

# Software to automate calibration and processing of flow cytometry data in clinical studies

Edwin van der Pol<sup>1-3</sup>, Frank A.W. Coumans<sup>1-3</sup>  
Leonie de Rond, Aleksandra Gasecka<sup>1,3,4</sup>  
Najat Hajji<sup>1,3</sup>, Rienk Nieuwland<sup>1,3</sup>  
and Ton G. van Leeuwen<sup>1,2</sup>



April 25<sup>th</sup>, 2019



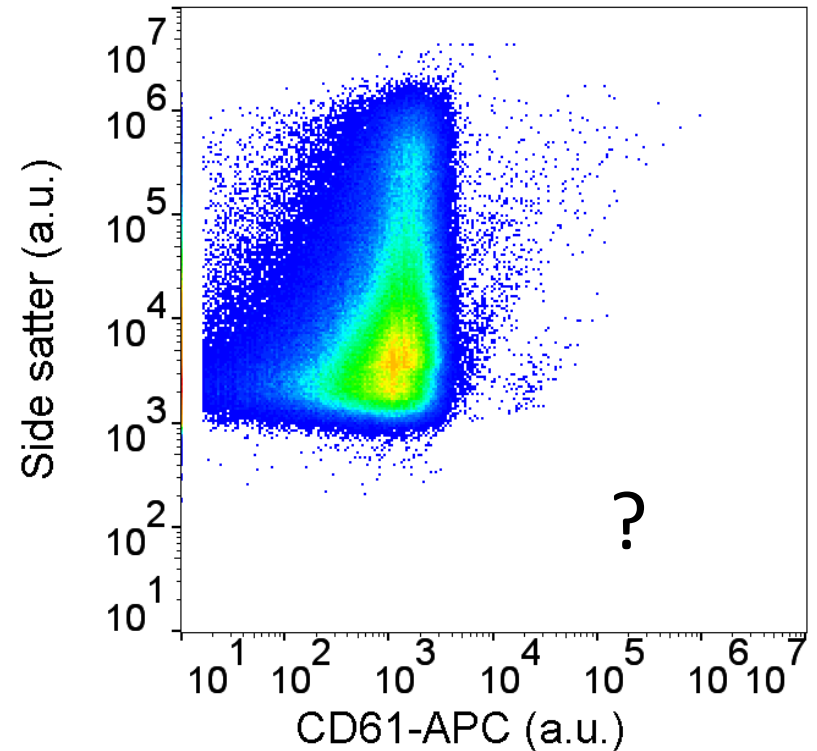
*<sup>1</sup>Vesicle Observation Center; <sup>2</sup>Biomedical engineering and Physics;  
<sup>3</sup>Laboratory Experimental Clinical Chemistry; Amsterdam University  
Medical Centers, The Netherlands; <sup>4</sup>1st Chair and Department of  
Cardiology, Medical University of Warsaw, Poland*

# Introduction

- Motive: develop an EV-based “liquid biopsy”
- Determine EV concentration by flow cytometry
- Case: clinical study AFFECT\*
  - 60 patients + 30 controls
  - 3 time points
  - 8 antibody panels + controls
  - 1,224 data files
  - >33 GB of data
  - >0.3 billion events

