High-throughput, High-resolution Interferometric Light Microscopy of Biological Nanoparticles

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Physics,

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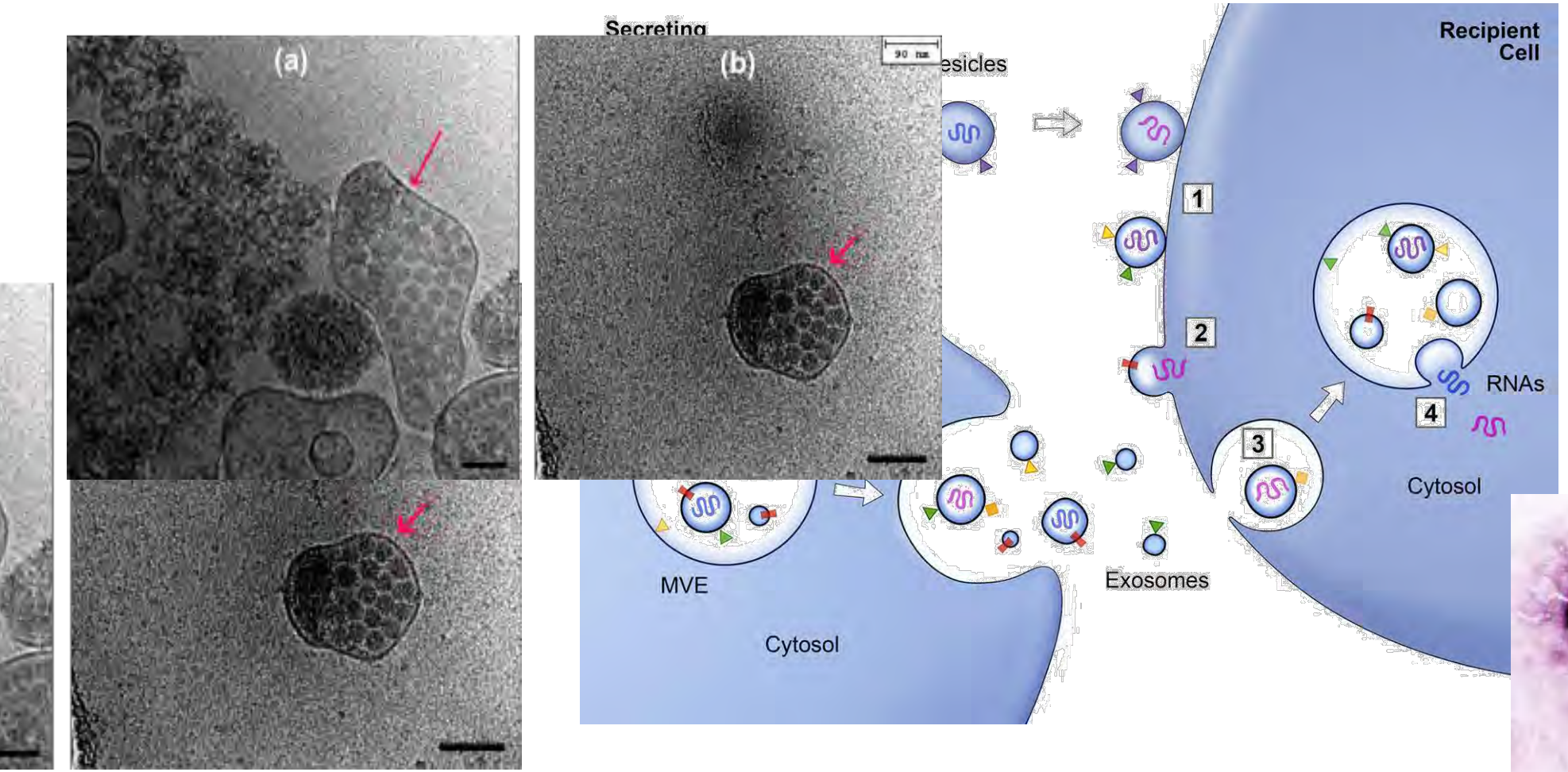
Photonics Center

Yurdakul, Avci, Matlock, Tian

Overview

- Motivation and problem identification
- Single particle interferometric reflectance (SPIR) microscopy
  - Enhancing sensitivity and Improving lateral resolution
    - Computational imaging
    - Pupil function engineering
- Summary

