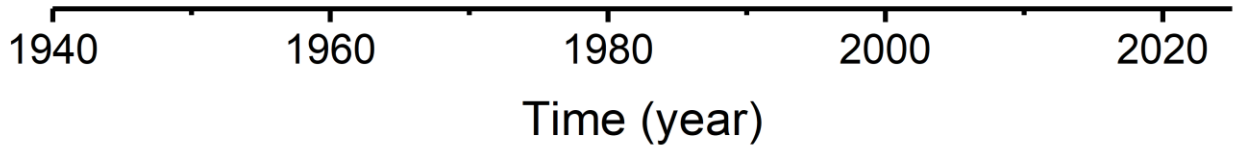
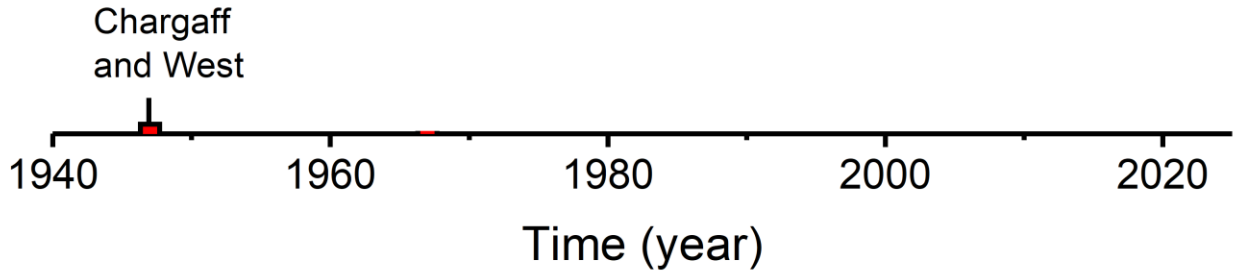
Detection of extracellular vesicles: from artifact to clinical act

Edwin van der Pol

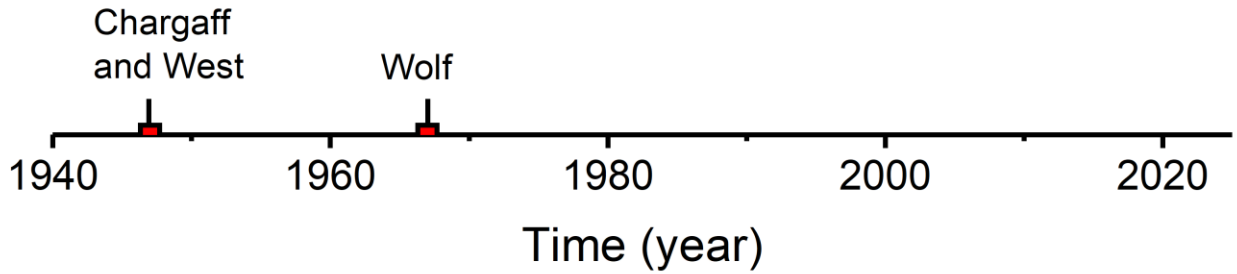
May 24th, 2022


1940
1960
1980
2000
2020
Time (year)

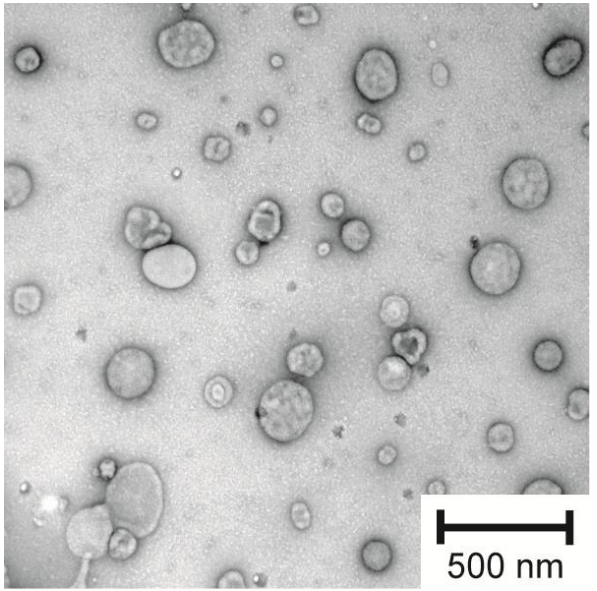
“cell-free plasma contains a subcellular factor that promotes clotting of blood”

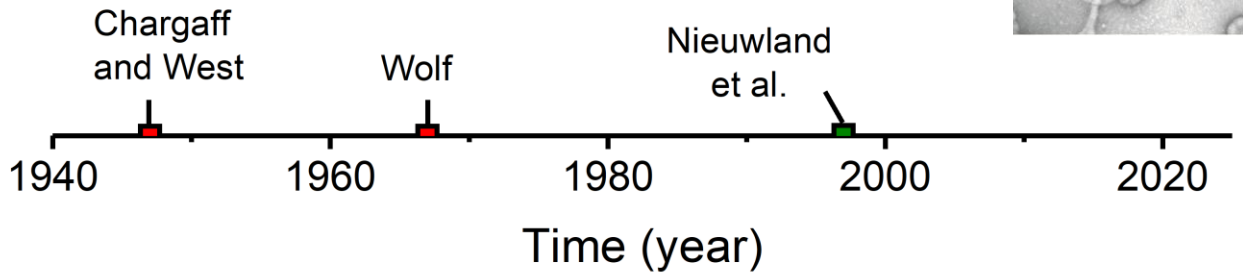


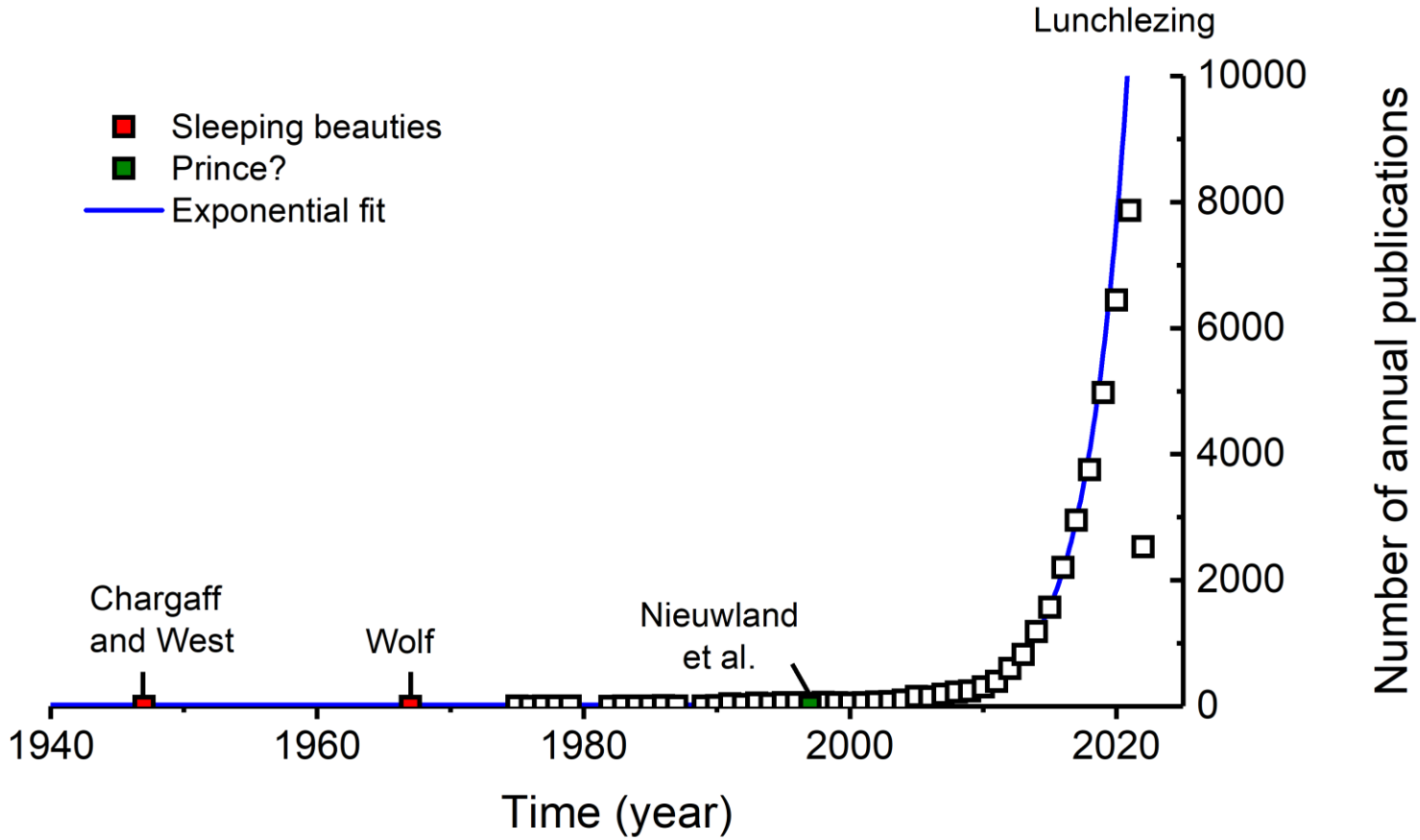
“small vesicles promote clotting of blood”



“Cells release extracellular vesicles (EVs) in body fluids”






