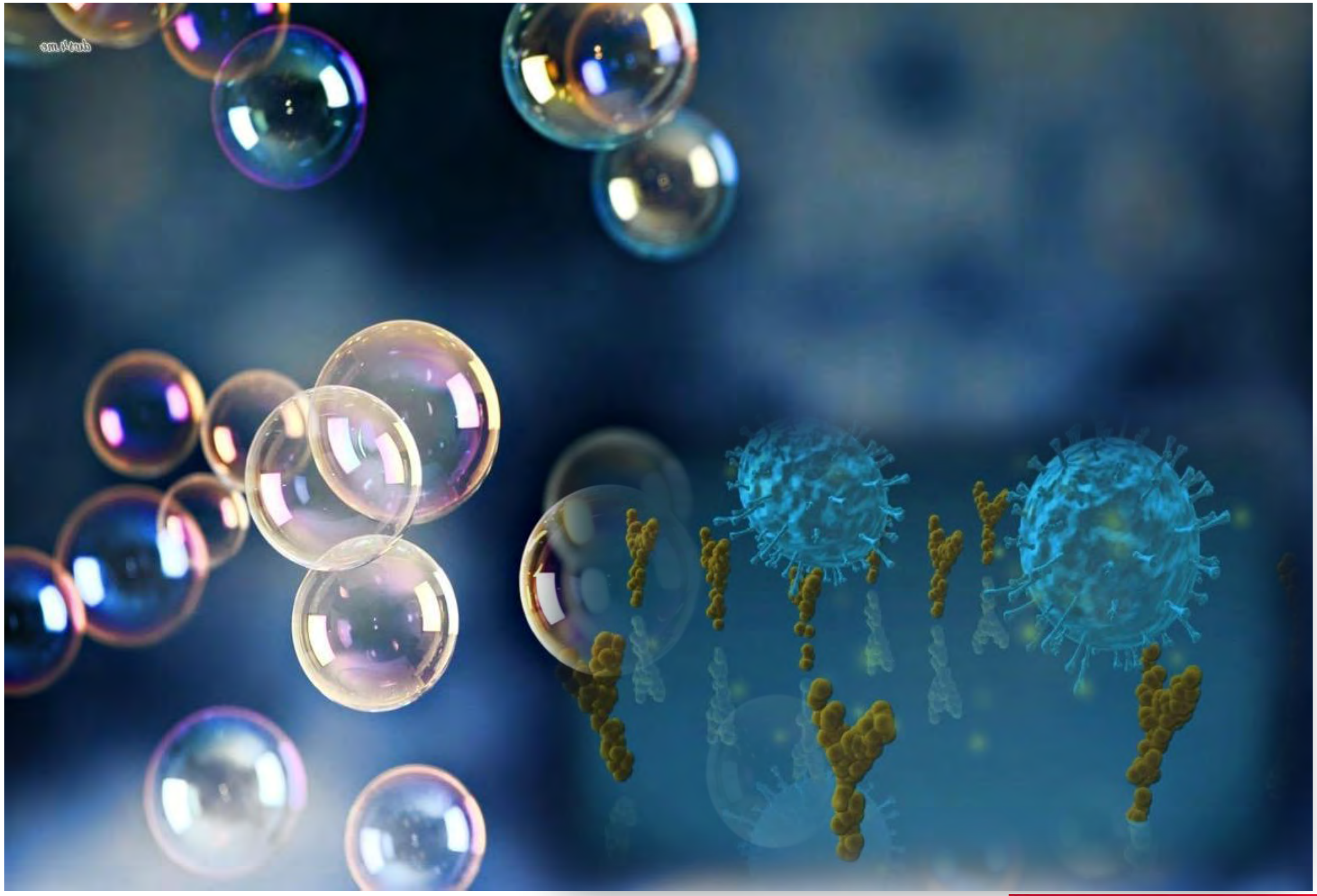
Reconstruction in wide-field interferometric microscopy for imaging weakly scattering biological nanoparticles with super-resolution



M. Selim Ünlü

Electrical Engineering,

Physics,

Biomedical Engineering

Graduate Medical Sciences

BUNano

Photonics Center

O. Avci, C. Yurdakul, D. Sevenler, F. Ekiz-Kanik, Lei Tian

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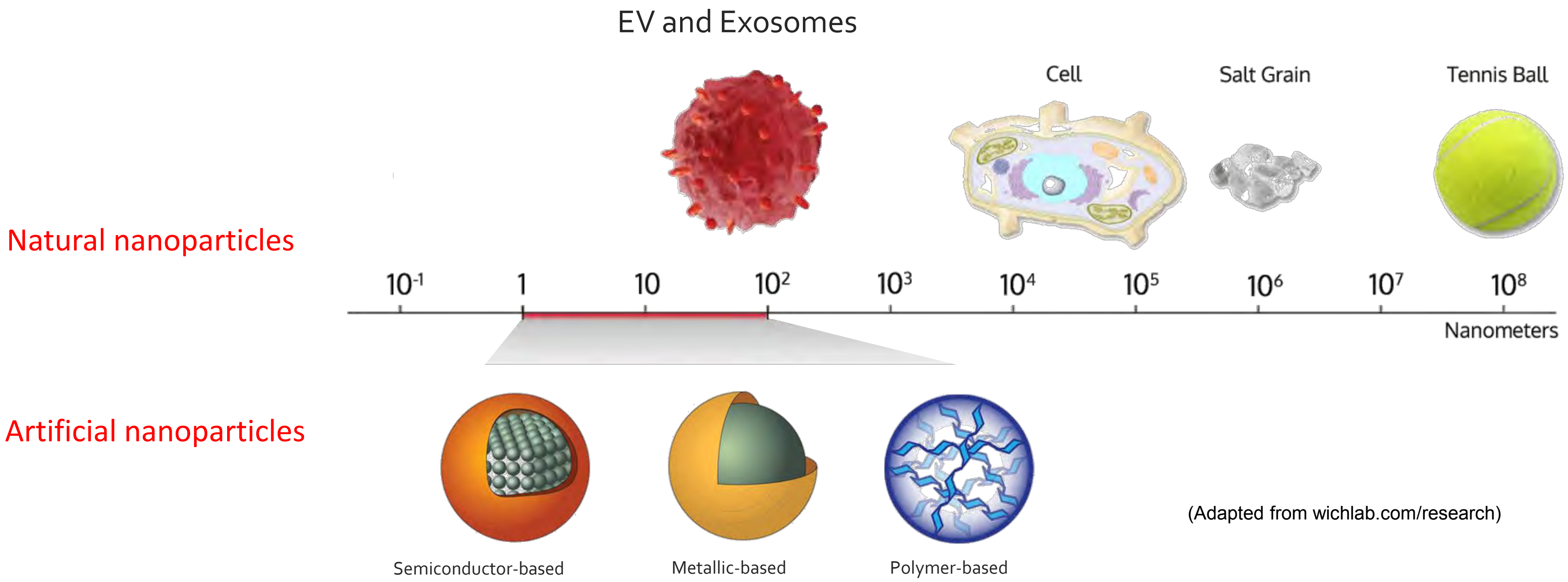
Outline

- 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

Motivation - Nanoparticles



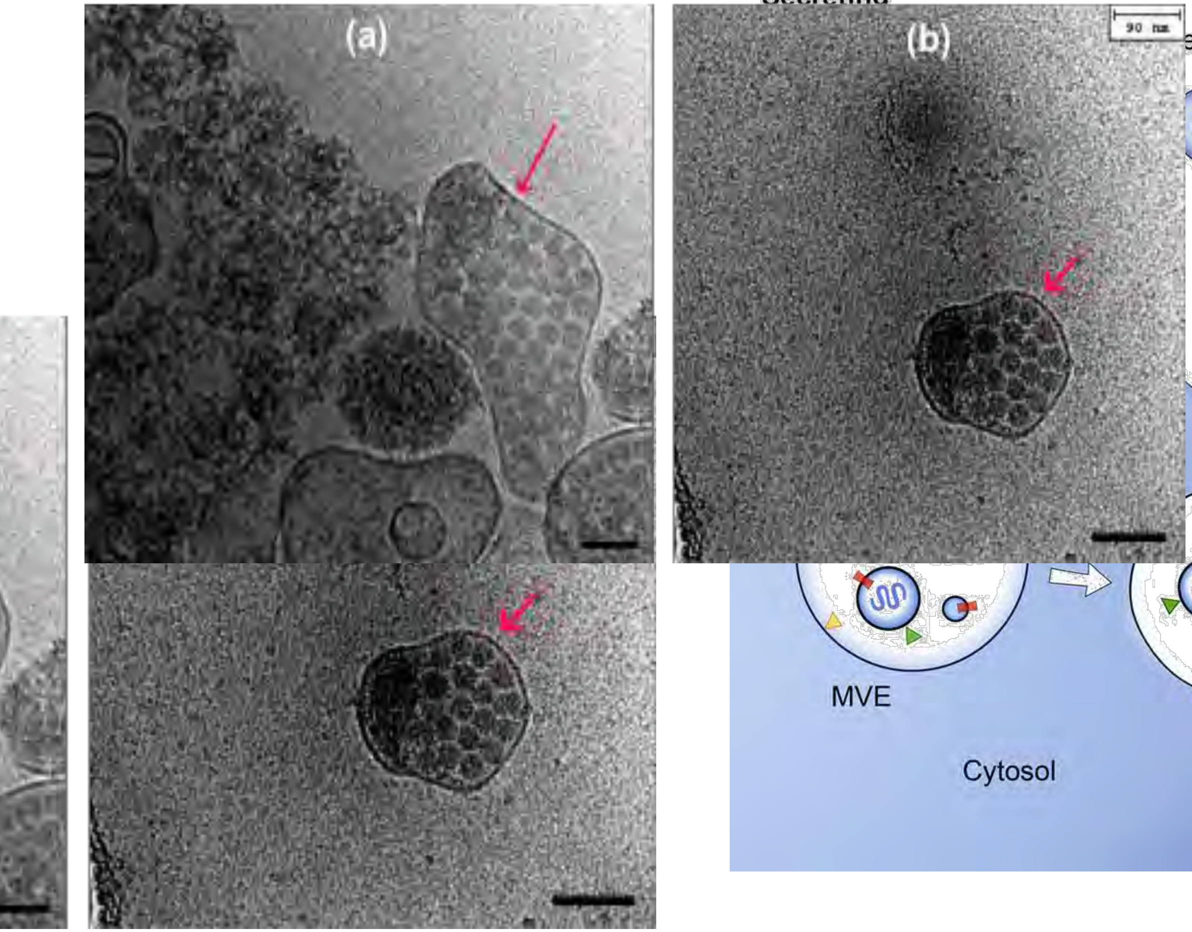
Artificial nanoparticles

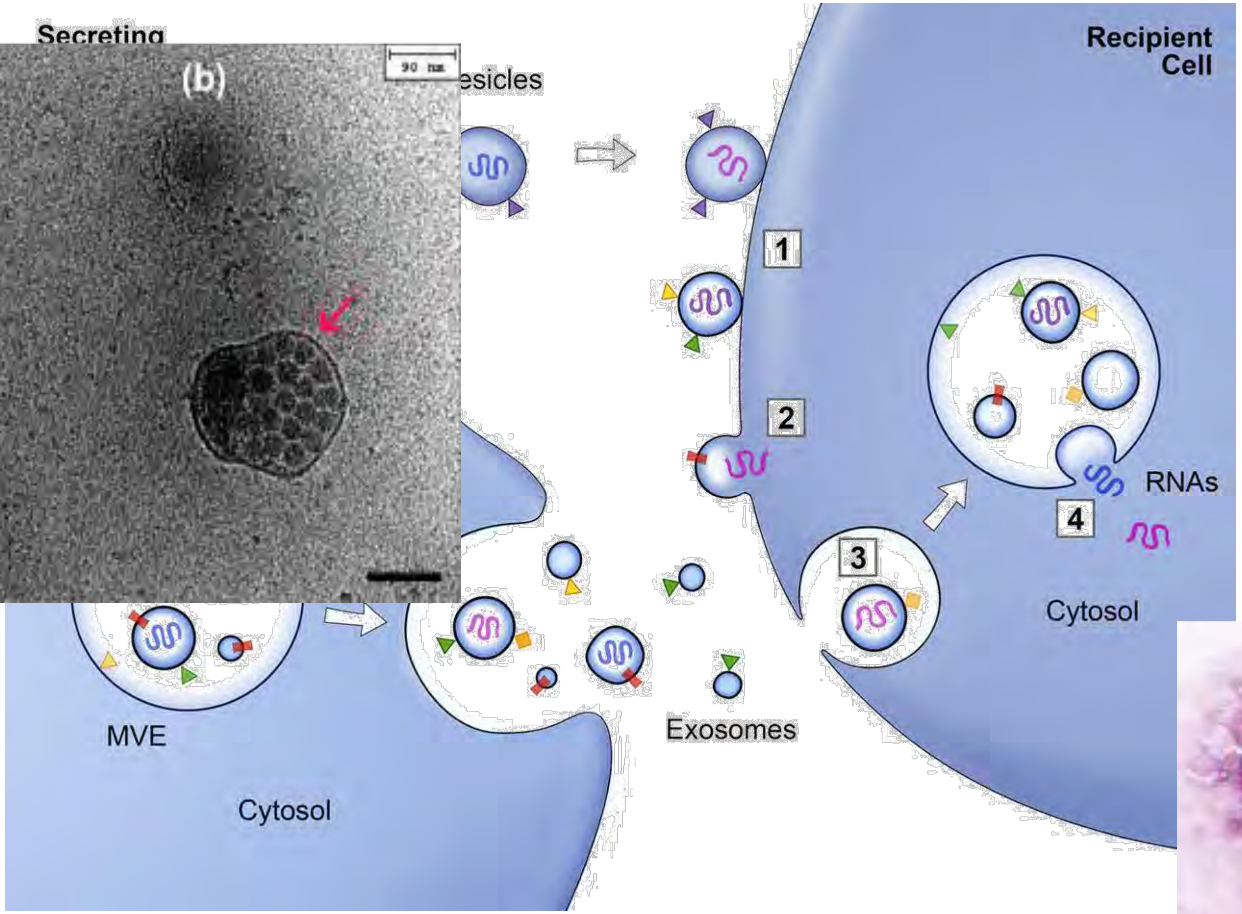
- Optically & physically engineered
- Used as labels or vehicles in diagnostics, therapeutic applications
- Gold, polystyrene NPs, QDs

Natural nanoparticles

- Low-index, complex-shaped
- Hard to detect without labels
- *Virus* – infectious diseases and cancer
- *Exosome* – secreted from cancer cells

