Measurements, applications, and impact of the refractive index of extracellular vesicles

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Disclosures for Edwin van der Pol

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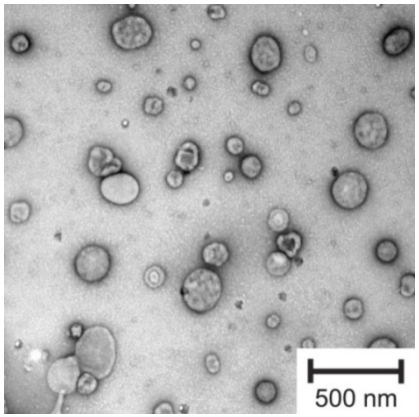
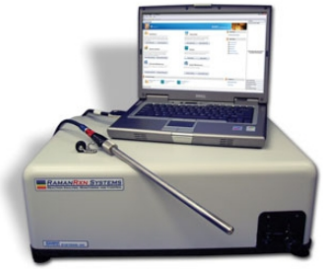
Presentation includes discussion of the following off-label use of a drug or medical device:
<N/A>

Vesicle refractive index is essential





Timeline vesicle detection with optical methods

2010	2011	2012	2013	2014
Flow cytometry standardization *Lacroix et al.*	Flow cytometry calibration *Chandler et al.*	Scanning flow cytometry *Konokhova et al.*	Flow cytometry artifact *Nolan et al.*	Flow cytometry standardization by vesicle size approximation *Coumans et al.*
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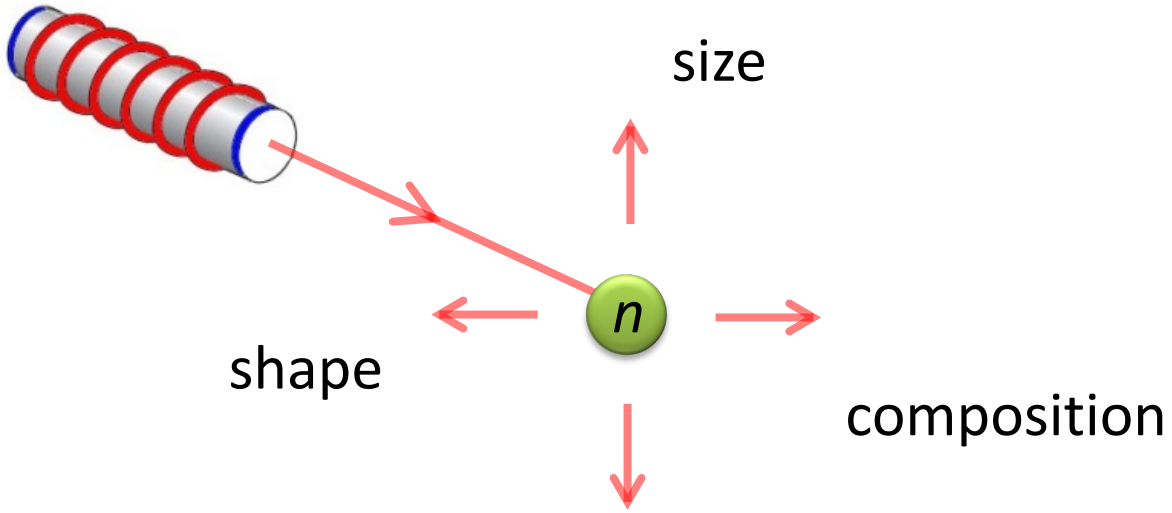
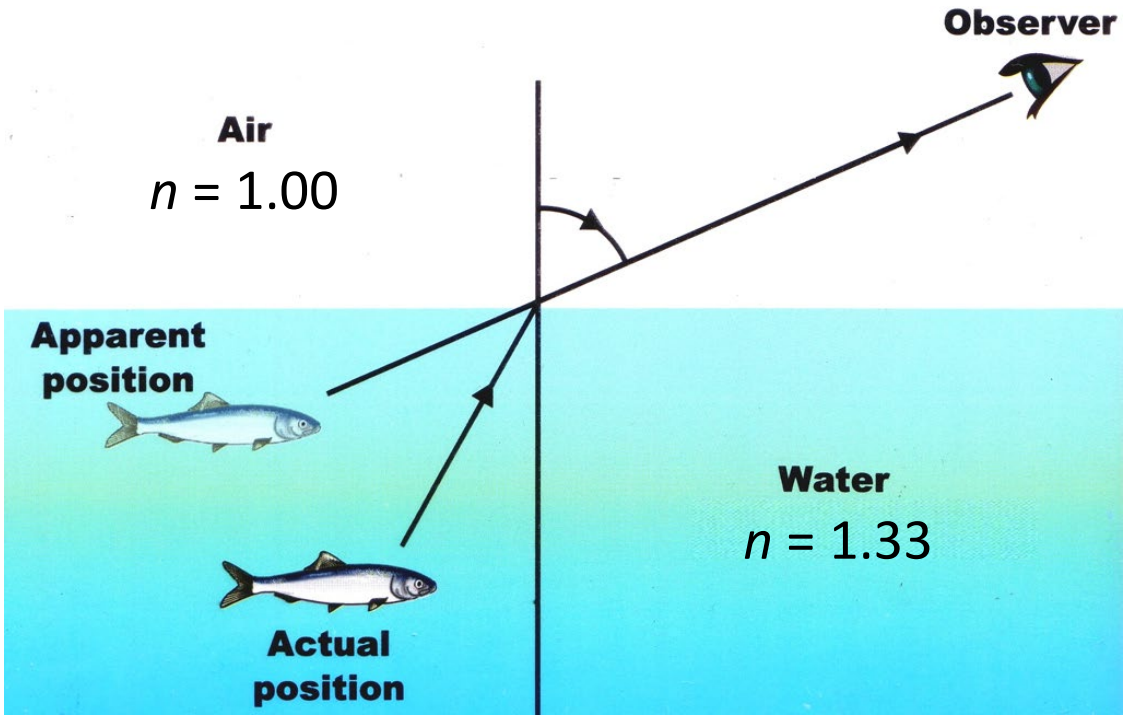
Introduction to light scattering



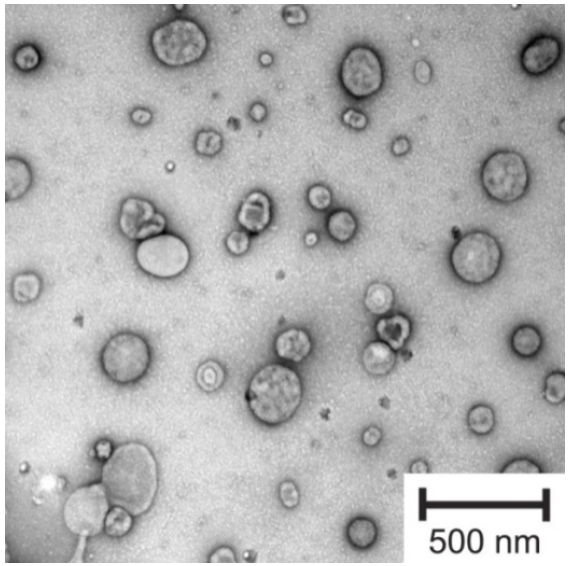
- light illuminating a vesicle is partly absorbed and partly scattered (deflected)
- light scattering depends on size and refractive index (*n*)

Introduction to the refractive index (n)



Problem

- no technique is capable of determining the refractive index of particles being
  - <500 nm
  - heterogeneous in size
  - heterogeneous in refractive index
  - in suspension



