Optical Trapping and Manipulation of a Single Human Virus

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Optical Trapping and Manipulation
in Molecular and Cellular Biology
Technical Group

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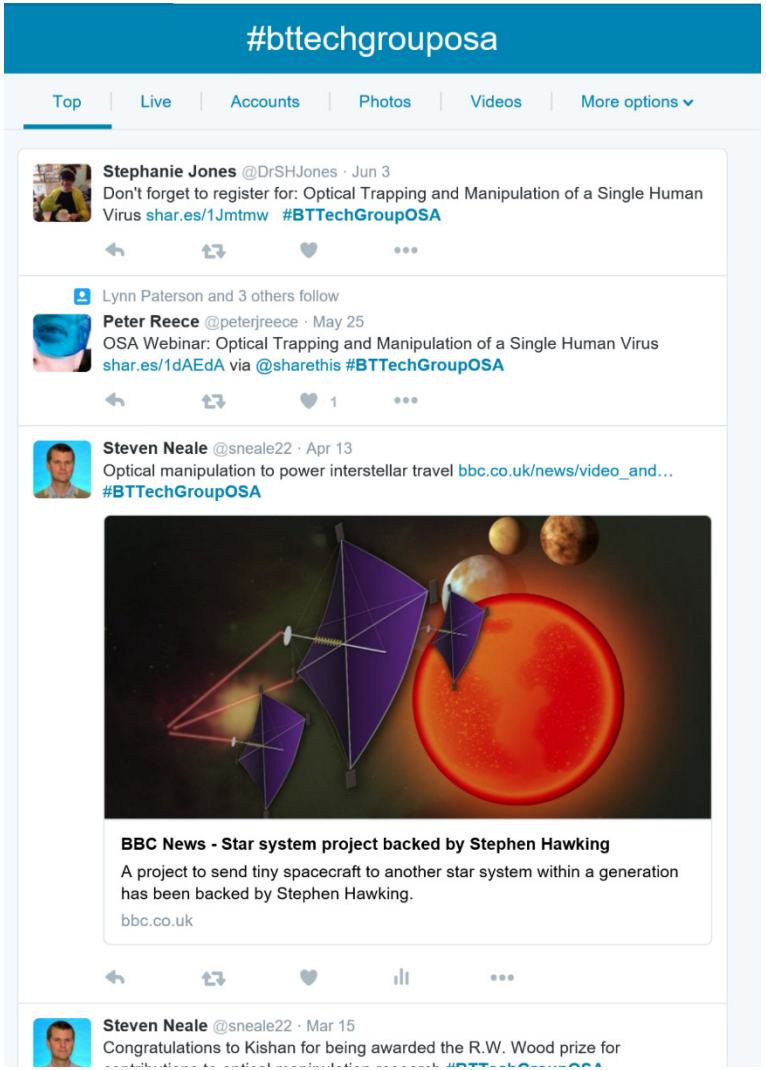

Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group

Webinars (today is our first!)
Previous webinars will be available for viewing at the OSA Technical Group website

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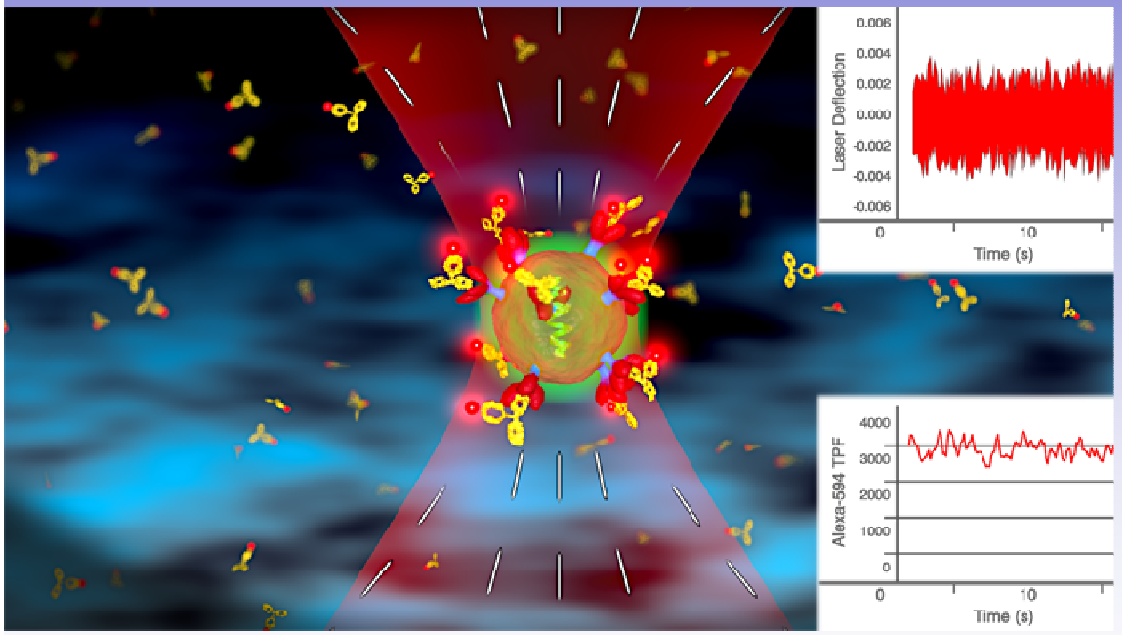
Facebook page
Optical Cooling and Trapping (OT) Technical Group; https://www.facebook.com/groups/1874 51984746395/

OSA Optical Trapping and Manipulation in Molecular and Cellular Biology Technical Group

Welcome to Today's webinar!

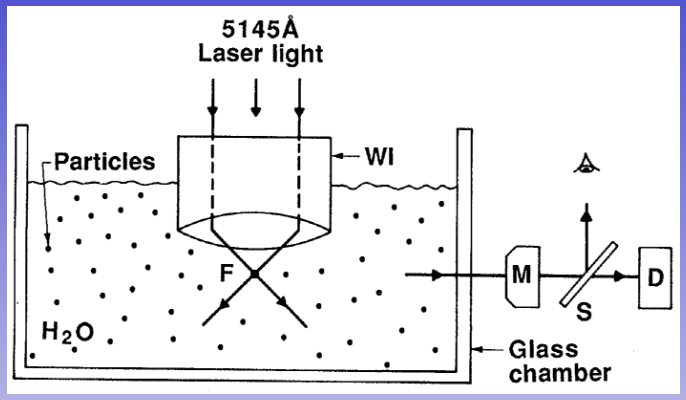
Dr Wei Cheng – University of Michigan

Optical Trapping and Manipulation of a Single Human Virus



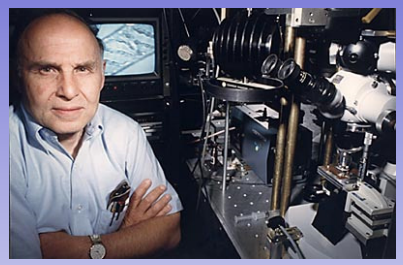
Wei Cheng
Associate Professor
Pharmaceutical Sciences
Biophysics
Biological Chemistry
University of Michigan, Ann Arbor

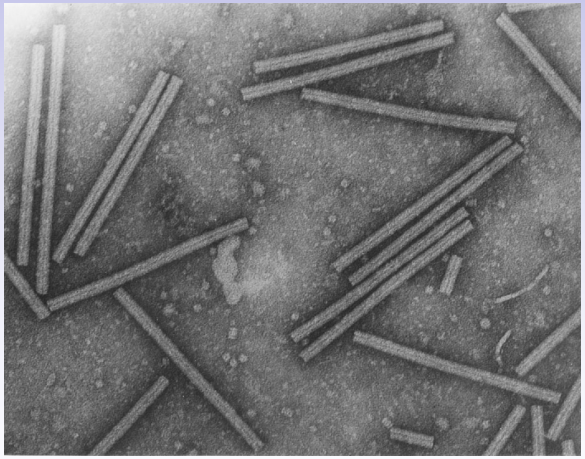
Trapping of Single Tobacco Mosaic Virus



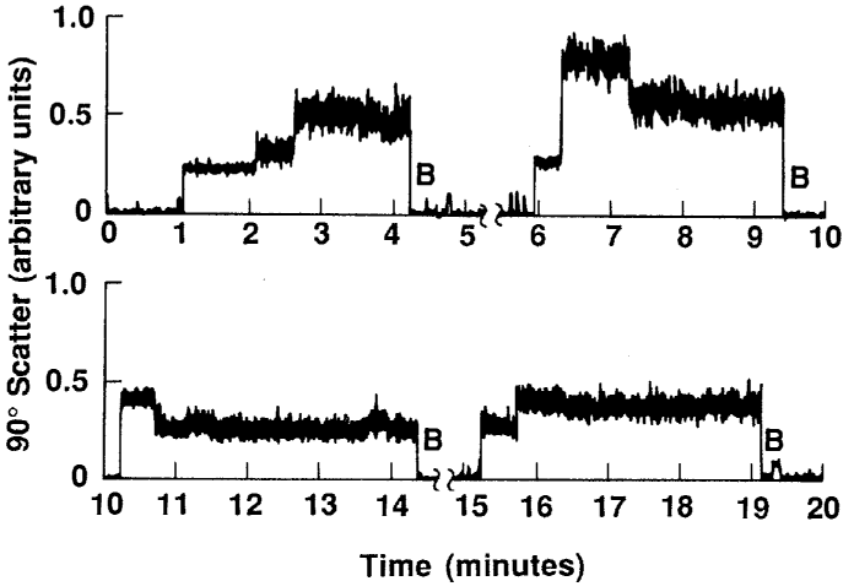
Optical Trapping and Manipulation
of Viruses and Bacteria