Extra cellular vesicles, exosomes, and viruses



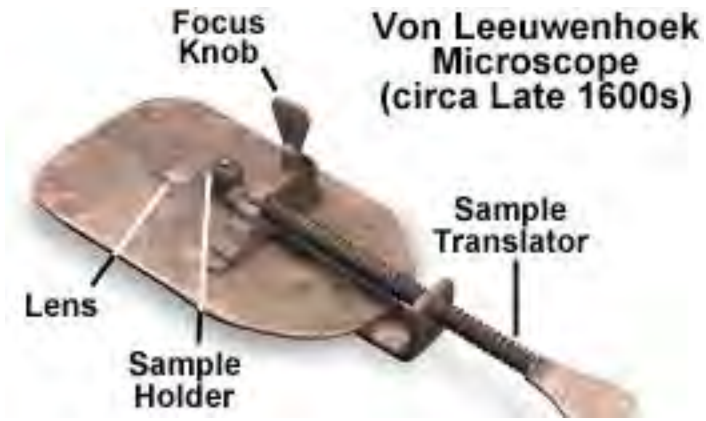


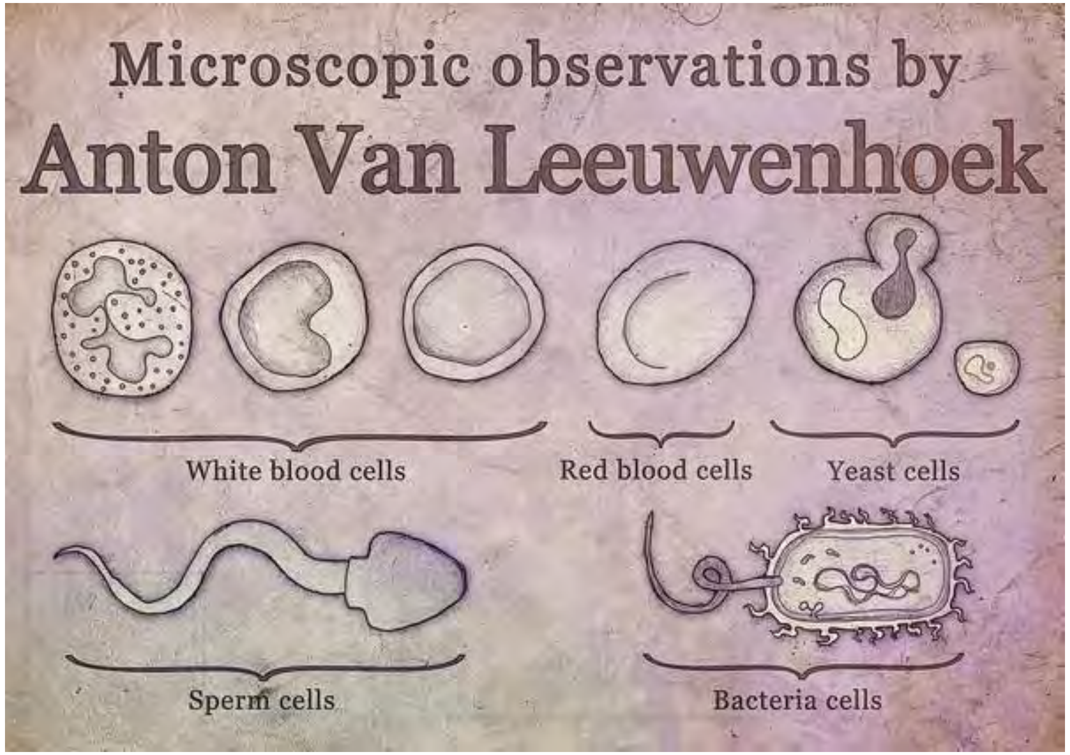
Example cryo-EM images of infectious extracellular vesicle (Bullitt Lab – BU MED)

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/

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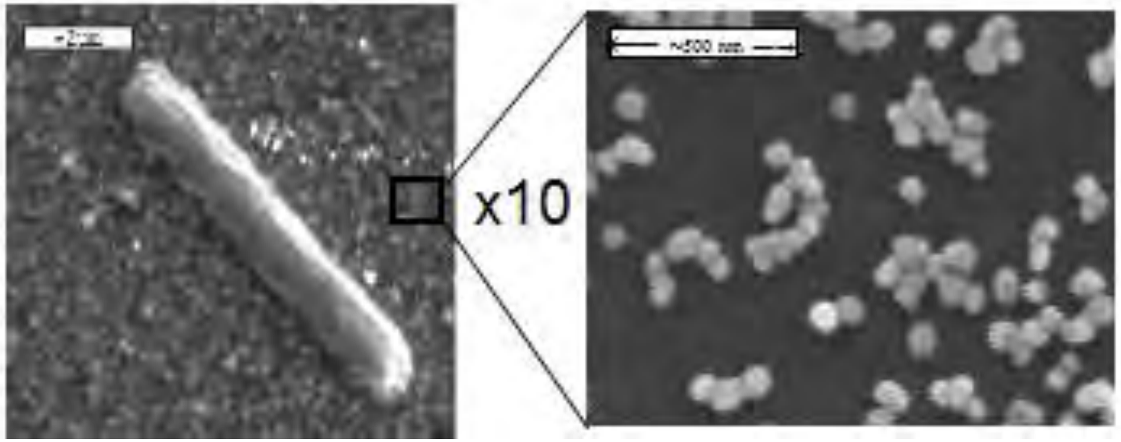
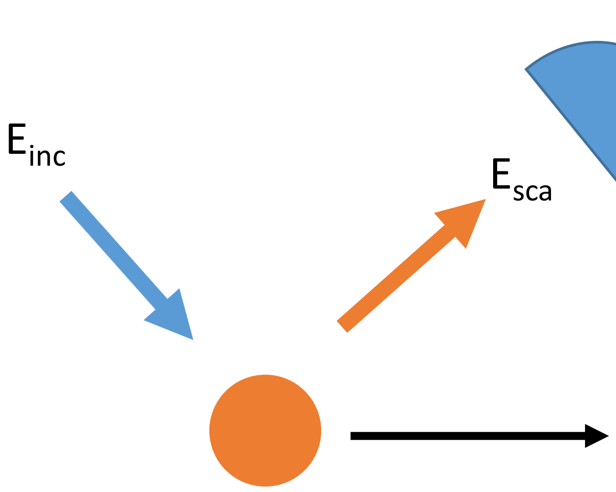
Biological Nanoparticle Detection Challenges – size and dielectric contrast



Ziegler



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

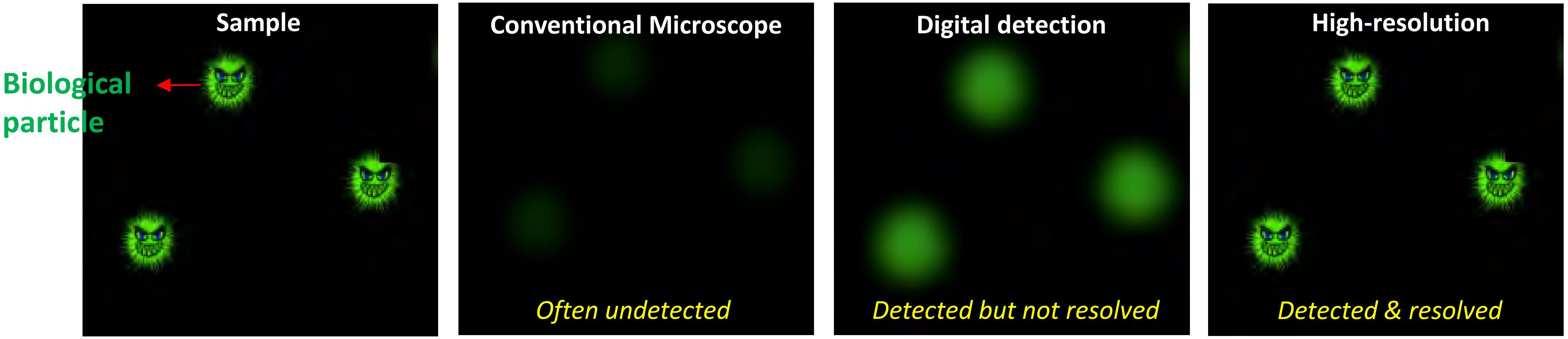
Size

contrast

Signal ~ R^6

Single nanoparticle detection / visualization

- **High-resolution** imaging systems provide visualization of nanoparticles – detailed structural information
  - Low-throughput, expensive and laborious
- **Digital detection** systems provide sensing of nanoparticles without visualization – limited or no structural information
  - High-throughput, often inexpensive and straightforward



Current State of the Art Technology

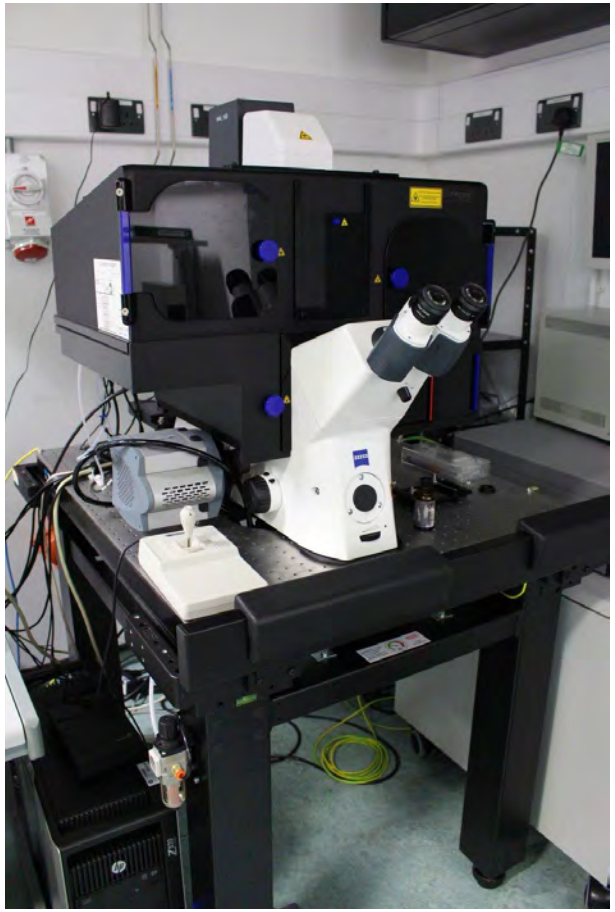
Electron microscopy

Zeiss Libra 200

- Great resolution

- Laborious
- Sample prep
- Expensive
- Not label-free
- Low-throughput

Fluorescence microscopy (STED/PALM)



clf.stfc.ac.uk

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Outline – IRIS

- Motivation – Biological Nanoparticles everywhere
- Problem definition – contrast and size
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- Interferometric Reflectance **Imaging** Sensor
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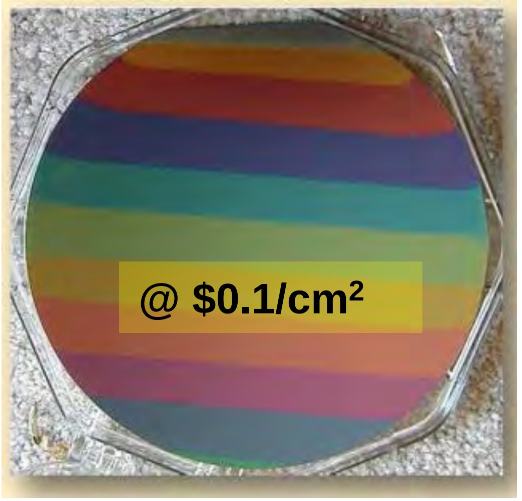
- Resolution improvement by oblique illumination and reconstruction
- Conclusions and Future

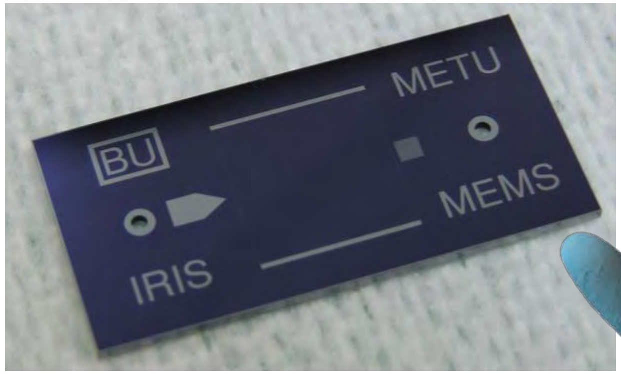
Interferometric Reflectance Imaging Sensor (IRIS)

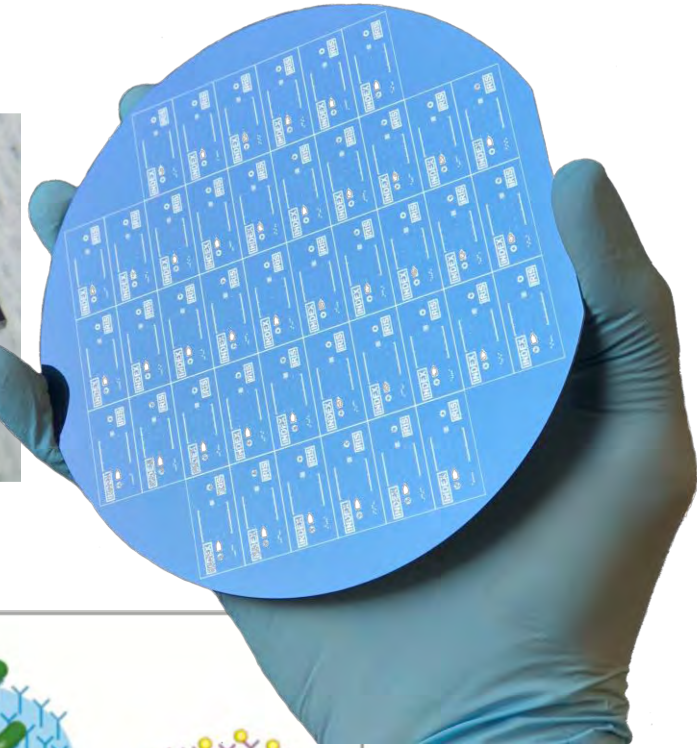
a high throughput biosensing platform

soap film

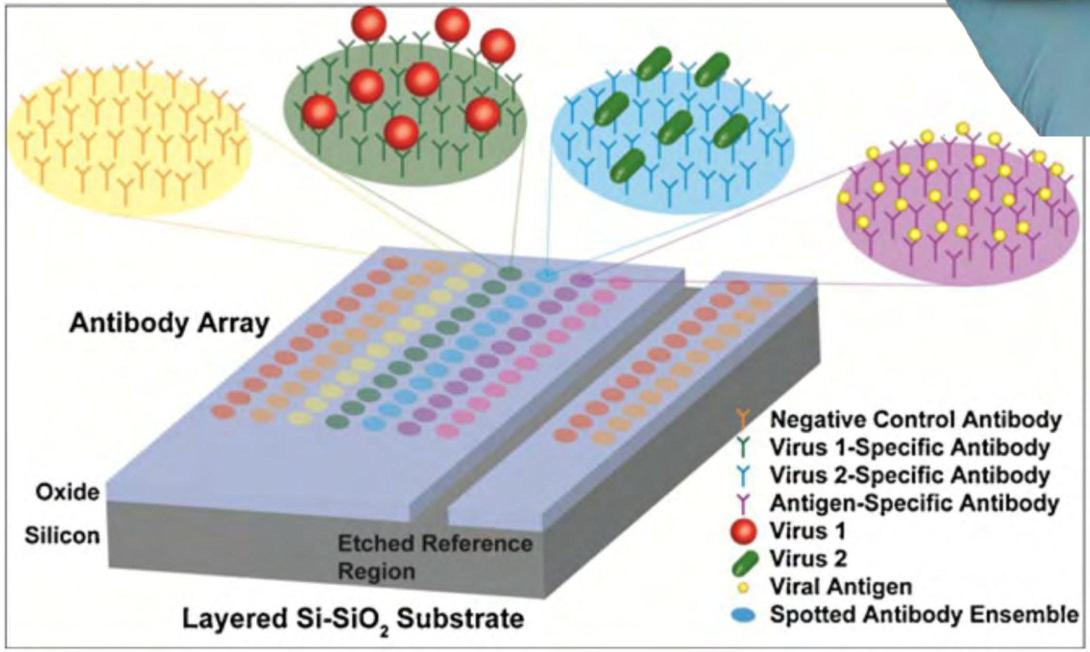
Oxide coated Si







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



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

pg/mm² sensitivity 1,000s of spots

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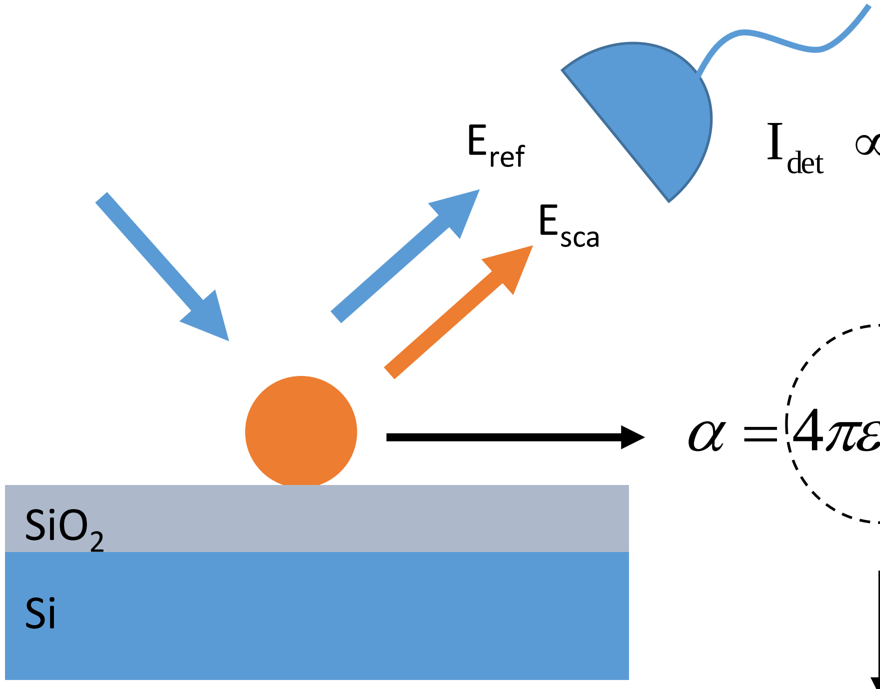


$$I_{det} \propto |E_{ref}|^2 + |E_{sca}|^2 - 2|E_{ref}||E_{sca}|\sin\theta$$

Phase Term ($\sin\theta$)

