15,16]

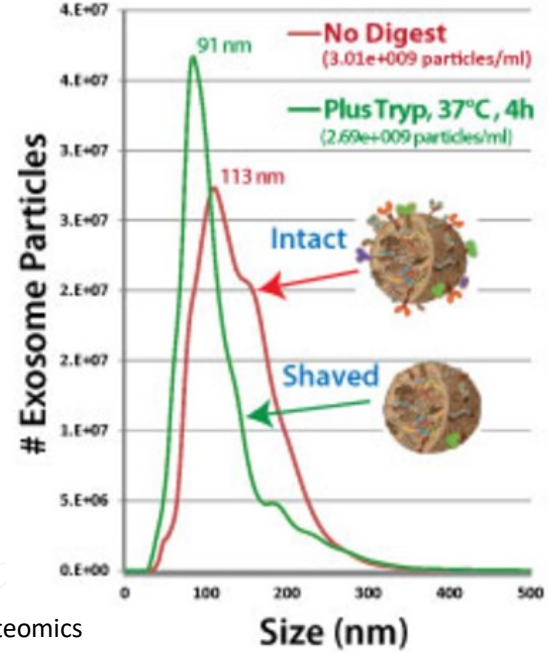


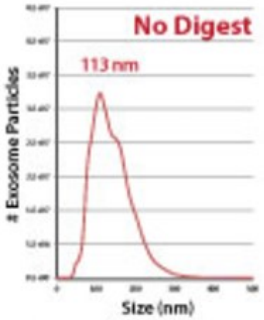
› **Quase nenhuma diferença** em Tamanho e Concentração de exossomas de pacientes em controle VS Grupos do Estágio III e IV

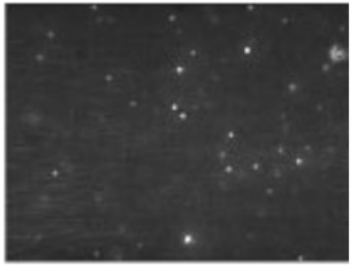
Sensibilidade da técnica NTA

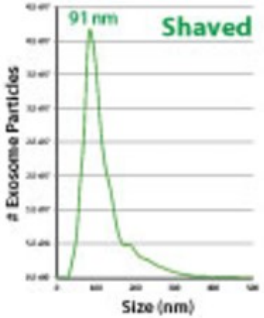
→ funcionalização/partículas na superfície

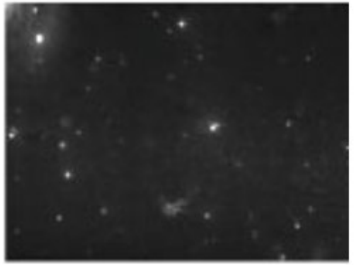








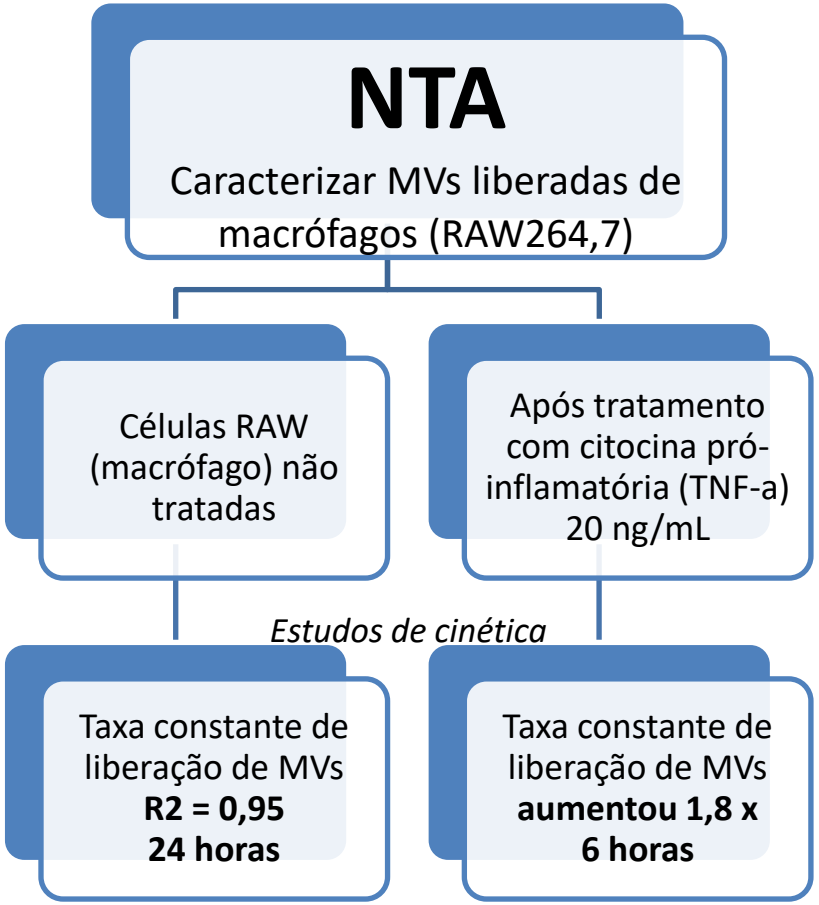




https://www.systembio.com/services/exosome-proteomics

ISEV
INTERNATIONAL SOCIETY FOR EXTRACELLULAR VESICLES

Hutcheson et Al .. (2013) identificou o papel da formação de microcalcificações em placas vulneráveis de aterosclerose durante liberação regulada de vesículas da matriz derivadas de macrófagos