Goal

- determine the refractive index of extracellular vesicles <500 nm in suspension

Methods - setup

- Commercial instrument
  - Nanosight NS-500

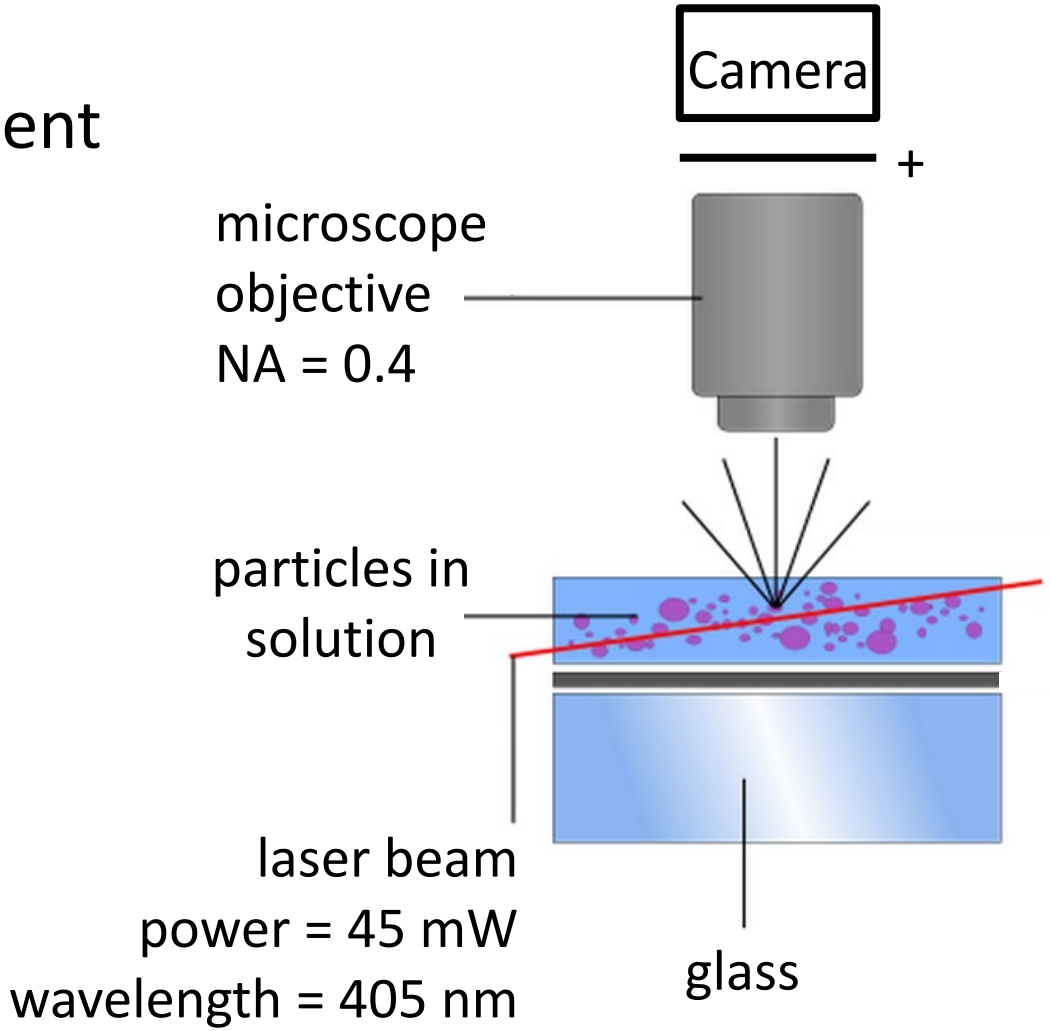
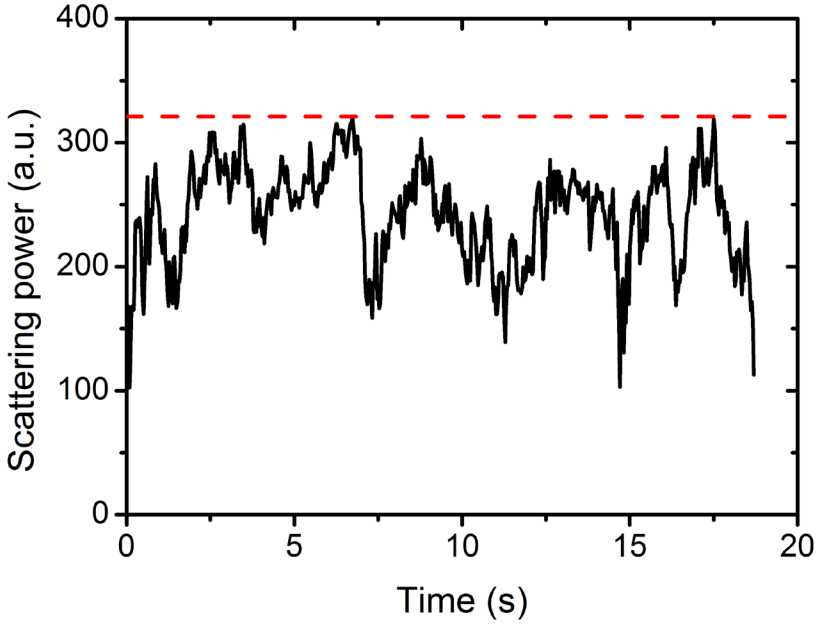


figure adapted from Nanosight Ltd, UK

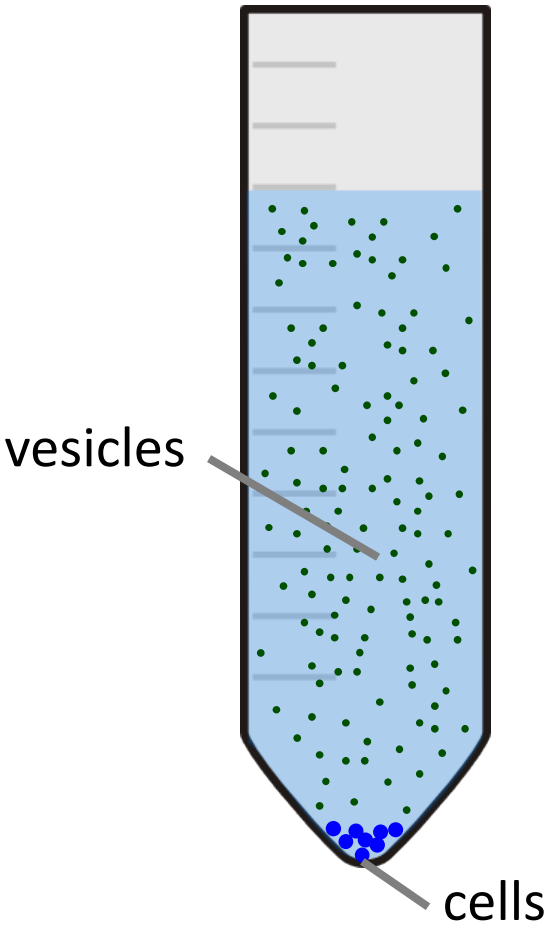
Methods – NTA



- obtain **particle diameter** *d* by tracking the Brownian motion of single particles (Stokes-Einstein equation)
- measure **scattering power** *P*
- derive particle **refractive index** *n(P,d)* from Mie theory

Methods - samples

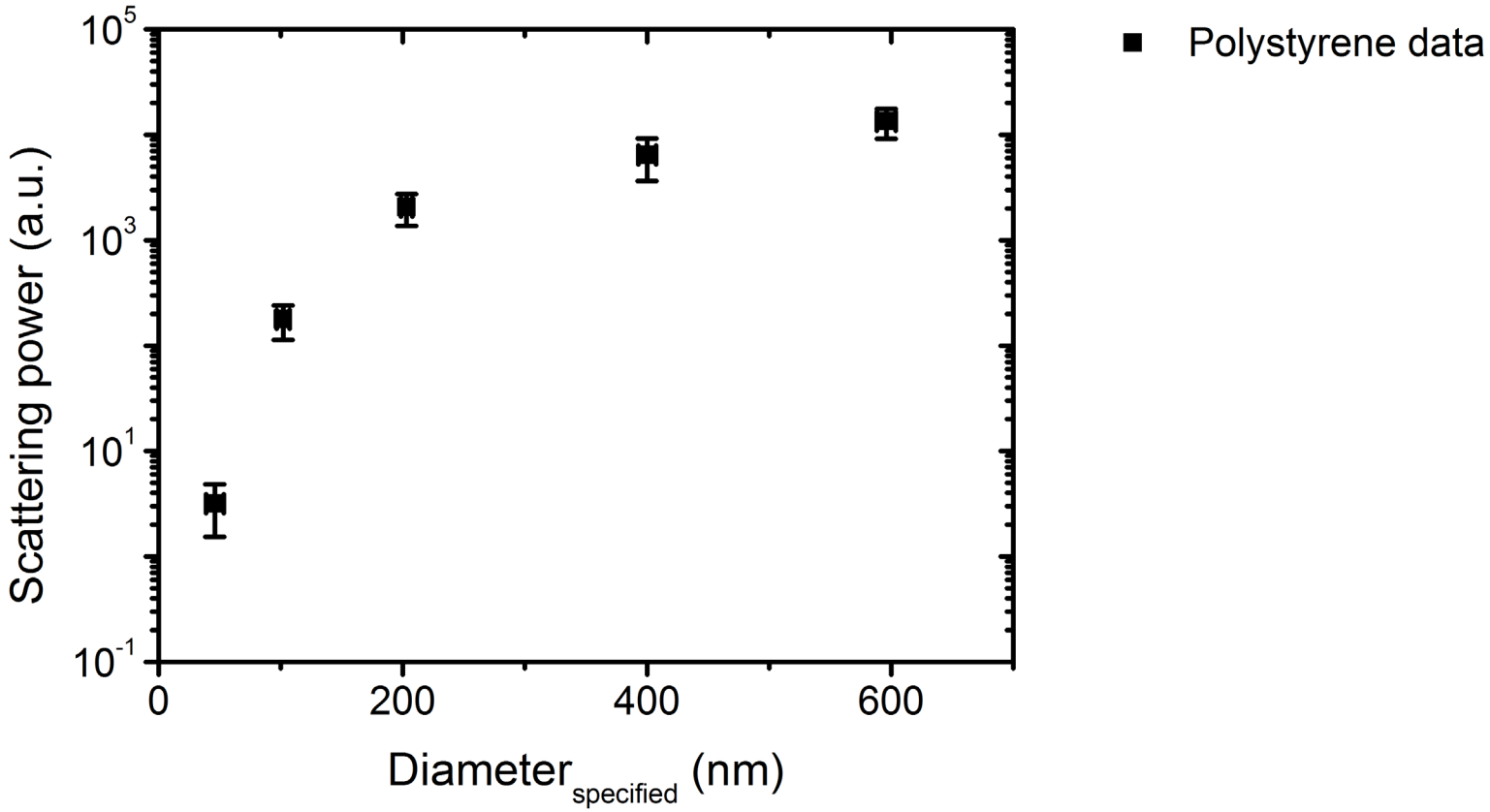
- Polystyrene beads (*n*=1.63)
  - Thermo Fisher Scientific, USA
- Silica beads (*n*=1.45)
  - Kisker Biotech, Germany
- Human urinary vesicles
  - differential centrifugation
  - protocol from metves.eu