Extra cellular vesicles, exosomes, and viruses



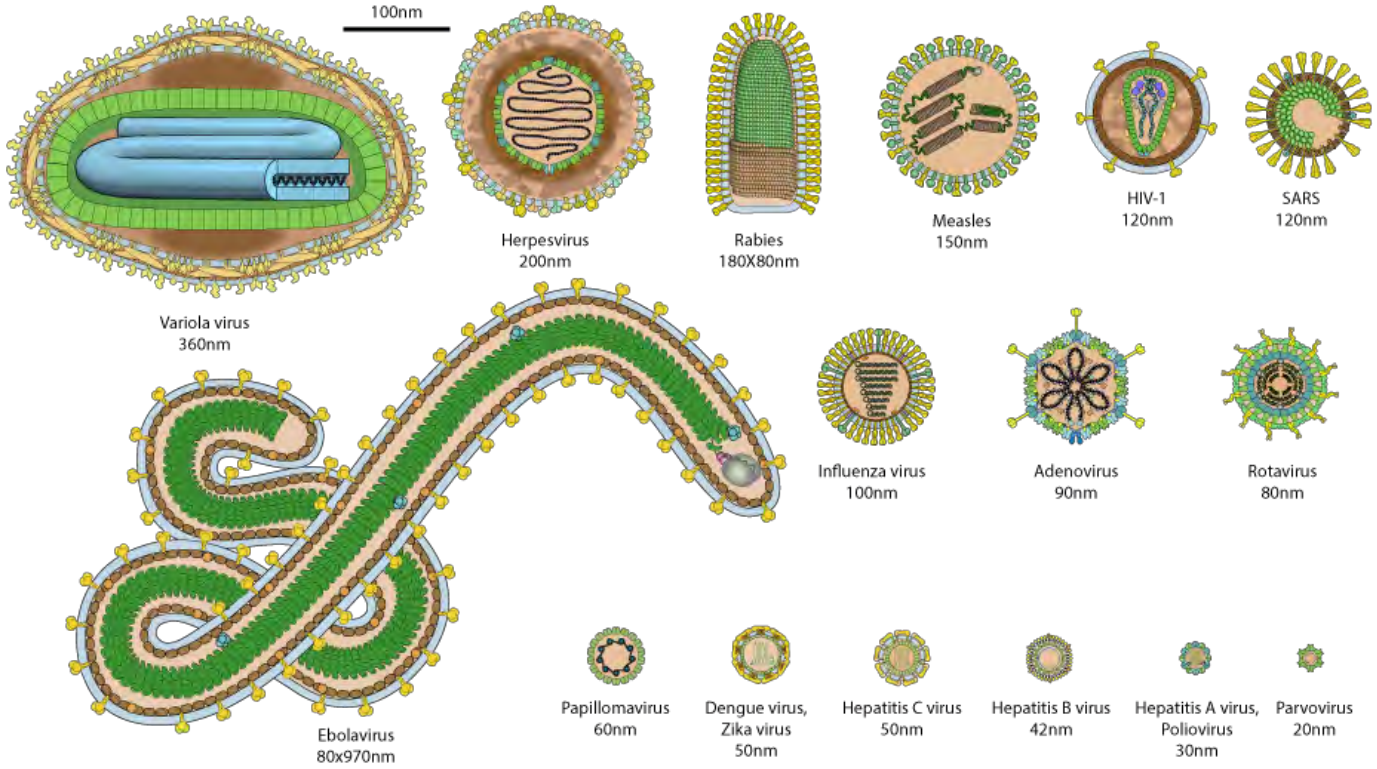
Example cryo-EM images of infectious extracellular vesicle (Bullitt Lab – BU MED)

SEM image of Ebola virion

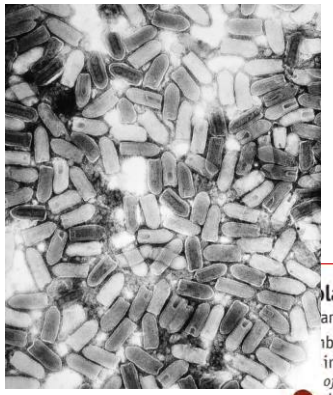
Viruses are the most abundant species on earth.
~10^{32} phages in the biosphere
~10^{7} viruses on average in a mL of seawater

Motivation / Significance

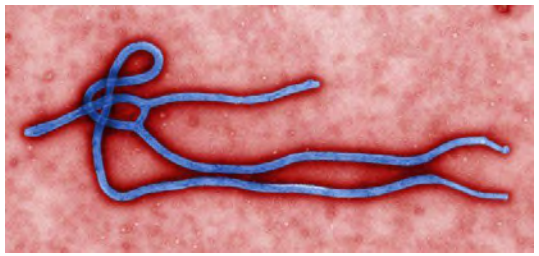
Size, morphology, and chemical composition of biological nanoparticles have strong implications on their functionality including cellular uptake, circulation, and biodistrubition

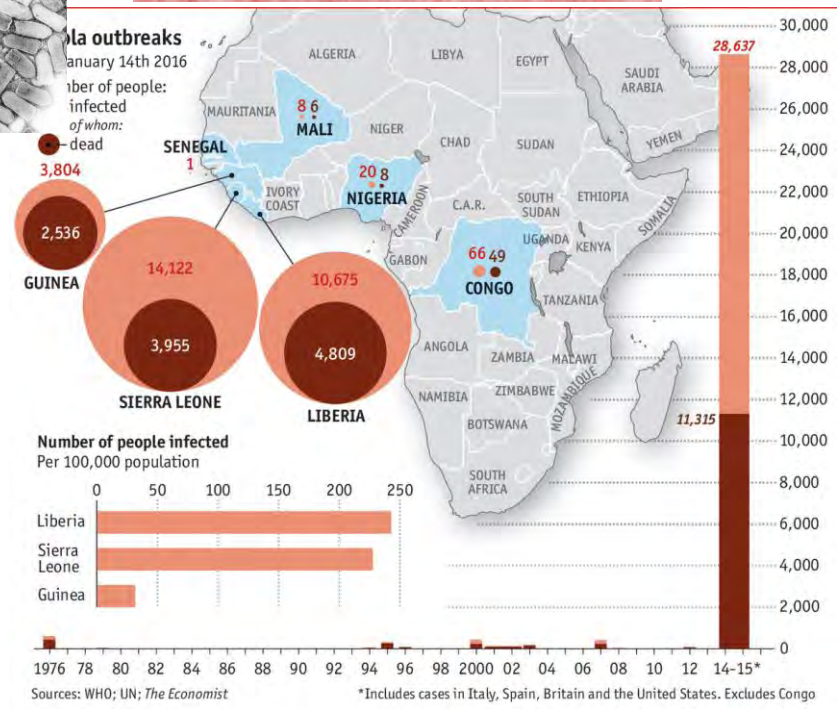


VSV (80x180 nm)



Ebola Virus (~80x1000 nm)