Ashkin & Dziedzic, ***Science*** 1987

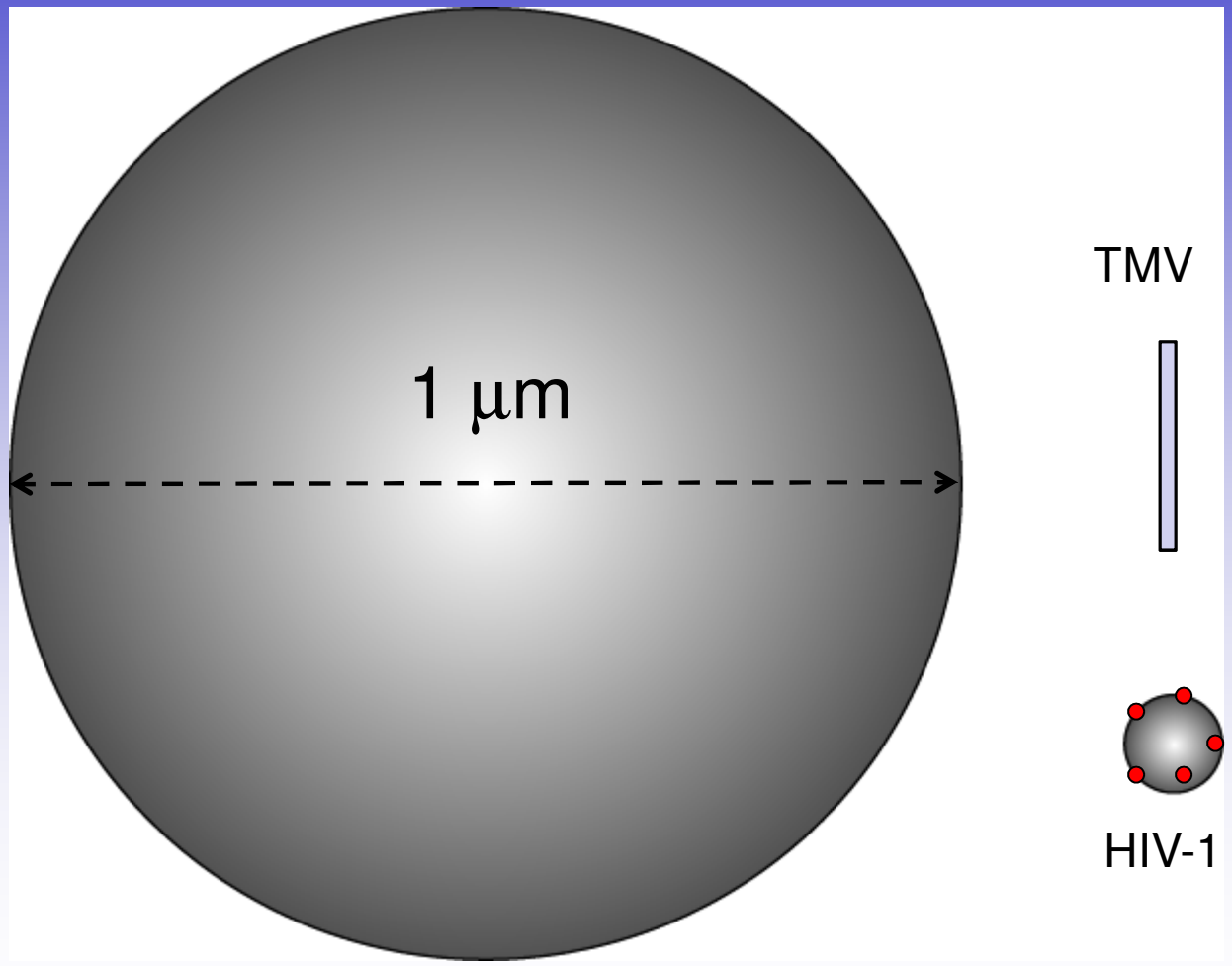




TMV, 300 nm long, 18 nm diameter



Challenges for Trapping Animal Viruses



(1) Small size
small dipole moment

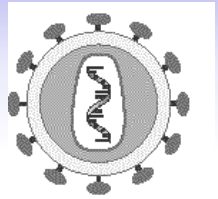
$$\left|\vec{P}\right| \propto d^3$$ **RI**

TMV has permanent dipole Moment,
Biopolymers (1976) 15: 301

(2) Index of refraction unknown

Technical Elements to Prepare for Trapping of a Single Virus

(1) The choice of trapping laser wavelength: 830 nm instead of 1064 nm
Less heating, free of oxygen-mediated photo damage



DNA or RNA genome

(2) Back-focal-plane interferometry with high accuracy:
Diffusion coefficient, corner frequency, particle diameter, trap stiffness

(3) Simultaneous two-photon fluorescence excitation by the 830 nm trapping laser with single-fluorophore sensitivity

830 nm CW laser can excite **GFP**

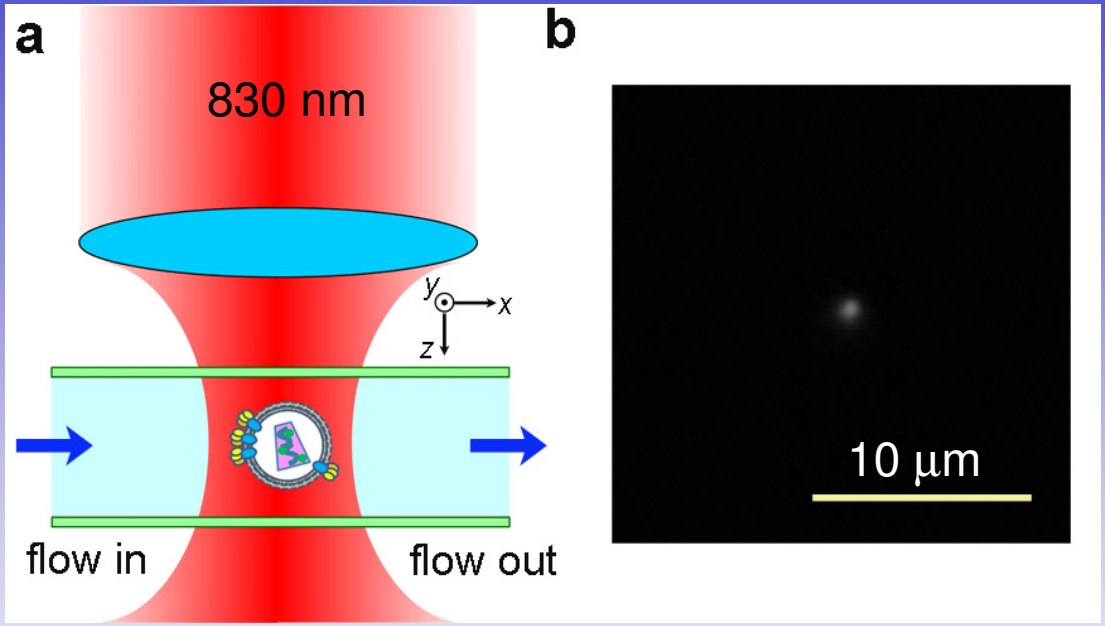
Cheng, Hou & Ye, ***Opt. Lett*** (2010) 35: 2988

Hou & Cheng, ***Opt. Lett*** (2011) 36: 3185

Hou & Cheng, ***Biomed. Opt. Express*** (2012) 3: 340

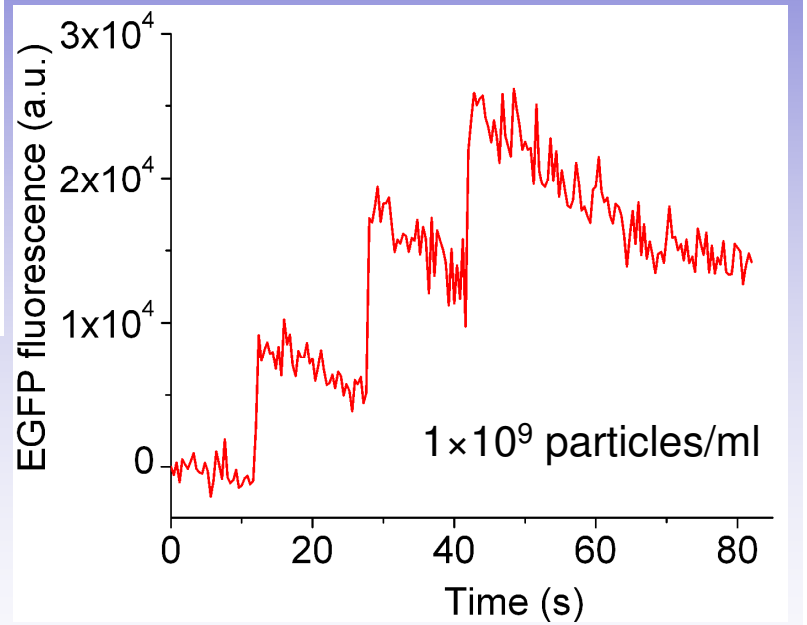
Optical Trapping of HIV-1 Virions in Culture Media