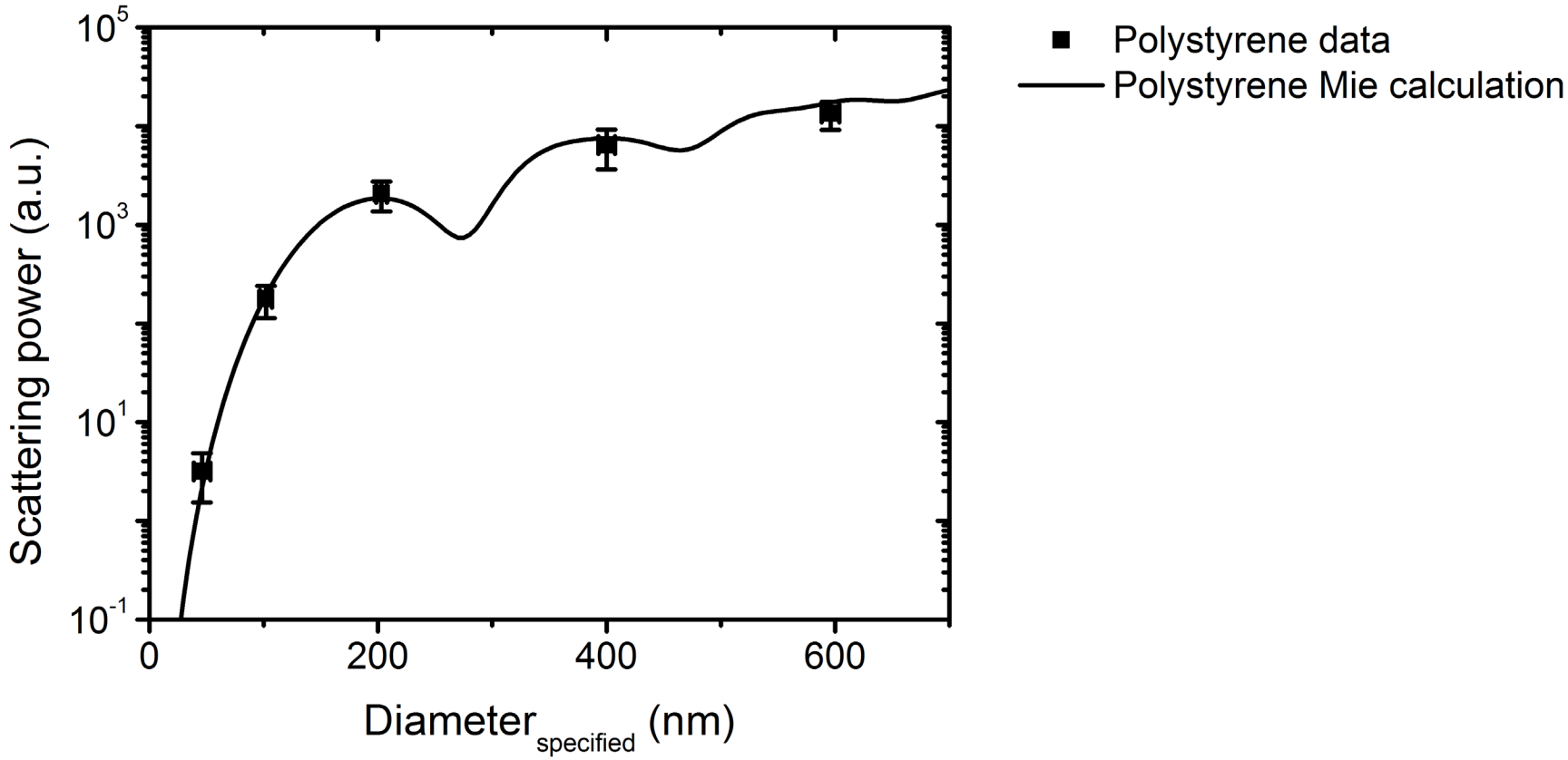
Methods - approach

- calibration
  - measure light scattering of beads
  - describe measurements by Mie theory
- validation
  - measure light scattering and diameter of beads mixture
- application
  - determine the refractive index of vesicles

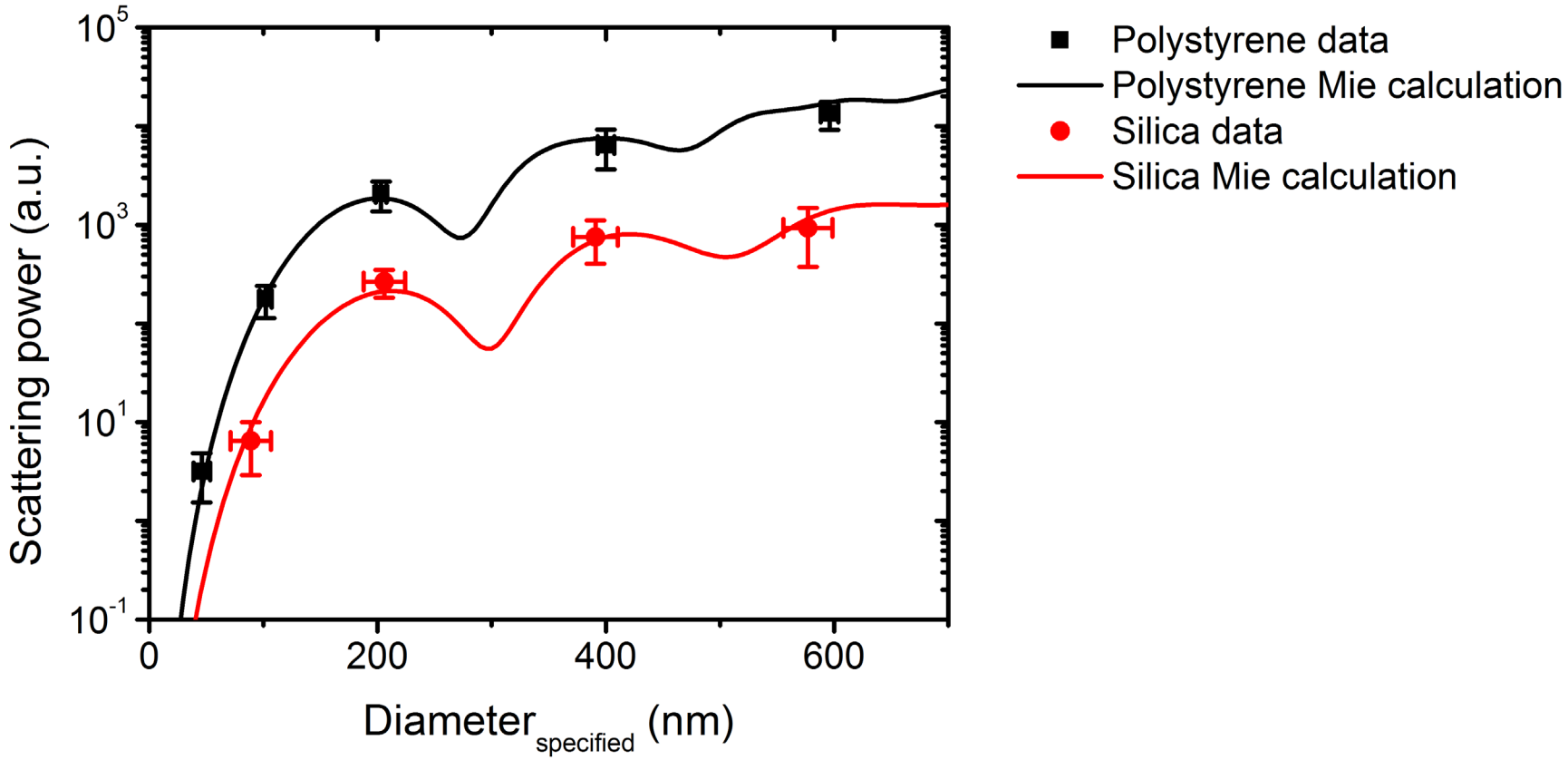
Results - scattering power versus diameter of polystyrene beads



Results - scattering power versus diameter of polystyrene beads described by Mie theory