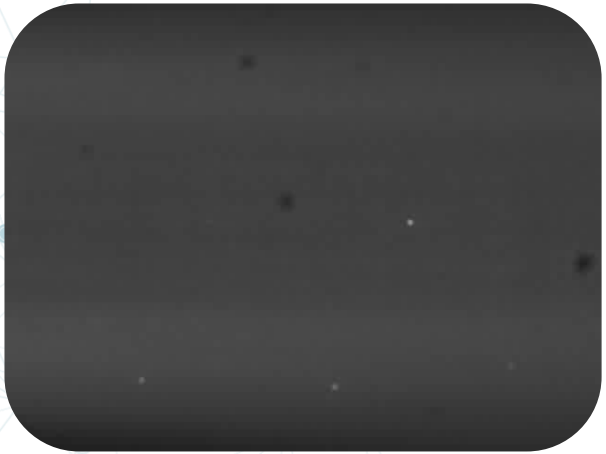


Hutcheson JD, New SEP, Goettsch C, Rogers MA and Aikawa E (2013) Regulated release of macrophagederived matrix vesicles from lipid raft domains: a role for formation of microcalcifications in vulnerable plaques, Second International Meeting of ISEV 2013, Boston, USA, April 17th-20th, 2013 , Journal of Extracellular Vesicles 2013, 2: 20826 - http://dx.doi.org/10.3402/jev.v2i0.20826

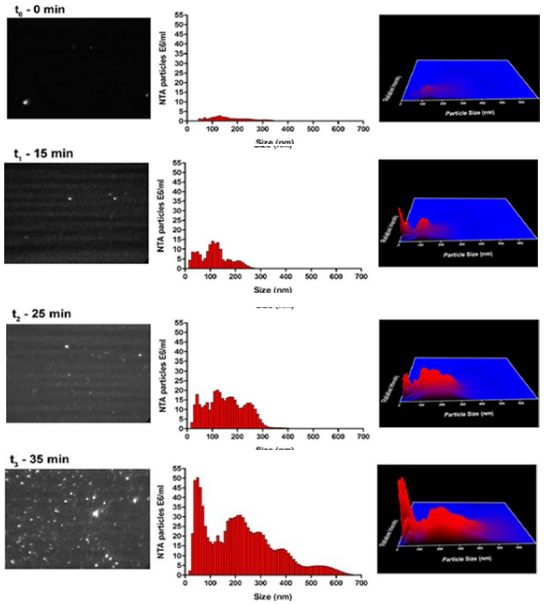
Cinética de Agregação

– Controle integrado de temperatura

- Monitoramento em tempo real / 5°C abaixo do ambiente até 55°C
  - fornece importantes informações sobre a cinética de agregação tanto de proteínas quanto de nanopartículas

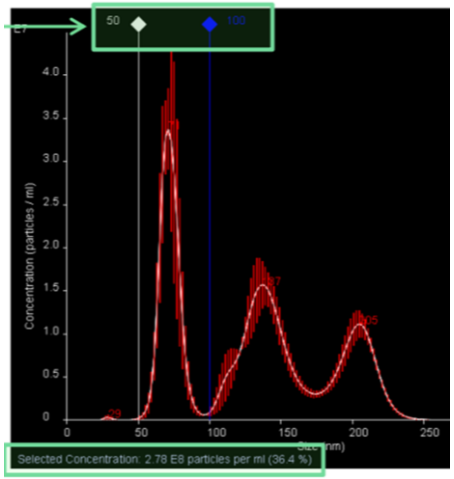


1 mg/mL de IgG – 50ºC durante 35 min



NTA CONFIRMOU: agregados submicrométricos na faixa de 50 e 500 nm se formaram no estresse térmico tanto da insulina quanto da insulina peguilada.

CONCLUSÃO: PEGuilação não protegeu a insulina contra a agregação forçada.

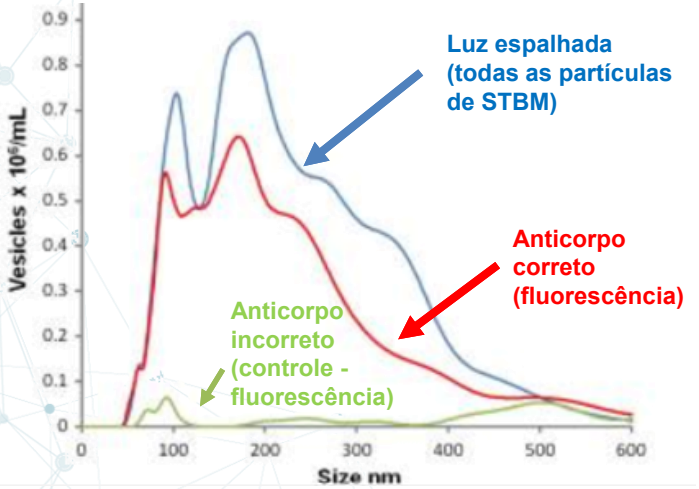


Torosantucci R, Kukrer B, Mero A, Winsen MV, Tantipolphan R and Jiskoot W (2011) Plain and monopegylated recombinant human insulin exhibit similar stress-induced aggregation profiles. Journal of Pharmaceutical Sciences.

Fenotipagem de Vesículas Extracelulares

O NanoSight é capaz de identificar através de fenotipagem as variedades de vesículas extracelulares secretadas com marcação

