Preliminary results from the METVES II interlaboratory comparison study

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METVES II

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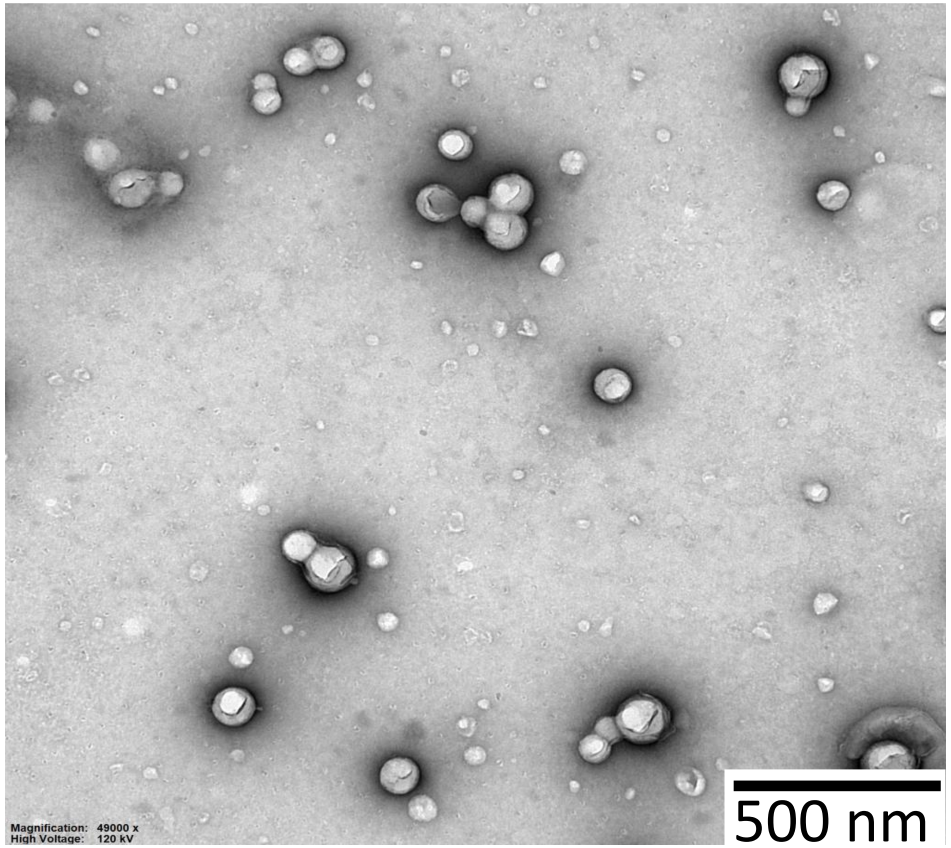
Conflict of interest

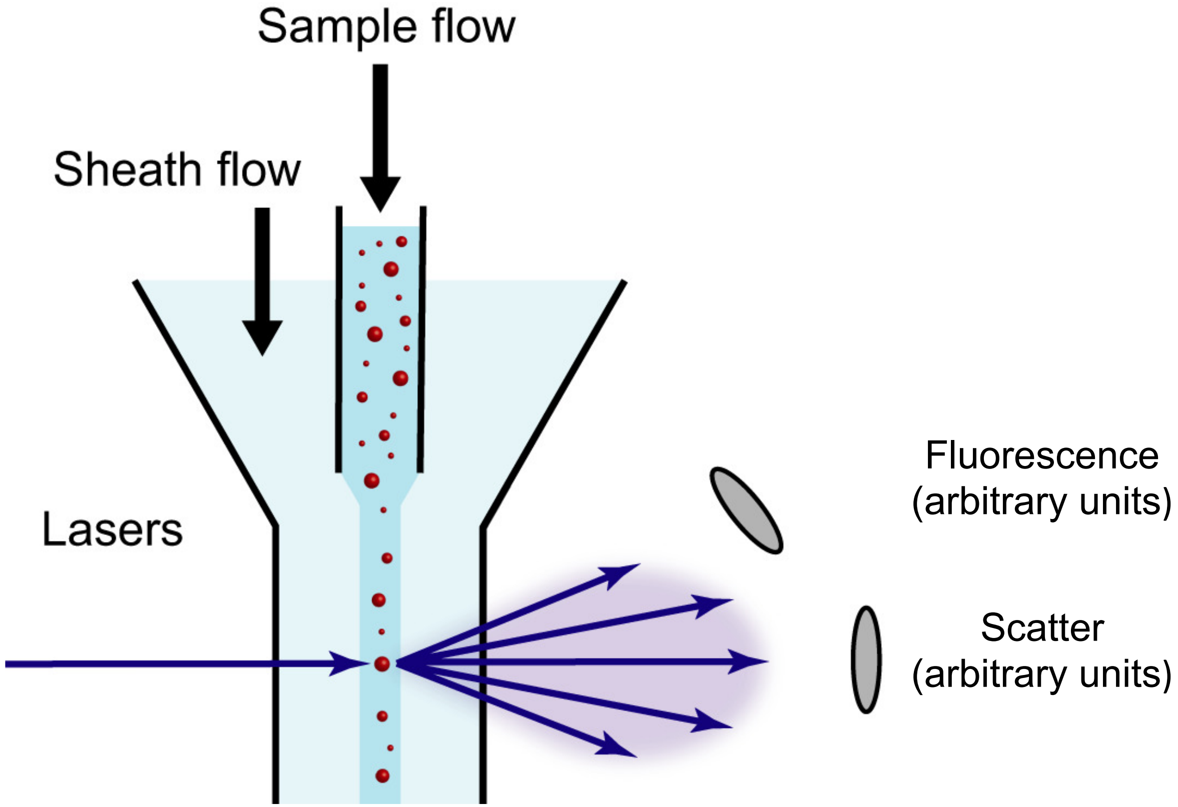
- Cofounder and shareholder of EXOMETRY

Outline

- Extracellular vesicles as biomarker
- Flow cytometry
  - Challenges
  - Calibration
  - History
- METVES II
  - Aim and approach
  - Analysis
  - Preliminary data