Results - scattering power versus diameter of polystyrene and silica beads



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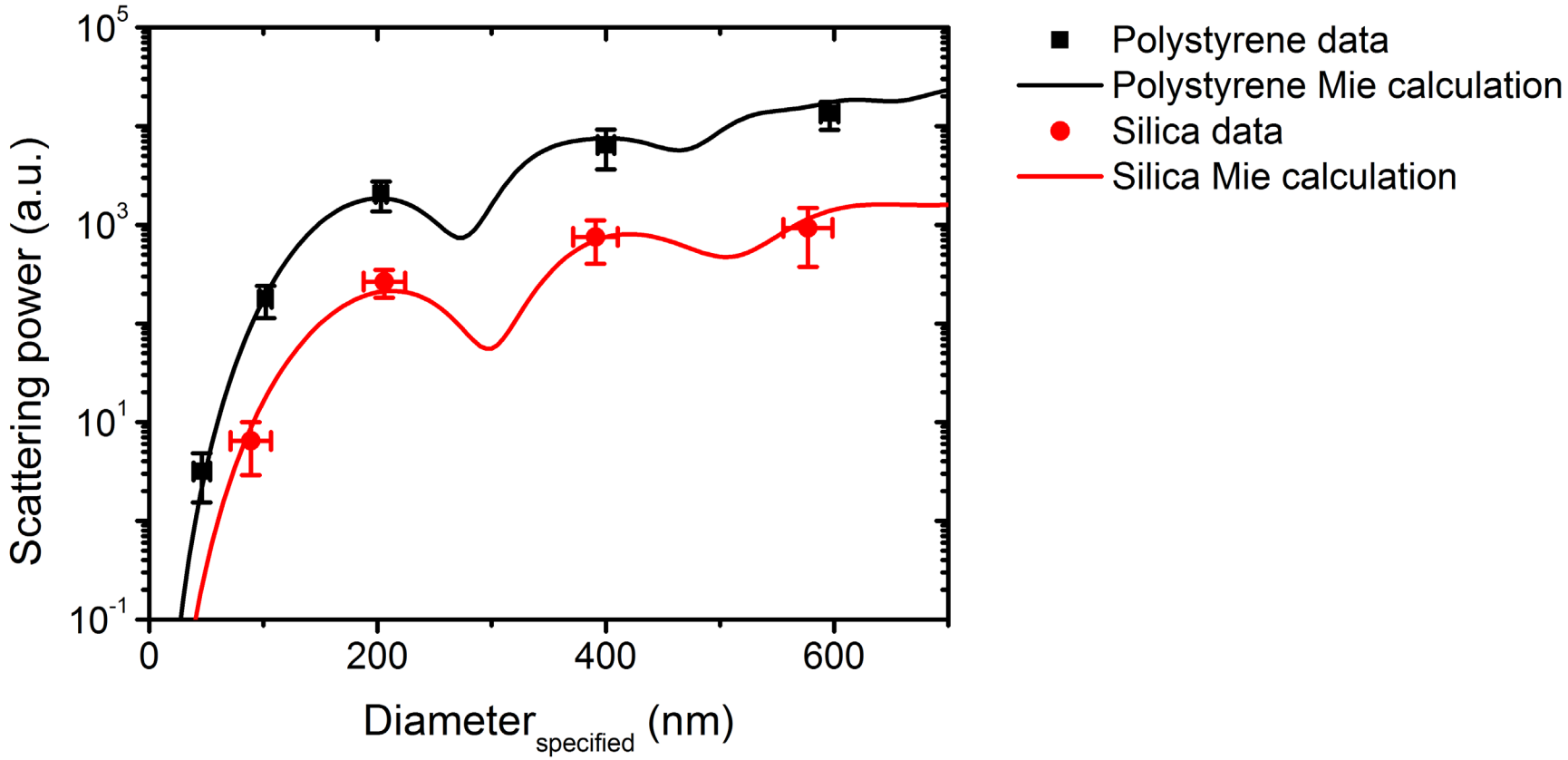
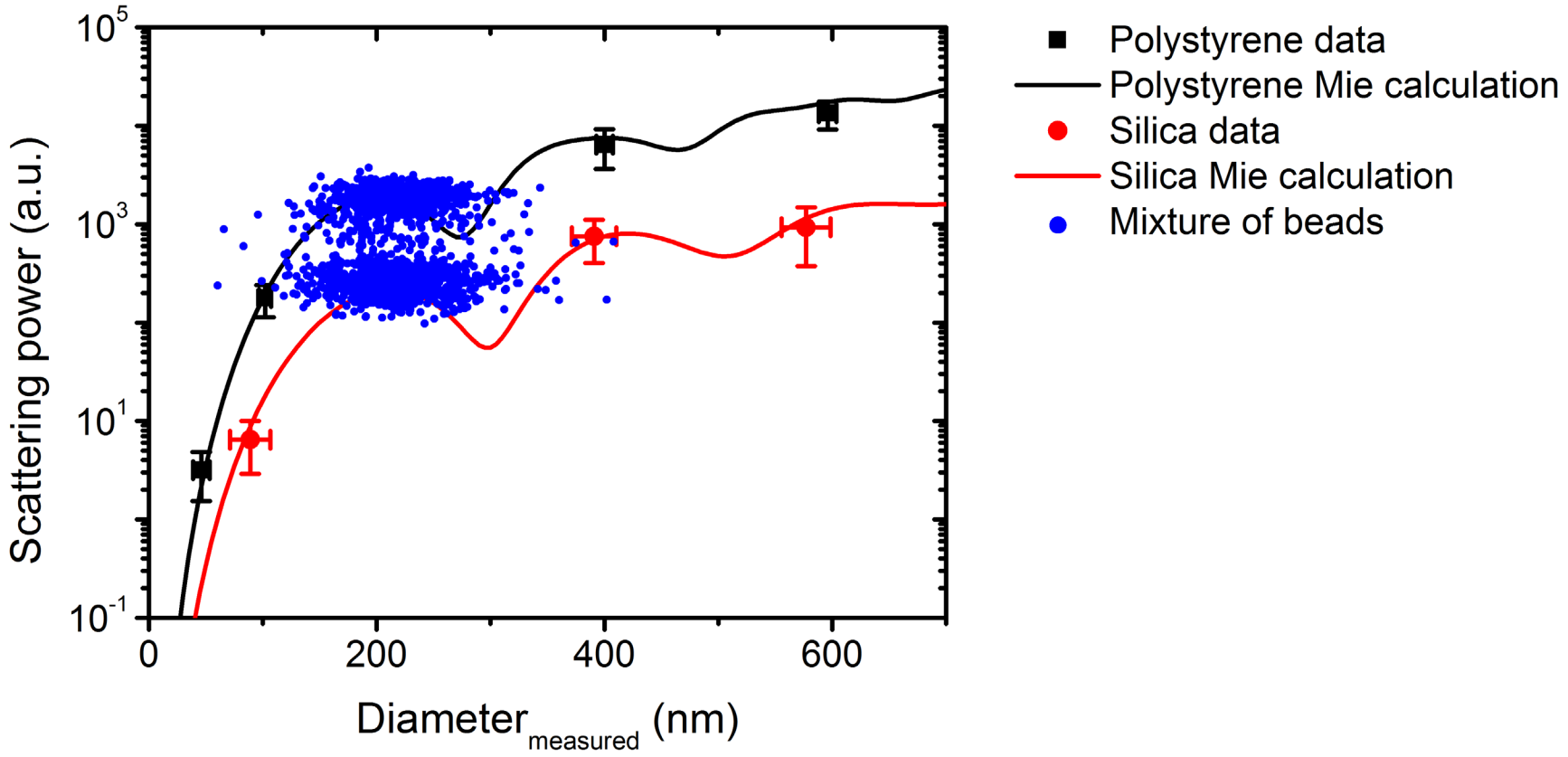


Results - scattering power versus diameter of a mixture of polystyrene and silica beads