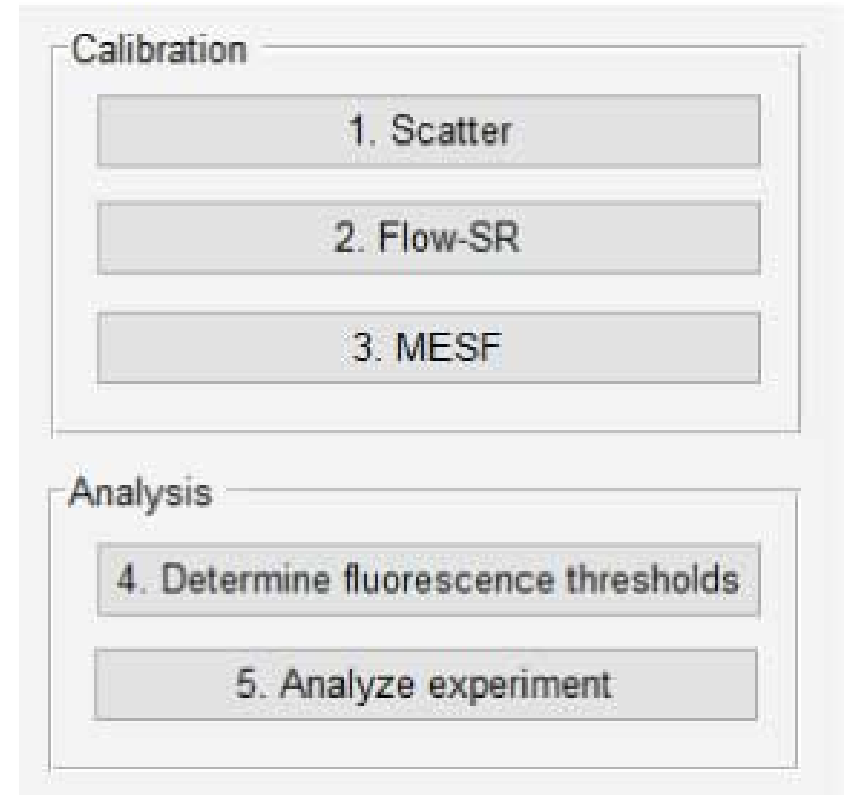
# Problems

- Flow cytometry data are in arbitrary units, hampering:
  - data comparison
  - data interpretation
  - standardization
- (semi) Manual calibration and data analyses
  - elaborate and slow
  - human mistakes
  - inter-user variability