Lunchlezing
Sleeping beauties
Prince?
Exponential fit
Chargaff
and West
Wolf
Nieuwland
et al.
10000
8000
6000
4000
2000
0
Number of annual publications
1940
1960
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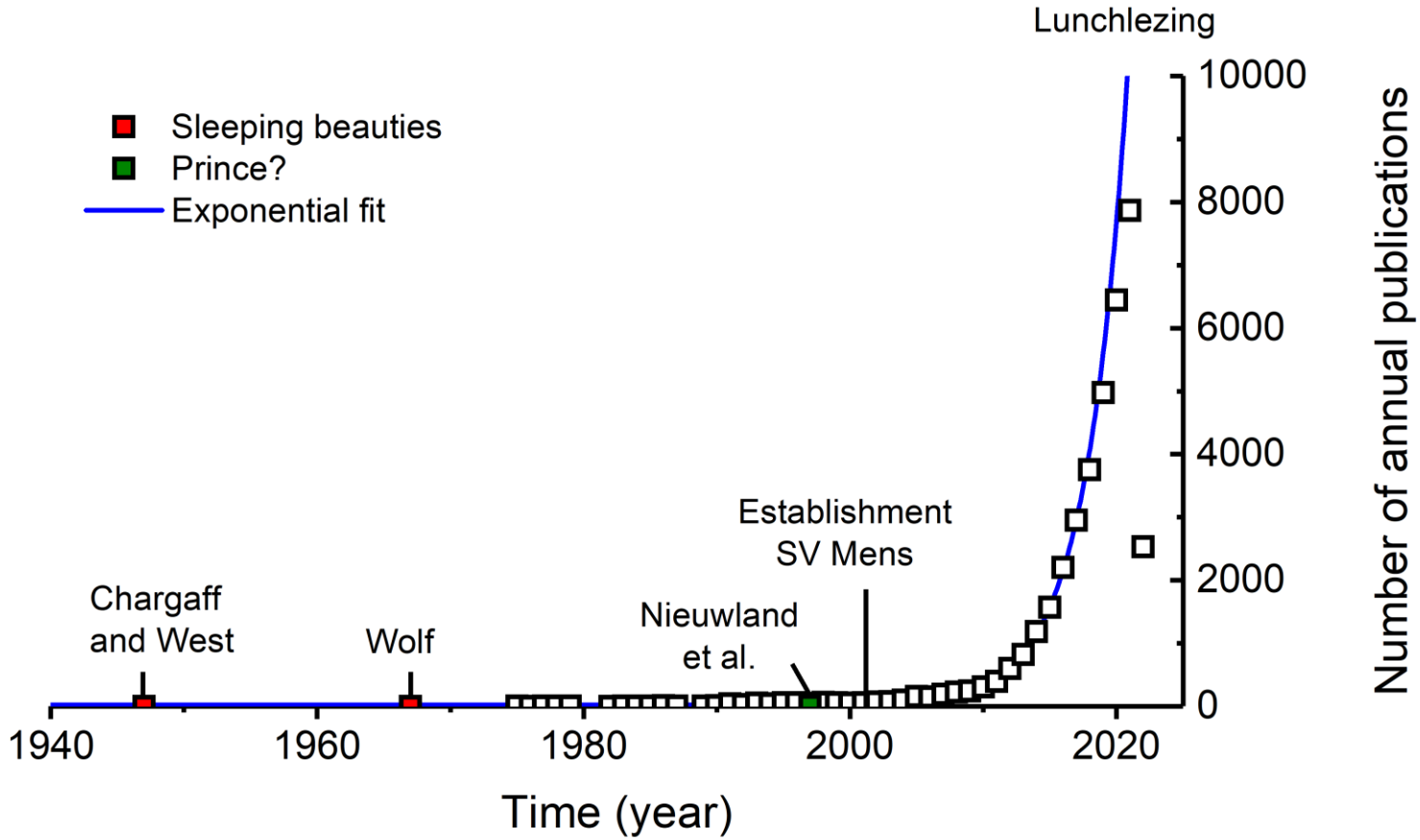
Lunchlezing
Sleeping beauties
Prince?
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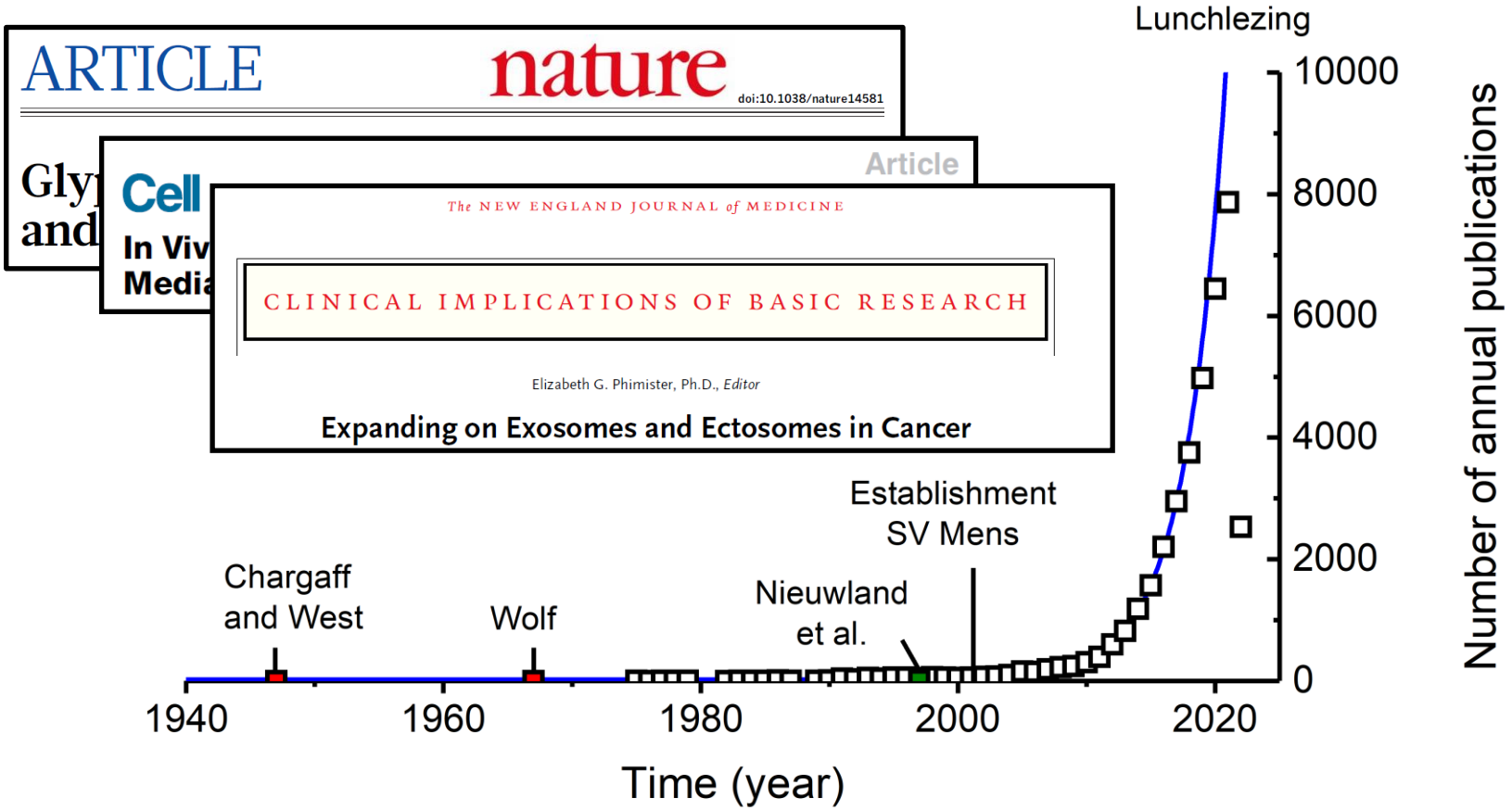
ARTICLE
nature
doi:10.1038/nature14581
Article
The NEW ENGLAND JOURNAL of MEDICINE
CLINICAL IMPLICATIONS OF BASIC RESEARCH
Elizabeth G. Phimister, Ph.D., Editor
Expanding on Exosomes and Ectosomes in Cancer
Lunchlezing
Establishment
SV Mens
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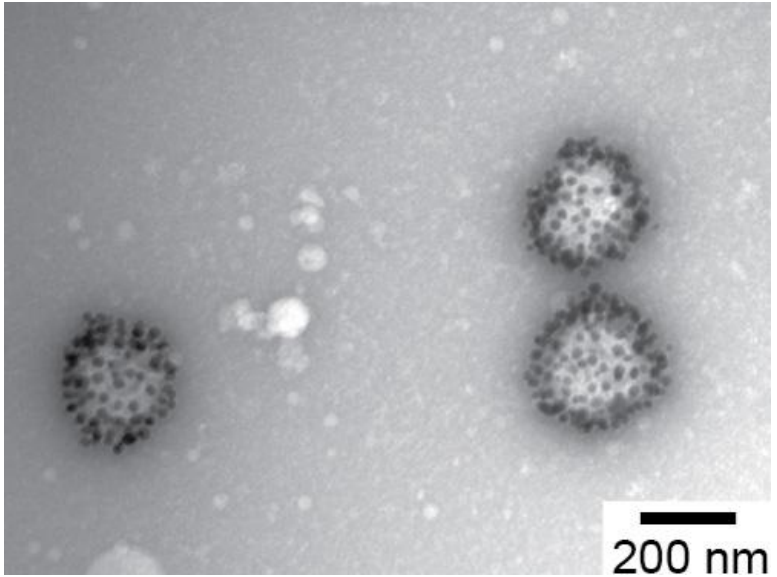
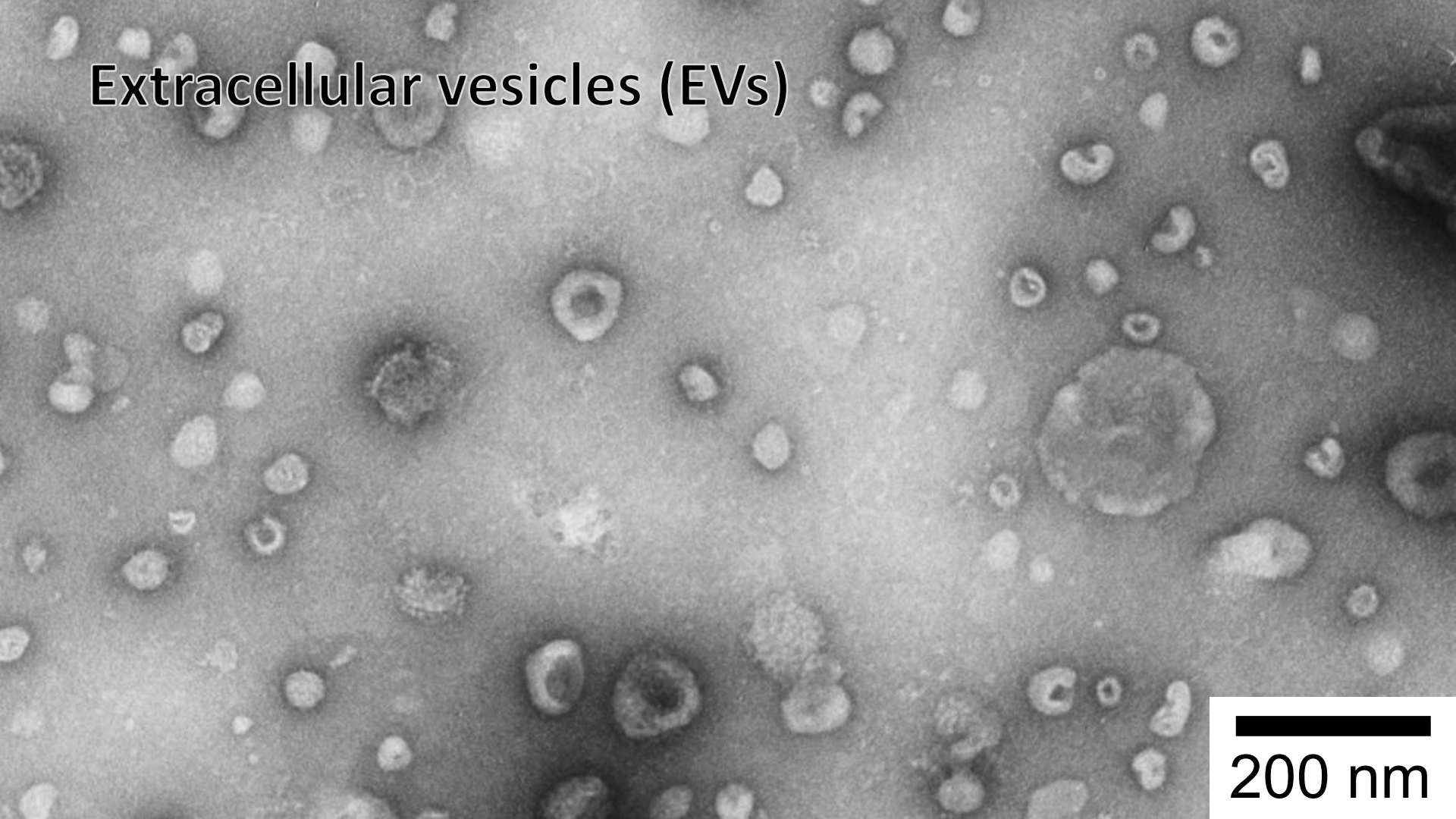
Outline lunchlezing

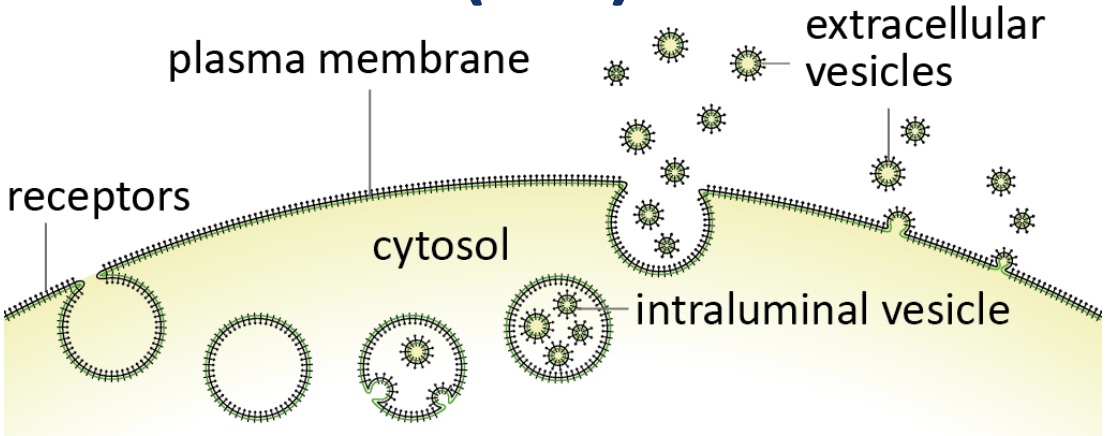
- Extracellular vesicles
- From artifact… *(2012)*
- …to artifact... *(2022)*
- …to clinical act



Extracellular vesicles (EVs)



Extracellular vesicles (EVs)