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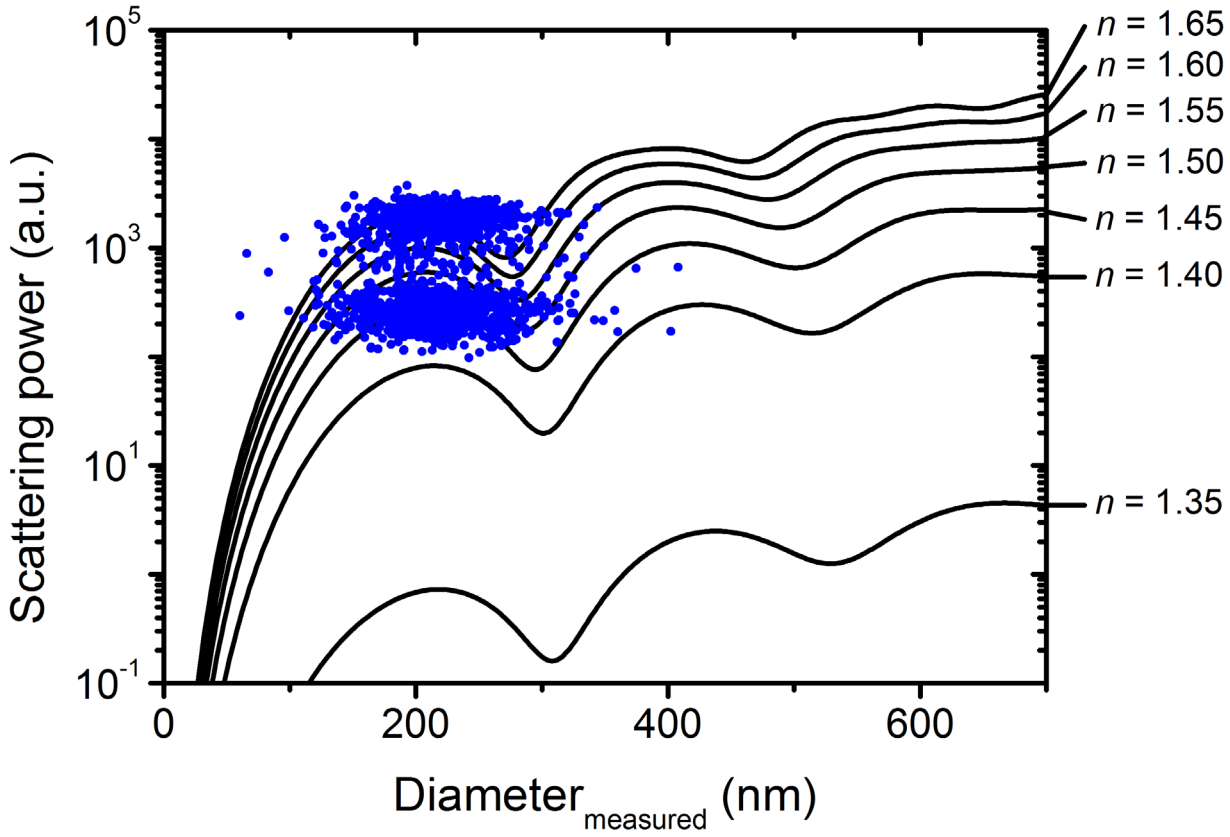
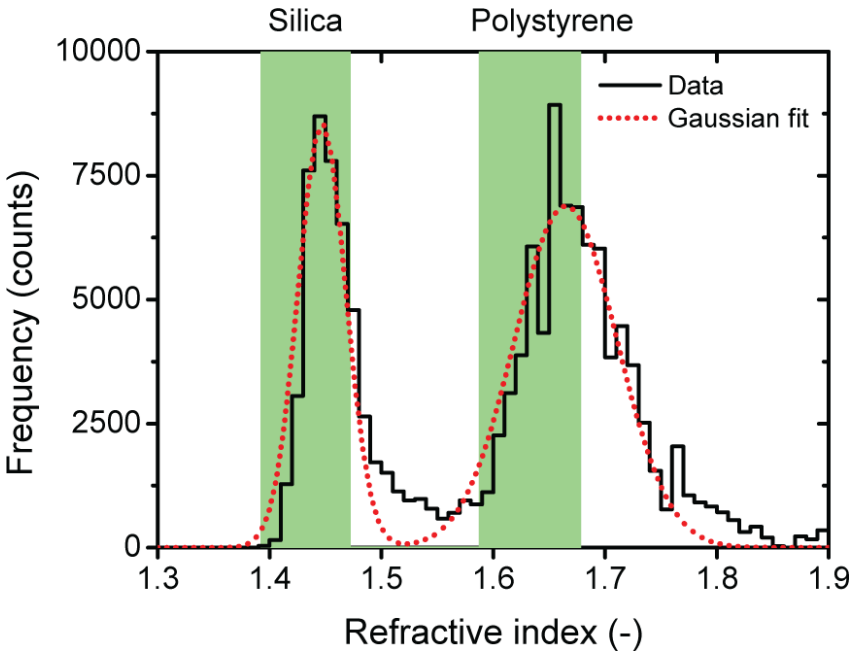
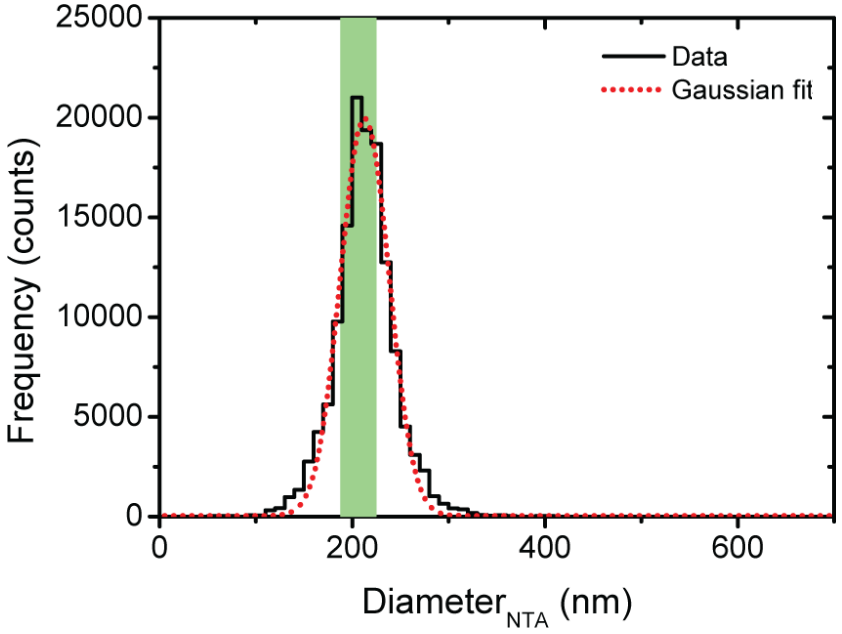


Results - refractive index and size distribution of a mixture of polystyrene and silica beads

