Flow Cytometry for Studying Exosomes and Extracellular Vesicles

SelectBIO Webinar

March 7, 2024

Giacomo Vacca, Ph.D.
Founder & CEO, Kinetic River

Prof. Christopher Ward
U. of Kansas Medical Center

Today's Webinar

Introduction to the *Delaware* Flow NanoCytometer® G. Vacca

- Features and capabilities
- Results to date
- Configurations

Toward Flow-Based Rapid Diagnostics of Kidney Disease C. Ward

- Polycystic Kidney Disease (PKD)
- Current methods and unmet needs
- Urine extracellular vesicles (EVs) as PKD biomarkers
- Noninvasive PKD dia/prognostics with ultrasensitive flow cytometry

Q&A

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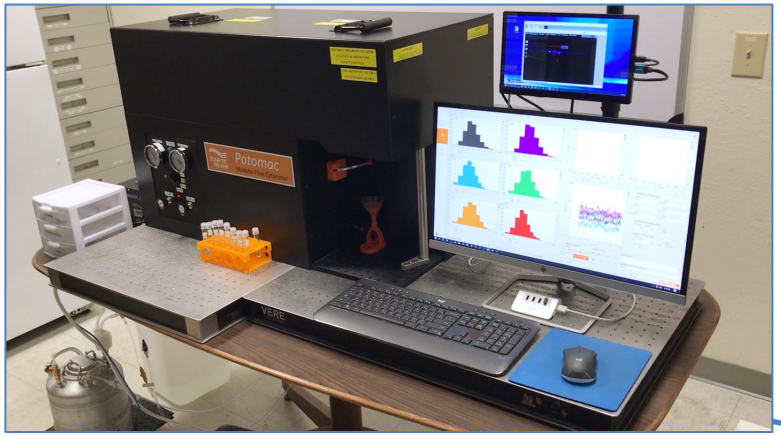

- flow cytometers
- optical instrumentation
- design & development
- 24 issued patents, 7 pending
- past customers include: NIH-NCI, Stanford, UC Davis, Mt. Sinai, Millipore, Sony, NMSU, National Research Council (Italy)

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Kinetic River Team

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Founder, President, & CEO

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Principal Scientist

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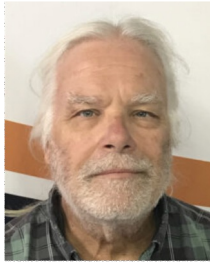
System Engineering Consultant



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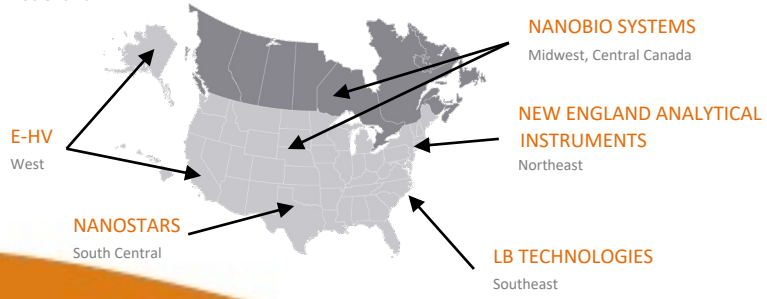
ASHLEY SLOAT, PHD
Advisor, Aurora Consulting

BONNIE SUN, BS
Office Administrator

CytoFlow Service
Distribution / Service Partner, Europe



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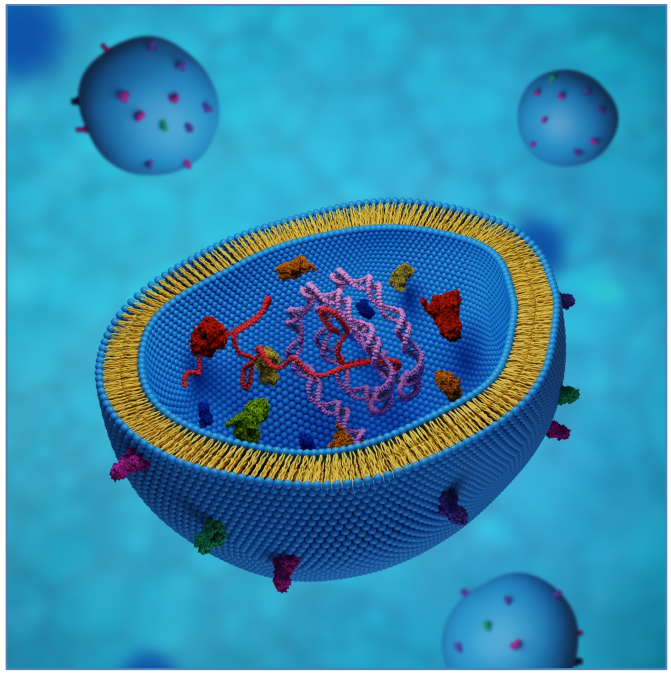
Delaware Flow NanoCytometer®

KINETIC RIVER®

What Matters in EV Analysis?

- Single-particle detection (not bulk/averages)
- Sensitivity (smaller particles)
- Multiparametric detection (simultaneous biomarkers)
- Throughput (greater accuracy)
- Flexibility (measuring a wide range of particle sizes)
- Stability (confidence in results)
- Ease of use (focus on measuring, not tweaking)



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Delaware Flow NanoCytometer® for EV Analysis

- high resolution *and* sensitivity
- stable, pressure-driven flow
- up to 5 lasers
- up to 6 fluorescence channels
- measures EVs *and* cells

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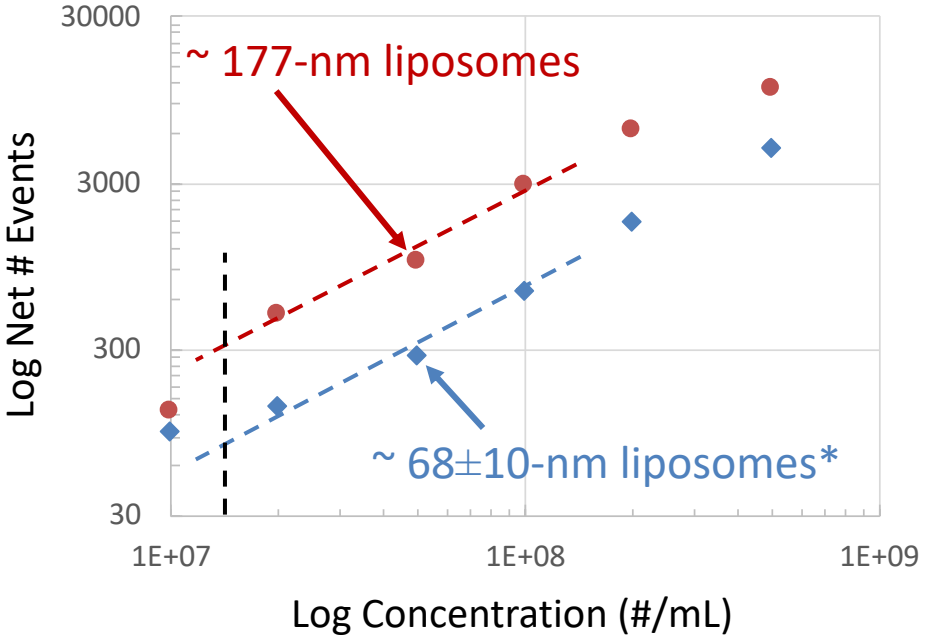
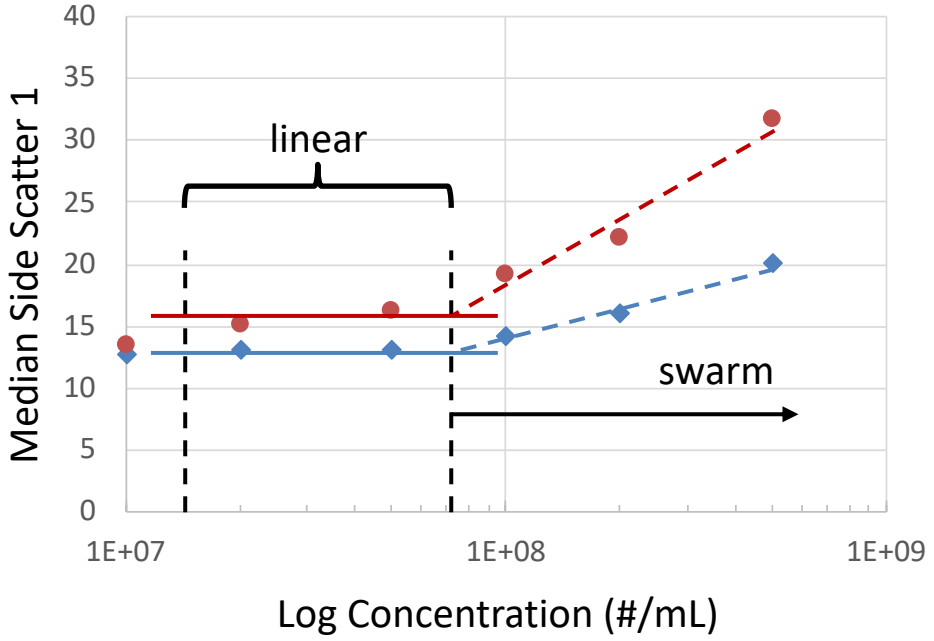
Delaware: Single-Particle Detection





- liposome standards: Acoerela AcoLS

ACOERELA

- sized by NanoFCM: 58 nm
- sized by ZetaView: 77 nm

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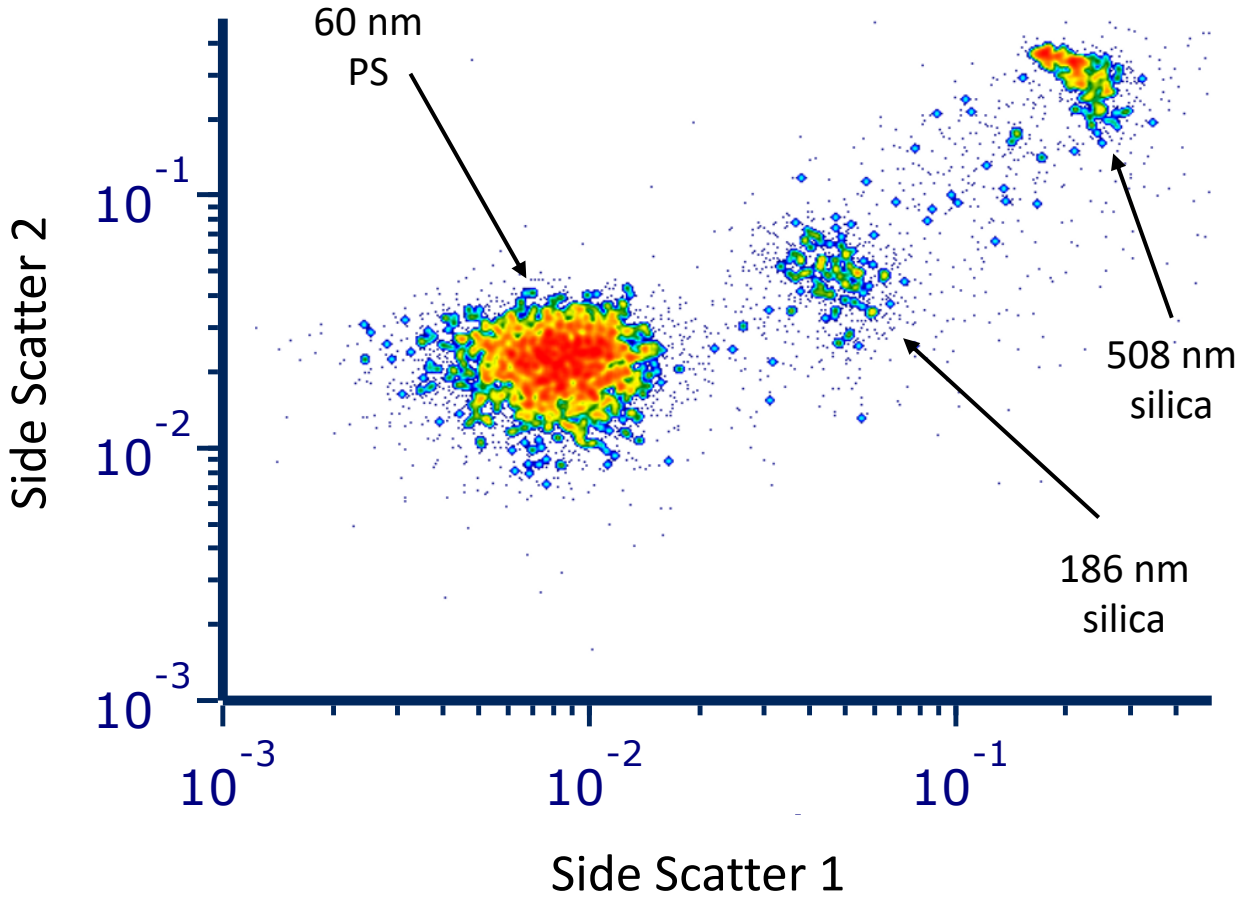
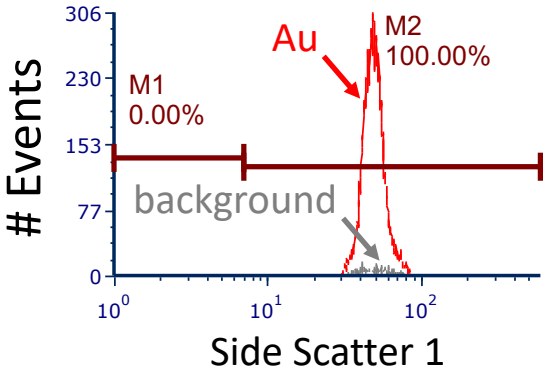
Delaware: 60-nm Scattering Sensitivity (PS NPs)