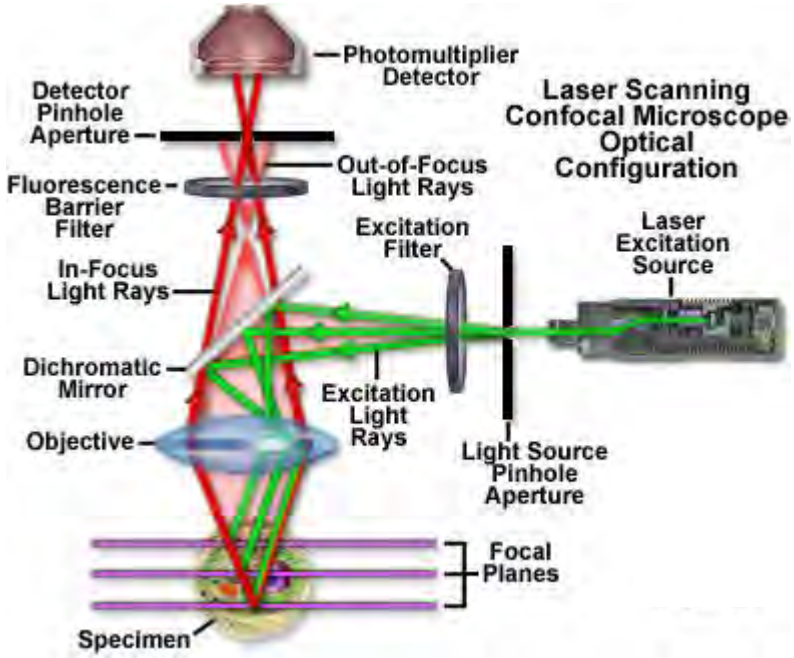




www.expasy.org/
phil.cdc.gov/
www.economist.com/

Challenges in optical microscopy of nanoparticles

Fluorescence microscopy



Have a great contrast **but** labelling:

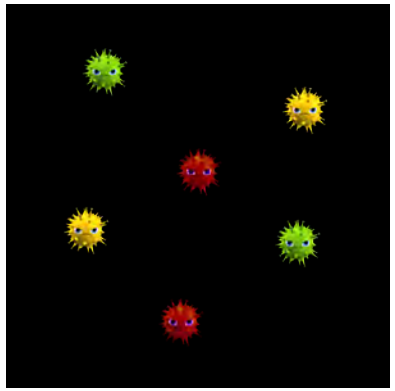
- Practically difficult
- May perturb sample functionality
- Limited by photobleaching and photoxiticity

http://zeiss-campus.magnet.fsu.edu/

Overarching Goals of Our Research

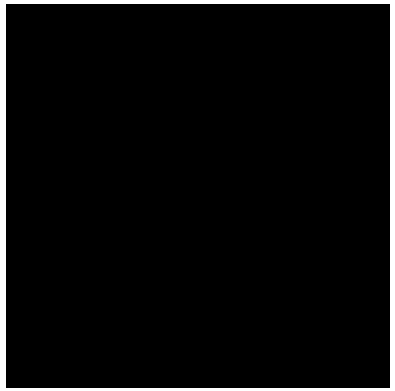
Aim: To develop a versatile optical microscopy platform that enables **high-throughput, high-resolution,** and **ultrasensitive** imaging and physicochemical characterization of a broad size range of biological nanoparticles **without any labelling**

Sample



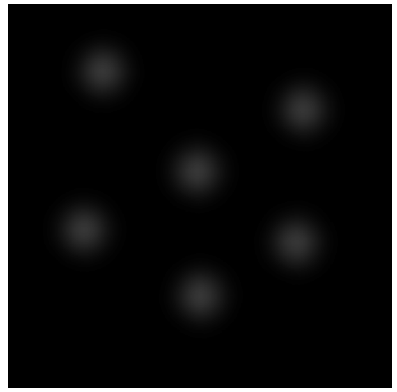
nanosized
(r < 50 nm)

