Interferometric Microscopy for Detection and Visualization of Biological Nanoparticles

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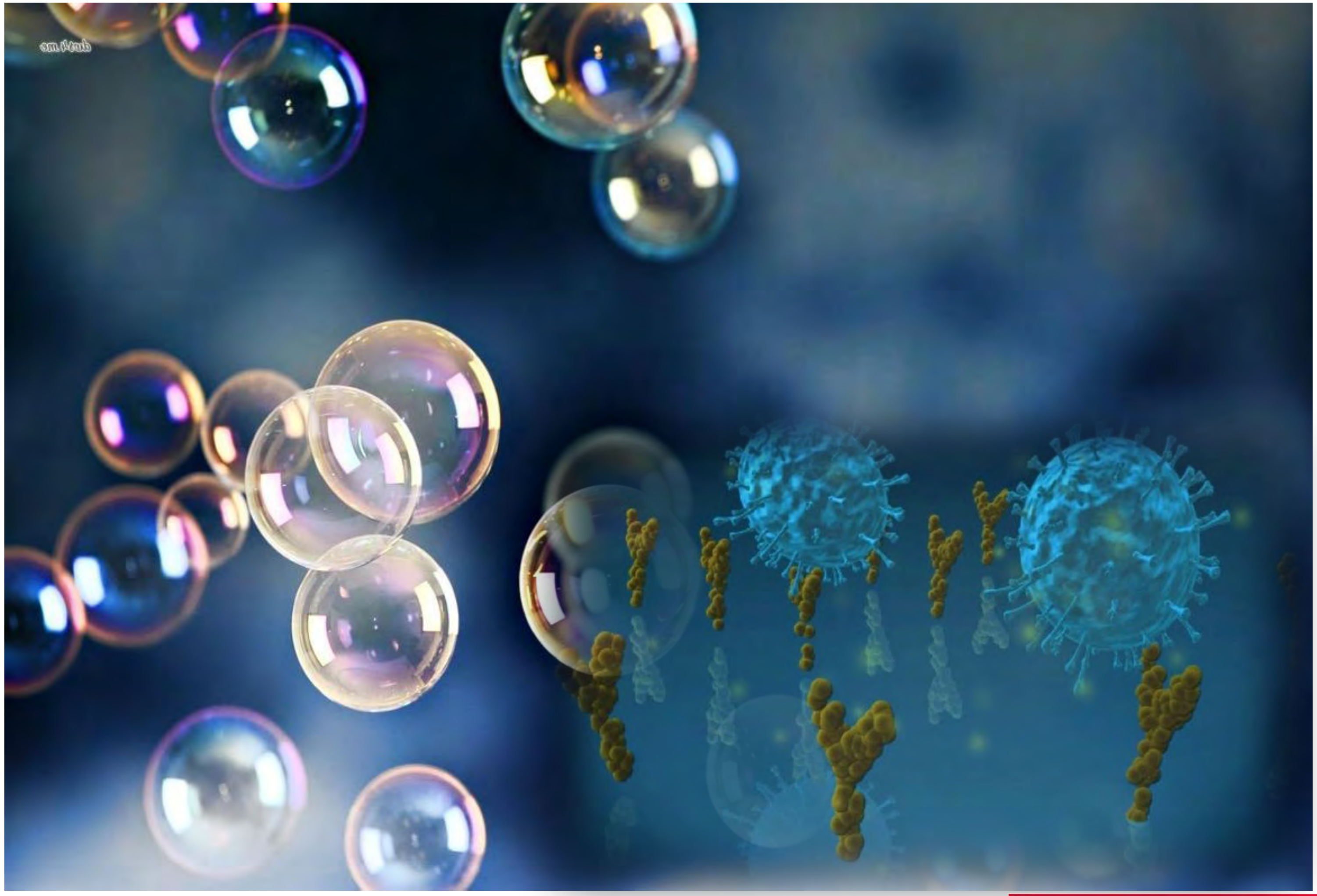

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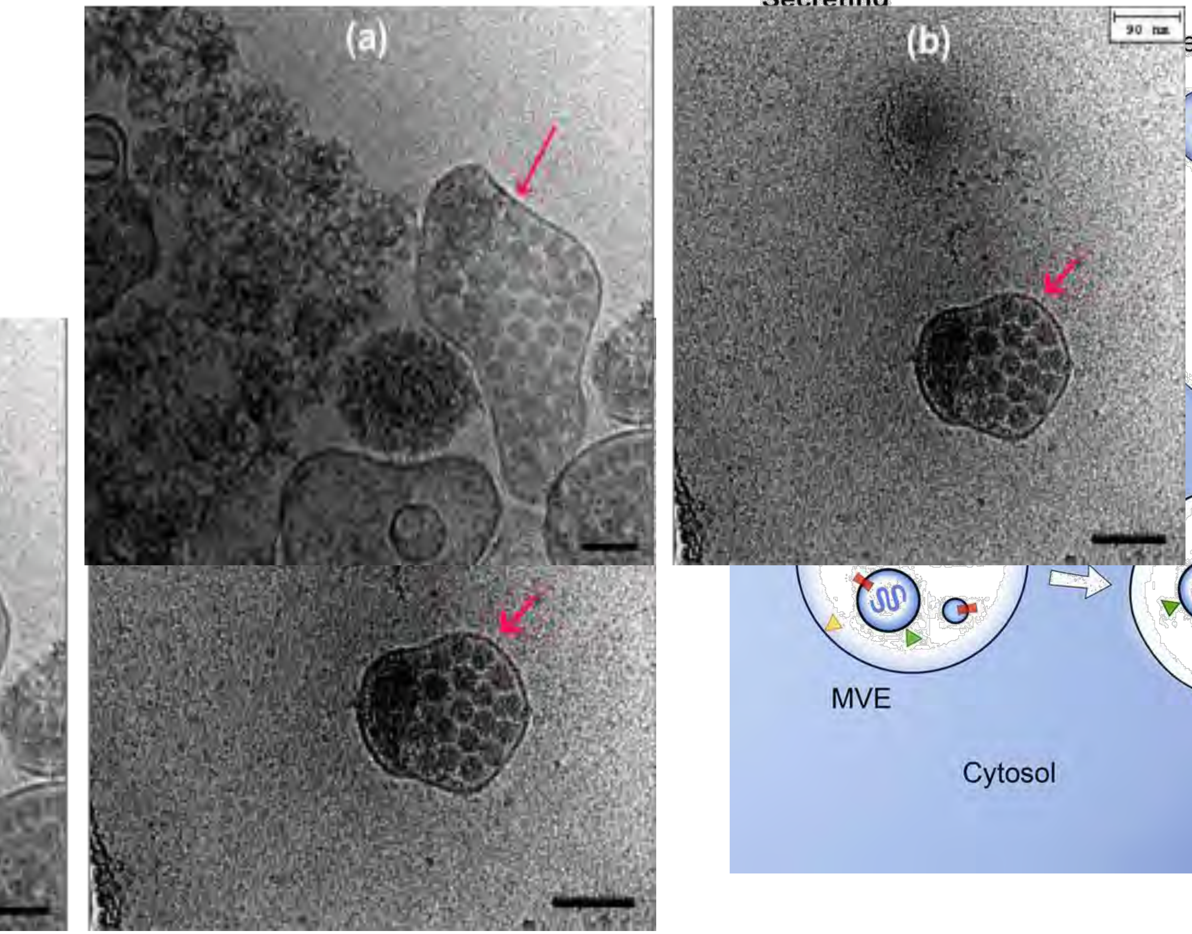
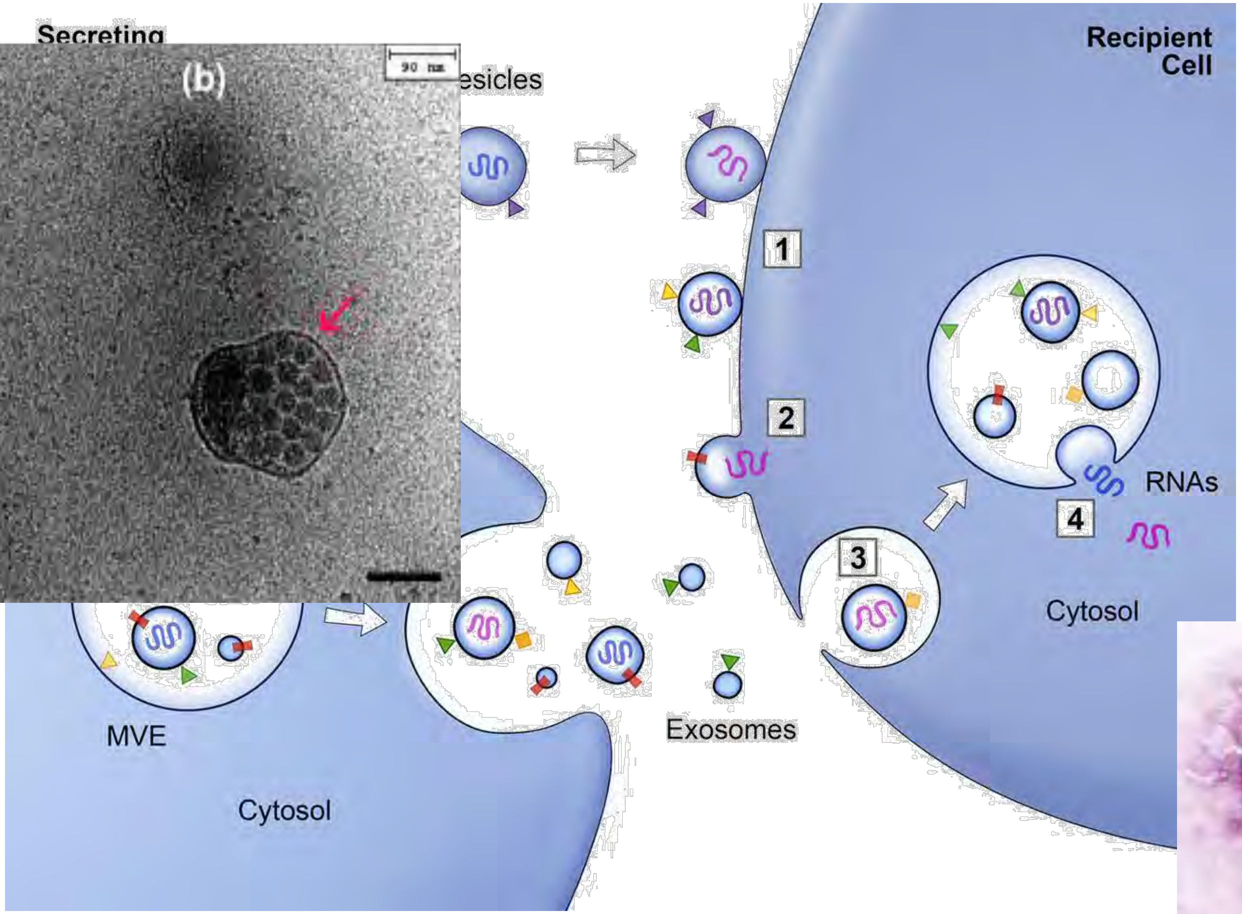
Outline

- Motivation – Biological Nanoparticles everywhere
- Problem definition – contrast and size
- Detection vs. visualization
- Interferometric Reflectance **Imaging** Sensor
- Biological Nanoparticle Detection and Sizing
- Pupil function engineering
- Resolution improvement by oblique illumination and reconstruction
- Towards 100nm in label-free visible light microscopy
- Conclusions and Future

Extra cellular vesicles, exosomes, and viruses



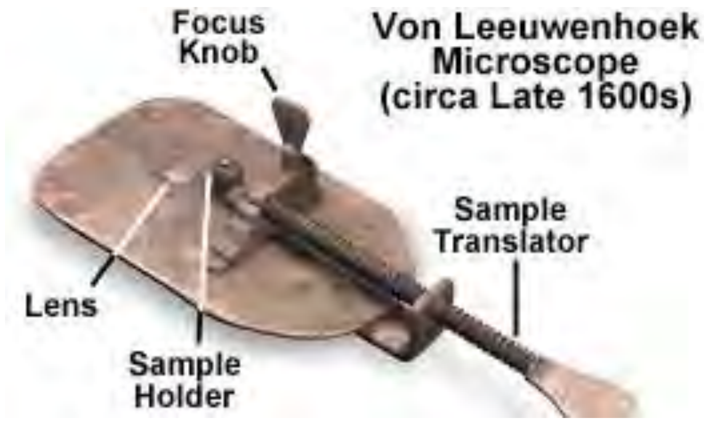
Example cryo-EM images of infectious extracellular vesicle (Bullitt Lab – BU MED)

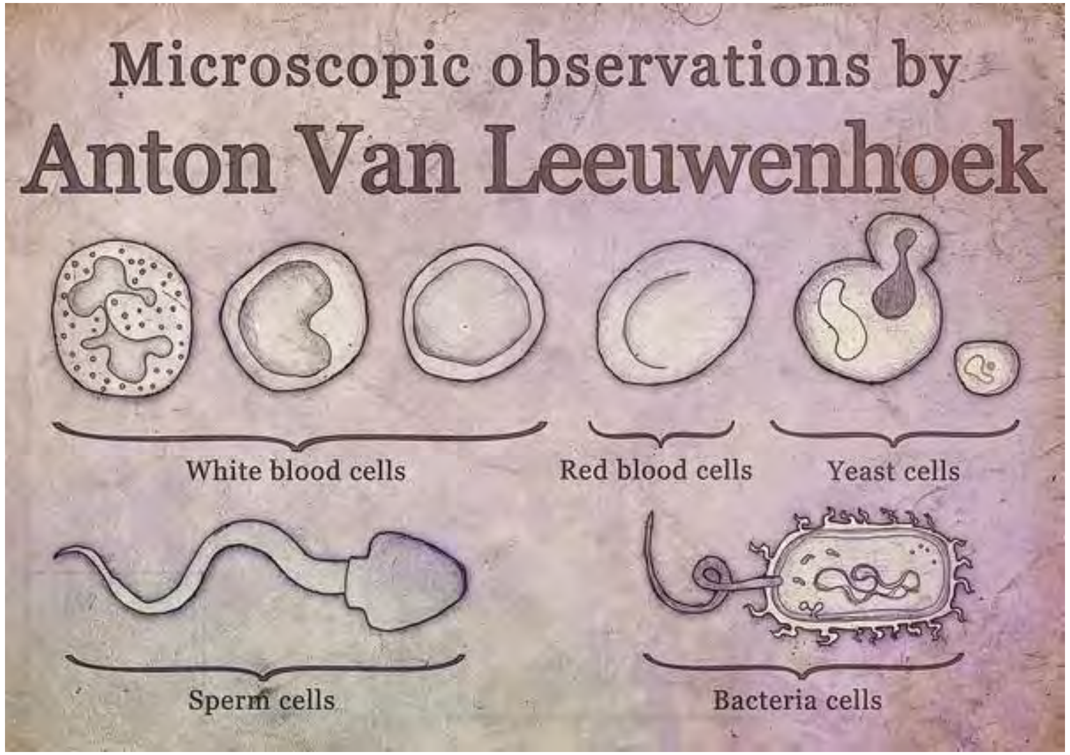


SEM image of Ebola virion

Viruses are the most abundant species on earth.
~10^{32} phages in the biosphere
~10^{7} viruses on average in a mL of seawater

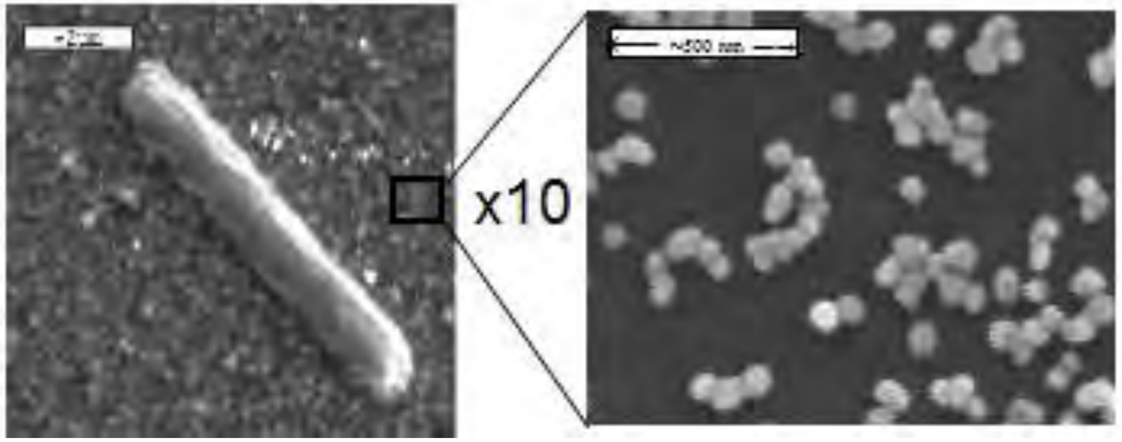
Optical microscopy can see small – but …



