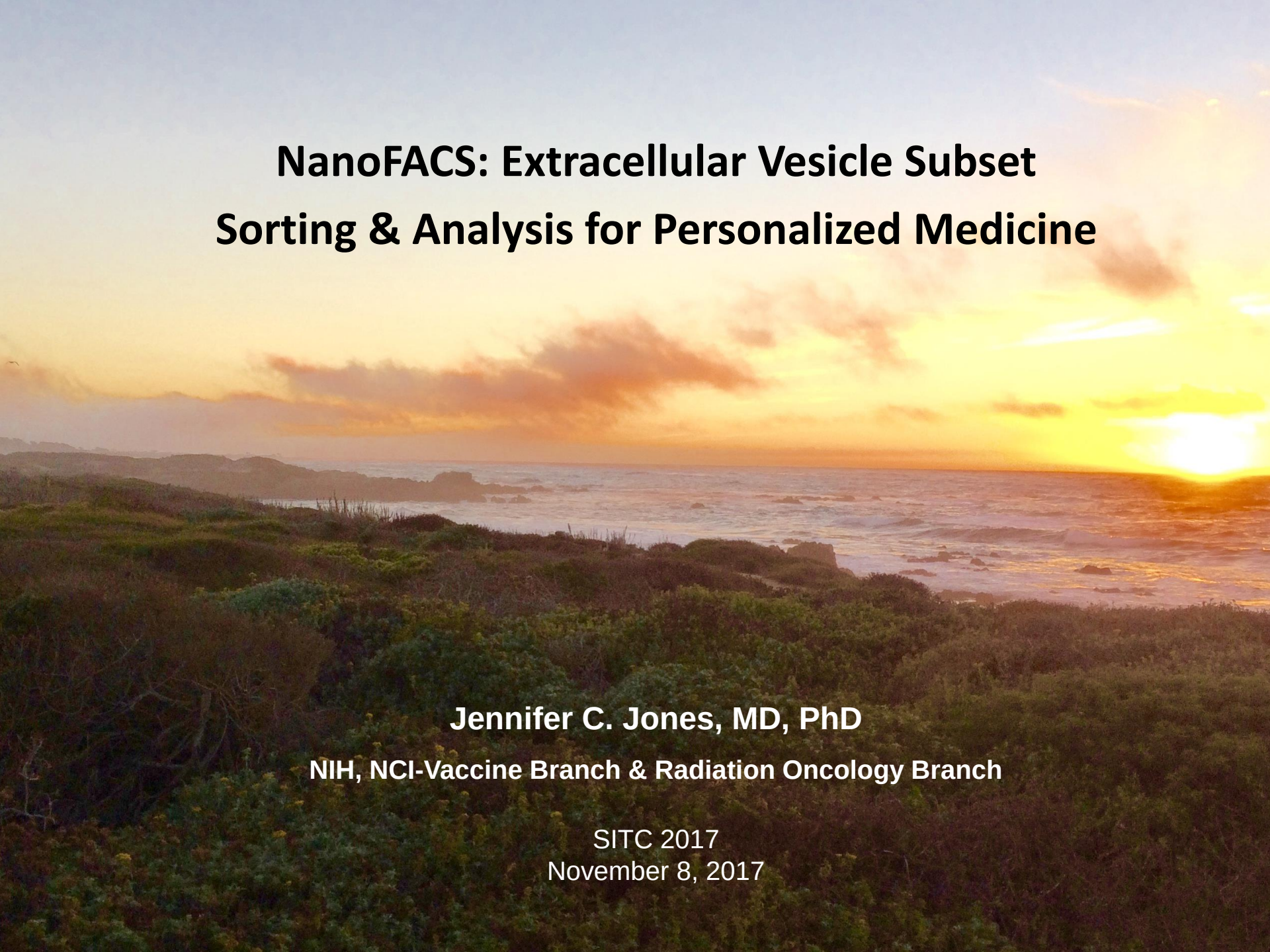

NanoFACS: Extracellular Vesicle Subset Sorting & Analysis for Personalized Medicine

Jennifer C. Jones, MD, PhD

NIH, NCI-Vaccine Branch & Radiation Oncology Branch

SITC 2017
November 8, 2017

The (Human) Clinical Problem

- Combining Radiation & Immunotherapy is very exciting
  - **Impact:** Cures not just "Treatments"
  - **Problems:**
    - Rad Onc: We don't know best doses & schedules
    - Immunology: We don't know best targets
      - Best choices = only 10-30% of treated patients respond
      - Best responses = not verifiable for months
    - Social: We don't know the cost:benefit outcomes ahead of time
      - Cancer Annual Drug Cost: $84 Billion.
      - $41 / $84 Billion is now "Immune-Targeted" Therapies
      - Opportunity cost: TIME for patients for whom IT isn't effective
      - Research Budgets: Billions being spent testing new combos

If 30% response rate, the annual cost of ineffective tx:
- $29 Billion Immune Tx
- $59 Billion all Medical Tx
- weeks/months for pts

- We need:
  - Pre-Tx Biomarkers to Personalize Therapy
  - Early-Tx Biomarkers to Adapt Treatments

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What if….

Every patient carried a Dossier with them?

Tumor

Immune

Normal Tissues

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What if….

Every patient carried a Dossier with them?

Tumor

Immune

Normal Tissues

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What if….

Every patient carried a Dossier with them?

Tumor

Immune

Normal Tissues

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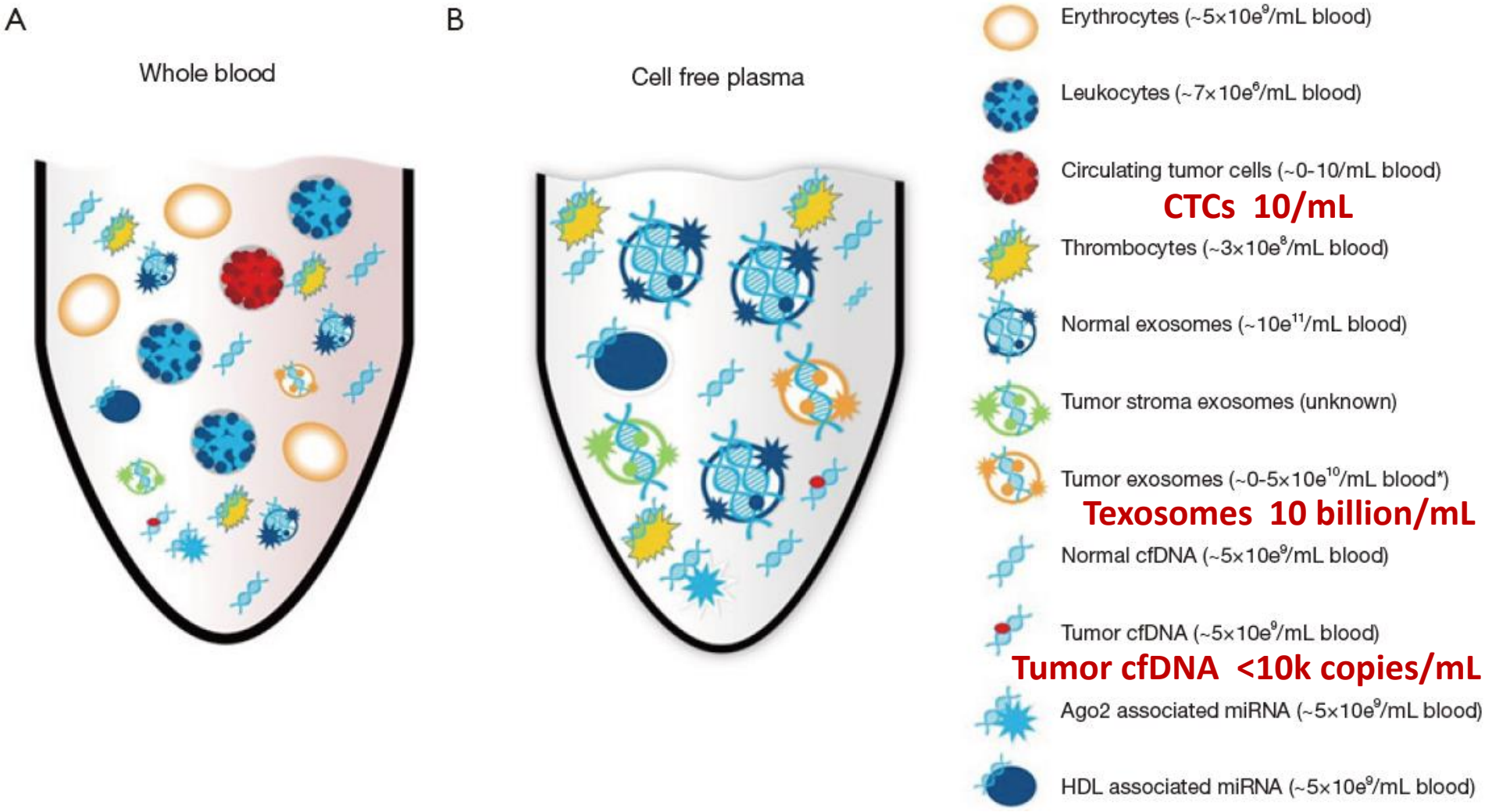
Blood Samples for Liquid Biopsies: More than Exosomes