Análise de fluorescência de vesículas no plasma

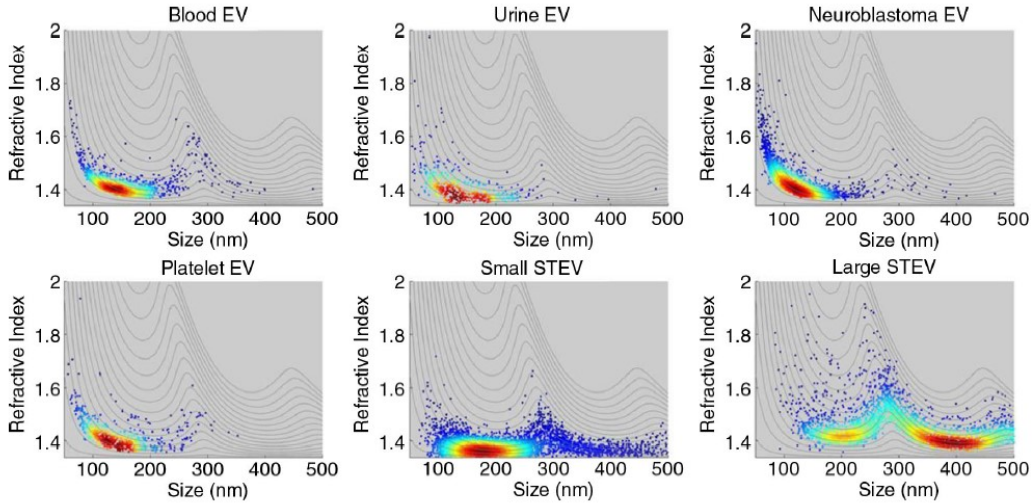


- Papel fundamental no estudo de mediadores da comunicação intercelular
- Saber o tamanho, concentração e comportamento de vesículas extracelulares em seu ambiente natural elucida a função que estas estruturas desempenham em mecanismos de doenças e as formas em que podem ser exploradas para aplicações diagnósticas e terapêuticas

Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJP, Hole P, Carr B, Redman CWG, Harris AL, Dobson PJ, Harrison P and Sargent IL (2011) Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis, Nanomedicine: Nanotechnology, Biology and Medicine (2011), doi:10.1016/j.nano.2011.04.003.

Índice de Refração e VE

- Análise por NTA revela heterogeneidade em vesículas extracelulares (VE)
- Útil para o desenvolvimento de calibradores apropriados para a medição EV



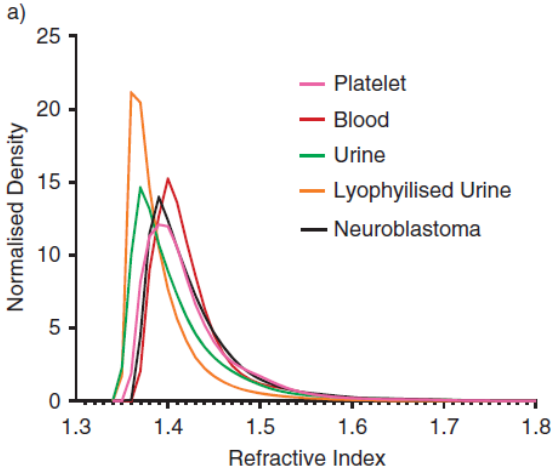


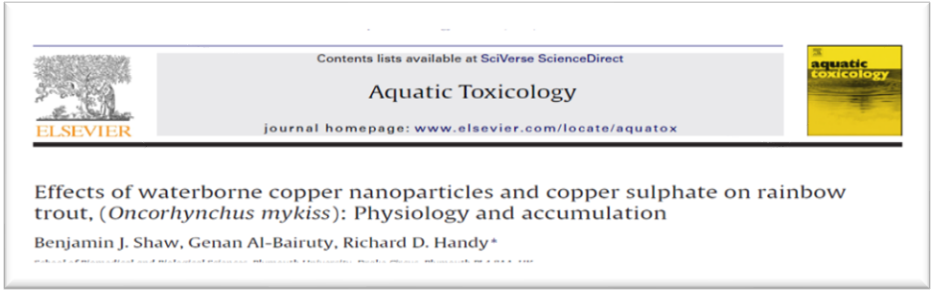
Fig. 2. Vesicle RI versus diameter. Representative data from one of each type of EV studied are shown. Colour of points indicates the density of the distribution. Red denotes dense, and blue sparse, with a rainbow scale between. Lines superimposed on the graphs indicate the projected RI of a particle with an intensity fixed for each line across the range of sizes (0–500 nm).

Fonte: Journal of Extracellular Vesicles 2014, 3: 25361 - http://dx.doi.org/10.3402/jev.v3.25361

EFEITO DO TEMPO NA ESTABILIDADE

Toxicologia

- Amostras podem mudar ao longo do tempo – efeito precisa ser estudado.
- Meio da amostra também pode mudar a amostra


Contents lists available at SciVerse ScienceDirect

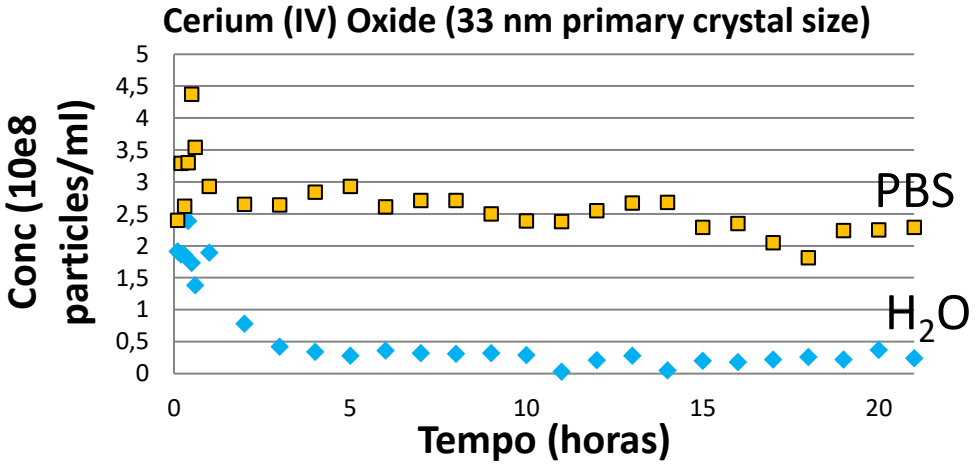
Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Effects of waterborne copper nanoparticles and copper sulphate on rainbow trout, (*Oncorhynchus mykiss*): Physiology and accumulation