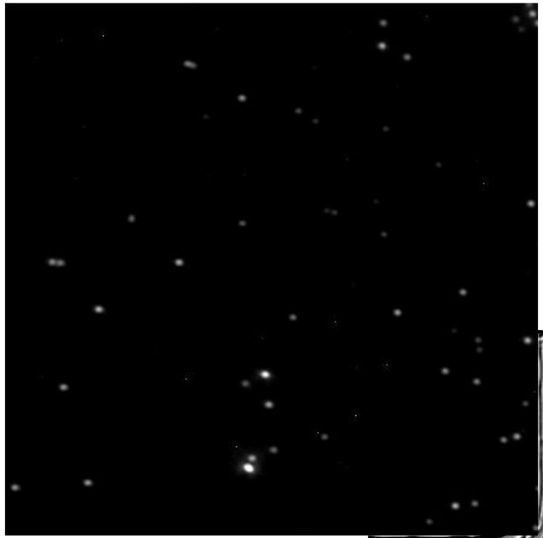
$$\alpha = 4\pi\varepsilon_0 R^3 \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}$$

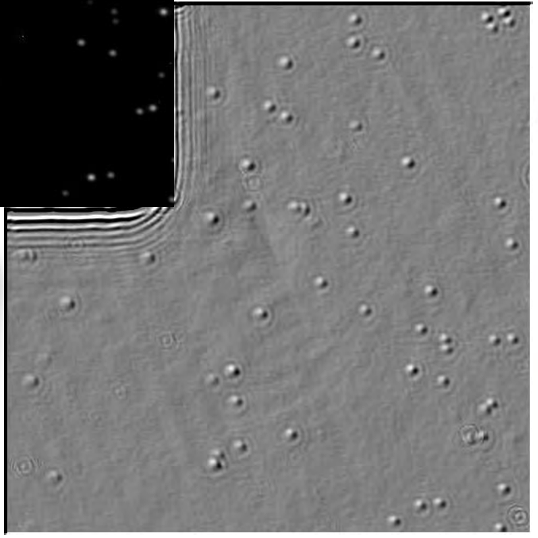
Size ($4\pi\varepsilon_0 R^3$)

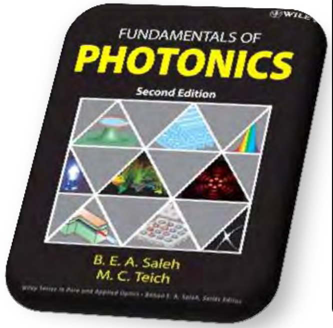
Material ($\frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}$)

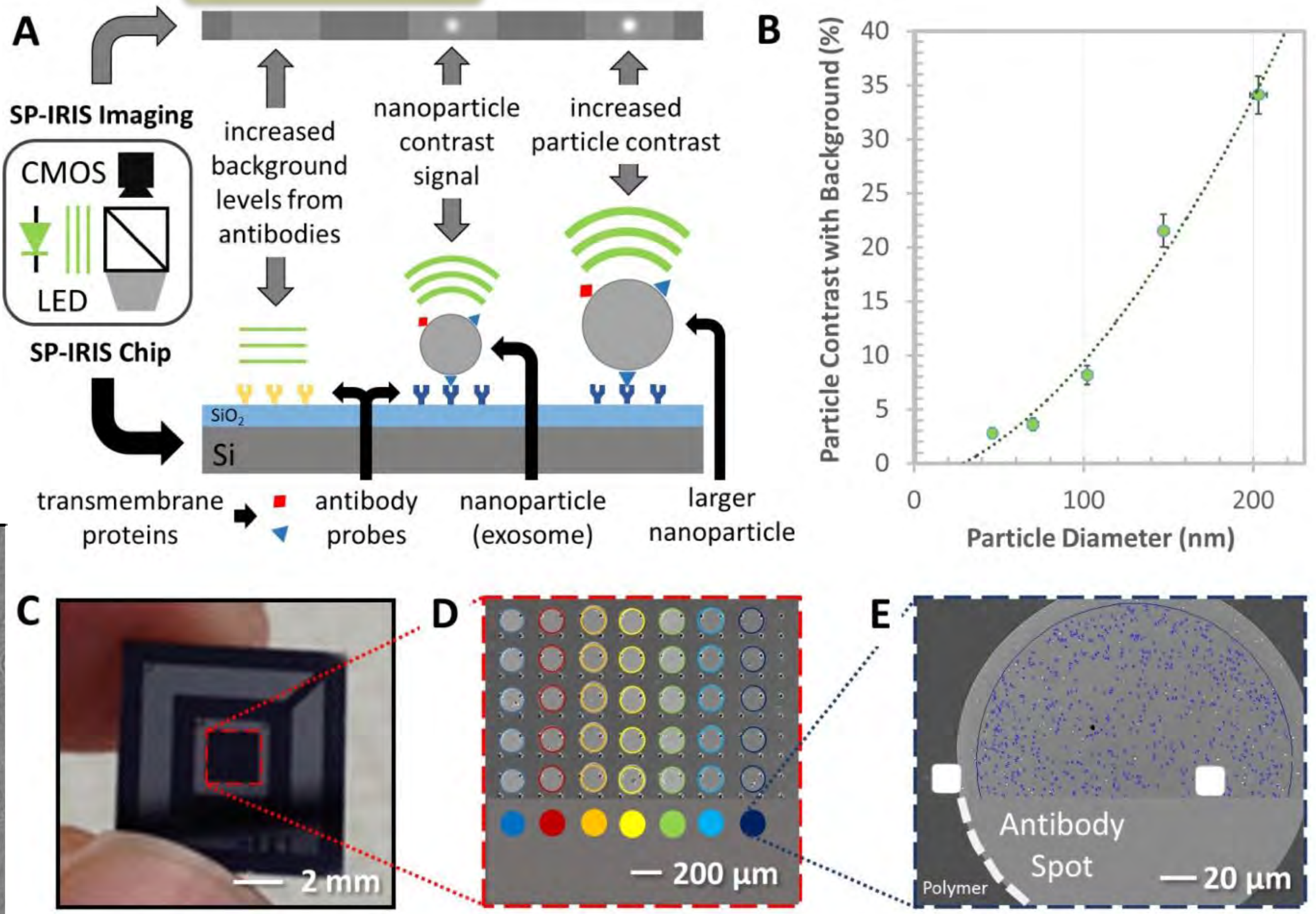
Simple Particle Detection

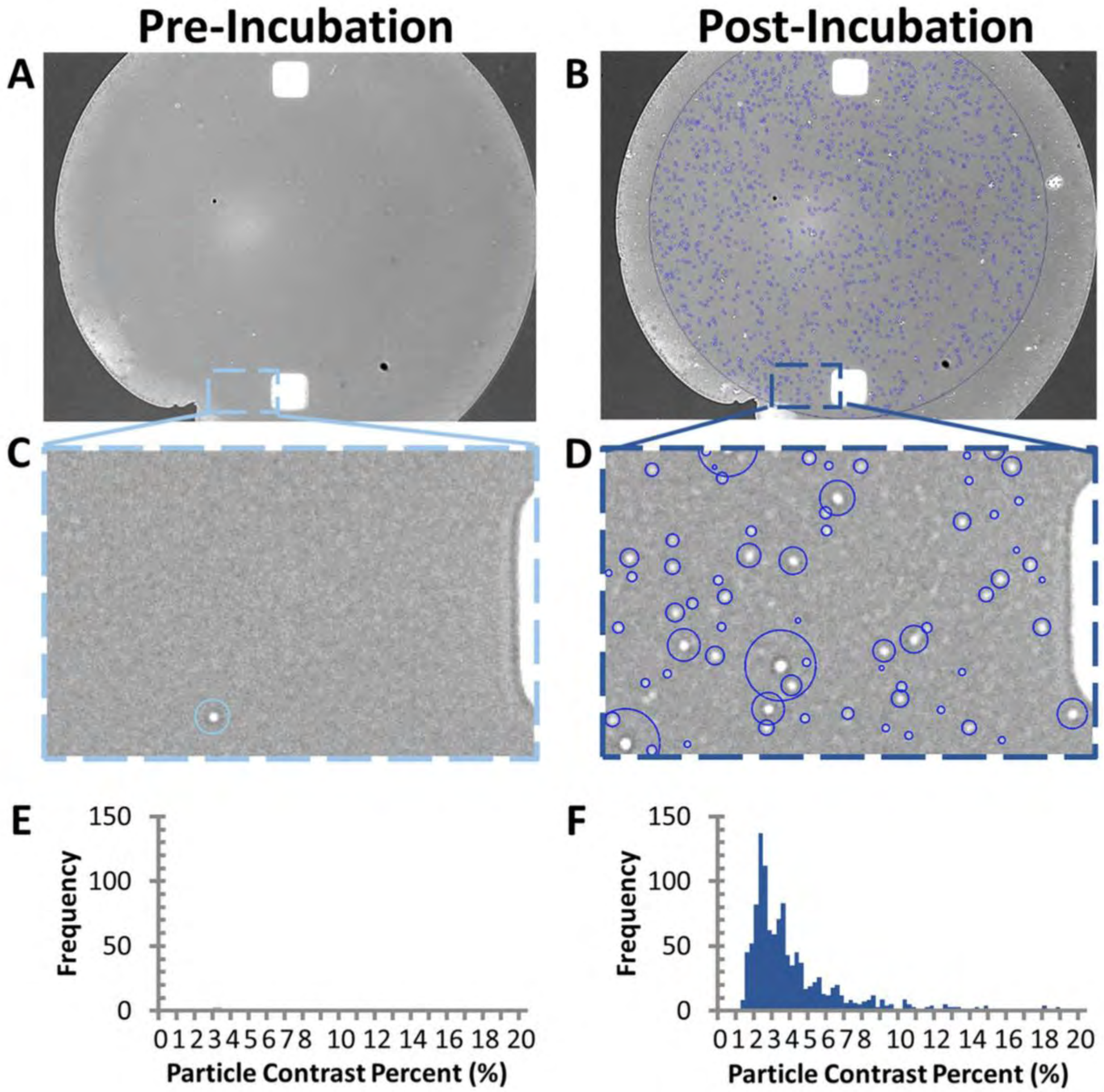
Rahul Vedula(MD) and George Daaboul, PhD '13











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

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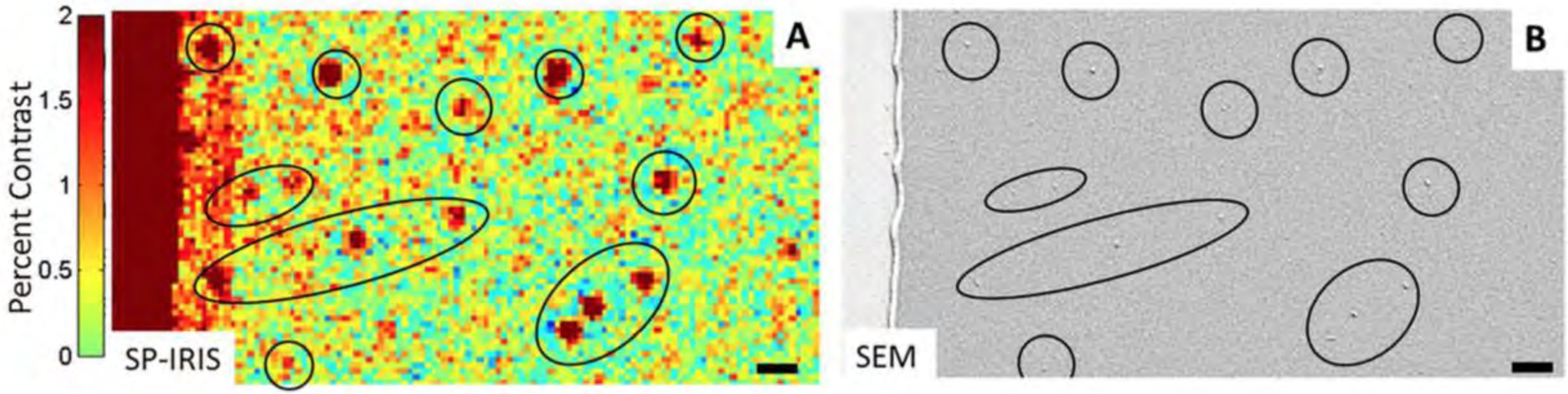
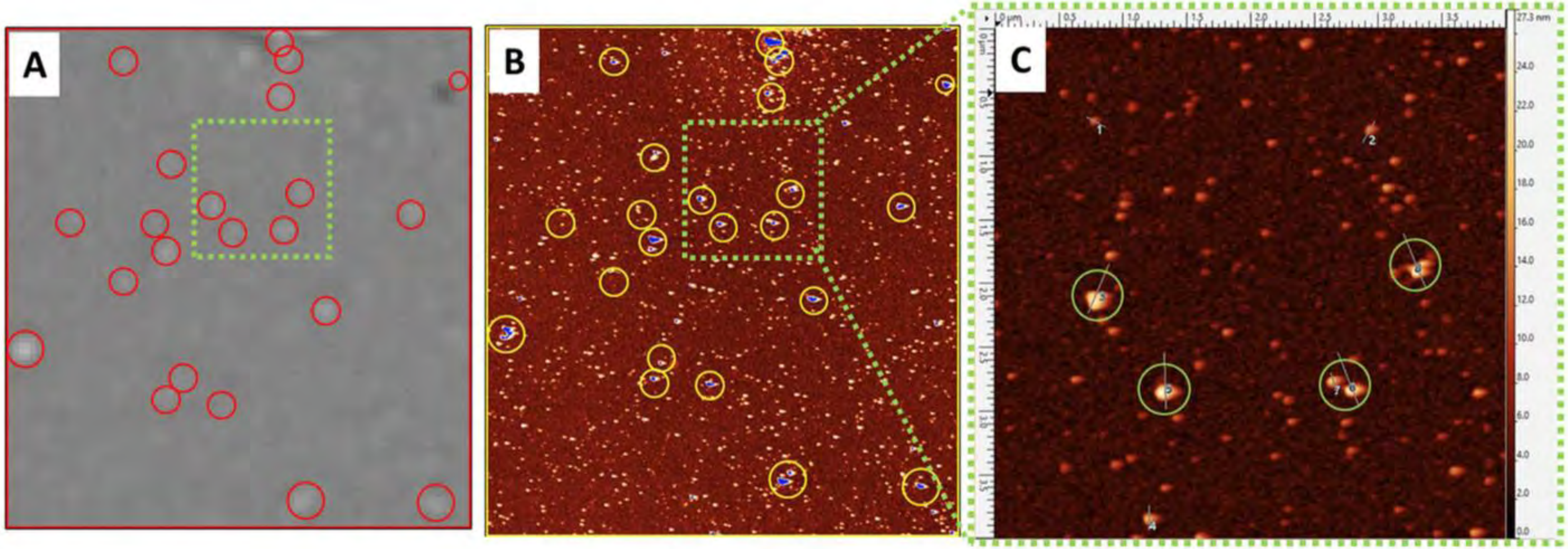
Verification by SEM and AFM – down to r=30nm dry