Conventional



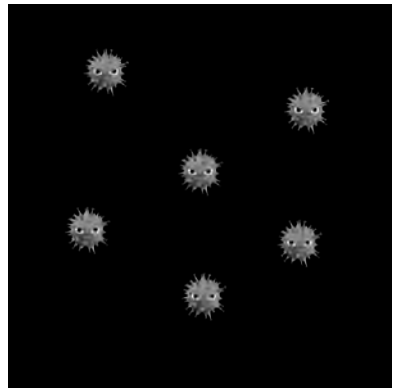
not detected

I. Interferometric microscopy



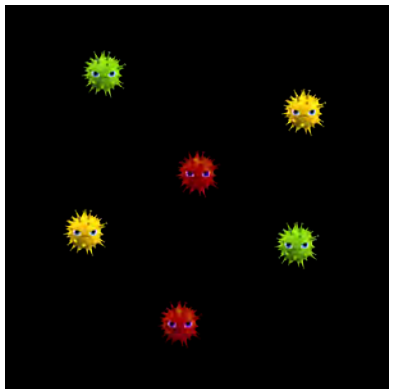
detected but not resolved

II. Computational interferometric microscopy



detected & resolved w/o chemical specificity

III. Interferometric photothermal microscopy



detected & resolved w/ chemical specificity

Colors represent different chemical compositions

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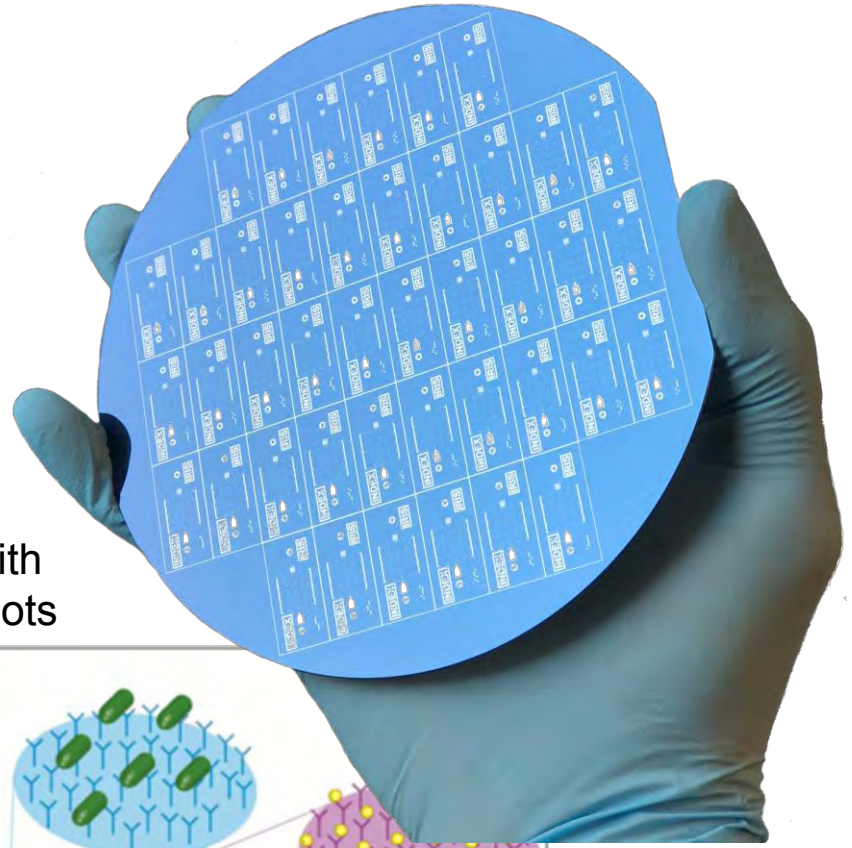
Interferometric Reflectance Imaging Sensor (IRIS)

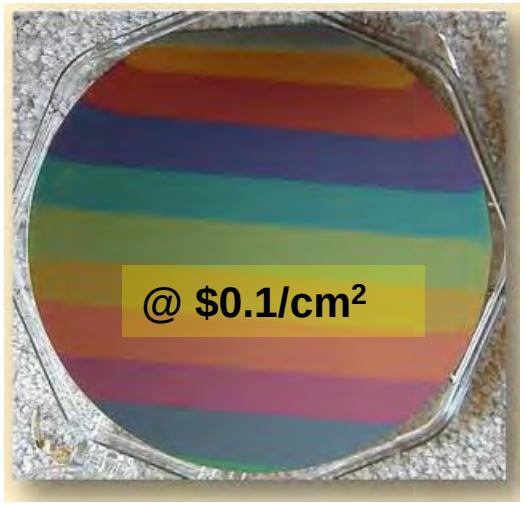
干涉反射成像传感器 (google translate)