Extracellular vesicles as disease biomarker

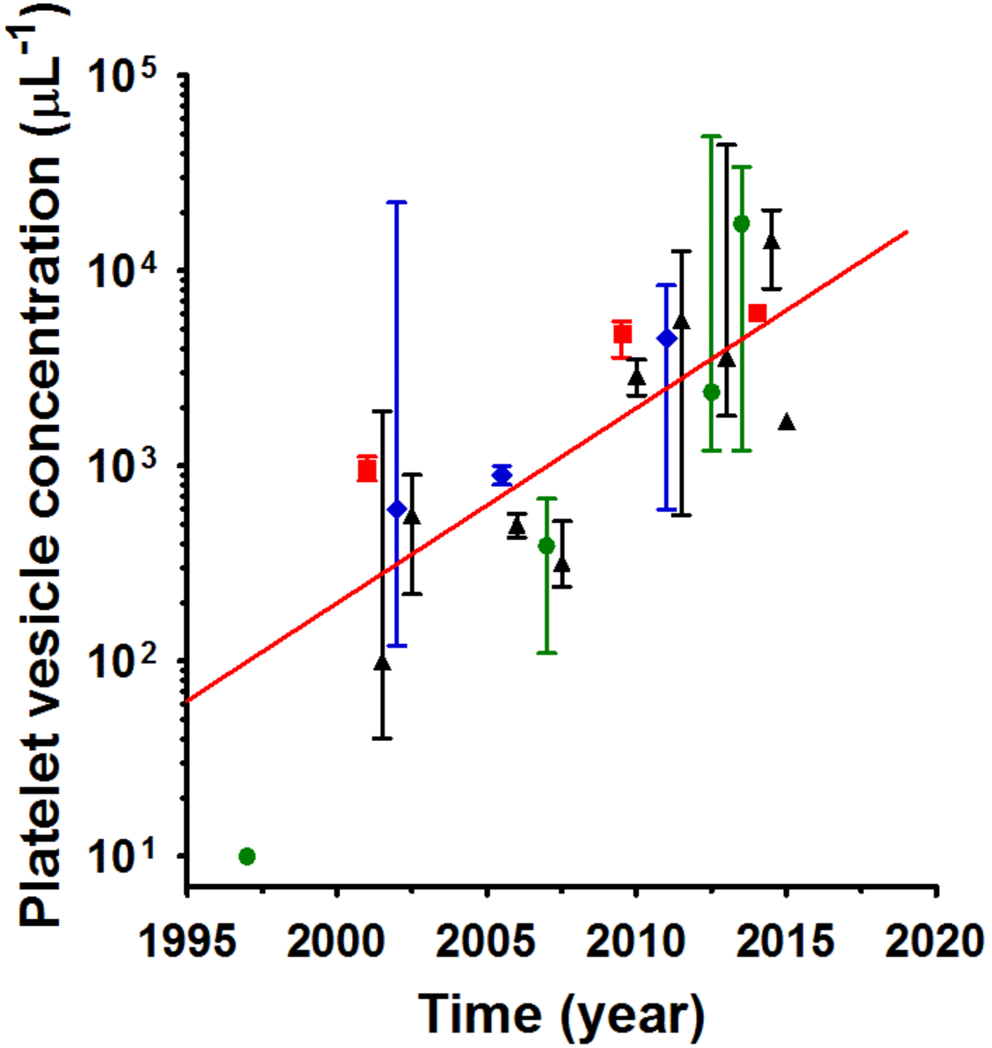




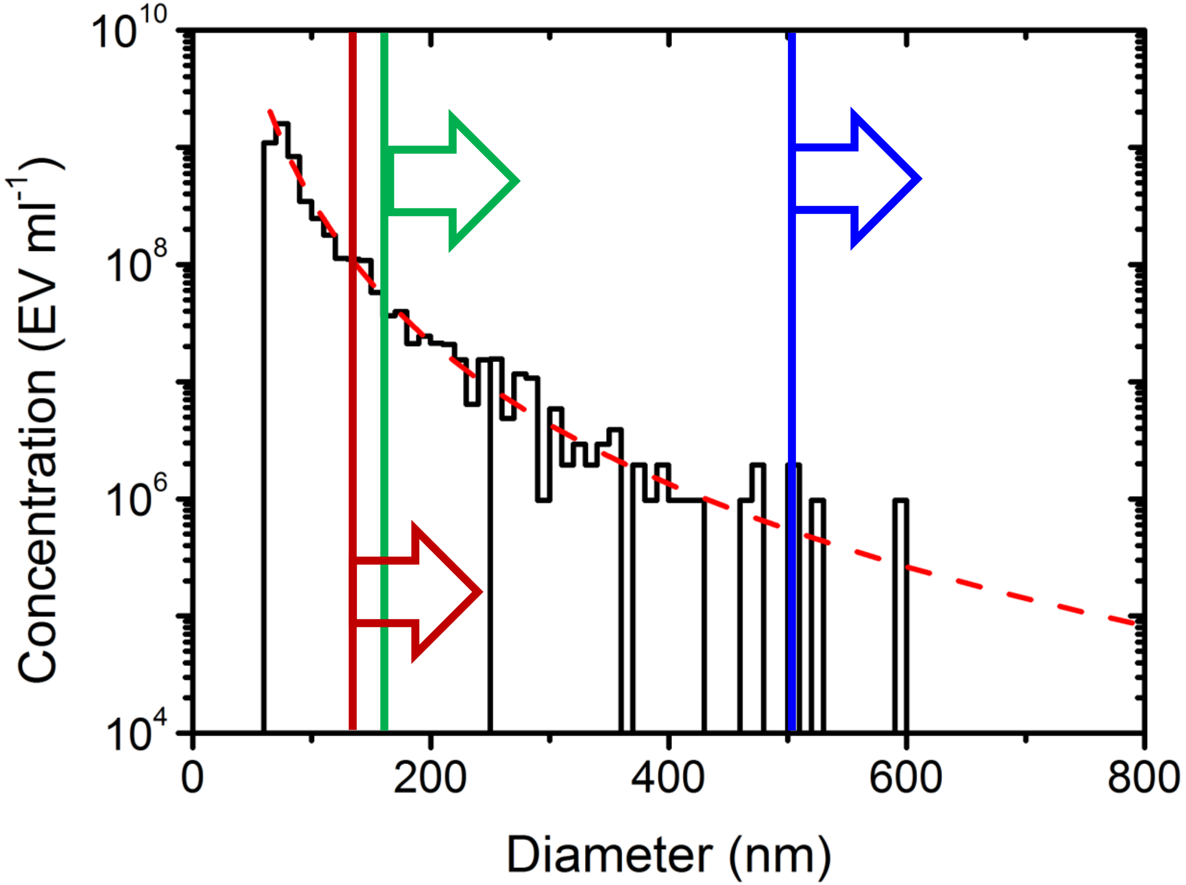
Clinical research reality

- Reported concentrations of blood plasma EV differ $>10^6$-fold
- Clinical data cannot be compared
- Standardization required

“Gasecka’s law”

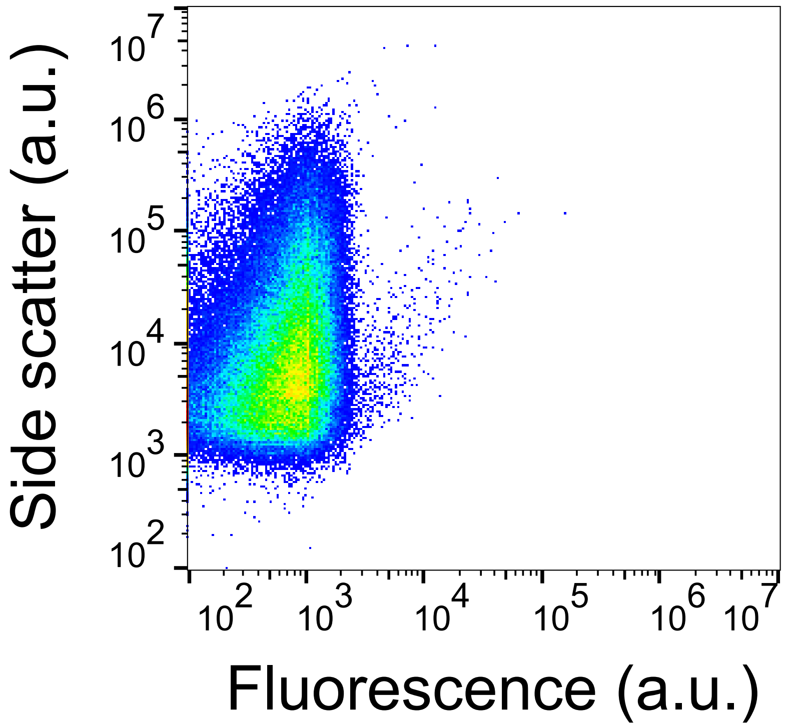


Challenge 1: flow cytometers differ in sensitivity



Challenge 2: data are in arbitrary units

- Data in arbitrary units (a.u.)
- Impossible to interpret/compare data
- No multi-center studies possible



Solution: calibration

- Conversion of arbitrary units to standard units
  - Fluorescence intensity (a.u.) → MESF
  - Light scattering intensity (a.u.) → Diameter (nm)
  - Sample volume (“unknown”) → µL
- Reference materials needed

MESF: molecules of equivalent soluble fluorochrome

Interlaboratory comparison studies

J Thromb Haemost publication	Standardization	>1 FCM	Traceable reference materials	Calibration			Stable test sample
				Flow rate	Fluorescence	Light scattering	
Robert et al. 2008	✓			✓			
Lacroix et al. 2010	✓	✓		✓			
Cointe et al. 2018	✓	✓		✓			
van der Pol et al. 2018	✓	✓	~	✓		✓	

State-of-the-art* results

- 46 flow cytometers
- EV concentration variability
  - bead diameter gate 139% CV
  - EV diameter gate 81% CV
- Part of the variation is caused by “test sample issues”