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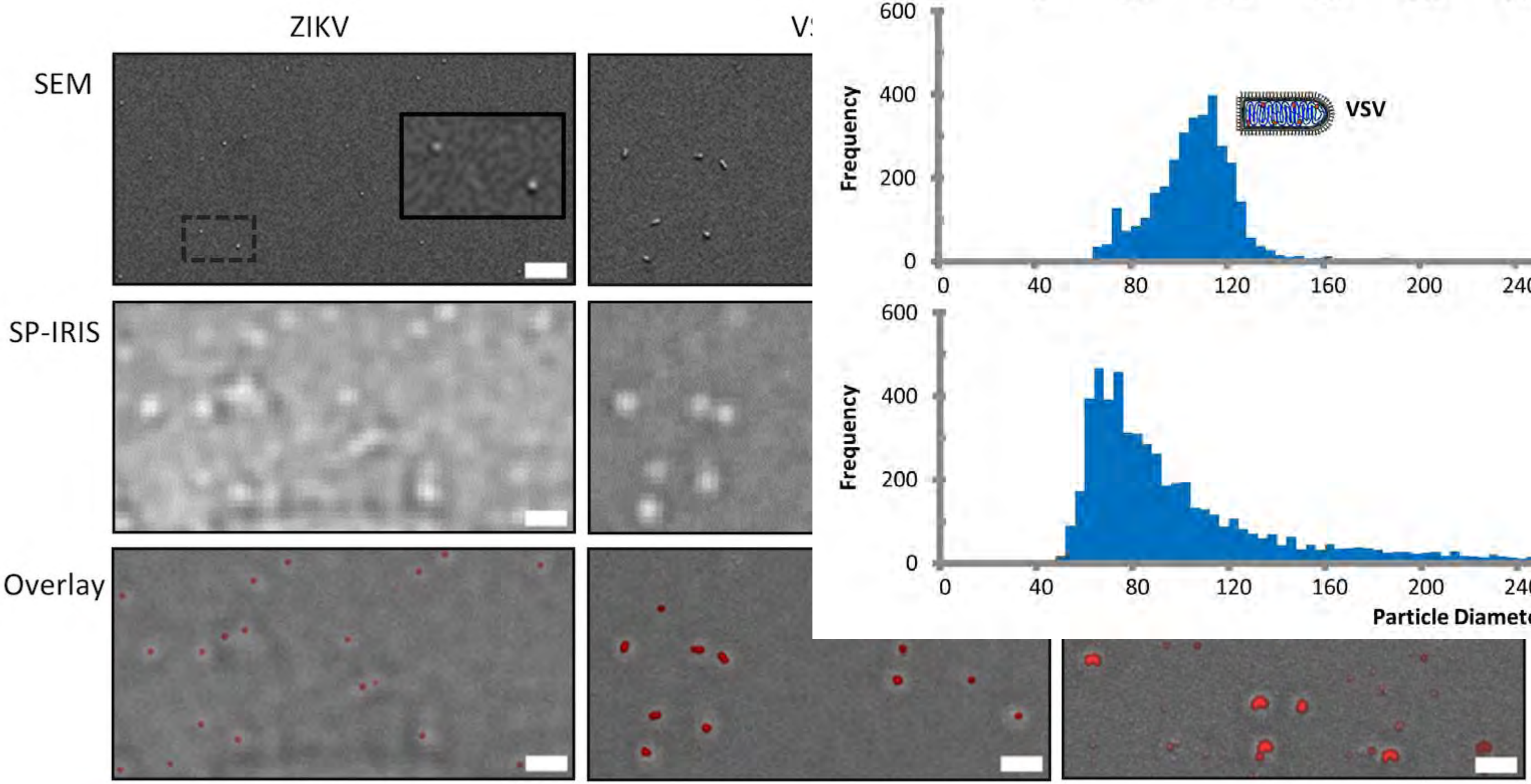
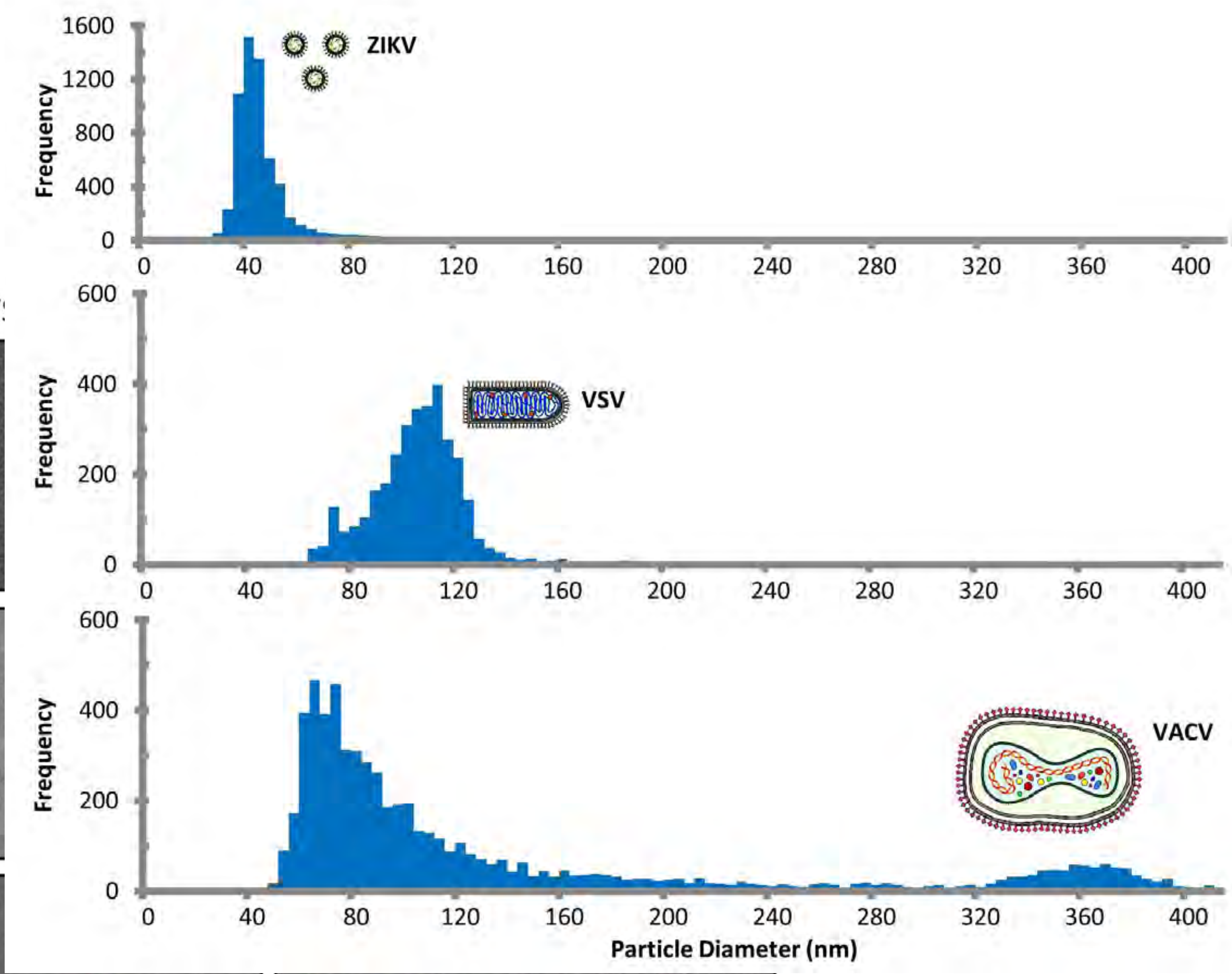
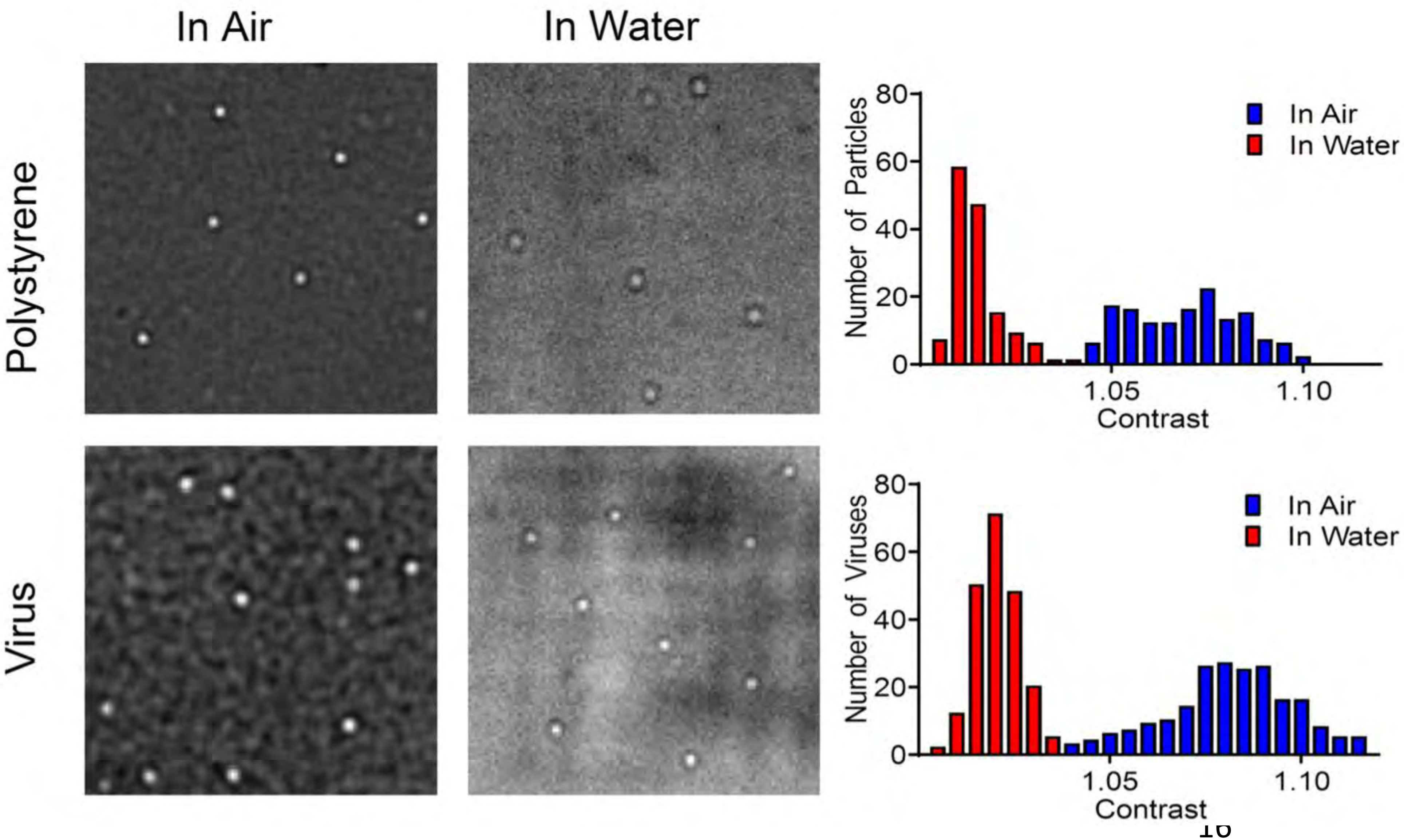
Various viruses



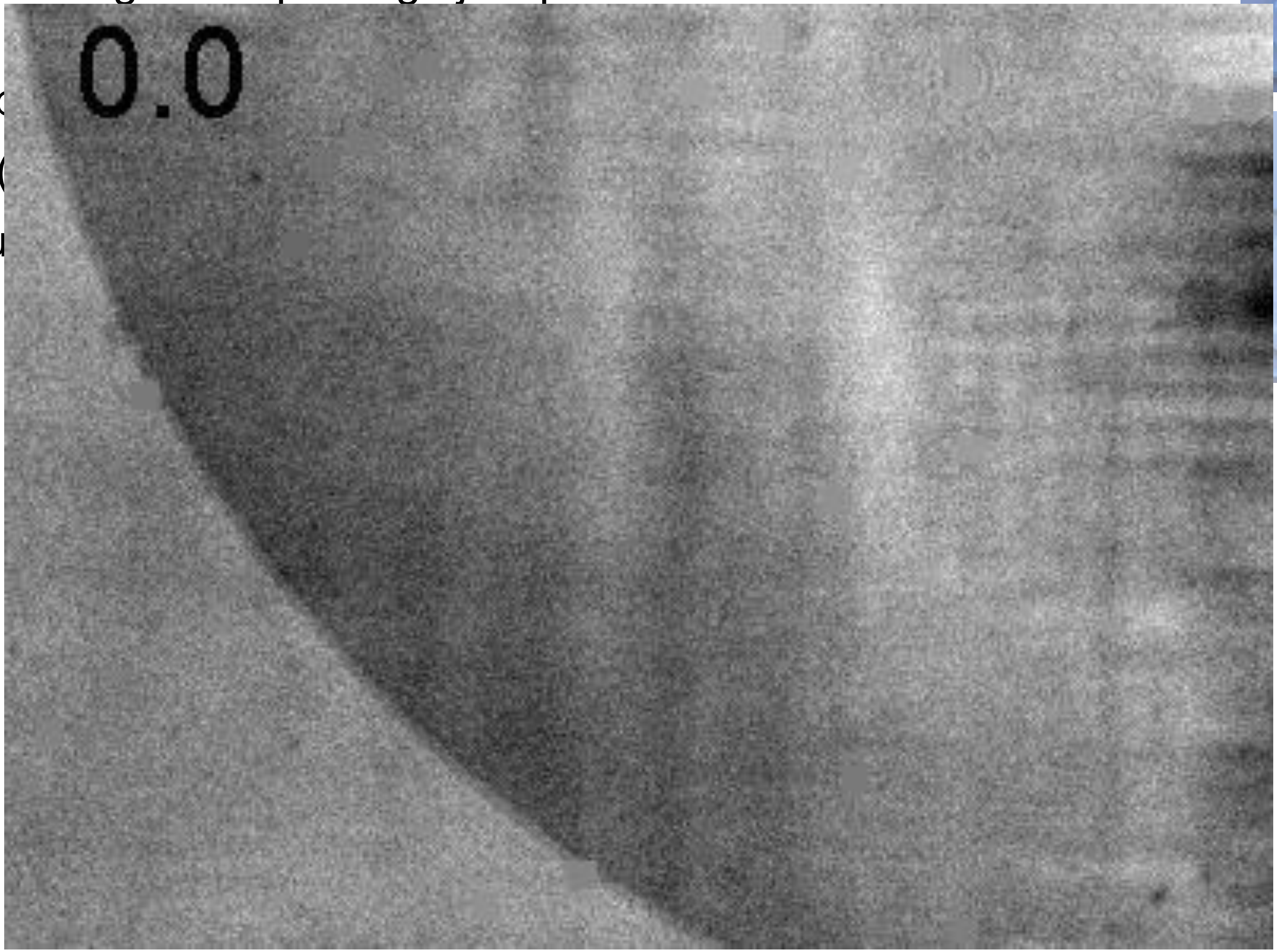


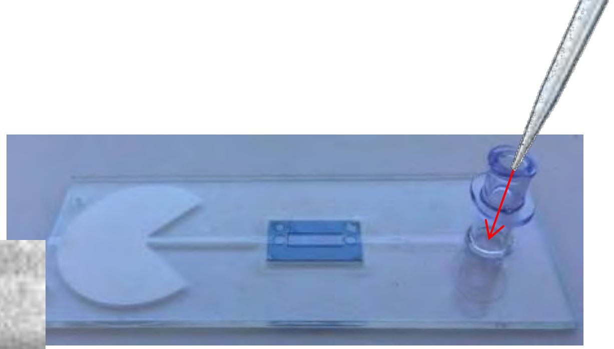
In-liquid detection to simplify the assay