Benjamin J. Shaw, Genan Al-Bairuty, Richard D. Handy*

Efeito das nanopartículas de óxido cério (IV)
- Água
- PBS



Zhou et al. Journal of Nanobiotechnology 2012, 10:18
http://www.jnanobiotechnology.com/content/10/1/18

JOURNAL OF NANOBIOTECHNOLOGY

RESEARCH Open Access

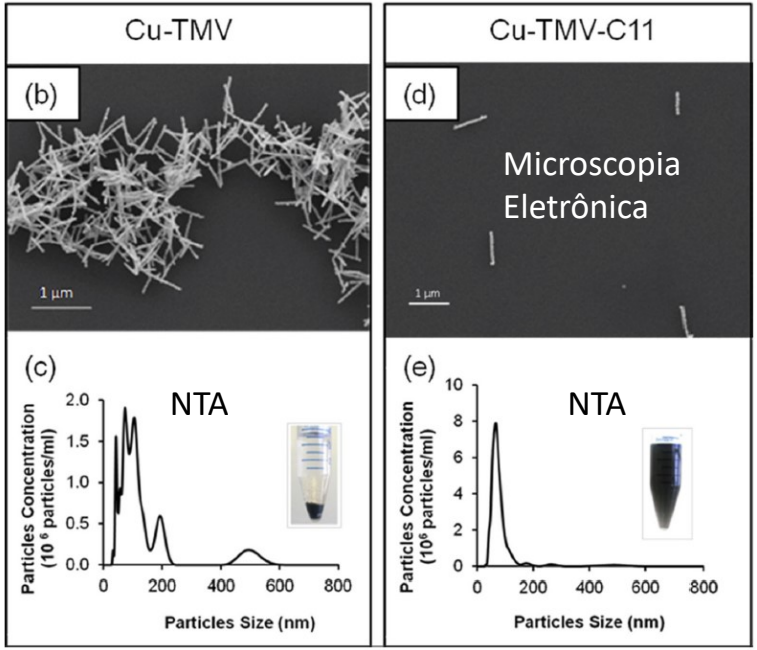
Biotemplating rod-like viruses for the synthesis of copper nanorods and nanowires

Jing C Zhou[1,5,6], Carissa M Soto[1*], Mu-San Chen[1], Michael A Bruckman[1,5], Martin H Moore[1], Edward Barry[2], Banahalli R. Ratna[1], Pehr E Pehrsson[3], Bradley R Spies[4] and Tammie S Confer[4]

Eficácia de Funcionalização

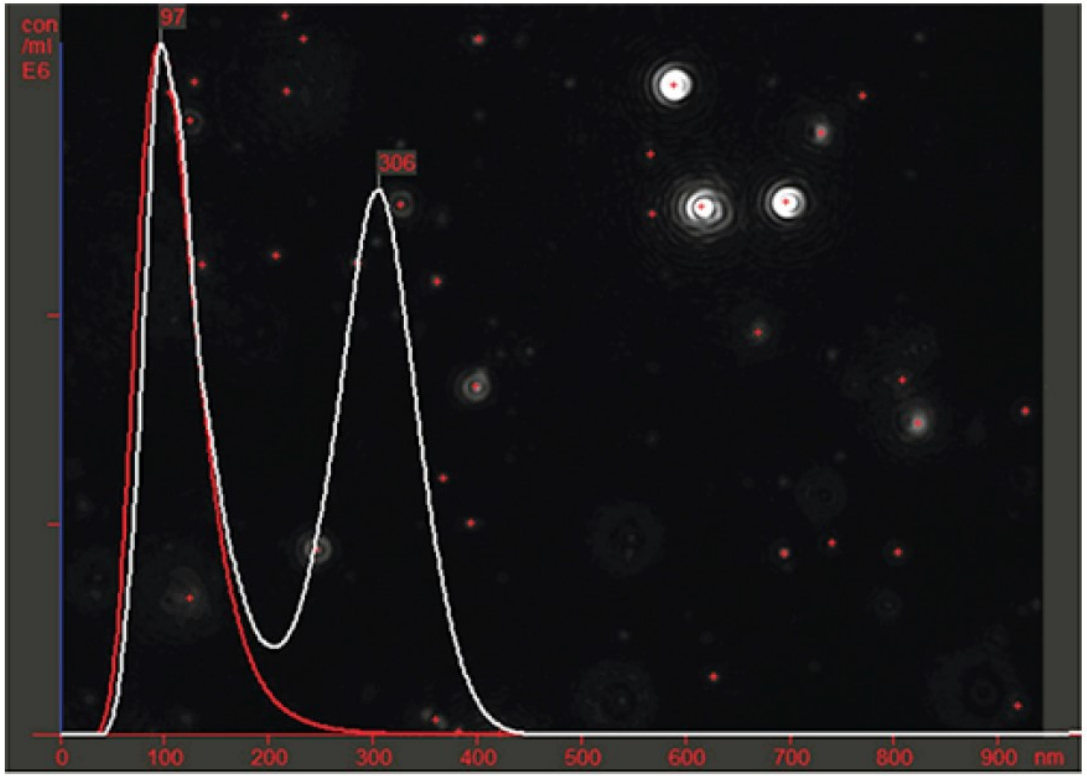
- Possibilidade de gerar uma variedade de nanorods e nanofios com tamanhos de 300 nm
- Tais materiais com base biológica podem ter amplo uso em nanoeletrônica, sensoriamento e terapia do câncer

- Dados obtidos diretamente pelo NanoSight demonstram a dispersão foi bem sucedida do Cu-TMV após o tratamento com C11-PEG-SH.