Translational Cancer Research 2016

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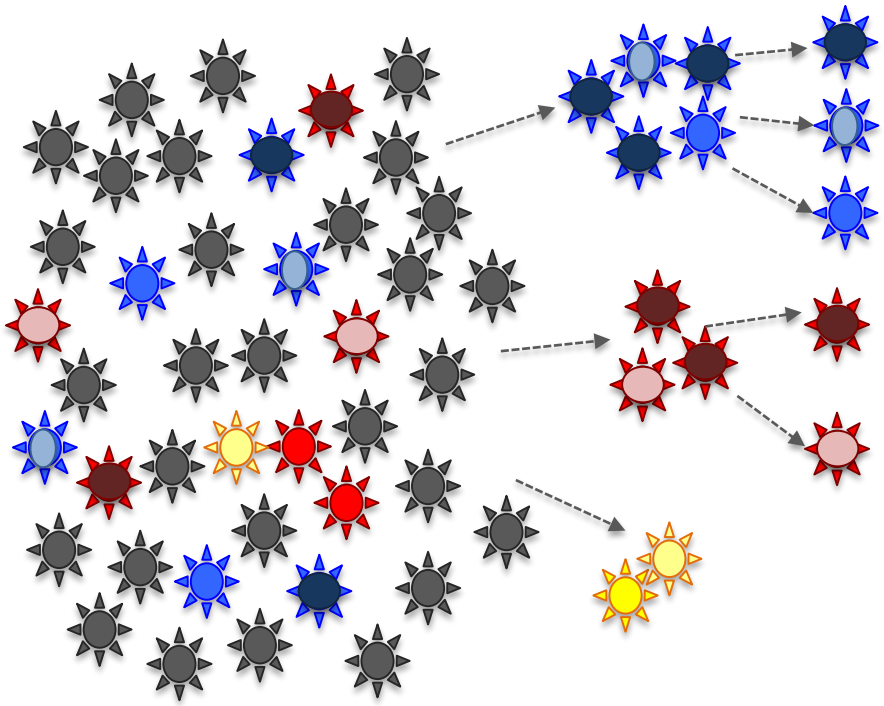
Jones Lab Program Priorities:

Extracellular Vesicles (EVs) for Personalized Medicine

NanoFACS & other Methods to Analyze, Sort, Decode EV Subsets



1. **Develop and refine methods needed to study EV subsets**

2. **Apply nanoFACS Program methods to the following clinical situations:**

   - Detect Tumor-Associated EVs
   - Detect Immune-Response EVs
   - Detect Radiation-Induced EVs
   - Define Abscopal EV Profile

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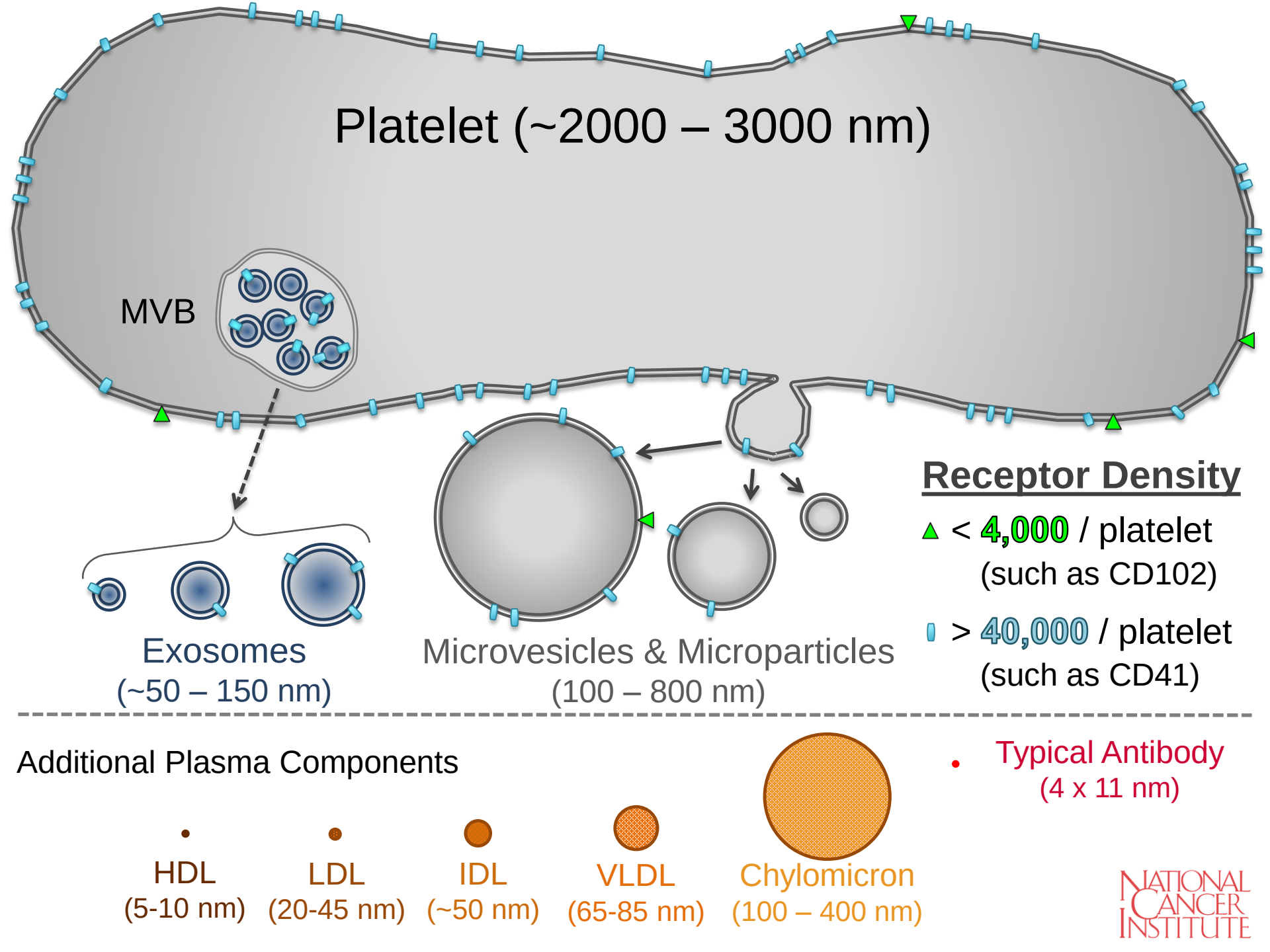
Platelet (~2000 – 3000 nm)
MVB
Exosomes
(~50 – 150 nm)
Microvesicles & Microparticles
(100 – 800 nm)
Receptor Density
< 4,000 / platelet
(such as CD102)
> 40,000 / platelet
(such as CD41)
Additional Plasma Components
HDL
(5-10 nm)
LDL
(20-45 nm)
IDL
(~50 nm)
VLDL
(65-85 nm)
Chylomicron
(100 – 400 nm)
Typical Antibody
(4 x 11 nm)
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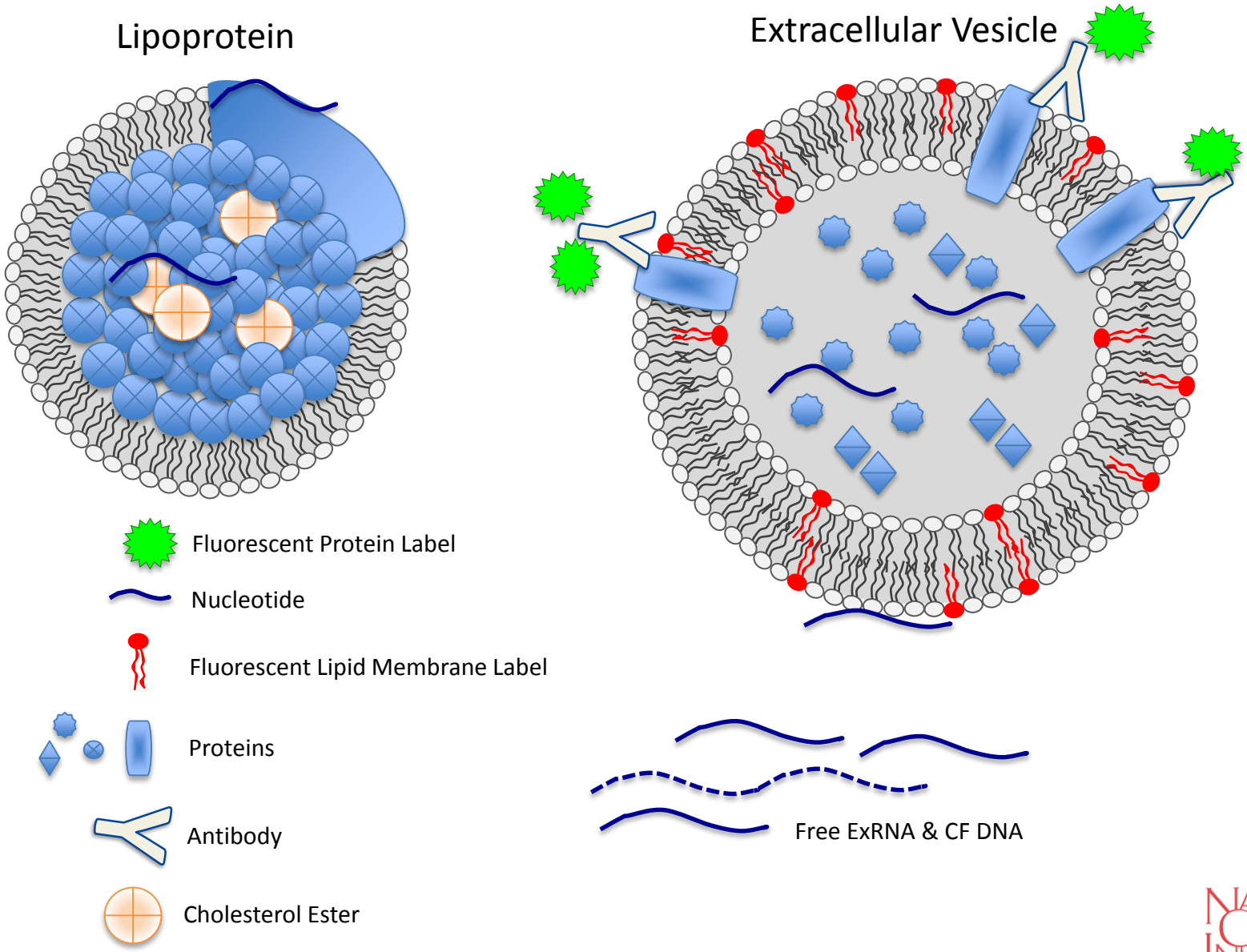
Nanoscale Biological Particles and Labels