a high throughput biosensing platform

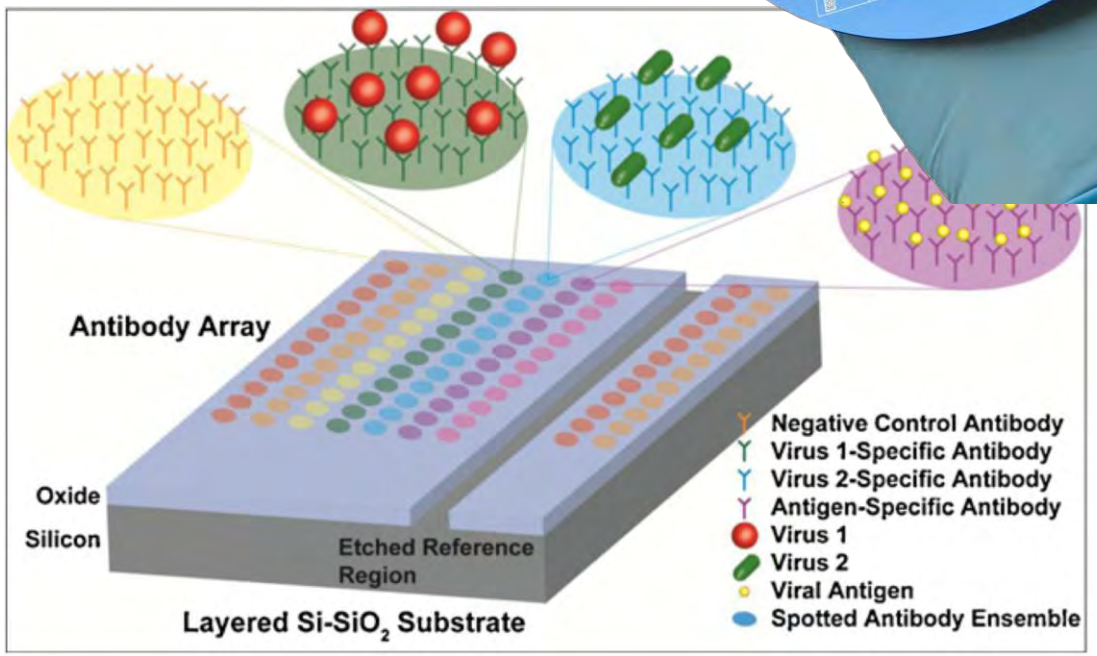


soap film

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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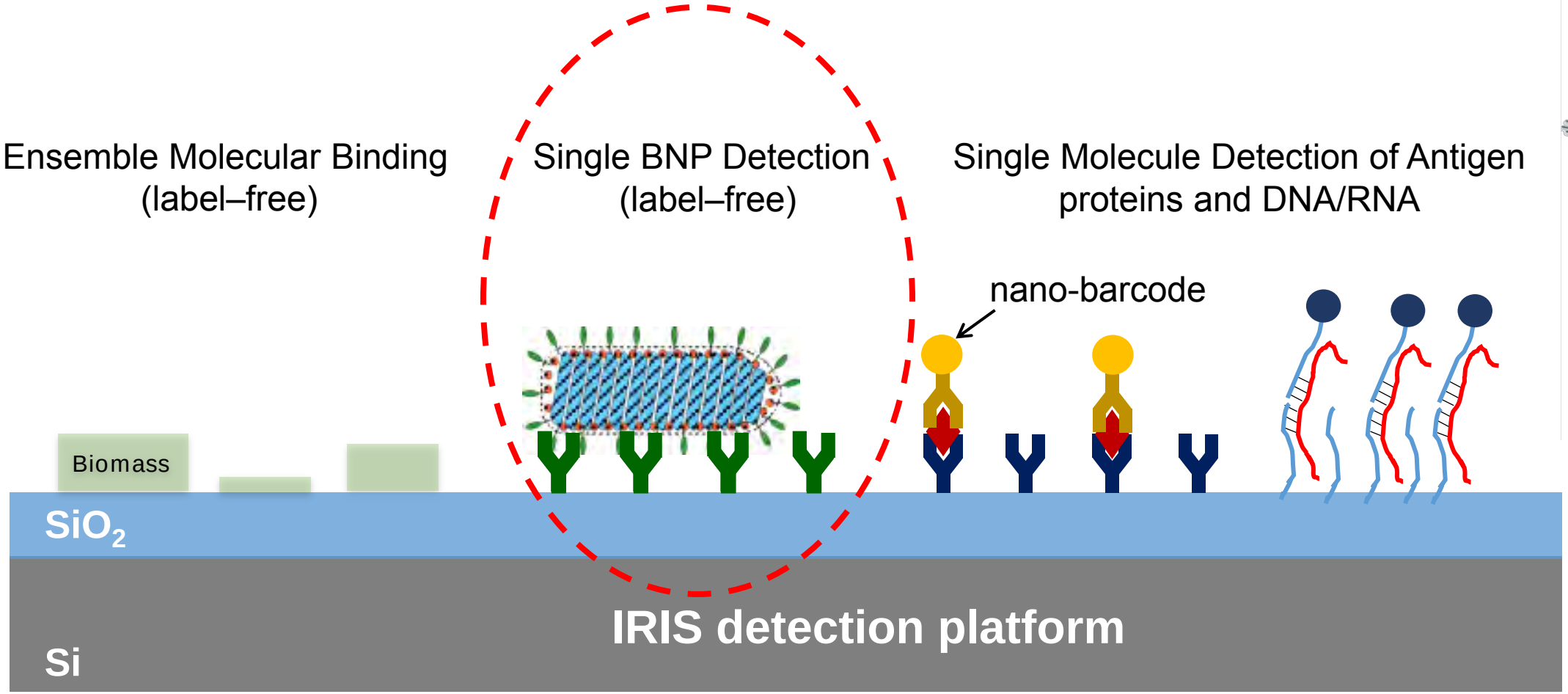
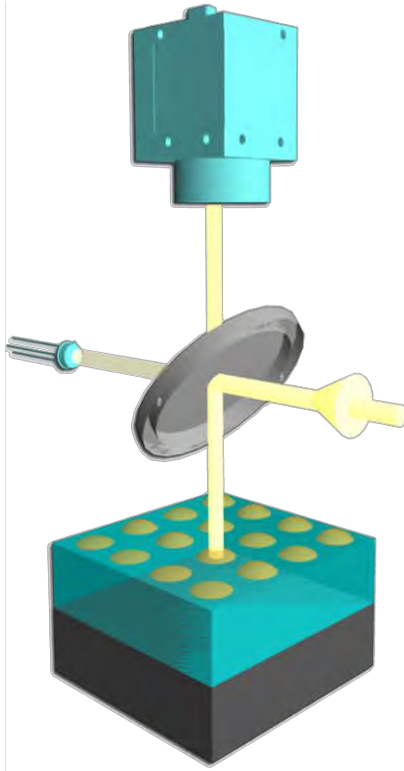
A versatile biosensing technology platform for microarrays





- Label-free molecular binding
- Label-free direct sensing of individual viruses and biological nanoparticles
- Digital Detection: Single molecule level detection of Nucleic Acids and Proteins