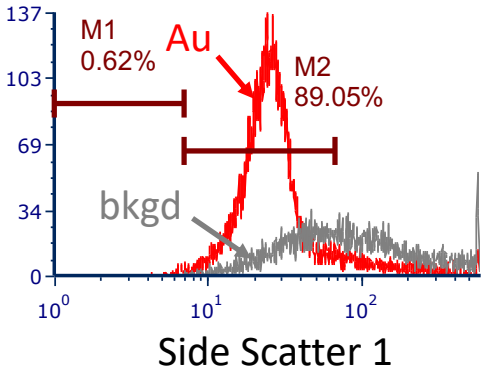
- silica: Alpha Nanotech
- polystyrene: Spherotech

Delaware: 28-nm Scattering Sensitivity (Au NPs)

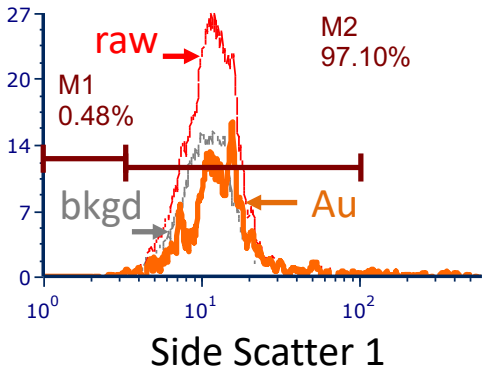
102 nm Gold

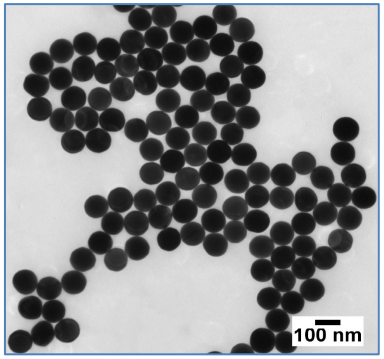


50 nm Gold



28 nm Gold





- Au NPs: nanoComposix
- sized by TEM

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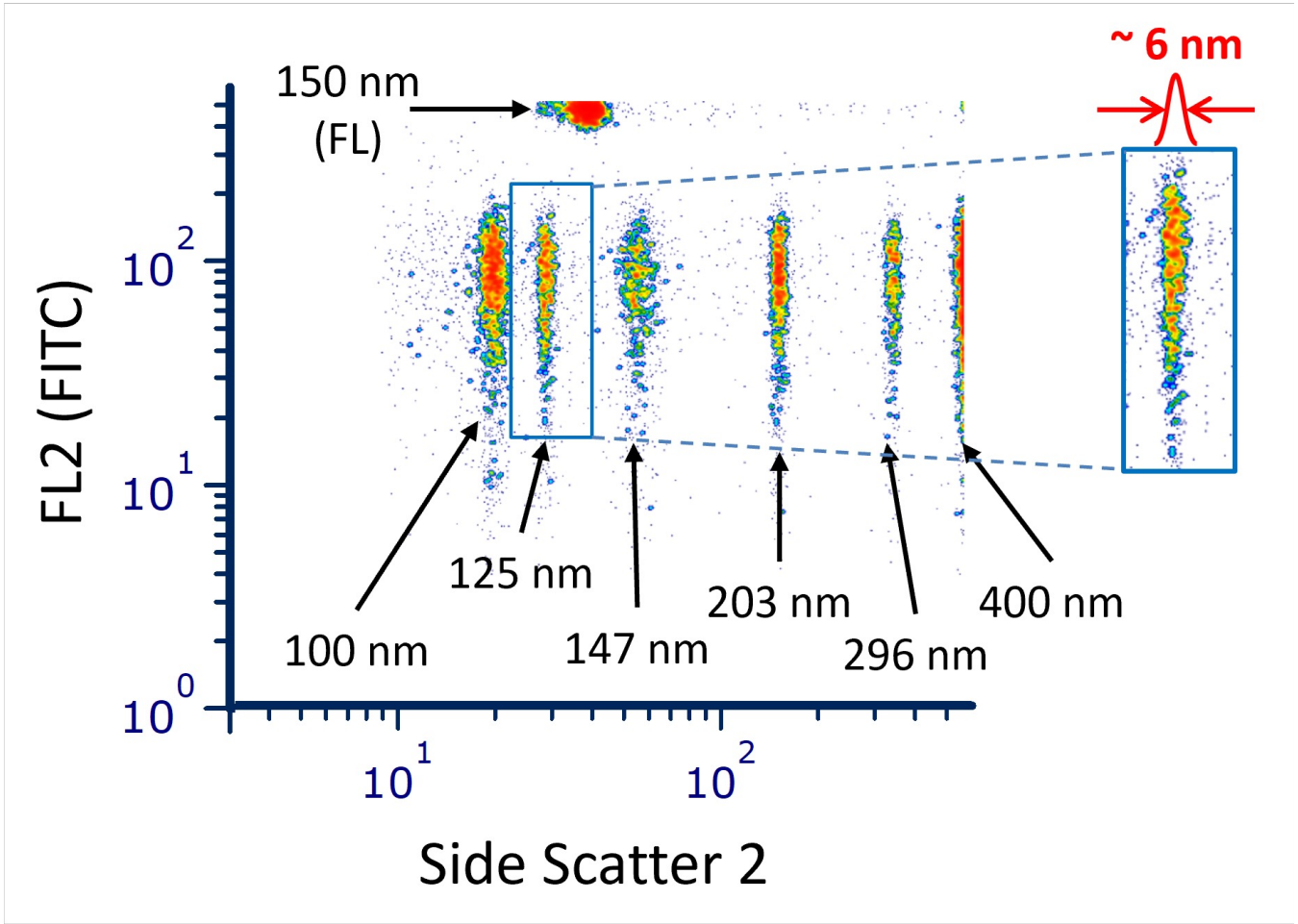
Delaware: 6-nm Resolution (2% CV)



- nanoparticles: Exometry Rosetta beads
- CV=σ/μ =FWHM/(2.36*μ)

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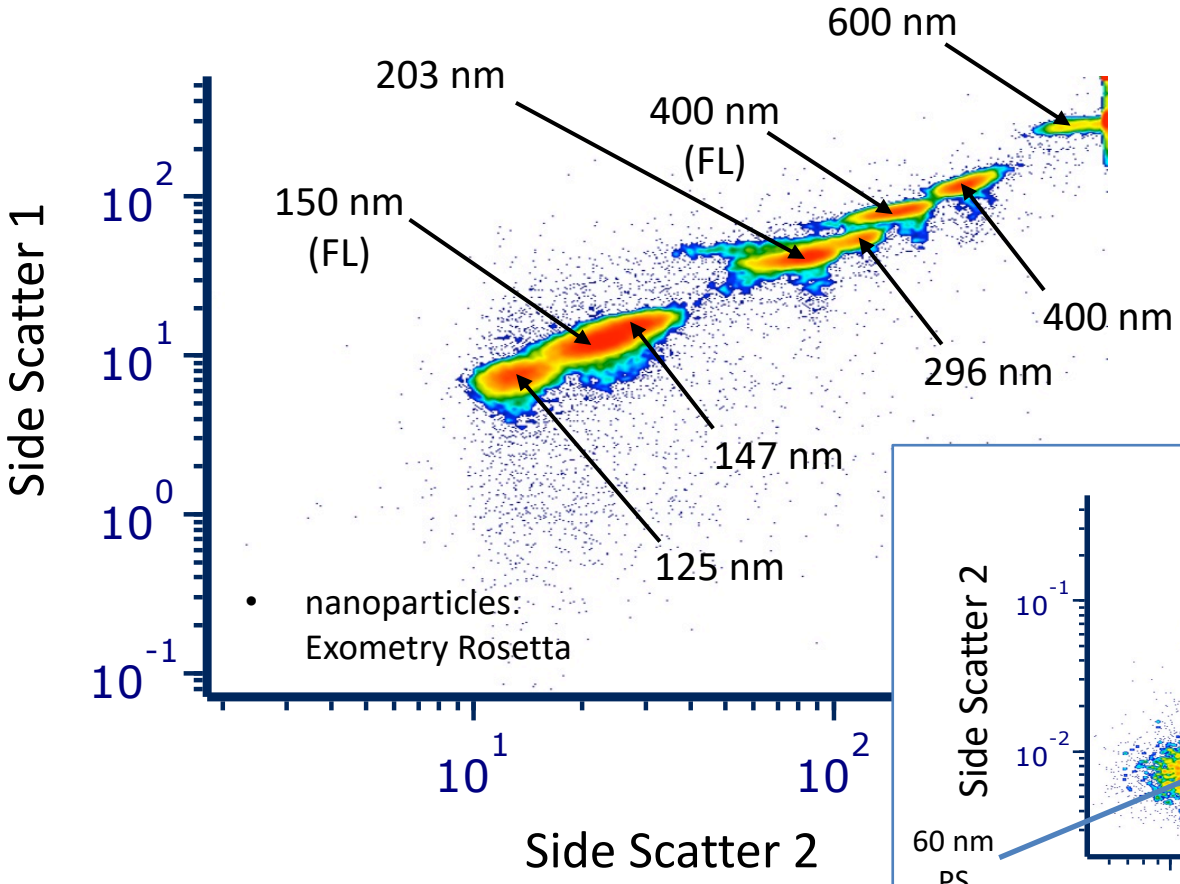
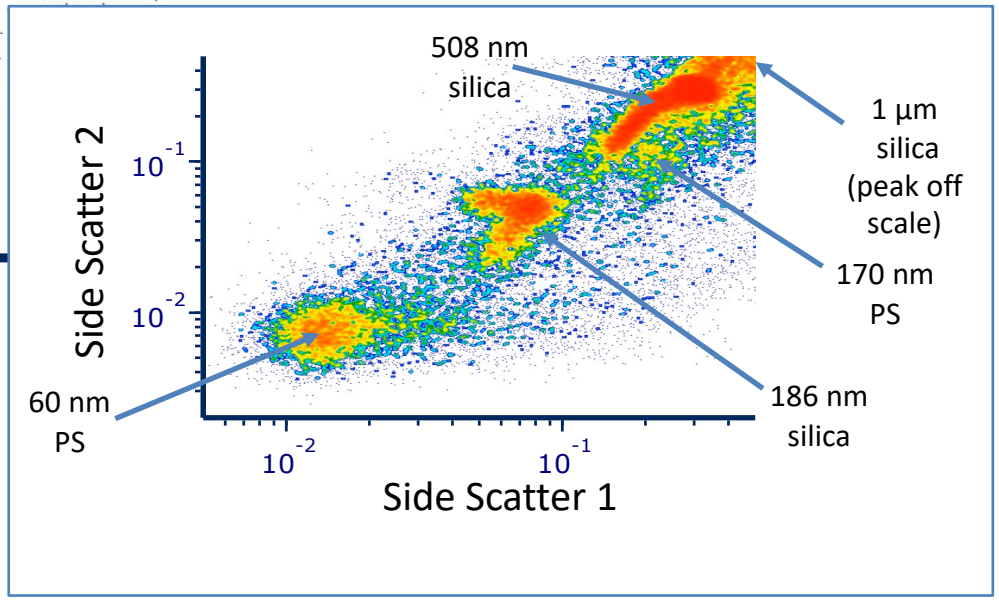
Delaware: Large Dynamic Range