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NanoFACS at NCI

	Cellular Flow Cytometry	EV Flow Cytometry Status Quo	NanoFACS Program @ NCI2016
Size Resolution (light scatter)	Standard	not possible <400nm	Resolve to ≤100nm
Size Resolution (fluorescence)	Standard	not possible <100nm	Resolve to ≤40nm
Calibration	Standard	No standards	Developed protocols
Preparative Sorting Protocols	Standard	Not possible (too slow, too blind)	Sorted DC-Tumor EVs Functional Sorts (HIV) Index Sorting: Copy #s
Staining	Standard	Bead-based (bkgd probs)	Single EV optimized
Counting	Standard	Not possible. Need NTA	nanoFACS: Method developed
Tumor Marker Detection	Standard	Not ever done for single EVs (GPC1 on EVs on beads)	PSMA+ prostate CA EVs detected Also other immune, tumor surface markers
Bio. Reference	Common	None	1st gen EV Stds

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SCIENTIFIC REPORTS

OPEN

Labeling Extracellular Vesicles for Nanoscale Flow Cytometry

Aizea Morales-Kastresana[1], Bill Telford[2], Thomas A. Musich[3], Katherine McKinnon[4], Cassandra Clayborne[1], Zach Braig[1], Ari Rosner[1,2], Thorsten Demberg[3], Dionysios C. Watson[5], Tatiana S. Karpova[6], Gordon J. Freeman[7], Rosemarie H. DeKruyff[8], George N. Pavlakis[5], Masaki Terabe[1], Marjorie Robert-Guroff[3], Jay A. Berzofsky[1] & Jennifer C. Jones[1]

Received: 13 October 2016
Accepted: 3 April 2017

JCI insight

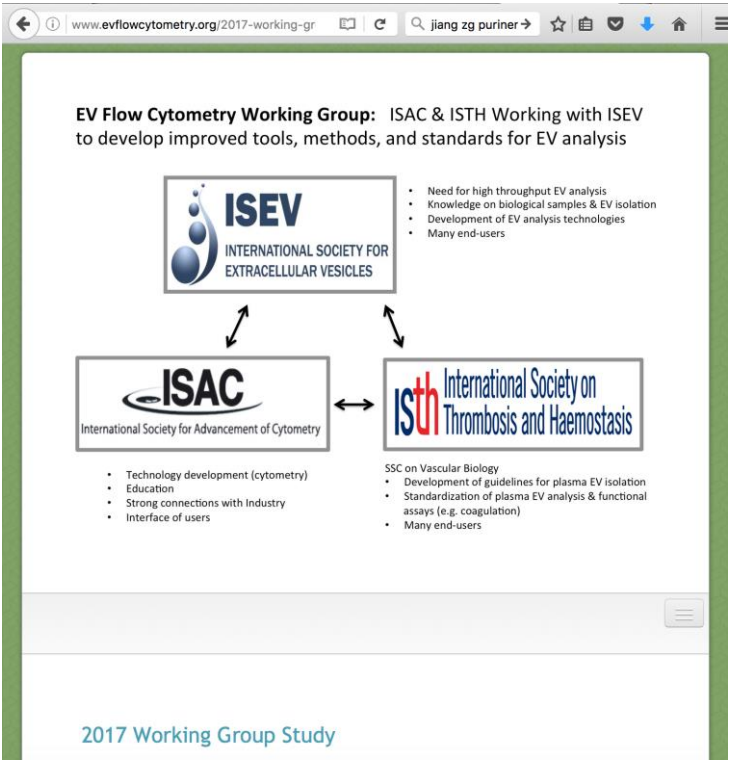
RESEARCH ARTICLE

Flow virometric sorting and analysis of HIV quasispecies from plasma

Thomas Musich,[1] Jennifer C. Jones,[2] Brandon F. Keele,[3] Lisa M. Miller Jenkins,[4] Thorsten Demberg,[1] Thomas S. Uldrick,[5] Robert Yarchoan,[5] and Marjorie Robert-Guroff[1]

[1]Immune Biology of Retroviral Infection Section and [2]Molecular Immunogenetics and Vaccine Research Section, Vaccine Branch, National Cancer Institute, Bethesda, Maryland, USA. [3]AIDS and Cancer Virus Program, Leidos Biomedical Research Inc., Frederick National Laboratory for Cancer Research, Frederick, Maryland, USA. [4]Collaborative Protein Technology Resource, Laboratory of Cell Biology, National Cancer Institute, Bethesda, Maryland, USA. [5]Retroviral Diseases Section, HIV and AIDS Malignancy Branch, National Cancer Institute, Bethesda, Maryland, USA.

2016-2017 Large Scale Efforts



JOURNAL OF EXTRACELLULAR VESICLES, 2017
VOL. 6, 1286095
http://dx.doi.org/10.1080/20013078.2017.1286095

Taylor & Francis
Taylor & Francis Group

ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

Obstacles and opportunities in the functional analysis of extracellular vesicle RNA – an ISEV position paper

Bogdan Mateescu[a*], Emma J. K. Kowal[b*], Bas W. M. van Balkom[c], Sabine Bartel[d], Suvendra N. Bhattacharyya[e], Edit I. Buzás[f], Amy H. Buck[g], Paola de Candia[h], Franklin W. N. Chow[g], Saumya Das[i], Tom A. P. Driedonks[j], Lola Fernández-Messina[k], Franziska Haderk[l,m], Andrew F. Hill[n], Jennifer C. Jones[o], Kendall R. Van Keuren-Jensen[p], Charles P. Lai[q], Cecilia Lässer[r,s], Italia di Liegro[t], Taral R. Lunavat[r,s], Magdalena J. Lorenowicz[u], Sybren L. N. Maas[v], Imre Mäger[w,x], Maria Mittelbrunn[y], Stefan Momma[z], Kamalika Mukherjee[e], Muhammed Nawaz[aa], D. Michiel Pegtel[ab], Michael W. Pfaffl[ac], Raymond M. Schiffelers[ad], Hidetoshi Tahara[ae], Clotilde Théry[af], Juan Pablo Tosar[ag], Marca H. M. Wauben[j], Kenneth W. Witwer[ah] and Esther N. M. Nolte-'t Hoen[j]

platelets

http://www.tandfonline.com/iplt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

Platelets, 2017; 28(3): 256–262
 DOI: 10.1080/09537104.2017.1280602

Taylor & Francis
Taylor & Francis Group

SPECIAL REVIEW: PLATELET MICROVESICLES

Detection of platelet vesicles by flow cytometry

John P Nolan[1] & Jennifer C Jones[2]

[1]Scintillon Institute, San Diego, CA, USA and [2]Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

PLOS ONE

RESEARCH ARTICLE

Diurnal Variations of Circulating Extracellular Vesicles Measured by Nano Flow Cytometry

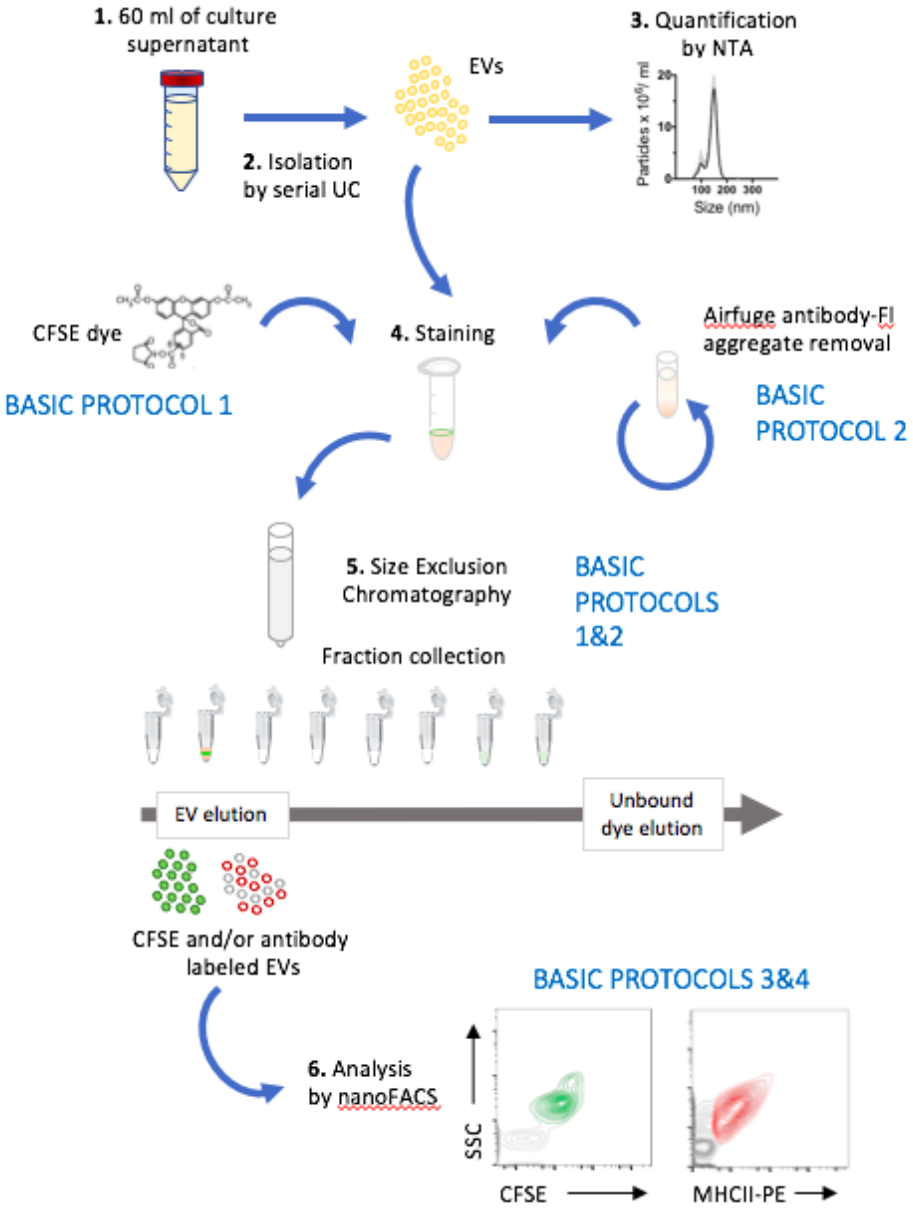
Kirsty M. Danielson[1☯], Jessica Estanislau[1☯], John Tigges[1], Vasilis Toxavidis[1], Virginia Camacho[1], Edward J. Felton[1], Joseph Khoory[1], Simion Kreimer[2,3], Alexander R. Ivanov[2,3], Pierre-Yves Mantel[1], Jennifer Jones[4], Praveen Akuthota[1], Saumya Das[1‡], Ionita Ghiran[1‡*]