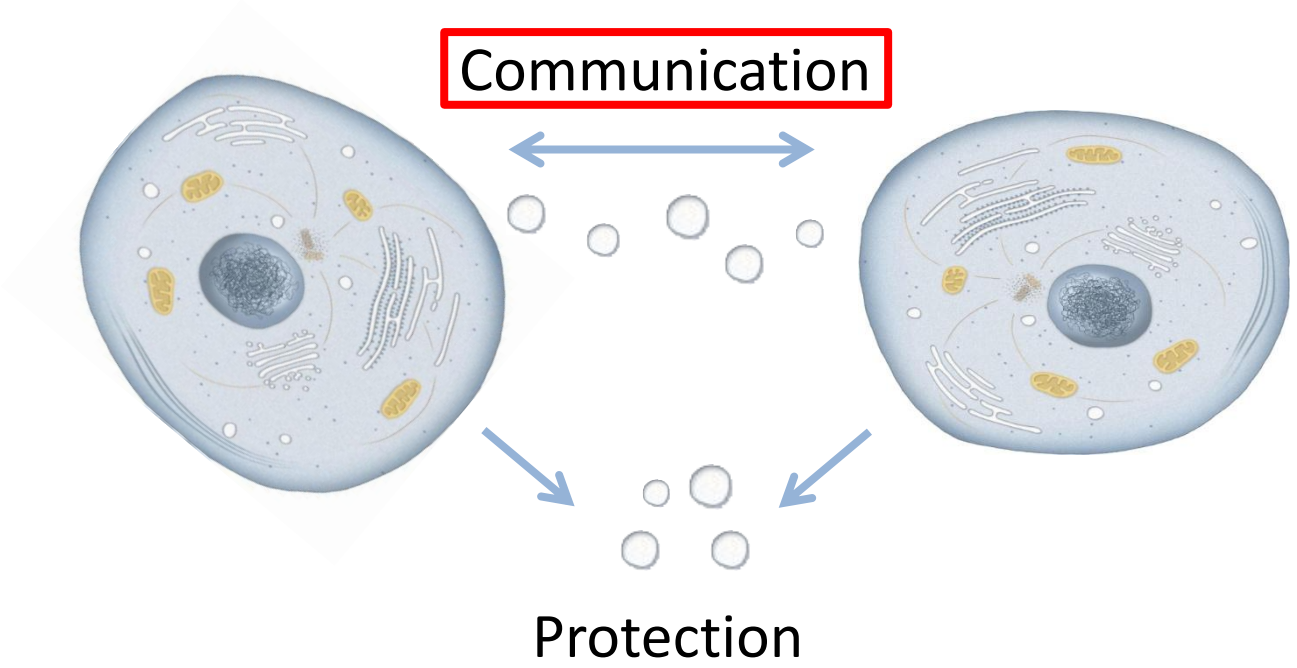


- cells release vesicles:
  biological nanoparticles with receptors, DNA, RNA
- specialized functions
- clinically relevant

Extracellular vesicles from yeast in unfiltered beer

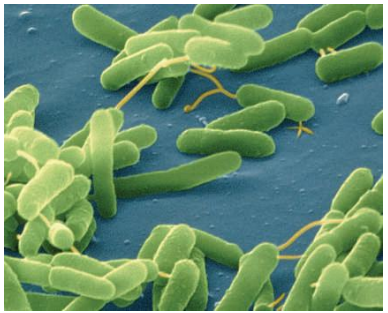
Stensballe et al. *JEV* (2014)

Functions of extracellular vesicles



van der Pol et al. *Pharmacol Rev* (2012)

Communication between prokaryotes



MICROBIOLOGY

Bacterial speech bubbles

Stephen C. Winans

Many bacteria socialize using diffusible signals. But some of these messages are poorly soluble, so how do they move between bacteria? It seems they can be wrapped up in membrane packages instead.

Communication between cells in mice and humans

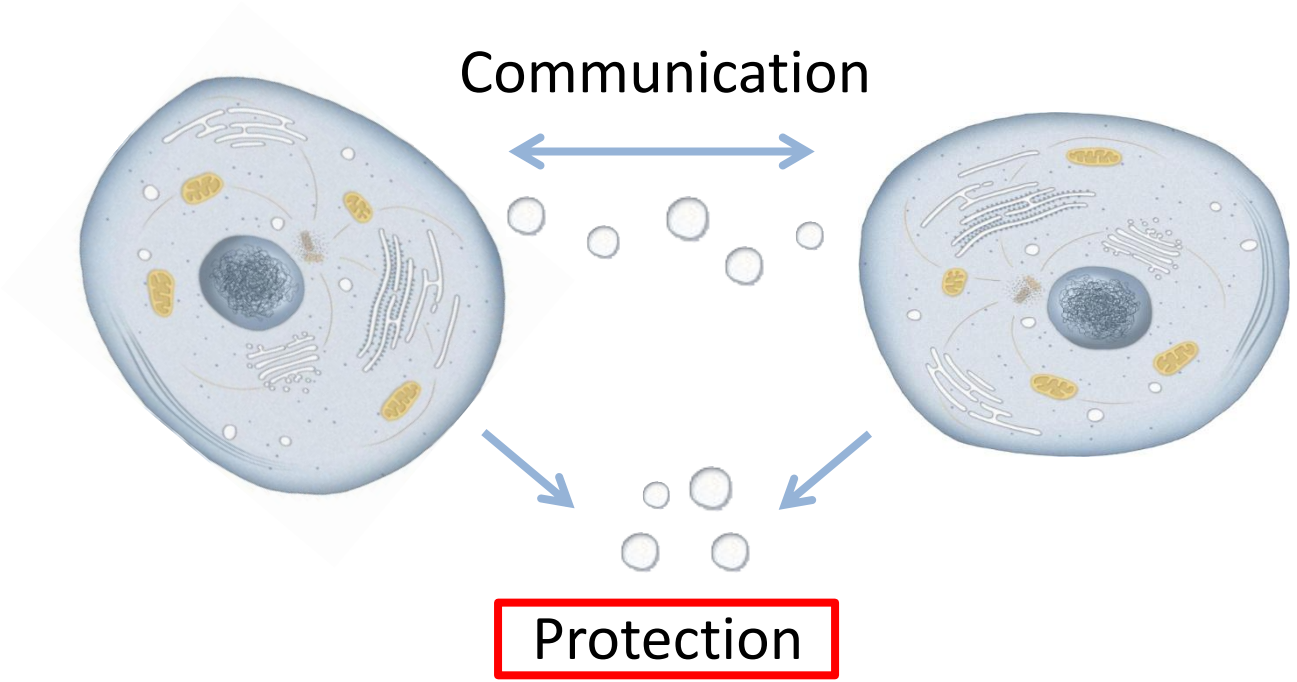
nature
cell biology

Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells

Hadi Valadi[1,3], Karin Ekström[1,3], Apostolos Bossios[1], Margareta Sjöstrand[1], James J. Lee[2] and Jan O. Lötvall[1,4]

Valadi et al. *Nat Cell Biol* (2007)

Functions of extracellular vesicles



van der Pol et al. *Pharmacol Rev* (2012)

Protection

O, THE OPRAH MAGAZINE

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VAL MONROE
MARTHA BECK
DR. KATZ

Saliva Heals Cuts Faster

By Catherine Guthrie

Photo: J Muckle/Studio D

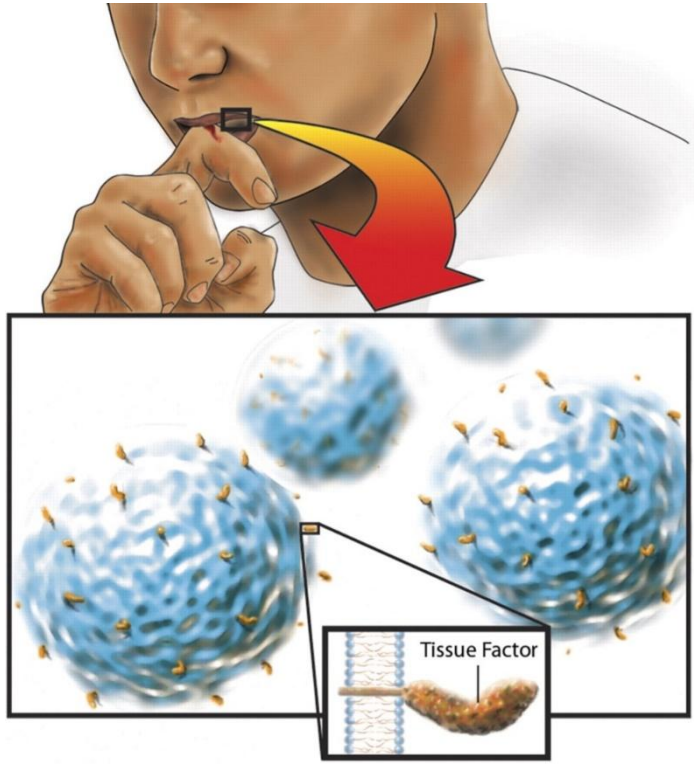
Go ahead: Lick your wounds. Your salivary glands are generating loads of curative compounds every day.

If you've ever cut your finger, your first impulse was probably to stick it in your mouth. That instinct serves you better than you might realize. Human saliva, it seems, speeds up wound healing.

Researchers at the University of Amsterdam in the Netherlands bathed injured skin in saliva or a saltwater solution. Sixteen hours later, they compared the samples and found that the cut treated with saliva had healed 30 percent faster.

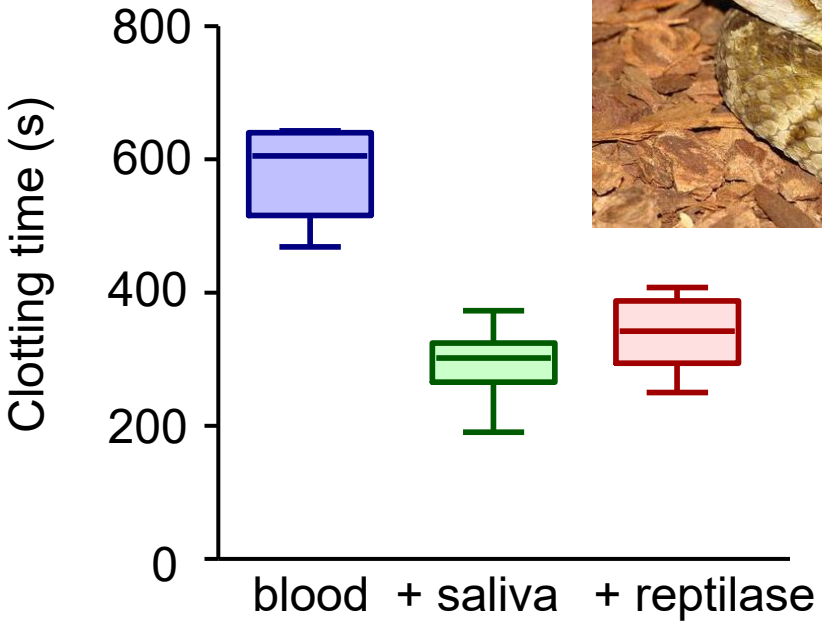
The finding wasn't a complete surprise; saliva contains more than 200 compounds, many of which have, in previous research, proved capable of preventing cavities, fighting fungi, and even protecting against

Tissue factor on extracellular vesicles in saliva can cause blood to clot



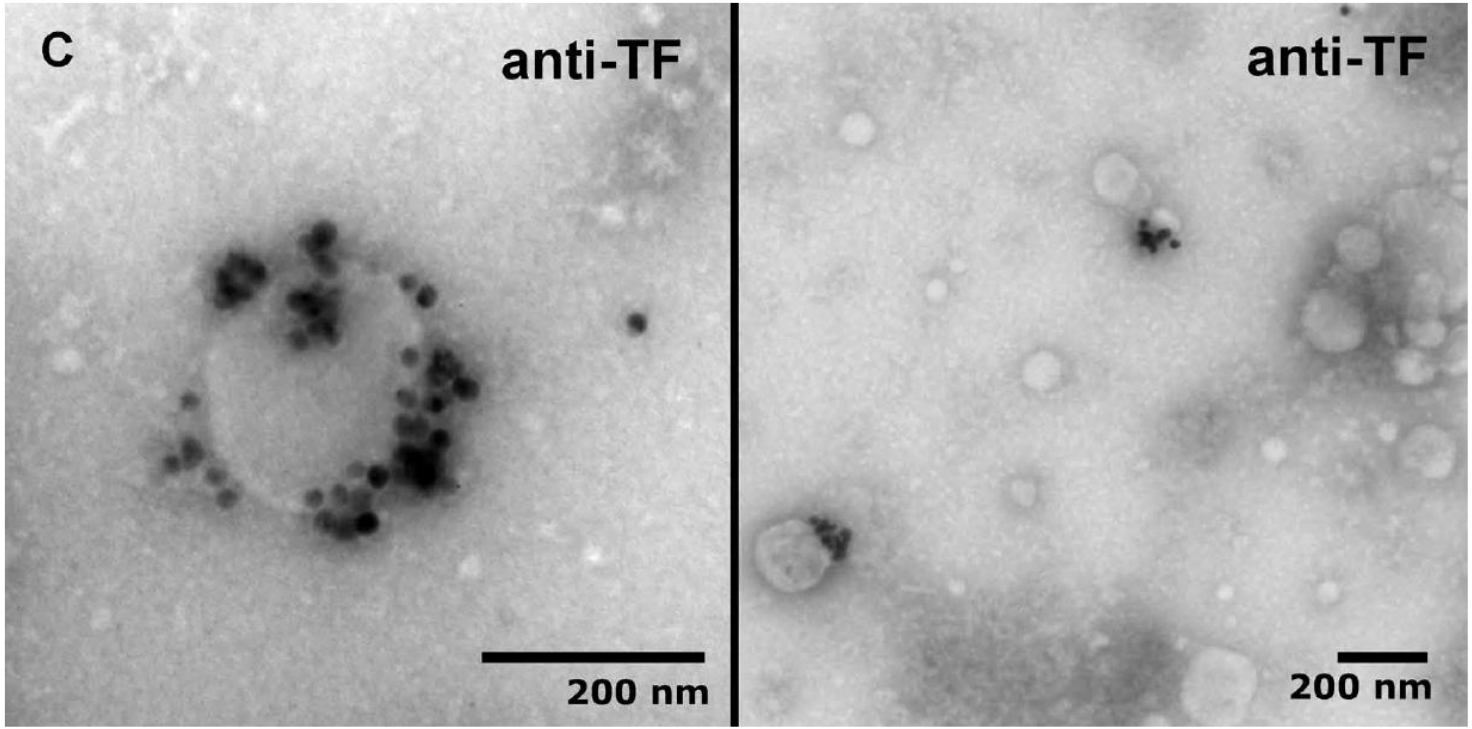
Gross *Blood* (2011)
Berckmans et al. *Blood* (2011)