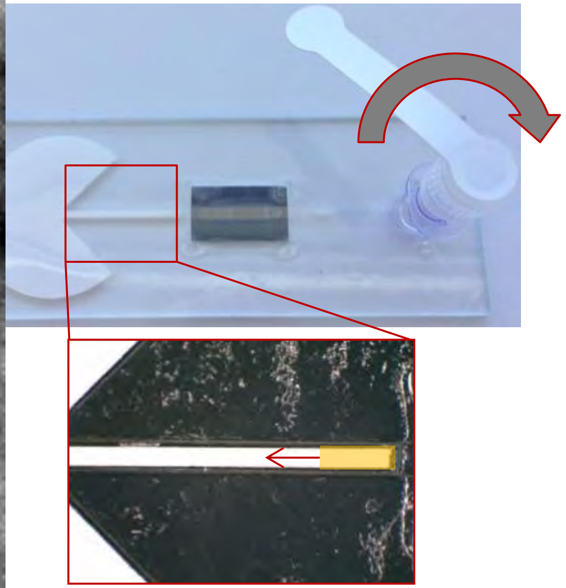


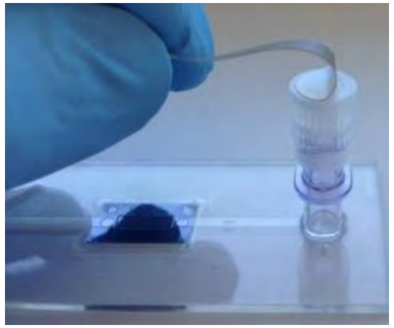
Passive Cartridge - Simple Workflow

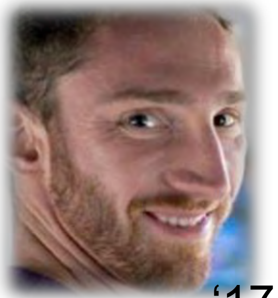
1. Remove cartridge from package just prior to use
2. 100 uL of
   needs to b
3. Luer cap (
4. When liqu
   removed)
5. Cartridge











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Lab on a Chip

PAPER

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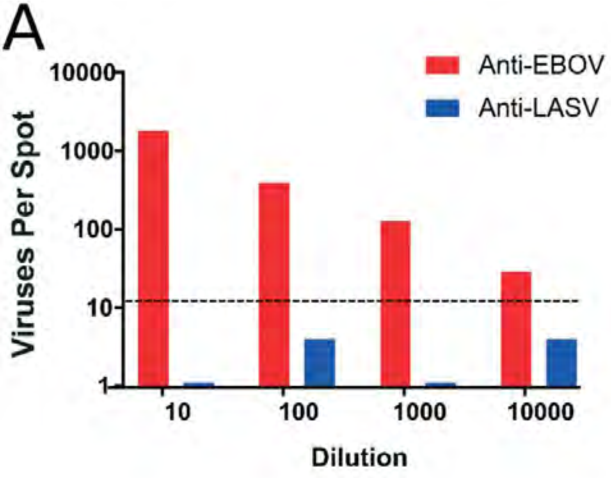
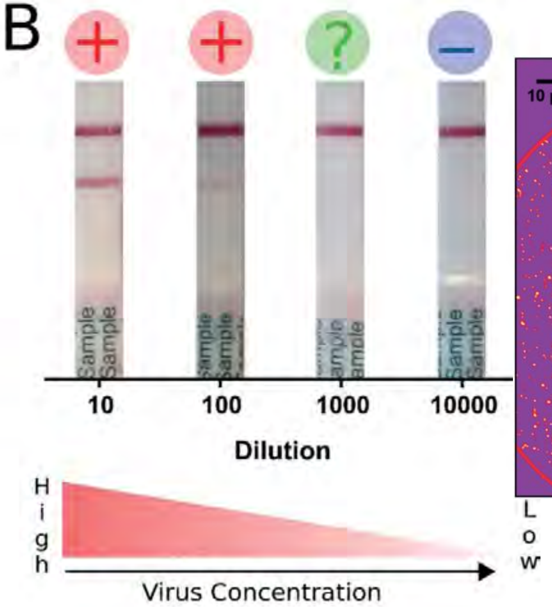
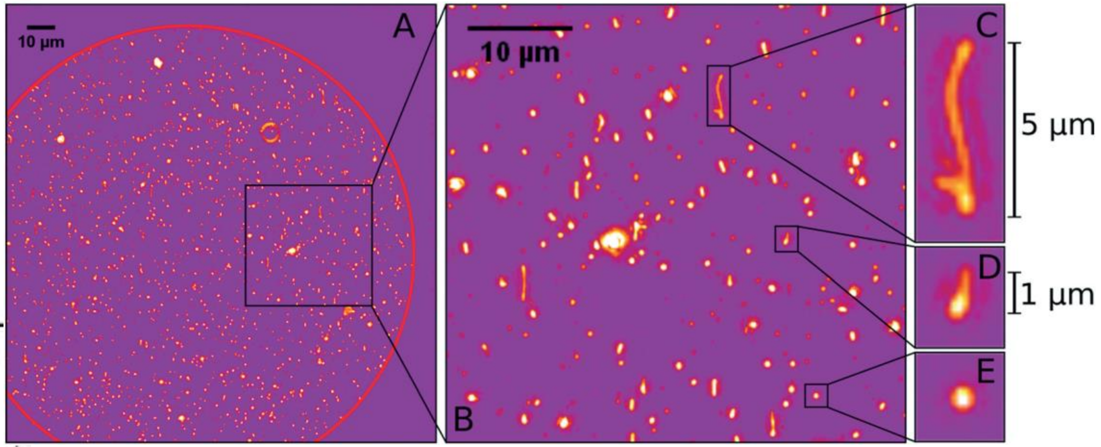
Cite this: DOI: 10.1039/c6lc01528j

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	-

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Outline – Going Beyond Detection and Sizing

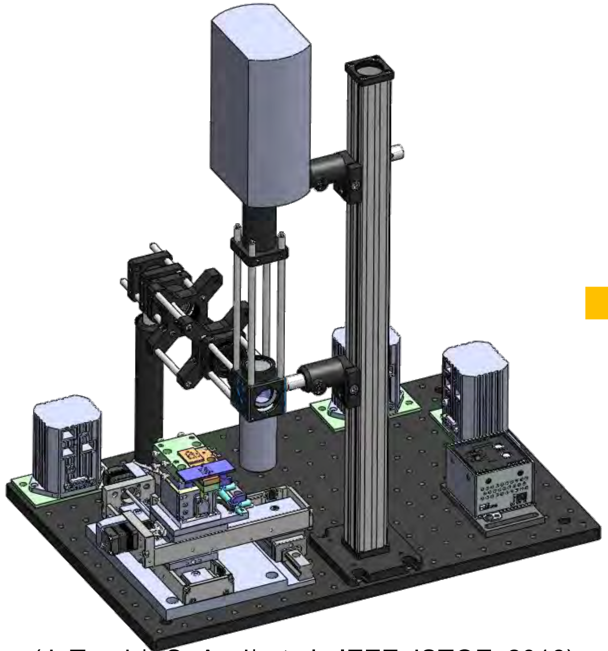
- Motivation – Biological Nanoparticles everywhere
- Problem definition – contrast and size
- Detection vs. visualization
- Interferometric Reflectance **Imaging** Sensor
- Biological Nanoparticle Detection and Sizing
- Pupil function engineering
- Resolution improvement by oblique illumination and reconstruction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

Reconstruction in Interference Microscopy

imaging system

object

in



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

out

observation

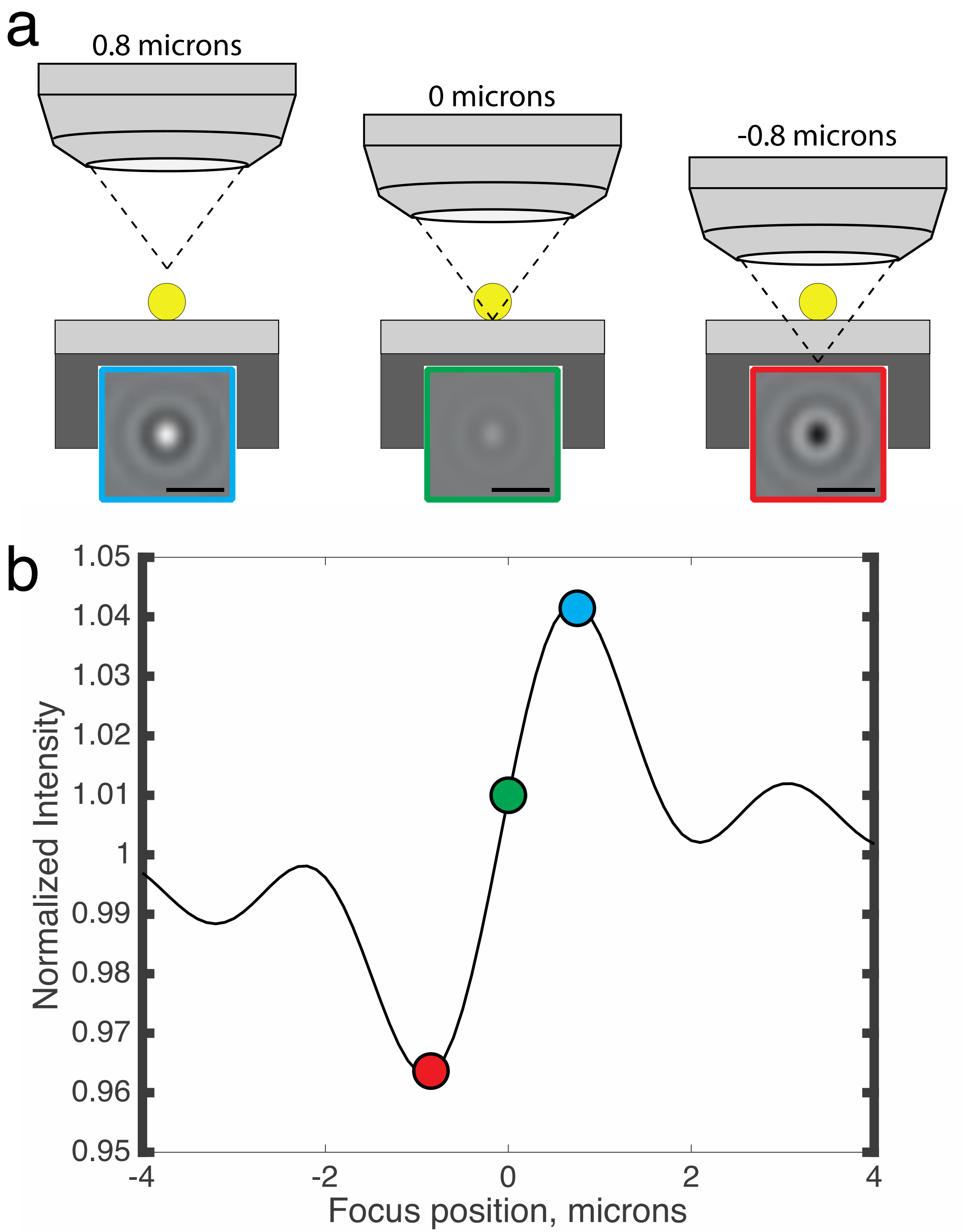
$$y = Ax + e$$

observation ← y

system response / convolution matrix ← A

object ← x

noise ← e



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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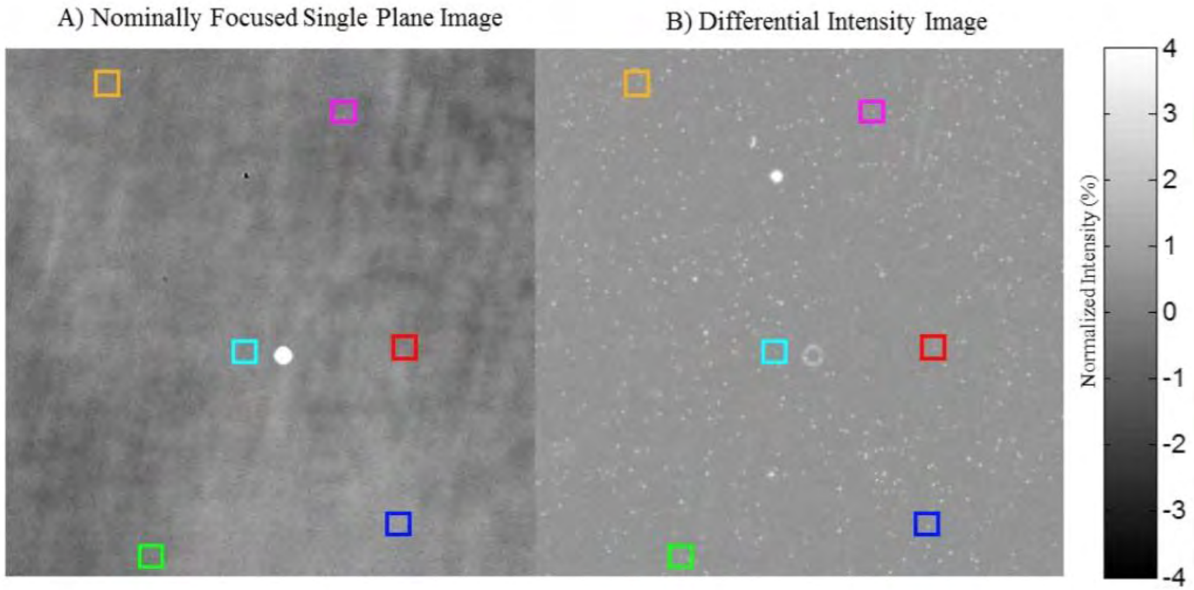
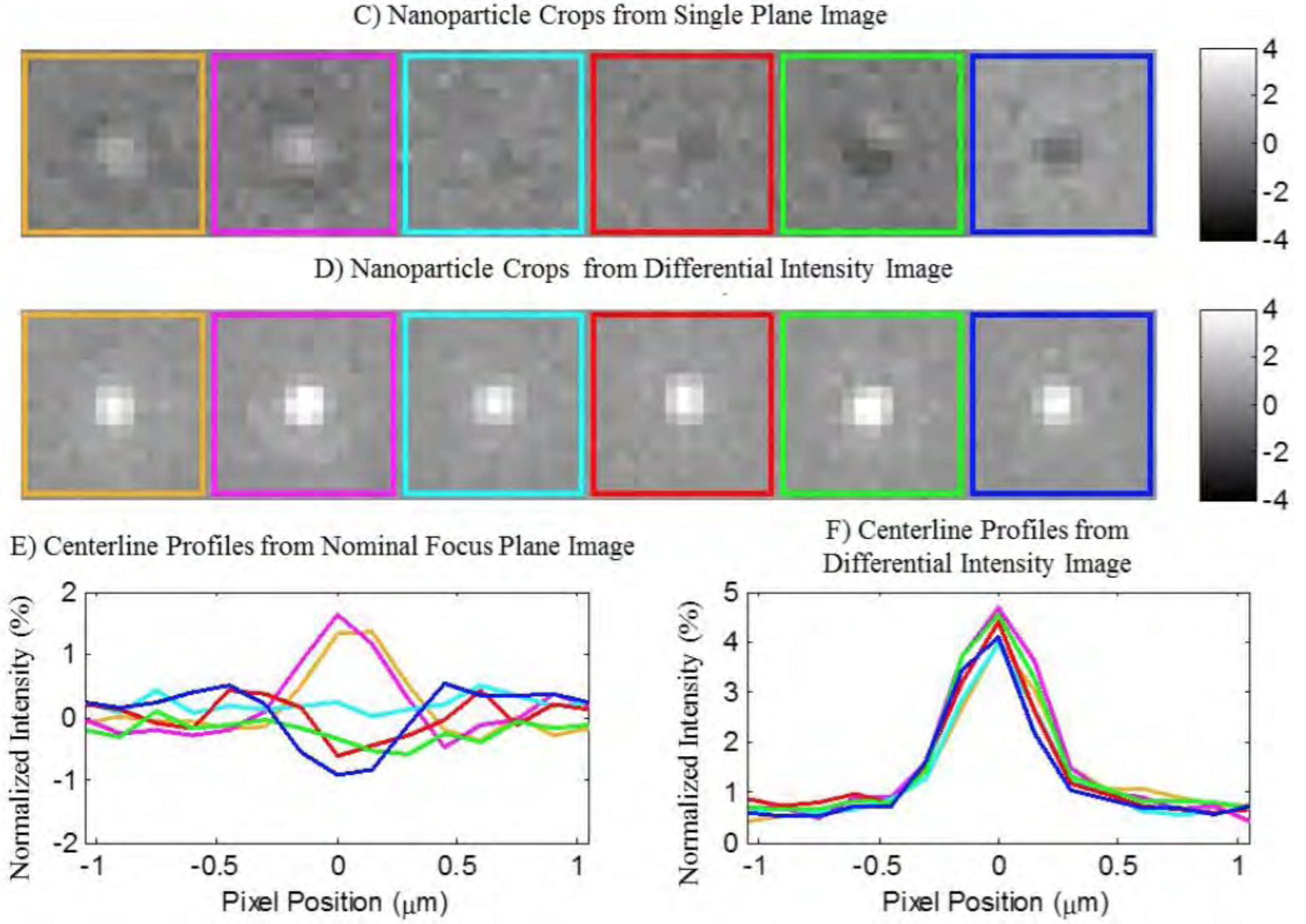
Robust Visualization and Discrimination of Nanoparticles by Interferometric Imaging