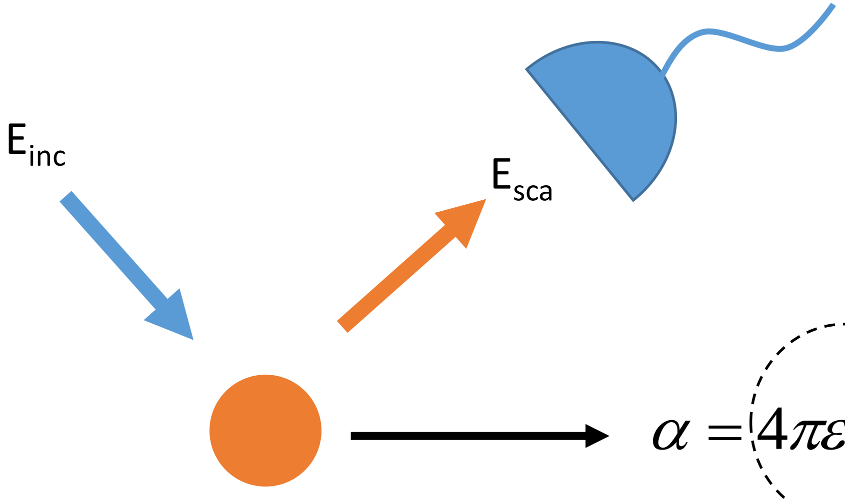


micro.magnet.fsu.edu/primer/

Biological Nanoparticle Detection Challenges – size and dielectric contrast



Ziegler



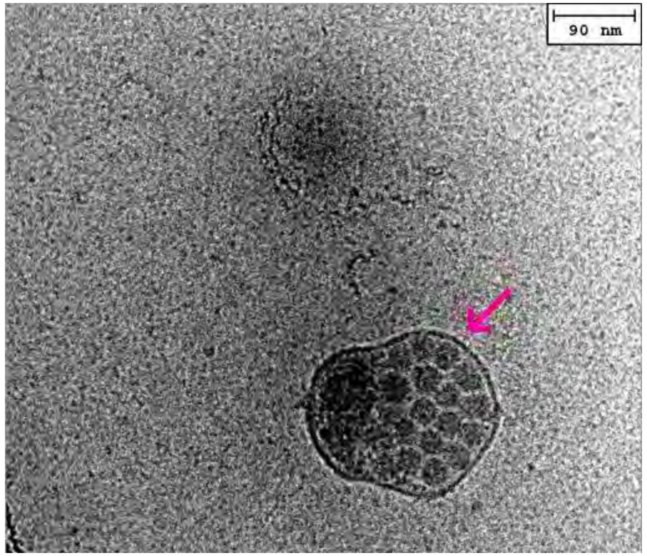
$$\alpha = 4\pi\varepsilon_0 R^3 \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}$$

Size   contrast

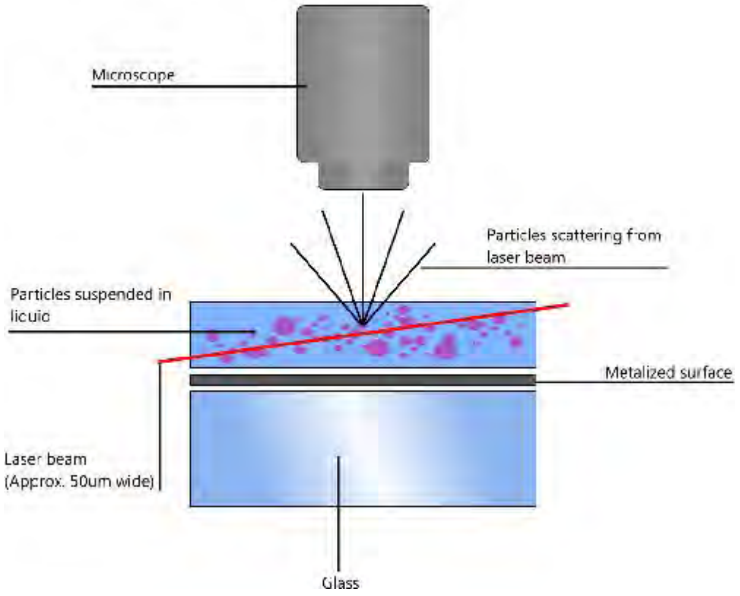
Signal ~ R^6

Technologies for bio-nanoparticle characterization

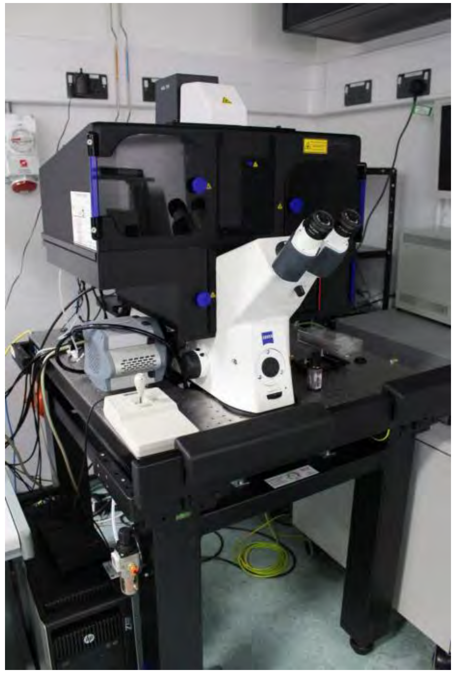
Cryo-TEM
- Fantastic resolution
- Low throughput and difficult



Nanoparticle Tracking Analysis
- Estimate size of particles based on Brownian motion
- Little/no molecular information



Fluorescence microscopy (STED/PALM)



Needed: High-throughput methods to measure the **size, shape** and **molecular profile** of biological nanoparticles

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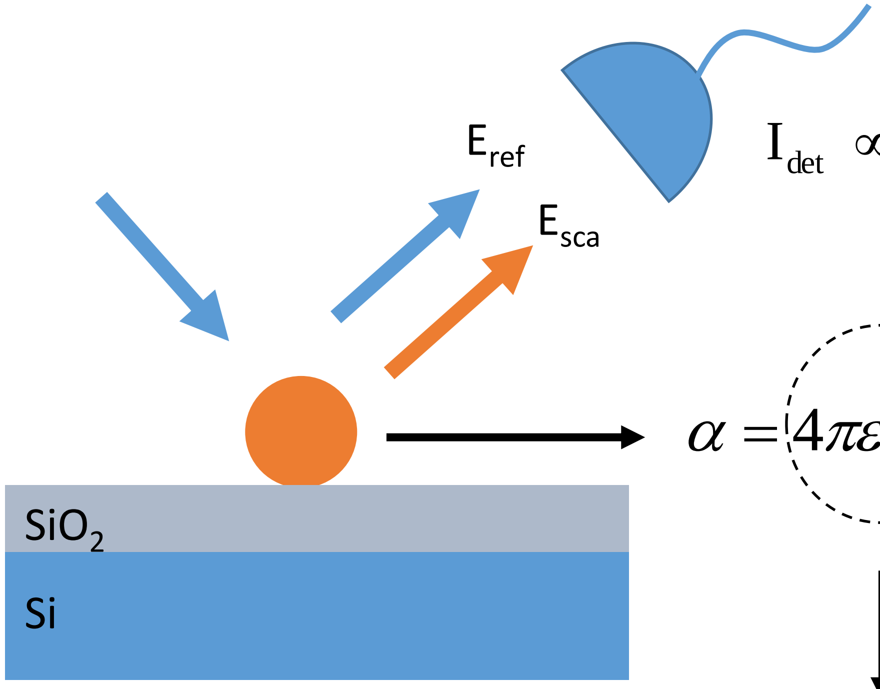


$$I_{det} \propto |E_{ref}|^2 + |E_{sca}|^2 - 2|E_{ref}||E_{sca}|\sin\theta$$

Phase Term ($\sin\theta$)

$$\alpha = 4\pi\varepsilon_0 R^3 \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}$$

Size ($4\pi\varepsilon_0 R^3$)

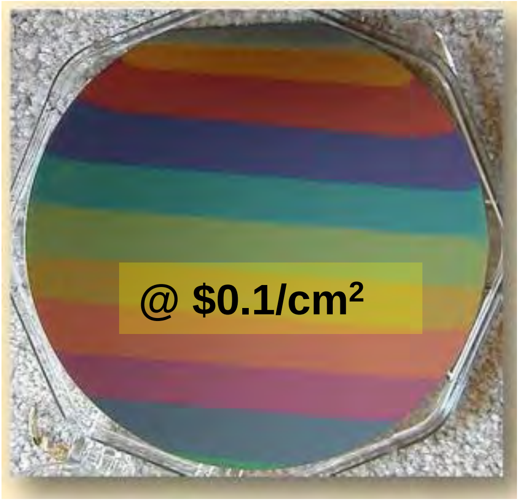
Material ($\frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}$)

Interferometric Reflectance Imaging Sensor (IRIS)

a high throughput biosensing platform

soap film

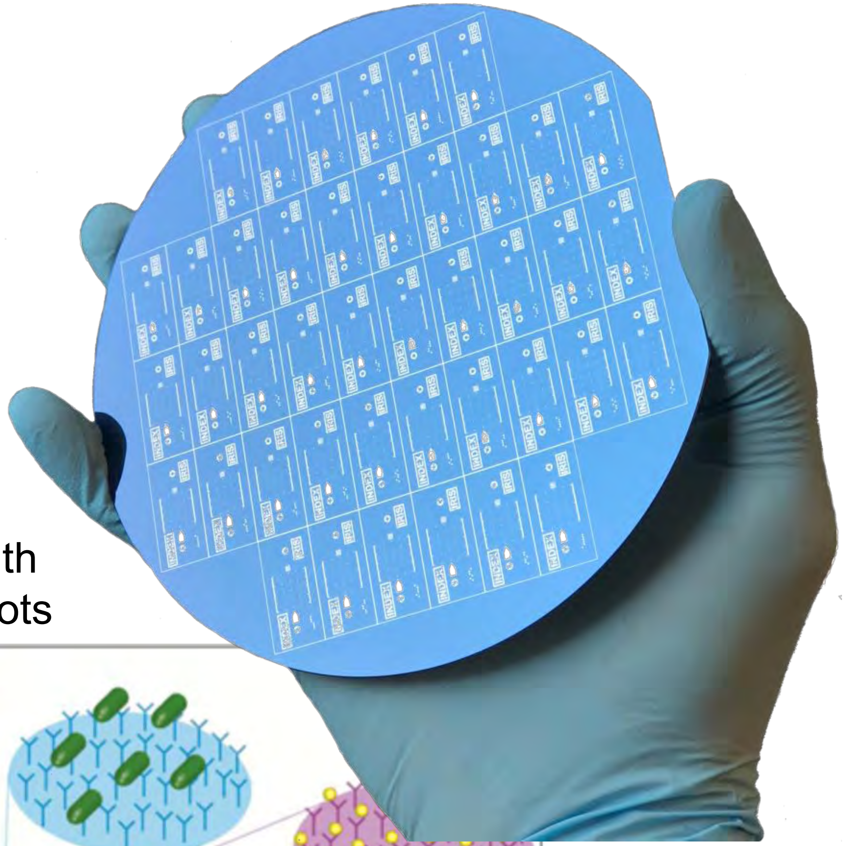
Oxide coated Si

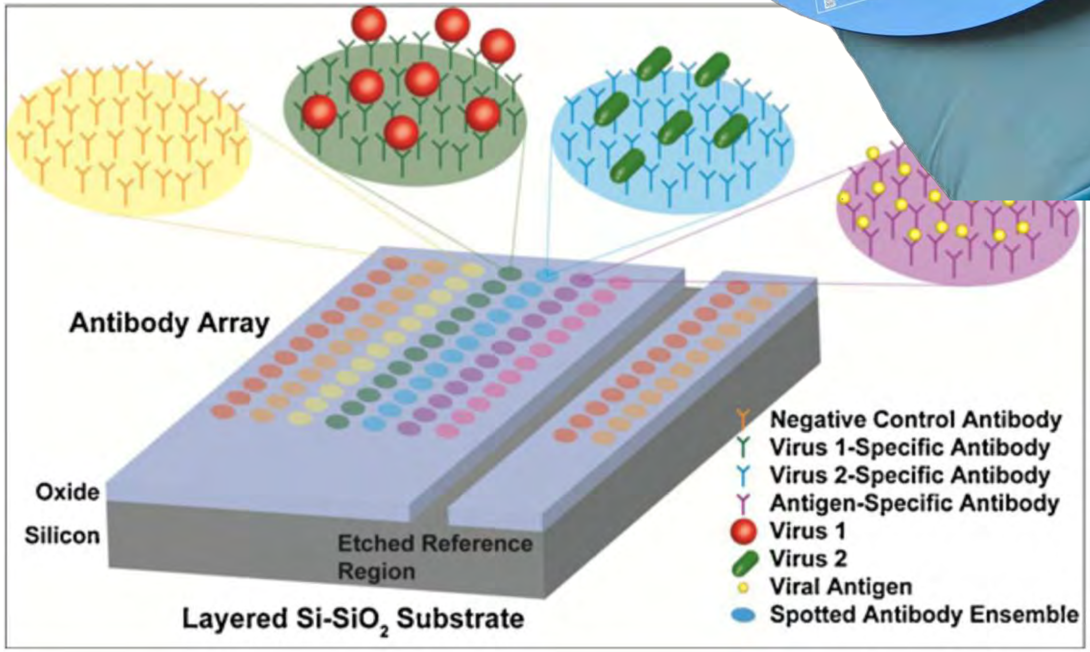


Ünlü et al, "STRUCTURED SUBSTRATES FOR OPTICAL SURFACE PROFILING,' US Patent No: 9599611, 2017

pg/mm² sensitivity 1,000s of spots

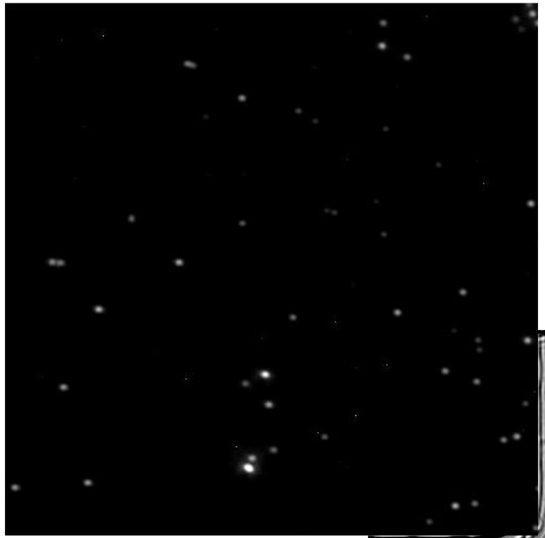
Protein microarray chips with 100s to 1,000s of probe spots