- nanoparticles: Exometry Rosetta



- silica: Alpha Nanotech
- polystyrene: Spherotech

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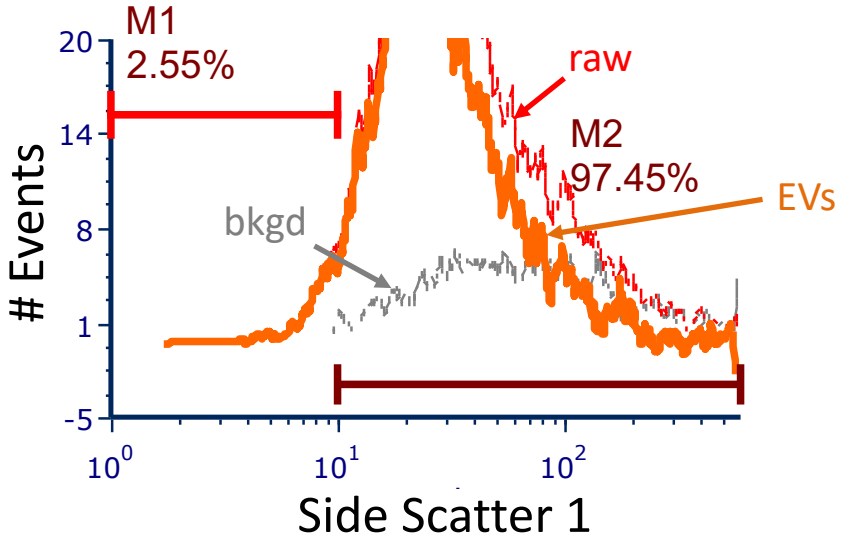
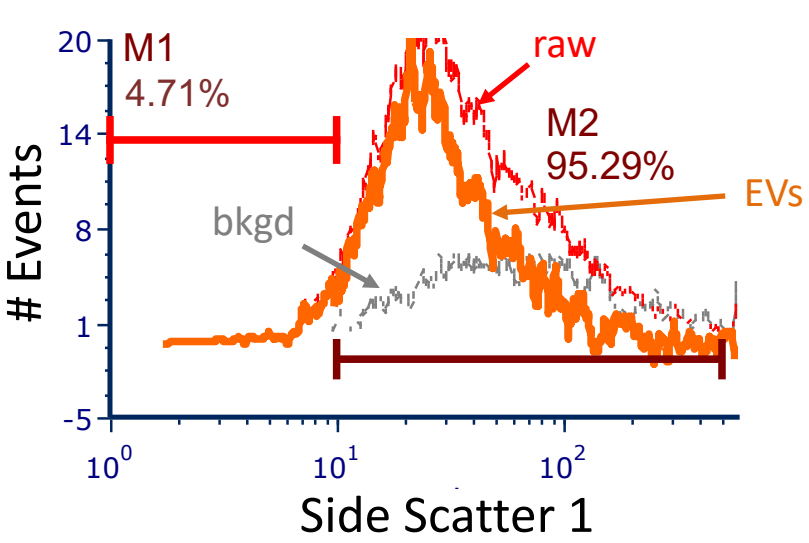
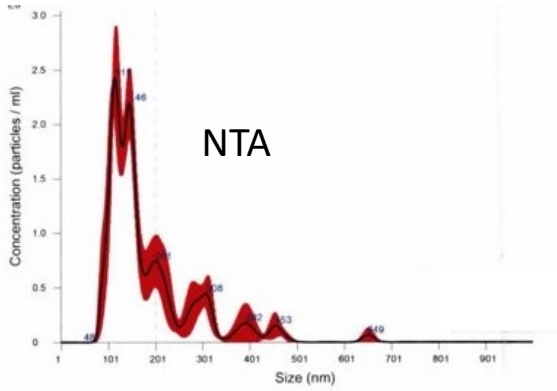
Delaware: Human Cell-Derived EVs (Scattering)

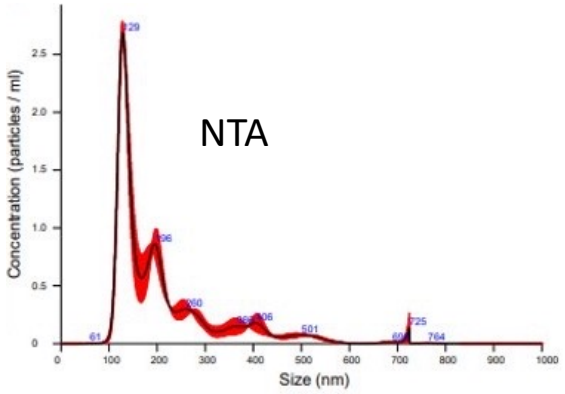
HeLa EVs



PC3 EVs



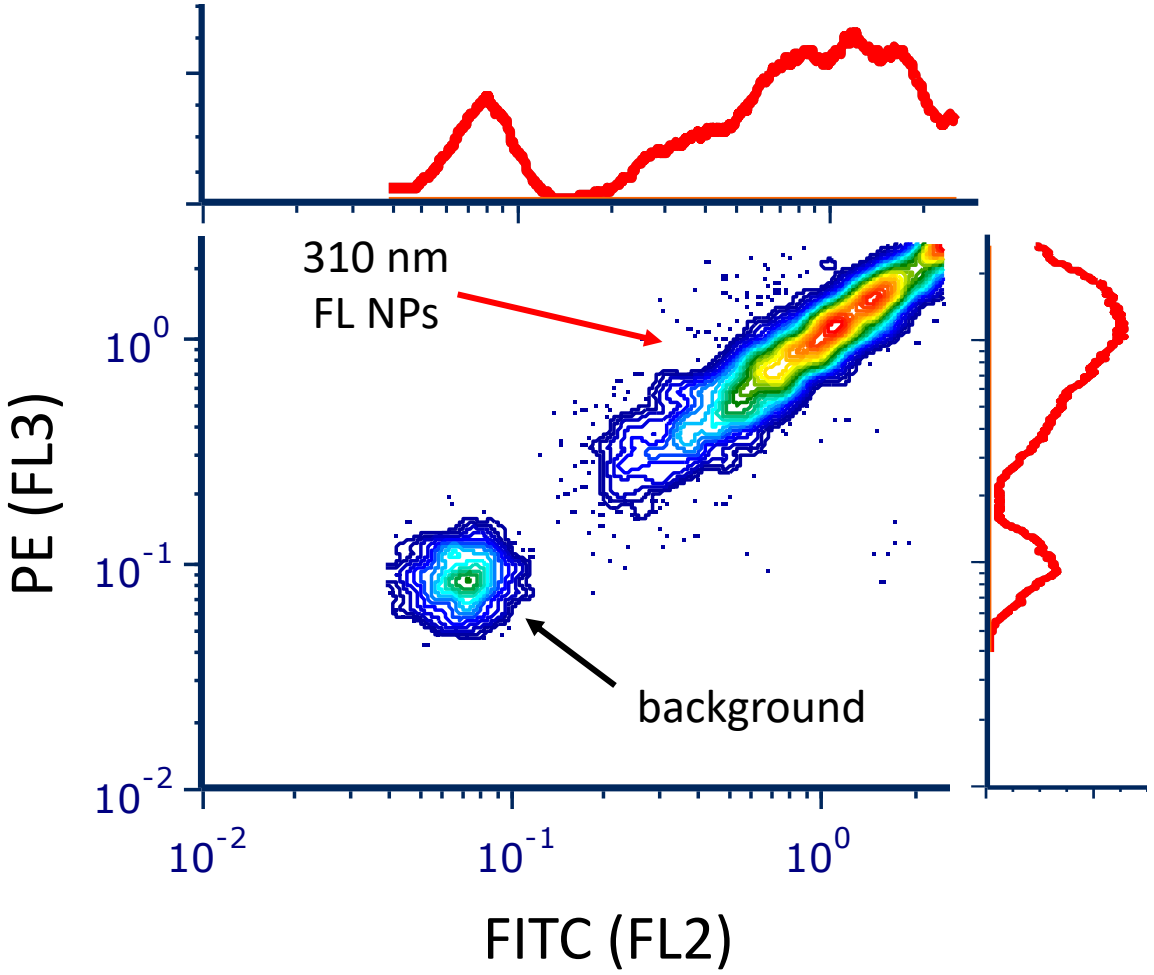




- EVs: Kashanchi Lab, GMU

Delaware: Up to 6 Fluorescence Channels

- multiparametric phenotyping
- flow: complete, simultaneous colocalization, all the time



- fluorescent nanoparticles: Spherotech UltraRainbow 310 nm

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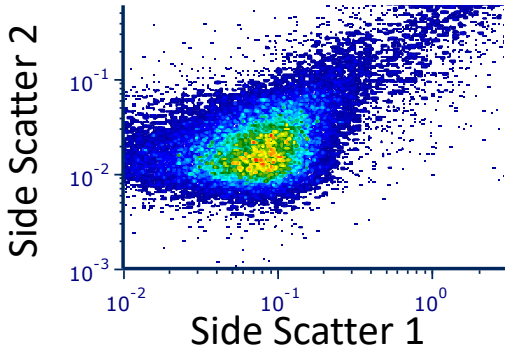
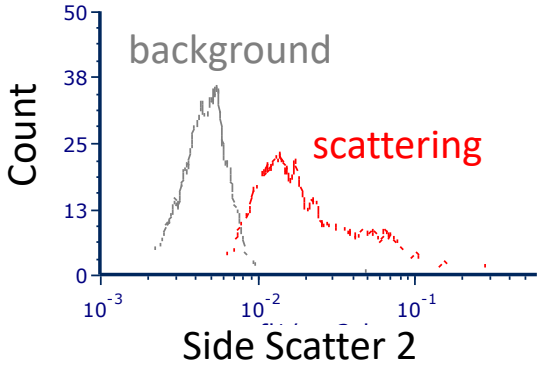
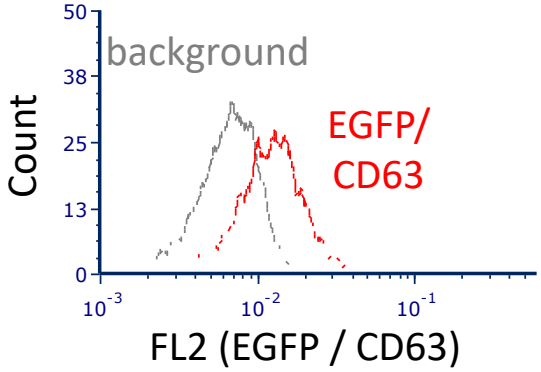
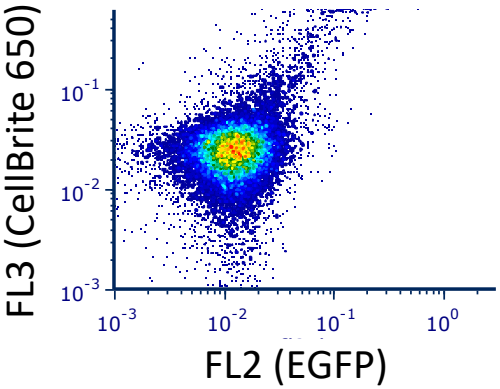
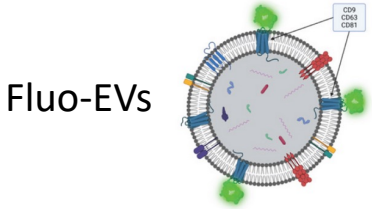
Delaware: Human Cell-Derived EVs (Scattering, FL)