Pang…Cheng, ***Nature Nanotech*** (2014) 9: 624



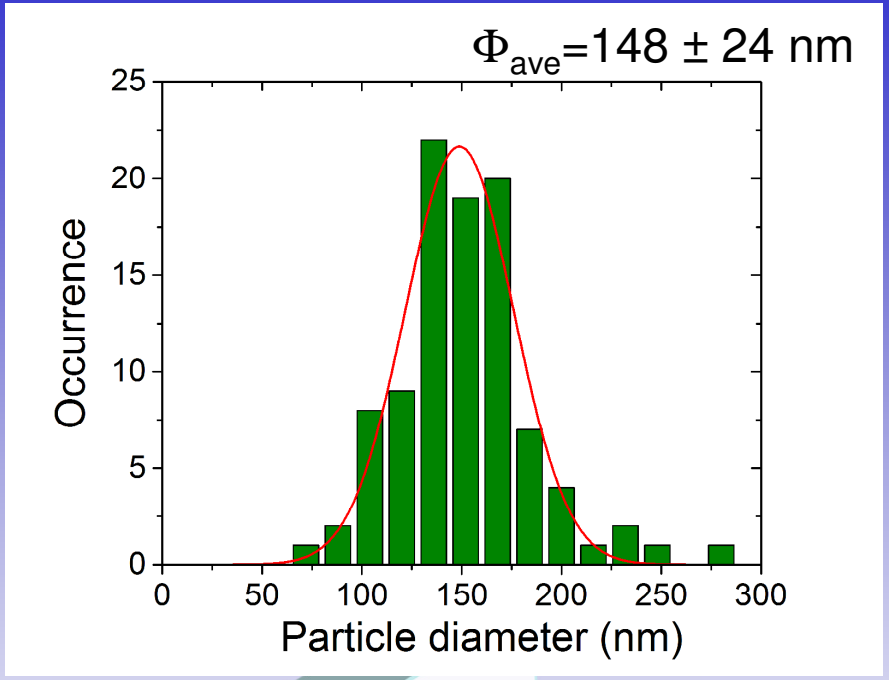
EGFP labeled HIV-1

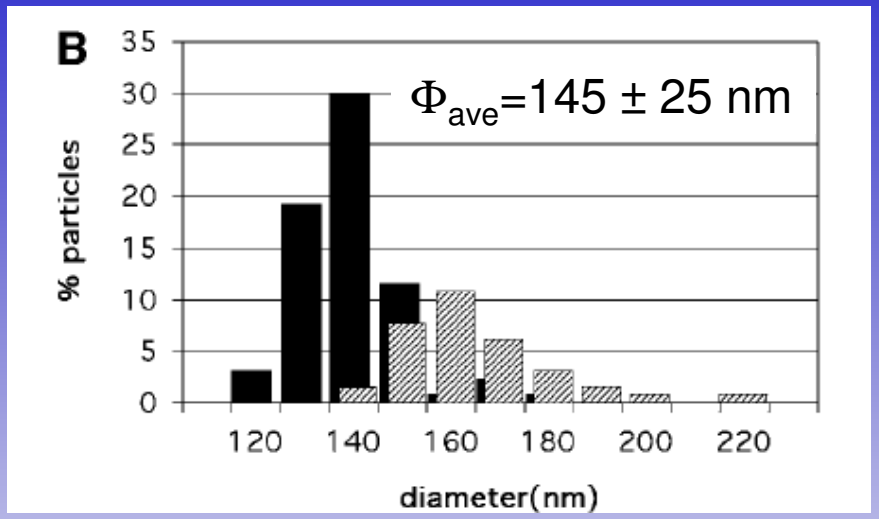
Kim…Cheng, ***PLOS One*** (2013) 8: e67170.

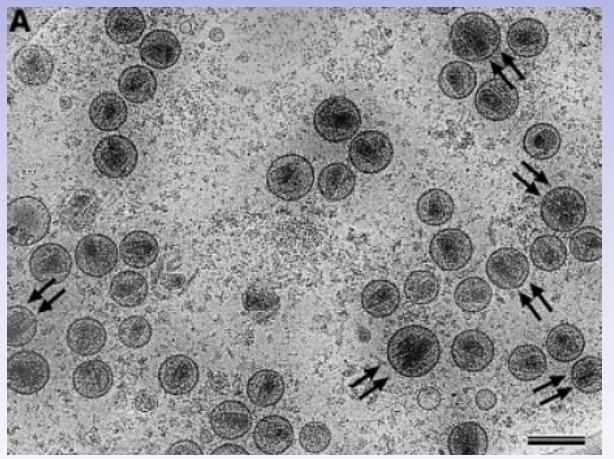


(in complete culture media: 90% DMEM + 10% FBS)