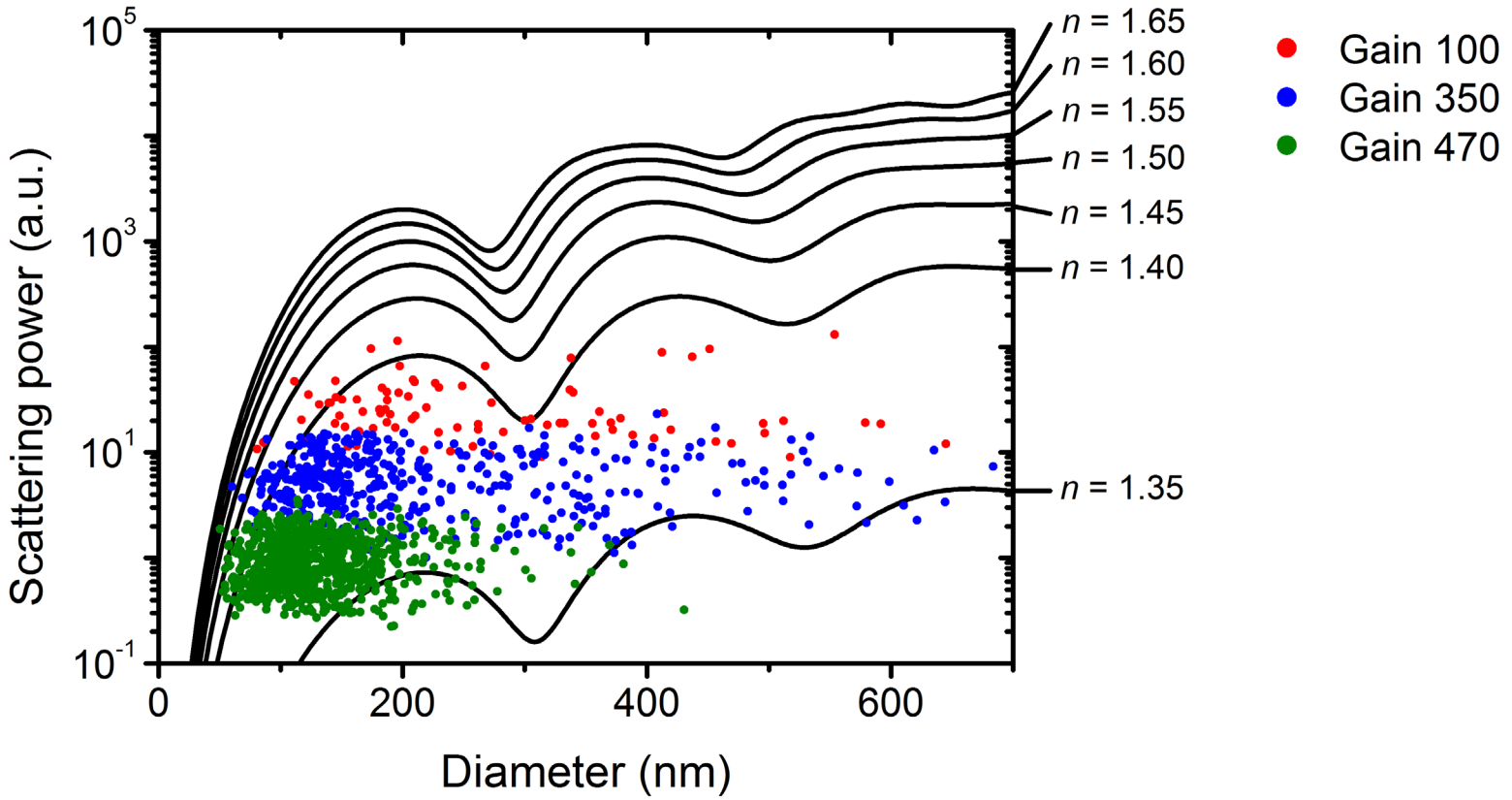




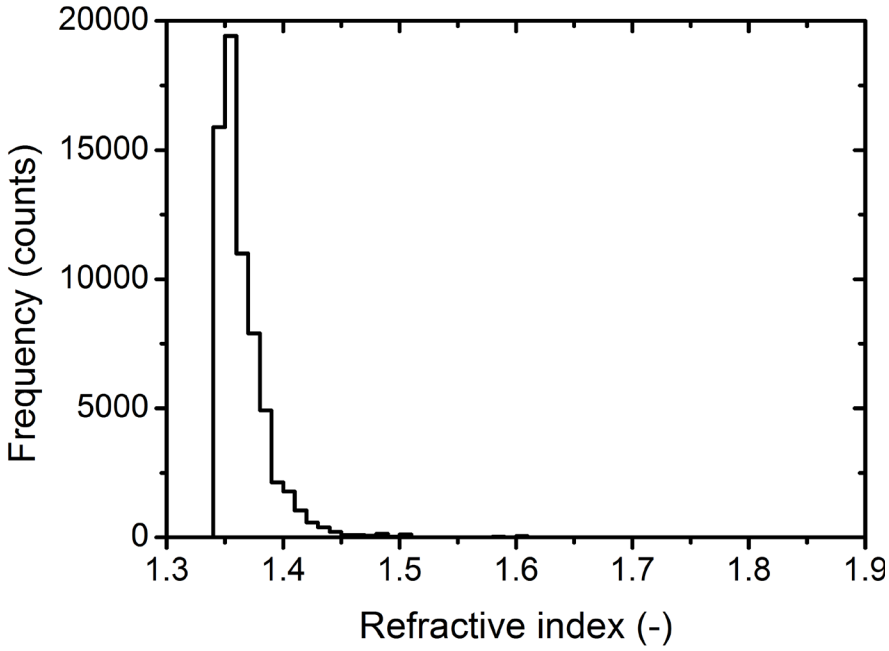
Methods - approach

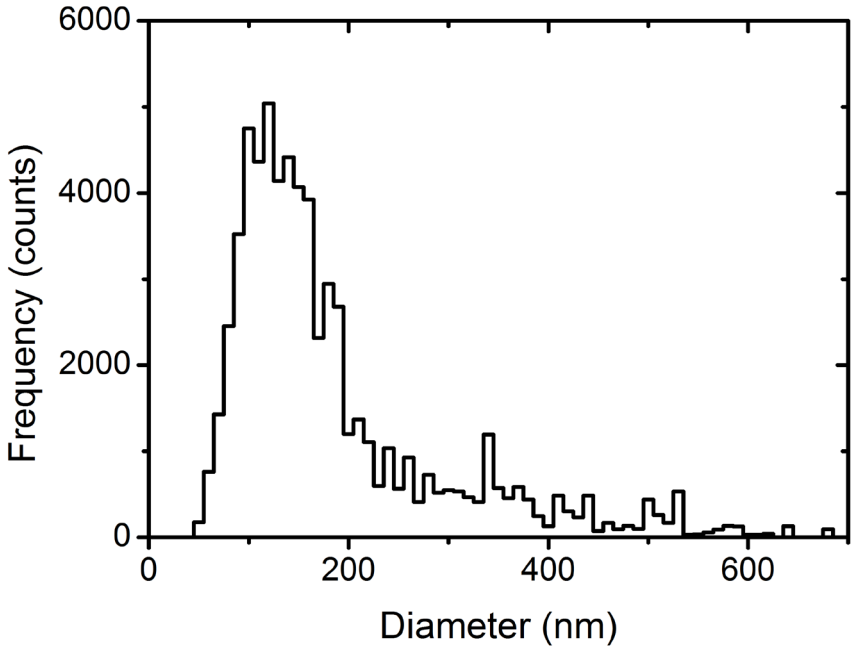
- calibration
  - measure light scattering of beads
  - describe measurements by Mie theory
- validation
  - measure light scattering and diameter of beads mixture
- application
  - determine the refractive index of vesicles

Results - scattering power versus diameter of urinary vesicles

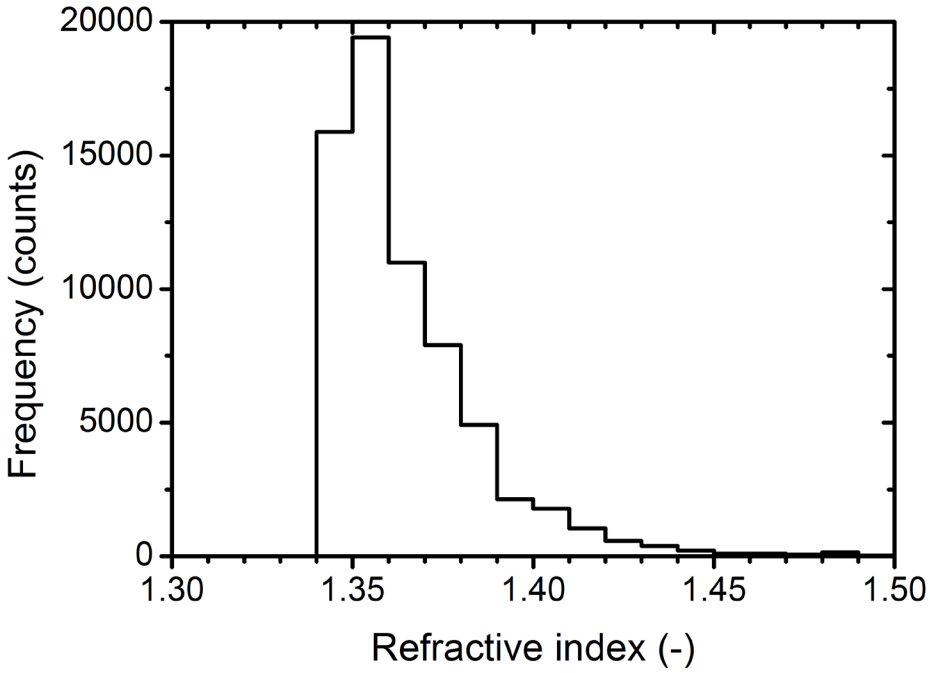


Results - size and refractive index distribution of urinary vesicles





Conclusions



- NTA can be used to determine the refractive index of single vesicles
- mean refractive index of urinary vesicles is 1.37

Timeline vesicle detection with optical methods

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Nanovesicles conference *Oxford*	ISTH *Kyoto*	ISEV *Gothenburg*	ISTH *Amsterdam*	ISEV *Rotterdam*
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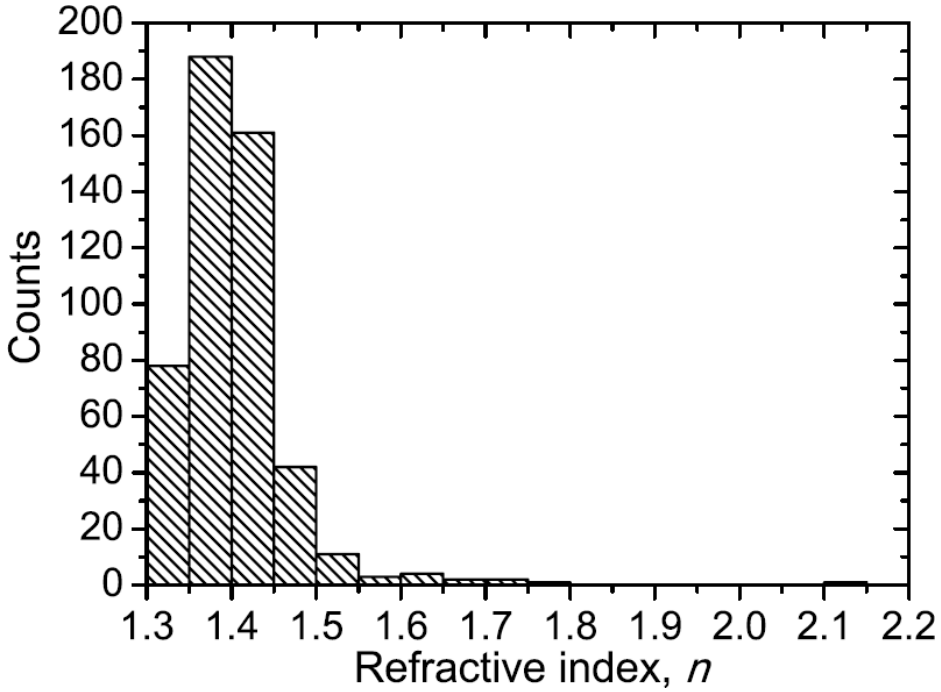
Refractive index of blood microparticles



- scanning flow cytometry
- microparticles >500 nm

Konokhova et al., J. Biomed. Opt. (2012)

Timeline vesicle detection with optical methods

Flow cytometry standardization
Lacroix et al.