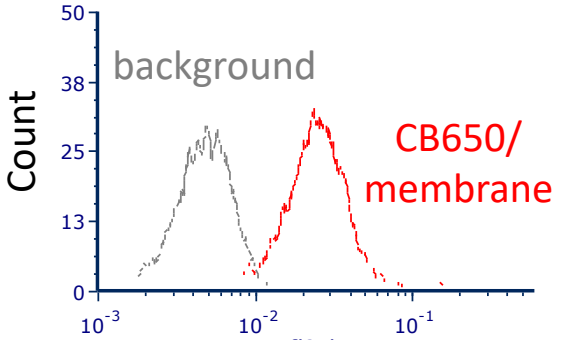


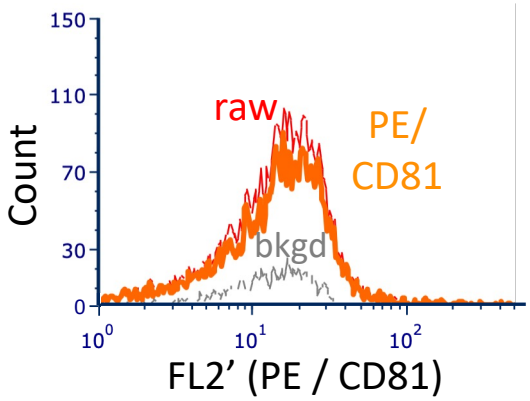












- EVs: HansaBioMed HEK293 FLuo-EVs
- ZetaView: ~ 90 nm
- run A: Biotium CellBrite Steady 650, EGFP/CD63
- run B: PE/CD81

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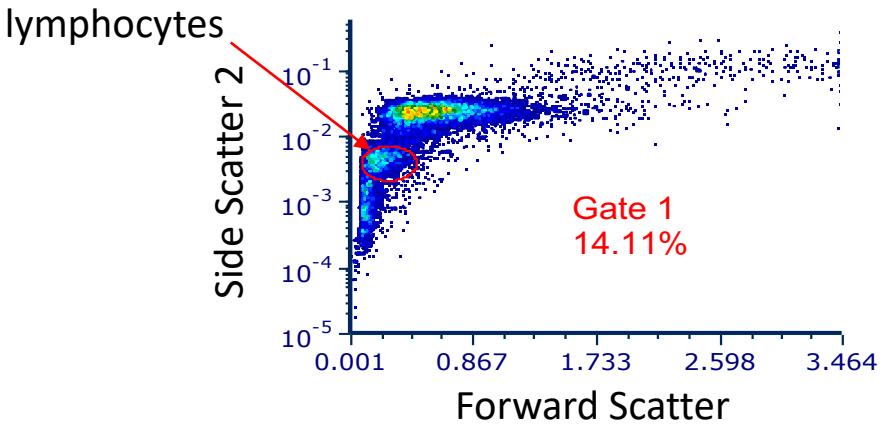
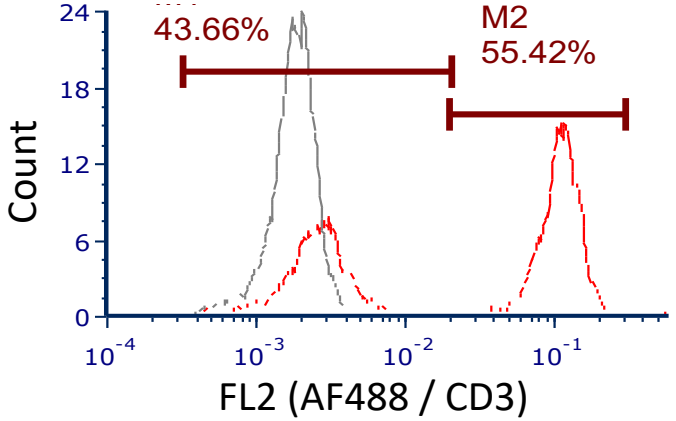
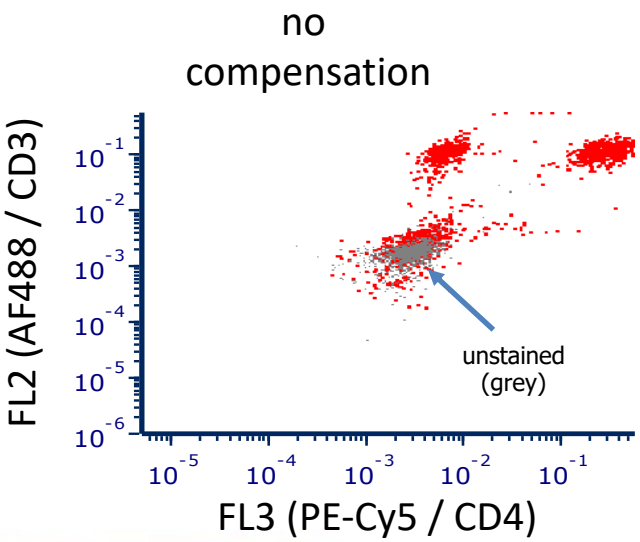
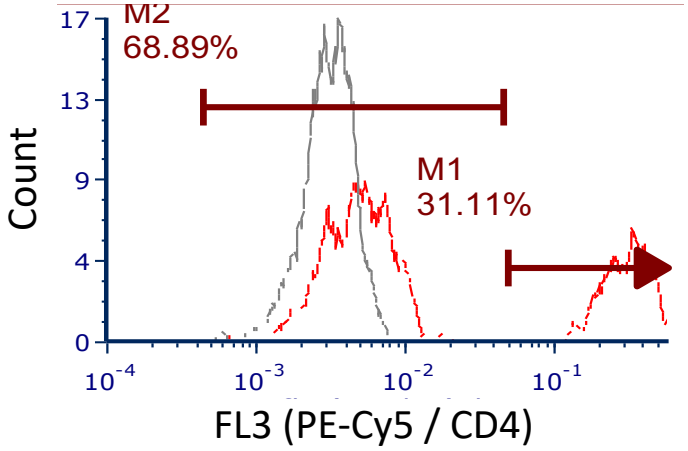
Delaware: Cell Assay: Stained VeriCell Leukocytes

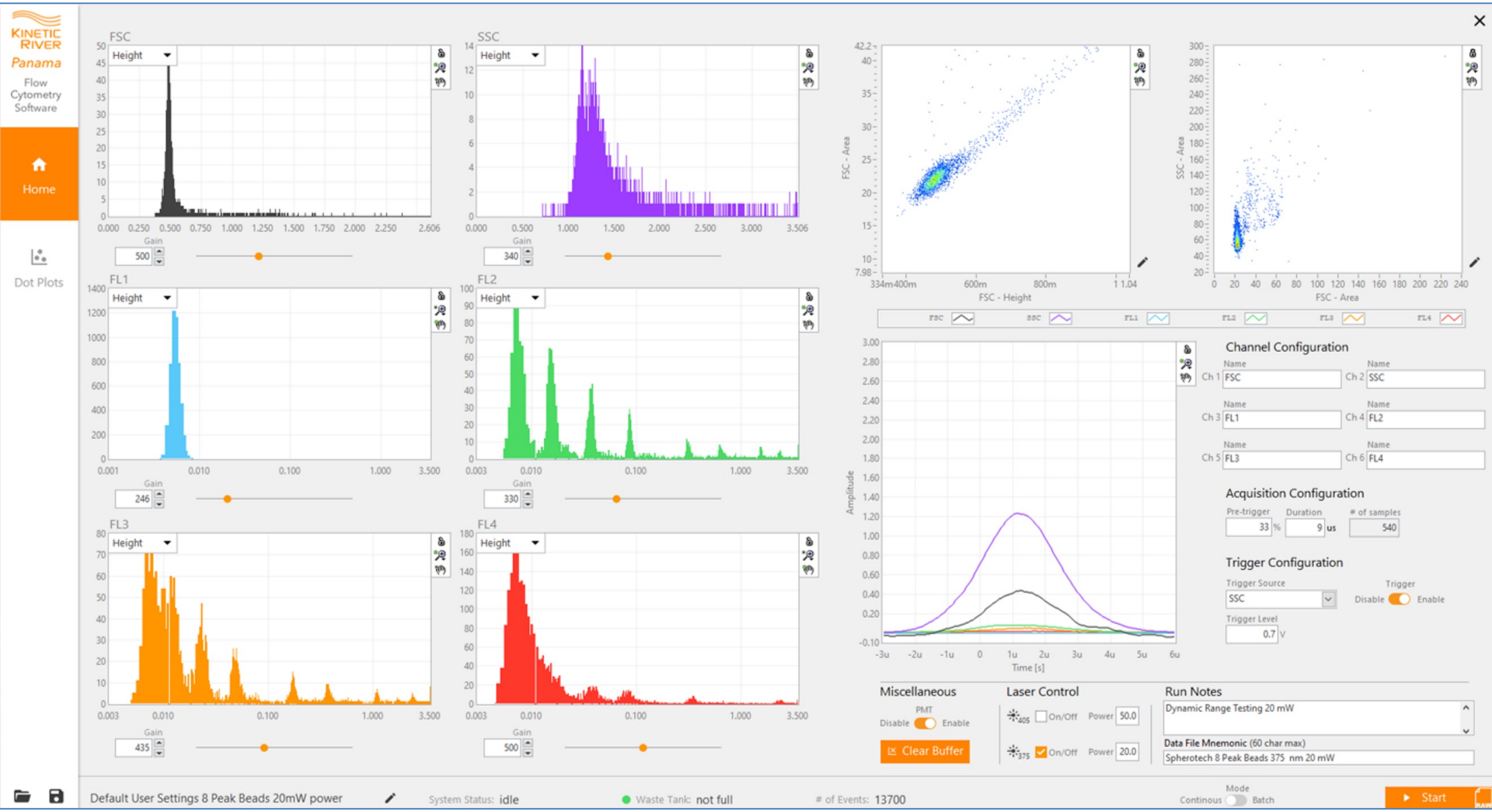








Panama Software: Instrument Control, Data Display



Delaware Technical Features and User Benefits

Features	*Benefits*
• highly stable, pressure-driven *Shasta* fluidics	→ more precise measurements
• proprietary probe wash design	→ lower background, carryover
• high-power lasers	→ higher signal-to-noise ratios
• UV/violet lasers	→ higher scattering signals
• proprietary collinear excitation architecture	→ improved scattering sensitivity
• three scattering detection channels	→ improved scattering resolution
• proprietary seven-element objective	→ improved light collection
• proprietary stray light rejection architecture	→ reduced background levels, more robust operation
• all-PMT detection	→ high sensitivity, low dark current
• proprietary signal processing algorithms	→ reduced background events
• intuitive, flexible user interface	→ easy to swap b/w different operating modes

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Delaware Configurations

Basic Configuration	High Sensitivity Configuration	Five-Laser Configuration
2 Lasers 405 nm, 250 mW 488 nm, 200 mW	**3 Lasers** 375 nm, 50 mW 405 nm, 250 mW 488 nm, 200 mW	**5 Lasers** 375 nm, 50 mW 405 nm, 250 mW 488 nm, 200 mW 561 nm, 50 mW 640 nm, 150 mW
Standard Scattering FSC, SSC (405 and 488 nm)	**Ultrasensitive Scattering** FSC, SSC (375, 405, 488 nm)	**Ultrasensitive Scattering** FSC, SSC (375, 405, 488 nm)
2 Fluorescence Channels 525/50 580/23	**4 Fluorescence Channels** 525/50 580/23 615/24 697/58	**6 Fluorescence Channels** 440/40 (optional) 525/50 580/23 615/24 697/58 755/35

KINETIC RIVER®

Delaware Flow NanoCytometer® for EV Analysis

- 3 configurations for broad range of needs
  - up to 5 lasers / 6 fluorescence channels
- 68-nm single-liposome detection
- 60-nm single-NP detection (PS)
- 28-nm single-NP detection (Au)
- 6-nm resolution
- 2% CVs
- HEK293, HeLa, PC3 EVs
- FL: tetraspanins, membrane, EGFP
- low background
- wide dynamic range
- up to 1,000+ events/sec
- intuitive user interface
- *also* measures cells

KineticRiver.com/Delaware
info@KineticRiver.com

KINETIC RIVER®
Where Light Meets Life®

KINETIC RIVER®

KU
THE KIDNEY INSTITUTE
The University of Kansas

Toward Flow-Based Rapid Diagnostics of Kidney Disease.

