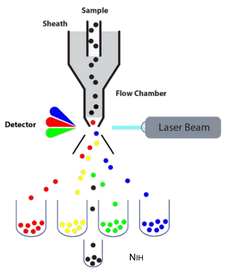
Phenotyping and Sorting of Individual Free Infectious HIV Particles by Flow Cytometry

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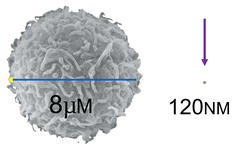
Motivation

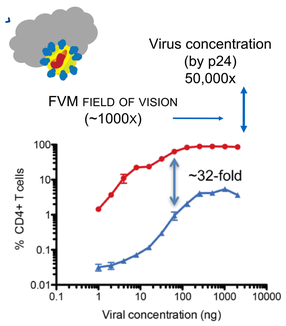


Flow cytometry (**cyto**=cell)
Flow virometry (**viro**=virus)

Analysis of physical and chemical characteristics of particles in a fluid as it passes through at least one laser. Subsets can be **sorted** away for functional studies.

Size comparison of cells and HIV. Modern cytometers have a detection cutoff of **~300nm** by light scattering alone (used to determine cell populations by size and granularity).

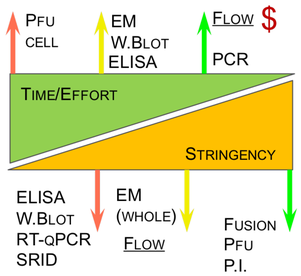




Why Virometry (FVM)?
~1 in 50,000 viruses fuse
- This is likely generous (more like 1 in 100,000)
- Unsuccessful viruses might be immune decoys

1 in 10-50 of those infect productively
- Latency plays a role
- Cell defenses (antiviral proteins)

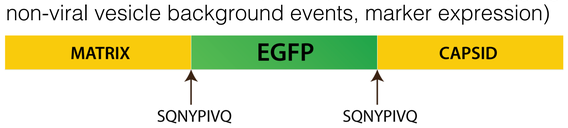
What is the pre-fusion block to infection?
Understand hurdles to fusogenicity and infectivity
Blocking them = Therapy
Enhancement = Gene Therapy



Things <1μm
Microvesicles
Exosomes
Picoplankton
Pollutants
Viruses
Immune complexes

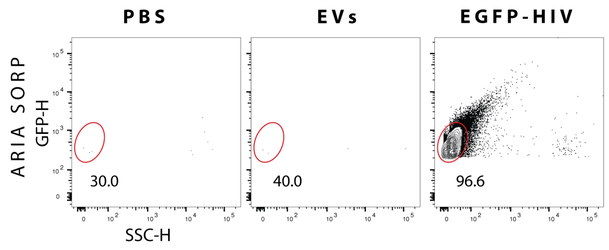
Areas of research
Environmental,
Marine Biology
Virology, Bacteriology
Cancer Biology
Extracellular vesicles

Using flow virometry for virus quantification offers advantages in a laboratory setting, but such a method has not been developed due to technical and biological limitations of the system (small genomes resist tagging; non-viral vesicle background events, marker expression)

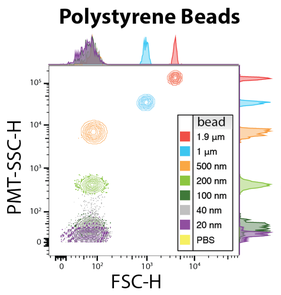


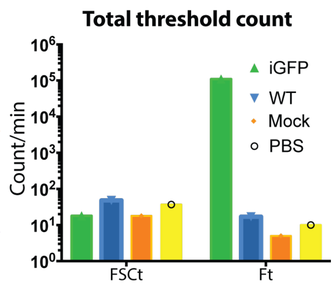
Introduction of a **fluorescent parameter** into the structural genes of the virus aids in the detection of nanoparticles which can't be picked up by light scatter alone.

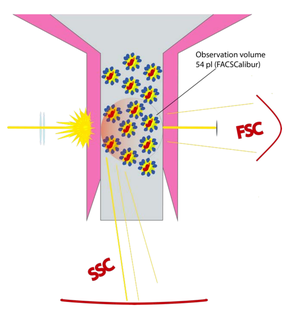
Results



Fluorescent virus detection by flow cytometry. Using an internal fluorescent specificity tag fused to the HIV structural proteins provides a low-background viral particle detection method.

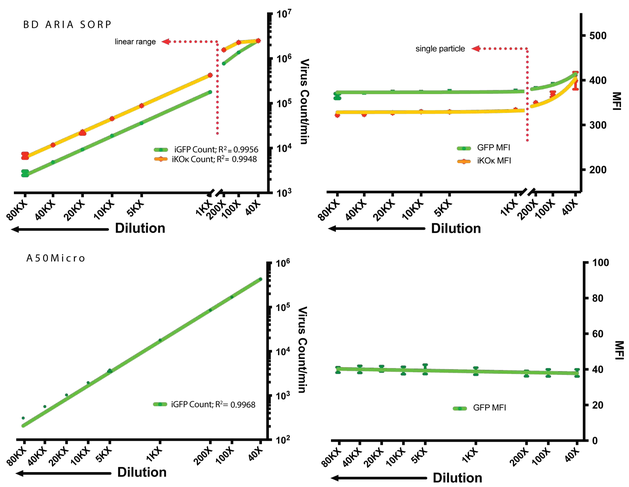






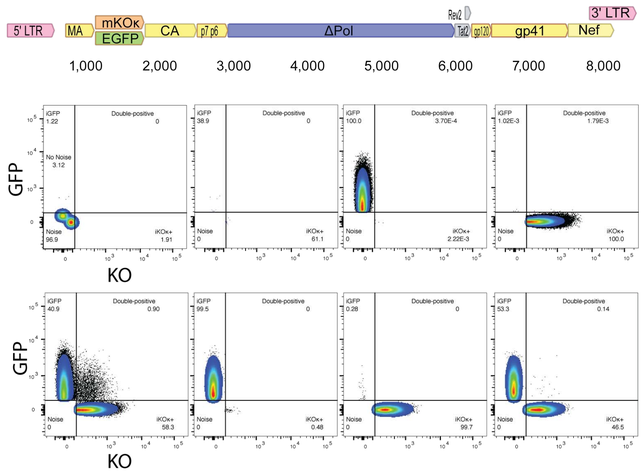
We have to guard against **coincidental (swarm) detection.** It's a phenomenon of multiple biological events being counted as one large, bright event at large concentrations due to their sub-micron size.