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





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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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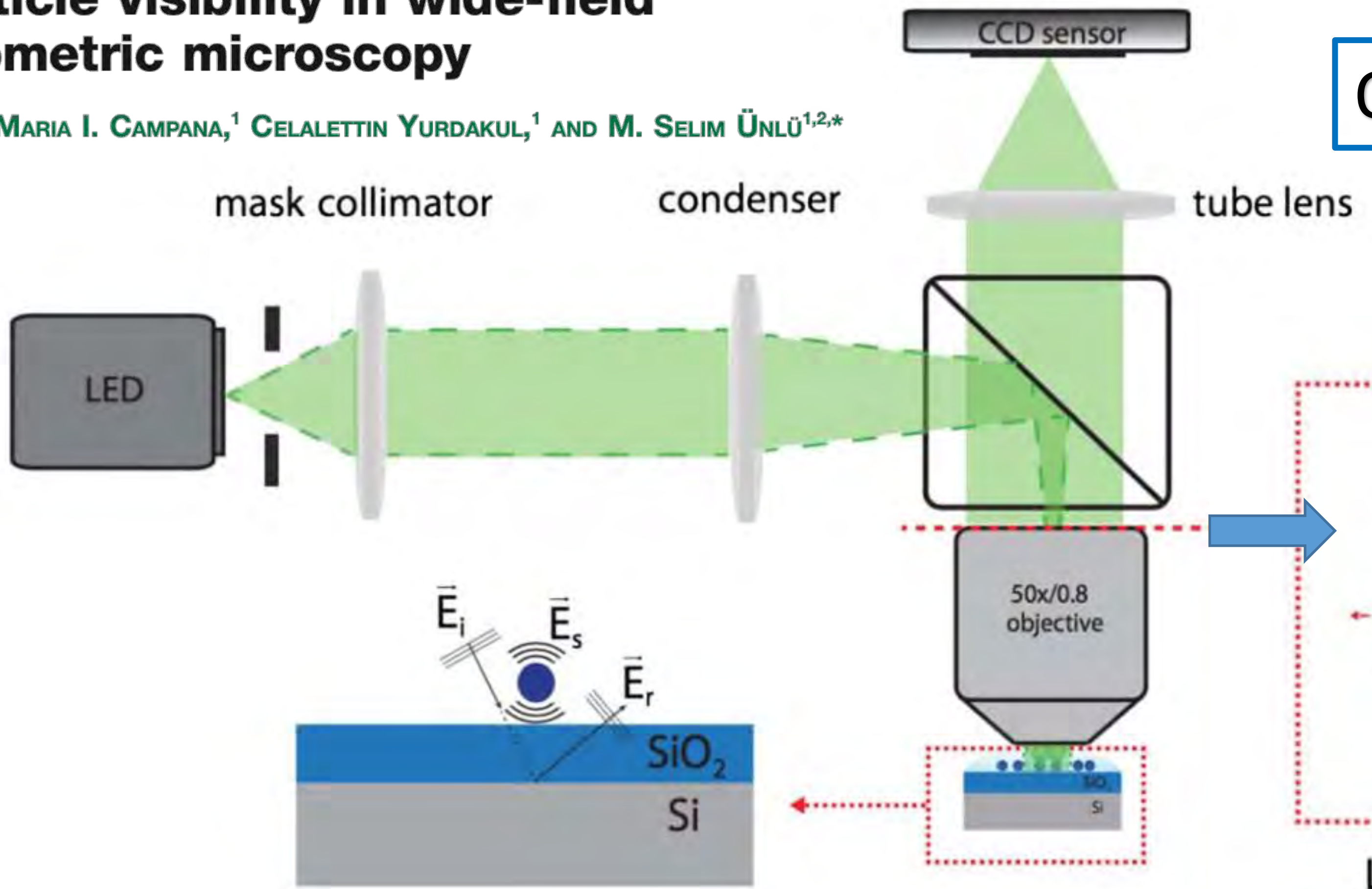
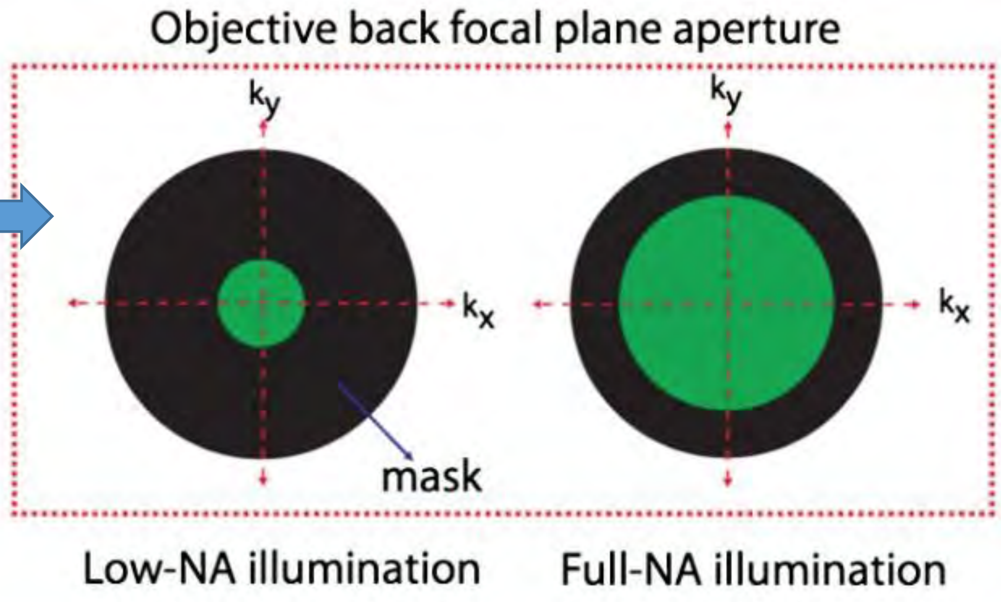
Overall of 10X enhancement





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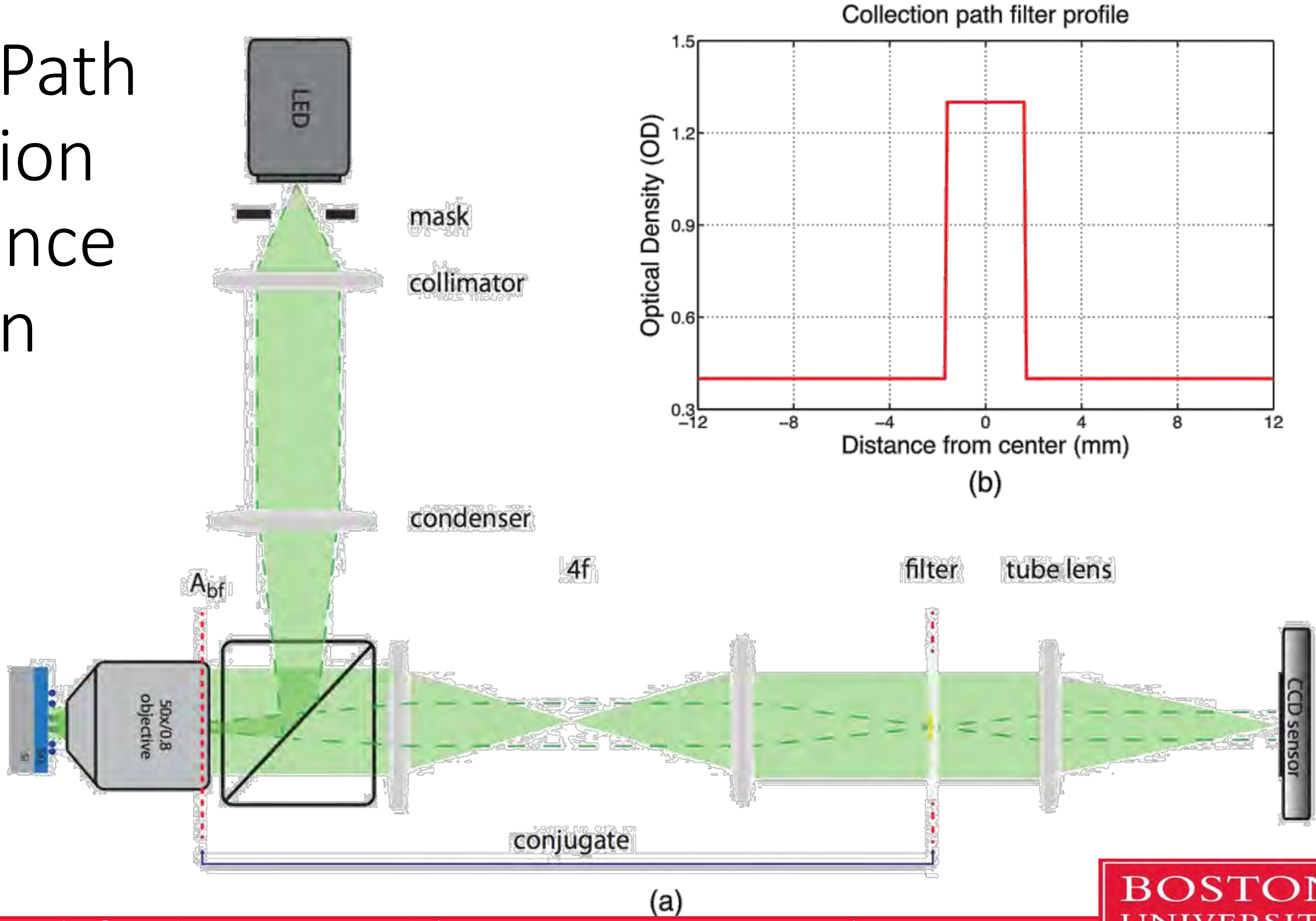
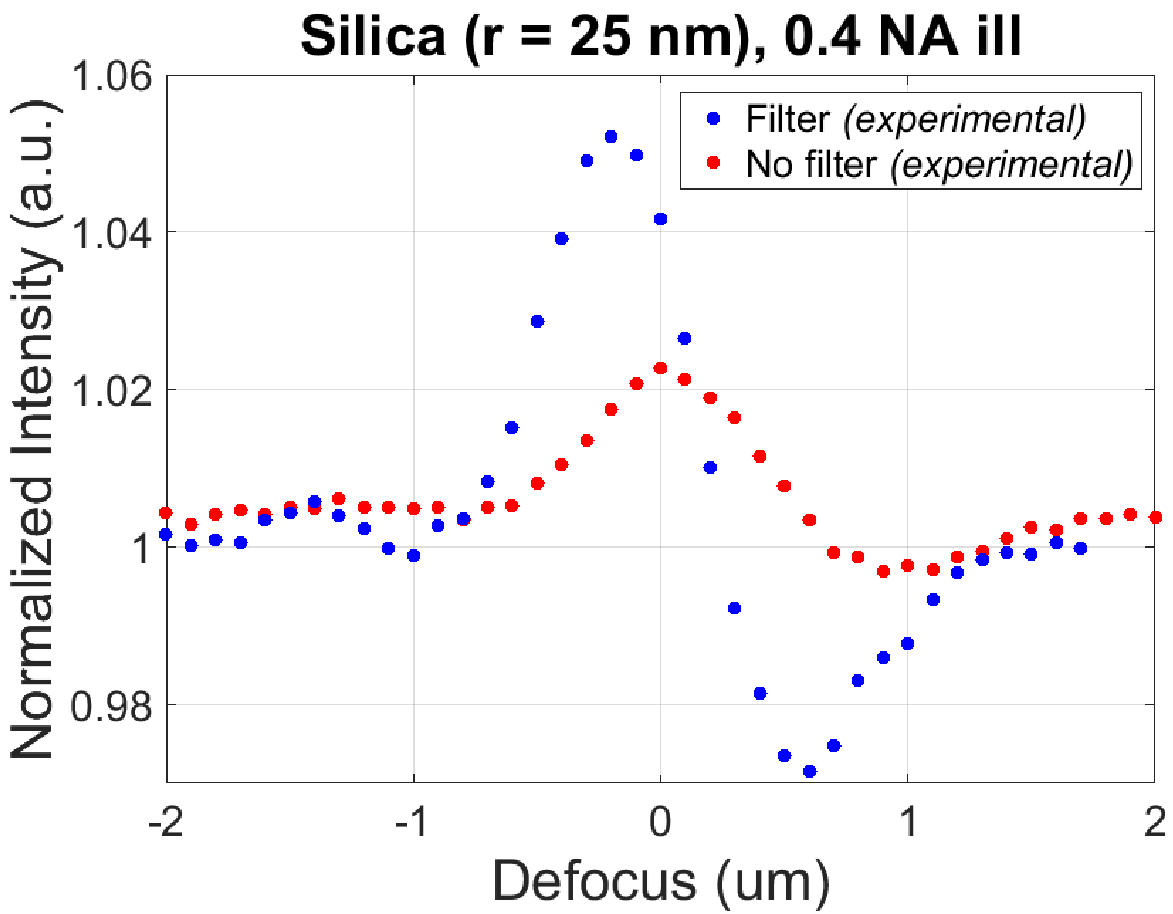
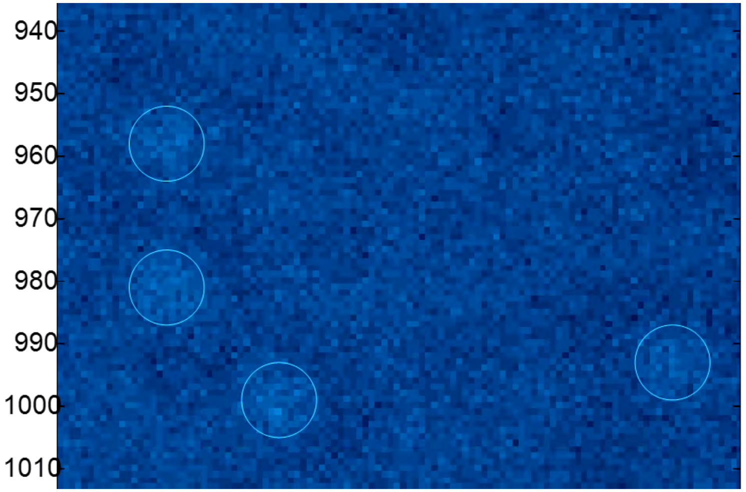
Collection Path – Apodization and Reference Attenuation