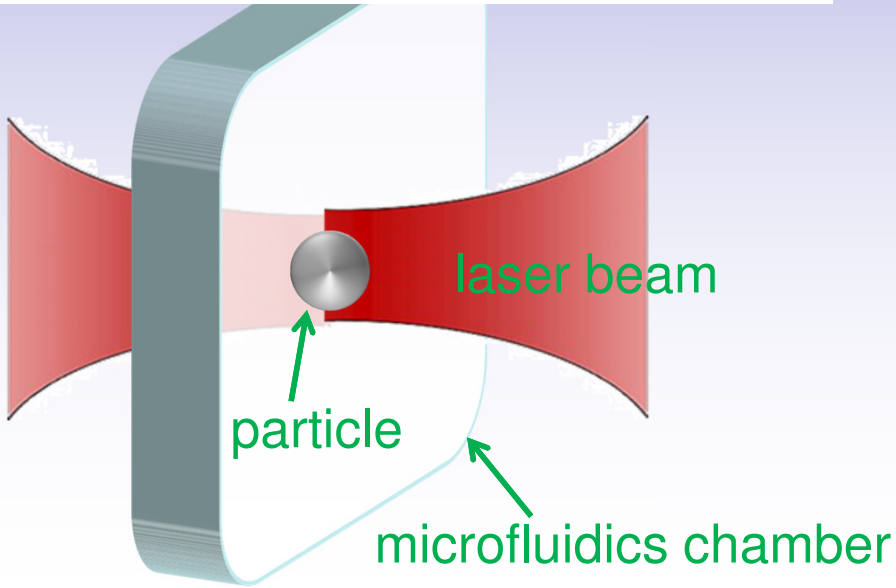
Polydispersity of HIV-1 Virions





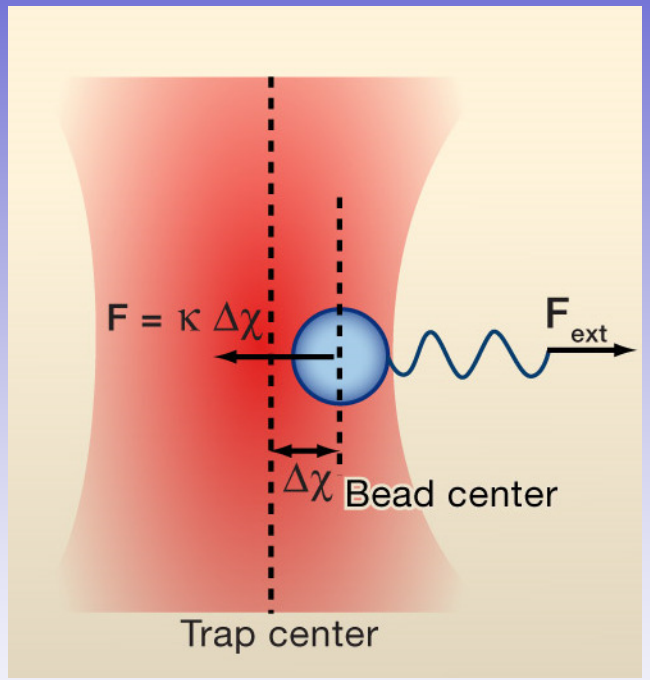


Briggs et al., ***EMBO J*** (2003) 22: 1707

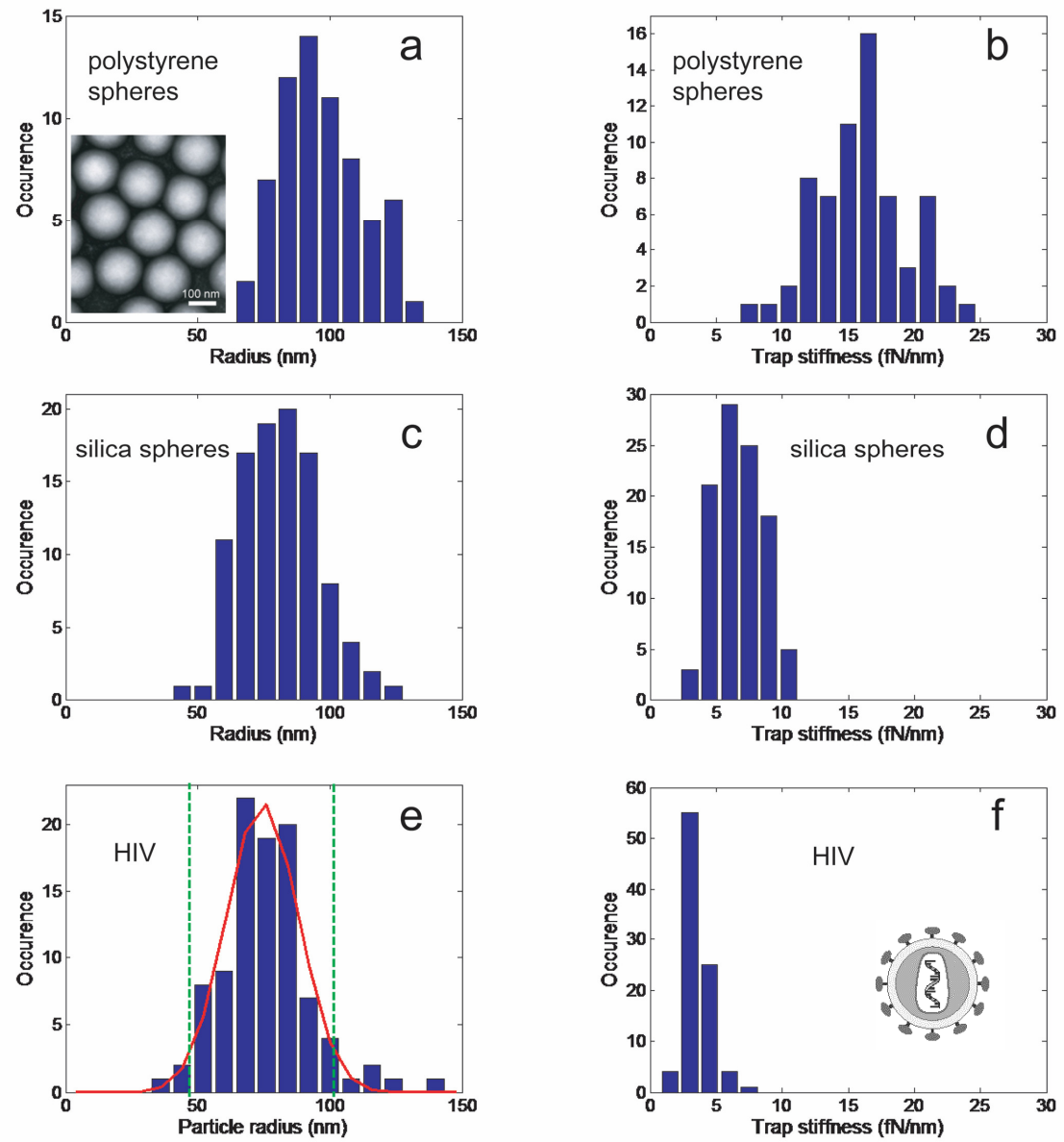


Tolić-Nørrelykke…Flyvbjerg, ***Rev. Sci. Instr.*** *(2006)* 77: 103101

Optical Trap Stiffness and Materials



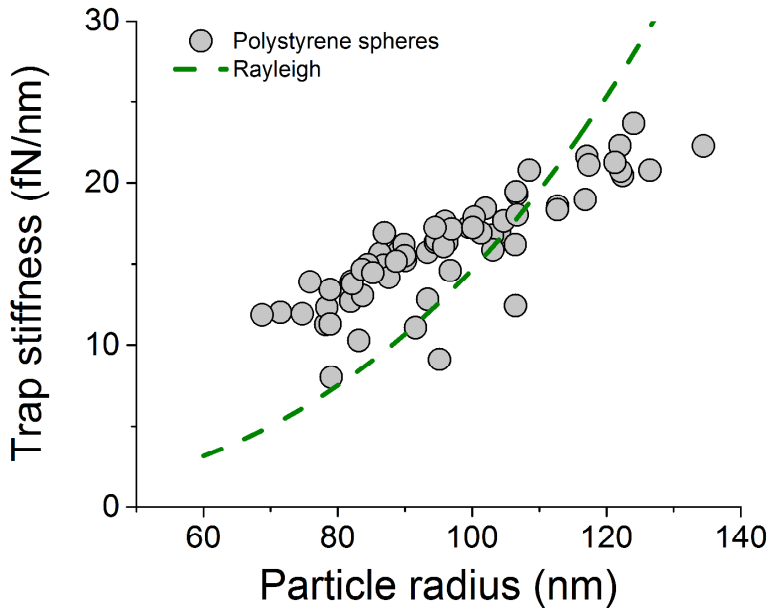
Bustamante, Cheng & Mejia, ***Cell*** (2011) 144: 480

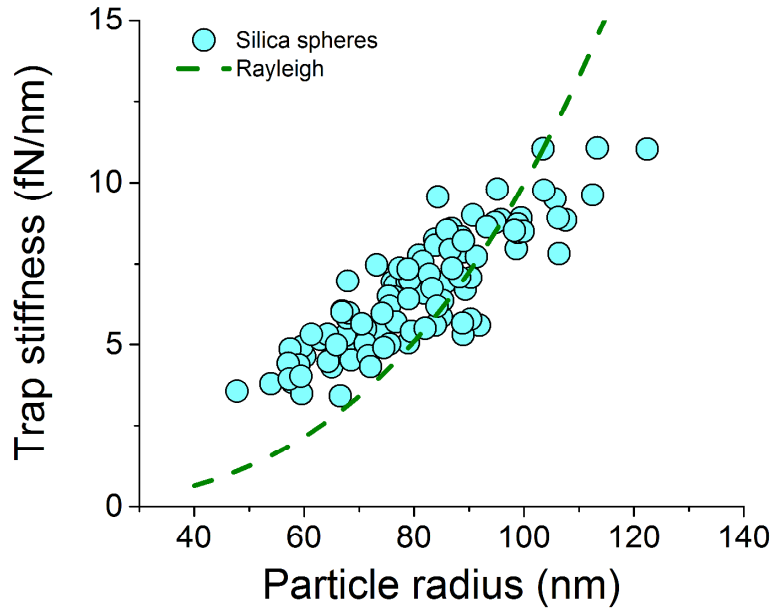


Relate Optical Trap Stiffness to Particle Size

Rayleigh particle, stiffness $\propto R^3$

$4\pi n_0 R/\lambda << 1$, in reality > 1



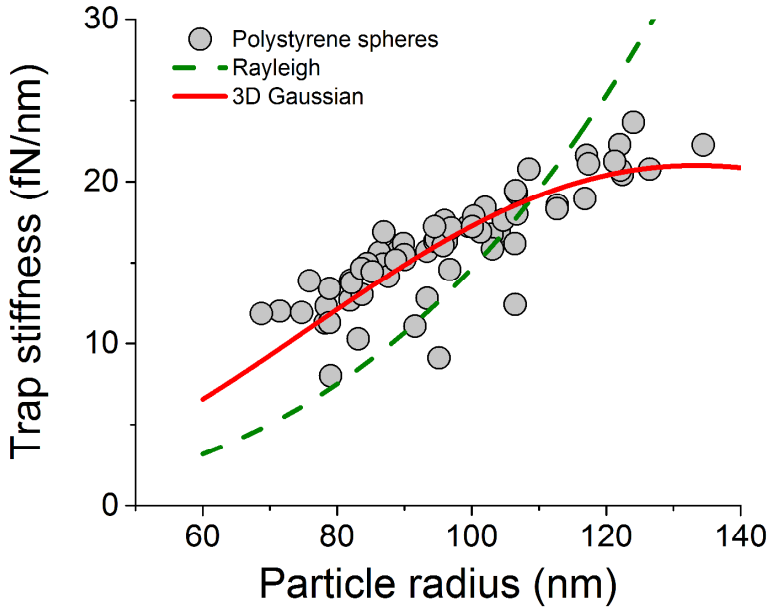


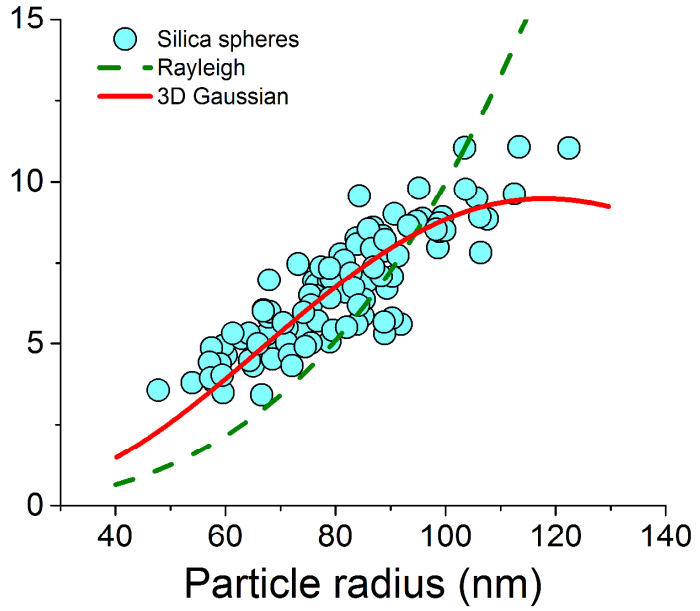
An Analytic Solution to Optical Trap Stiffness

$$\kappa = \alpha I_0 \omega_0 \frac{2\pi}{\xi^3}\left[\sqrt{2\pi}\left((\xi a)^2 + \frac{1}{4}\right)e^{-2a^2} erfi(\sqrt{2}a\xi) - \xi a e^{-2a^2/\varepsilon^2}\right.$$

Applies to spherical particles < 160 nm radius

Stiffness = F(**particle radius**, **particle refractive index**, **beam parameters**)