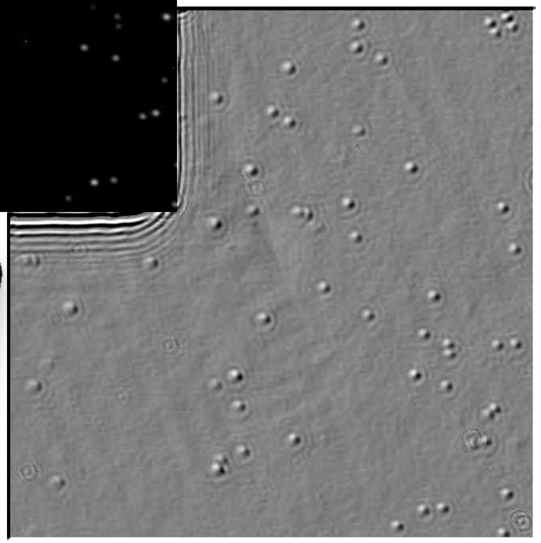


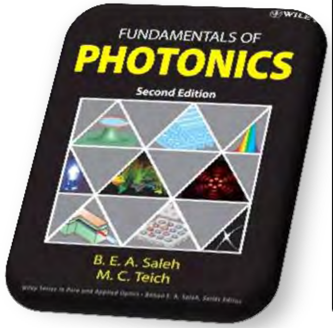


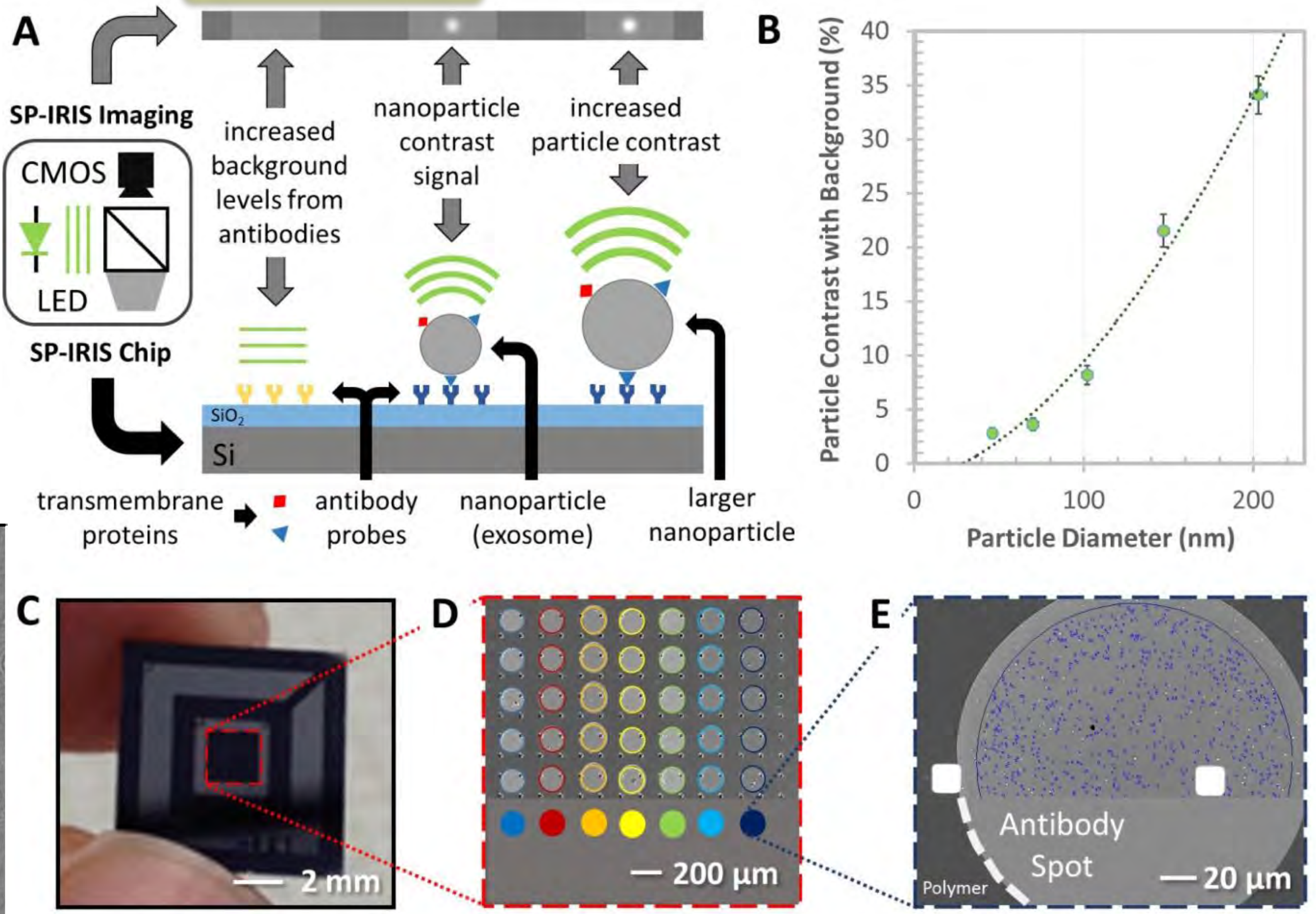
Simple Particle Detection

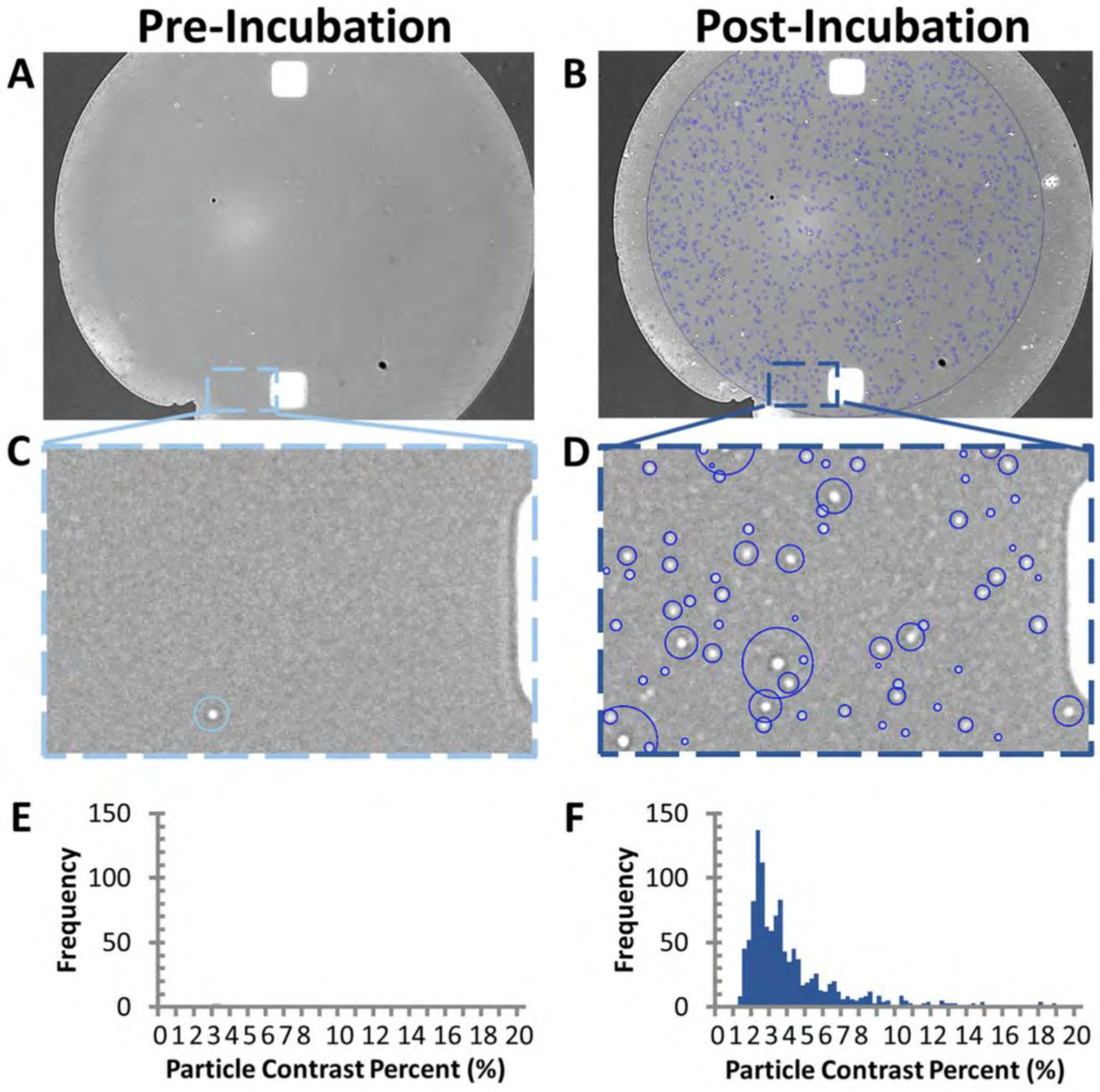
Rahul Vedula(MD) and George Daaboul, PhD ‘13











Exosome detection

Anti-CD81 capture probe image acquired before and after incubation with purified HEK293 cells derived exosomes.

SCIENTIFIC REPORTS

OPEN

Digital Detection of Exosomes by Interferometric Imaging

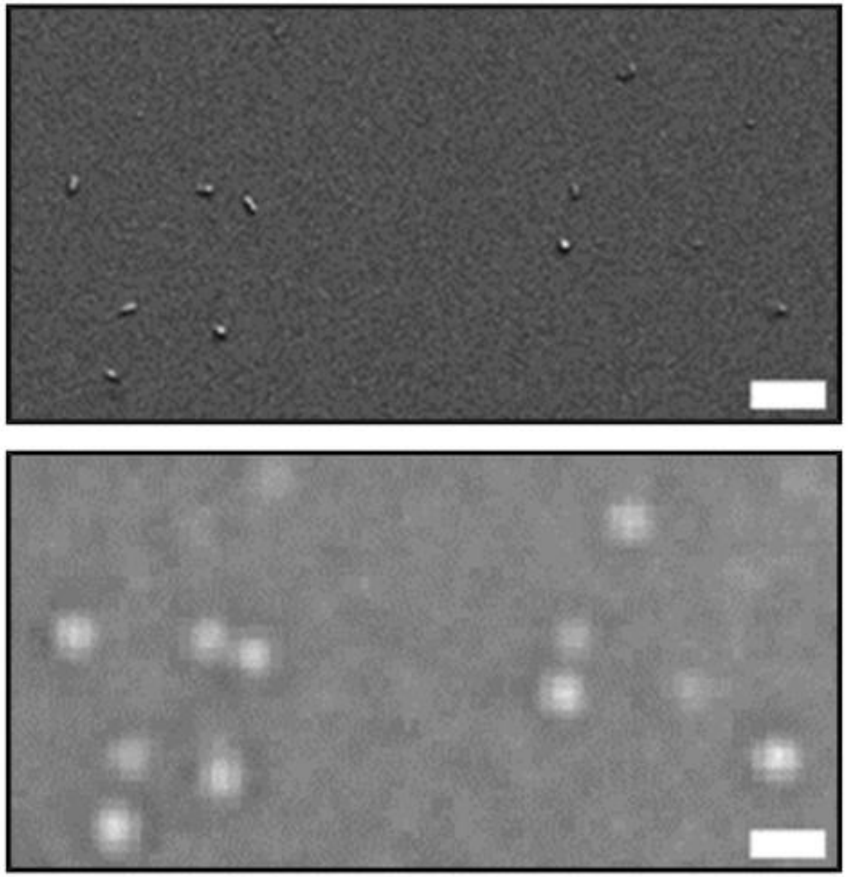
George G. Daaboul[1,*], Paola Gagni[2,*], Luisa Benussi[3], Paolo Bettotti[4], Miriam Ciani[3], Marina Cretich[2], David S. Freedman[1], Roberta Ghidoni[3], Ayca Yalcin Ozkumur[5], Chiara Piotto[4], Davide Prosperi[6], Benedetta Santini[6], M. Selim Ünlü[7] & Marcella Chiari[2]