A series of dilutions can test detection accuracy.

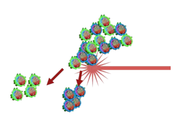


Dilution and observed particle count form a linear relationship, while the MFI (mean fluorescence intensity) remains constant. We are working with single viruses.

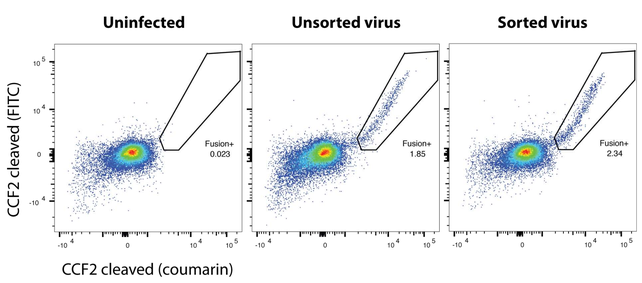
Results



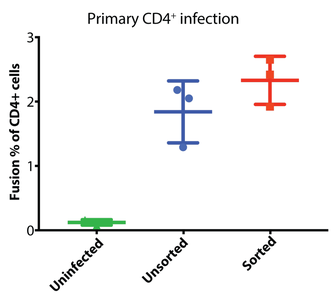
HIV containing internal fluorescent tags can be specifically detected in mixed populations. The component populations are then **sorted to high purity**. Minimal coincidental detection shown.

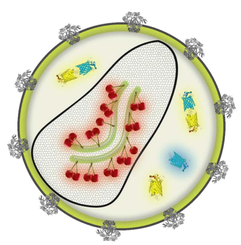


Isolation of live virus allows for standard **biochemical and functional testing** downstream – by TRPS, electron or confocal microscopy, RT activity (PERT), western blotting, etc.



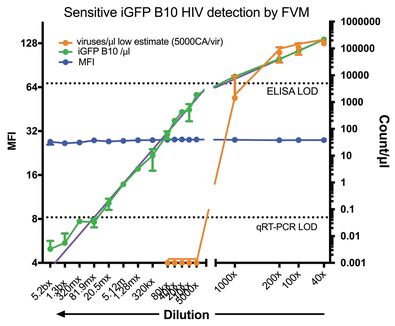
The sorted CXCR4-tropic HIV retains infectivity. Prior reports of HIV Env fragility necessitate rigorous functional testing of viruses subjected to sorting. The CCF2-AM/β-lactamase fusion assay shows no loss of fusogenicity using purified primary CD4+ cells.



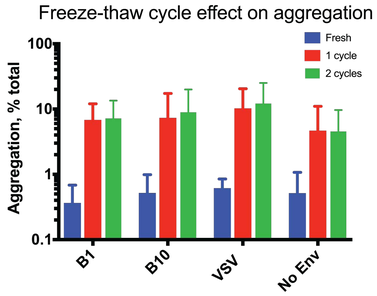


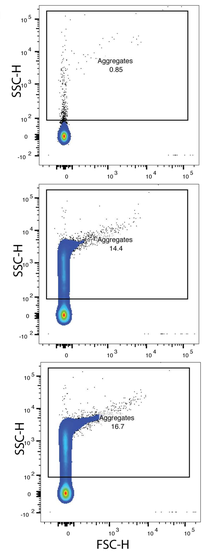
A fluorescent reporter virus may potentially contain bio-tags for **physical and functional components of HIV**, such as internal protein incorporation or protease activation, respectively. A green internal signal helps in detection of the virus by flow, while tags in other colors provide an infectivity predictor.

Results

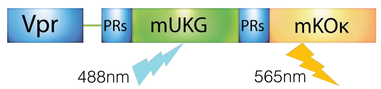


FVM as an HIV quantification method. A comparison of volumetric FVM (Apogee A50M) and ELISA shows high sensitivity of virus detection compared to standard methods. LOD=limit of detection.

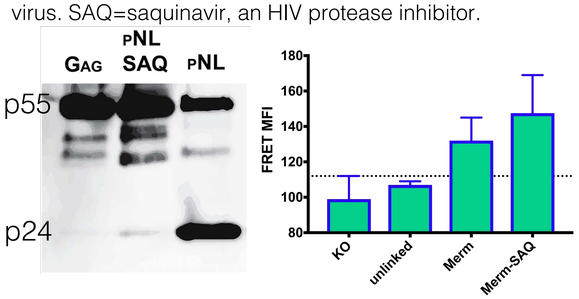




Aggregation rate analysis by FVM. Pseudotyped HIV can aggregate, and that behavior is easily quantified with a flow cytometer. This may be a useful parameter for rapid evaluation of clinical VLP preparation quality.



Fluorescent protease activity reporter. HIV maturation is required for infectivity. A reporter FRET construct was joined by an HIV protease site and specifically imported into virions as a Vpr fusion. The resulting FVM readout corresponds to the rate of p55 processing within the virus. SAQ=saquinavir, an HIV protease inhibitor.



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