1 Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, United States of America, **2** Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA, United States of America, **3** Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA, United States of America, **4** Molecular Immunogenetics & Vaccine Research Section Vaccine Branch, CCR, NCI, Bethesda, MD, United States of America

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* ighiran@bidmc.harvard.edu

CrossMark
click for updates

Basic Jones Lab Protocols / Workflow - I



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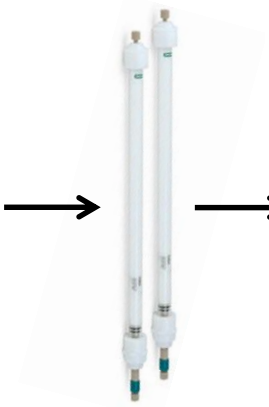
Basic Jones Lab Protocols / Workflow - II

A. Sample

B. Isolation

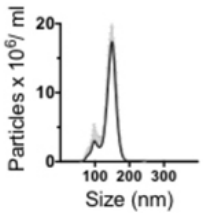


C. General EV Size, Concentration, and Cargo Assays

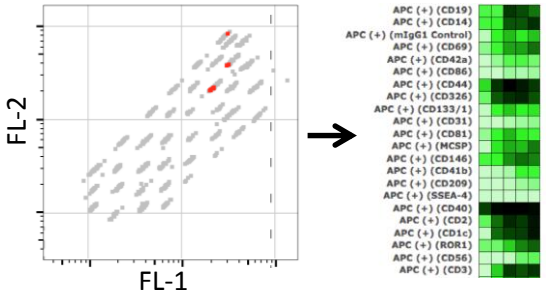
Protein Concentration

Western Blot (Tsg101, Alix)

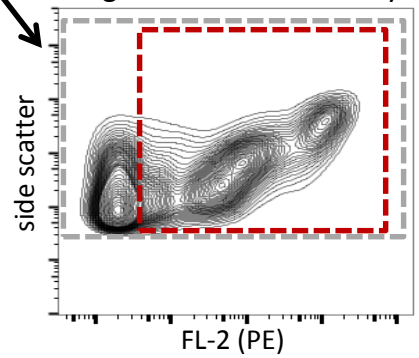
Nanoparticle Tracking Analysis



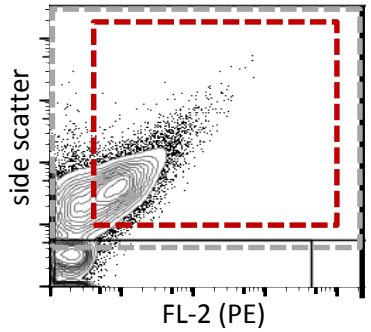
D. Multiplex EV Assay: > 20 Epitope Survey



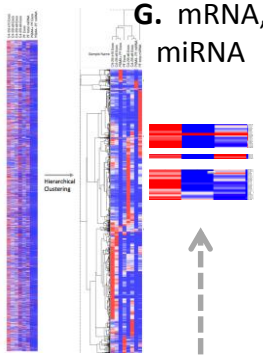
E. High Resolution EV Analysis



F. nanoFACS Sorting



G. mRNA, miRNA



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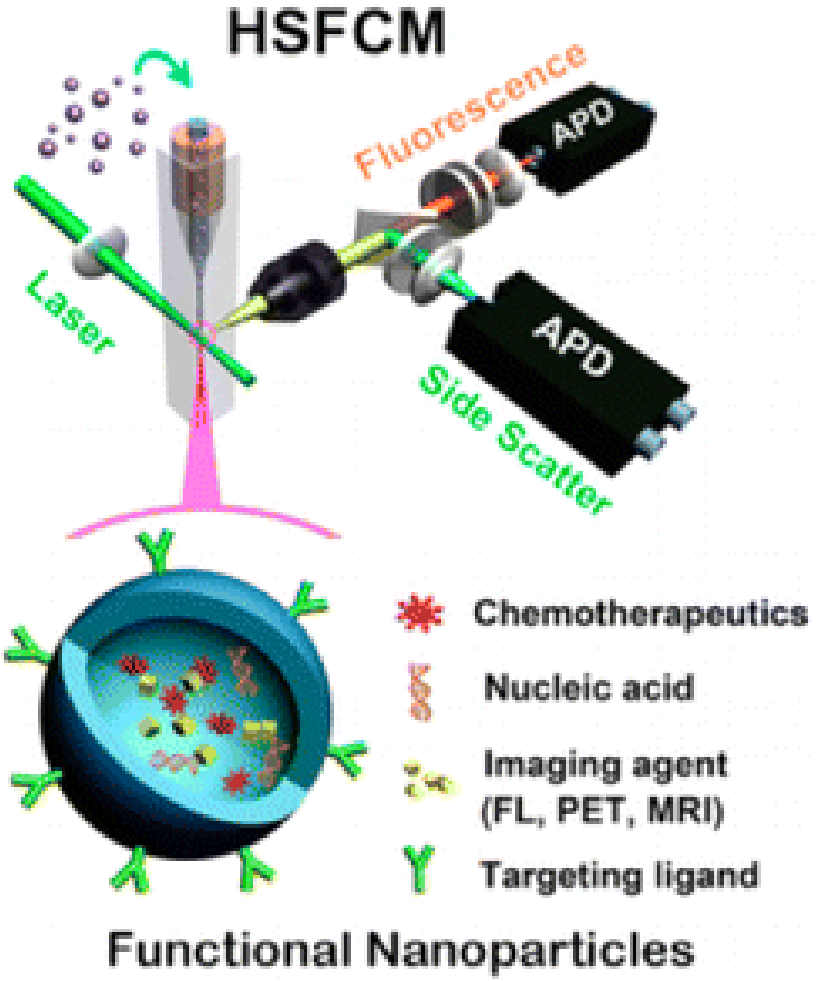
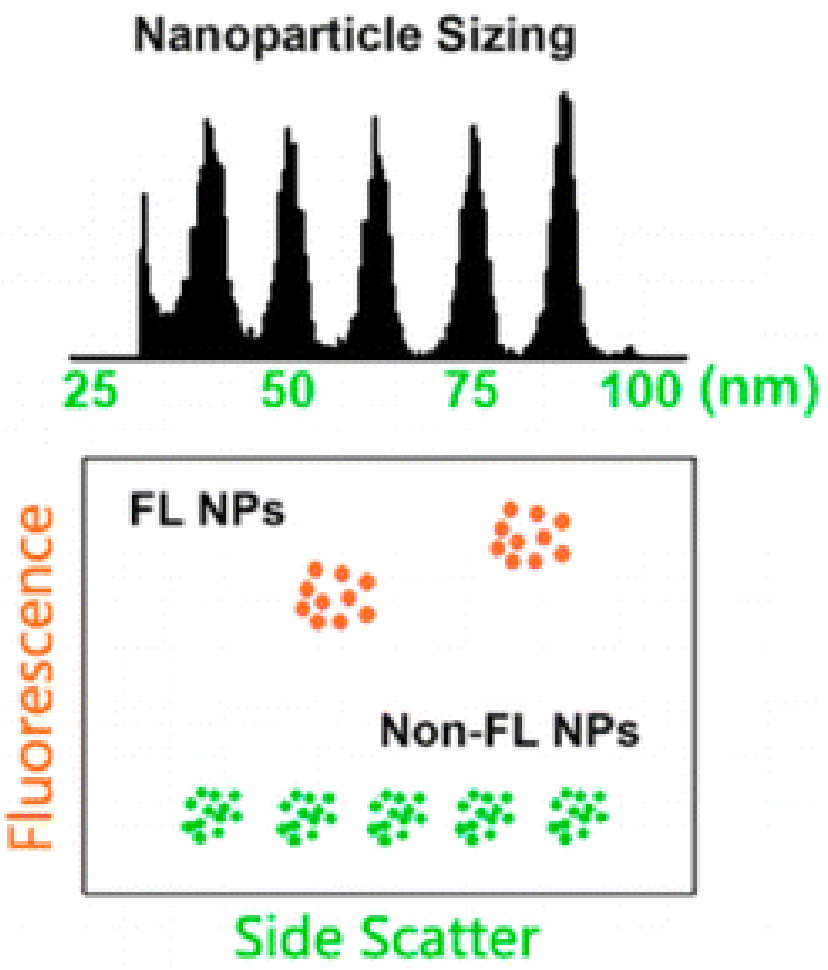
Next Generation High Sensitivity Flow Cytometers