Saliva *versus* reptilase



Gross *Blood* (2011)
Berckmans et al. *Blood* (2011)

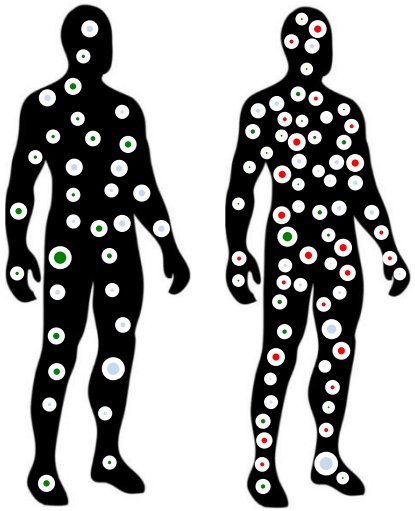
Saliva extracellular vesicles contain tissue factor



Gross *Blood* (2011)
Berckmans et al. *Blood* (2011)

Extracellular vesicles in body fluids

- during disease, EVs change in
  - concentration
  - cellular origin
  - biochemical composition
  - function
- novel biomarkers for disease



healthy *disease*

e.g. Hoshino et al. *Nature* (2015)

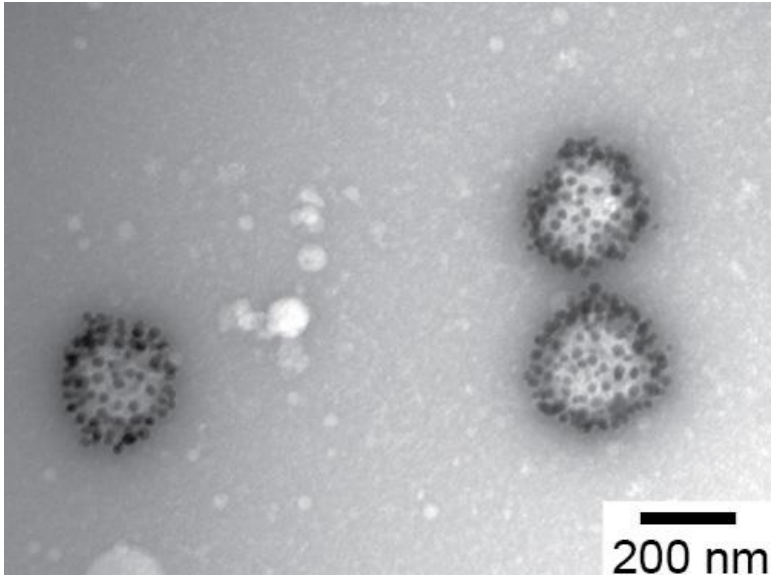
Personal ambition:
EV-based liquid biopsy

Hematology parameter	Concentration (vesicles mL^{-1})
Platelet vesicle count	$2.3 - 6.2 \cdot 10^9$
Erythrocyte vesicle count	$7.0 - 8.6 \cdot 10^{10}$
Reticulocyte vesicle count	$3.9 - 15.6 \cdot 10^8$
Leukocyte vesicle count	$6.2 - 16.4 \cdot 10^7$
Total vesicle count	$7.3 - 9.4 \cdot 10^{10}$

Fictive values, PhD thesis van der Pol *UvA* 2015

Outline lunchlezing

- ✔ Extracellular vesicles
- From artifact… *(2012)*
- …to artifact... *(2022)*
- …to clinical act



Flow cytometry

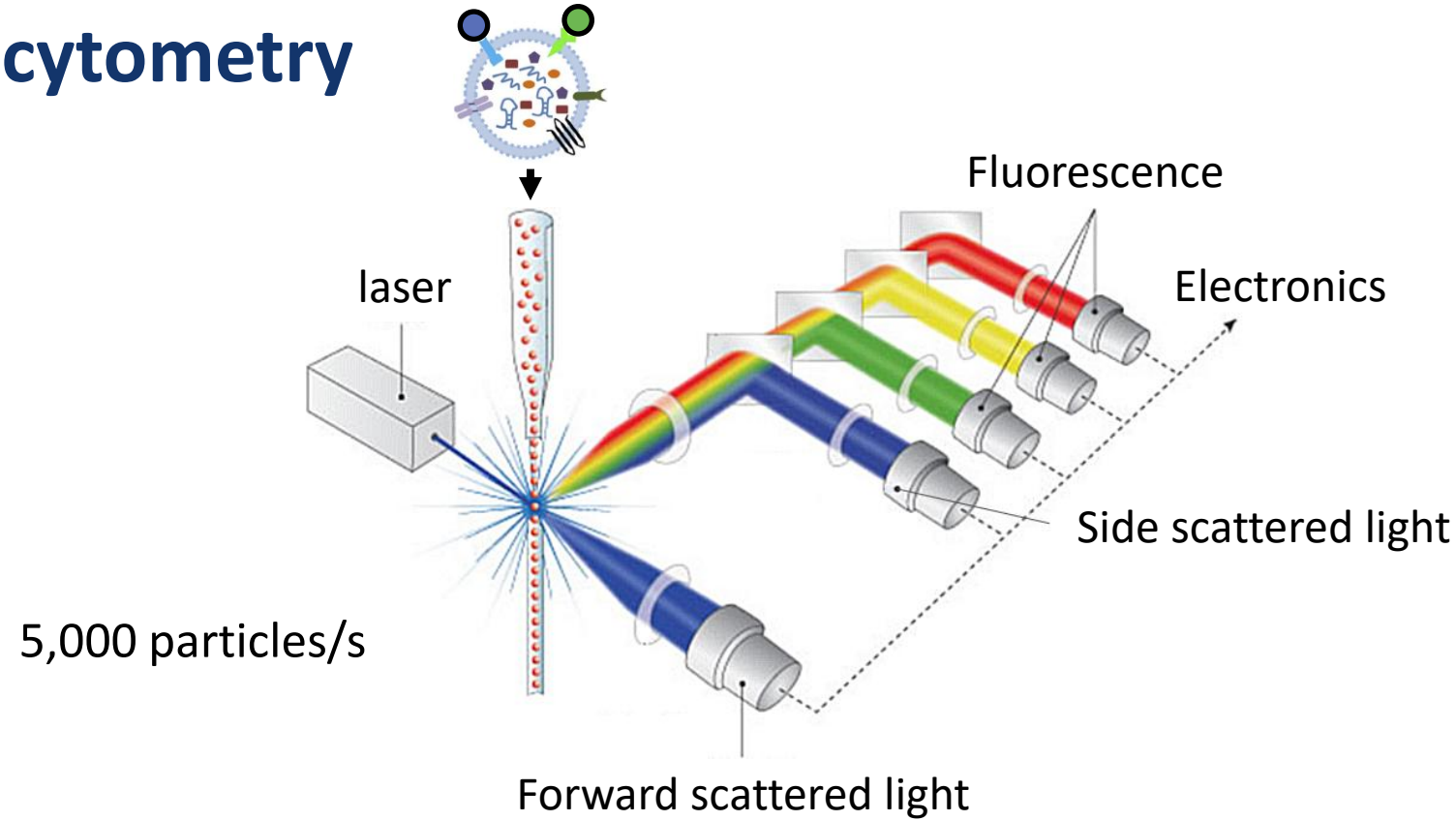
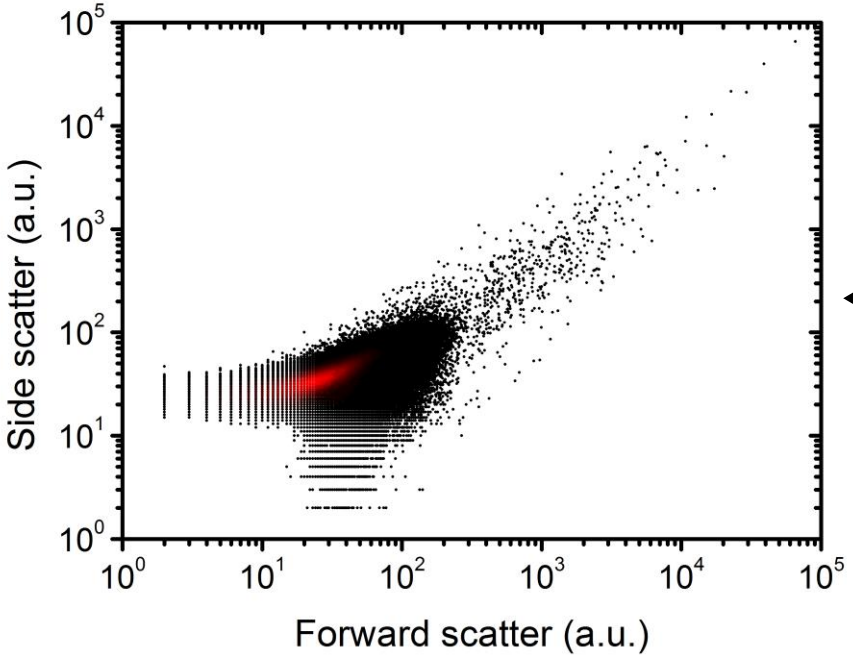
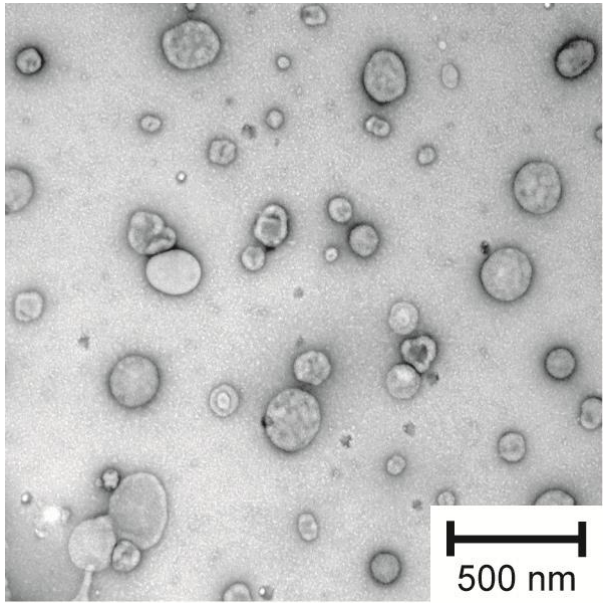


Figure adapted from semrock.com

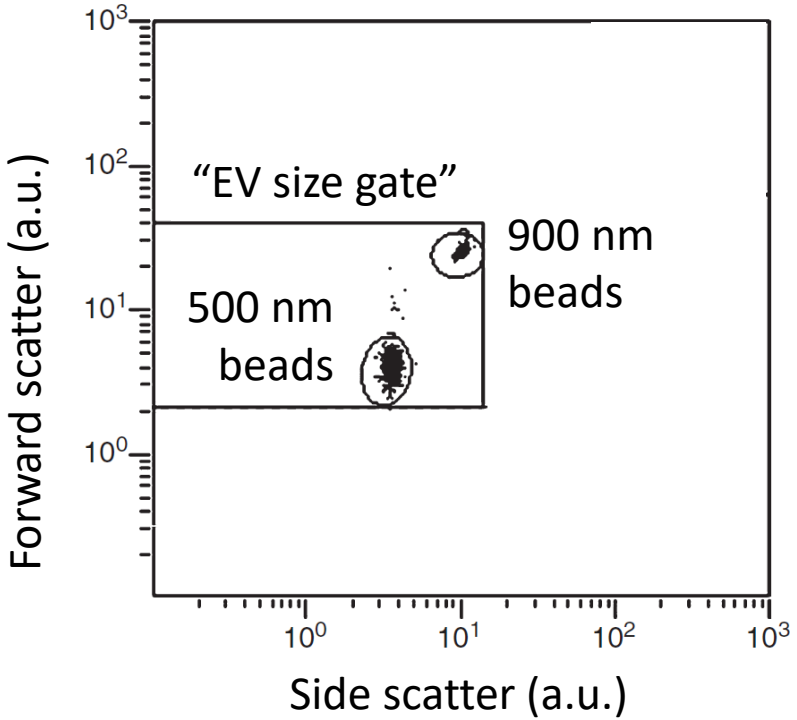
How do we know that we are counting EVs?