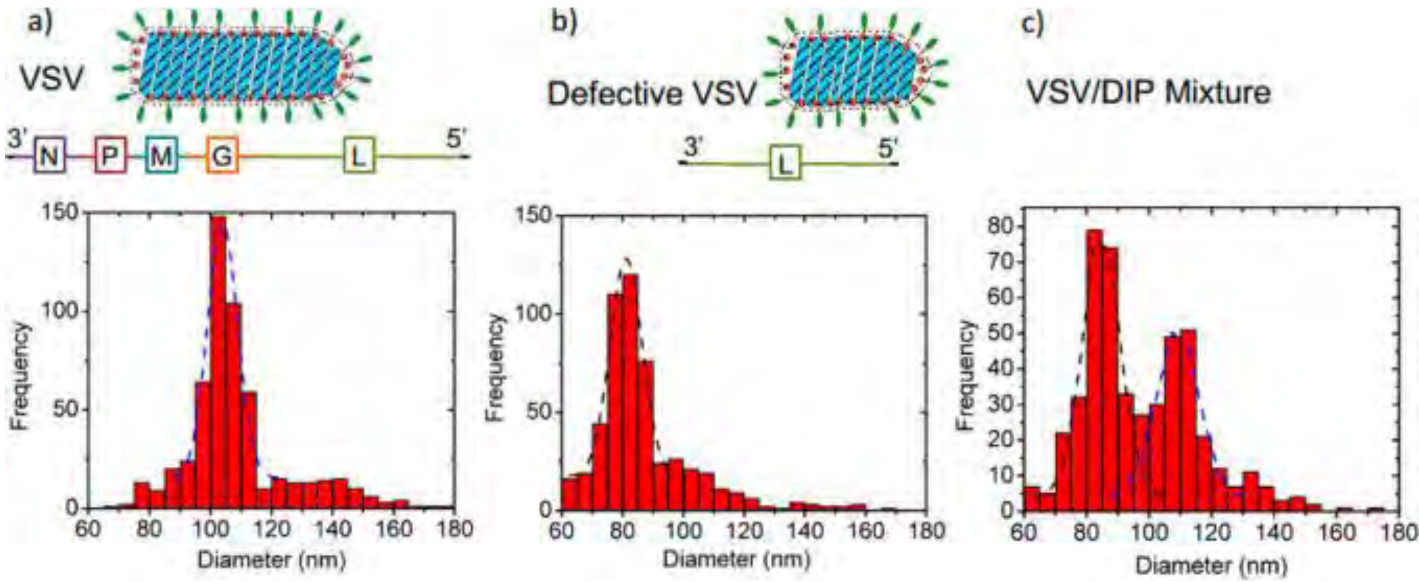
Received: 20 June 2016

Virus characterization: size determination

SEM

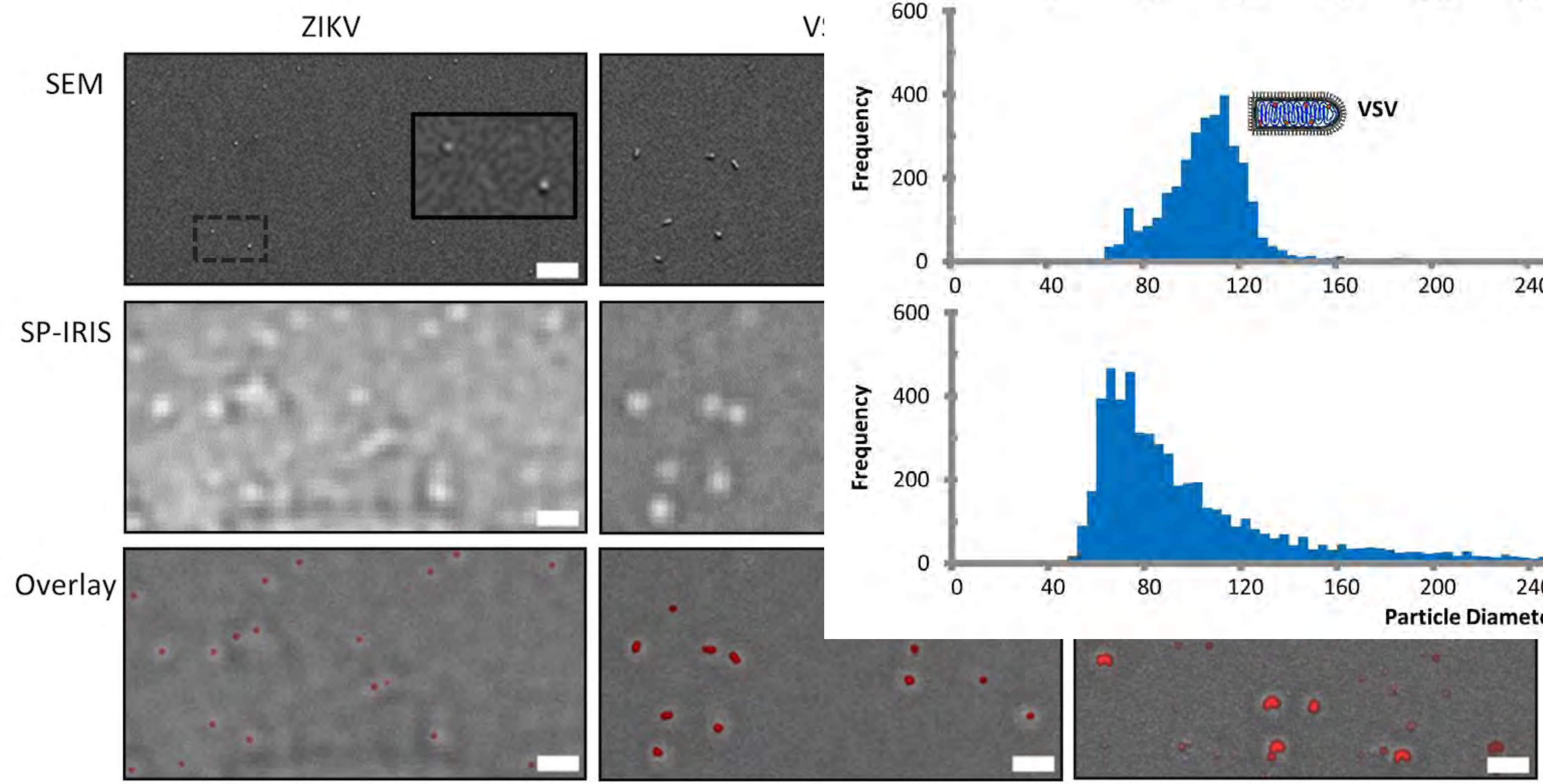
IRIS

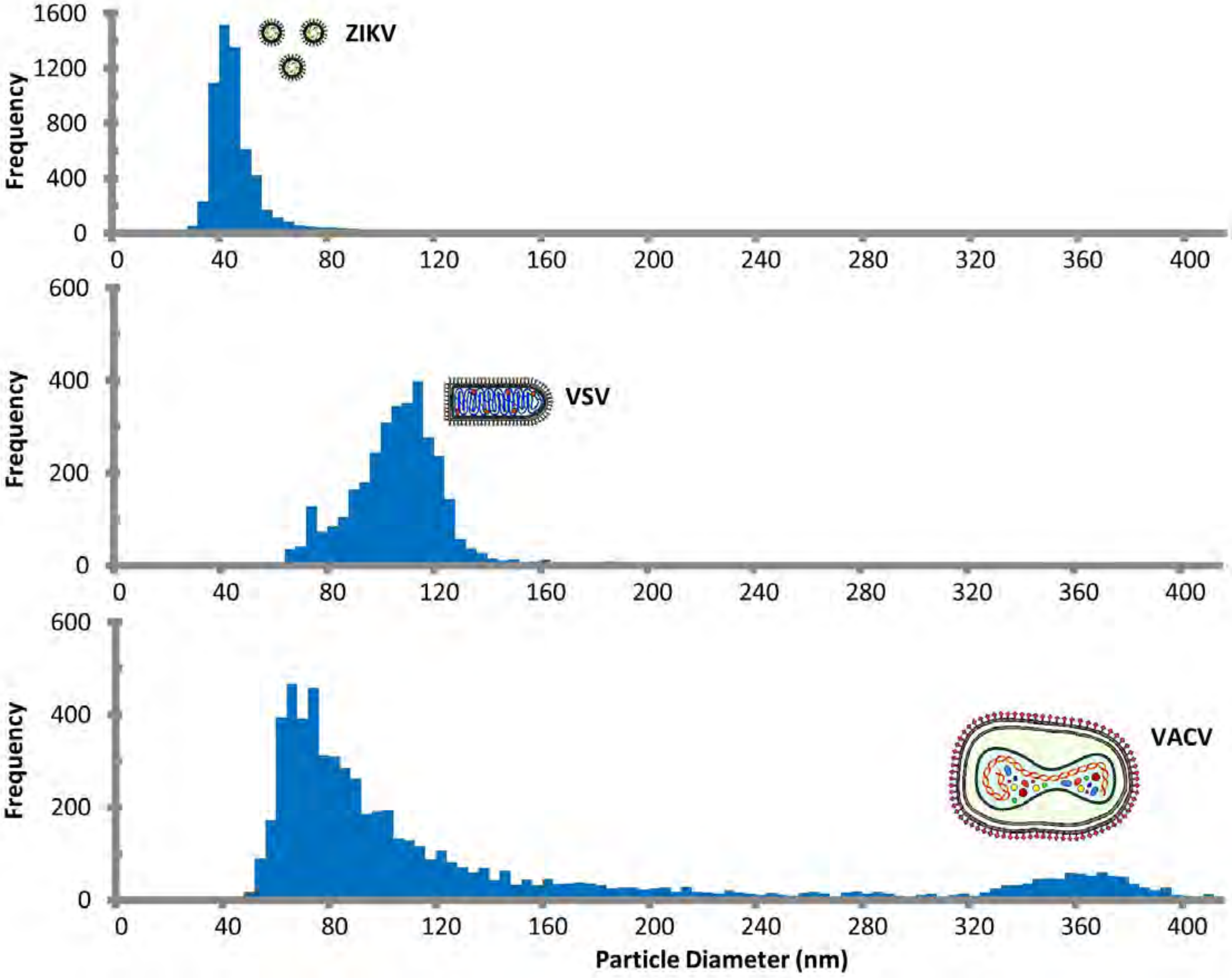




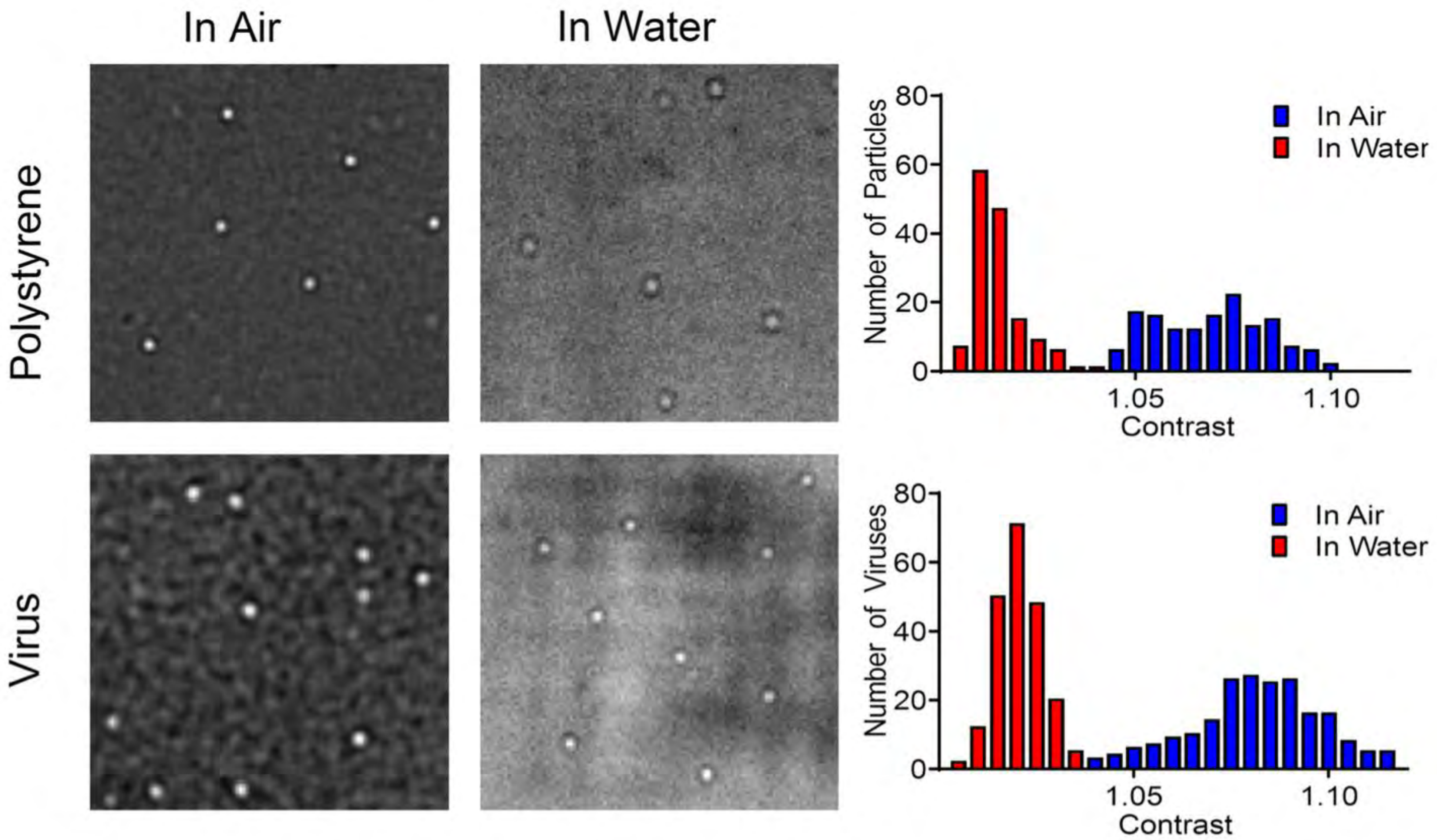
Daaboul et al, *ACS nano* 8 (2014)

Various viruses





In-liquid detection to simplify the assay



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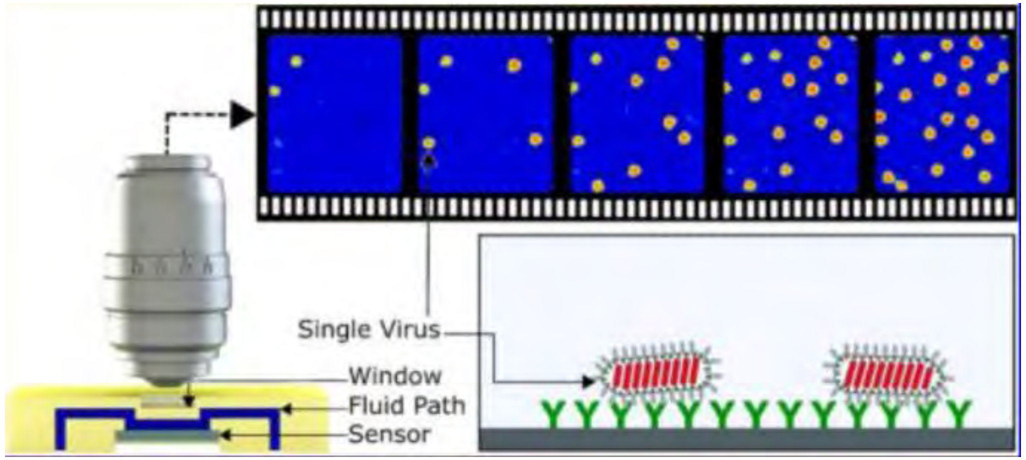
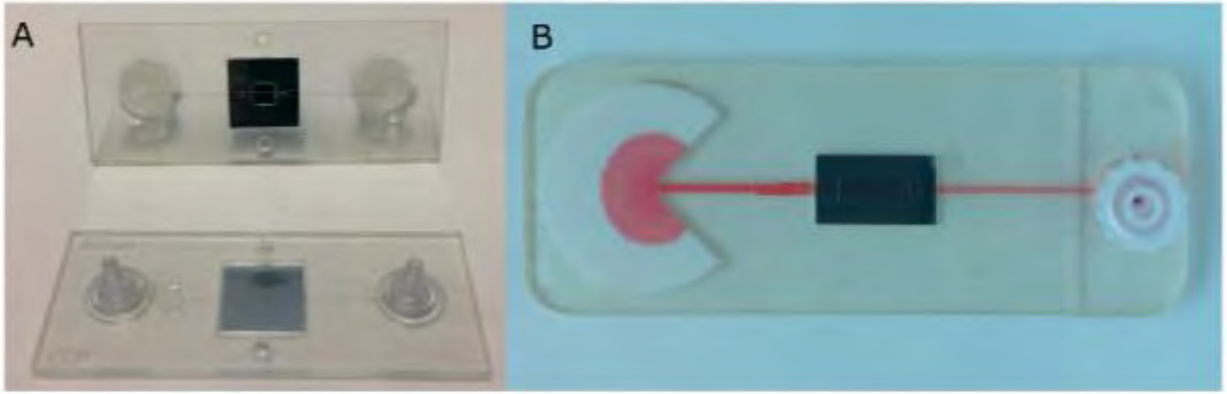
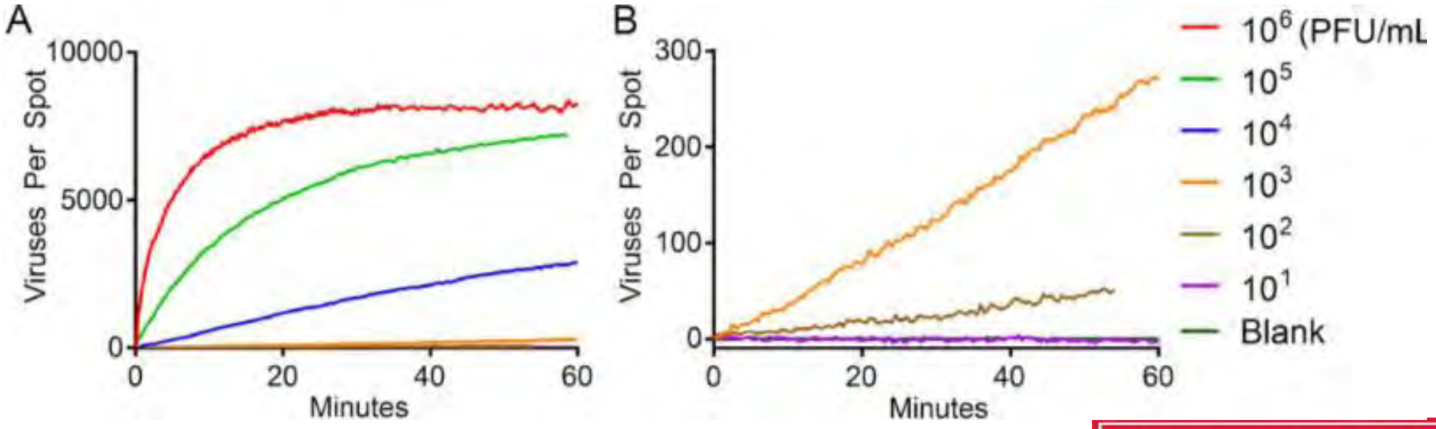
Virus detection for diagnostic applications





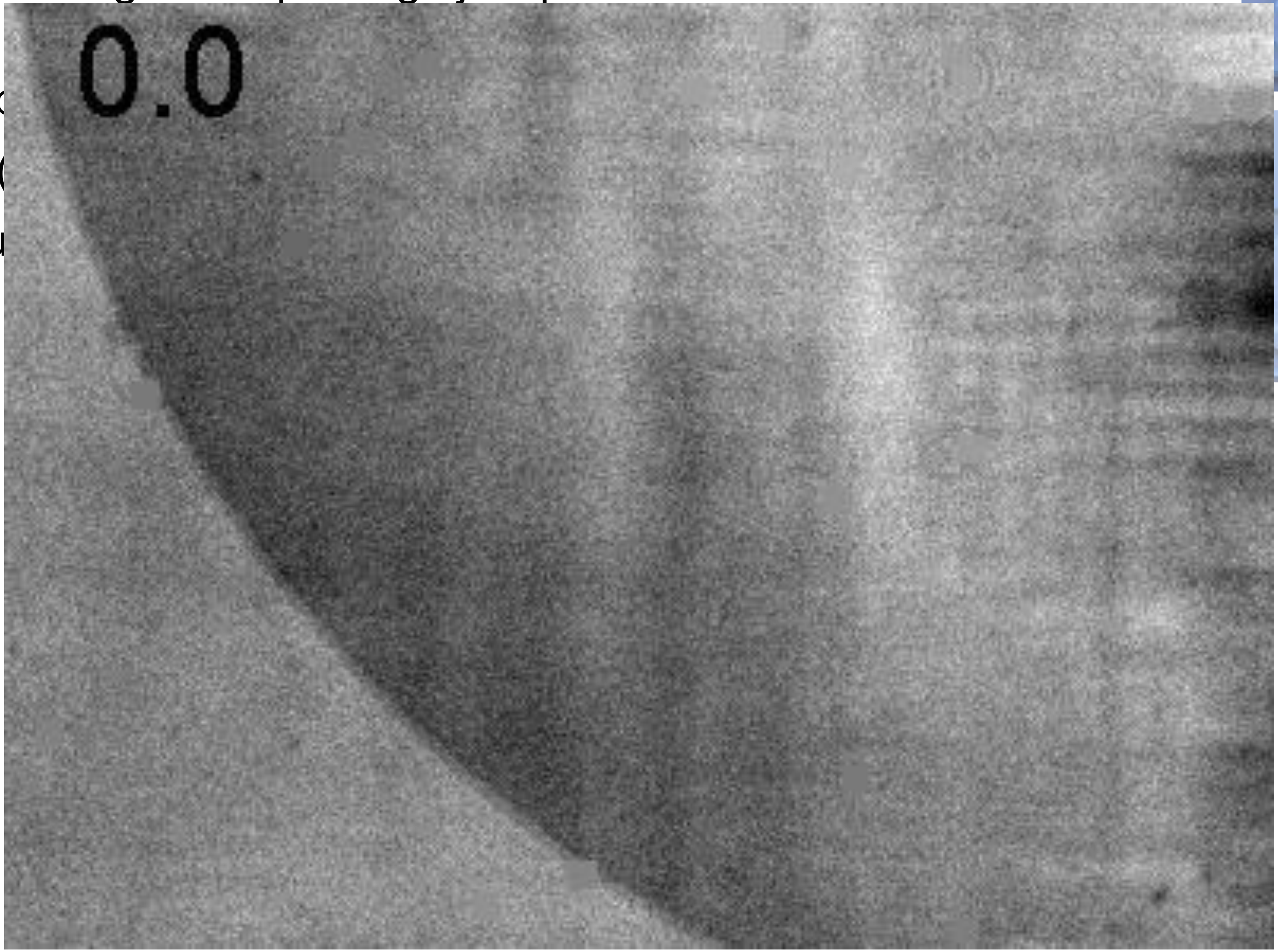
Highly-sensitive virus detection directly from blood serum

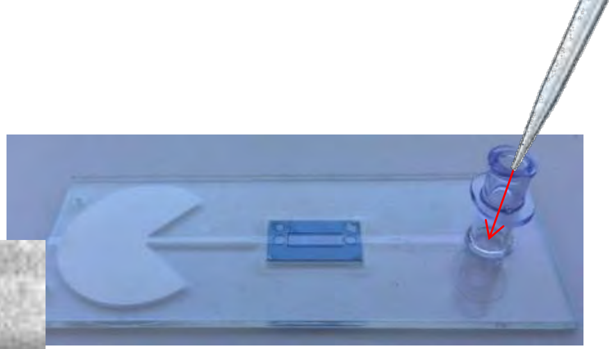


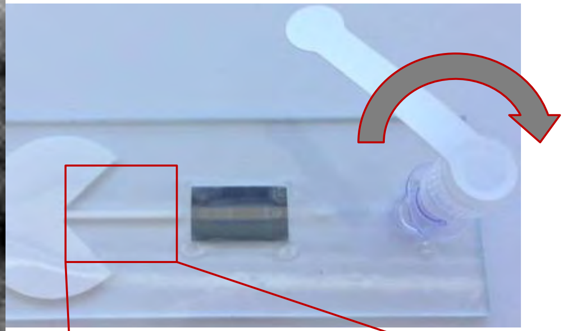
Scherr et al, *ACS Nano* 10 (2016) and Scherr et al, *Lab on a Chip* (2017) (2017)

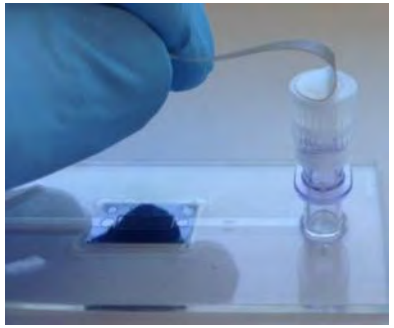
Passive Cartridge - Simple Workflow

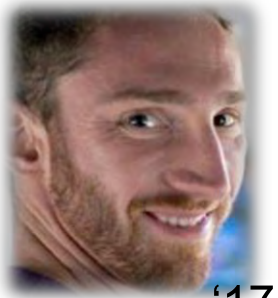
1. Remove cartridge from package just prior to use
2. 100 uL of
needs to b
3. Luer cap (
4. When liqu
removed)
5. Cartridge