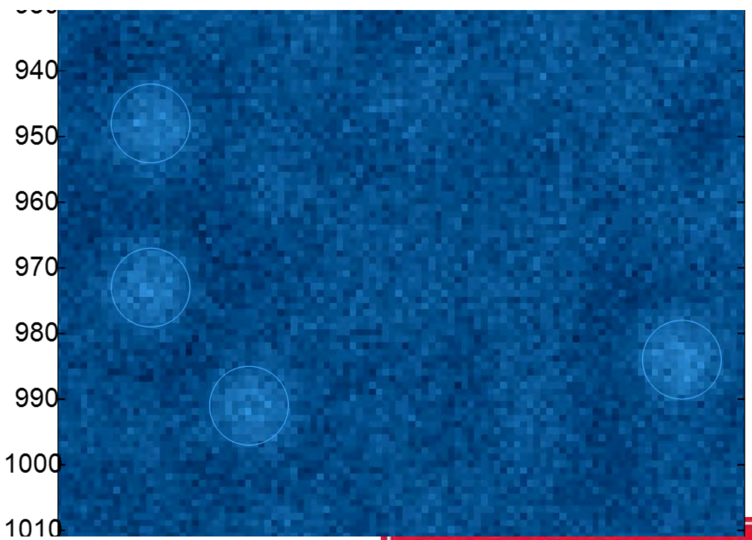


Registered silica particles defocus curve

~4X enhancement (2.2% → 8%)







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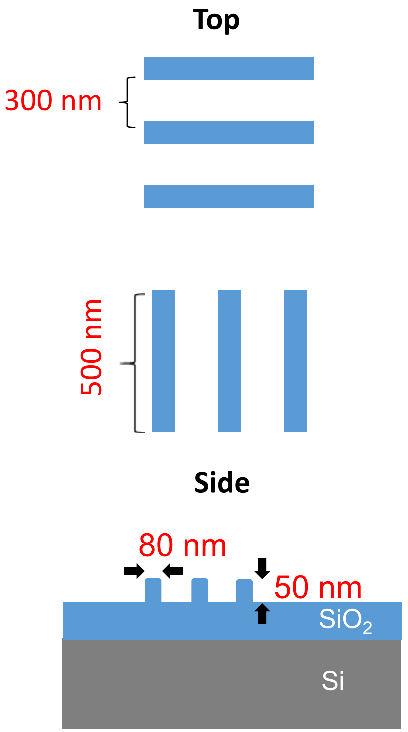
Reconstruction – first with defocus

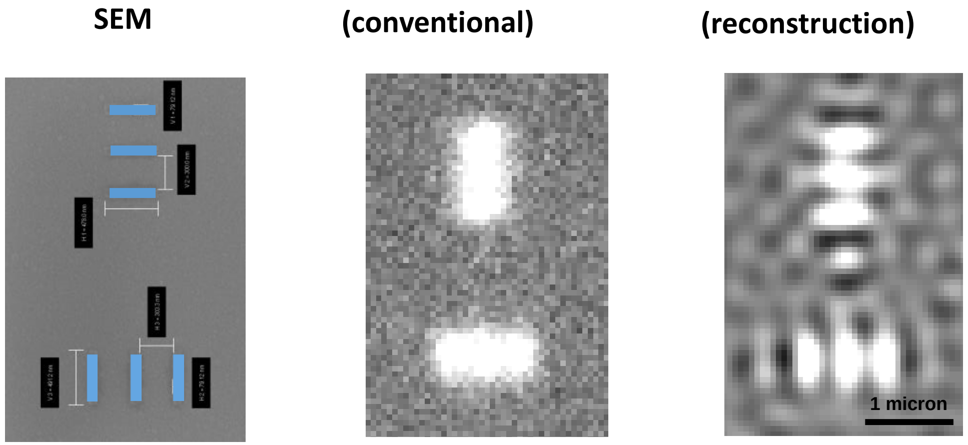
- **Tikhonov regularization:** Least-squares cost function with quadratic side-constraint

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

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

- **Defocus based reconstruction**





50x/0.8NA 525nm

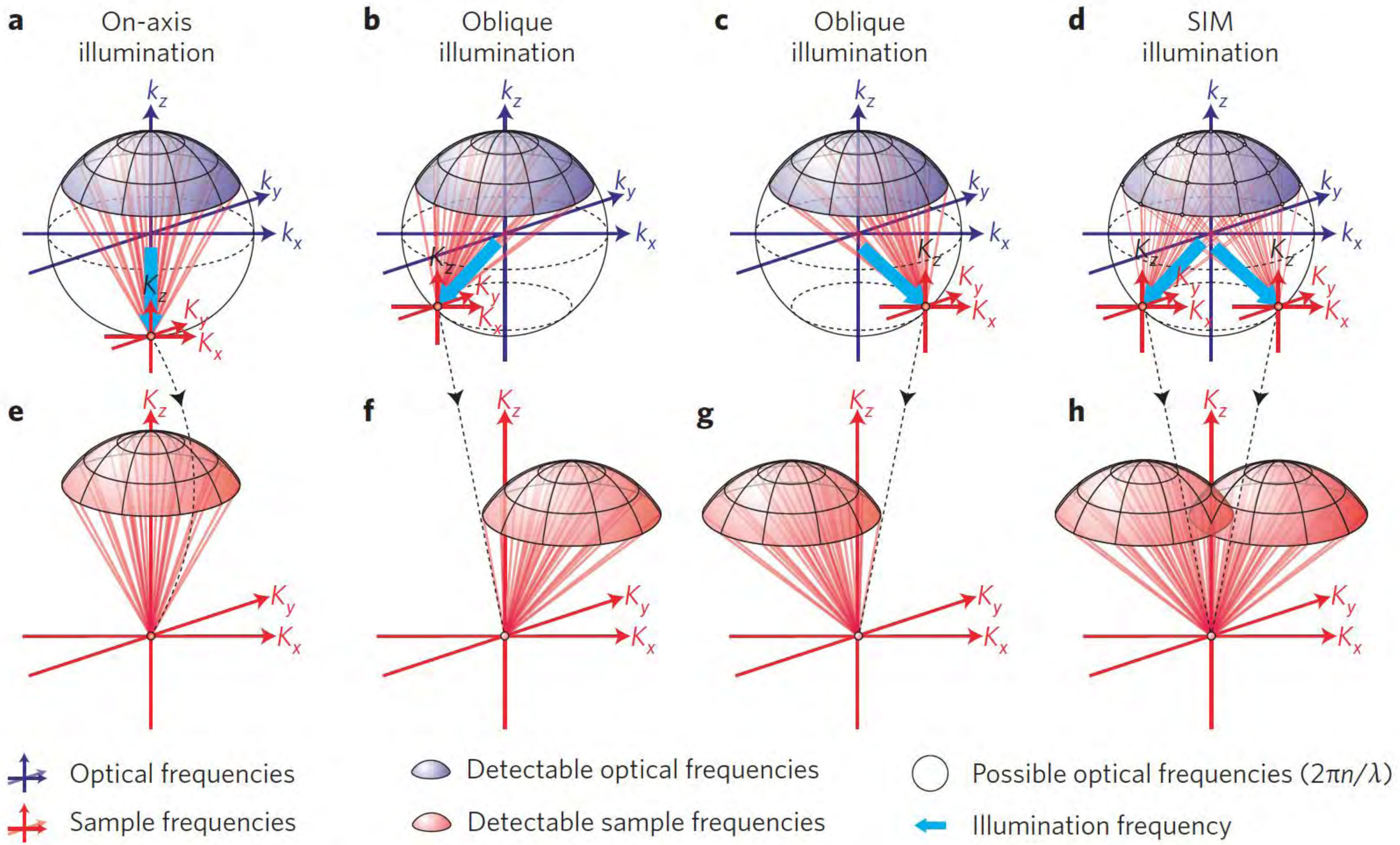
Reconstruction – Structured Illumination (?)

Resolving a misconception about structured illumination

Kai Wicker and Rainer Heintzmann

Applying structured illumination microscopy to coherent imaging modalities such as scattering does not yield any additional information beyond that provided by oblique illumination. It thus yields no resolution enhancement over the Abbe diffraction limit, which was derived precisely for that case.

NATURE PHOTONICS | VOL 8 | MAY 2014 |



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Super-resolution in wide-field interferometric microscopy

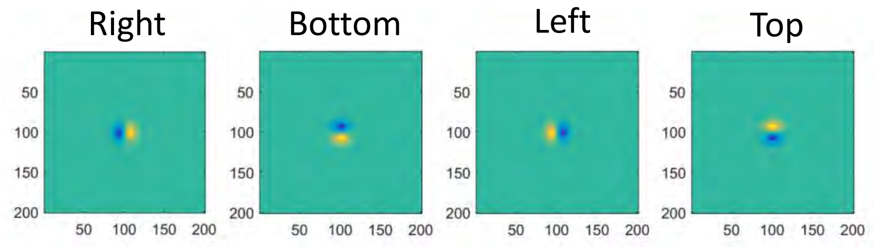
- ***Enhancing low-index nanoparticle resolution via reconstruction schemes***

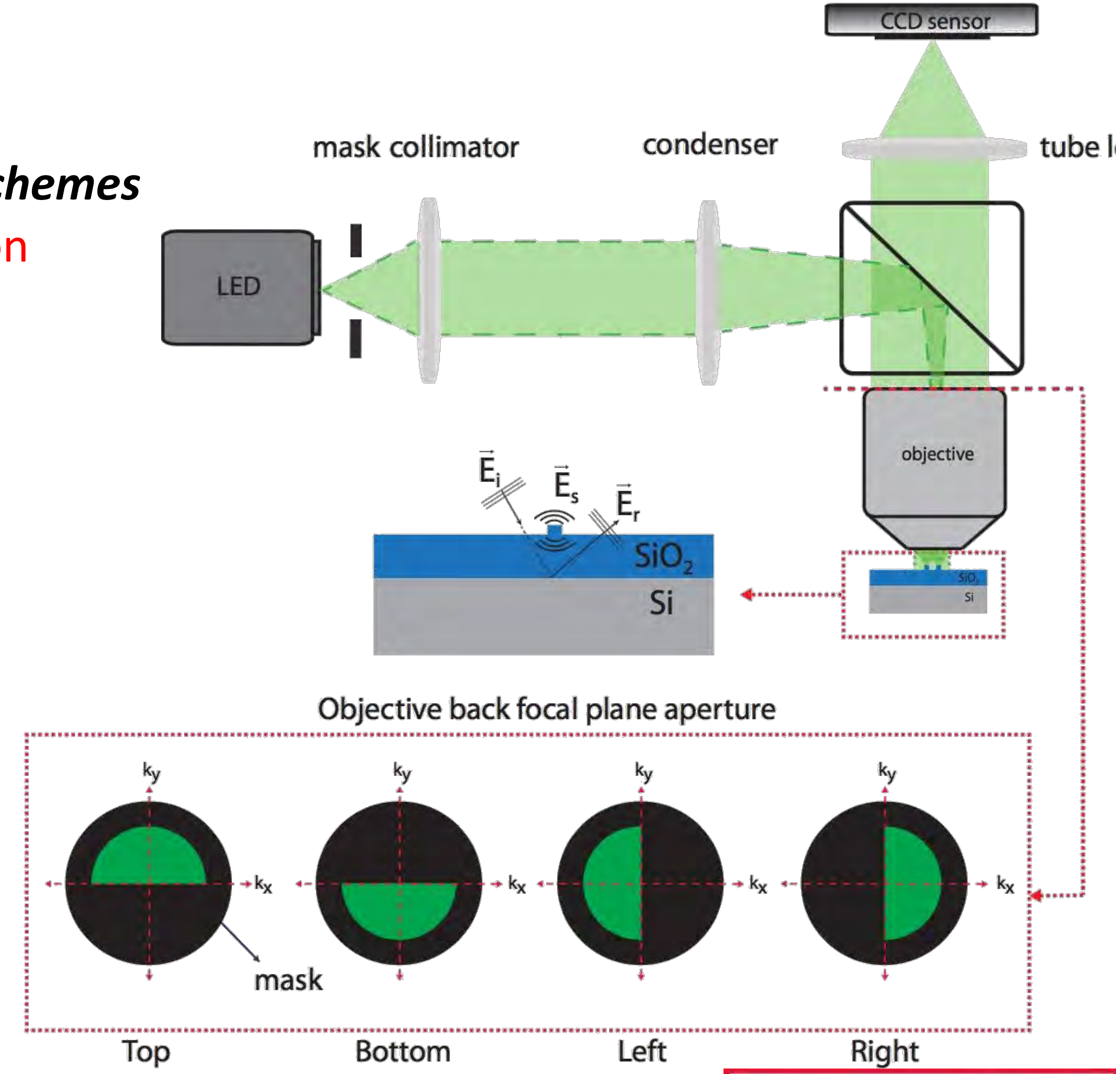
  Asymmetric illumination based reconstruction for super resolution (with Lei Tian)