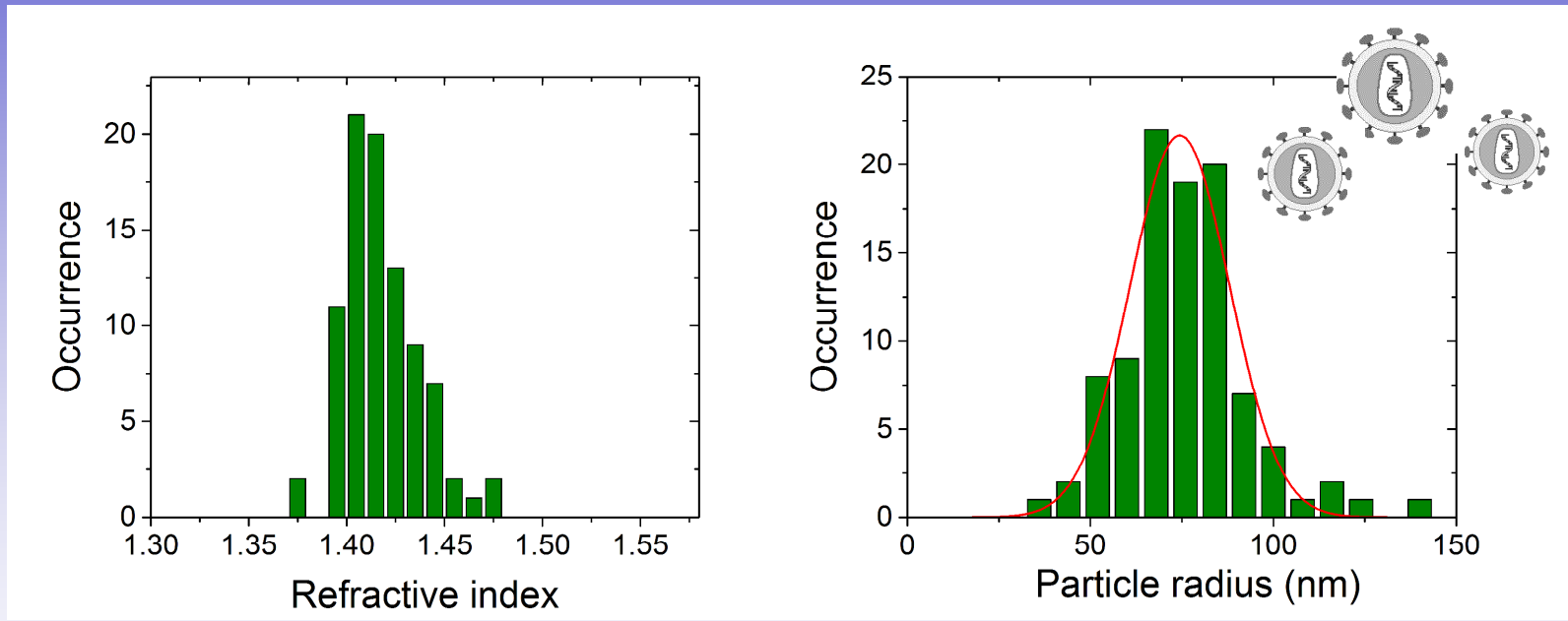




Pang, Song & Cheng, ***Biomed. Opt. Express*** (2016) 7: 1672

Measure Refractive Index for Single HIV-1 with High Precision

Stiffness = F(**particle radius**, **particle refractive index**, **beam parameters**)



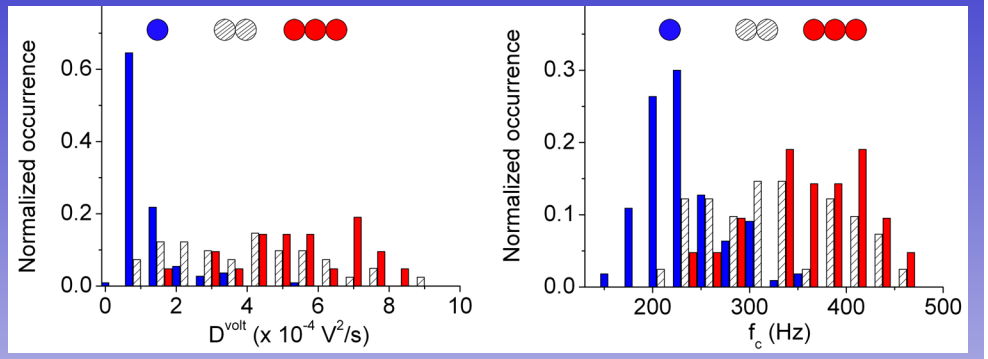
Mean ± SD	1.42 ± 0.02	74 ± 12 (nm)
CV	1.4%	16%

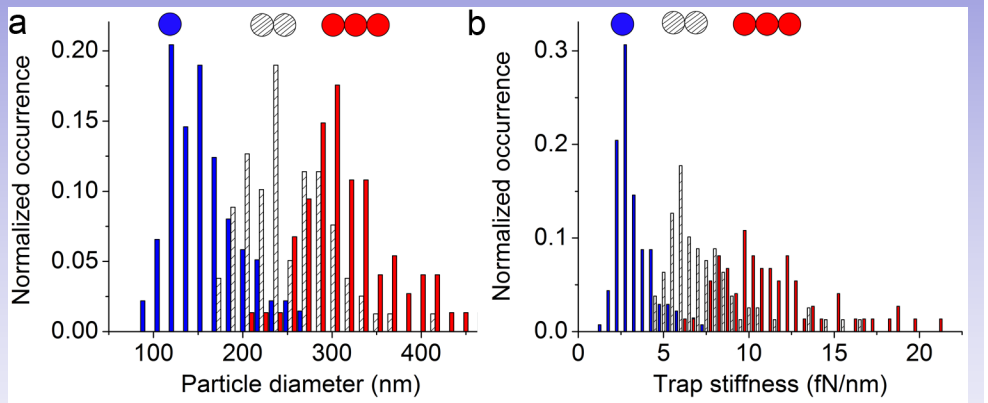
Compared to 1.58 of polystyrene; close to 2M sucrose; first time that the RI of a single virus was ever measured.

Pang, Song & Cheng, ***Biomed. Opt. Express*** (2016) 7: 1672

Parameters from Optical Trap

- Diffusion coefficient
- Corner frequency
- Particle diameter
- Trap stiffness
- Index of refraction





Pang, Song & Cheng, ***Biomed. Opt. Express*** (2016) 7: 1672

- Two-photon fluorescence

with single-molecule sensitivity

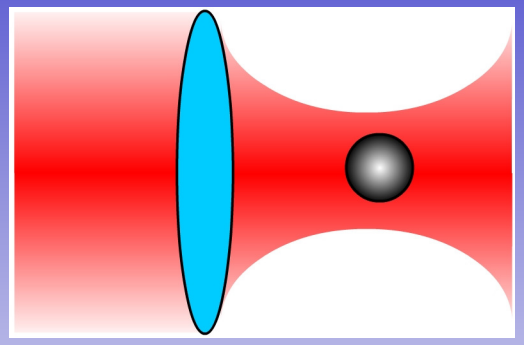
Pang…Cheng, ***Nature Nanotech*** (2014) 9: 624

Multi-parameter **analysis** and potential **sorting** of biological nanoparticles

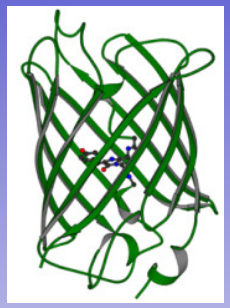
Current flow cytometry >300 nm

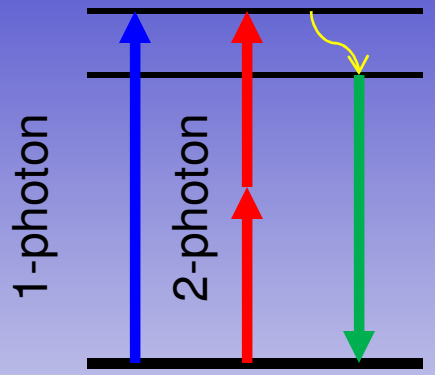
DeSantis & Cheng, ***WIRES Nanomedicine and Nanobiotechnology*** (2016)

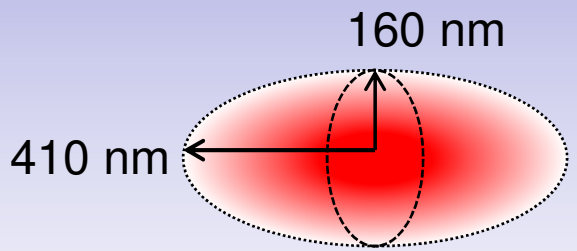
Two-photon Fluorescence with Single-Molecule Sensitivity