?



EV size gate based on polystyrene beads



- Introduced in 2008
- Common practice
- Bad practice

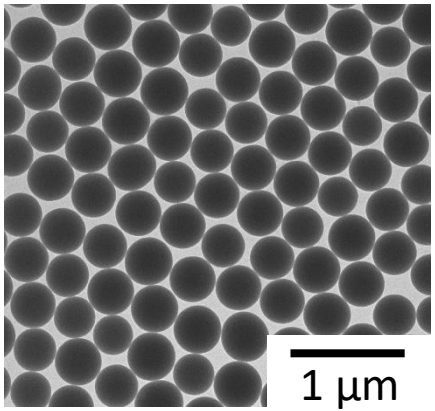
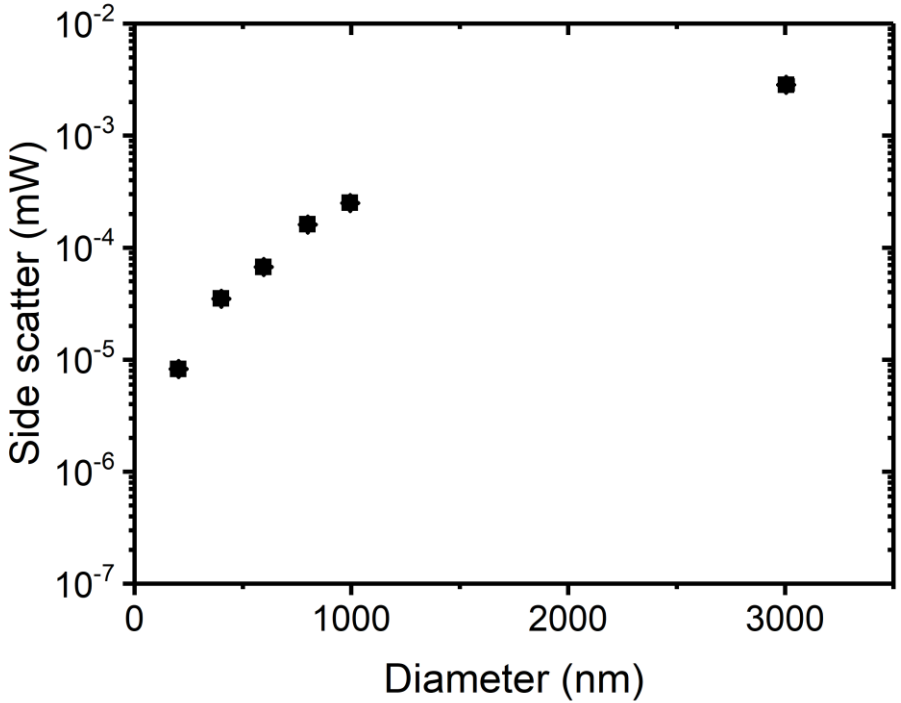
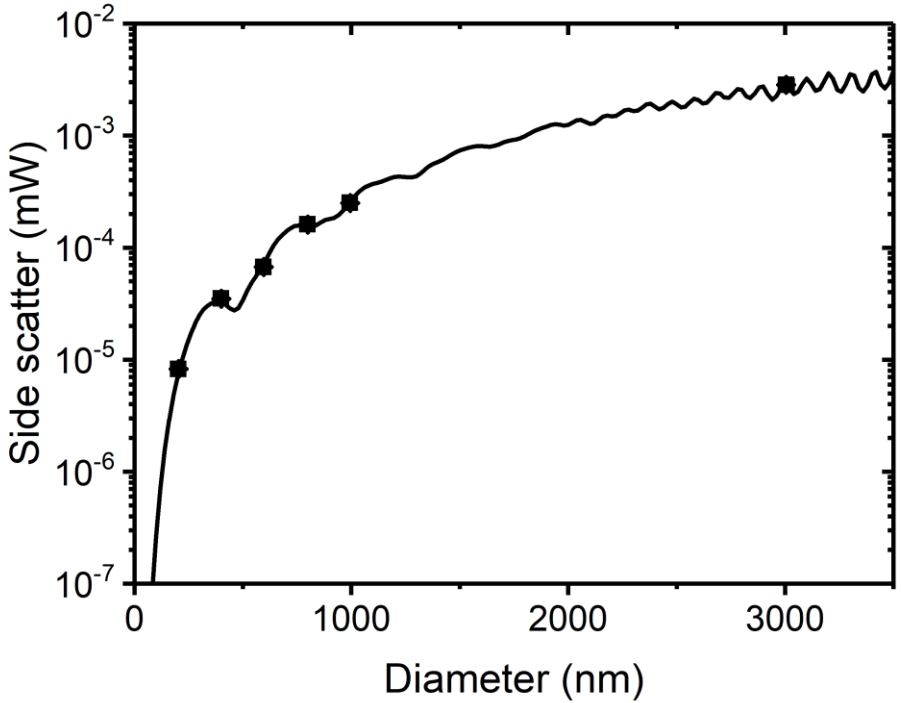


image adopted: Robert et al. *J Thromb Haemost* 2008

Relate scatter to diameter of beads



Relate scatter to diameter of beads

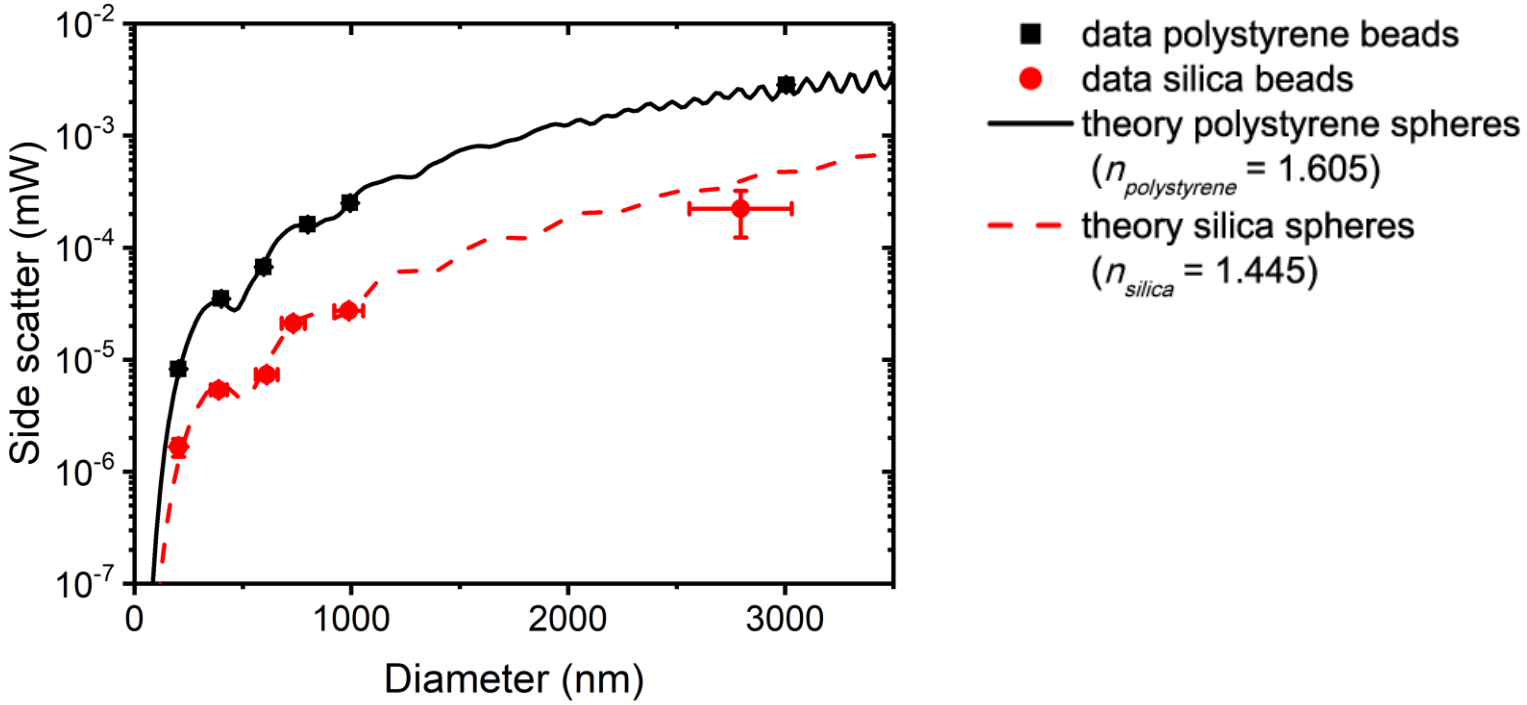


■ data polystyrene beads

—— theory polystyrene spheres ($n_{polystyrene}$ = 1.605)

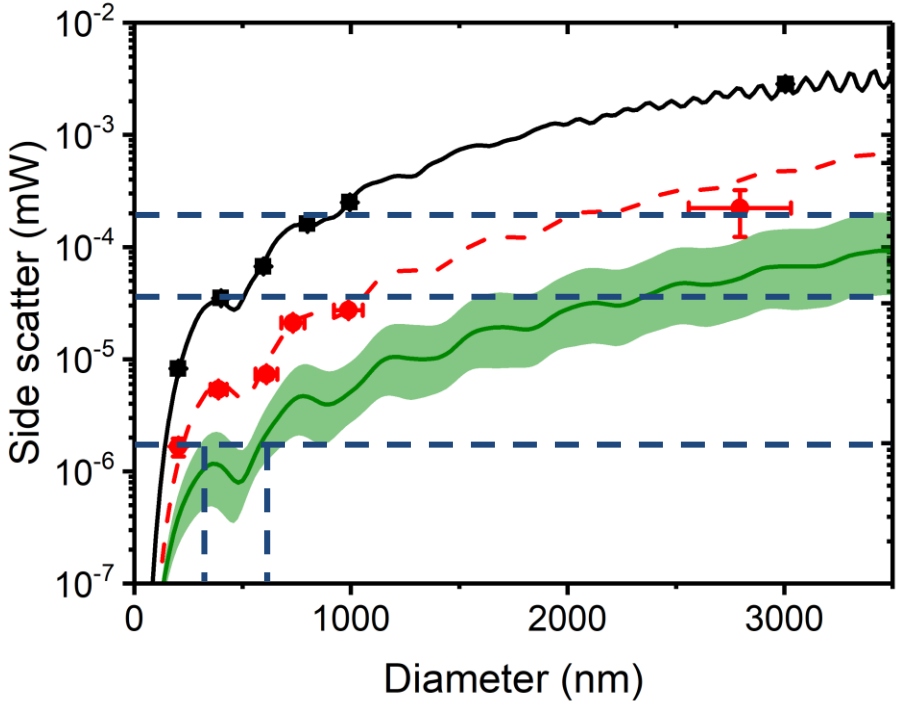
Model: de Rond et al. *Curr Protoc Cytom* 2018