Next Generation High Resolution Flow Cytometry

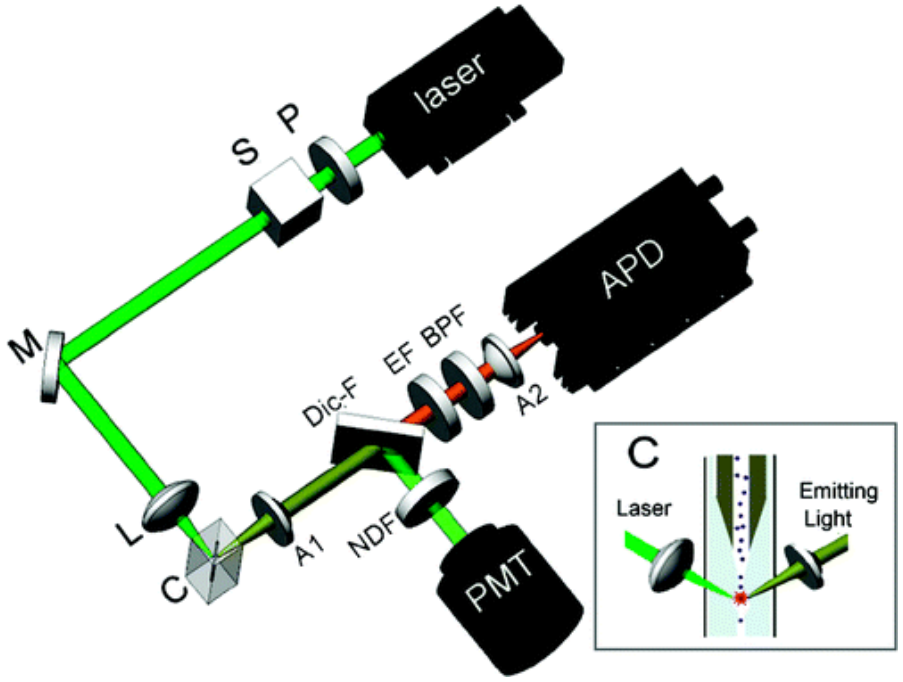


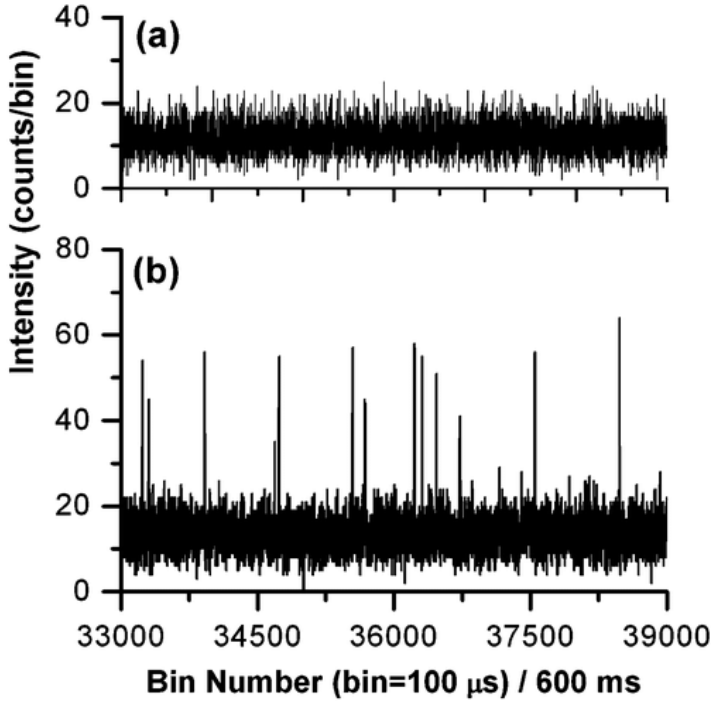


Szu, et al. *ACS Nano*, 2014, 8 (10), pp 10998–11006

ACSNANO

Next Generation High Resolution Flow Cytometry
Single Molecule Detection





Single-molecule fluorescence burst data from (a) a 20 nm filtered ultrapure water blank and (b) R-PE molecules in an 83 fM solution. The data were binned into 100 μs intervals, and the excitation laser power was 0.75 mW

Anal. Chem., 2009, 81 (7), pp 2555–2563

analytical chemistry

Why Does Single Epitope Sensitivity Matter?

NCI High Sensitivity EV Flow Cytometry Evaluations 2017:

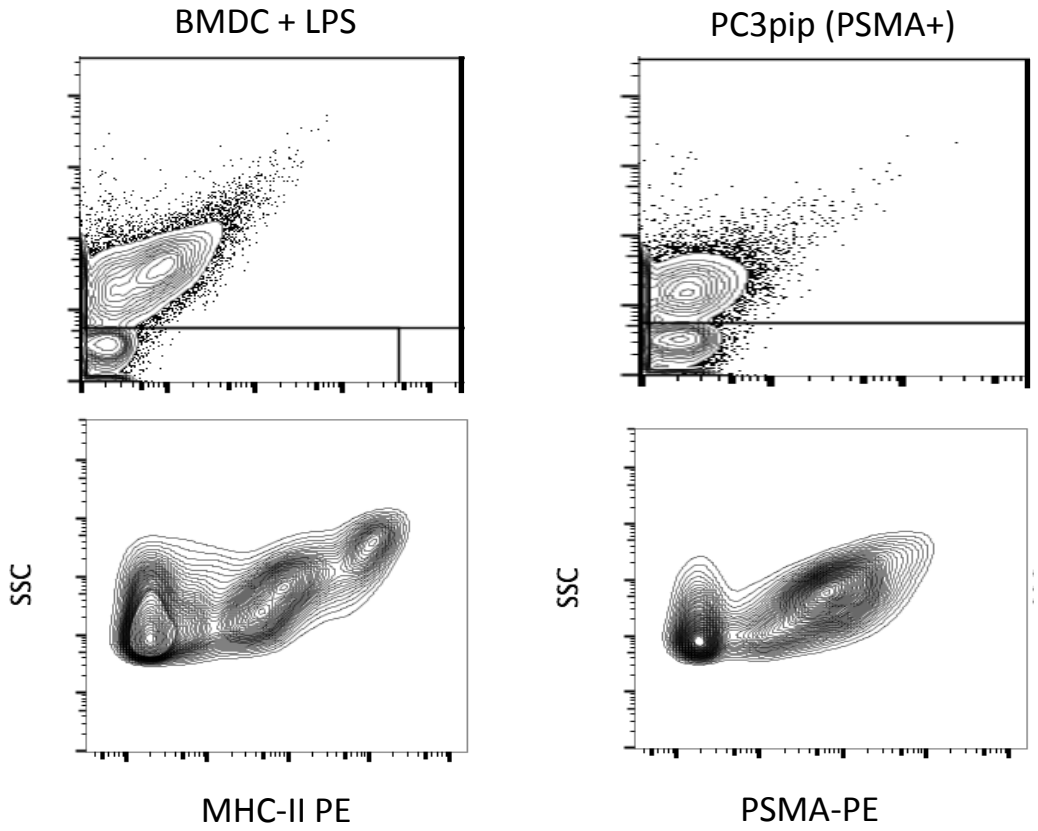
High resolution FCM versus Next Generation FCM

Astrios-EQ nanoFACS

high speed detection, sorting
detection limit estimate:
~30-200 epitopes

nanoFCM prototype May 2017

highly dedicated setup
limited dynamic range
detection limit estimate:
1-10 epitopes



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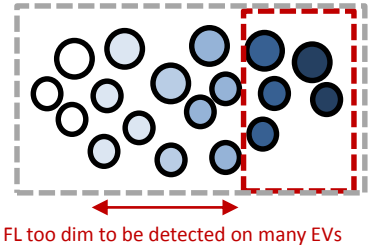
NCI High Sensitivity EV Flow Cytometry Evaluations 2017



Astrios-EQ nanoFACS

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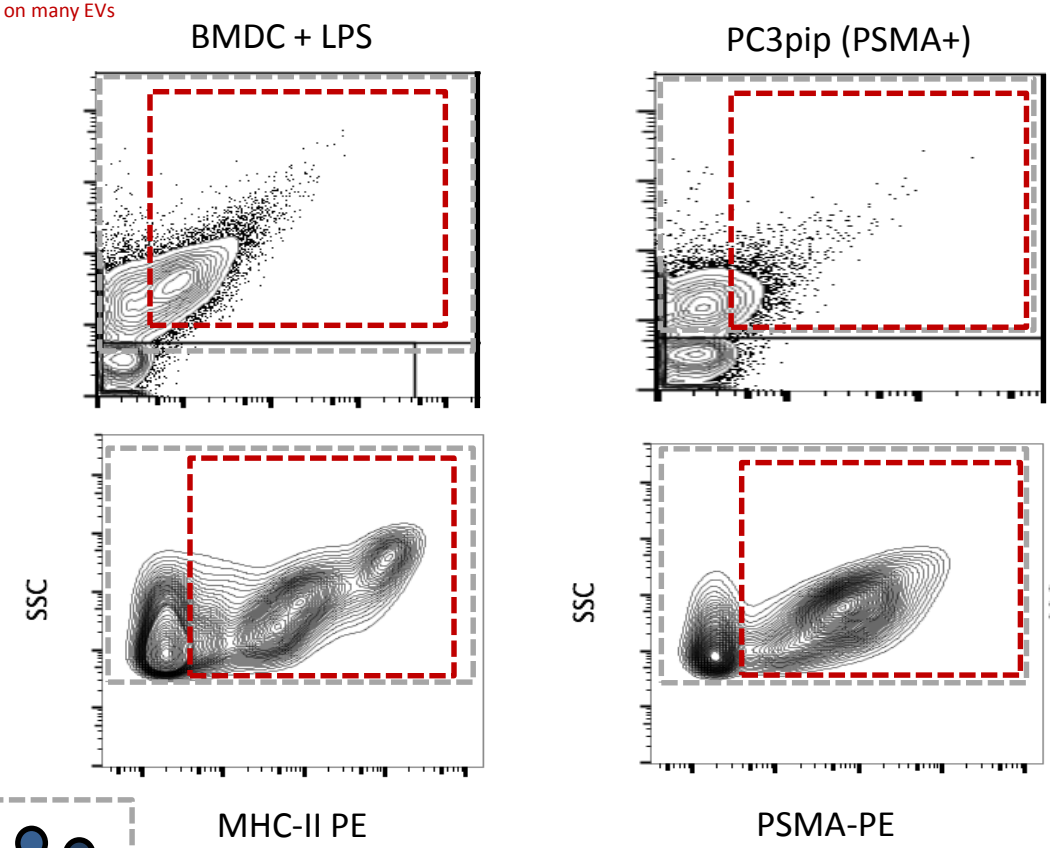