Zhou et al. Journal of Nanobiotechnology 2012, 10:18

Purificação de vírus



- Adenovirus
- Cytomegalovirus (MCMV)
- Porcine papillomavirus
- Lambda phage
- Tobacco Mosaic virus (TMV)
- Murine Leukaemia virus (MuLV)
- Japanese Encephalitis virus (JEV)
- Cyanovirus
- Herpes simplex virus
- Influenza virus
- Baculovirus
- M13
- M14

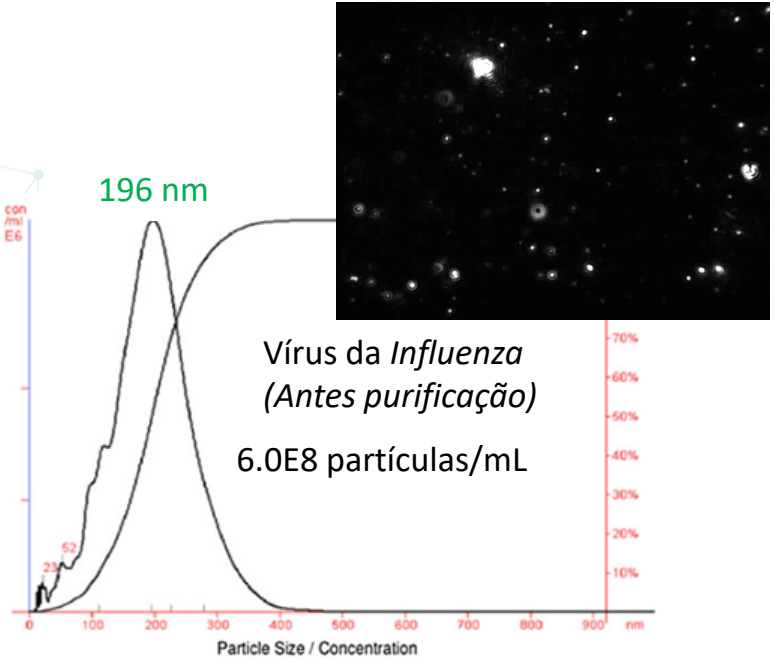
METHODOLOGY Open Access

VIROLOGY JOURNAL

Kramberger *et al. Virology Journal* 2012, **9**:265
http://www.virologyj.com/content/9/1/265

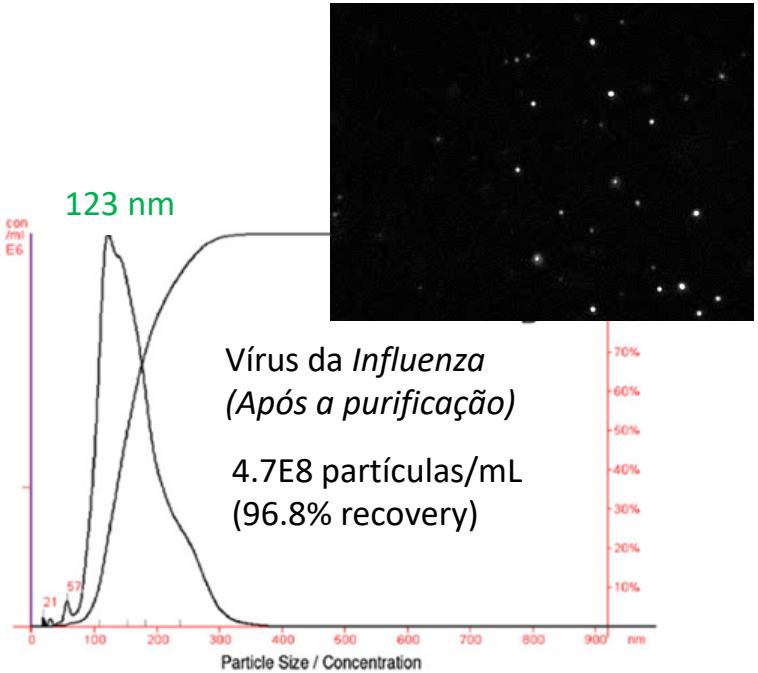
Evaluation of nanoparticle tracking analysis for total virus particle determination

Petra Kramberger[1,2*], Mateja Ciringer[2], Aleš Štrancar[1,2] and Matjaž Peterka[1,2]



Vírus da *Influenza*
(Antes purificação)

6.0E8 partículas/mL



Vírus da *Influenza*
(Após a purificação)

4.7E8 partículas/mL
(96.8% recovery)

- Fornece dados de concentração de forma rápida
- Mede diretamente a pureza e estado de agregação da dispersão de vírus

METHODOLOGY Open Access

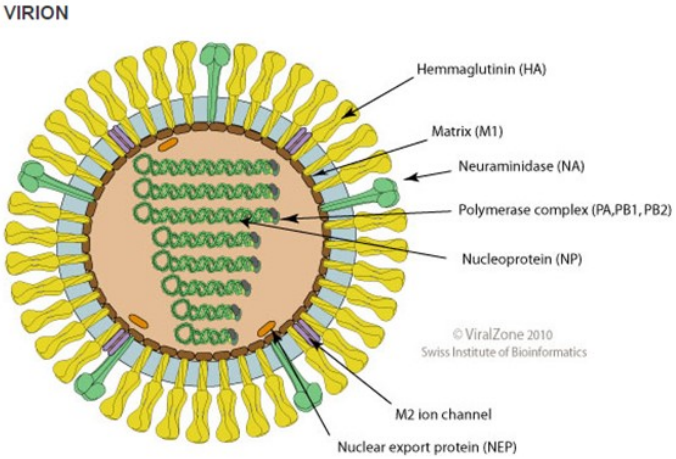
Evaluation of nanoparticle tracking analysis for total virus particle determination