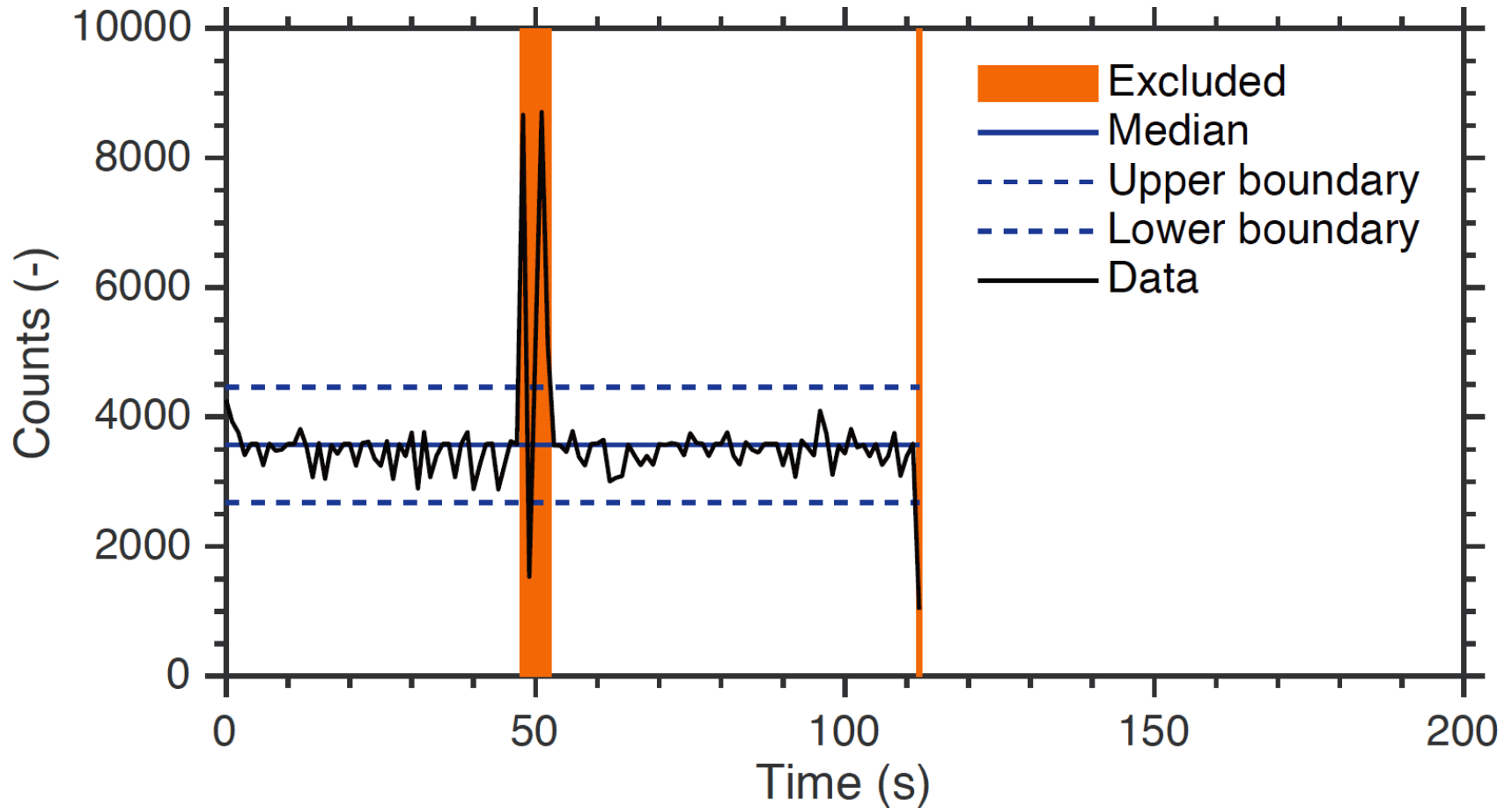


# Goal

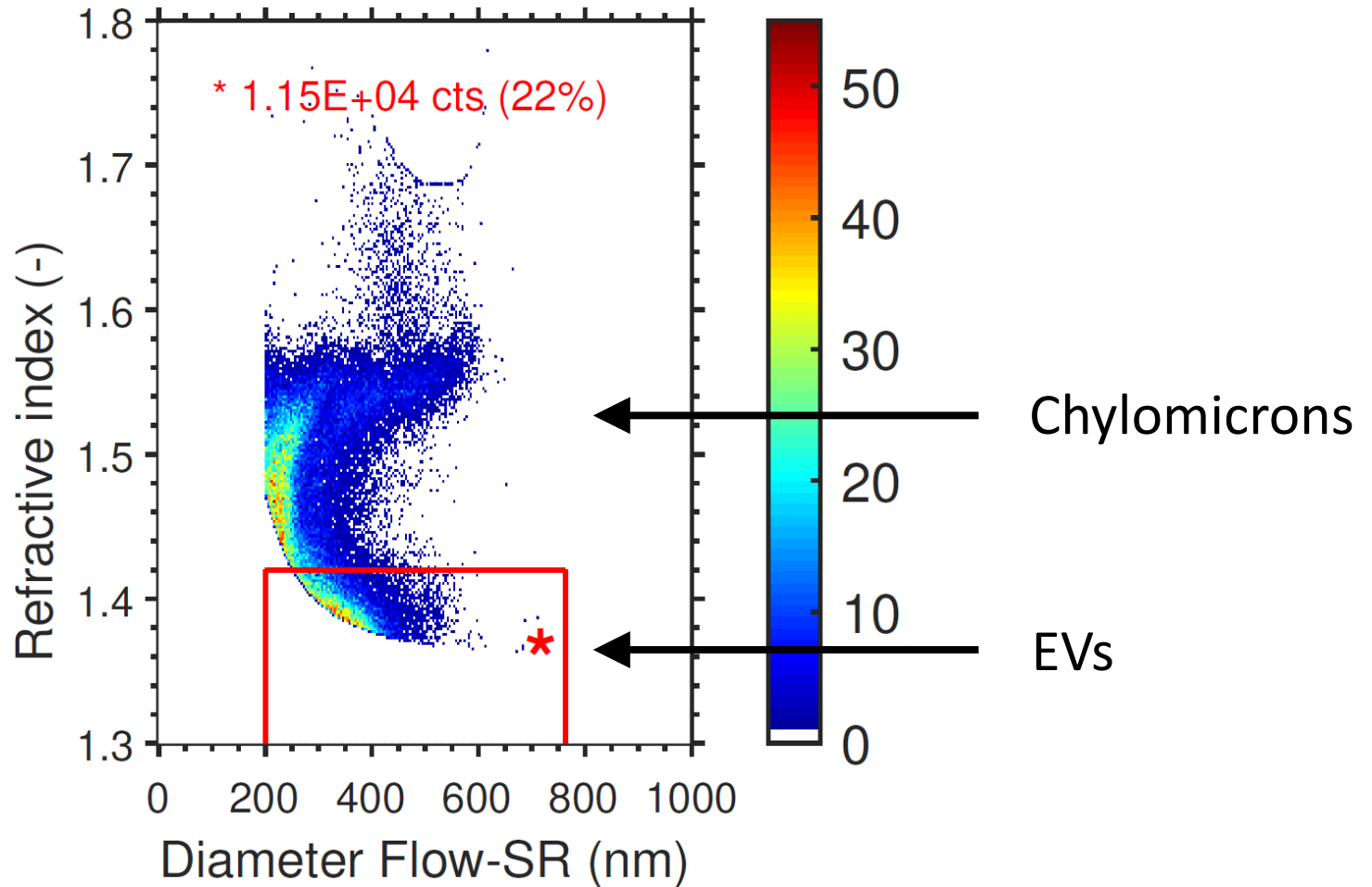
- Automate data calibration and processing
  - simple
  - all detectors are calibrated and exported to FCS files
  - scatter plots and gates are exported to PDF
  - data are summarized in one CSV file