EV diameter range (nm)	Number of flow cytometers
300 – 600	5
600 – 1,200	21
1,200 – 3,000	31
No utility to EV research	15

CV: coefficient of variation (standard deviation / mean)

EV: extracellular vesicle; *van der Pol et al. *J Thromb Haemost* 2018

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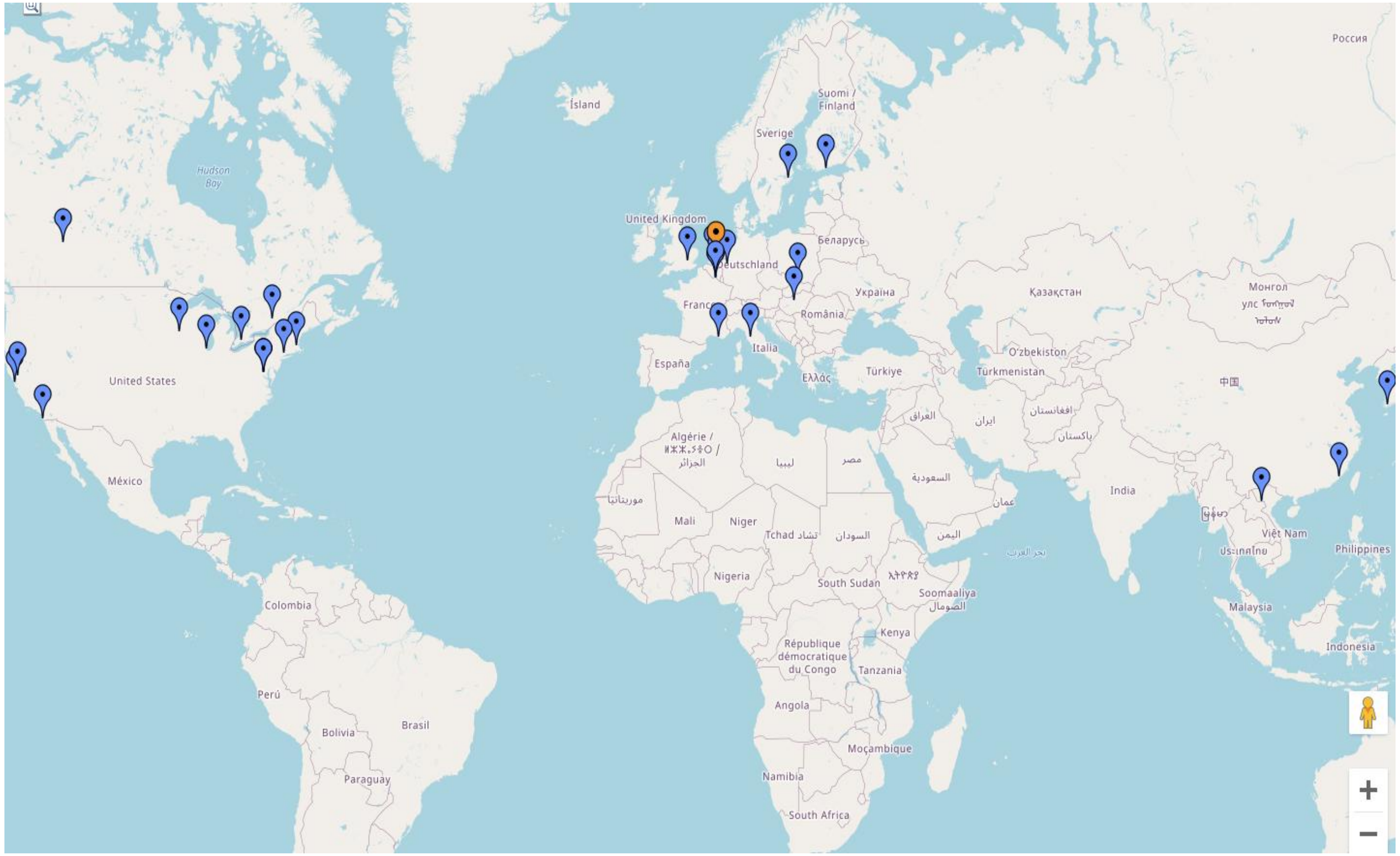
METVES II aim

To obtain reproducible measurements of the EV concentration using different flow cytometers

METVES II: 24 participants, 39 flow cytometers



Source: http://maps.google.com/

METVES II approach

Reference materials to calibrate flow cytometers

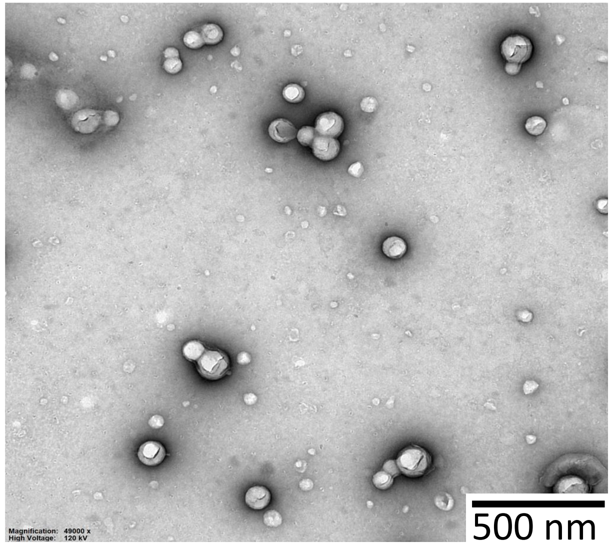
Ready-to-use stable biological test sample

Achieve reproducible measurements of EV concentration (CV<20%)

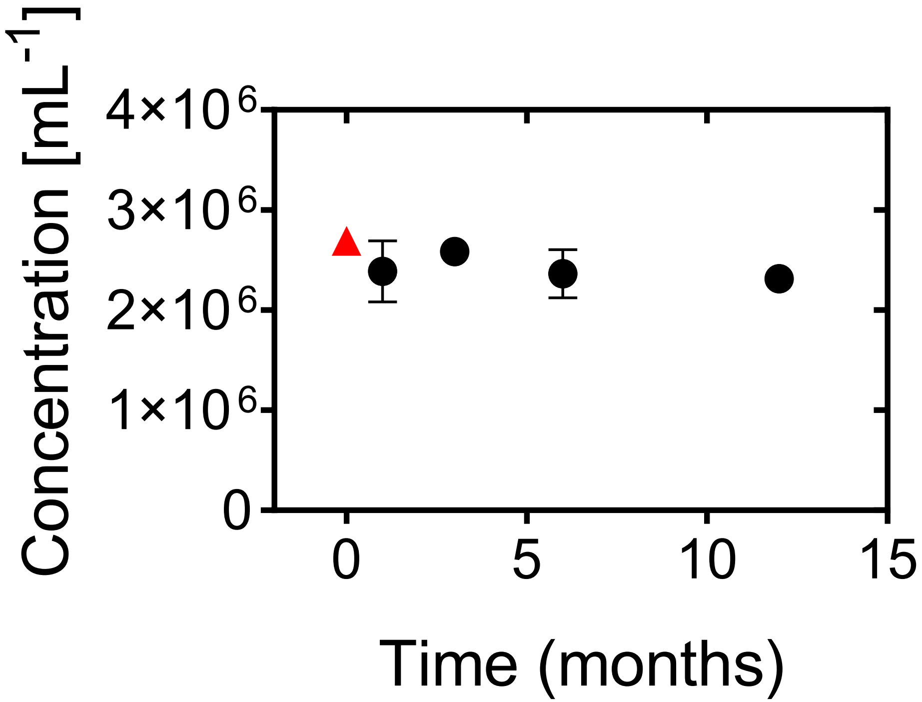
Plasma EV test sample (PEVTES)

Starting material

TEM image of pre-labelled EVs



Erythrocyte-derived EVs



Reference materials to calibrate

- Fluorescence intensity
  - Custom-made MESF beads (Becton Dickinson)
- Light scattering intensity
  - Polystyrene beads (Exometry)
  - Hollow organosilica beads (TTK Hungary)
- Sample volume
  - Silica nanoparticle counting beads (TTK Hungary)