Christopher J. Ward, MBChB, PhD

Division of Nephrology and Hypertension

Jared Grantham Kidney Institute

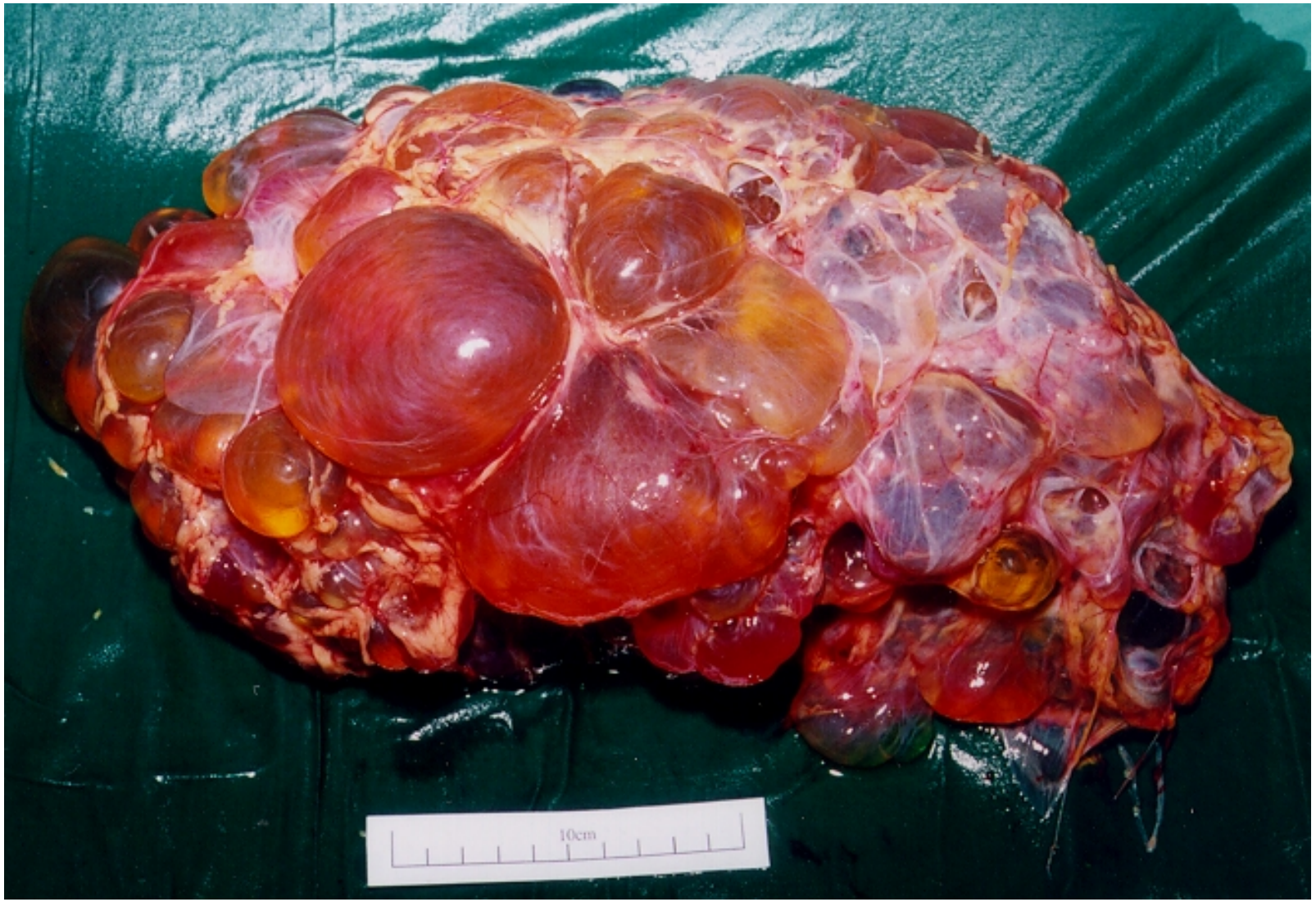
University of Kansas Medical Center

030724

Speaker Bio

- Trained as a medical doctor 1981-86 (Edinburgh, Scotland).
- PhD Birmingham University UK 1986-1990.
- Worked on ADPKD from 1991-present (33 years).
- Involved in the cloning of *PKD1*, *TSC2* (Oxford) and *PKHD1* (Mayo Rochester).
- Reagent development (monoclonal antibodies).
- Exosome hypothesis of ADPKD.
- Development of assays for diagnosis and prognosis of ADPKD.

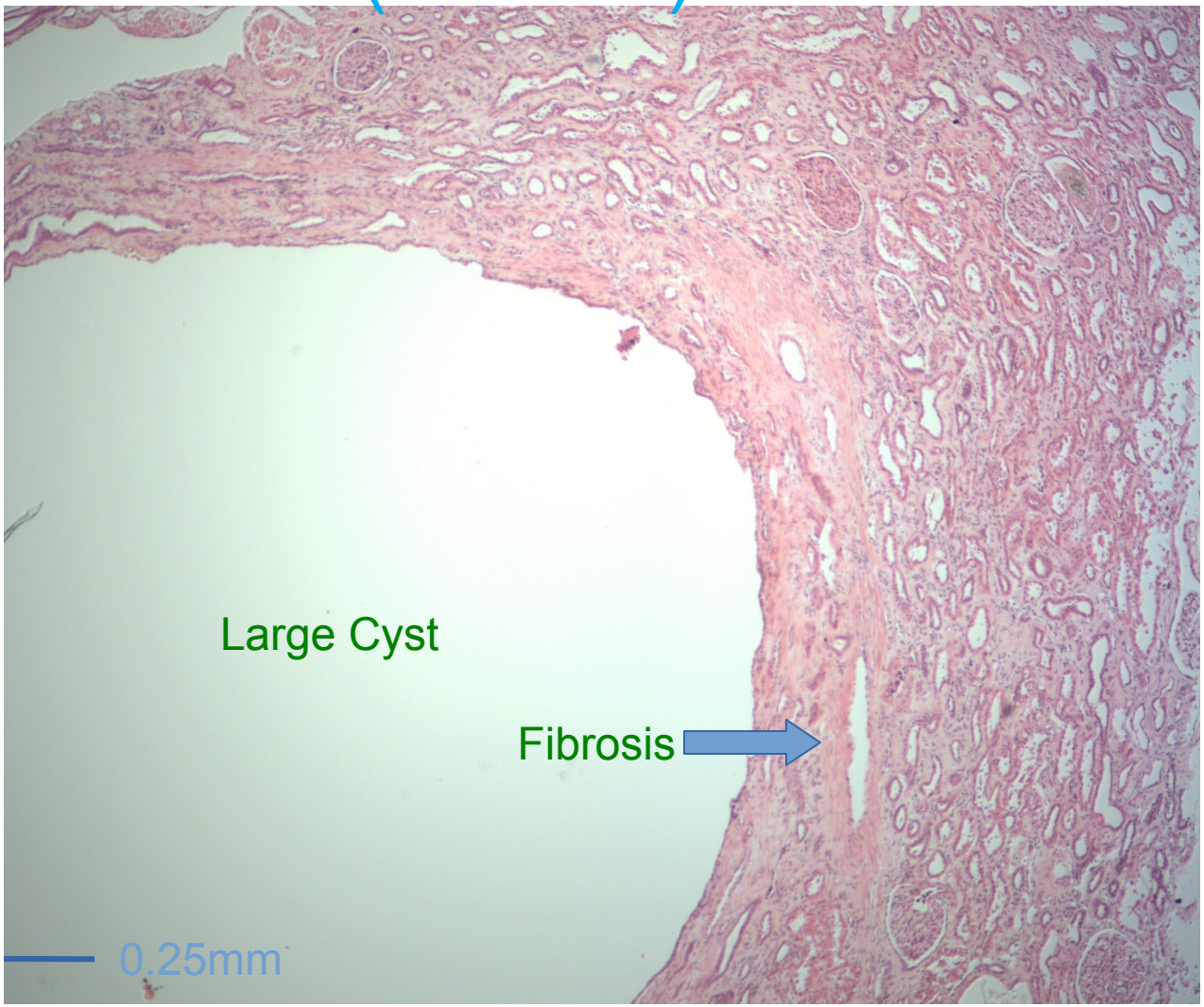
Autosomal dominant polycystic kidney disease (ADPKD)



About PKD

- Affects 1:800 people, 425,000 in the US.
- Dominant disease.
- Mainly private mutations.
- Long therapeutic window.
- Multiple treatment in the pipeline, including strategies to increase the gene expression.
- Need for a rapid, low cost and non invasive monitoring test, better than MRI.

Autosomal dominant polycystic kidney disease (ADPKD)



