Detection of microparticles by flow cytometry

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vesicles are studied mostly by flow cytometry
mechanism causing detection incompletely understood
Introduction to flow cytometry

- smallest detectable polystyrene bead is 200 nm
  \[ n = 1.61 \]
Light scattering and the refractive index

Polystyrene bead
\[ n = 1.61 \]

Silica bead
\[ n = 1.45 \]

Vesicle
\[ n_{\text{core}} = 1.38 \]
\[ n_{\text{membrane}} = 1.48 \]

100 nm
Problem

- diameter of vesicles is <300 nm
- against expectations, vesicles are detected by flow cytometry
Goals

- optimize detection settings
- measure light scattering power of beads
- describe measurements by Mie theory
- determine size of smallest detectable *single* vesicle
- investigate role of *multiple* particles in detection volume by dilution series
- prospects
Methods – optimize flow cytometer settings

- Cell: $d = 500 \text{ nm}$
- Microparticle: $d = 50 \text{ nm}$

Illumination: $1.4 \times 10^7 \text{ Wm}^{-2}$

Results based on BD FACSCalibur
Goals

- optimize detection settings
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- determine size of smallest detectable single vesicle
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Results – scattering power of polystyrene beads

\[ \text{SSC} \times 1.3 \times 10^6 = \]

- Frequency (counts min\(^{-1}\))
- Power (a.u.)
- Diameter (nm)
- Power (mW)
Results – scattering power of silica beads

\[ \times 1.3 \times 10^6 = \]
Goals

- optimize detection settings
- measure light scattering power of beads
- describe measurements by Mie theory
- determine size of smallest detectable single vesicle
- investigate role of multiple particles in detection volume by dilution series
- prospects
Results – scattering power vs. diameter

van Manen et al., Biophys J (2008)
Konokhova et al., J Biomed Opt (2012)
Results – scattering power vs. diameter

![Graph showing scattering power vs. diameter for different materials and theoretical models.]

- **Data polystyrene beads**
- **Data silica beads**
- **Theory polystyrene spheres** \( n_{\text{polystyrene}} = 1.605 \)
- **Theory silica spheres** \( n_{\text{silica}} = 1.445 \)
- **Theory vesicles** \( n_{\text{core}} = 1.38 \pm 0.02, n_{\text{shell}} = 1.48 \)
Results – scattering power vs. diameter

![Graph showing power vs. diameter for different materials and theoretical models. The graph includes data points for polystyrene beads and silica beads, as well as theoretical curves for polystyrene spheres, silica spheres, and vesicles. The scattering power is given in milliwatts, and the diameter is in nanometers.](image)

- **Data Polystyrene Beads**
- **Data Silica Beads**
- **Theory Polystyrene Spheres** ($n_{polystyrene} = 1.605$)
- **Theory Silica Spheres** ($n_{silica} = 1.445$)
- **Theory Vesicles** ($n_{core} = 1.38 \pm 0.02$, $n_{shell} = 1.48$)

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Goals

- optimize detection settings
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- describe measurements by Mie theory
- determine size of smallest detectable *single* vesicle
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- prospects
Results – *multiple* vesicles as single count

89-nm silica beads at concentration $10^{10}$ beads ml$^{-1}$

urine filtered with 220-nm filter concentration $\geq 10^{10}$ vesicles ml$^{-1}$
beam volume $\approx 54$ pl

At a concentration of $10^{10}$ vesicles ml$^{-1}$, >800 vesicles are simultaneously present in the beam.
Results – counts from mixtures of beads

![Graph showing concentration (counts ml⁻¹) on the y-axis and concentration (particles ml⁻¹) on the x-axis. The graph includes data points for 610 nm beads and a detection range.](image-url)
Results – counts from mixtures of beads

![Graph showing concentration vs. concentration](image)

- Detection range
- **610 nm beads**
- **610 nm + 89 nm beads (1/100)**
Results – counts from mixtures of beads

![Graph showing counts from mixtures of beads](image)

- **Concentration**
  - **Flow cytometer** (counts ml$^{-1}$)
  - **Prepared** (particles ml$^{-1}$)

- **Detection range**
- **610 nm beads**
- **610 nm + 89 nm beads (1/100)**
- **610 nm + 89 nm beads (1/10,000)**
Results – counts from mixtures of beads

![Graph showing concentration of flow cytometer counts vs. prepared concentration of particles.]

- Detection range
- 610 nm beads
- 610 nm + 89 nm beads (1/100)
- 610 nm + 89 nm beads (1/10,000)
- 610 nm + 89 nm beads (1/100,000)
Results – counts from mixtures of beads

Concentration_{flow cytometer} (counts ml\(^{-1}\))

Concentration_{prepared} (particles ml\(^{-1}\))

- Detection range
- 610 nm beads
- 610 nm + 89 nm beads (1/100)
- 610 nm + 89 nm beads (1/10,000)
- 610 nm + 89 nm beads (1/100,000)
- 89 nm beads
Results – counts from urinary vesicles

Concentration (counts ml$^{-1}$) vs Concentration$_{\text{prepared}}$ (vesicles ml$^{-1}$)

- Detection range
- Cell-free urine filtered with 220-nm filter
Results – counts from urinary vesicles
Conclusion

- Vesicle detection by flow cytometry
  - Scattering power related to diameter and refractive index for *single* beads and vesicles
  - Single event signal attributed to scattering from *multiple* vesicles

van der Pol et al., J Thromb Haemost (2012)
Prospects of vesicle detection by flow cytometry

- calibration should be based on experiments \textit{and theory}
  - size distribution
  - refractive index
- flow cytometry is good
- increase sensitivity
Sensitivity should be increased

“A flow cytometer is unable to detect the smallest vesicle as long as you can detect cells with it.”
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More on vesicle detection: edwinvanderpol.com