MESF: molecules of equivalent soluble fluorochrome
SNP: silica nanoparticles

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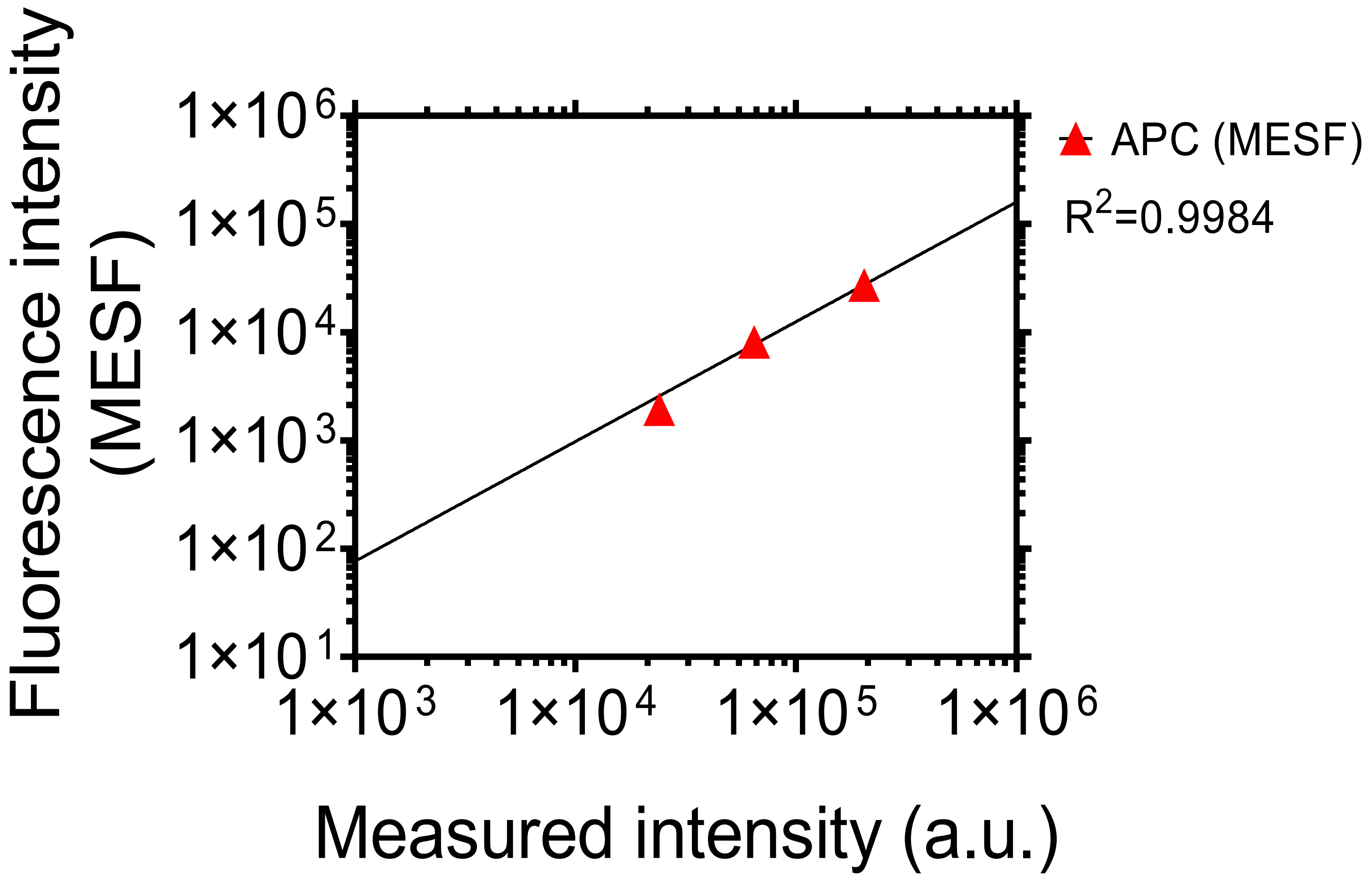
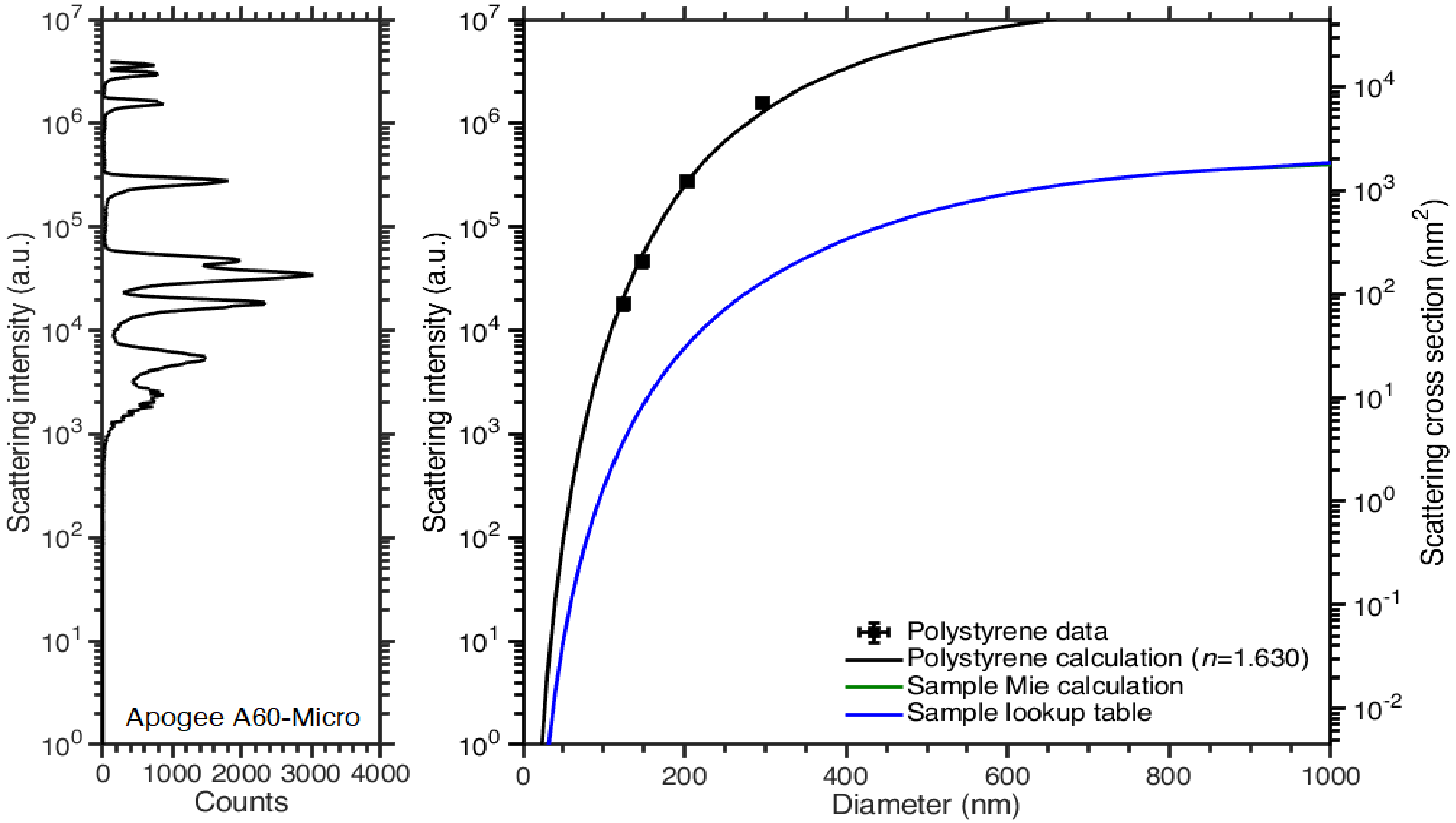
Fluorescence calibration