Simple Nanoparticle Detection

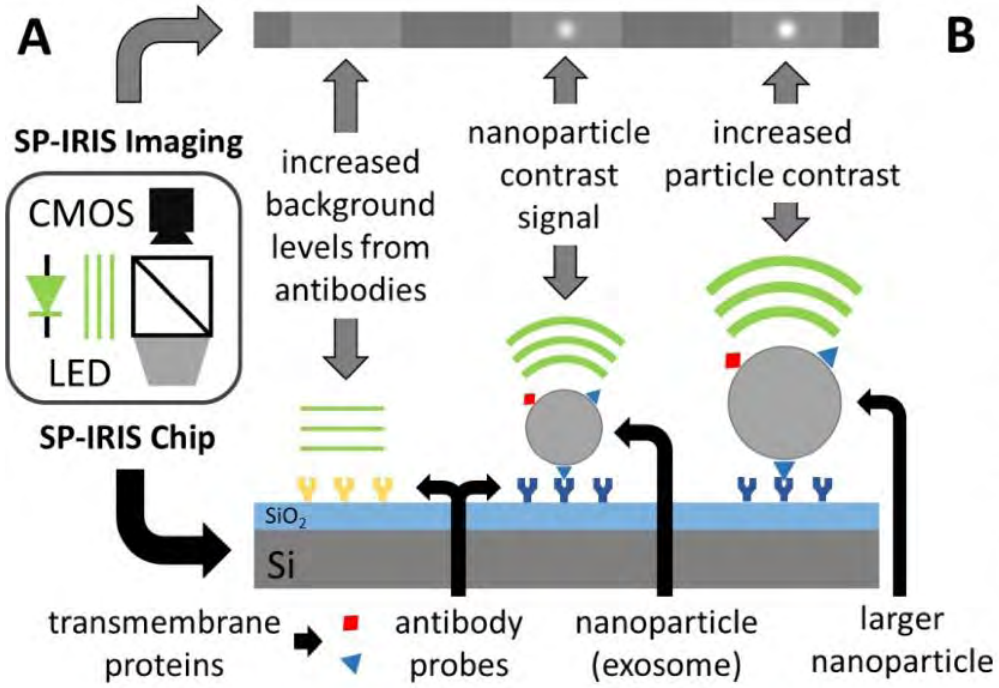
SCIENTIFIC REPORTS

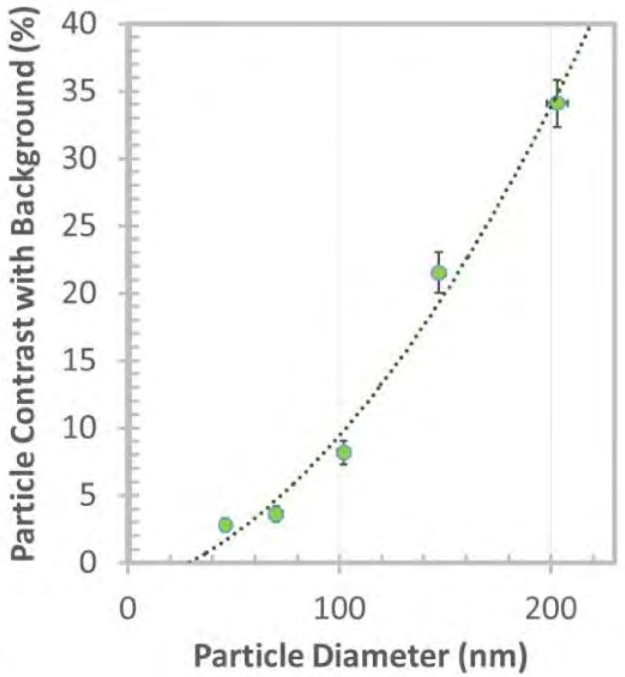
OPEN

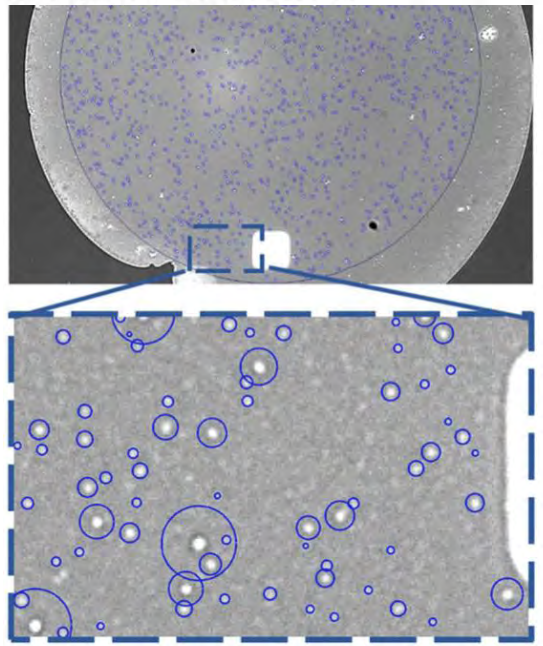
Digital Detection of Exosomes by Interferometric Imaging

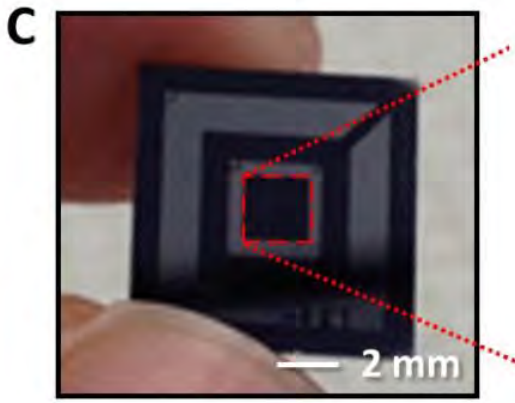
George G. Daaboul[1,*], Paola Gagni[2,*], Luisa Benussi[3], Paolo Bettotti[4], Miriam Ciani[3], Marina Cretich[2], David S. Freedman[1], Roberta Ghidoni[3], Ayca Yalcin Ozkumur[5], Chiara Piotto[4], Davide Prosperi[6], Benedetta Santini[6], M. Selim Ünlü[7] & Marcella Chiari[2]