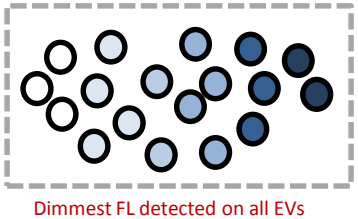

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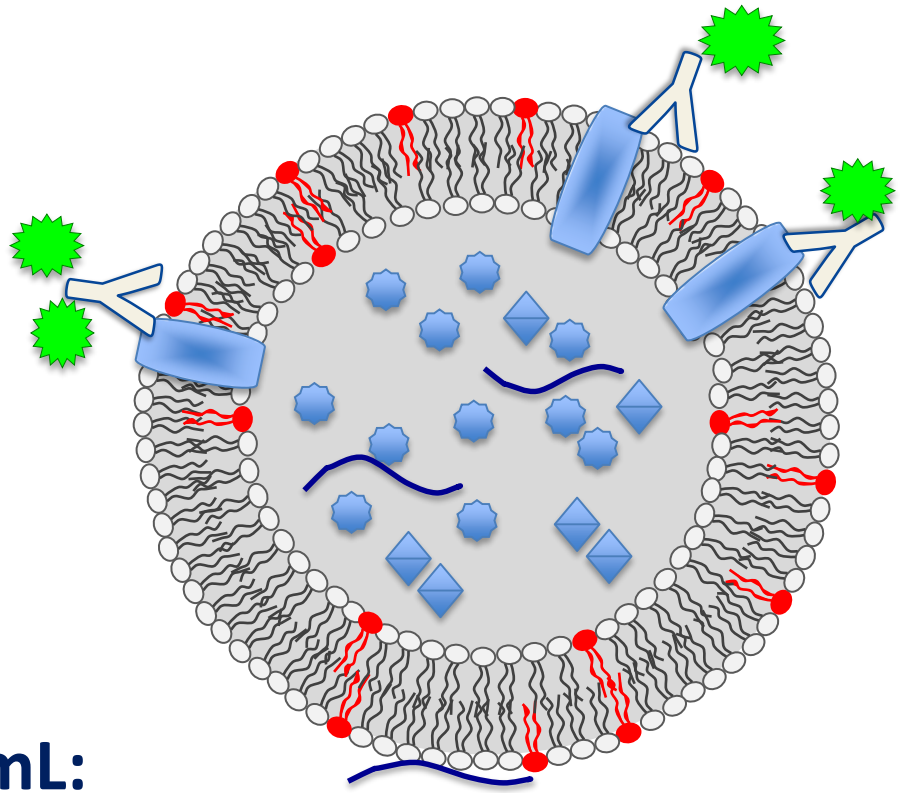
OMD
20-16
NO PARKING
CRAP METAL
MPA

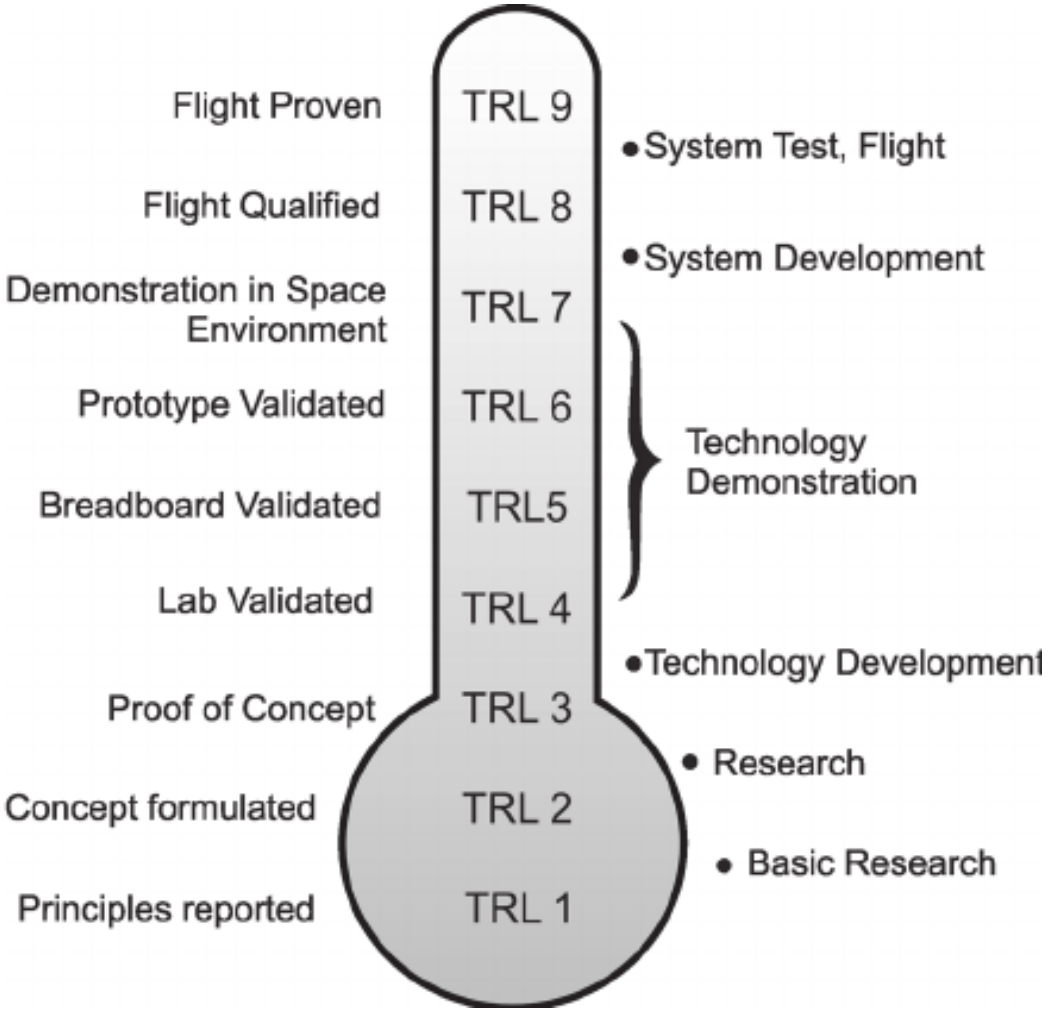

CRAP METAL

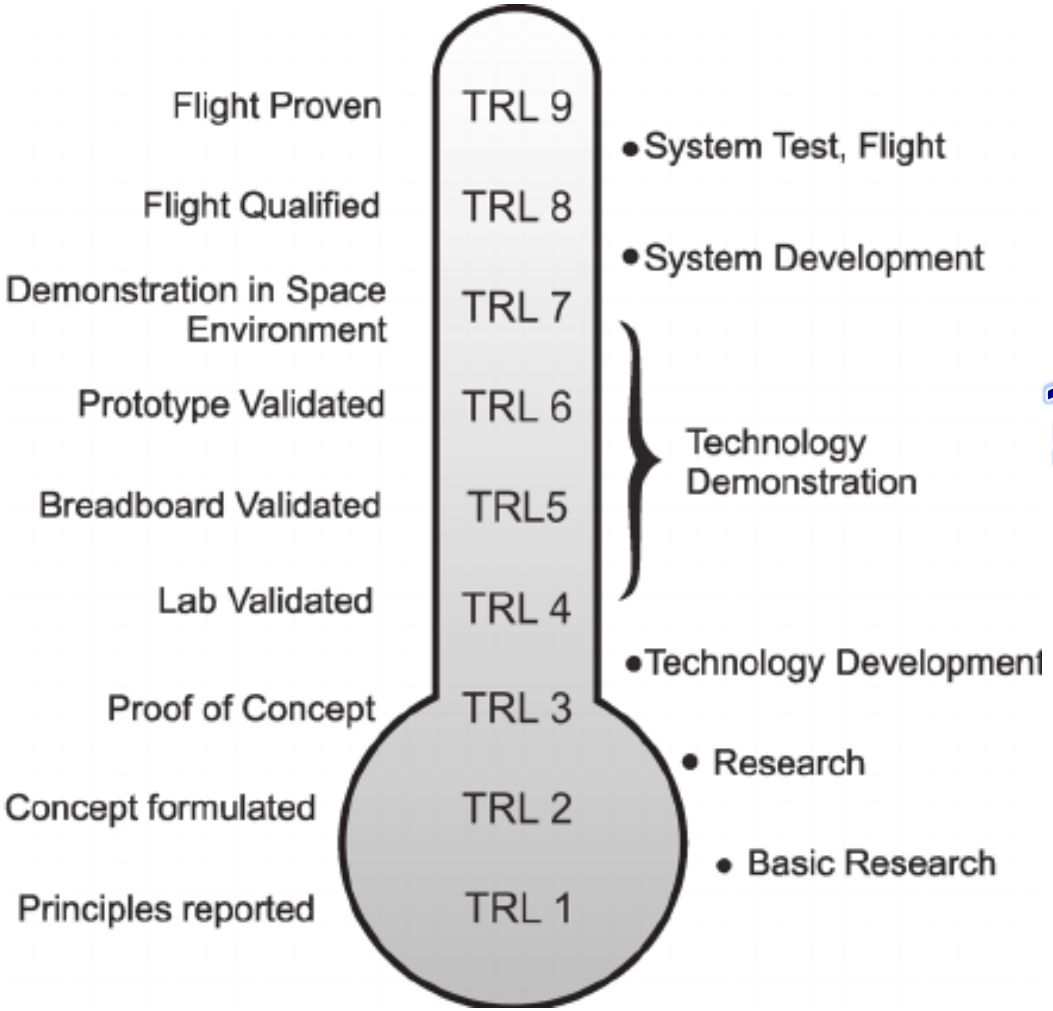

SCRAP METAL



To report X+ EVs per mL:

Accurate single vesicle analysis with **single epitope and small EV sensitivity** is essential for accurate reporting of how many EVs/mL are positive for X