IR laser;
>100 mW

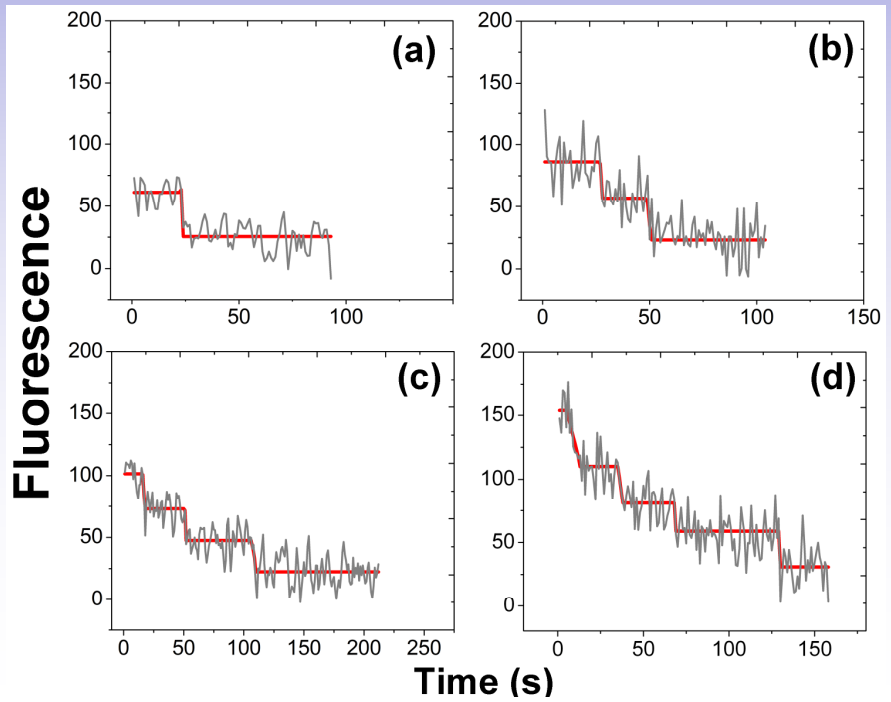






Excitation volume = 44 aL

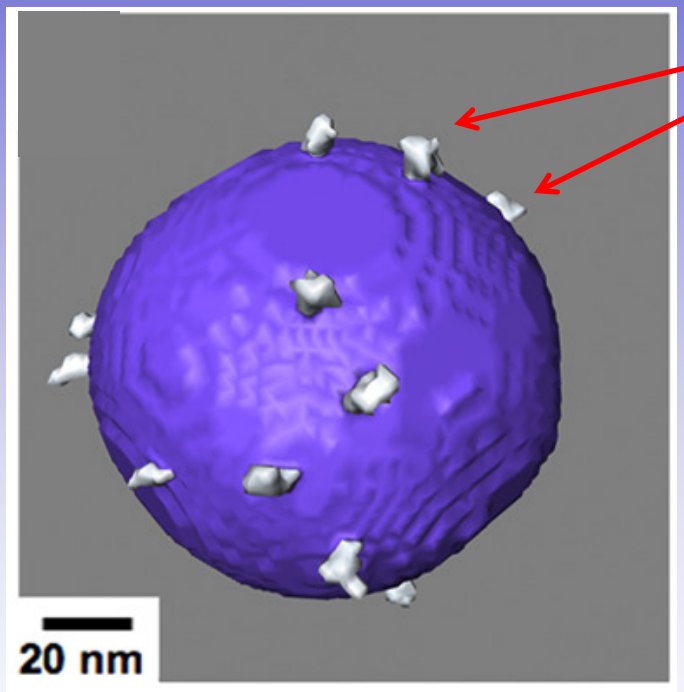
1 molecule / 44 aL: 38 nM



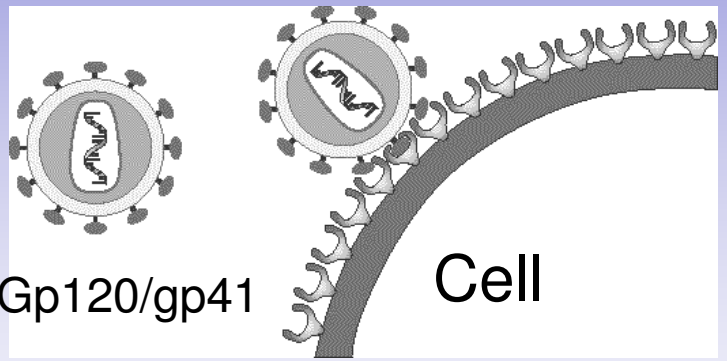
Hou & Cheng, ***Opt. Lett.*** (2011) 36: 3185

Heterogeneity Matters for Viruses

Model of HIV-1 virion

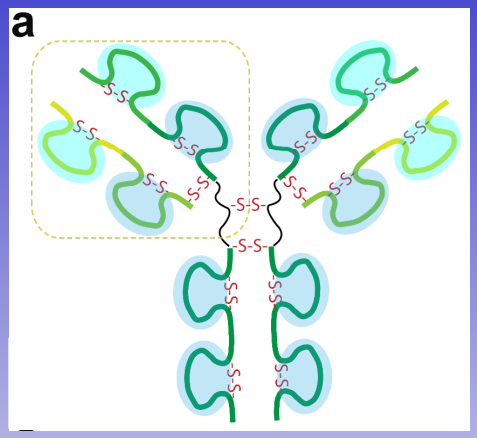


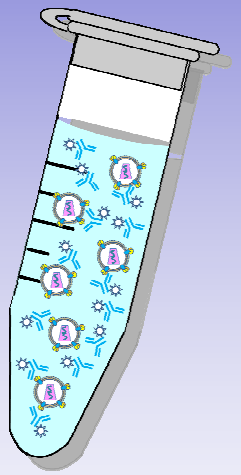
Presumed gp120/gp41

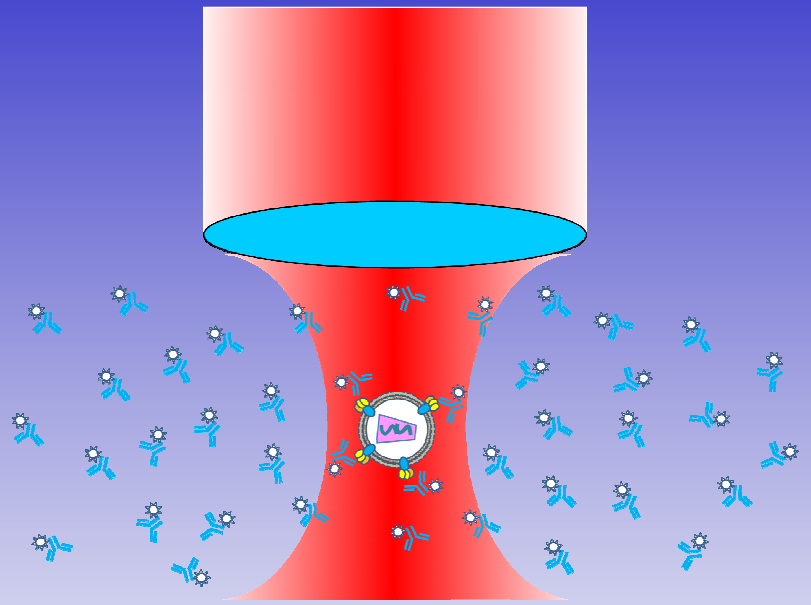


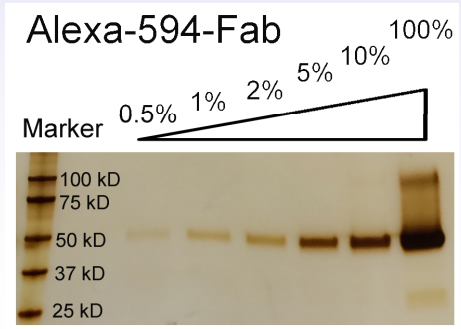
Zhu…Roux, ***Nature*** (2006) 441: 847

Optical Trapping ‘Virometry’