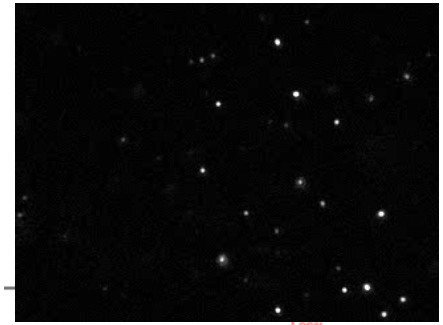
Petra Kramberger[1,2*], Mateja Ciringer[2], Aleš Štrancar[1,2] and Matjaž Peterka[1,2]

VIROLOGY JOURNAL

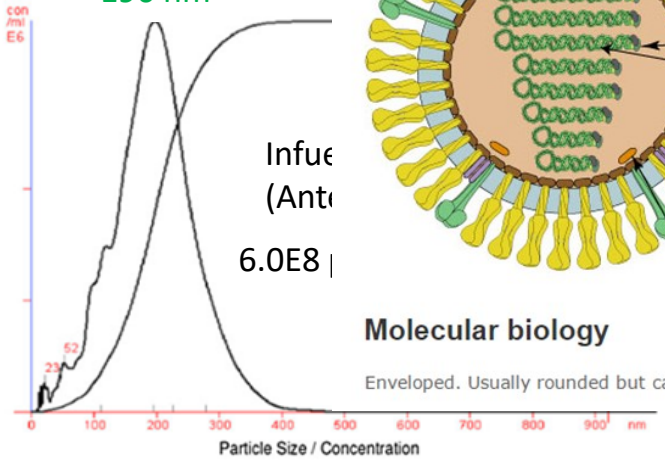
Kramberger *et al. Virology Journal* 2012, **9**:265
http://www.virologyj.com/content/9/1/265

H1N1 subtype





196 nm



Infue
(Ant

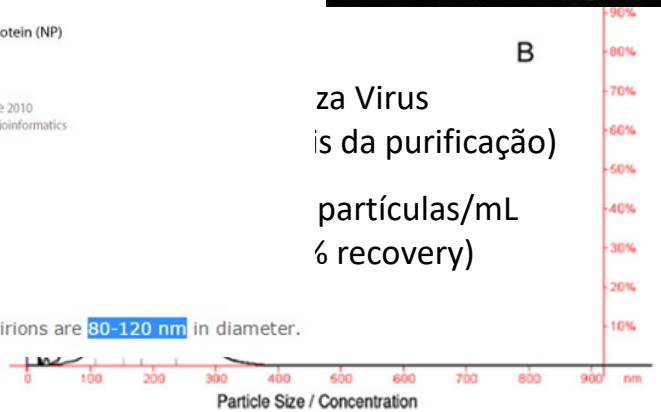
6.0E8 |

za Virus
is da purificação)

partículas/mL
% recovery)

Molecular biology

Enveloped. Usually rounded but can be filamentous. The virions are 80-120 nm in diameter.



❖ ZIKA

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

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Biology of Zika Virus Infection in Human Skin Cells

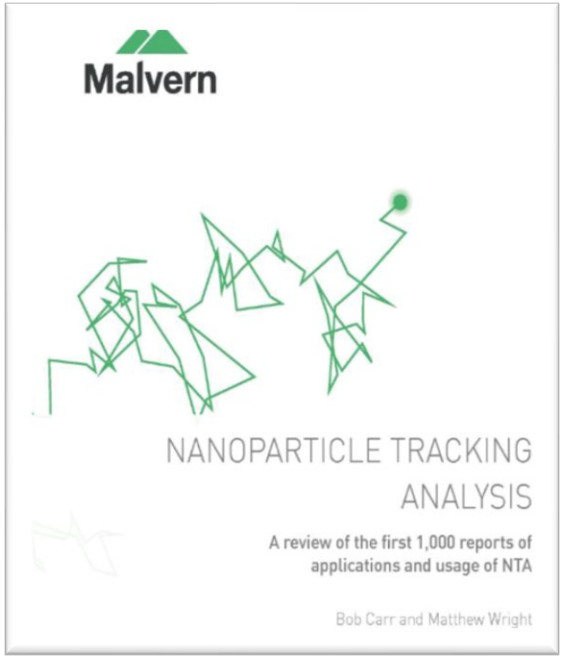
- ❑ Agregação de partículas
- ❑ Vantagem importante na análise de vacinas com vírus **atenuado (1%)** vs **inativado**.

Electron microscopic imaging of ZIKV–infected primary fibroblasts. (A) Membrane vesicles with sizes between 70 and 100 nm, observed in intimate association with the endoplasmic reticulum, are indicated by a white arrow. Black arrows indicate the presence of spherical capsids detected in intracellular vacuoles or docked to intracellular membranes. (B) Enlargement