Relate scatter to diameter of beads

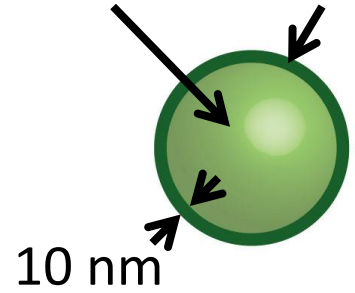


Model: de Rond et al. *Curr Protoc Cytom* 2018

Relate scatter to diameter of EVs

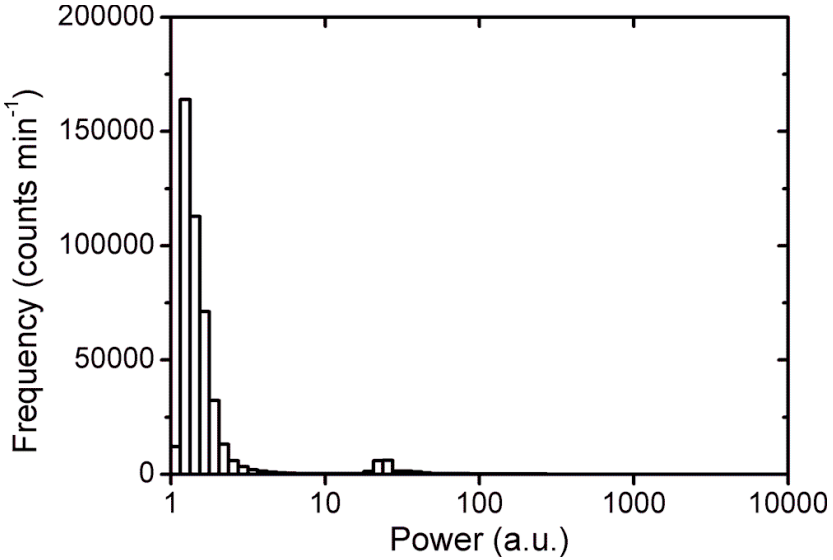


data polystyrene beads
data silica beads
theory polystyrene spheres ($n_{polystyrene}$ = 1.605)
theory silica spheres (n_{silica} = 1.445)
theory vesicles (n_{core} = 1.38±0.02, n_{shell} = 1.48)

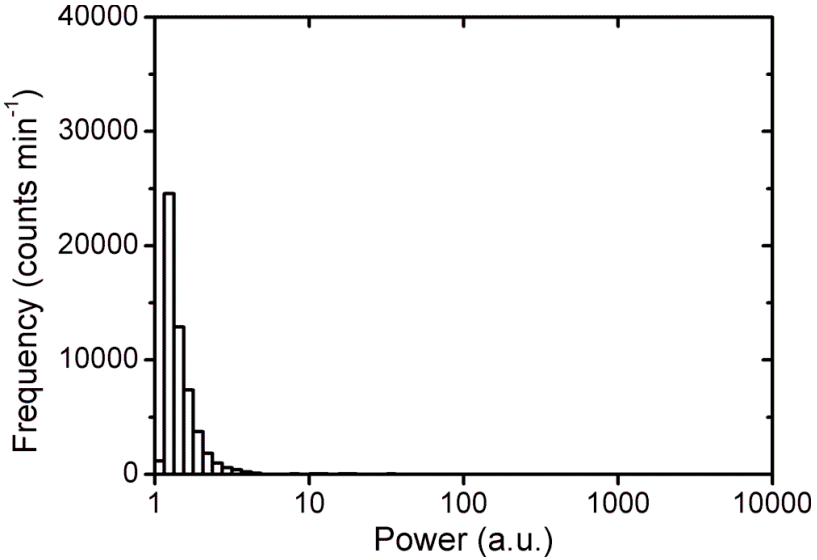


Particles below detection limit are detected

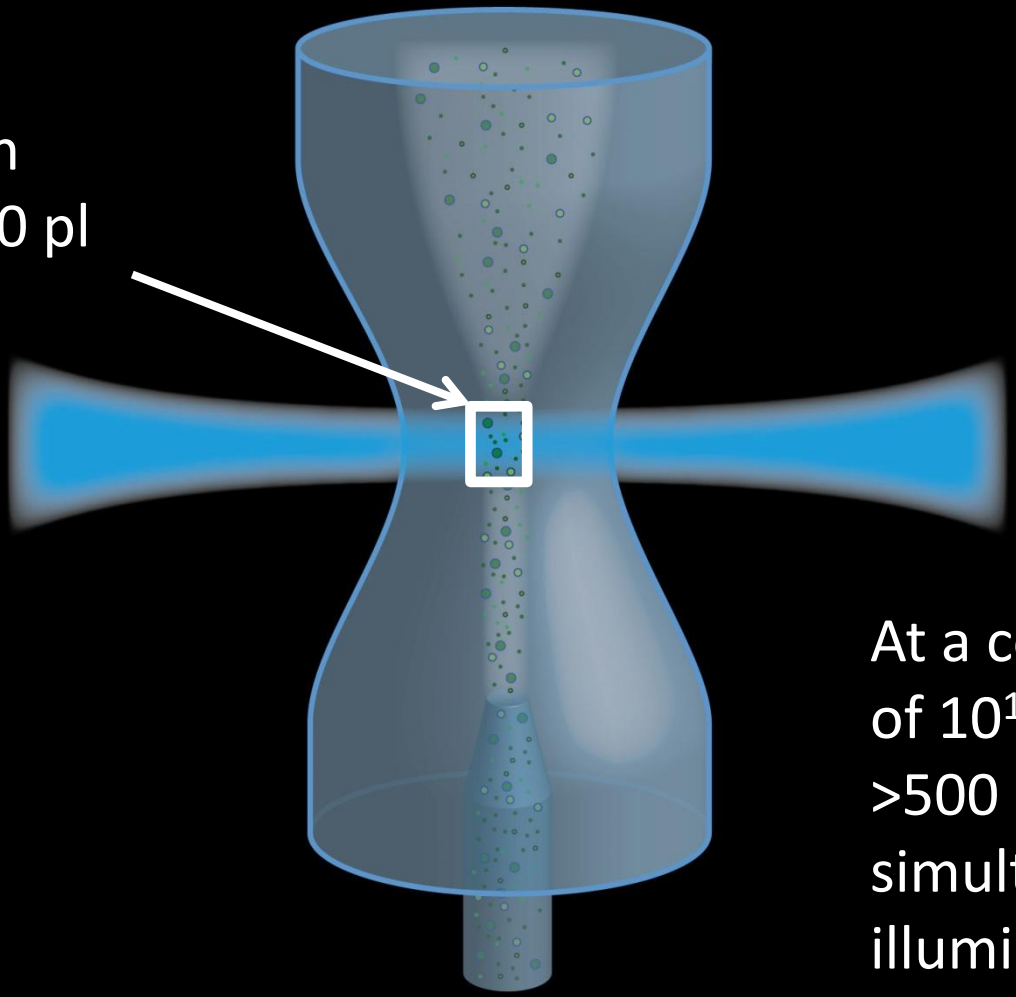
89 nm silica beads
(10^{10} ml^{-1})



220 nm filtered urine
(10^{10} EVs ml^{-1})



illumination
volume ≈ 50 pl



At a concentration of 10^{10} EVs ml^{-1}, >500 EVs are simultaneously illuminated

Outline lunchlezing

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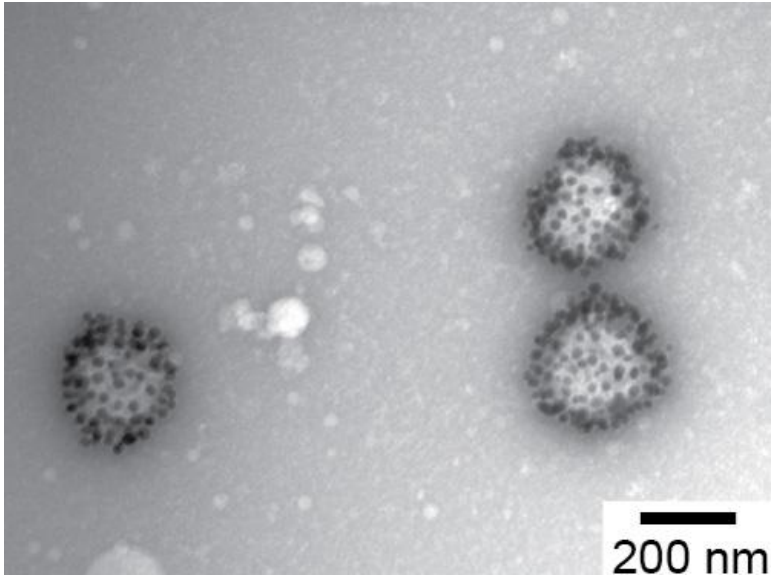
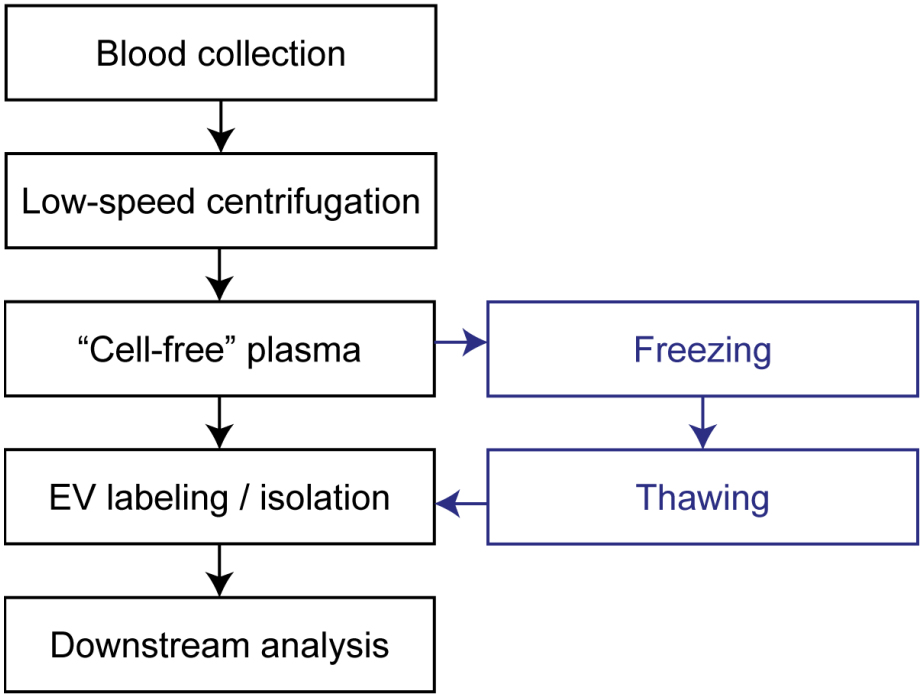
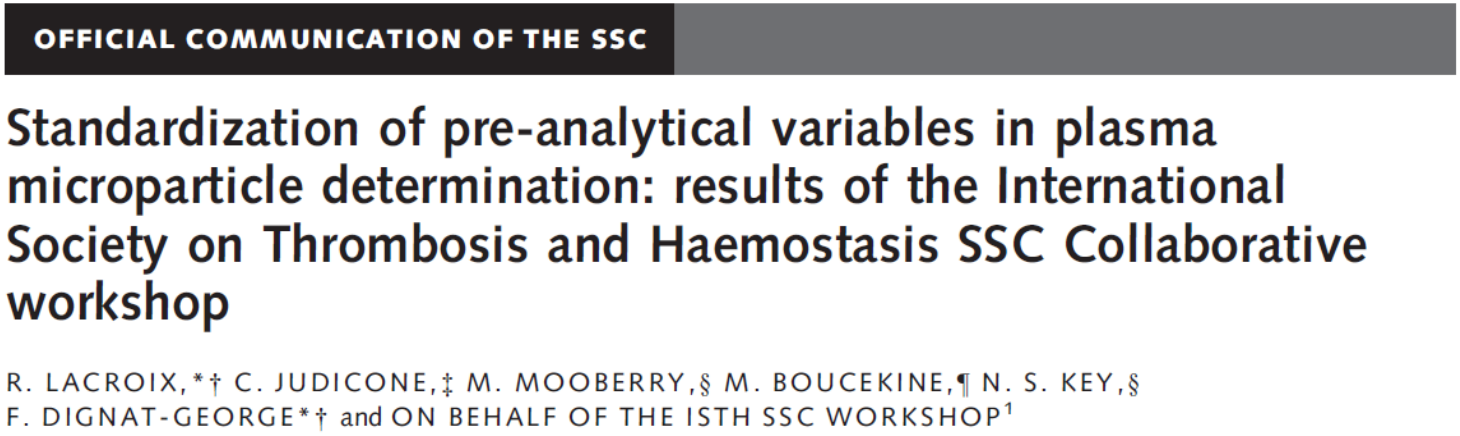


Experiment EVs in human blood anno 2022



Plasma preparation



OFFICIAL COMMUNICATION OF THE SSC

Standardization of pre-analytical variables in plasma microparticle determination: results of the International Society on Thrombosis and Haemostasis SSC Collaborative workshop

R. LACROIX,*† C. JUDICONE,‡ M. MOOBERRY,§ M. BOUCEKINE,¶ N. S. KEY,§
F. DIGNAT-GEORGE*† and ON BEHALF OF THE ISTH SSC WORKSHOP[1]

- Remove cells and platelets by using 2 subsequent centrifugations steps of 2,500 *g* for 15 minutes

Lacroix et al. *J Thromb Haemost* 2013
Coumans et al. *Circ Res* 2017

Are all cells and platelets removed?