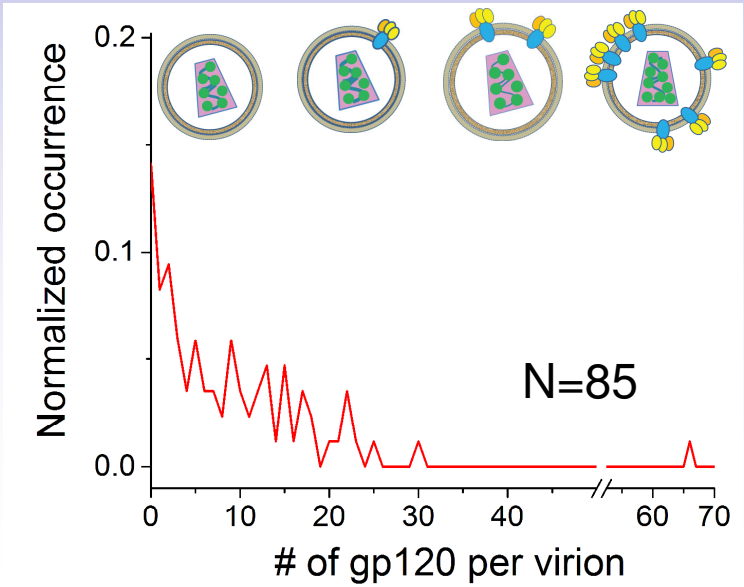








DeSantis…Cheng, ***J. Biol. Chem*** (2016) 291: 13088

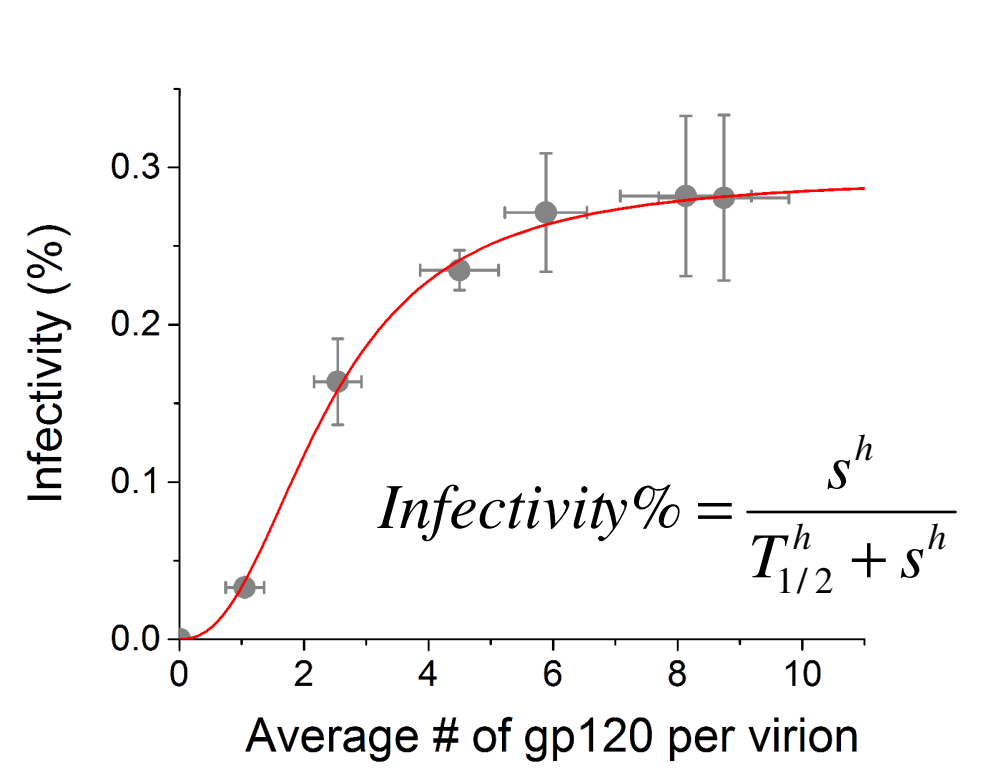


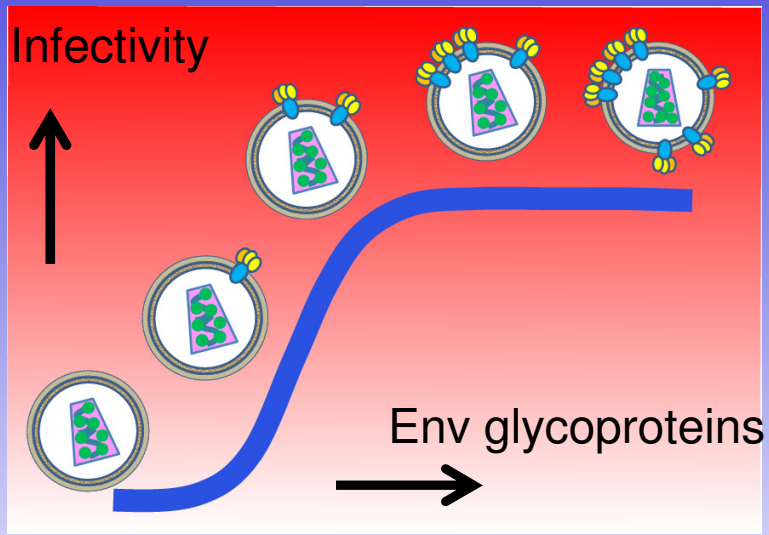
Cooperativity among Gp120 Molecules

Prepared seven populations of HIV-1, each population with on average different density of gp120 molecules

→ Virometry to measure # of gp120

→ Cell culture assay for infectivity



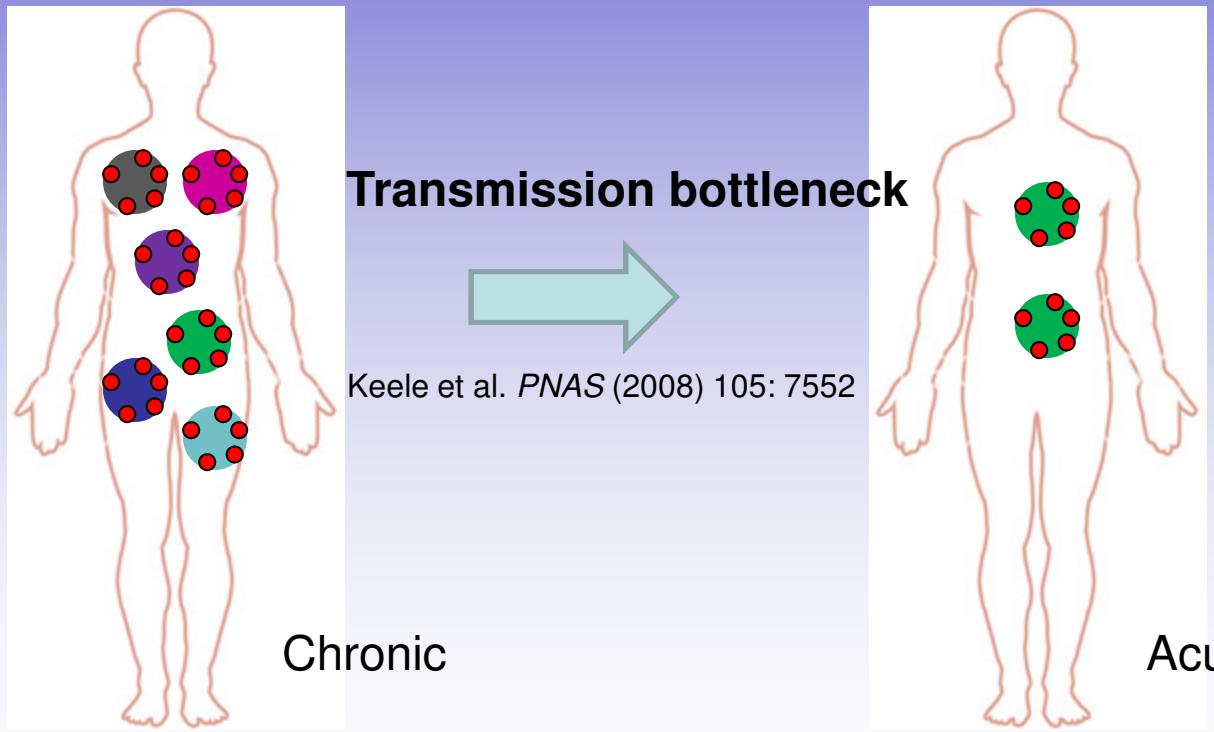


DeSantis…Cheng, ***J. Biol. Chem*** (2016) 291: 13088

Clinical Implications

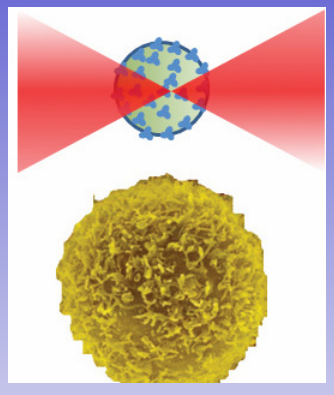
- Are they prone to transmission?
- Transmitted virus are enriched for higher gp120 content.

Parrish et al. *PNAS* (2013) 110: 6626

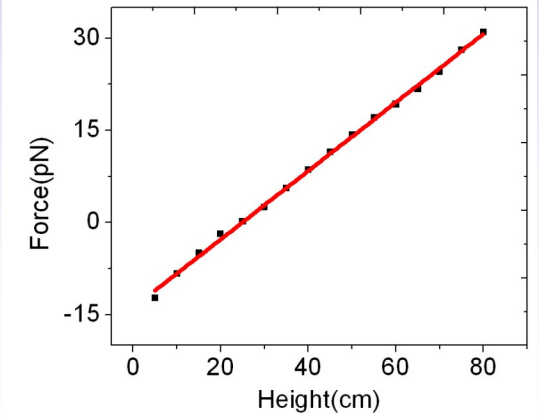


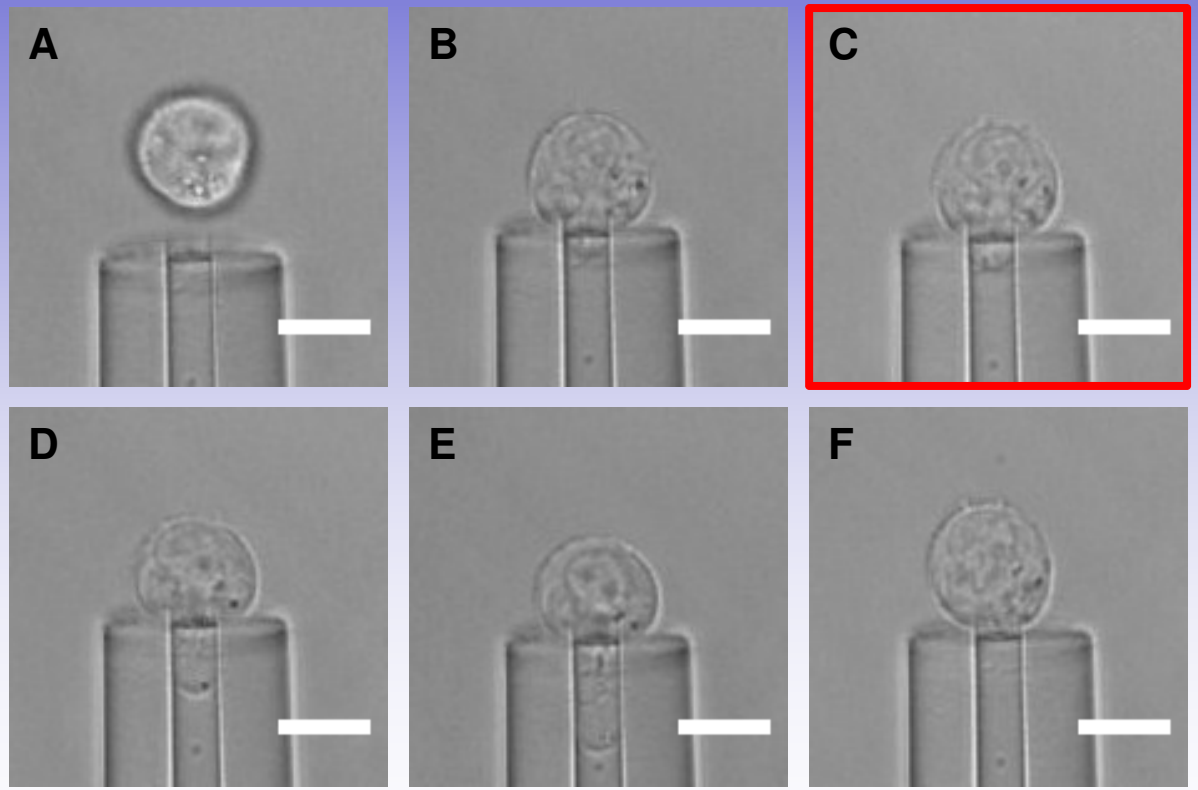
Not every HIV-1 virion is created equally!