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Lab on a Chip

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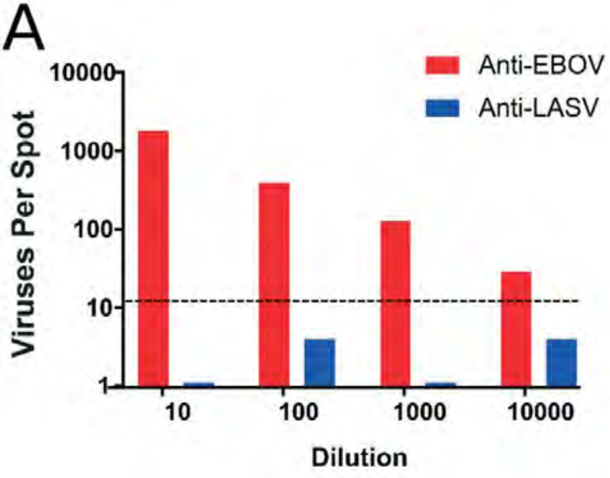
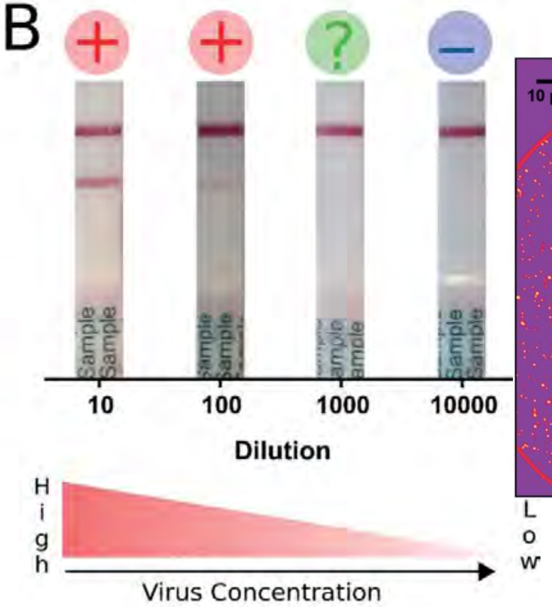
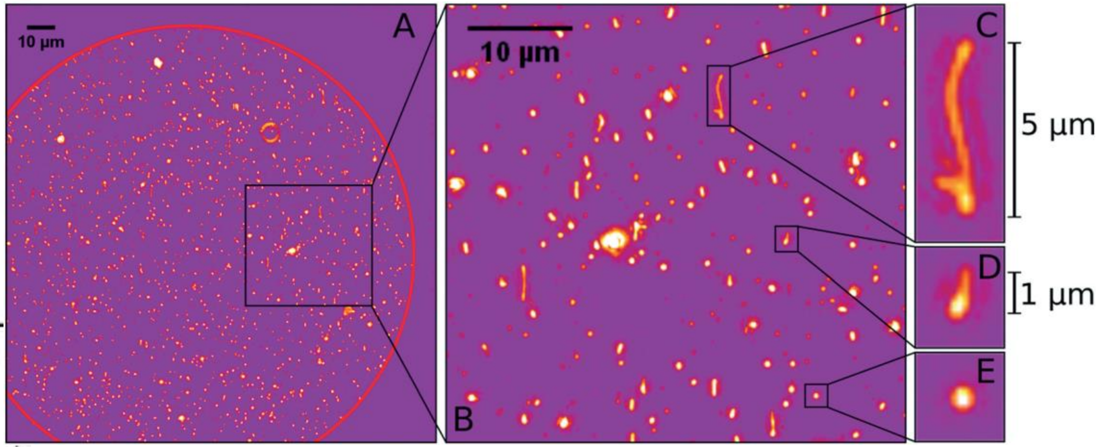
Cite this: DOI: 10.1039/c6lc01528j

Disposable cartridge platform for rapid detection of viral hemorrhagic fever viruses†

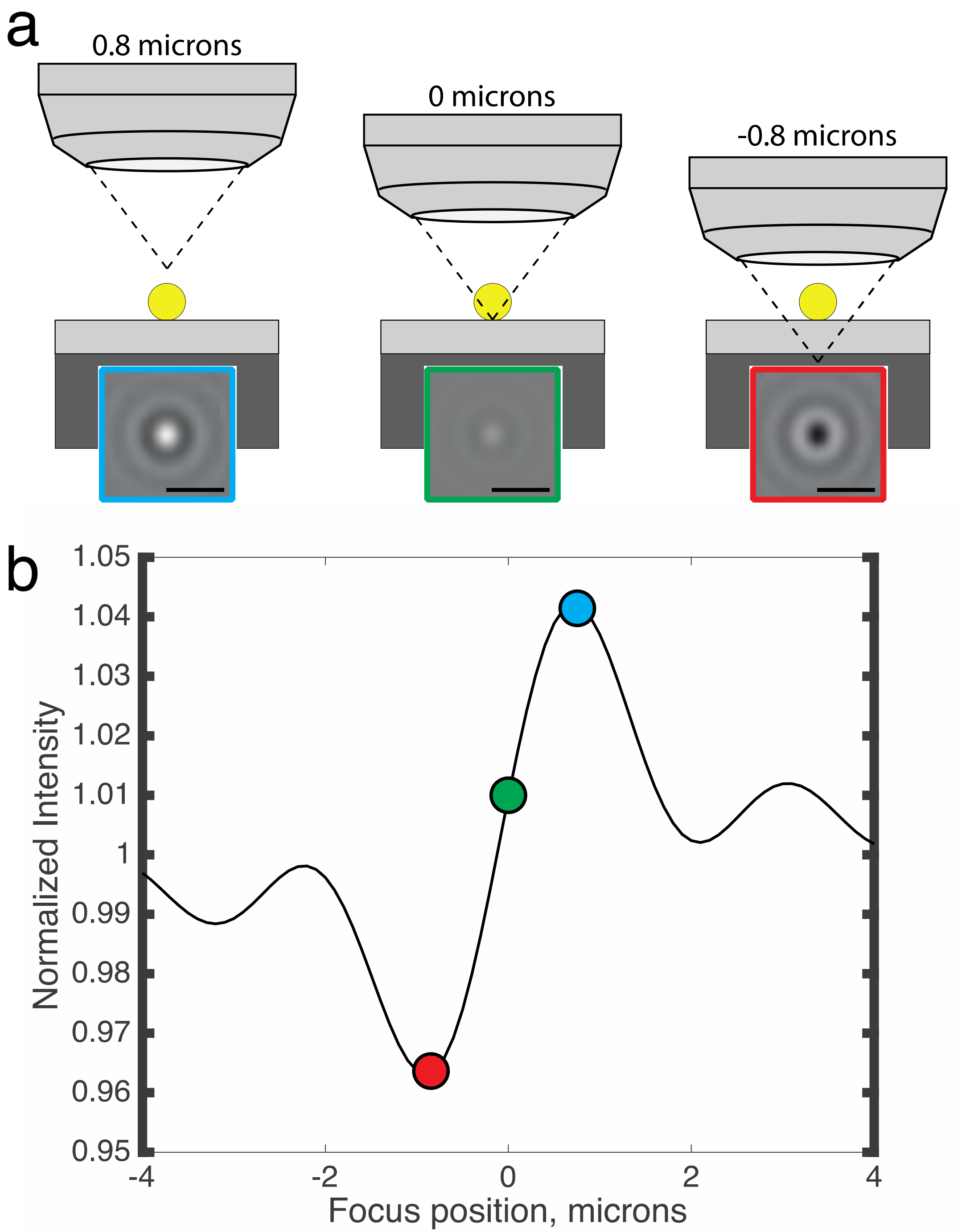
Steven M. Scherr,[a] David S. Freedman,[b] Krystle N. Agans,[cd] Alexandru Rosca,[b] Erik Carter,[e] Melody Kuroda,[f] Helen E. Fawcett,[a] Chad E. Mire,[cd] Thomas W. Geisbert,[cd] M. Selim Ünlü[ghi] and John H. Connor*[eh]







Dilution	SP-IRIS Anti-EBOV (Viruses Per Spot)	SP-IRIS Anti-LASV (Viruses Per Spot)	ReEbov Test Strip
1:10	1824	**	+
1:100	393	4	+
1:1000	126	-4	?
1:10000	29	4	-



Interferometric fringes – defocus profile

Changing the focus position changes the path length to the detector differently for reference reflection and scattered light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D. Sevenler et al, **"Quantitative interferometric reflectance imaging for the detection and measurement of biological nanoparticles,"** *Biomedical Optics Express*, 2017
O. Avci, et al., **"Physical Modeling of Interference Enhanced Imaging and Characterization of Single Nanoparticles,"** *Optics Express*, 2016
O. Avci, et al. **"Pupil function engineering for enhanced nanoparticle visibility in wide-field interferometric microscopy,"** *Optica*2017

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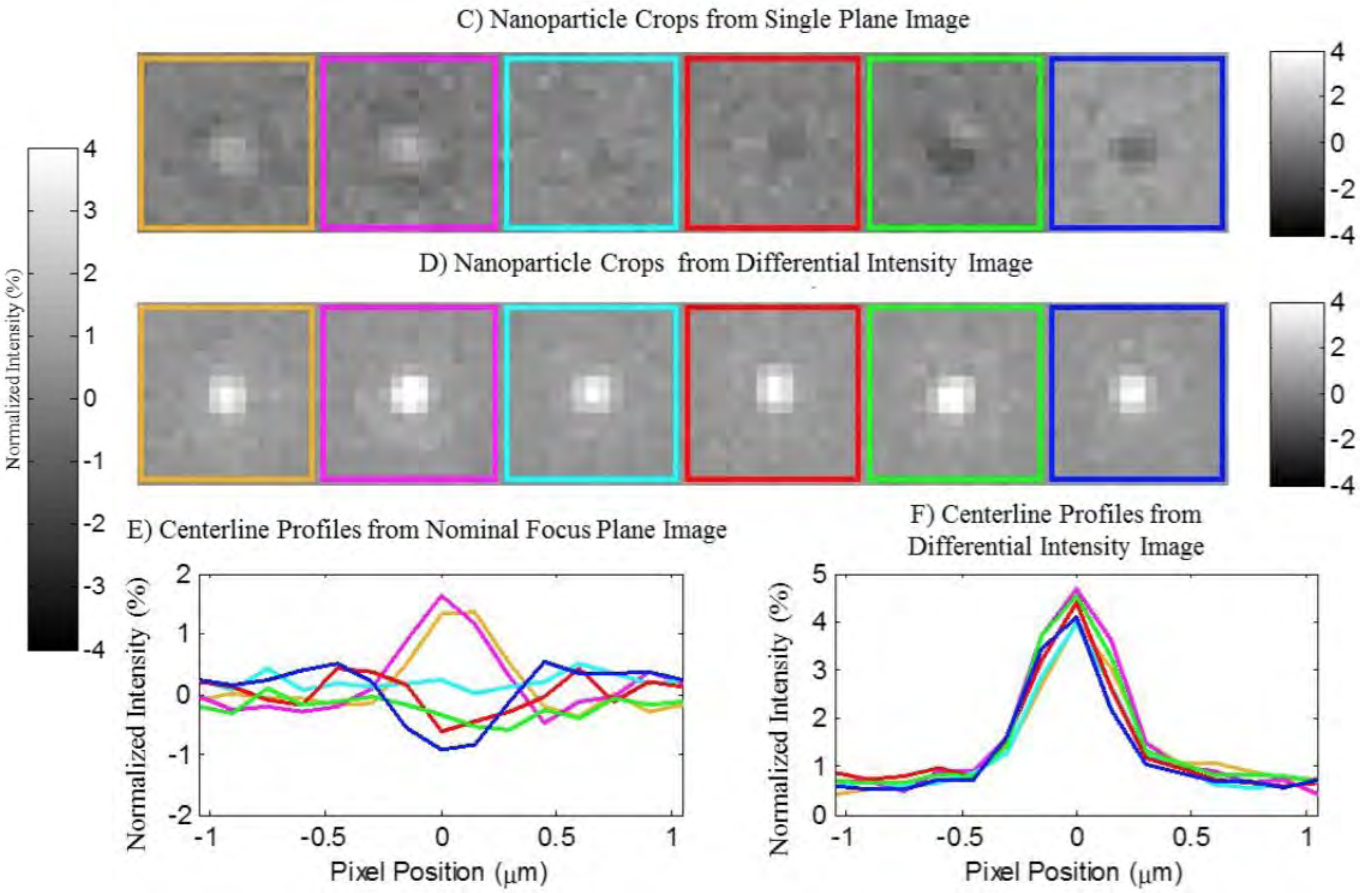
Robust Visualization and Discrimination of Nanoparticles by Interferometric Imaging

Jacob Trueb ‡, Oguzhan Avci ‡, *Student Member, IEEE*, Derin Sevenler, John H. Connor, and M. Selim Ünlü, *Fellow, IEEE*



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Outline – Going Beyond Detection and Sizing

- Motivation – Biological Nanoparticles everywhere
- Problem definition – contrast and size
- Detection vs. visualization
- Interferometric Reflectance **Imaging** Sensor
- Biological Nanoparticle Detection and Sizing
- Pupil function engineering
- Resolution improvement by oblique illumination and reconstruction
- Towards 100nm in label-free visible light microscopy
- Conclusions and Future

Research Article Vol. 4, No. 2 / February 2017 / *Optica* 247

optica

Pupil function engineering for enhanced nanoparticle visibility in wide-field interferometric microscopy

Oguzhan Avci,[1] Maria I. Campana,[1] Celalettin Yurdakul,[1] and M. Selim Ünlü[1,2,*]

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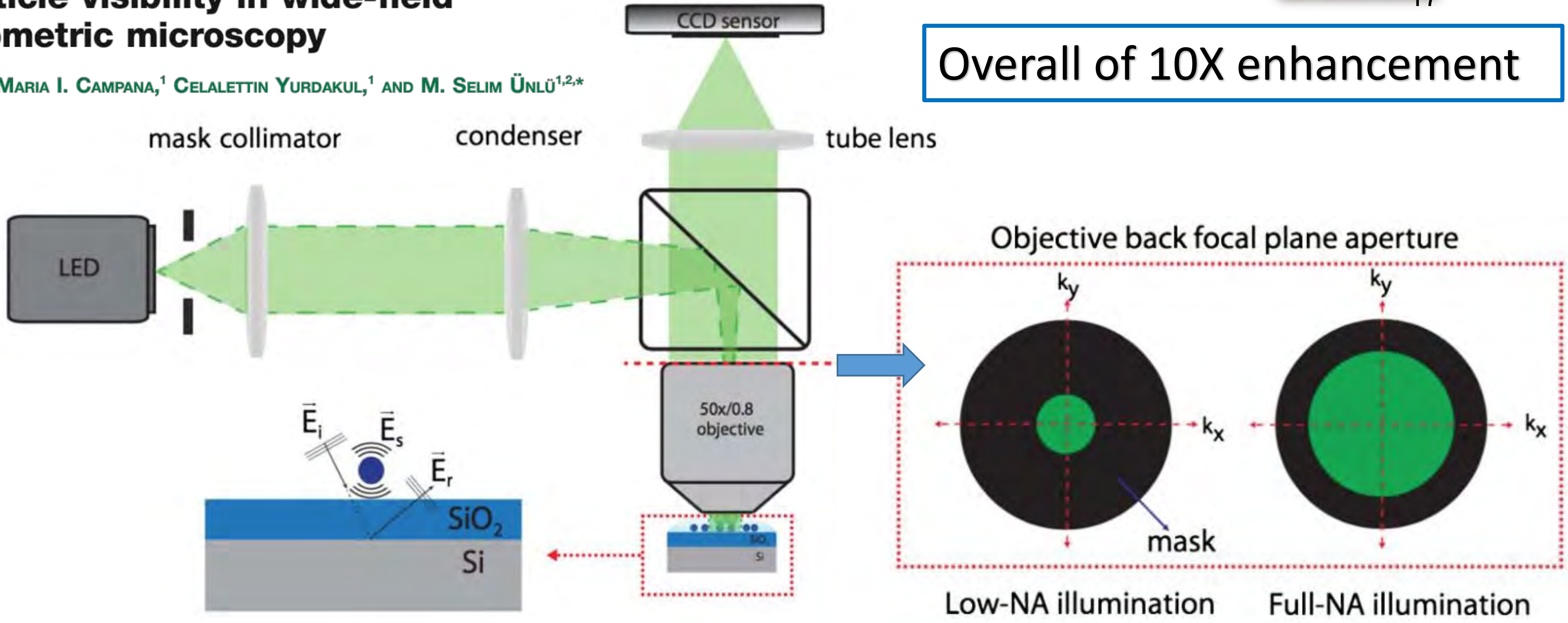
Overall of 10X enhancement



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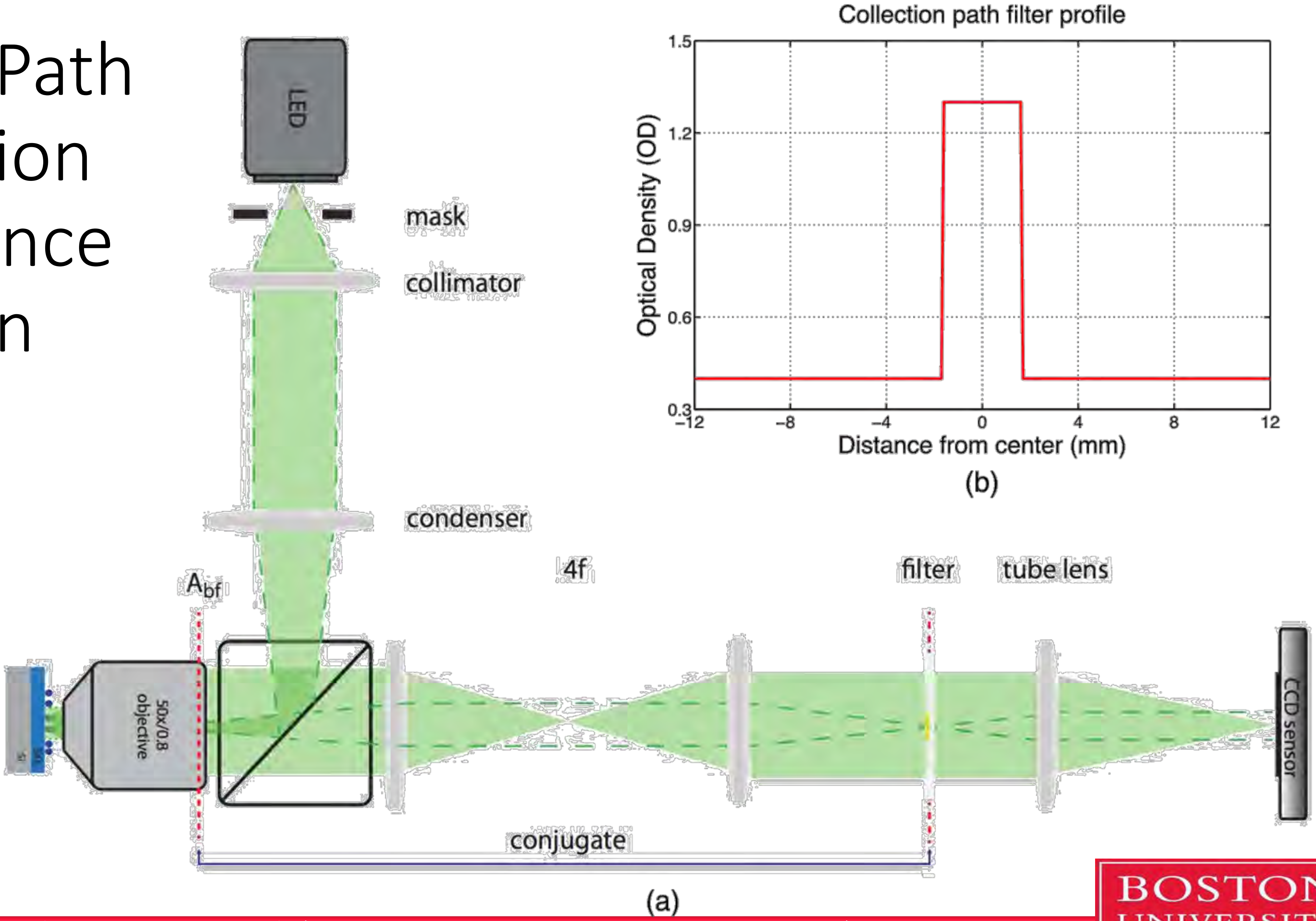
Collection Path – Apodization and Reference Attenuation