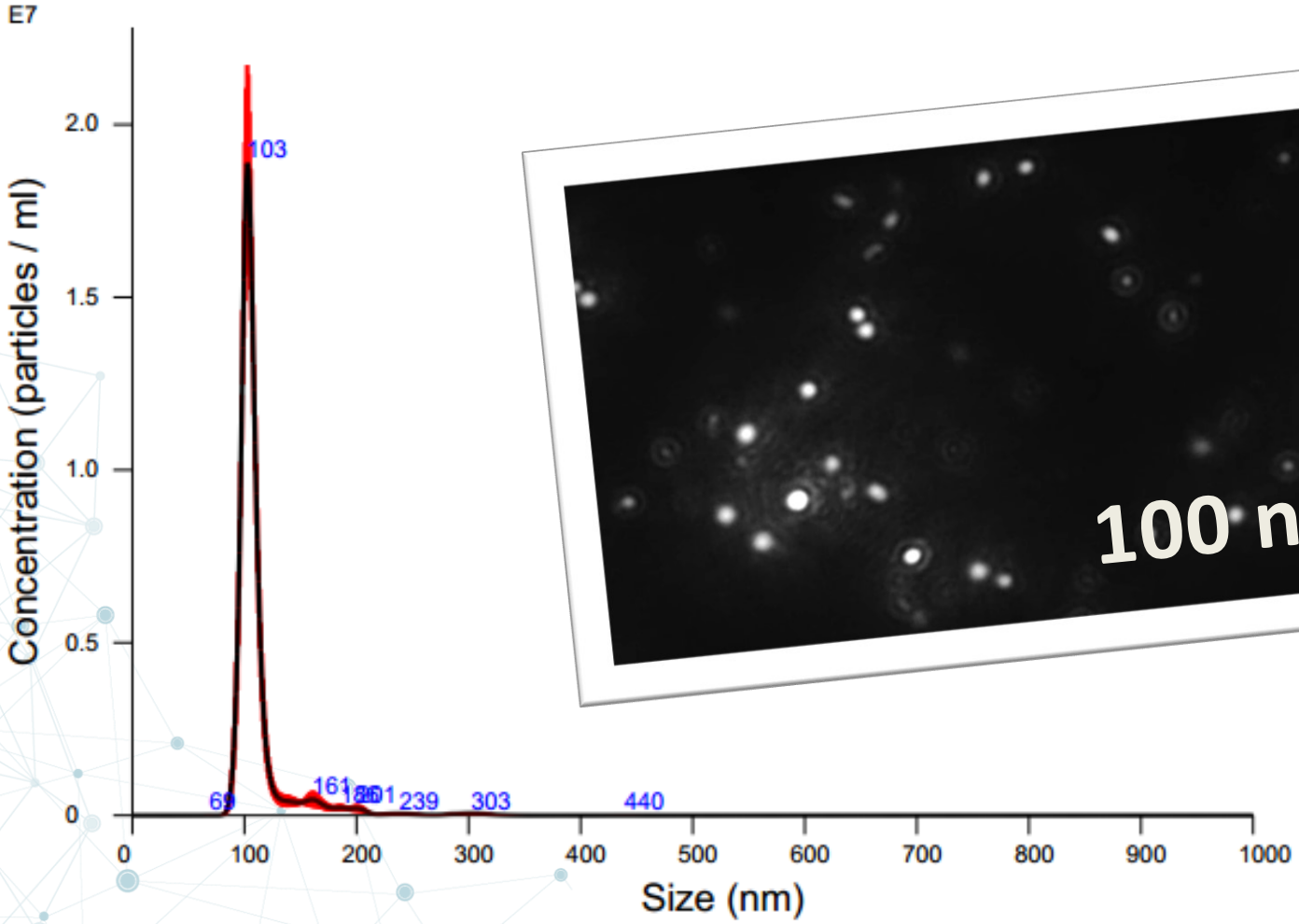
Fonte: http://jvi.asm.org/content/89/17/8880/F10.expansion.html

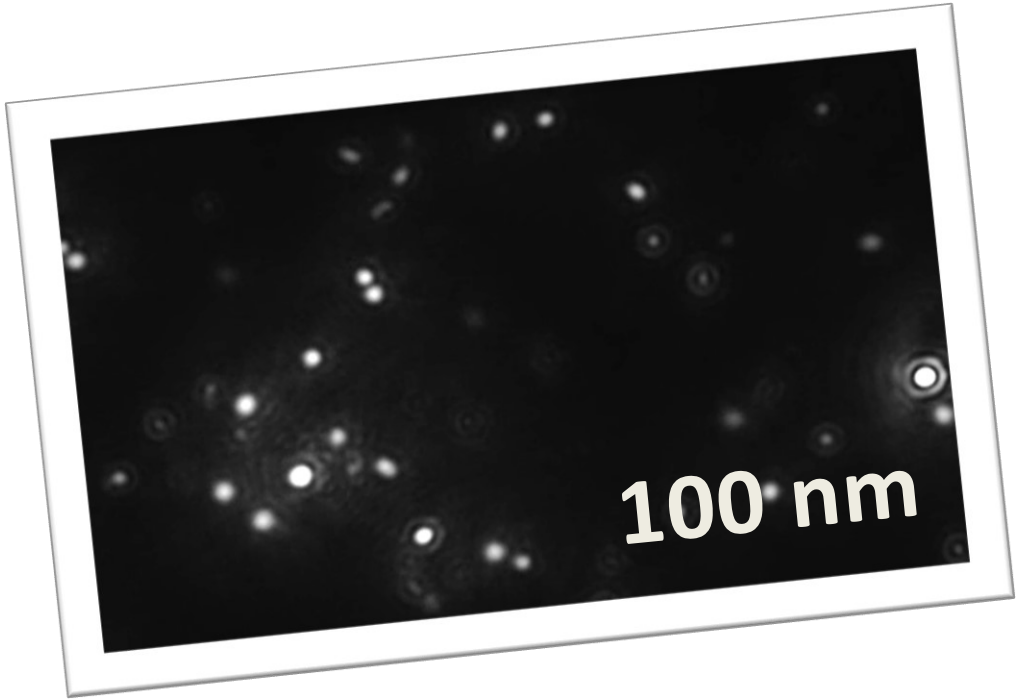
Livro de Referências do NanoSight

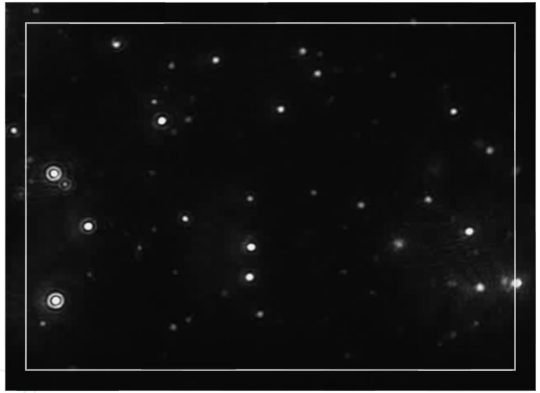
- Conteúdo:
  - Exossomos
  - Agregação de Proteínas
  - Toxicologia de Nanopartículas
  - Carreamento de fármacos
  - Produção de nanopartículas