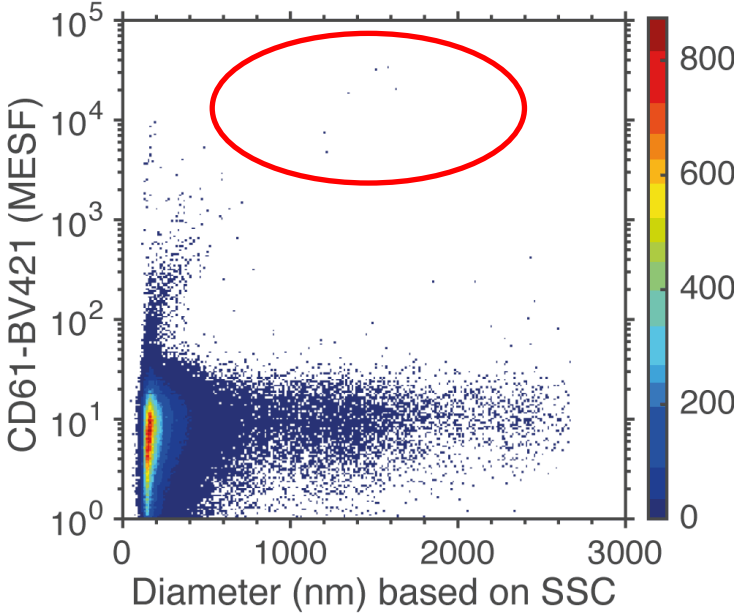
- Hematology analyzer
  - 0 cells
  - 0 platelets
- Concentration detection limit
  - $3.0 \cdot 10^6$ platelets mL^{-1}

Serendipity: study with a broken centrifuge

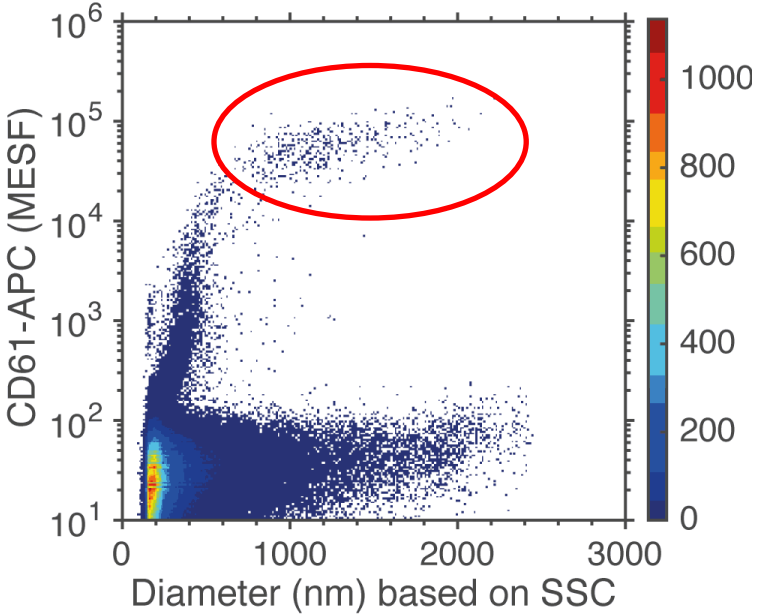
Left image: upenn.edu

Residual platelets in plasma identified

Normal

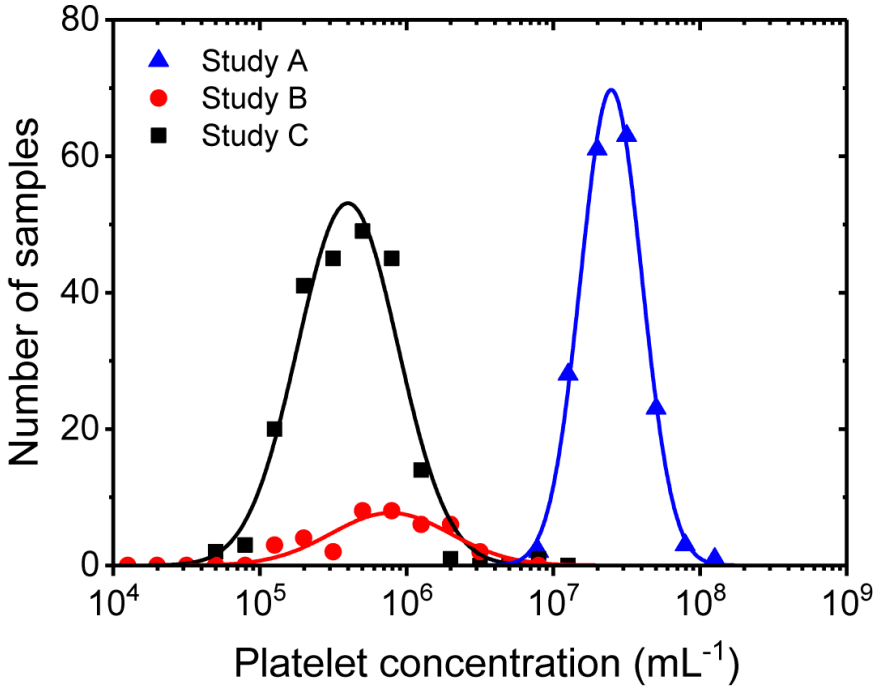


Unmaintained centrifuge



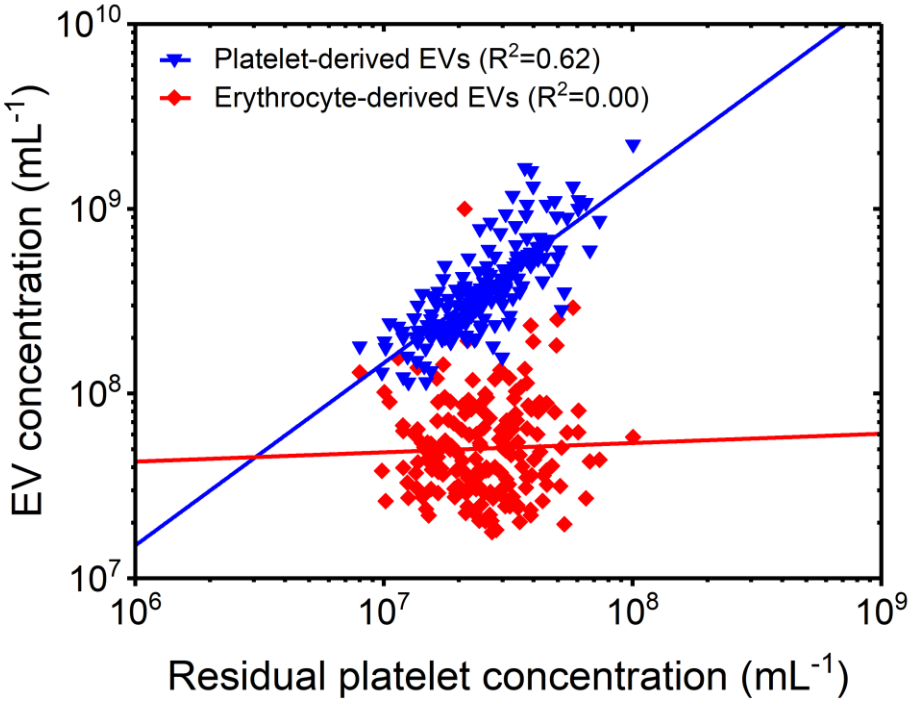
Right data: Gasecka et al. *J Thromb Haemost* 2020

Blood plasma contains residual platelets



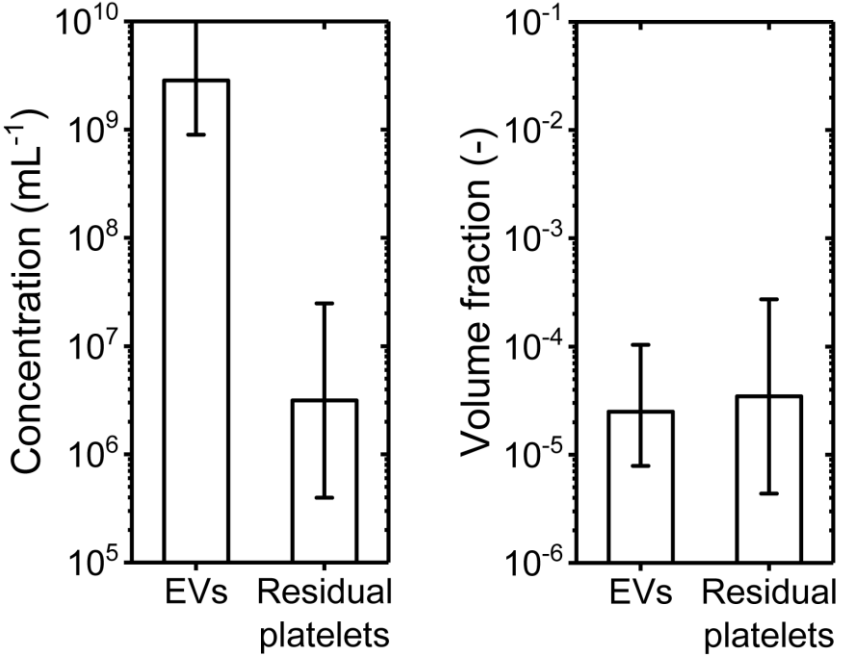
- Problem for:
  - EV flow cytometry?
  - Other down-stream analyses?

Residual platelets affect EV flow cytometry results



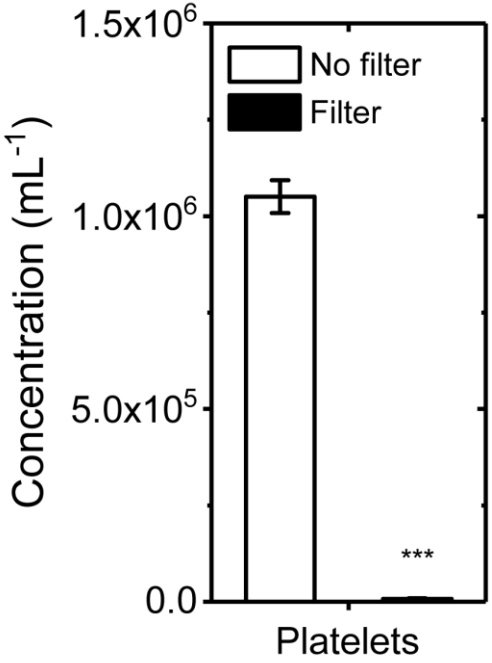
- After freeze thawing:
  - 16 CD61+ EVs per mL per platelet
  - Diameter >160 nm
  - CD61-APC >150 MESF

Residual platelets likely affect –omics results



- ISEV 2022 late breaking
  - Jillian Bracht et al. *Residual platelets affect EV-associated miRNA results*