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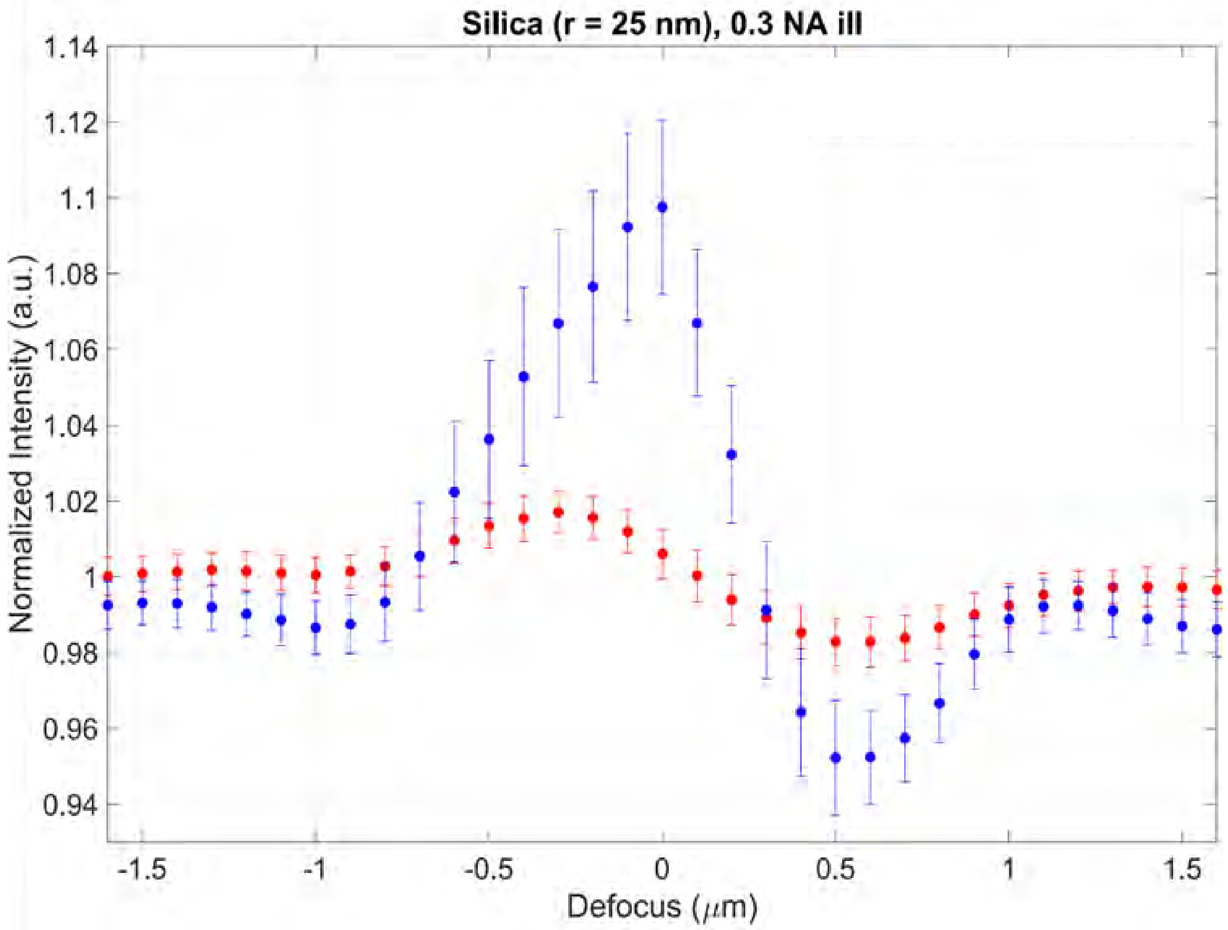
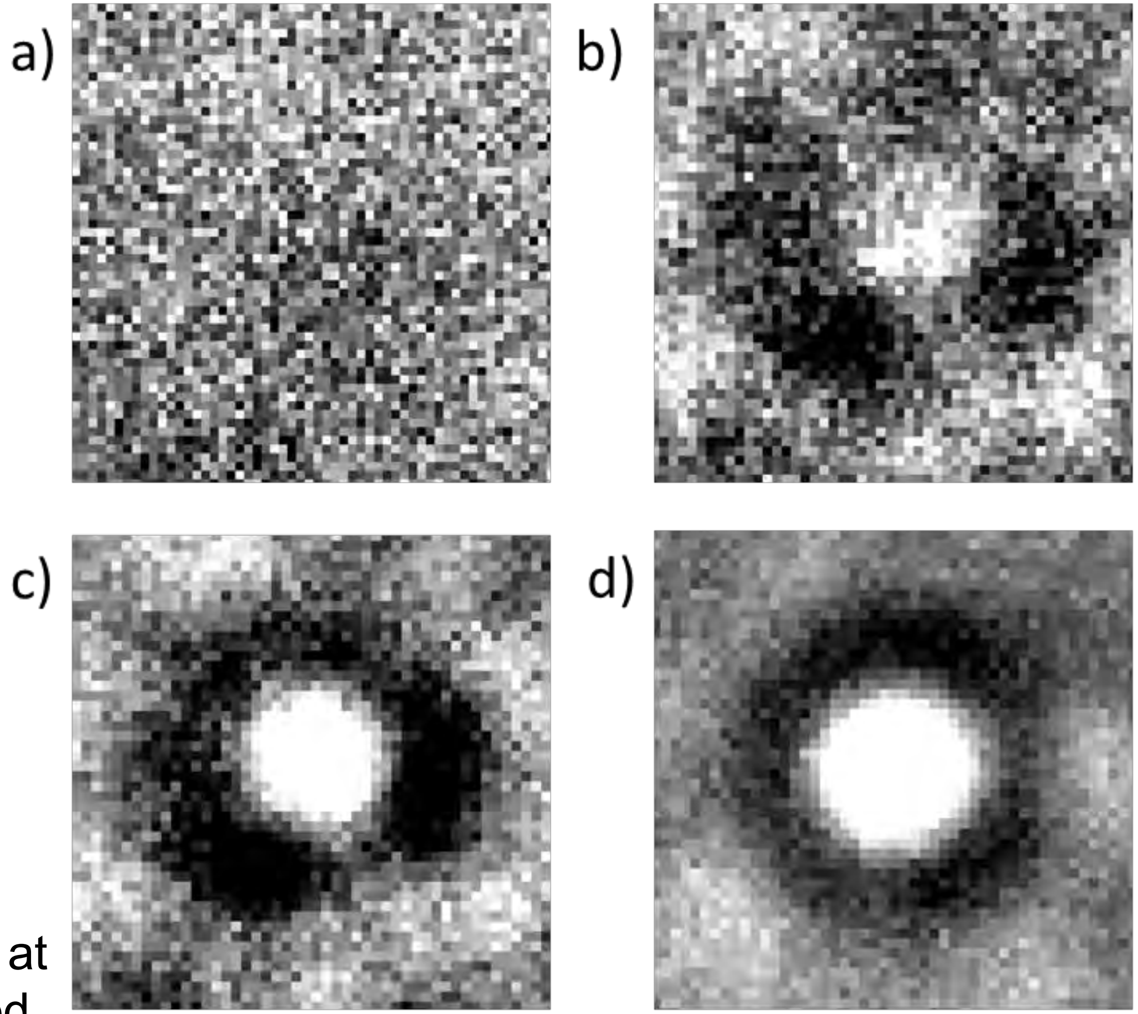
Silica particles defocus curve **~5X enhancement (3% → 15%)**





a) full-NA illumination at λ=530 nm, b) apodized illumination at λ=530 nm, c) apodized illumination at λ=460 nm, d) apodized illumination with amplitude filter in the collection path at λ=460 nm.

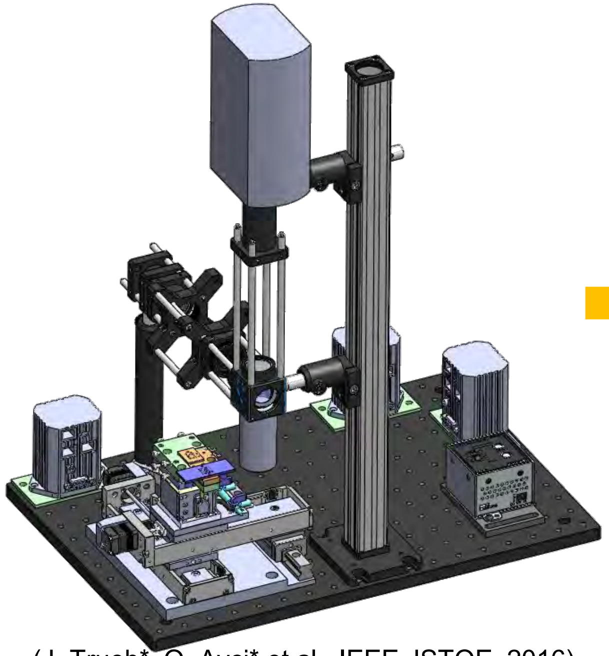
Reconstruction in Interference Microscopy

imaging system

object

in

out



(J. Trueb*, O. Avci* et al., IEEE JSTQE, 2016)

observation

$$y = Ax + e$$

- y → observation
- A → system response convolution matrix
- x → object
- e → noise

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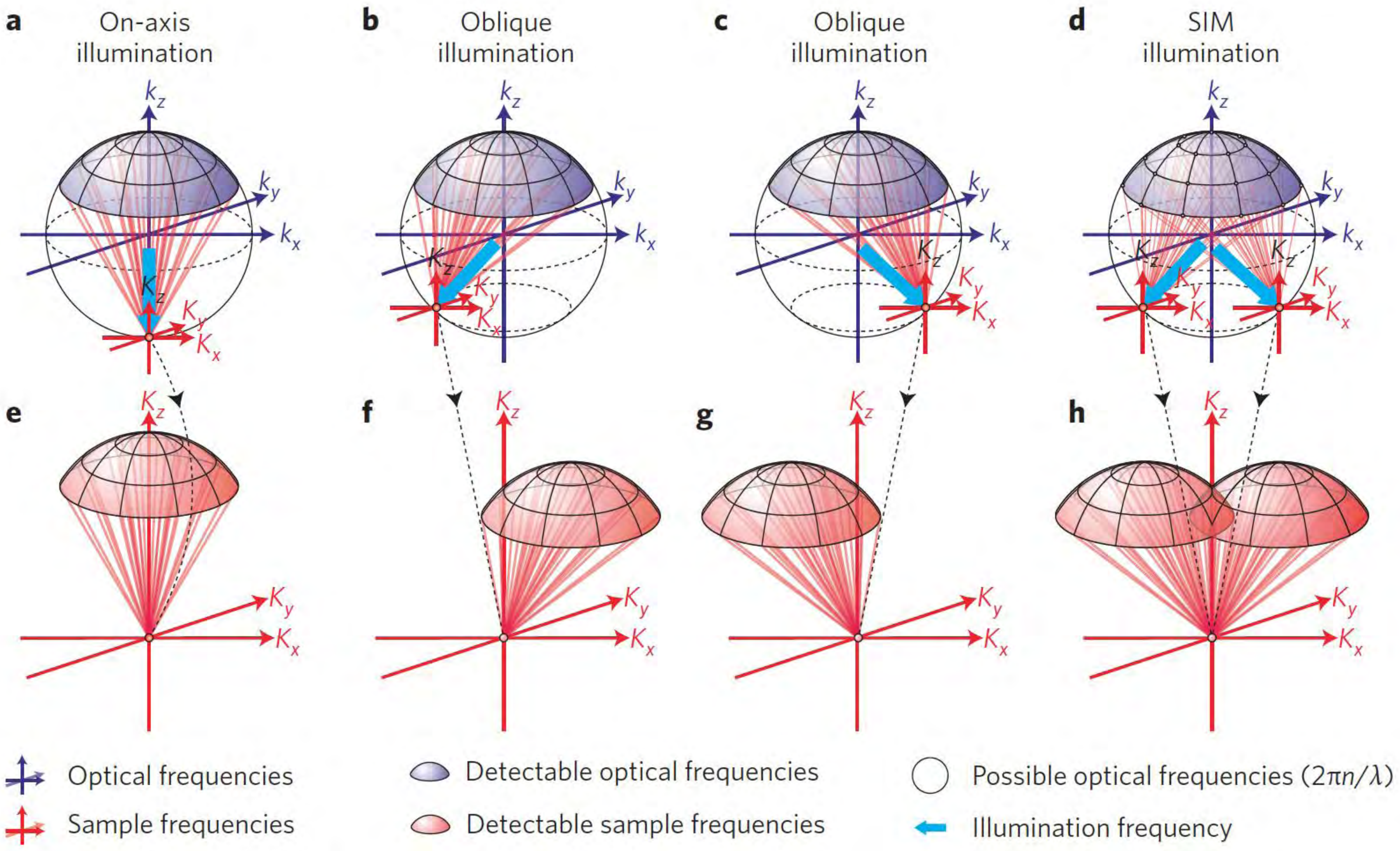


NATURE PHOTONICS | VOL 8 | MAY 2014 |

Super-resolution in wide-field interferometric microscopy

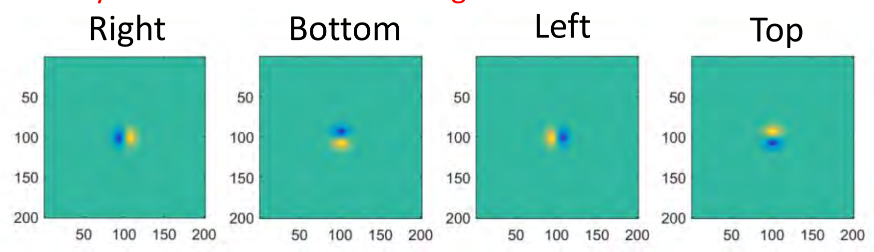
- ***Enhancing low-index nanoparticle resolution via reconstruction schemes***

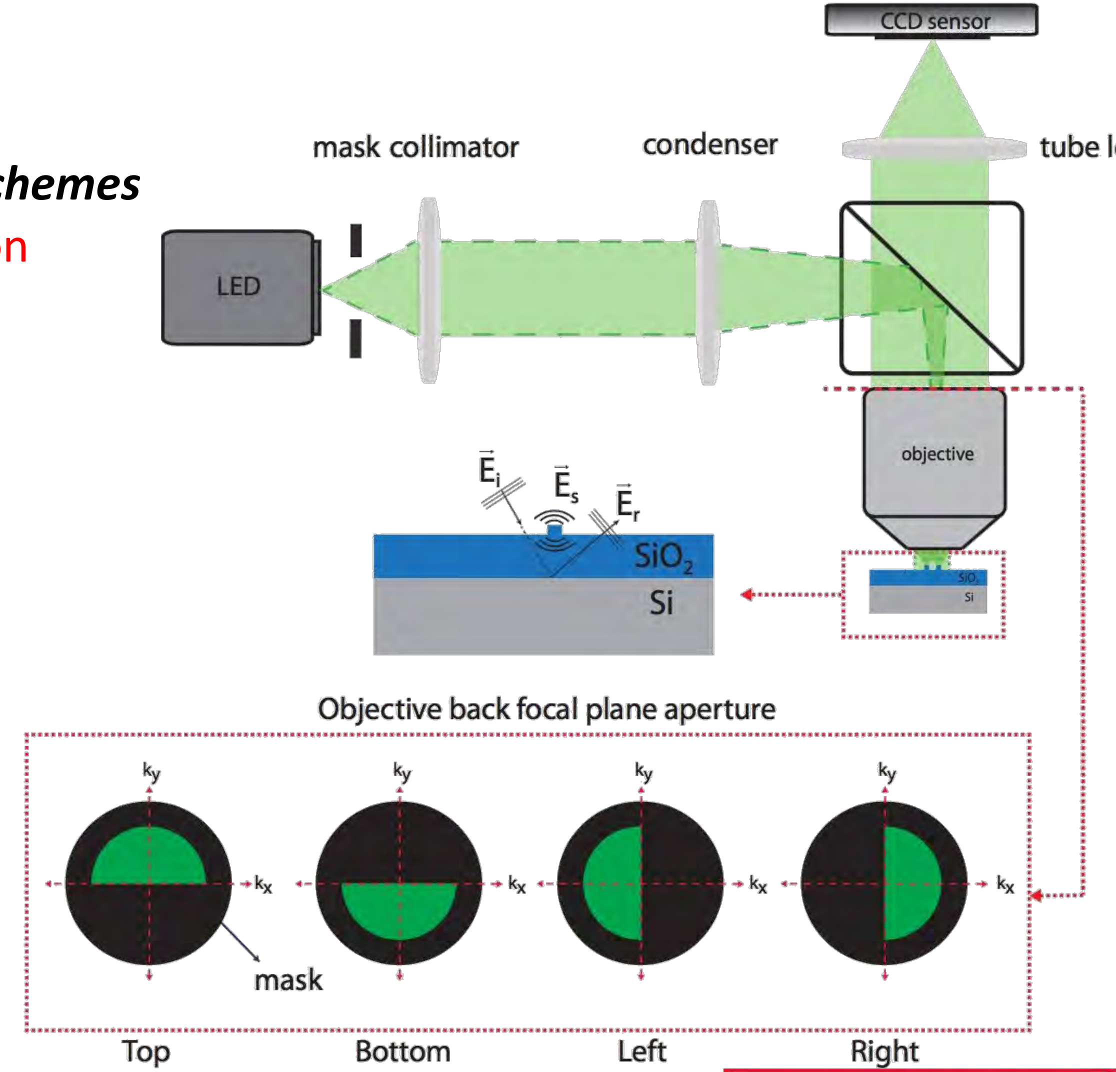
  Asymmetric illumination based reconstruction for super resolution (with Lei Tian)

$$\min_{x} \sum_{j=1}^{N} \|A_j x - y_j\|_2^2 + \alpha \|x\|_2^2$$

$$x = \mathcal{F}^{-1}\left\{ \frac{\sum_{j=1}^{N} H_j^* y_j}{\sum_{j=1}^{N} |H_j|^2 + \alpha} \right\}$$