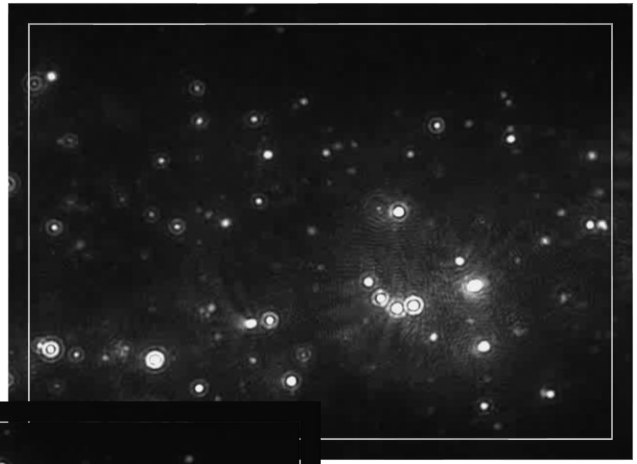


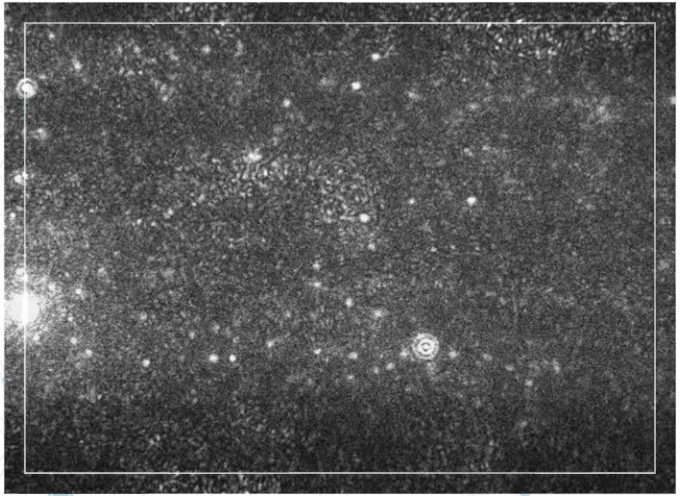
› PDF é gratuito e pode baixar pelo link: http://www.malvern.com/en/support/resource-center/Whitepapers/WP150320NTABook.aspx

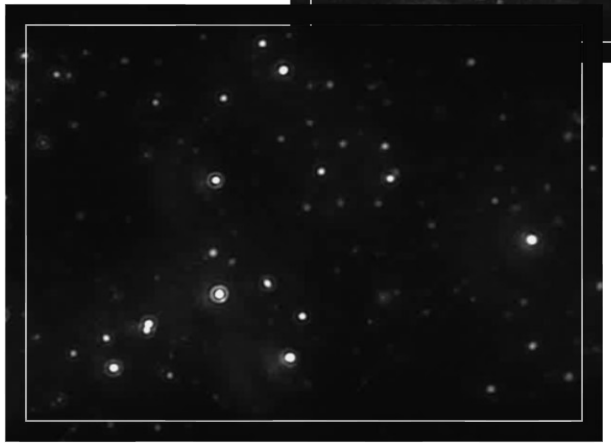

E7
Concentration (particles / ml)
2.0
1.5
1.0
0.5
0
103
69
161
239
303
440
0
100
200
300
400
500
600
700
800
900
1000
Size (nm)


100 nm



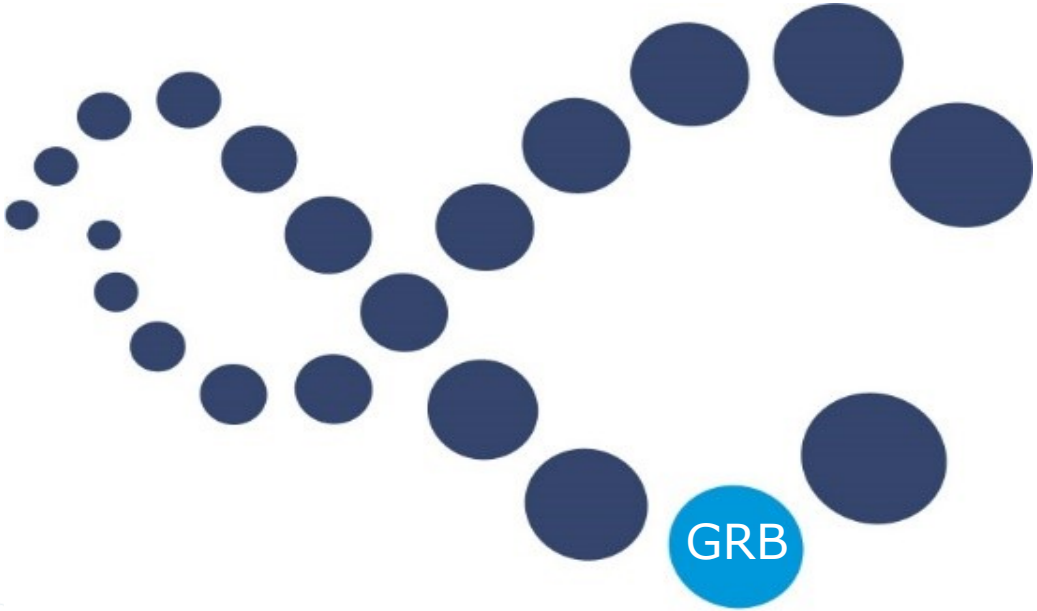






Exemplos de Exossomos e Microvesículas com Concentração e Pureza diferentes

Muito obrigada!



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