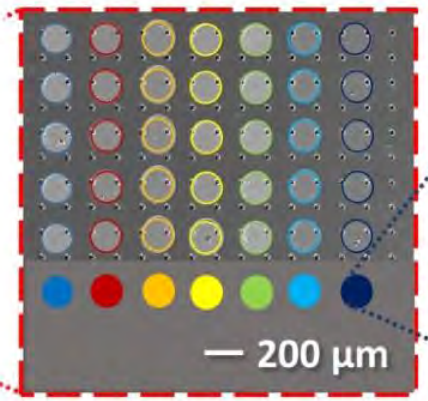
2016

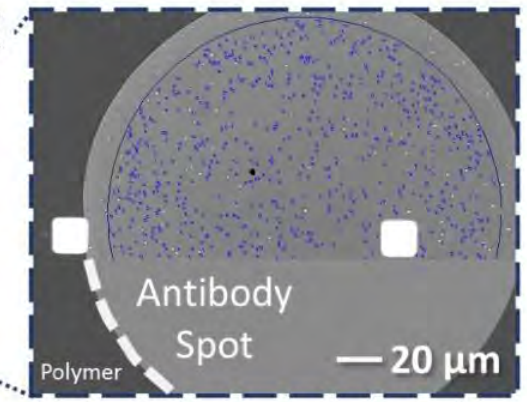




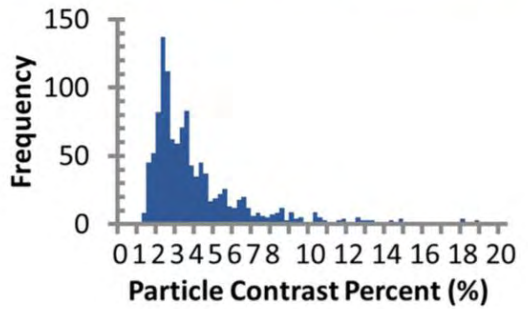


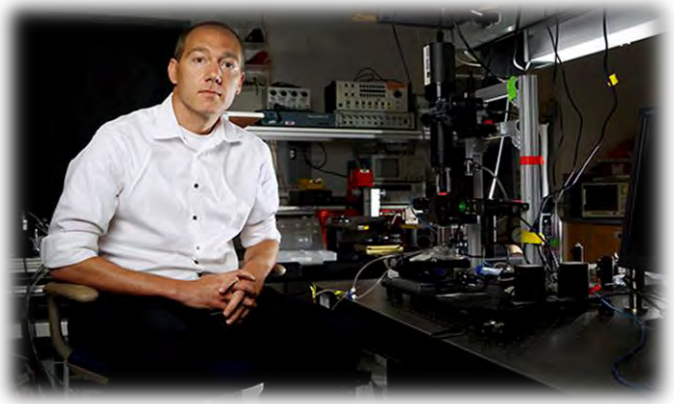






Daaboul '13





Lab on a Chip

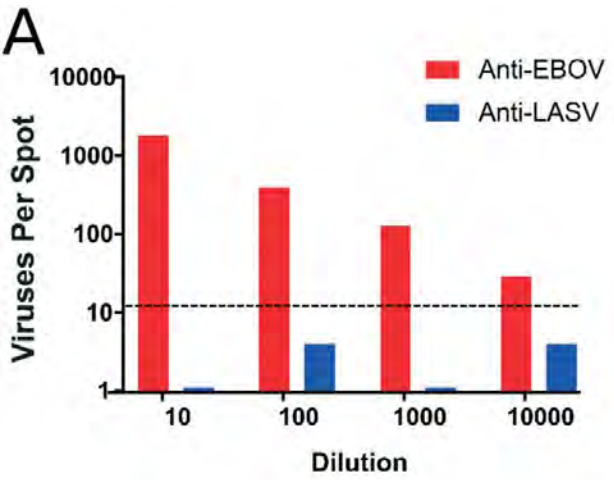
PAPER

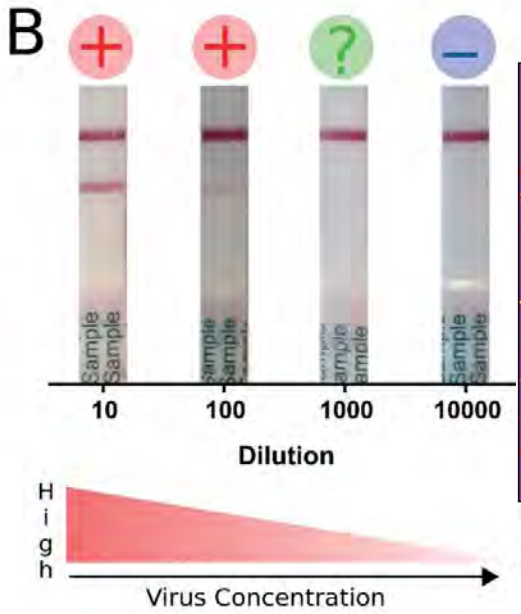
View Article Online
View Journal

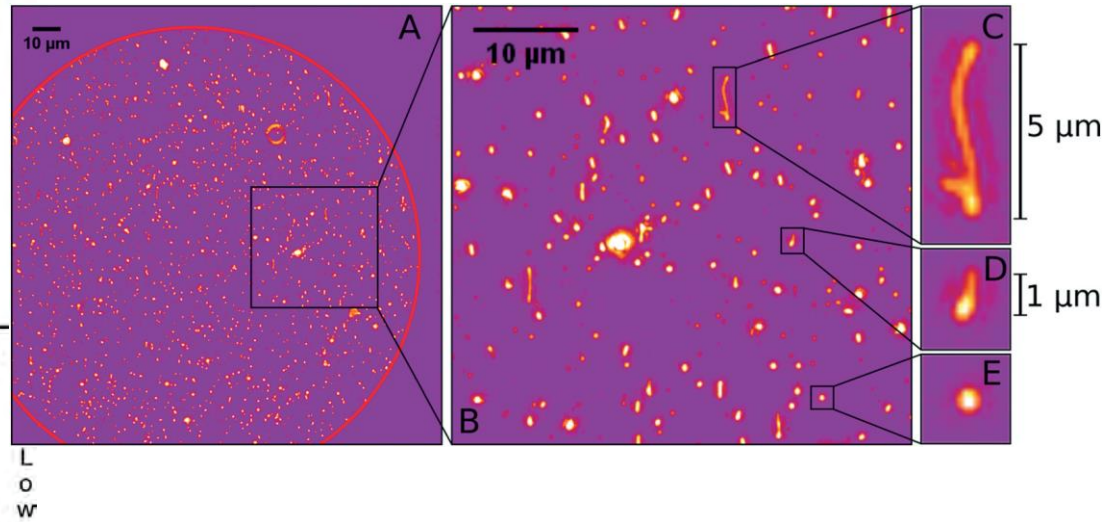
Disposable cartridge platform for rapid detection of viral hemorrhagic fever viruses†