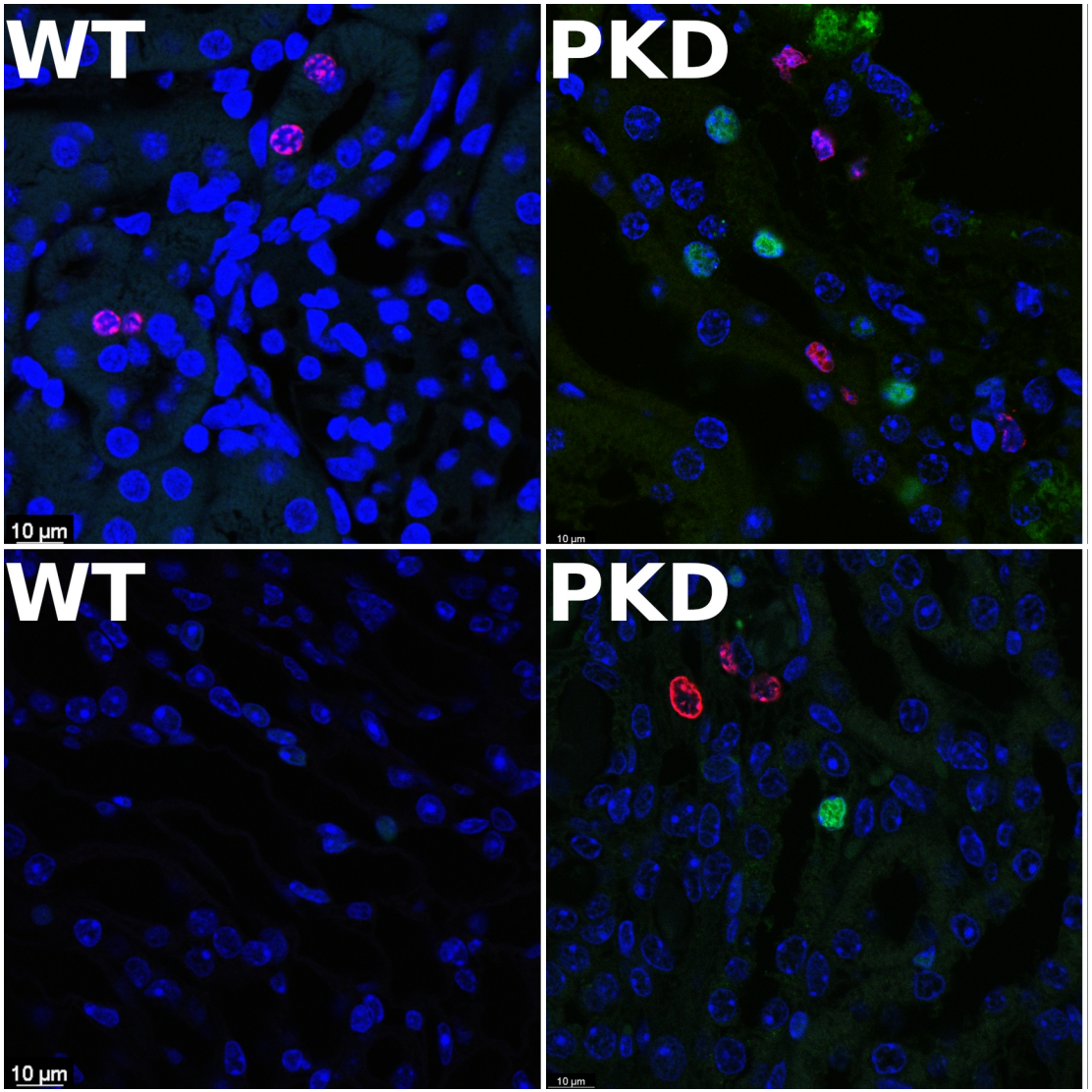
Kidney from normal (WT) and ADPKD mice

RED:

S-phase

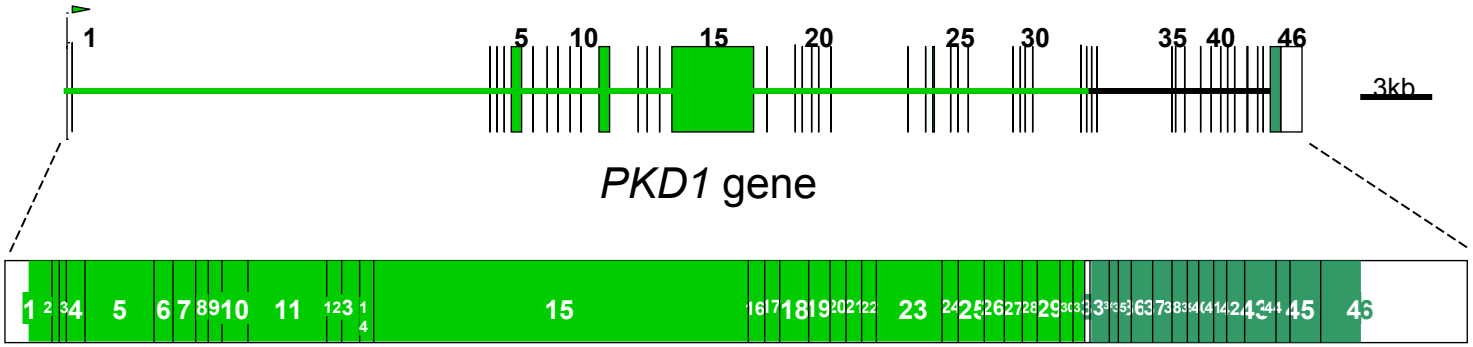
GREEN:

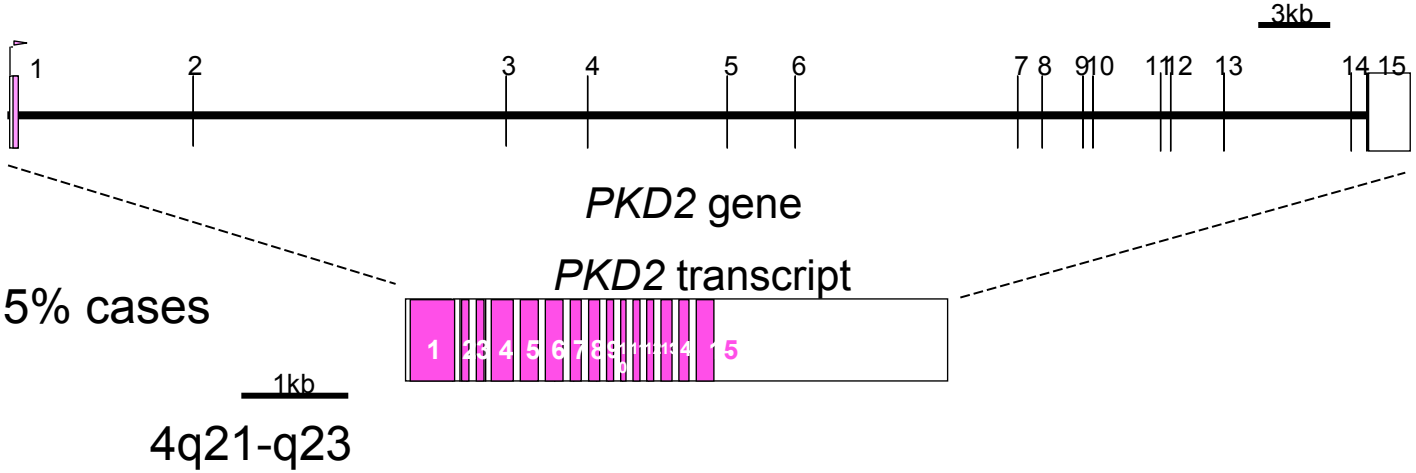
Apoptotic



Polycystic kidney disease genes

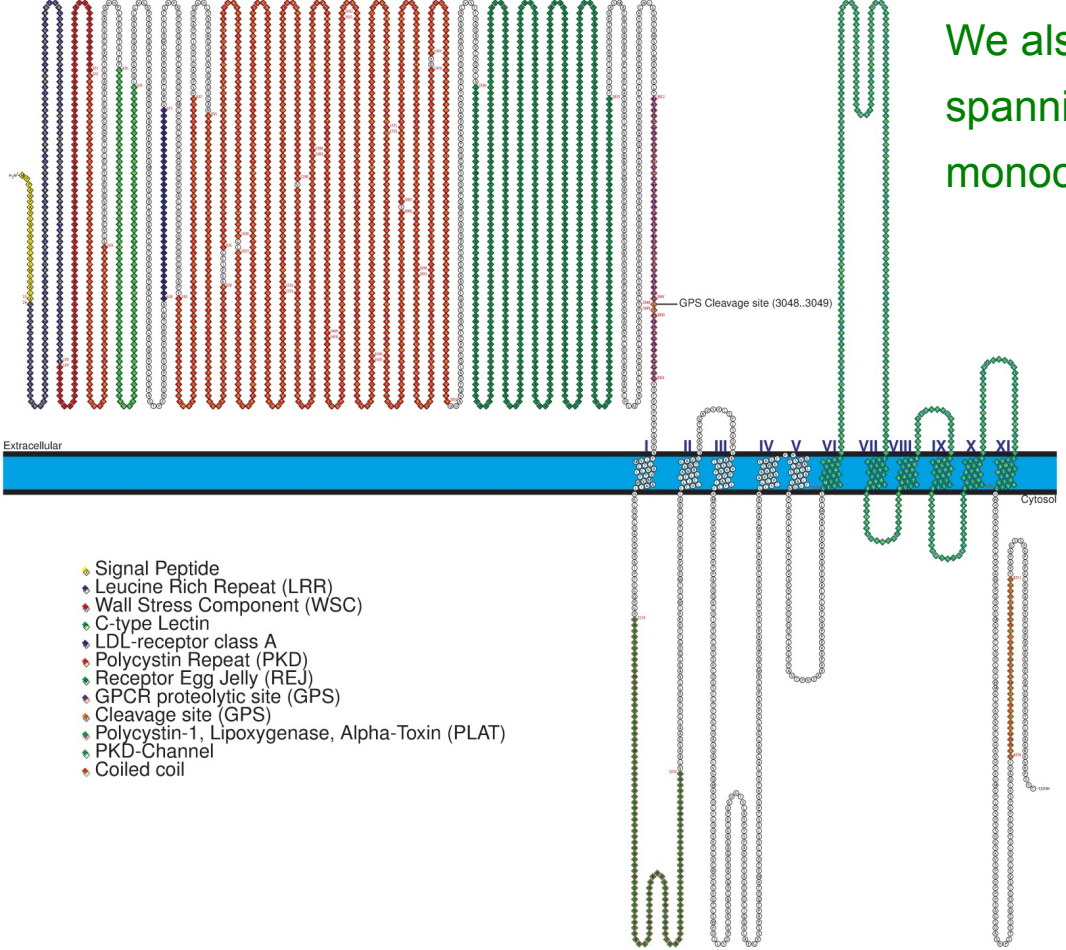
We were responsible for identifying the PKD1 gene (1994)

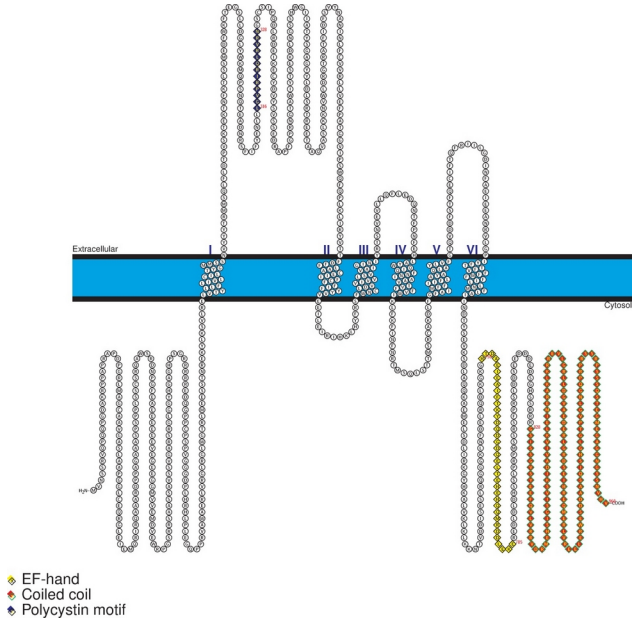




European PKD1 Consortium, Ward CJ *et al.* Cell 1994

We also developed a model of the proteins spanning the membrane and developed monoclonal antibodies to the proteins.