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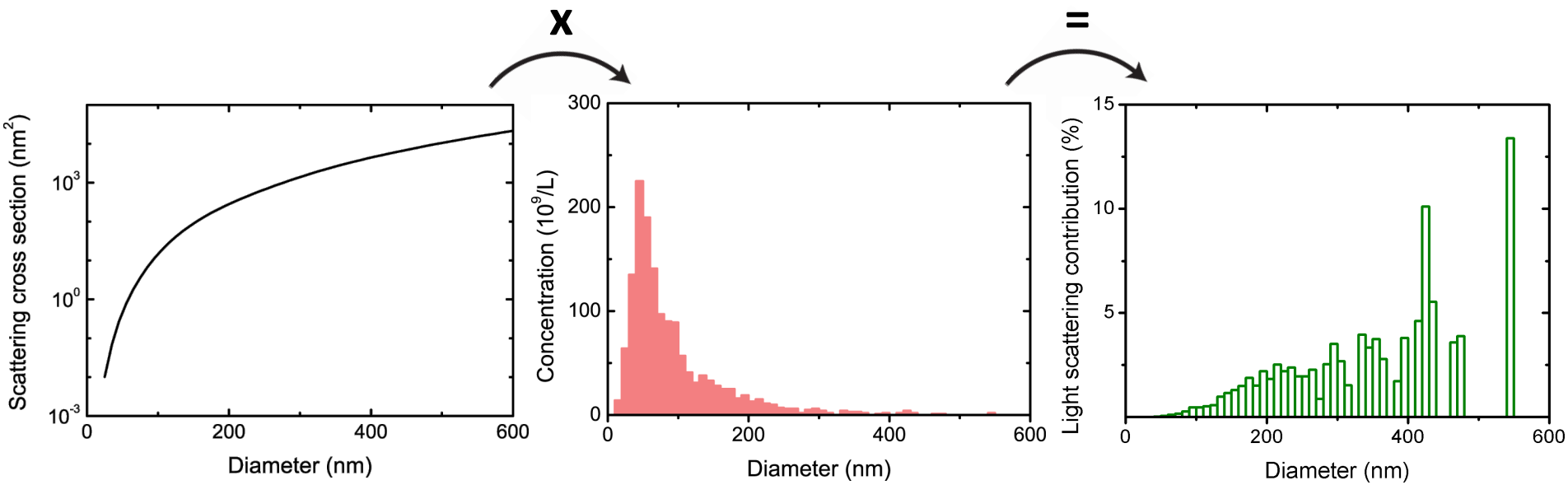
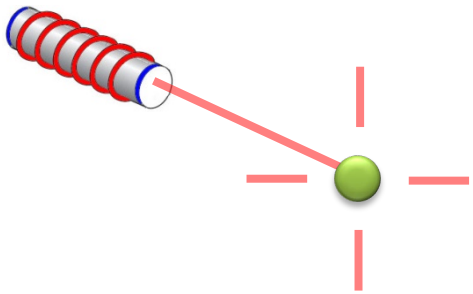
2010

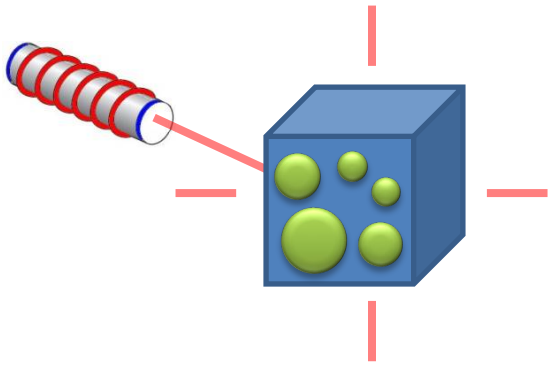
Nanovesicles conference
Oxford

Large vesicles dominate light scattering signal





$n = 1.38$



Timeline vesicle detection with optical methods

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scheff et al.

Flow cytometry
artifact
Nolan et al.

Flow cytometry
standardization
by vesicle size
approximation
Coumans et al.

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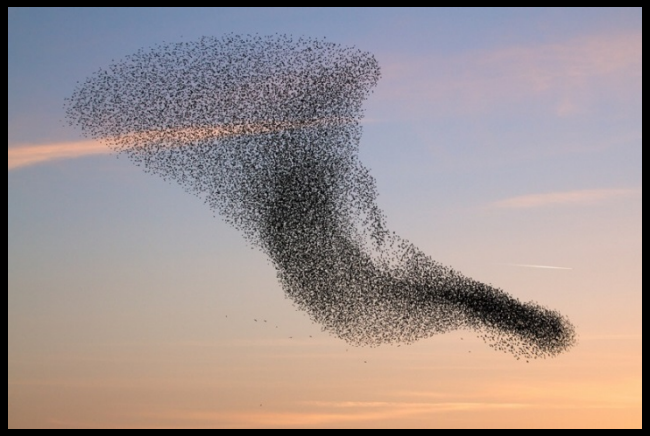
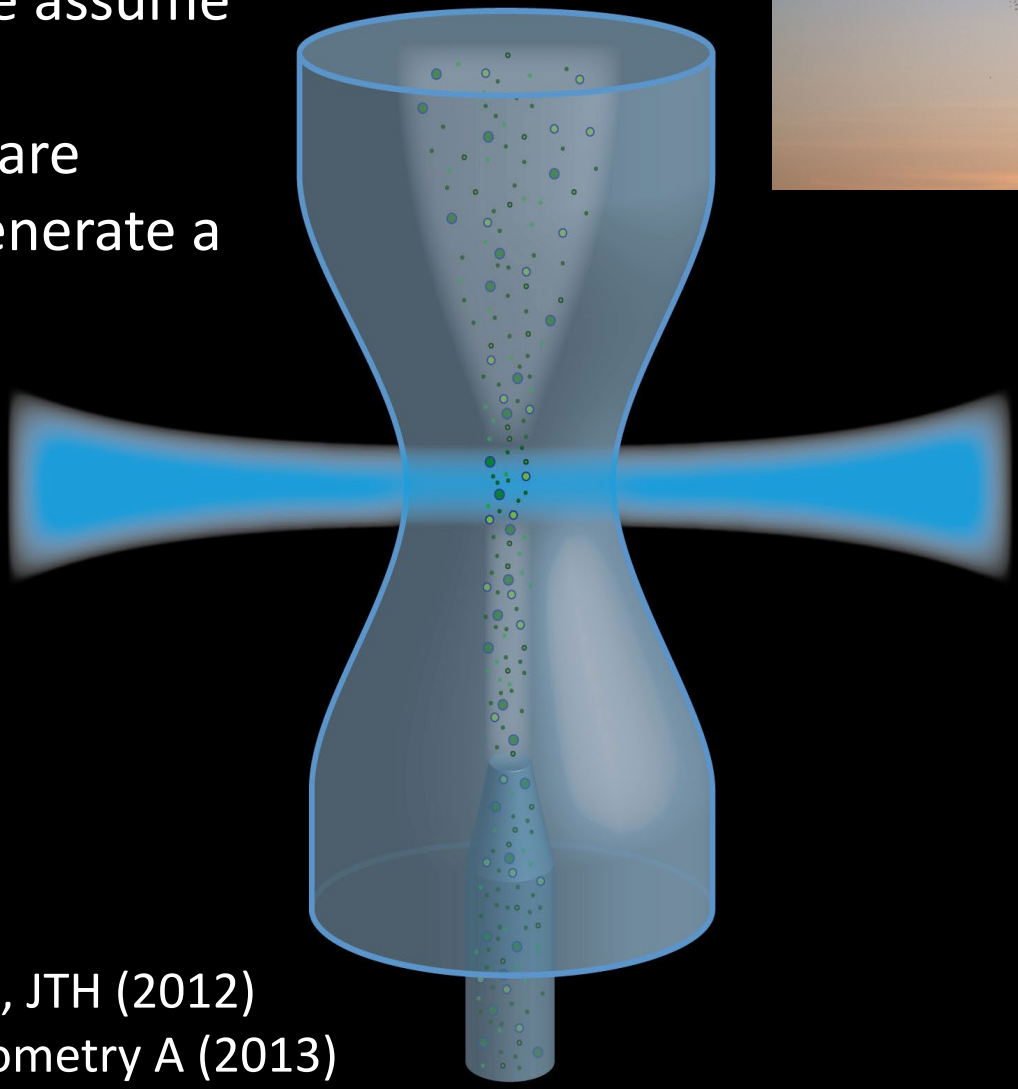
Swarm detection



for vesicles we assume
n = 1.38
>150 vesicles are required to generate a signal event



van der Pol et al., JTH (2012)
Nolan et al., Cytometry A (2013)