a.u.: arbitrary units

MESF: molecules of equivalent soluble fluorochrome

Scatter calibration



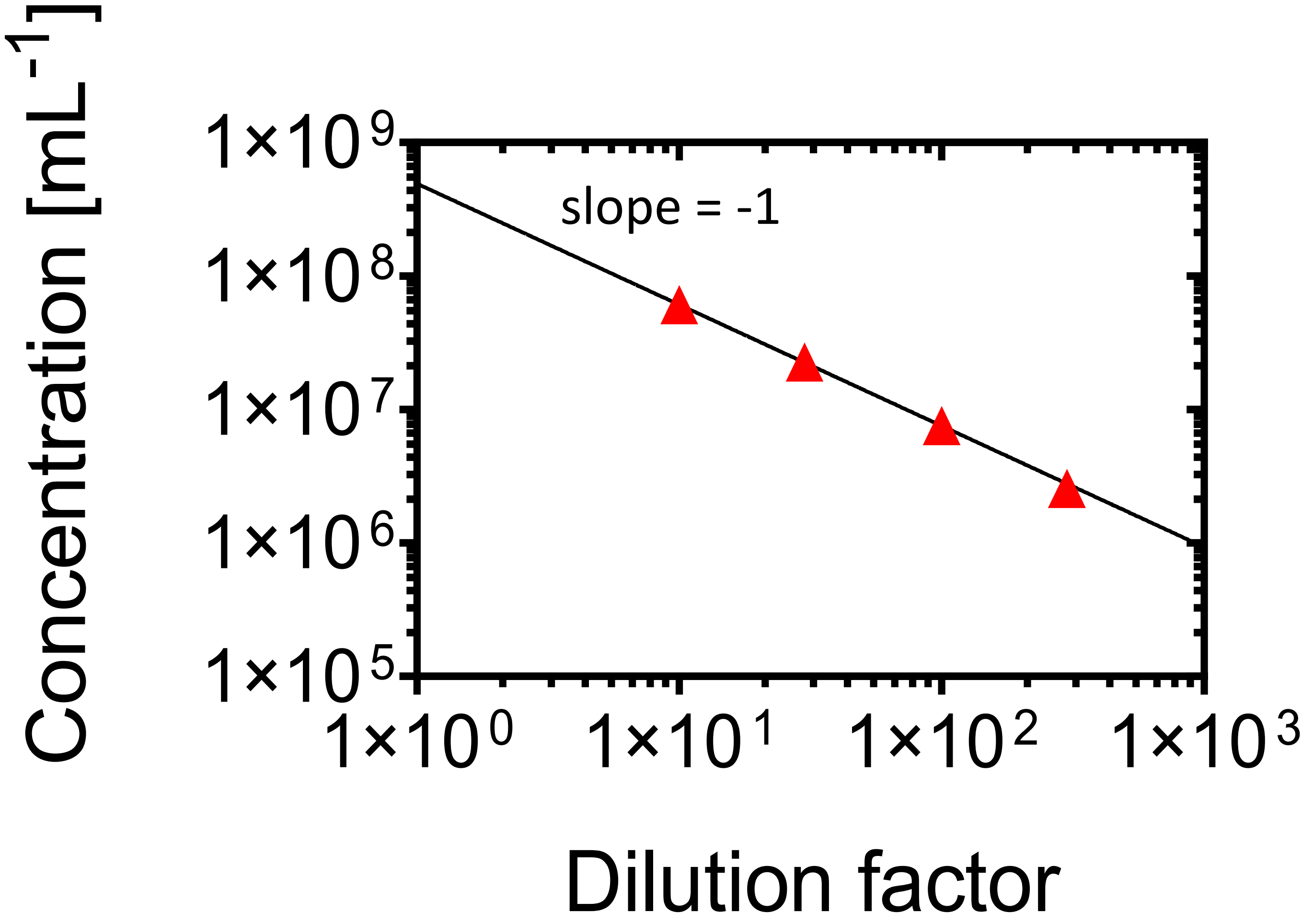
Flow rate calibration

$$\text{Sample volume} = \frac{\text{SNP counts} \cdot \text{sample dilution}}{\text{SNP stock concentration}}$$

Assumption: sample volume is stable between subsequent measurements

SNP: silica nanoparticles with traceably determined concentration

Avoid swarm detection



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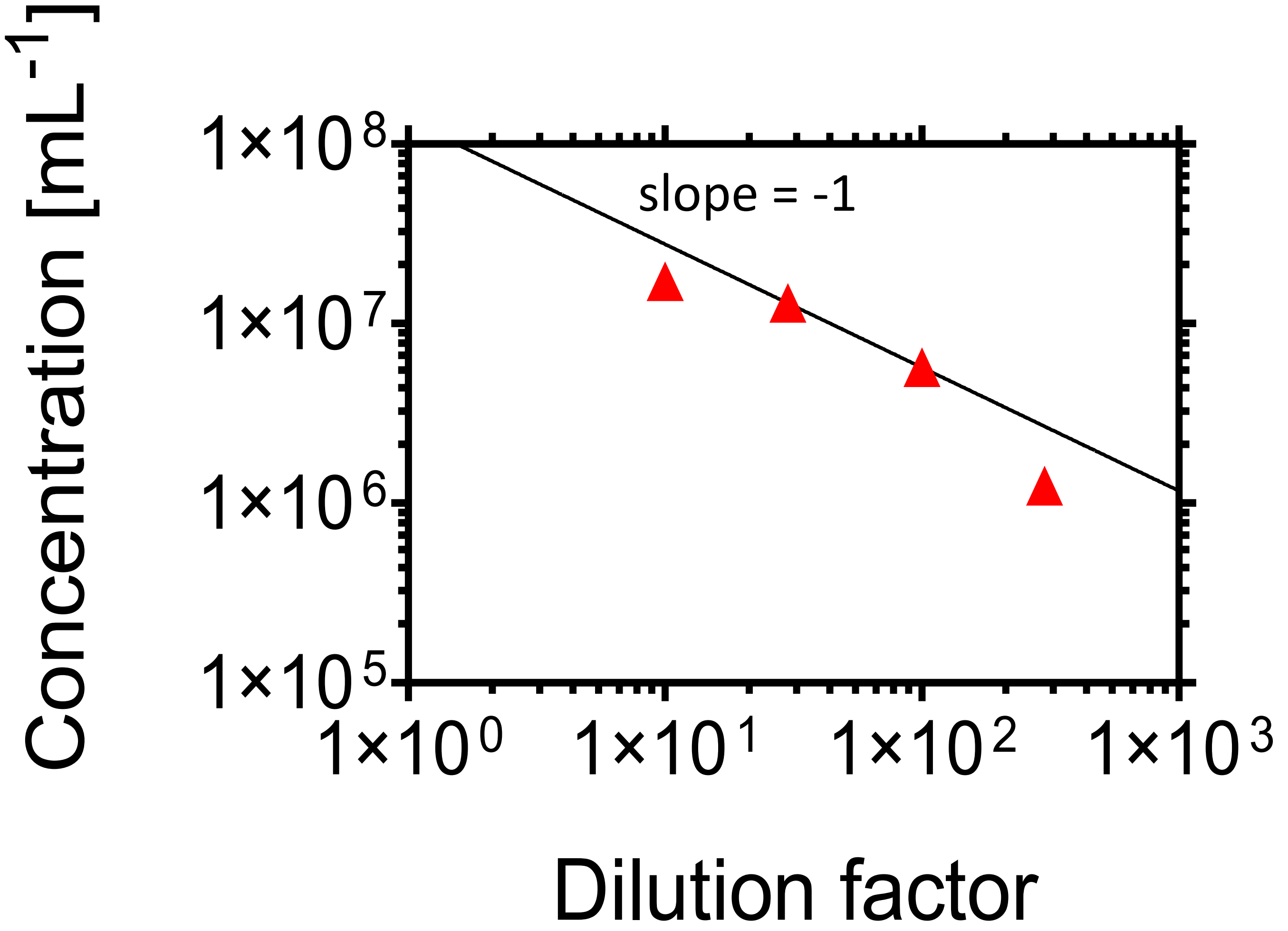
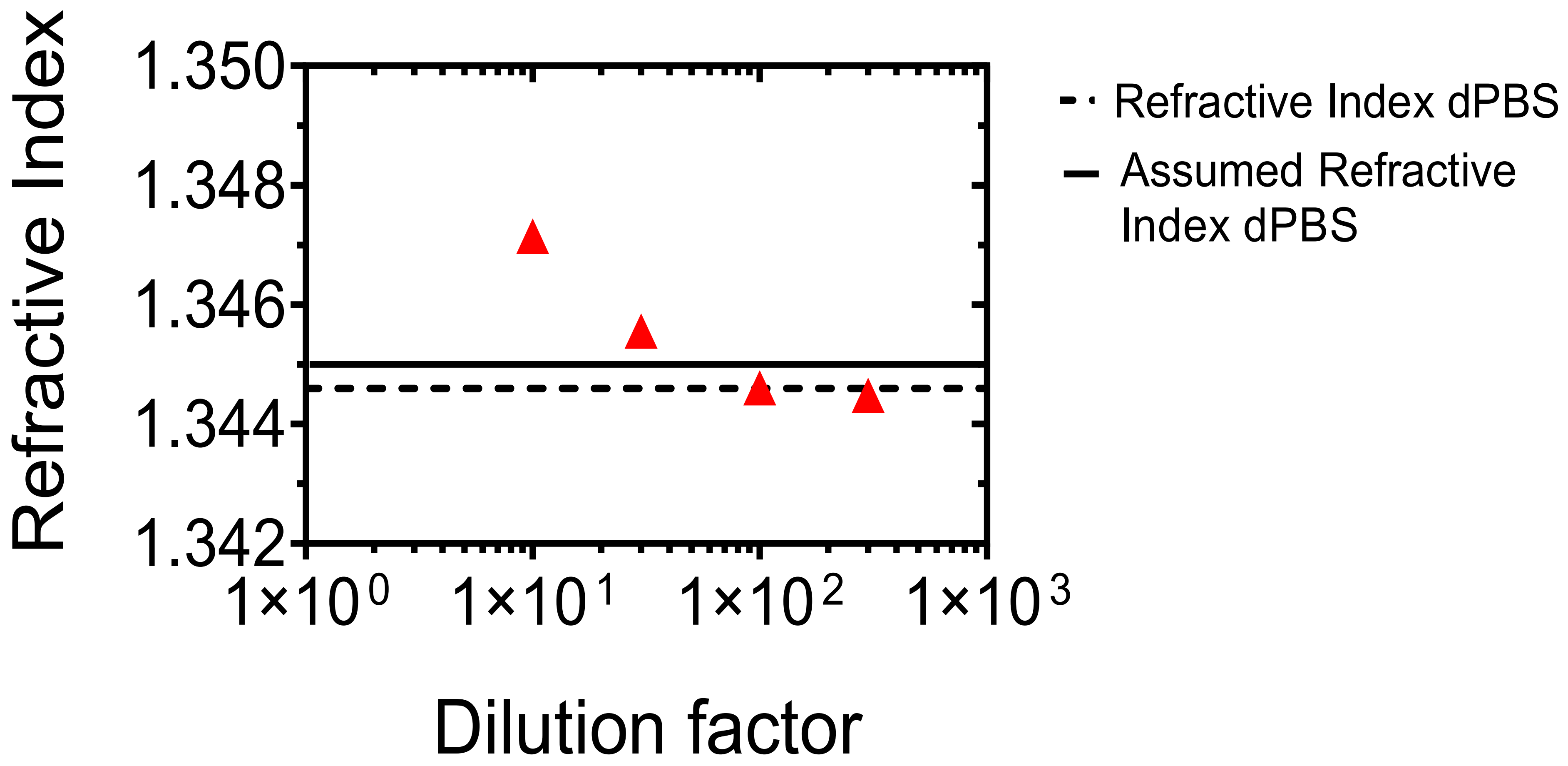