Fourier transforms of the transfer functions (H) for each asymmetric illumination configuration





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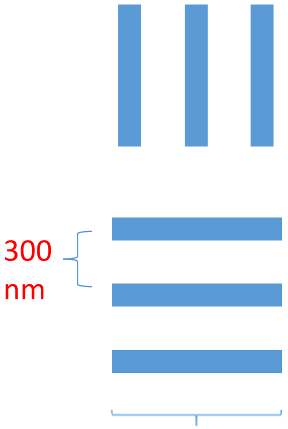
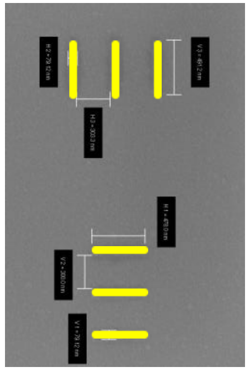
Experimental Results

Sample – E-beam fabricated

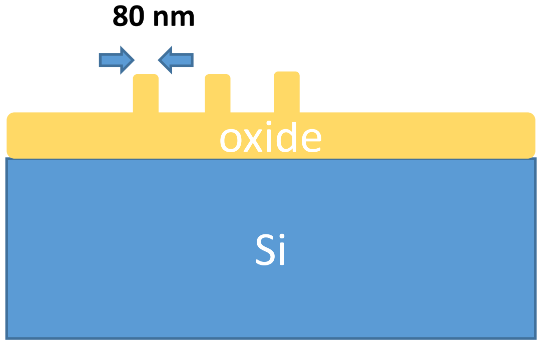
Sketch

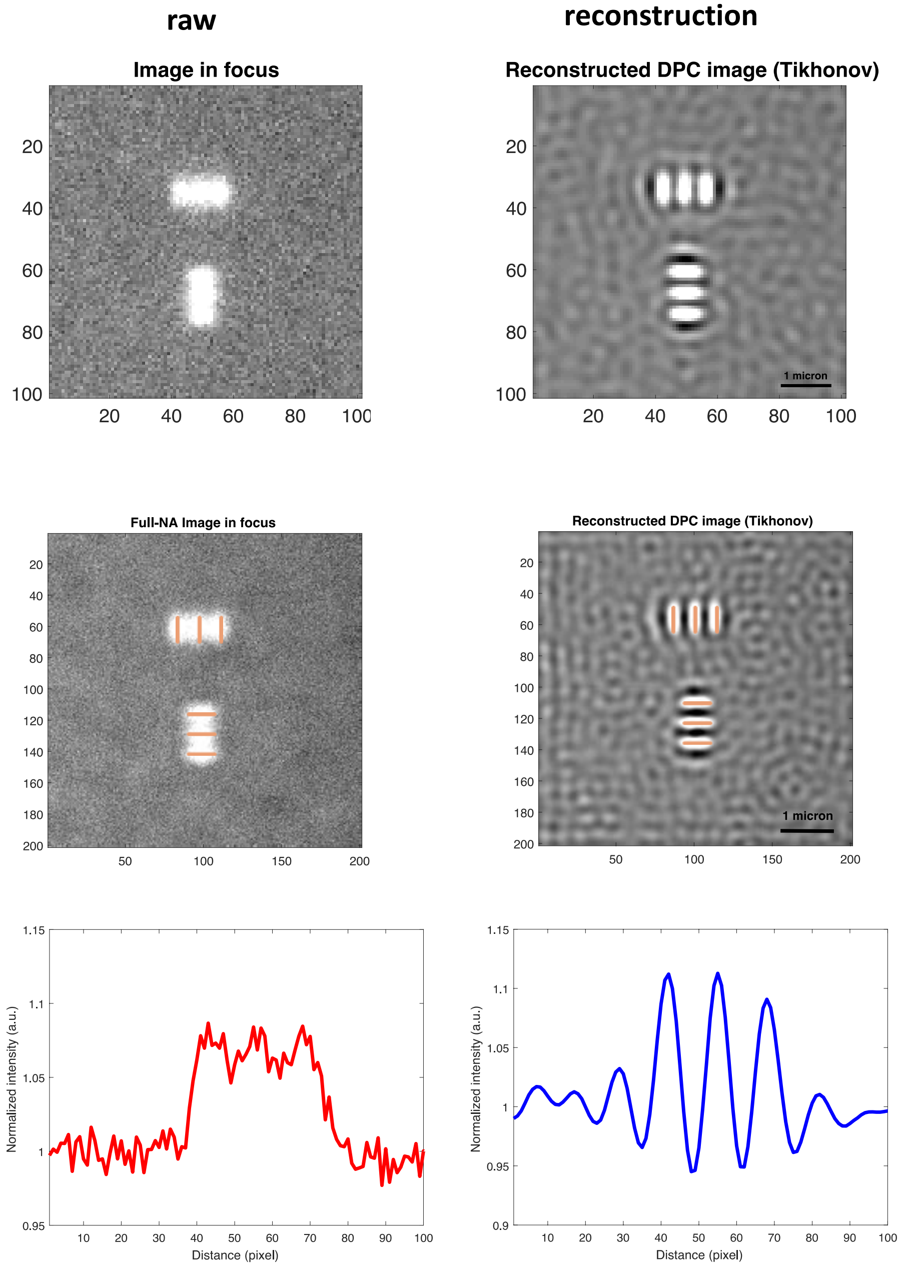


SEM



SEM

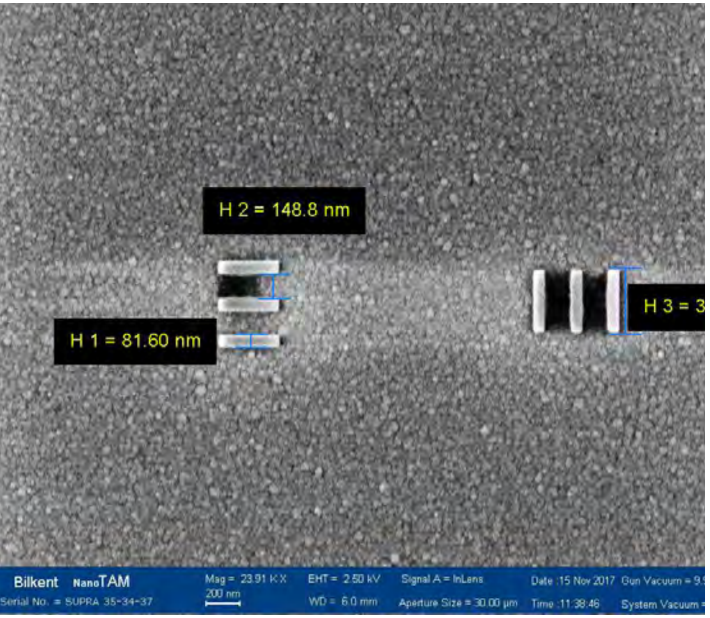




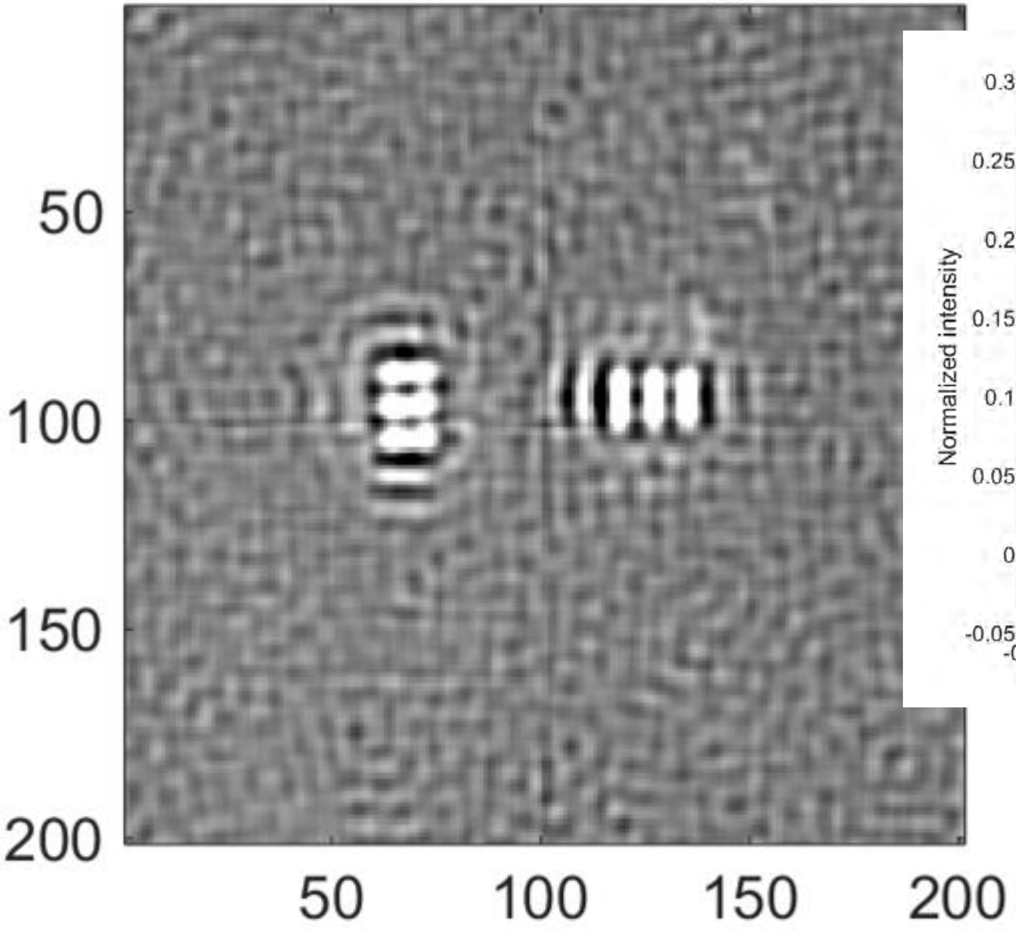
50x/0.8NA 525nm

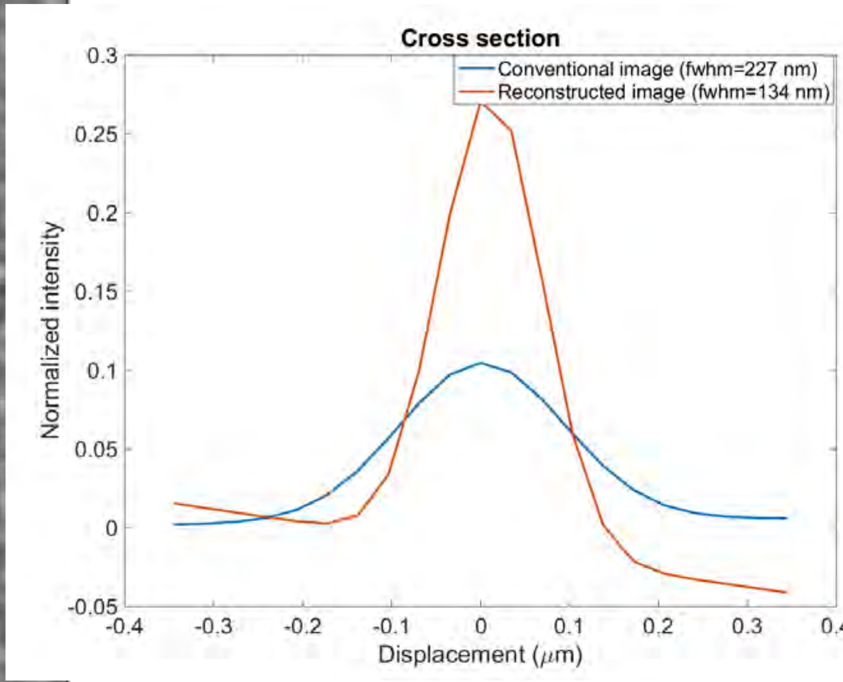
100x/0.9NA 525nm

150 nm separation, 0.9 NA, λ=420nm



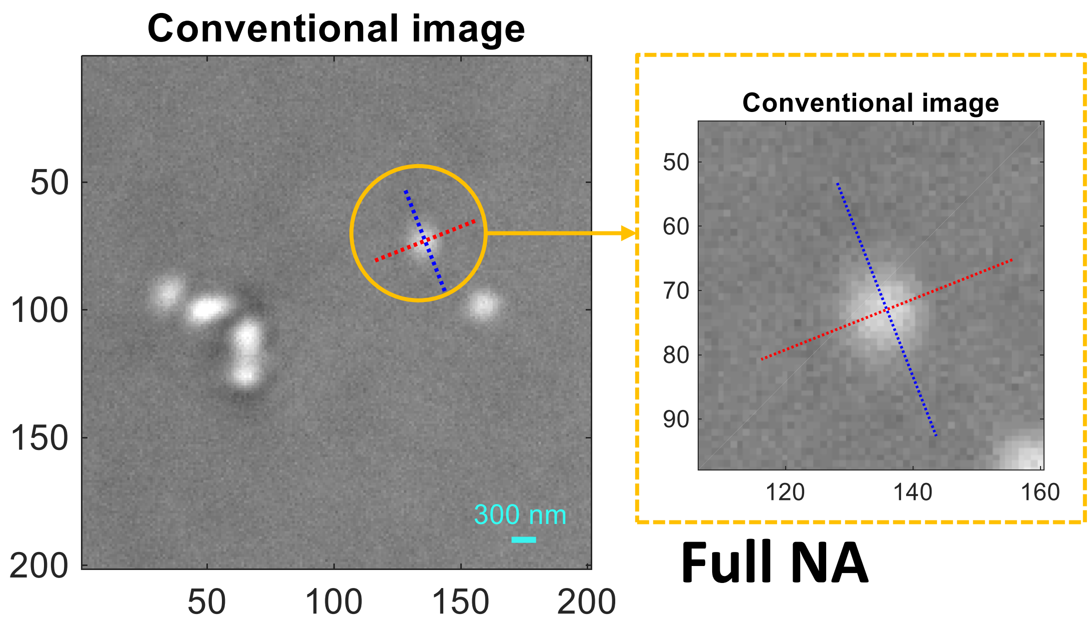
Reconstructed DPC image (Tikhonov)



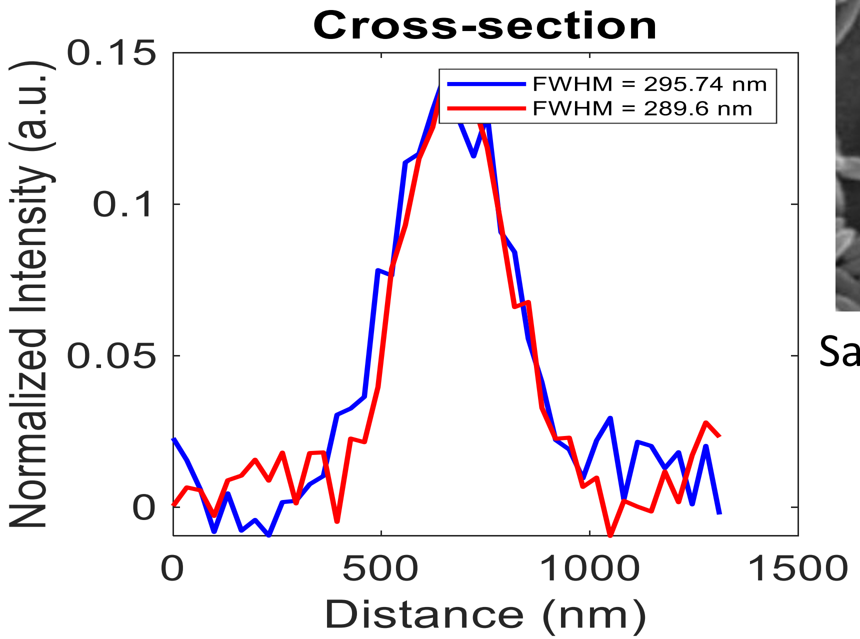


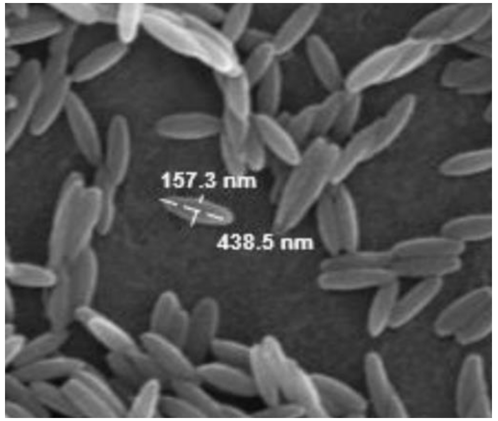
FWHM ~ 130nm < (λ / 3)