Timeline vesicle detection with optical methods

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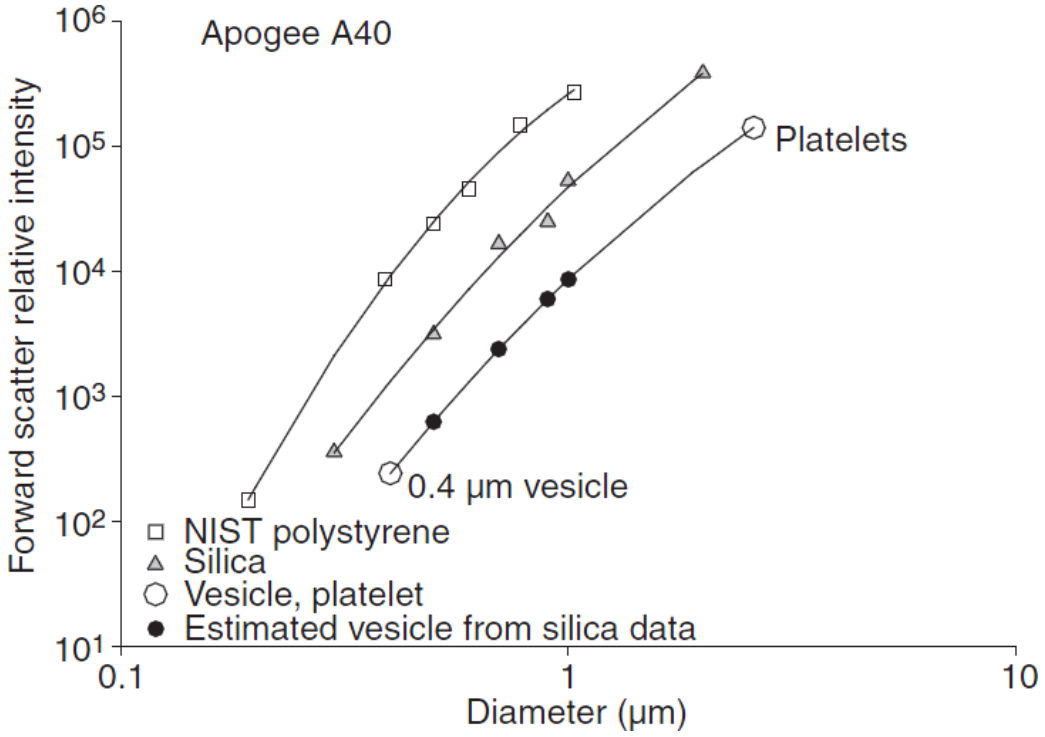
Enhanced insight in flow cytometry data

"The use of plastic beads for size calibration in flow cytometry remains an imperfect model. Many factors other than size influence scatter, among which are the refractive index…"



Lacroix et al.,
Semin. Thromb. Hemost. (2010)

Chandler et al., JTH (2011)

Timeline vesicle detection with optical methods

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2010

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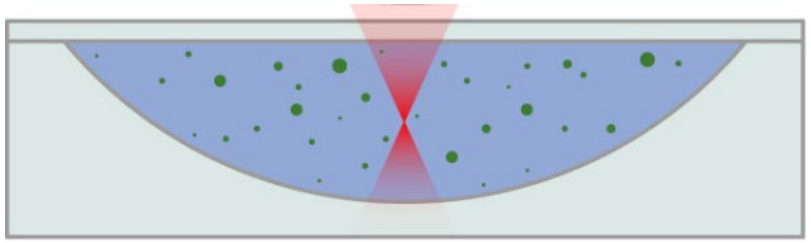
ISTH
Milwaukee

Refractive index affects smallest detectable size of vesicles

- Nanoparticle analysis range: typically 10 nm - 2000 nm, dependent on particle material



"Cellular vesicles have a low refractive index, and the smallest detectable size using nanoparticle tracking analysis is in the order of 50 nm…."

Dragovic et al.,
Nanomedicine (2011)

"It is difficult to estimate whether one big vesicle or a cluster of several smaller vesicles have been optically trapped…"

Tatischeff et al.,
J. Extracellular Vesicles (2012)

Outlook vesicle detection with optical methods

Flow cytometry standardization
Lacroix et al.

Flow cytometry cali… *Chan…*

Nan… tracki… *Drag…*

Scanning flow …

Flow cytometry …

Flow cytometry standardization by vesicle size approximation
Coumans et al.

2010

Nanovesicles conference
Oxford

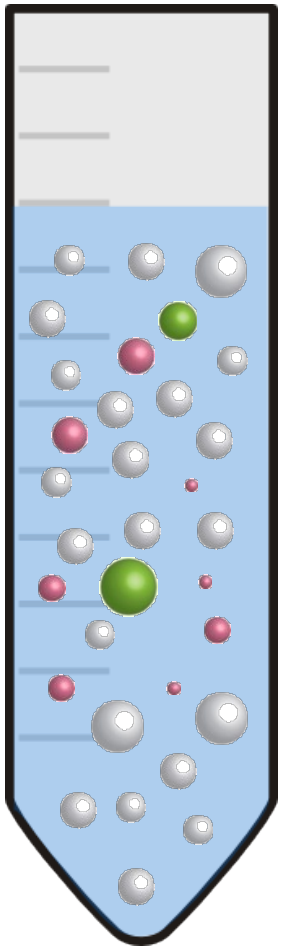
2014

ISEV
Rotterdam

ISTH
Milwaukee

Refractive index enables flow cytometry standardization

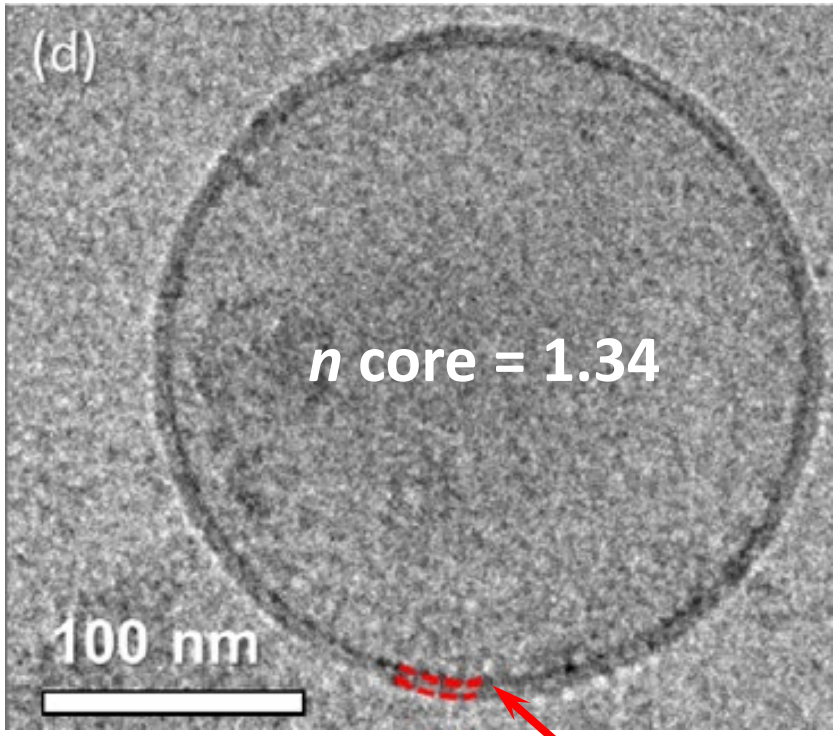
Distinguish vesicles from other particles



- lipoproteins (*n* = 1.45-1.60)
- protein aggregates (*n* = 1.53-1.60)
- vesicles (n < 1.45)

* Konokhova et al., J. Biomed. Opt. (2012)

Refractive index reveals vesicle composition

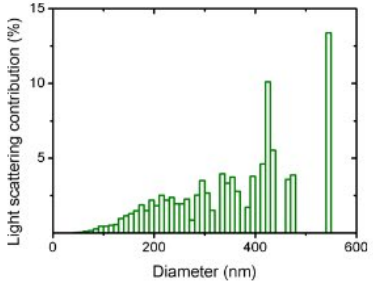


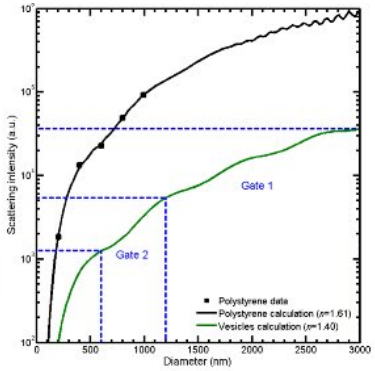
thickness = 5 nm
n membrane = 1.46 *

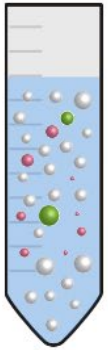
image courtesy of Issman et al., PLoS ONE (2013)
* van Manen et al., Biophys. J. (2007)

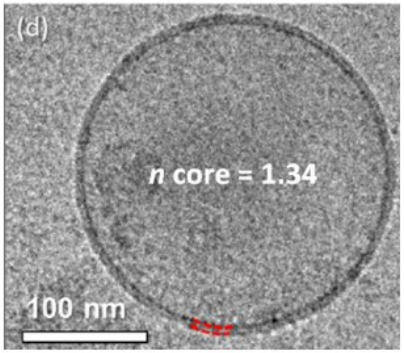
Summary: implications of the vesicle refractive index

- insight
  - large vesicles dominate scattering
  - swarm detection
- relate scatter to size
  - detection limit
  - flow cytometry standardization
- vesicle identification
- vesicle composition









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  - Rienk Nieuwland
  - Ton van Leeuwen
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- University of Birmingham
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- NanoSight Ltd.
  - Andrew Malloy
  - Patrick Hole
  - Jonathan Smith

More on vesicle detection:
edwinvanderpol.com