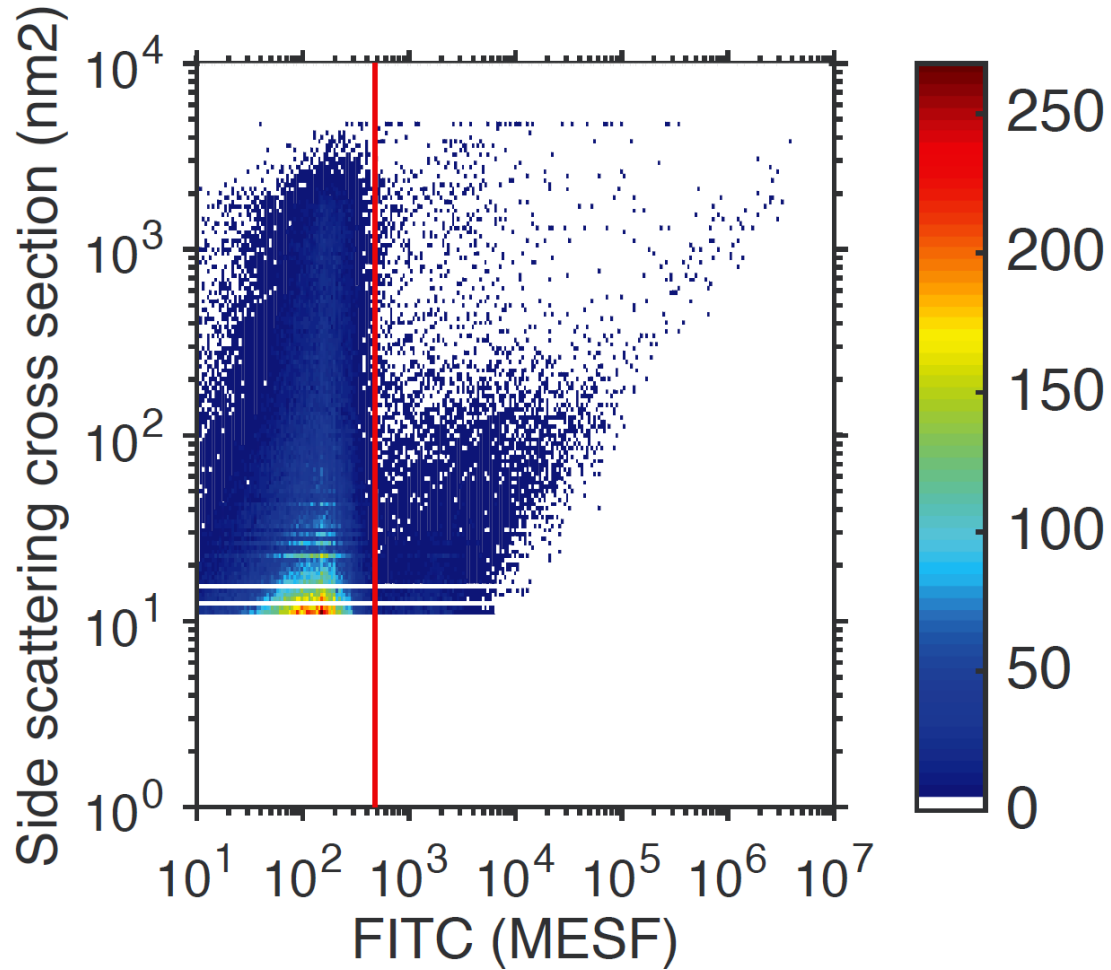
# Automatic count rate filtering



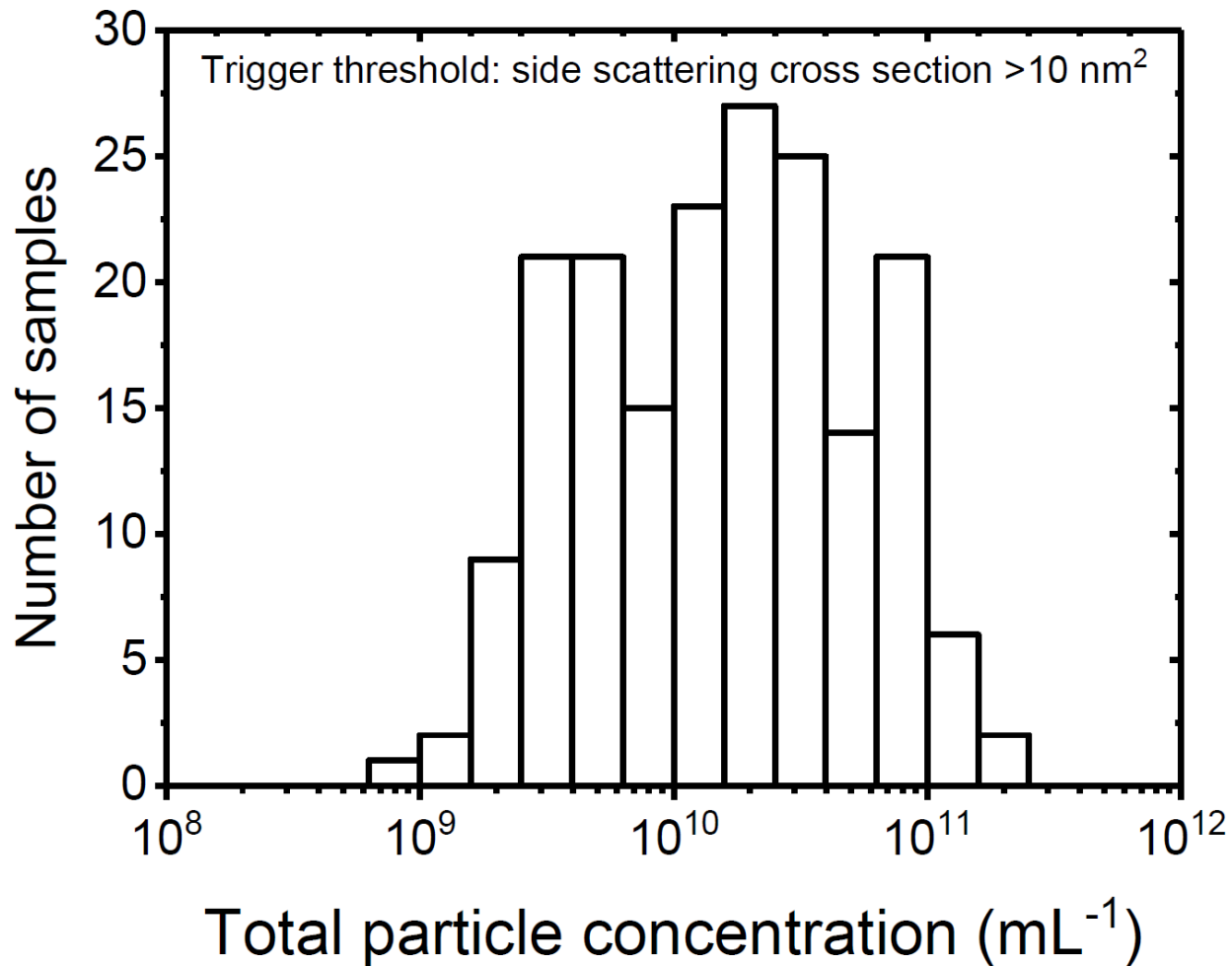
# Size and refractive index determination