Single-Cell Manipulation for Single Virion Delivery



Suction force controlled by hydrostatic pressure

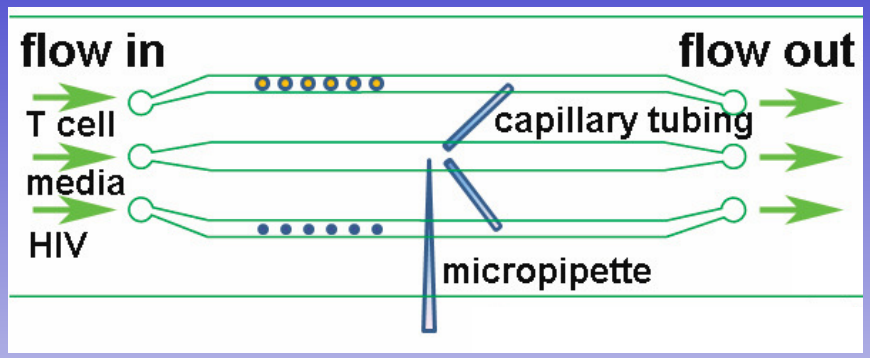


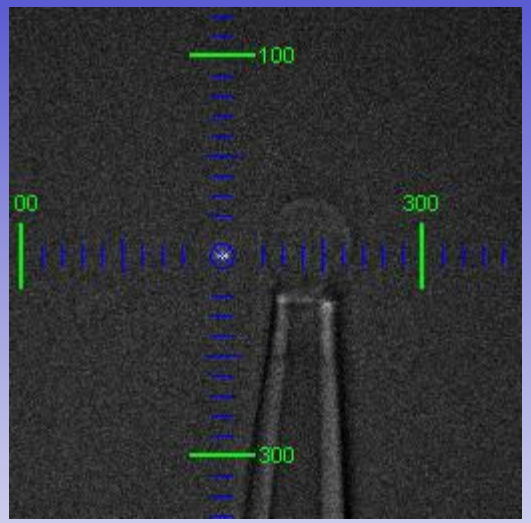


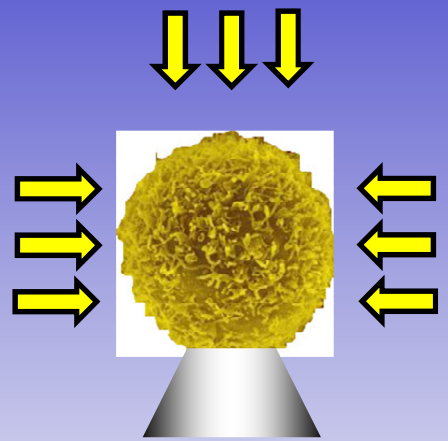
Scale bar: 10 μm

Hou & Cheng, unpublished

Specific Association upon a Single Collision is Rare







"**Forced**" delivery of HIV to CD4+ T cells

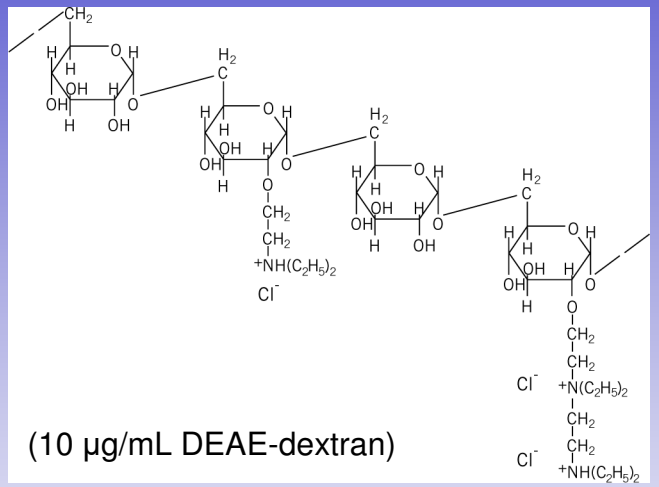
- An optically trapped virion is slowly brought into contact with a cell
- Attachment is easy to detect as an immobilized virion remains in focus
- The laser is turned off once the virion escapes

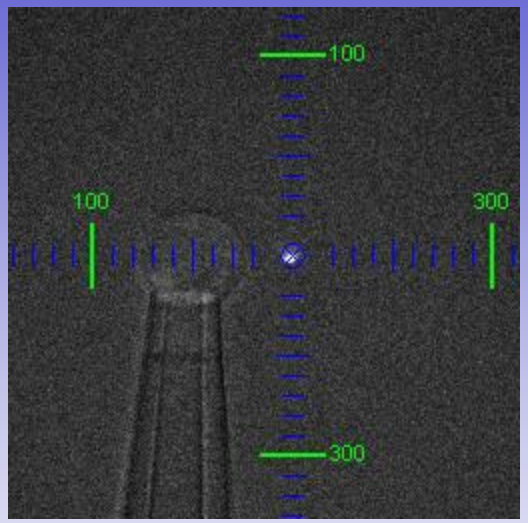
Trials	Attachments	Attachment Probability (%)
73	0	0

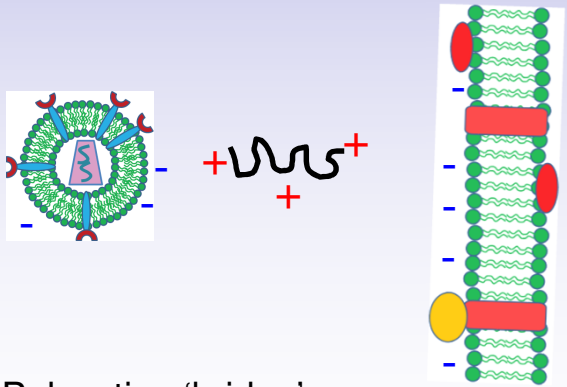
GFP labeled HIV-1, SUP-T1 cell, 20°C PBS

DeSantis & Cheng, unpublished

Frequent but Nonspecific Association Promoted by DEAE-dextran







Polycation ‘bridge’

Koh & Cheng, ***Langmuir*** (2014) 30: 10899

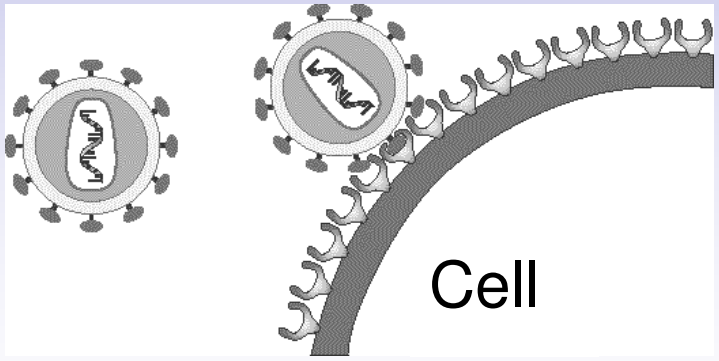
Trials	Attachments	Attachment Probability (%)
77	59	>77

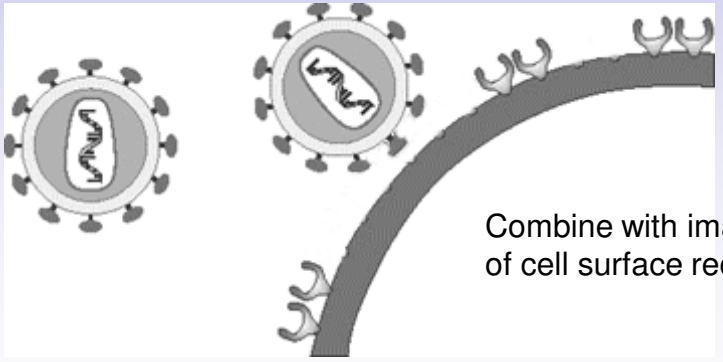
GFP labeled HIV-1, SUP-T1 cell, 20°C PBS

DeSantis & Cheng, unpublished

Possible Reasons for Lack of Attachment and Potential Future Development

- The virion may have low gp120; Combine with virometry
- Cell surface receptor may be low or distribution of receptors is heterogeneous





To form specific contact upon a single collision is a rare event!!

Conclusions

- For the first time, optical trapping of a single human virus
- For the first time, measurement of the index of refraction for a single virus particle
- Optical trapping virometry for multi-parameter analysis of biological particles.
- HIV-1 gp120 displays a positive cooperativity in mediating HIV-1 infection.
- The technique to deliver a single virus to a single cell.

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