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

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

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





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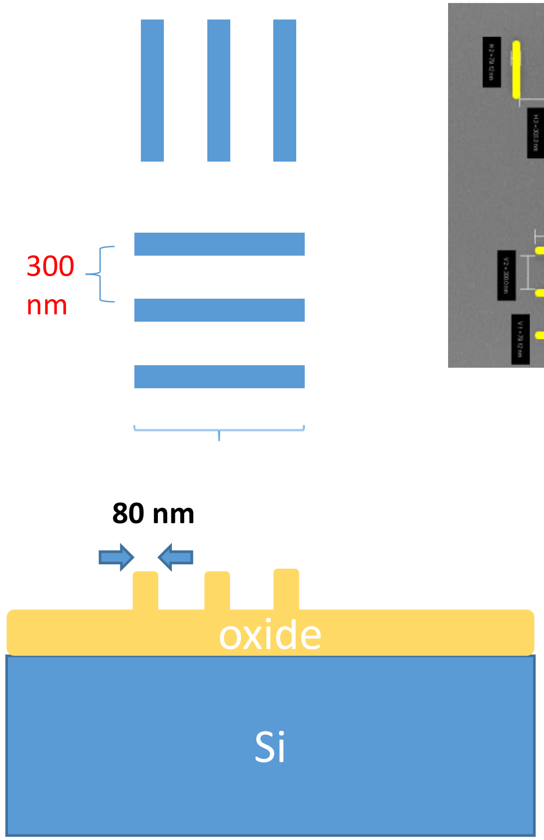
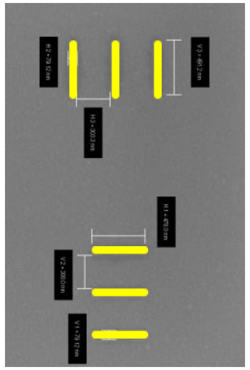
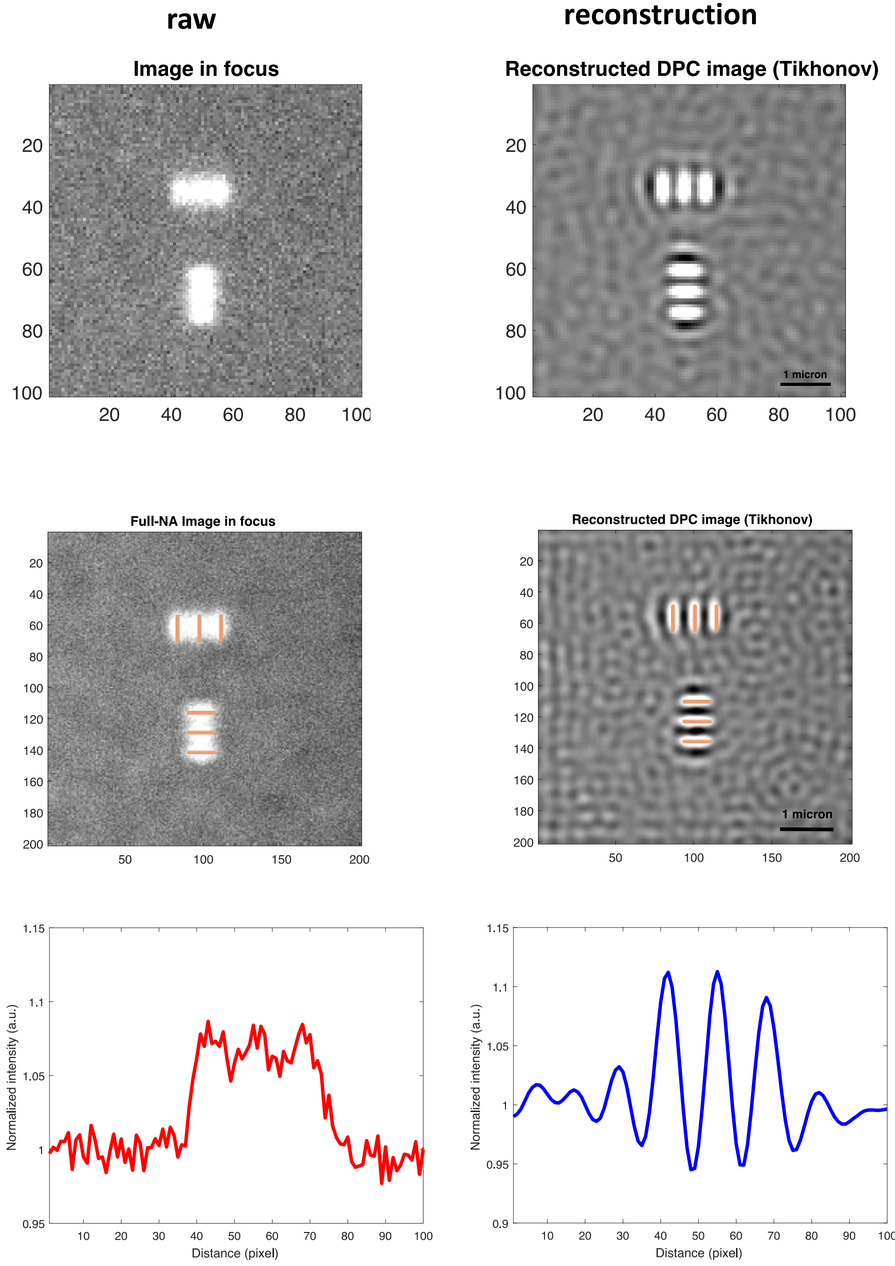
Experimental Results

Sample – E-beam fabricated





SEM



50x/0.8NA 525nm

100x/0.9NA 525nm

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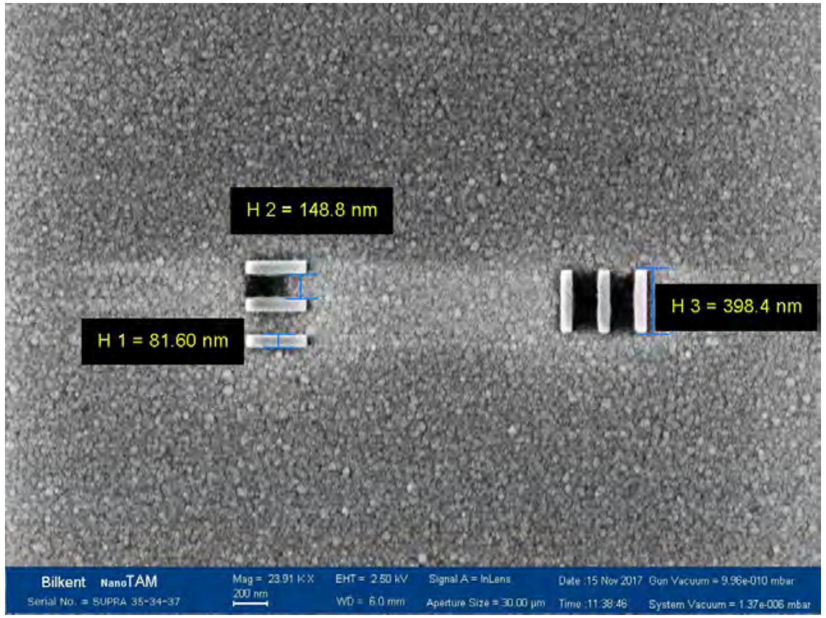
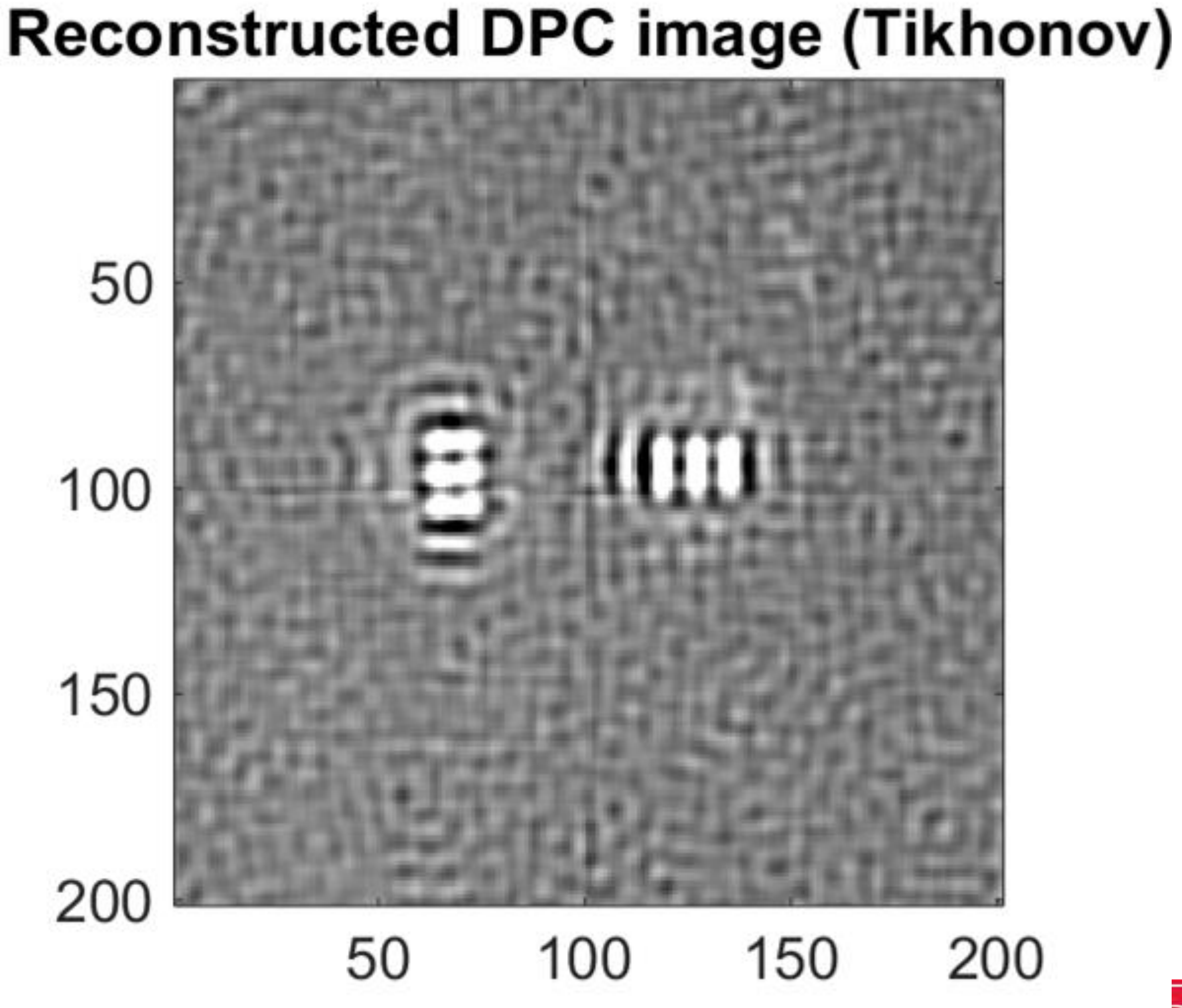
150 nm separation, 0.9 NA, **λ**=420nm





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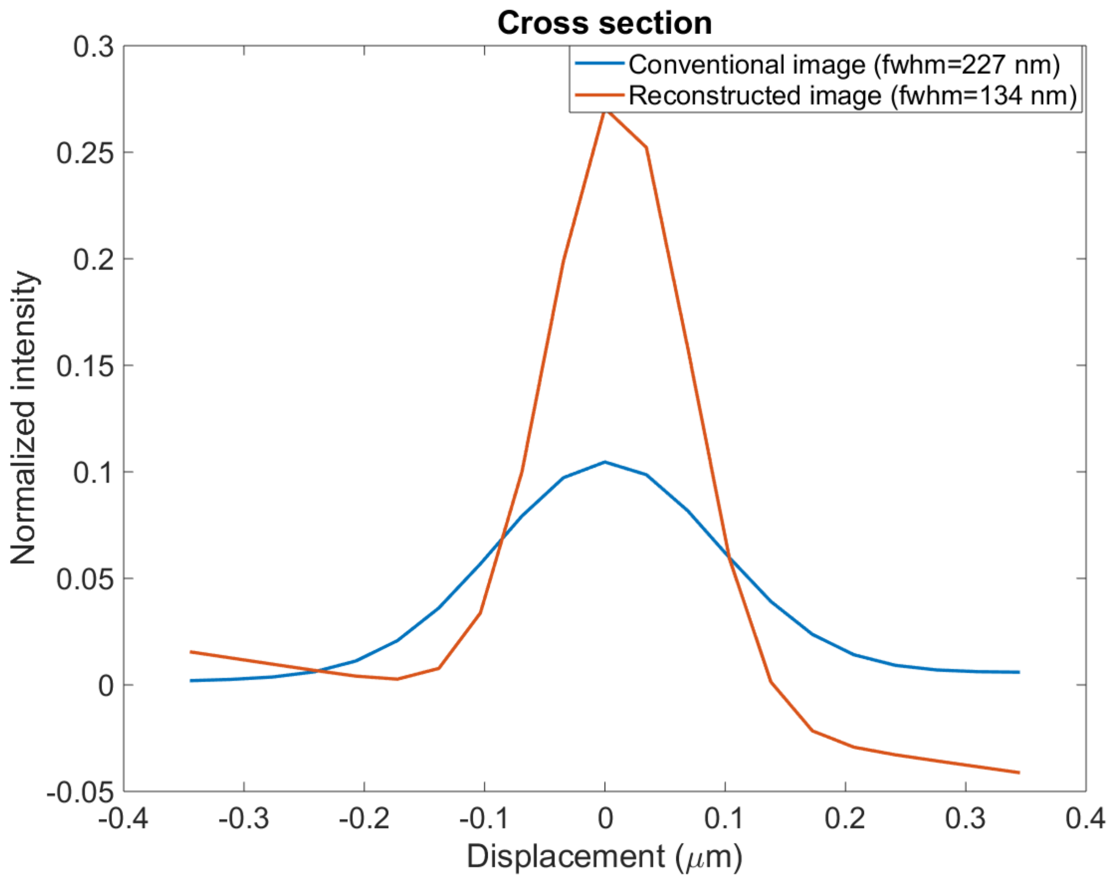
FWHM ~ 130nm < (λ / 3)



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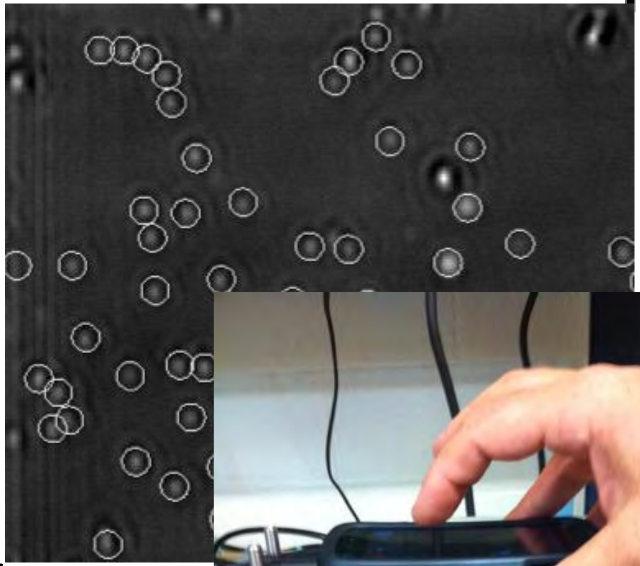
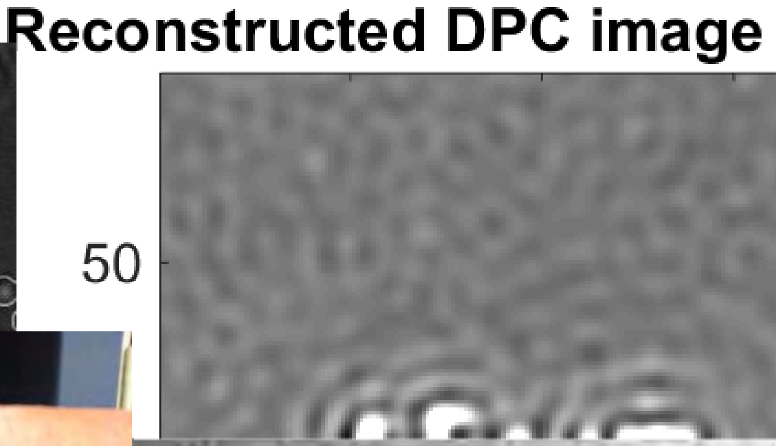
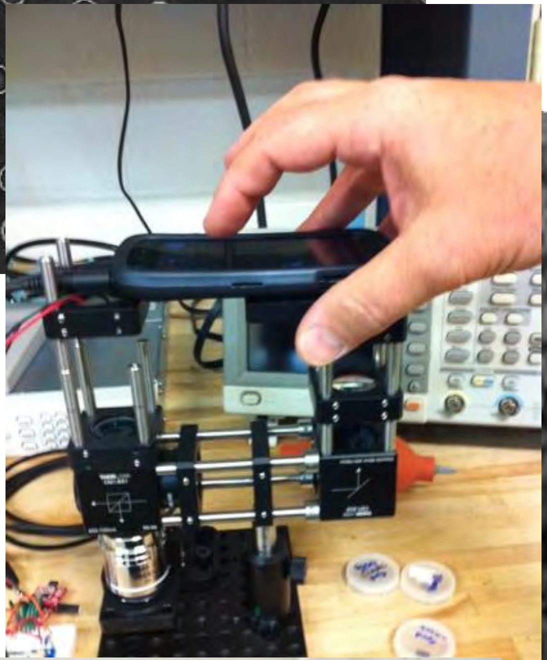
CONCLUSIONS & FUTURE

- Optical interference is a very powerful sensing technique.
- Multi-disciplinary innovation
- Single biological nanoparticle detection / counting / size and shape discrimination / visualization
- **Goals:** 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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