# Automatic fluorescence thresholding



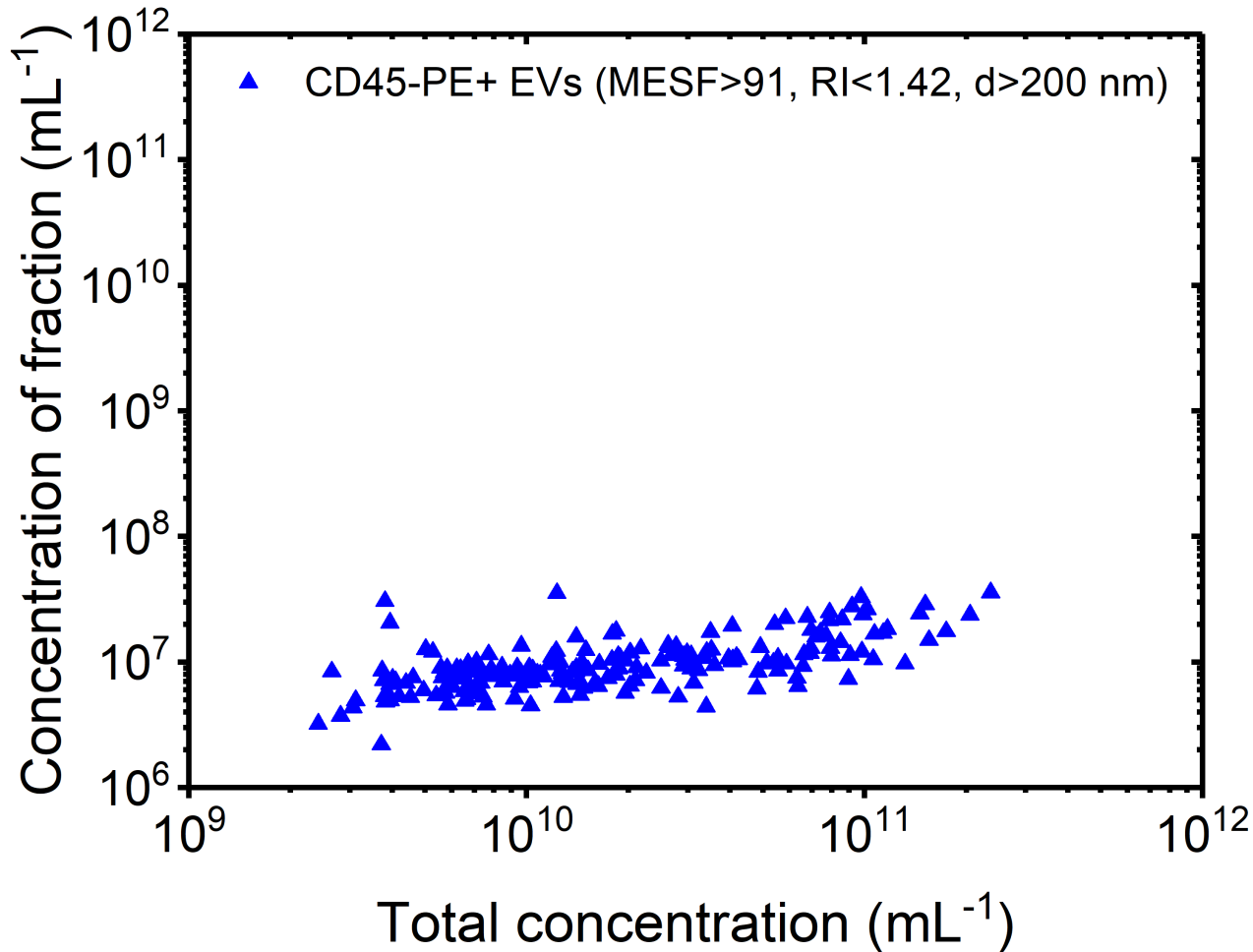
# Results AFFECT: particle concentration plasma