Solution: track-edged polycarbonate 800-nm filters



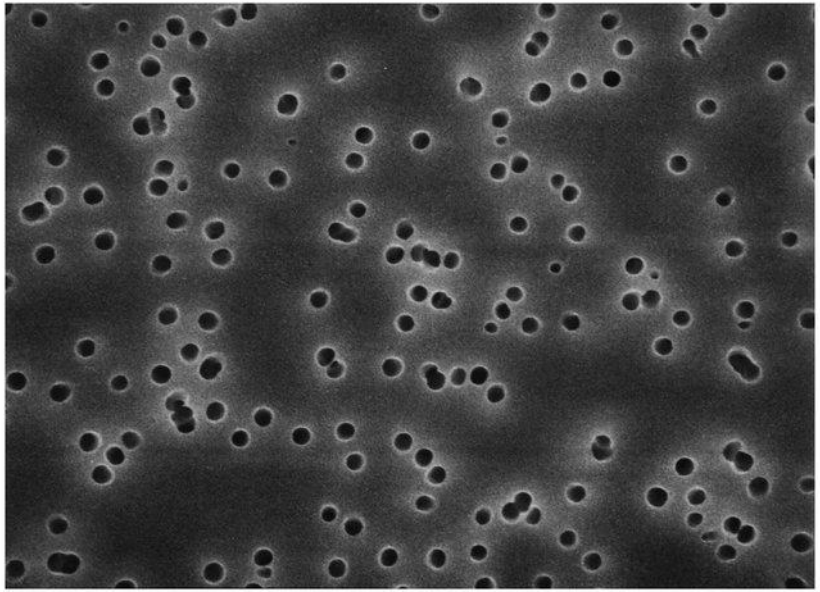
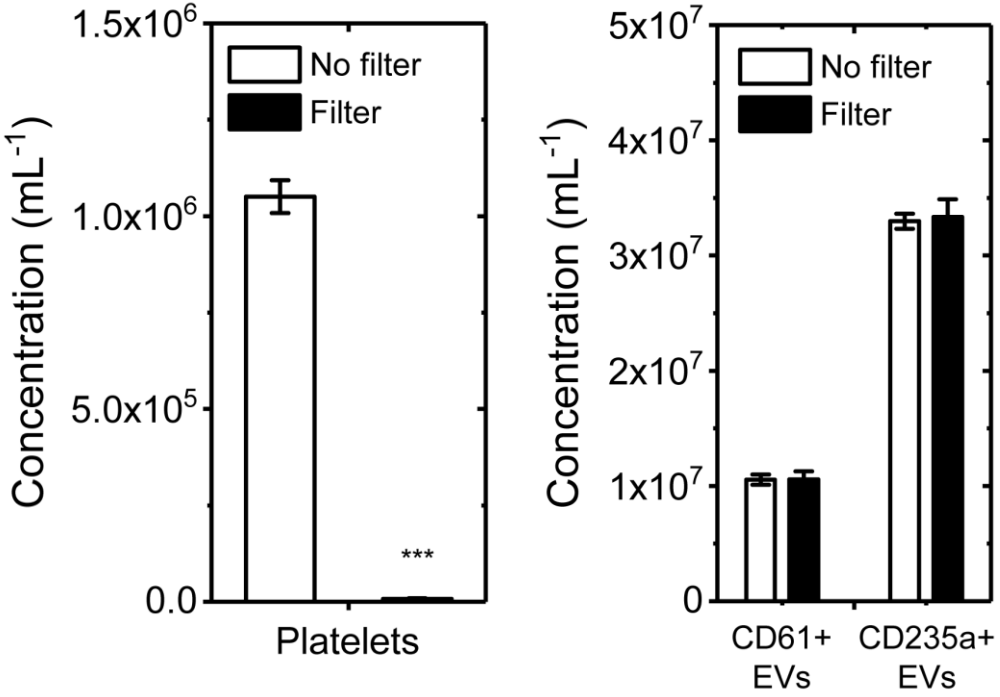


Image: fishersci.com, showing 10-µm pores

Solution: track-edged polycarbonate 800-nm filters

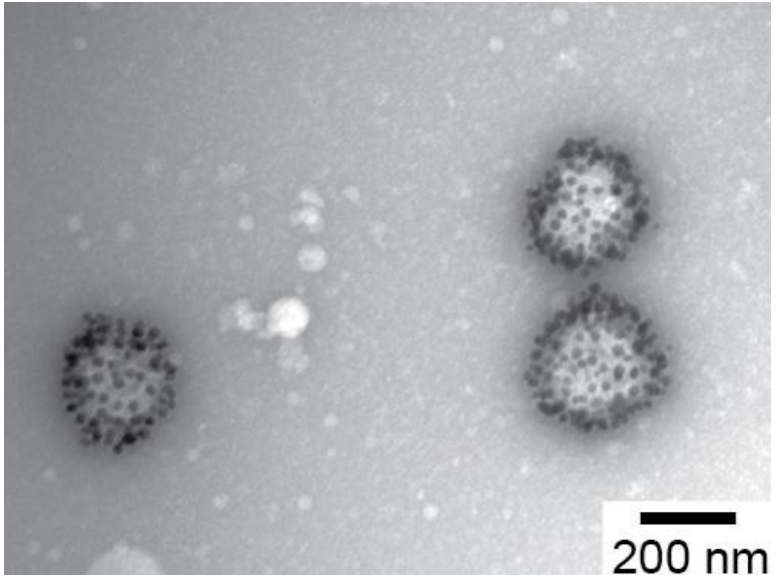


ISEV 2022

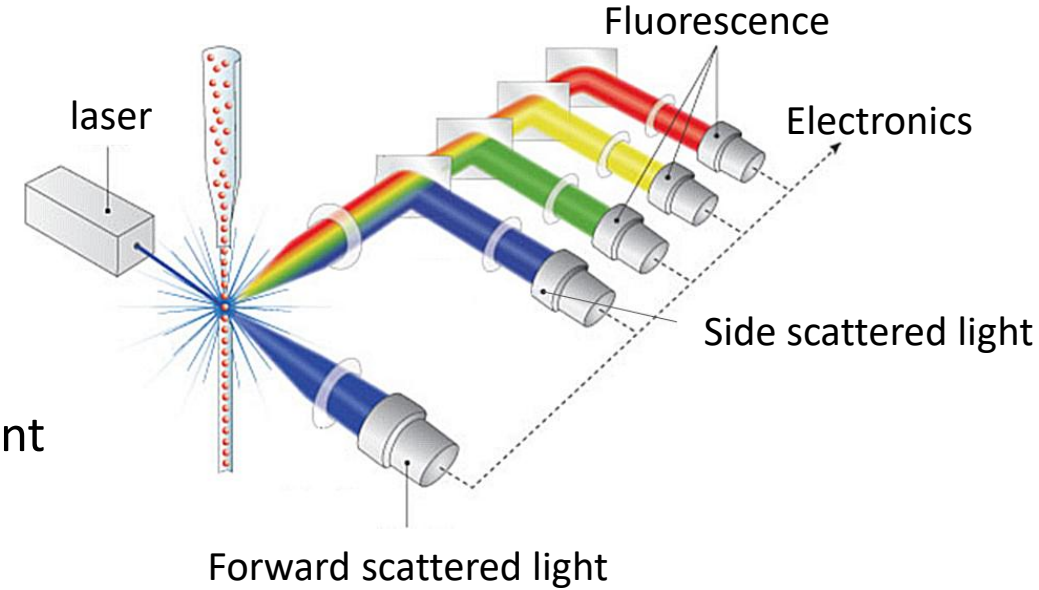
- Britta Bettin et al. *Remove all residual platelets from blood plasma*

Outline lunchlezing

- Extracellular vesicles
- From artifact… *(2012)*
- …to artifact... *(2022)*
- …to clinical act



Flow cytometry

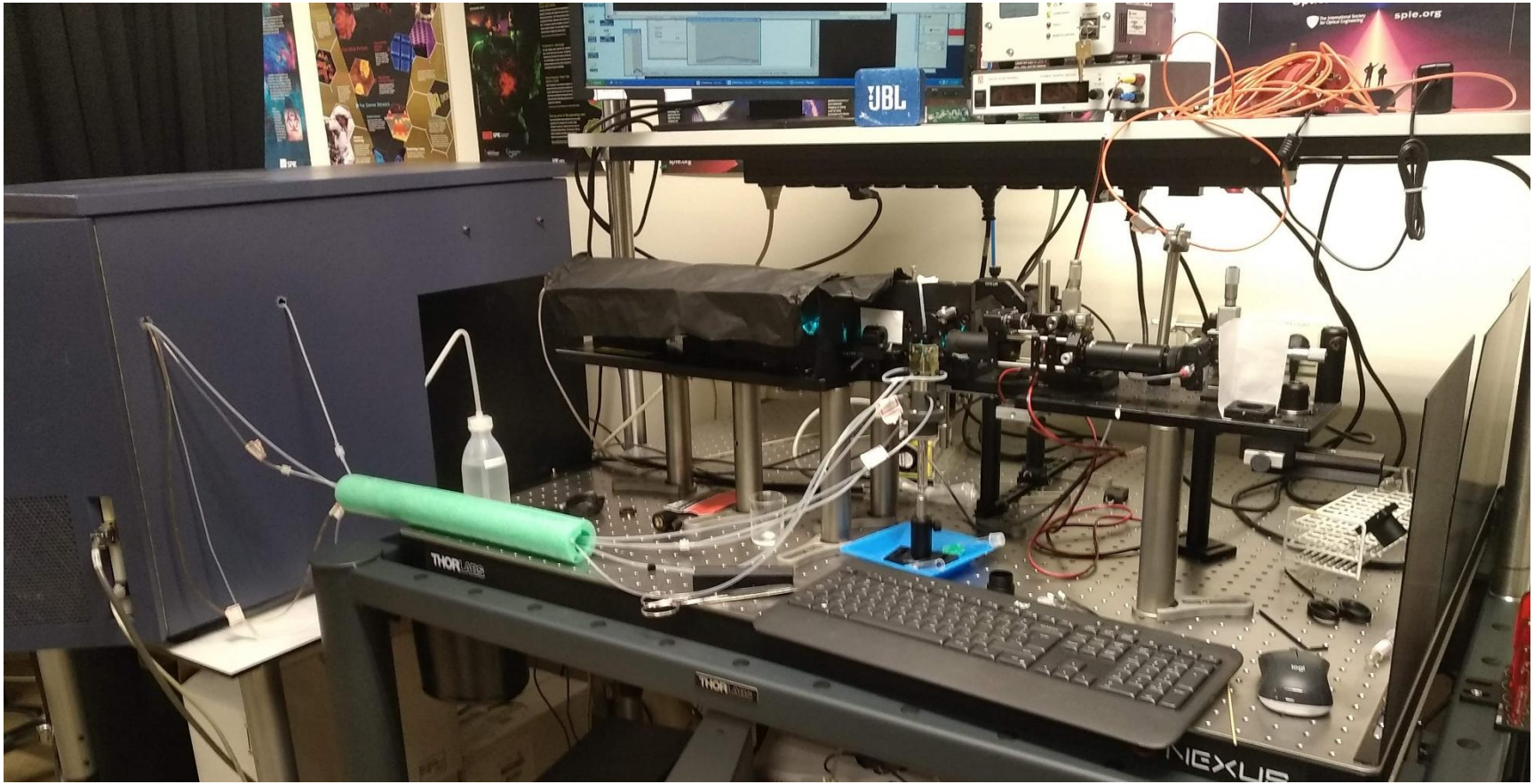


100 clinical samples per day

500,000 particles per measurement

- Diameter (nm)
- Refractive index
- Cellular origin and state
- Concentration (mL^{-1})

Figure adapted from semrock.com


JBL
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THORLABS
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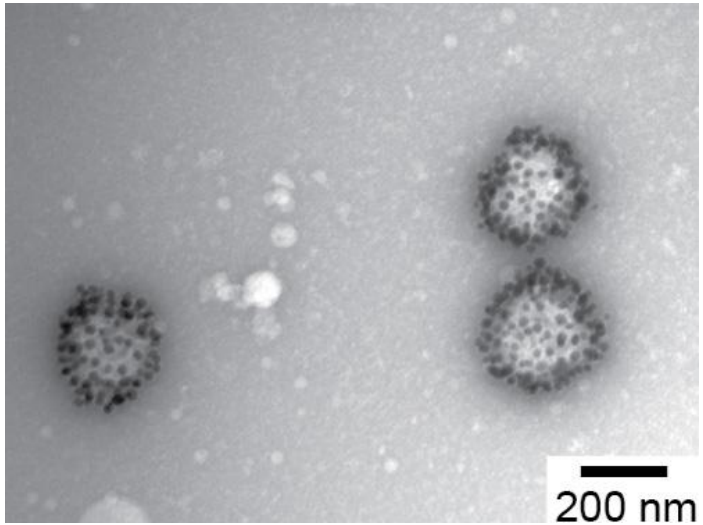
Outlook

- Hematology reference values of EV concentrations
- Early recognition of cause of stroke (Naomi Buntsma)

- Standardization of EV measurements
  (Martine Kuiper, Britta Bettin, De Qing Görtzen)
- Quantifying quality of EV isolation methods
  (Heleen ter Kuile)
- Detect EVs 1,000-fold faster (Biomedical Engineering)

Take home messages

- Body fluids contain extracellular vesicles (EVs): potential disease biomarkers
- Before applying assay in clinic: understand measured signals!
  - Calibrate: avoid arbitrary units
  - Standardize
  - Use controls



Acknowledgements

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  - Biomedical Engineering & Physics
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- More information
  - edwinvanderpol.com
  - exometry.com
  - metves.eu
  - isev.org