Polycystin-1

4300aa

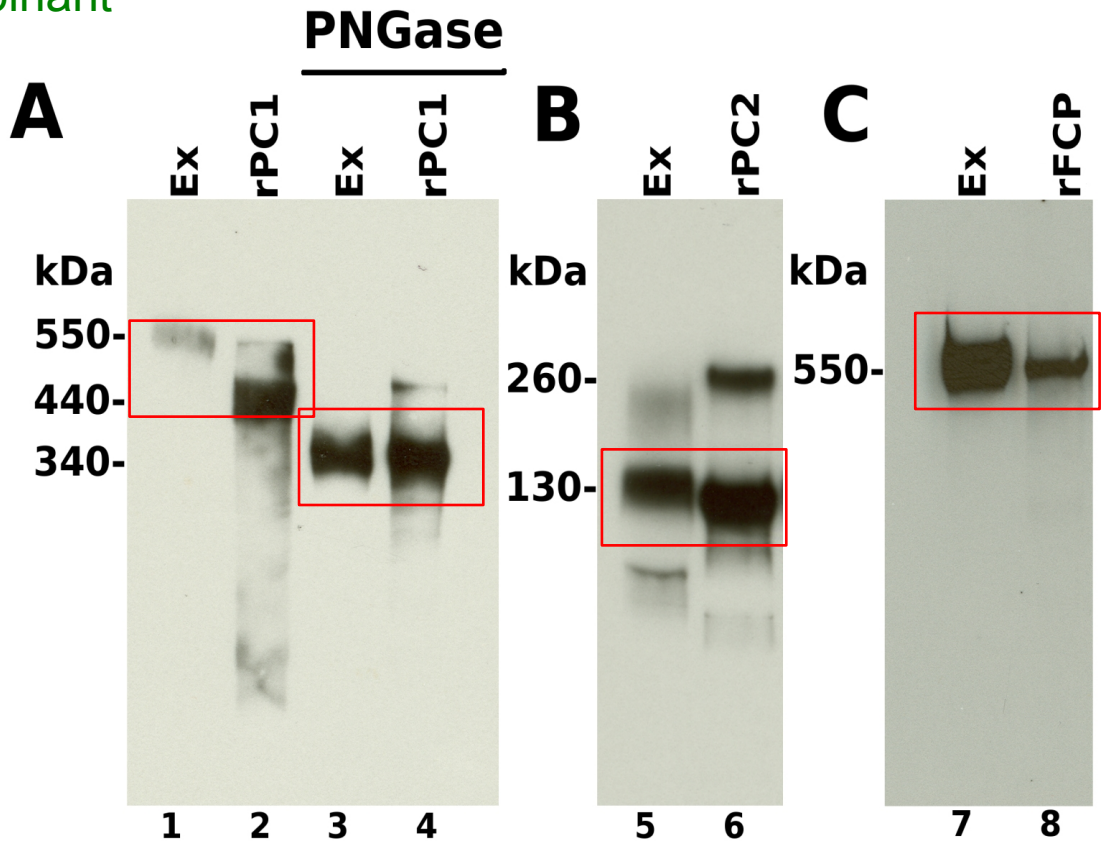
Polycystin-2

1000aa

PC1, PC2 and fibrocystin are found in urine Extracellular vesicles (EVs)

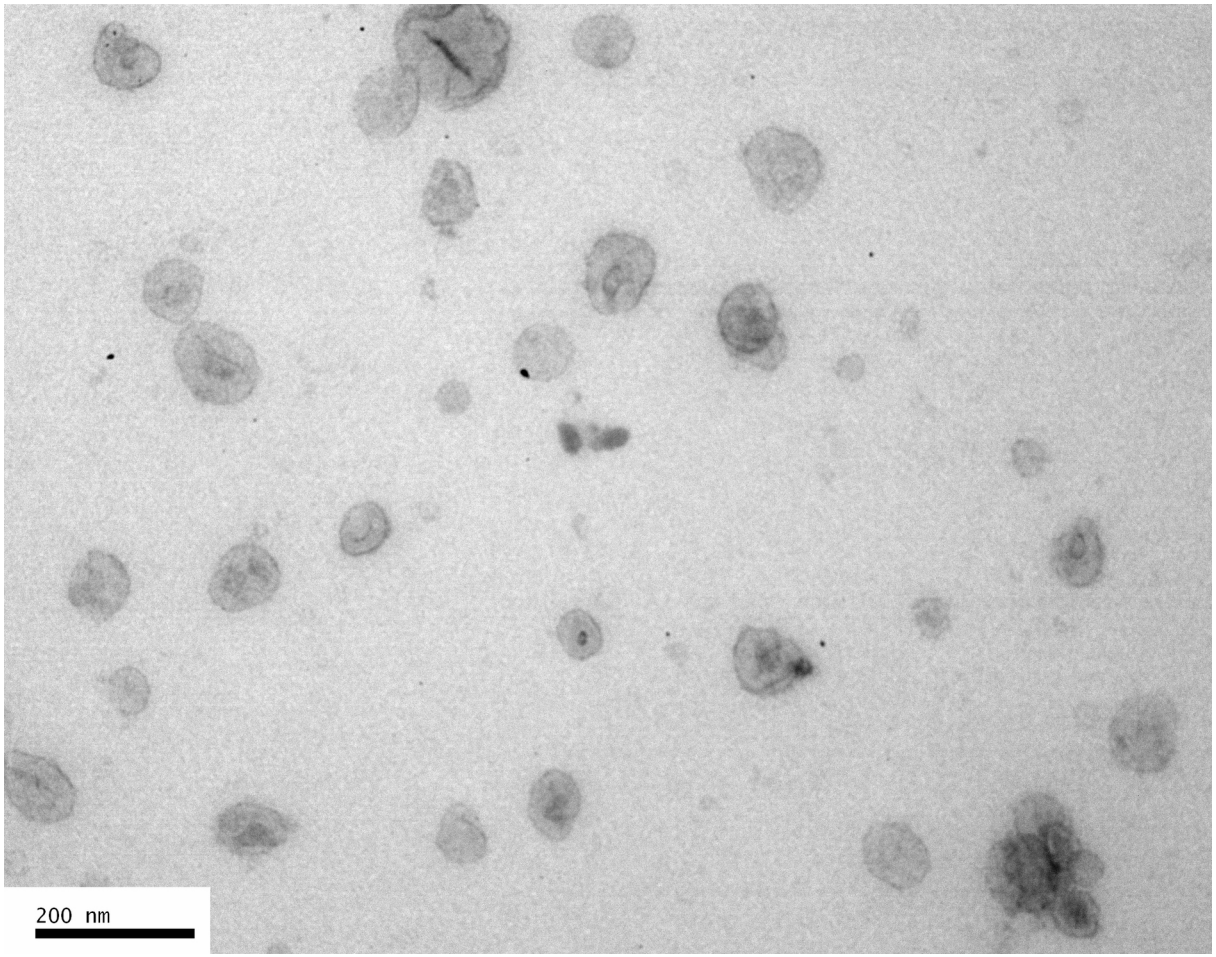
Western blots for polycystins and fibrocystin, Ex = urine exosomes (EVs)

r=recombinant



Hogan *et al.* 2009

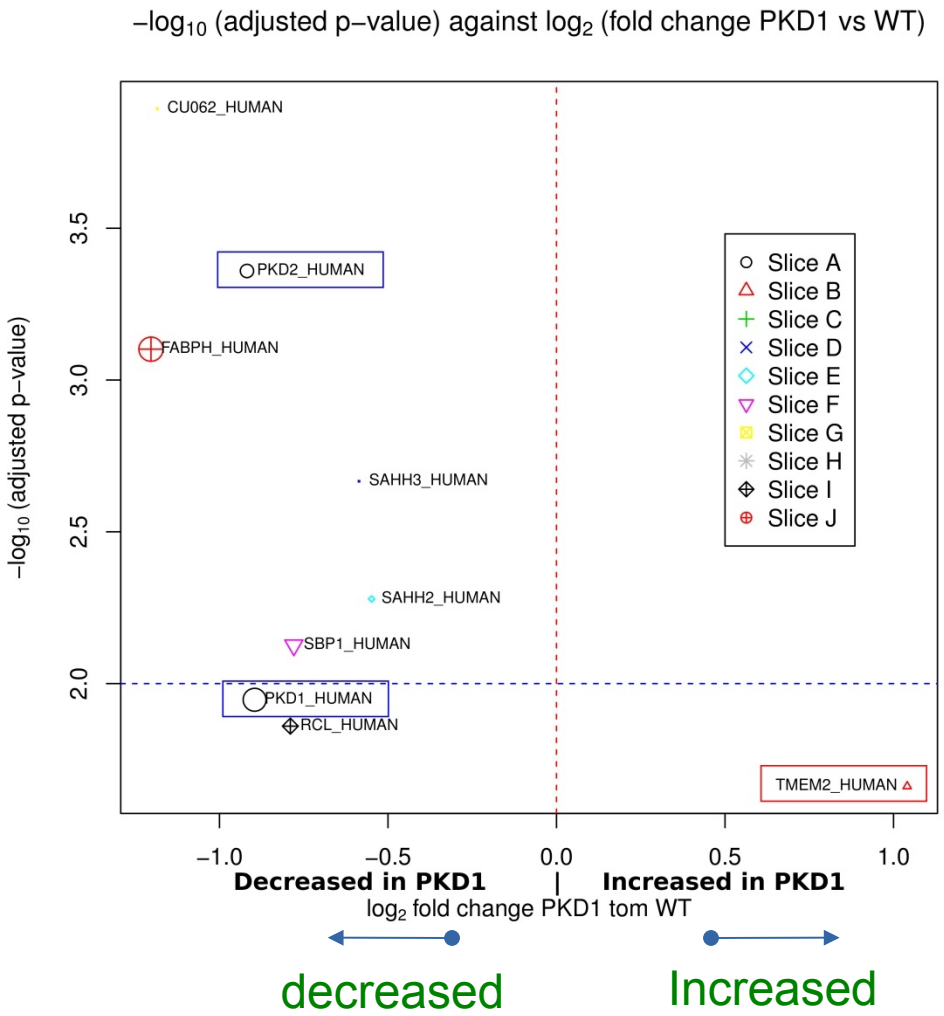
PC1 enriched urinary exosomes are 100-150nm diameter