Flight Proven
TRL 9
• System Test, Flight
Flight Qualified
TRL 8
• System Development
Demonstration in Space Environment
TRL 7
Prototype Validated
TRL 6
Technology Demonstration
Breadboard Validated
TRL5
Lab Validated
TRL 4
• Technology Development
Proof of Concept
TRL 3
• Research
Concept formulated
TRL 2
• Basic Research
Principles reported
TRL 1


Flight Proven
TRL 9
•System Test, Flight
Flight Qualified
TRL 8
•System Development
Demonstration in Space Environment
TRL 7
Prototype Validated
TRL 6
Technology Demonstration
Breadboard Validated
TRL5
Lab Validated
TRL 4
•Technology Development
Proof of Concept
TRL 3
• Research
Concept formulated
TRL 2
• Basic Research
Principles reported
TRL 1

?

sitc

Technology Readiness Levels: Overview of Jones Lab EV Biomarker Program in the NASA TRL Framework

Publication / Patent

- Jones Lab
- Jones Lab Collaboration

Ongoing Studies

- "Flow Virometric Sorting of infections HIV quasispecies from plasma for genomic and proteomic analysis." ***JCI Insight***, Feb 2017 (Marjorie Robert-Guroff Lab, NCI-VB)
- "Labeling Extracellular Vesicles for Nanoscale Flow Cytometry," ***Scientific Reports,*** May 2017
- "Manumycin A suppresses exosome biogenesis and secretion via targeted inhibition of Ras/Raf/ERK1/2 in castration-resistant prostate cancer cells," ***Cancer Letters,*** August 2017 (Asim-Abdel Mageed Lab, Tulane University)
- ***PLoS One,*** 2016 (a precursor to robust nanoFACS methods)
- **Provisional Patent 2017:** "Novel Methods for Submicron Vesicle or Particle Processing, Purification, and Detection"
- **PCT# 62/411,324 2016** "Molecualr NanoTags"
- "Efficient production and enhanced tumor delivery of engineered EVs." ***Biomaterials*** 2017 (Pavlakis Lab, NCI-VB)
- "Obstacles and opportunities in the functional analysis of extracellular vesicle RNA." ***Journal of Extracellular Vesicles*** 2017
- "Detection of Platelet Vesicles by Flow Cytometry" ***Platelets*** 2017
- "Serum Exosome Biomarkers for Immunotherapy and Radiation Responses" (abstract) ***Int J Radiation Biology & Physics*** 2011

TRL 9
Actual system "flight proven" in successful missions
Prospective Clinical Trial Validation

TRL 8
Actual system completed and "flight qualified"
CLIA - ready

TRL 7
System prototype demonstration in space
Exploratory Tests of Pipeline for Clinical Samples

TRL 6
System prototype in relevant environment
Use of Pipeline to test Biorepository Samples

TRL 5
Component validation in a relevant environment
Assay validation with Healthy Serum, Plasma EVs

TRL 4
Component validation in a laboratory environment
Assay validation: Cancer/Immune Cell EV Subsets

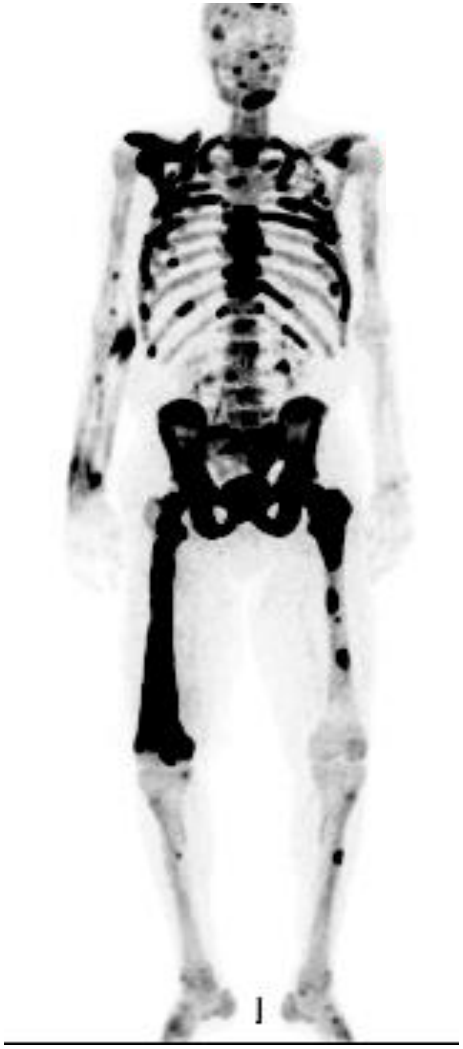
TRL 3
Analytical and experimental proof-of-concept
Assay validation: General EVs and HIV Subsets

TRL 2
Technology concept and/or application formulated
Formulation of EV Subset Hypothesis & Identification of Technological Requirements

TRL 1
Basic principles observed and reported
Observed increased CD81 with Radiation and Absence of early metrics for treatment response

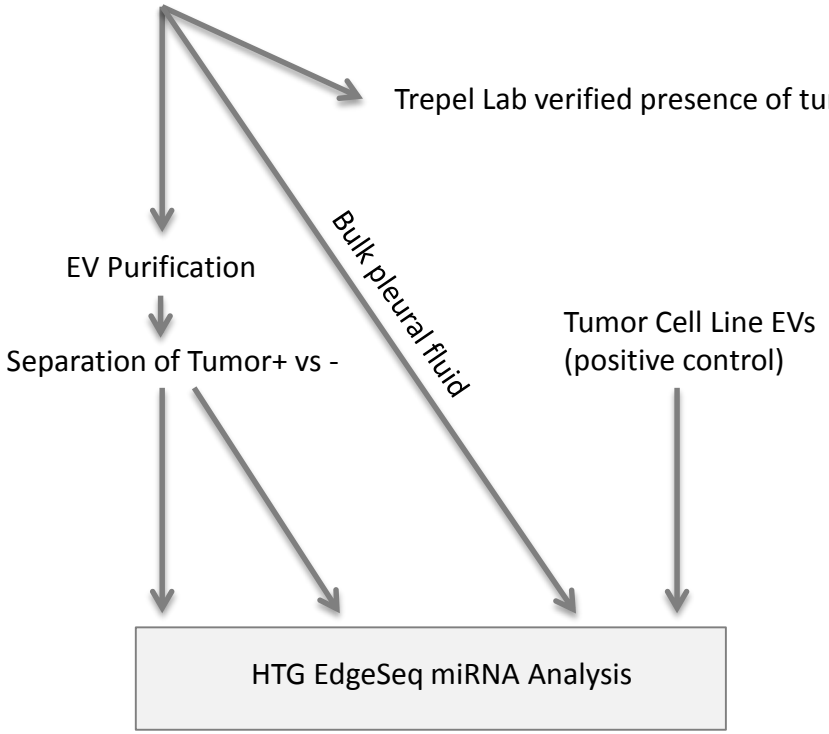
Clinical Example: GMB/ROB Patient w Metastatic Tumor

Tumor Scan



Malignant Pleural Effusion

Large volume clinical bio-fluid sample. Useful for nanoFACS method validation, refinement.



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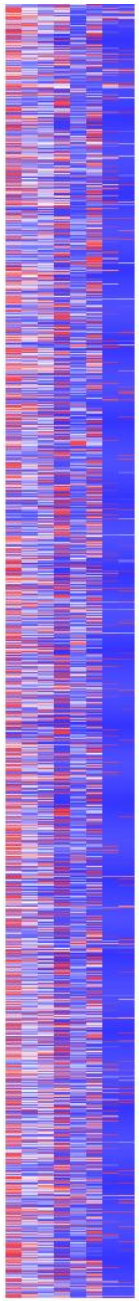
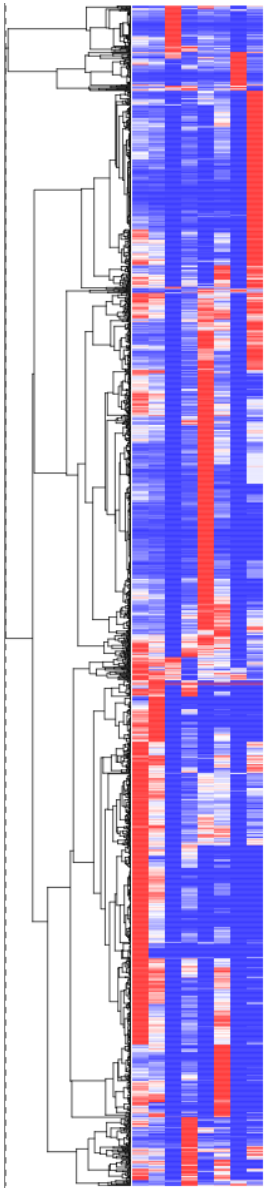
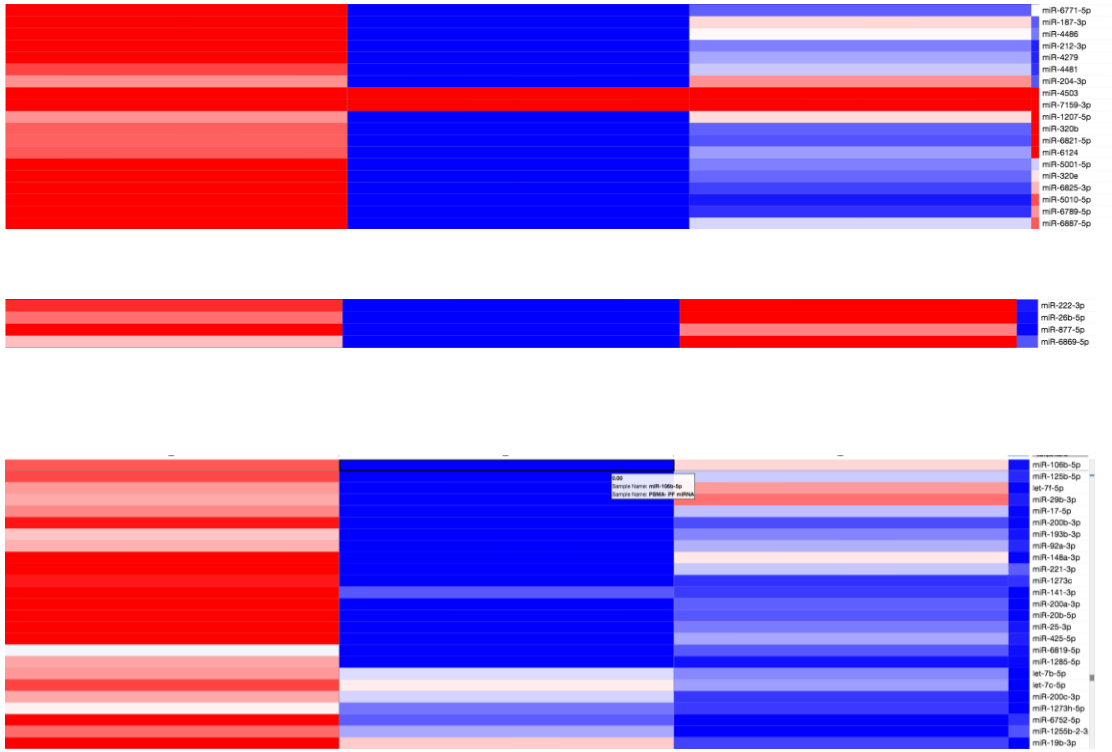
Tumor miRNA Enriched in PSMA+ Exosomes