PEVTES medium refractive index



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Instruments

Enrolled

24 sites, 39 FCMs

Reason for discontinuation

Instrument sensitivity
Multiple instruments
Maintenance
No response to mails

Complete data sets

21 FCMs

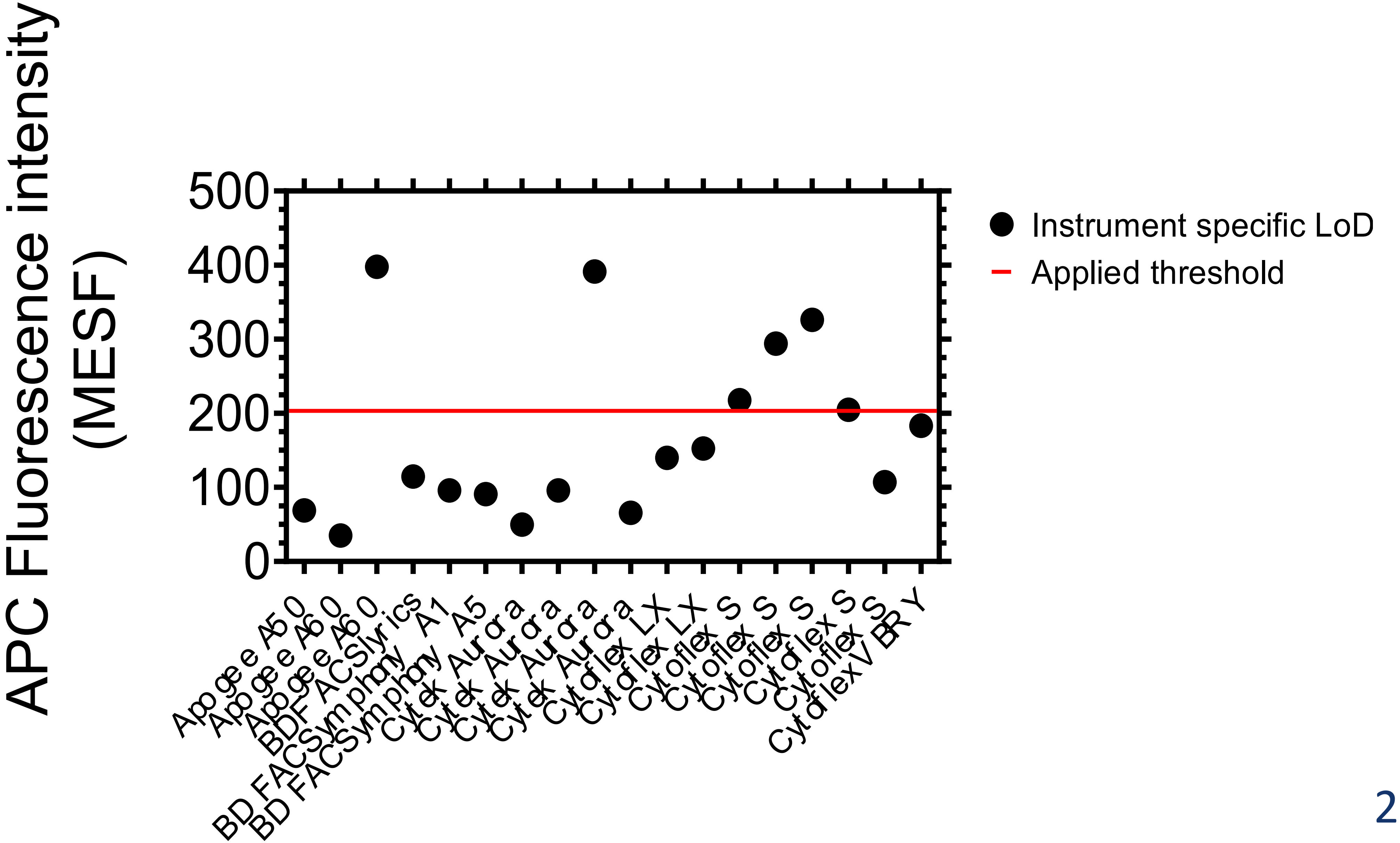
Gates
Analysis challenges

Preliminary data

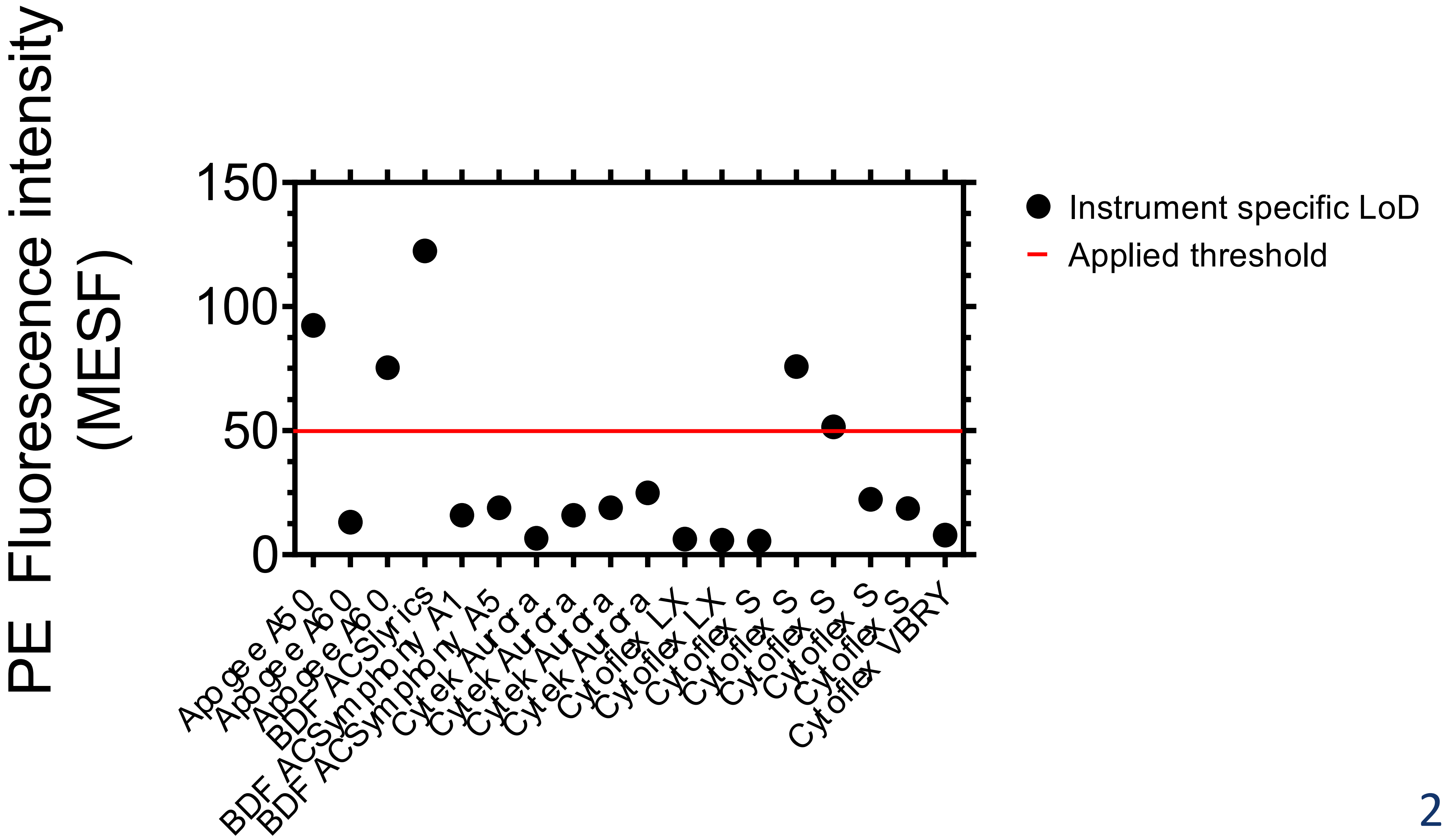
9 FCMs

FCMs: flow cytometers

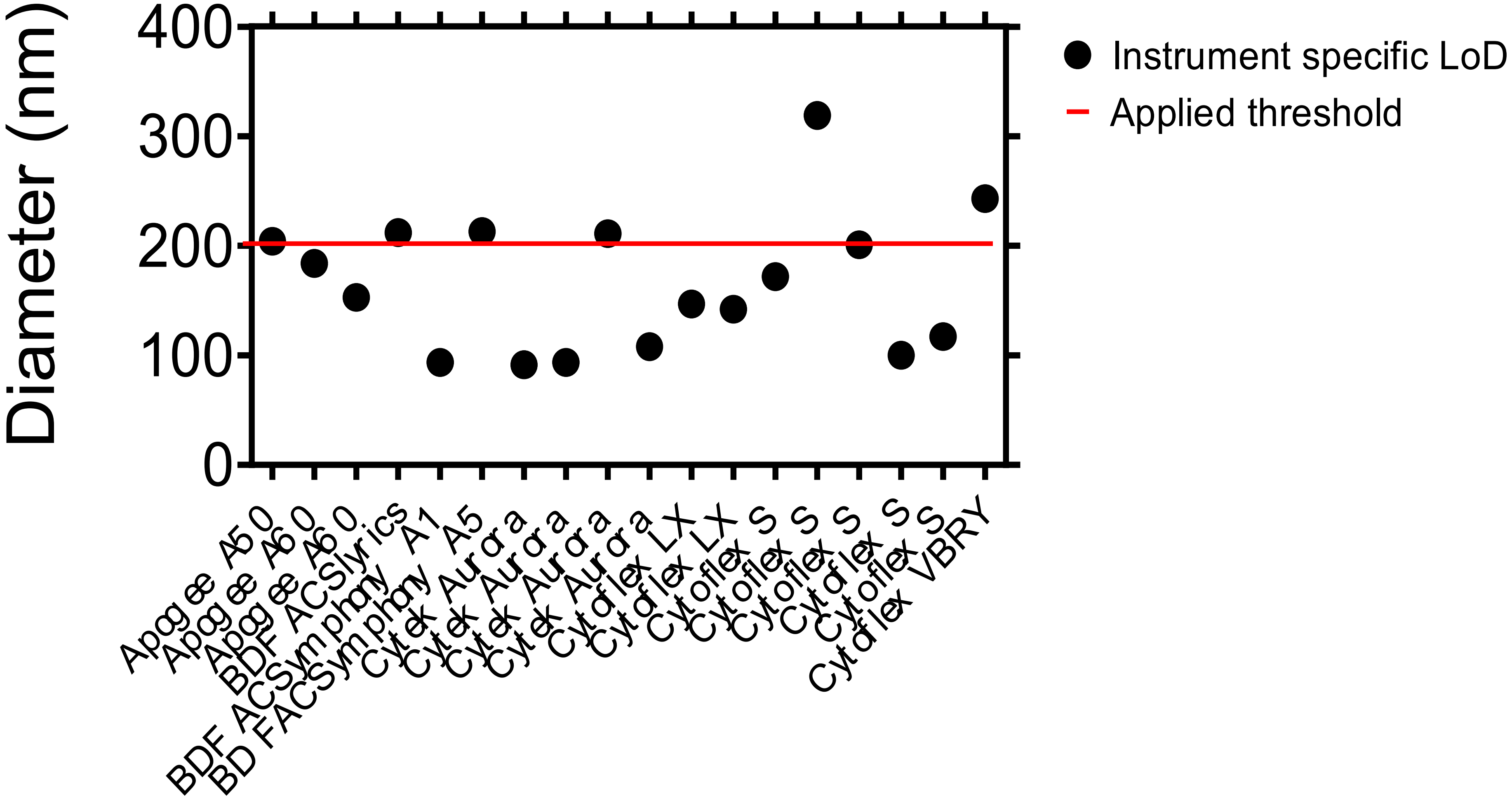