CV = 122%

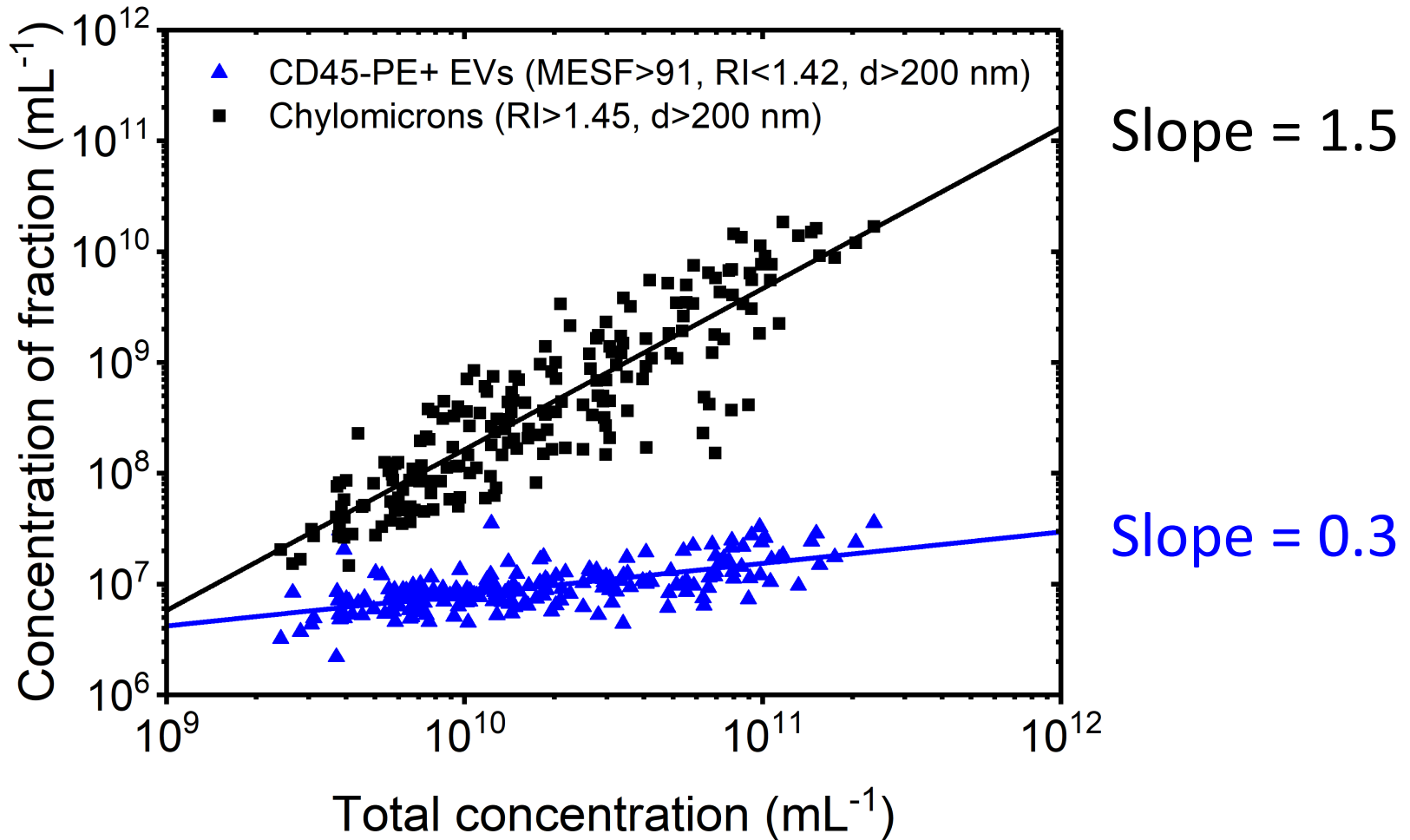


# Results AFFECT: CD45+ EV concentration



CV = 55%

# CD45+ EV vs. chylomicron concentration



# Conclusions

- We developed software to fully **automate** flow cytometry data **calibration** and **analyses** for clinical studies on EVs
- Software provides new insights, e.g.
  - chylomicrons dominate counts in blood plasma

# Outlook

- Software will be
  - extended with new functions
  - used for in-house analyses of clinical studies
  - made compliant with other flow cytometers
  - used in METVES II standardization study\*
- MIFlowCyt-EV will be published soon\*
- Posters
  - AFFECT: PS03.11
  - “Software”: PS08.09
- More: [edwinvanderpol.com](http://edwinvanderpol.com)