Purified from human urine

Volcano Plot of *PKD1* vs Normal

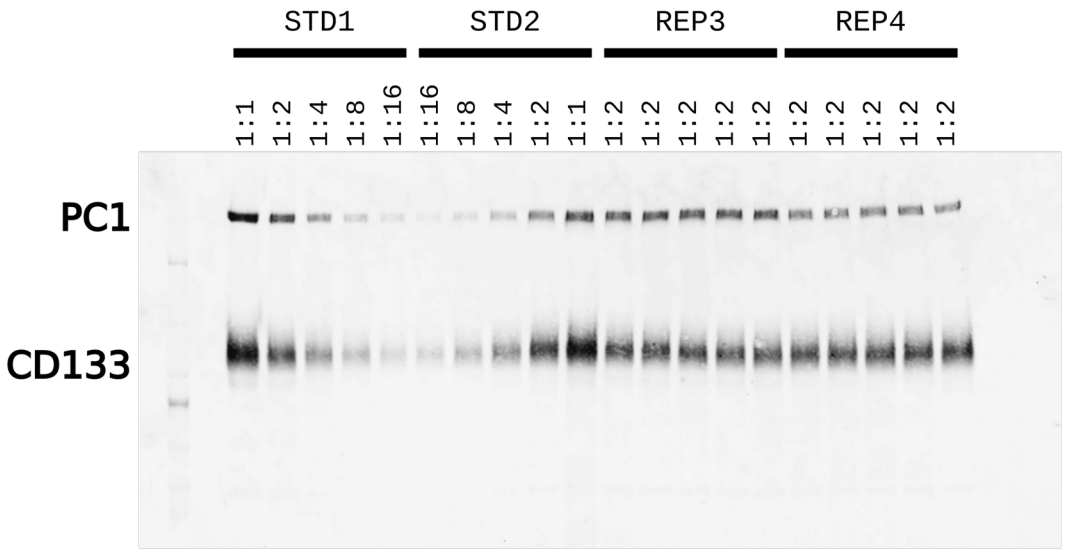
Increase statistical significance



decreased

Increased

Western blot analysis of PC1 levels controlled by CD133

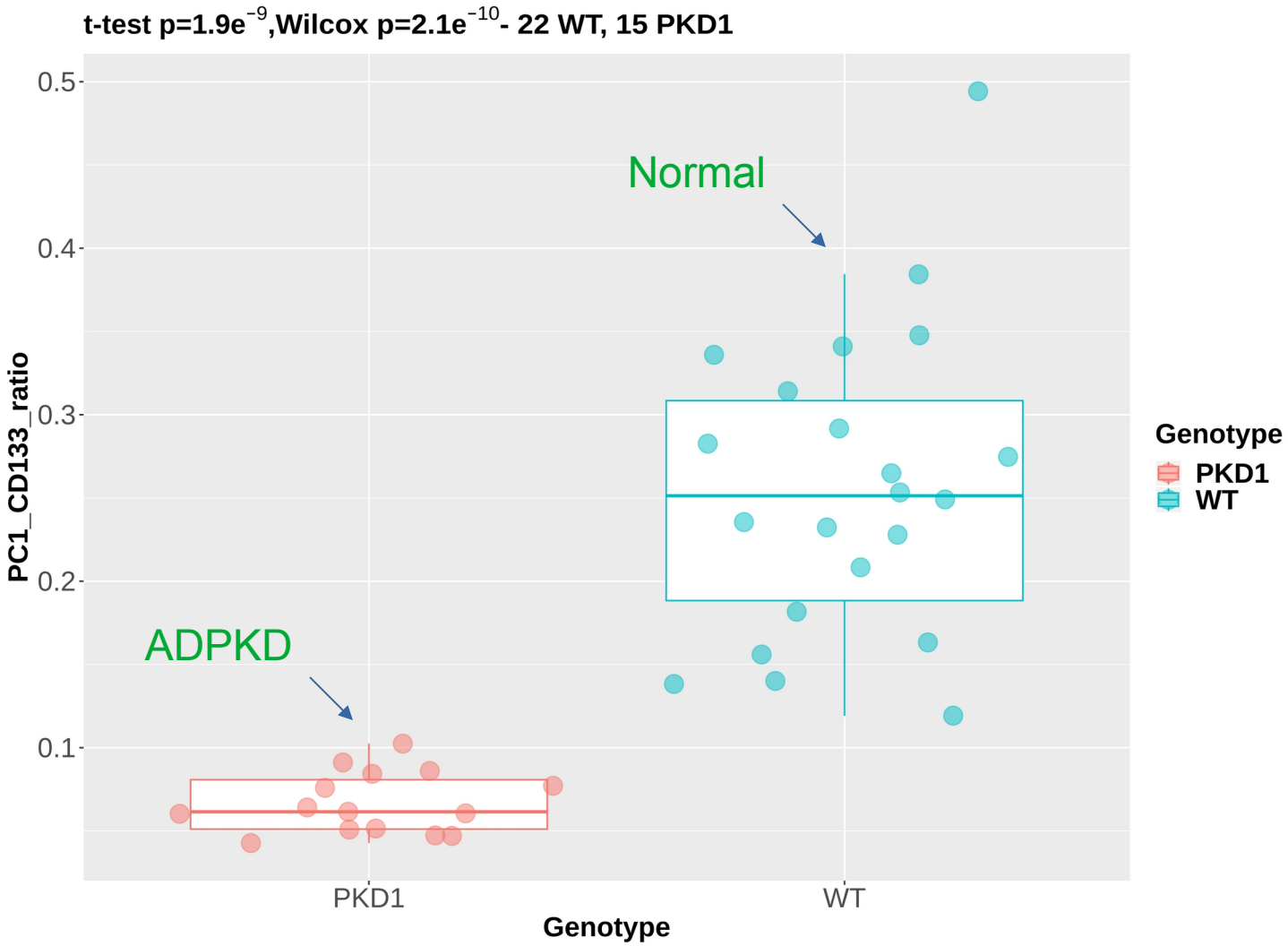


Linear dose response in batch CV 12%

This Western shows a titration of EVs STD1 & 2 and two repeat loadings to determine the variation in the assay.

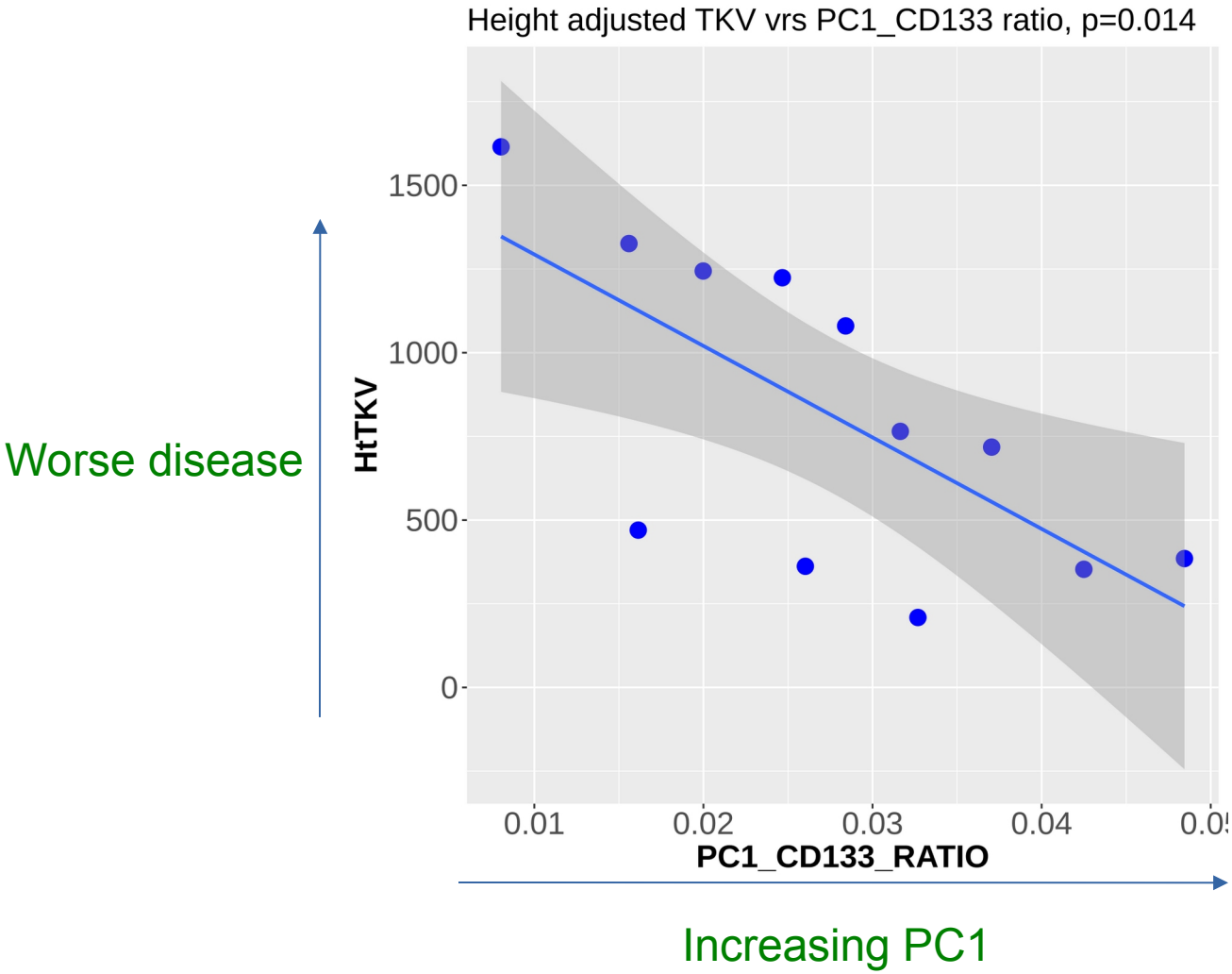
A Western blot can be used to diagnose ADPKD but the variability inherent in the assay is such that we cannot determine severity or prognosis.

PC1/CD133 ratio is a promising biomarker for ADPKD.

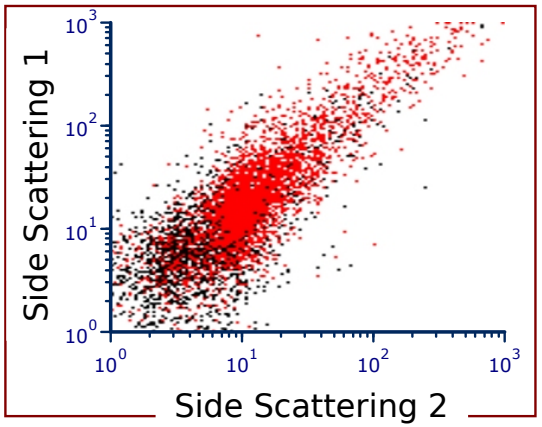


MS/MS data HtTKV against PC1_CD133 ratio, R^2=0.63

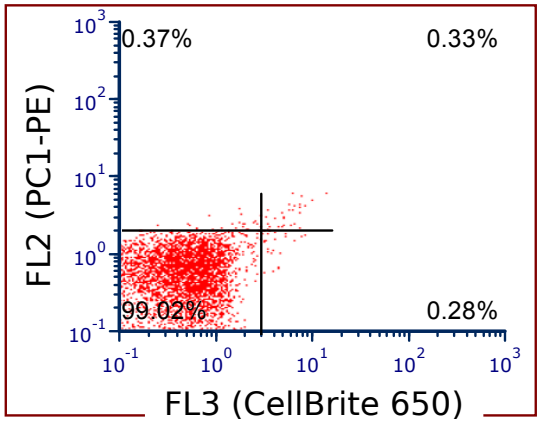
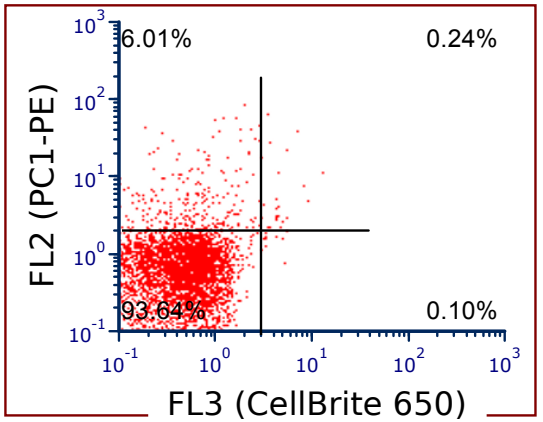
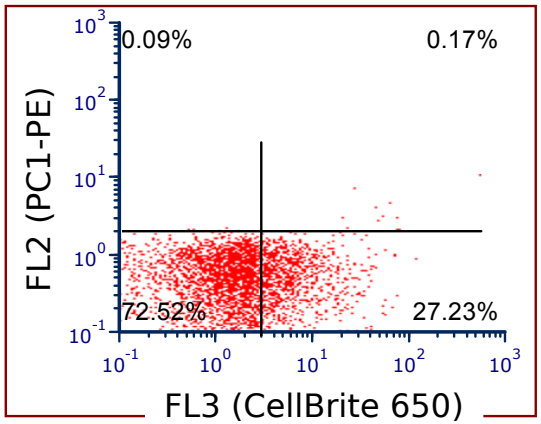
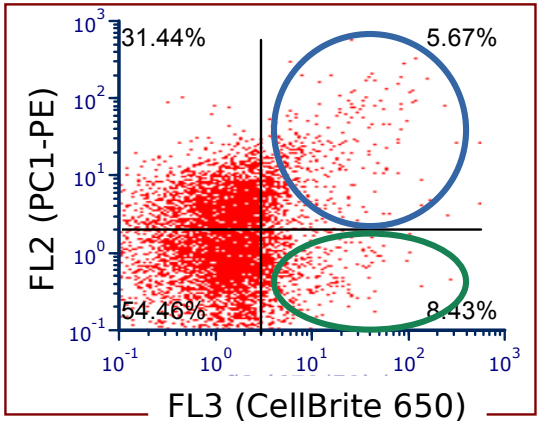
This shows that urine EVs contain important information about disease severity.



The anti-PC1 monoclonal antibody can detect urinary exosomes.



Results collected on the *Delaware* Flow NanoCytometer

	no PC1-PE	with PC1-PE
no membrane stain		
with membrane stain		

Other possibilities:

Proteins decreased in ADPKD, for which we have mAbs:

- Polycystin-2 (PC2)
- Exosomal polycystin-1 interacting protein (EPCIP)
- *C16orf89* protein
- Fibrocystin

There is one protein that is increased in ADPKD:

- TMEM2 (CEMIP2) work in progress.

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