Instrument sensitivity



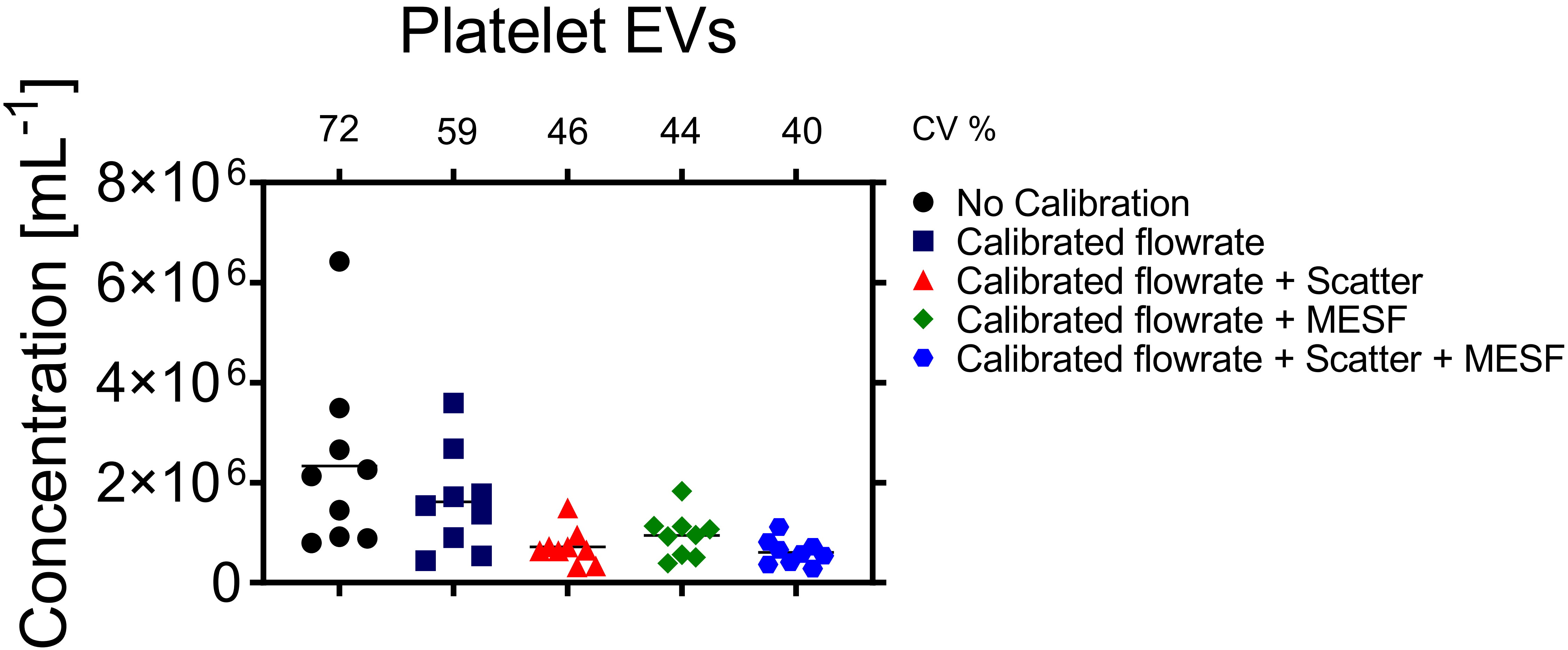
Instrument sensitivity



Instrument sensitivity

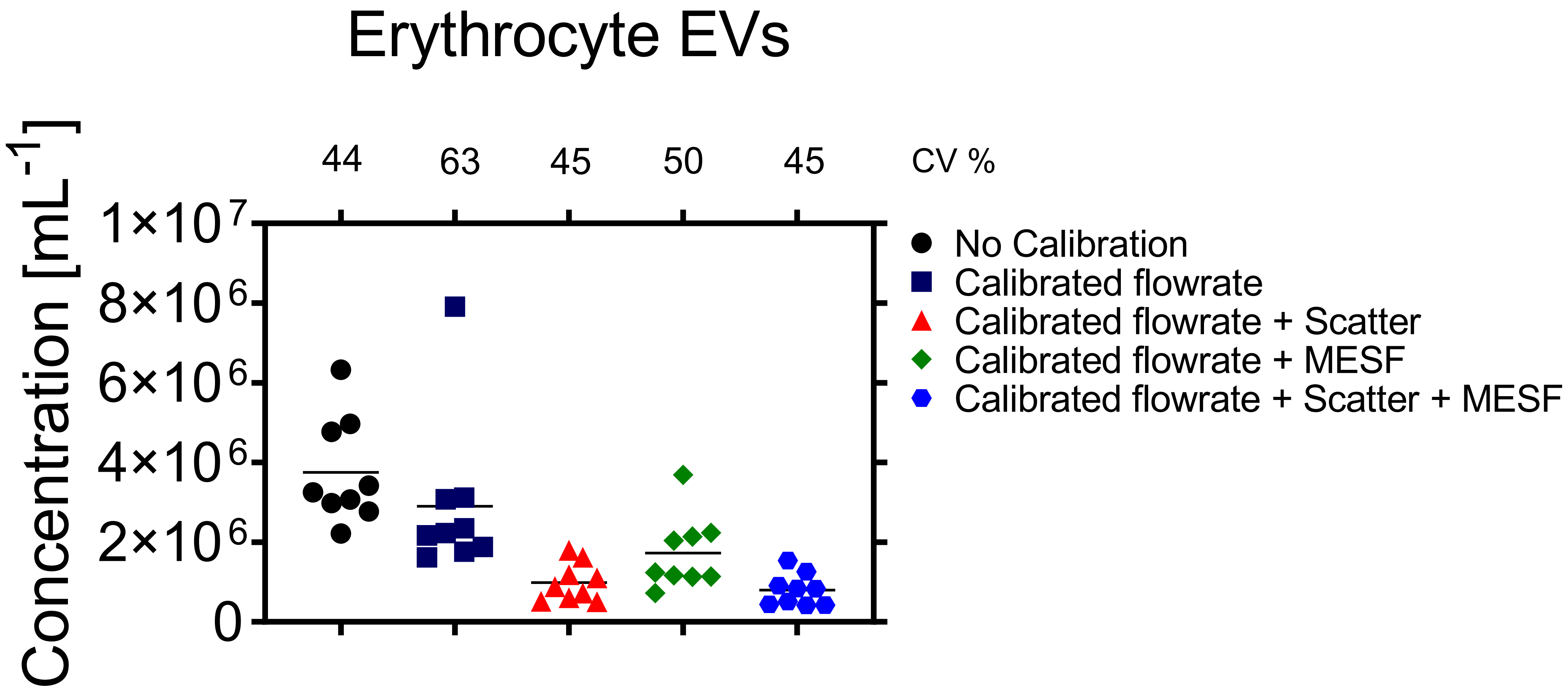


Instrument preliminary data



%CV = standard deviation / mean *100%;
MESF: molecules of equivalent soluble fluorochrome

Instrument preliminary data



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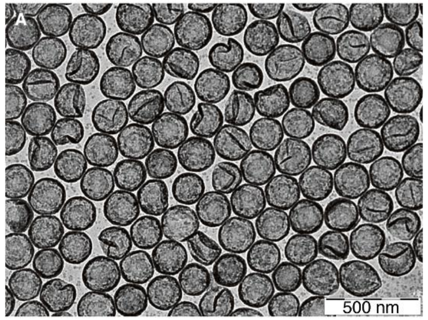
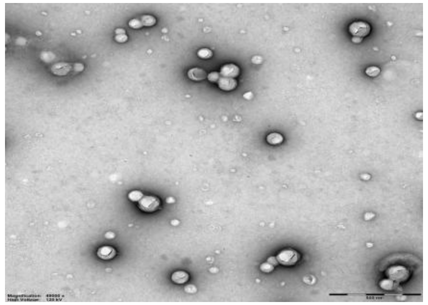
METVES II	✓	✓	~	✓	✓	✓	✓

Summary





- Aim: Measure comparable EV concentrations in EV test sample
- Preliminary results: CV of 40% on measured EV concentrations
- Starting point for reliable multi-center studies

What’s next?

- Complete analysis
  - Multiple gates
  - Traceable refractive index
  - Quality controls
- Hollow silica beads as scatter calibrators
- Calibration of imaging flow cytometers

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