Hierarchical Clustering



Tumor+ Exo miRNA | **Tumor- Exo miRNA** | **Total miRNA**



miRNA-204
miRNA-1207
miRNA-222
miRNA-26b
miRNA-877
miRNA-6869
miRNA-106b
Let7f
miRNA29b
miRNA-148
miRNA-221

miRNA-204
miRNA-1207
miRNA-222
miRNA-26b
miRNA-877
miRNA-6869
miRNA-106b
Let7f
miRNA29b
miRNA-148
miRNA-221

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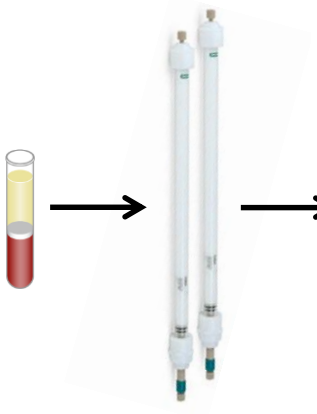
Critical Steps for EV/Exosome Analysis in Immunotherapy

A. Sample

B. Isolation

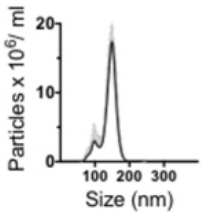


C. General EV Size, Concentration, and Cargo Assays

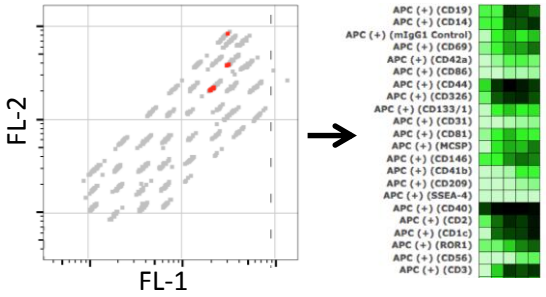
Protein Concentration

Western Blot (Tsg101, Alix)

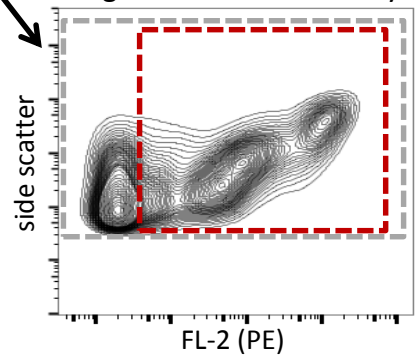
Nanoparticle Tracking Analysis



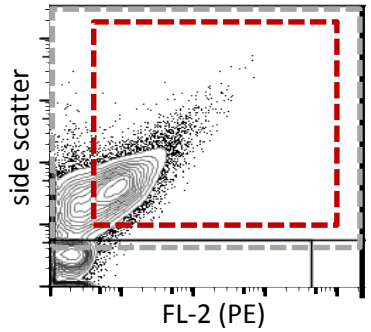
D. Multiplex EV Assay: > 20 Epitope Survey



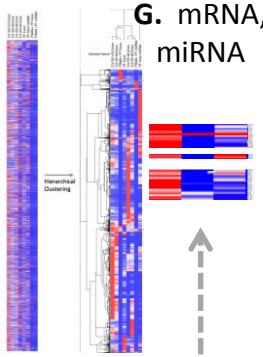
E. High Resolution EV Analysis



F. nanoFACS Sorting



G. mRNA, miRNA



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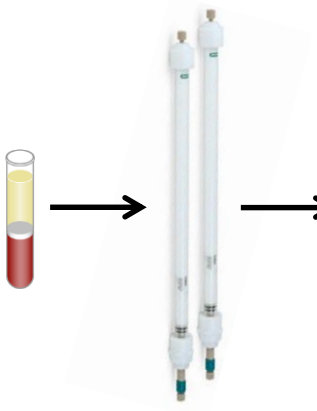
Critical Steps for EV/Exosome Analysis in Immunotherapy

A. Sample

B. Isolation

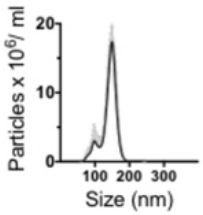


C. General EV Size, Concentration, and Cargo Assays

Protein Concentration

Western Blot (Tsg101, Alix)

Nanoparticle Tracking Analysis



Each Step in Sample Collection, Processing and EV Isolation is Critical

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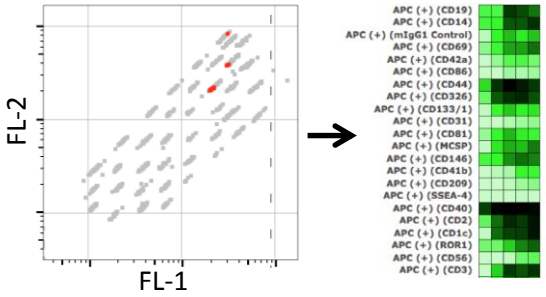

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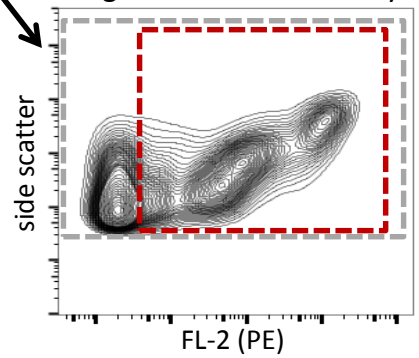

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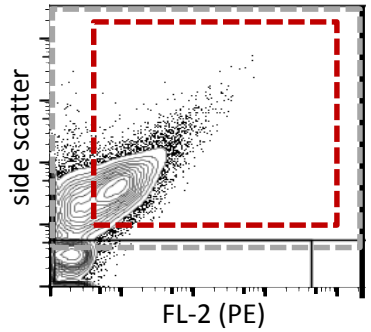

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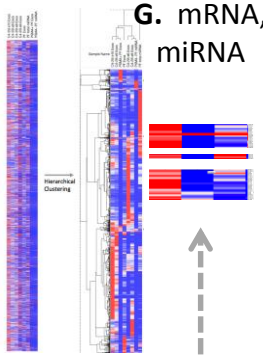

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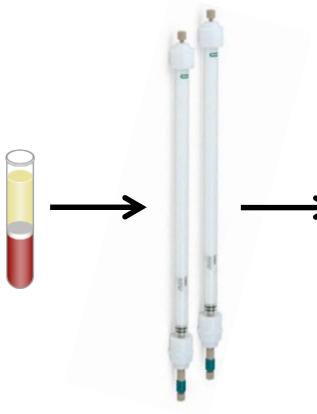

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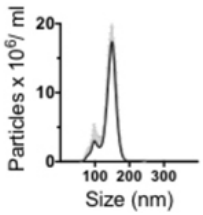

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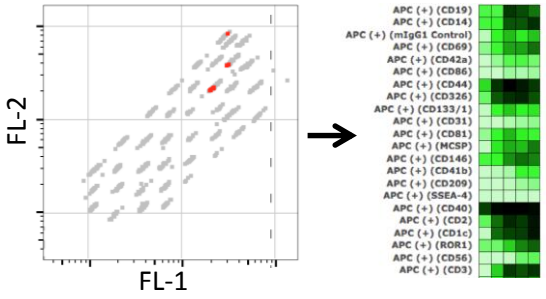

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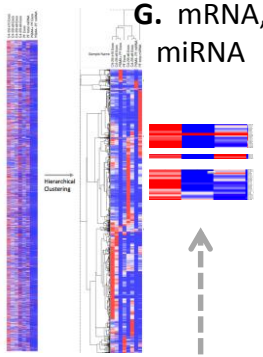

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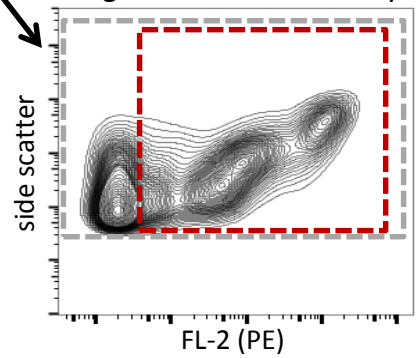

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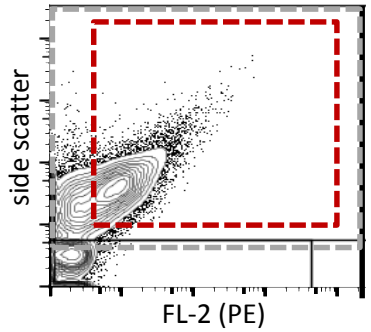

Specific analytical approach depends on what information is being interrogated

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NCI – Vaccine Branch
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