Elongated polystyrene rods

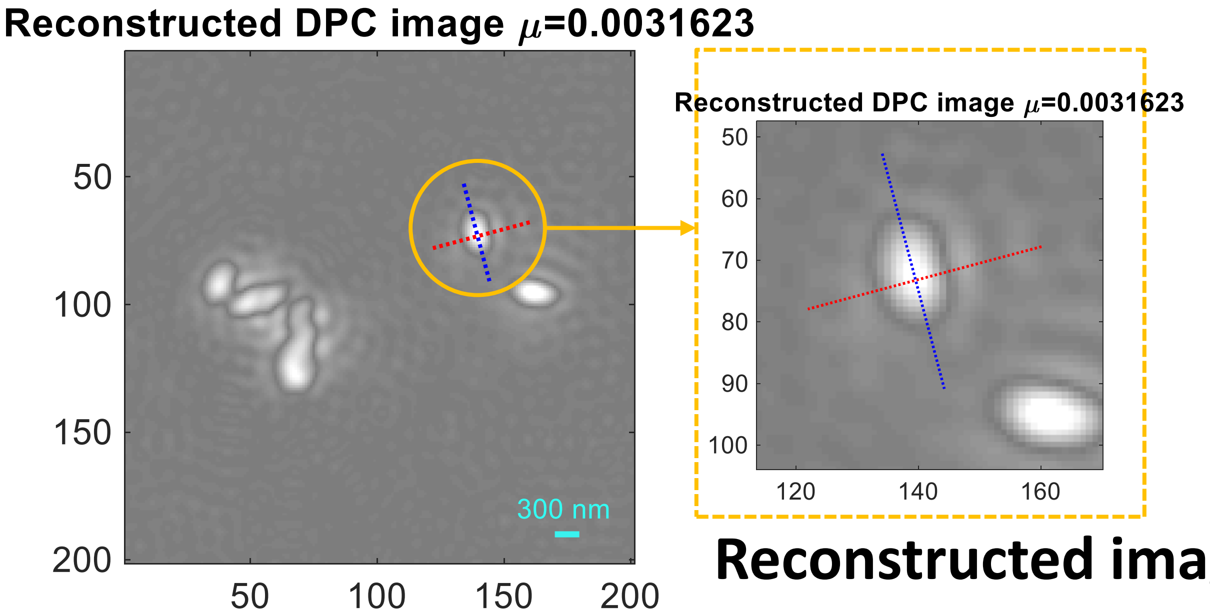


Full NA

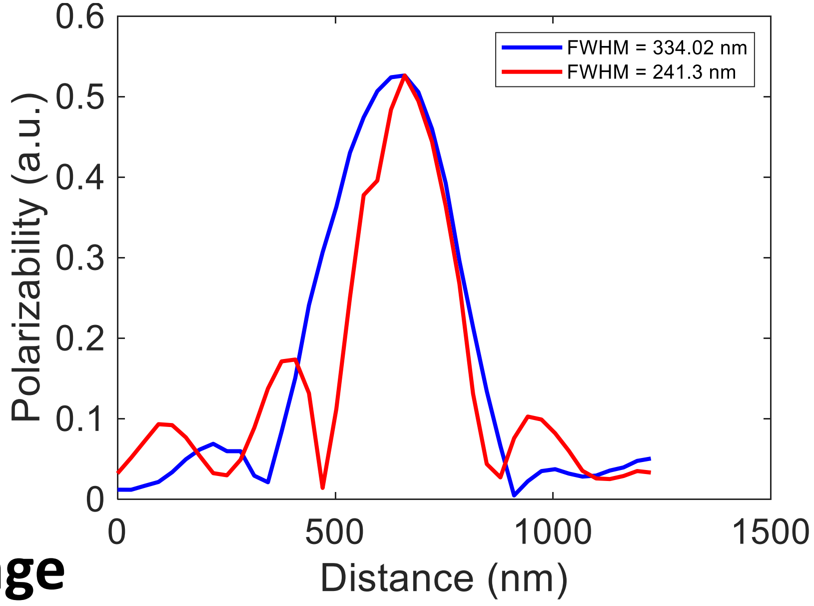




Samir Mitragotri (Harvard)



Reconstructed image



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Commercialization

NanoView
BIOSCIENCES

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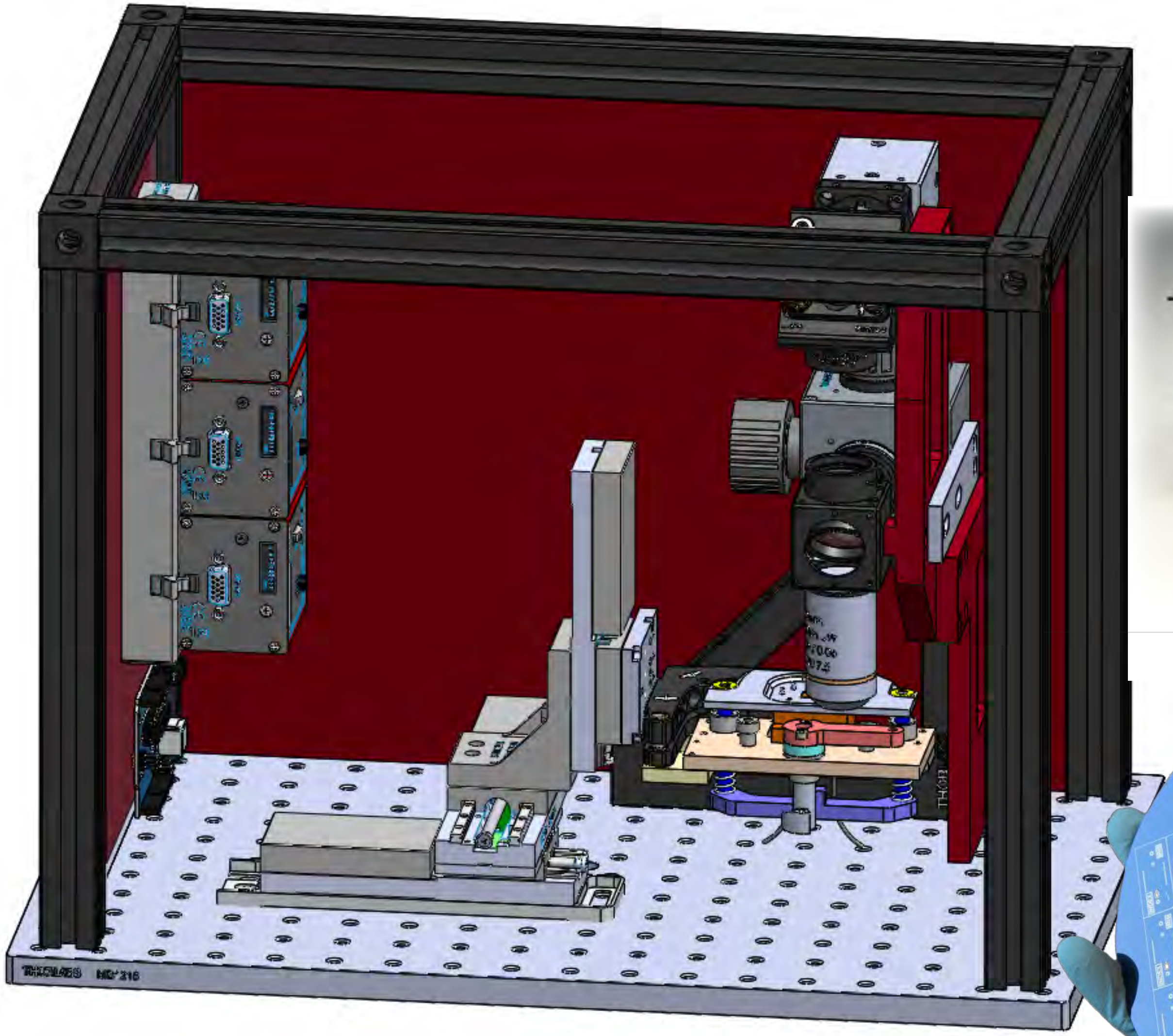

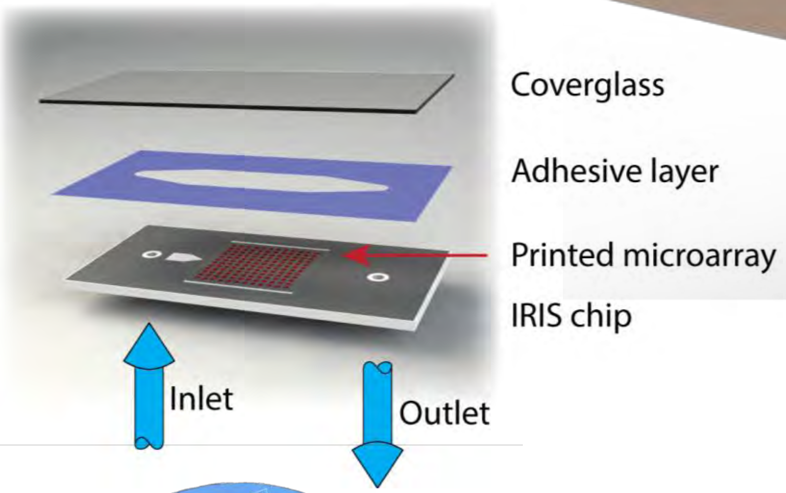

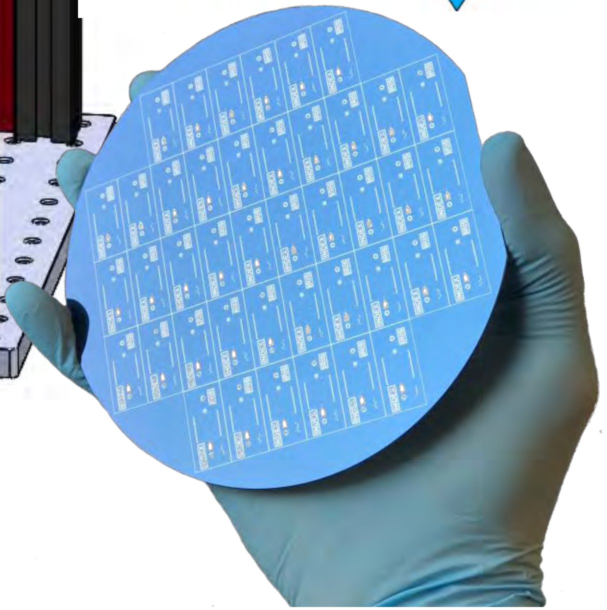

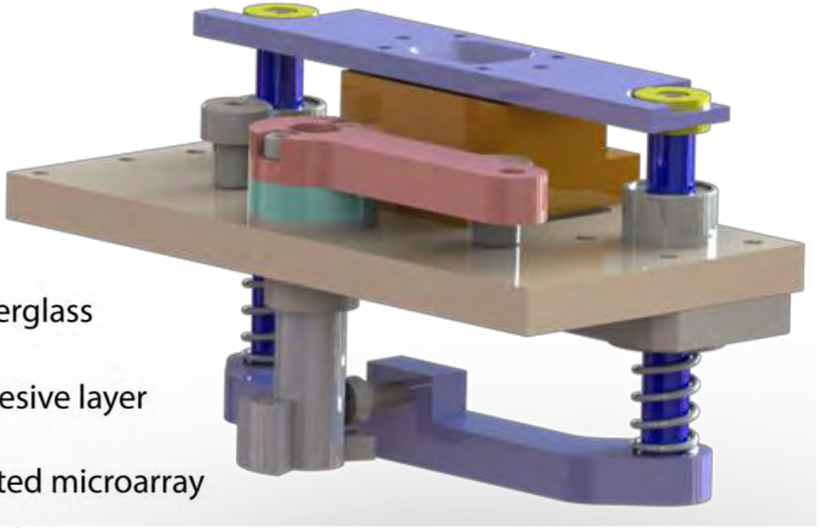

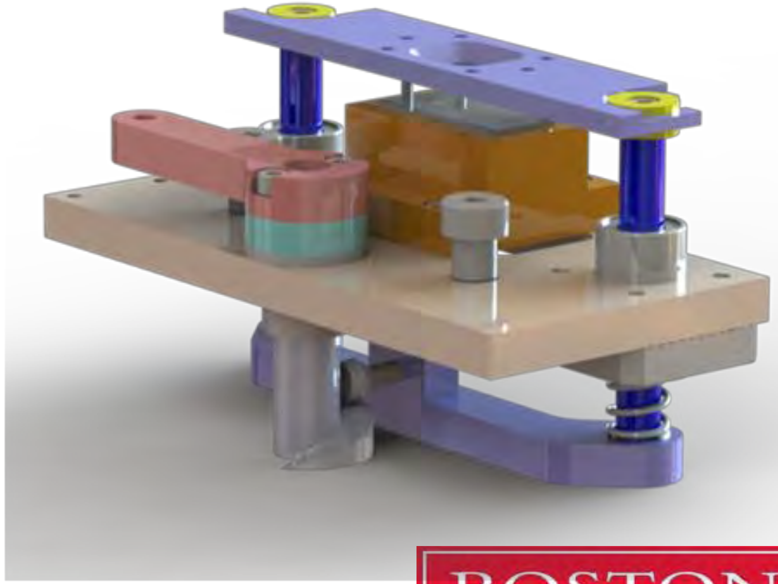

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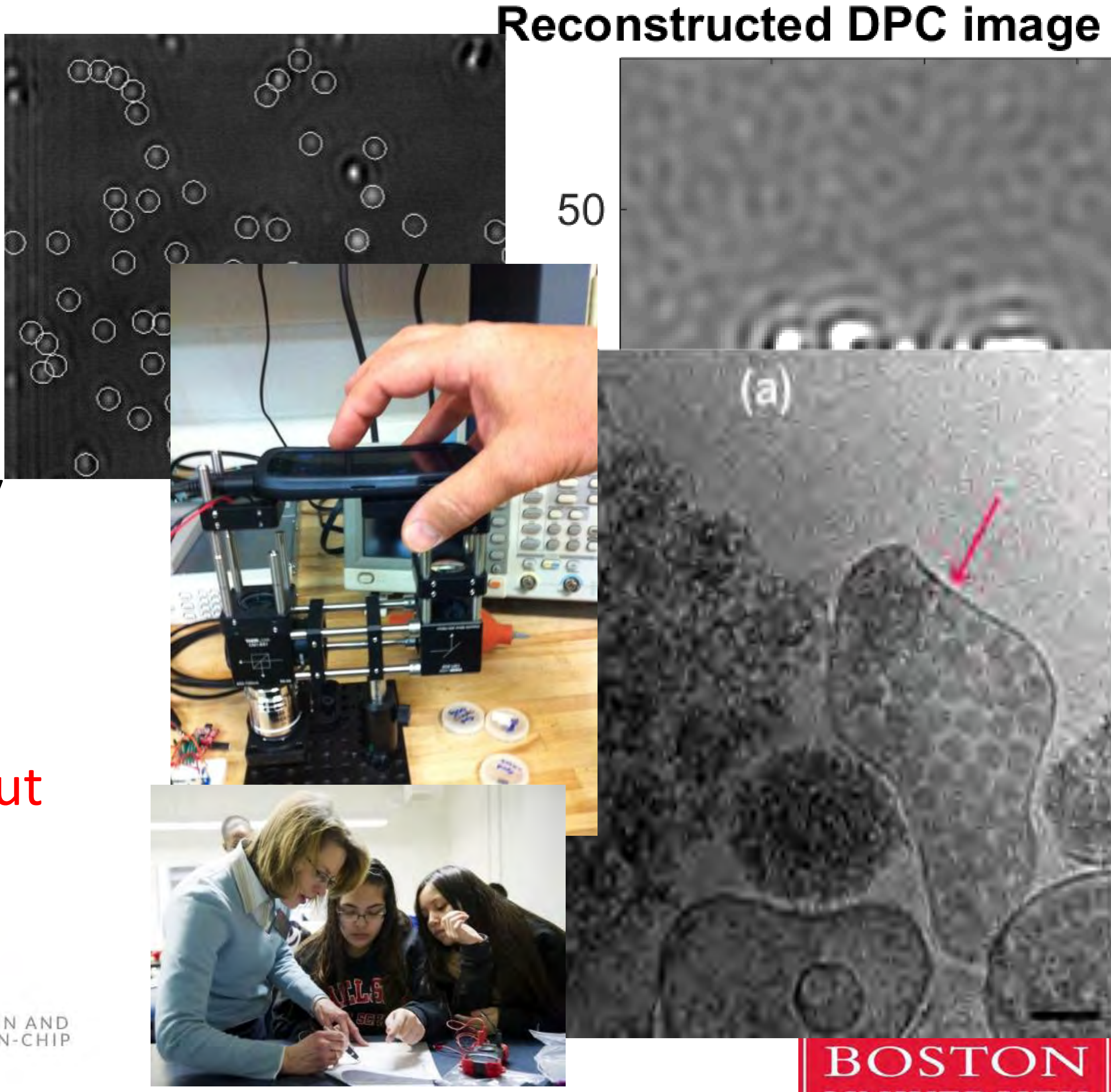
CONCLUSIONS & FUTURE

- Optical interference is a very powerful sensing technique.
- Multi-disciplinary innovation
- Single biological nanoparticle detection / counting / size and shape discrimination / visualization
- **<u>Goals:</u>** Down to r=20nm Biological nanoparticle detection in liquid
- Lateral resolution of ~100nm without labeling



INTEGRATED NANOPARTICLE ISOLATION AND DETECTION SYSTEM FOR COMPLETE ON-CHIP ANALYSIS OF EXOSOMES

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