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]

Cite this: DOI: 10.1039/c6lc01528j







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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    - Pupil function engineering

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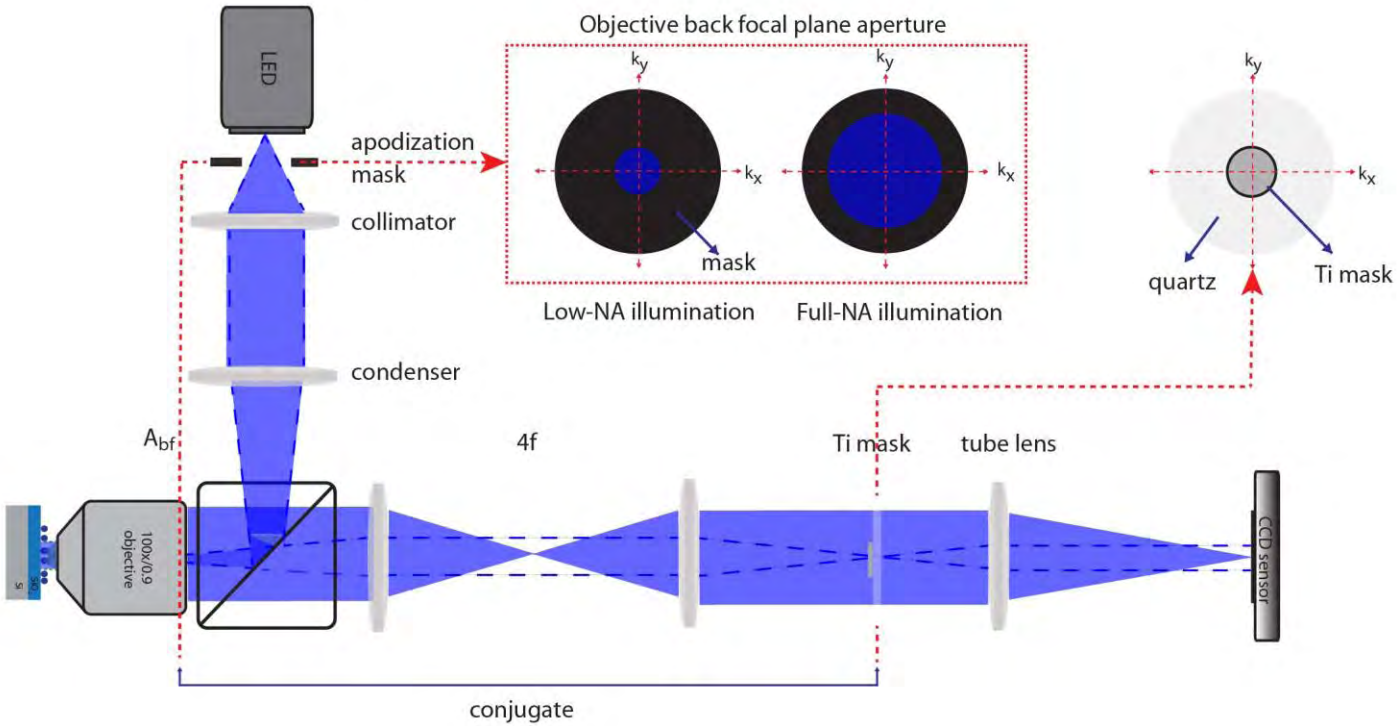
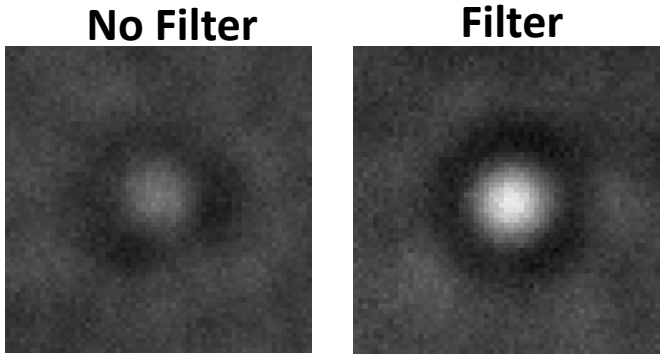
Research Article Vol. 4, No. 2 / February 2017 / Optica 247

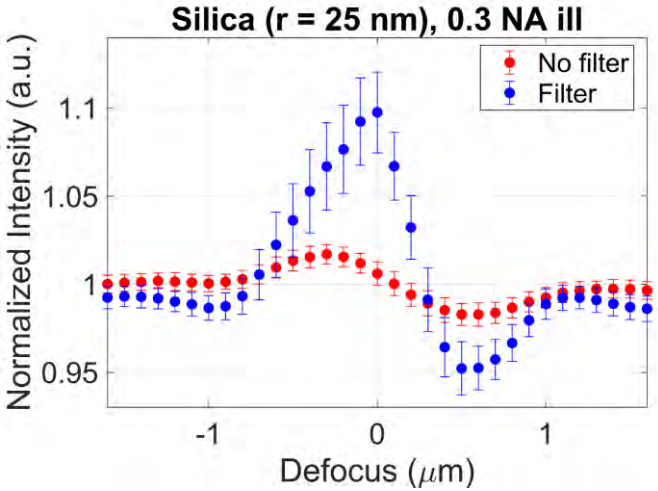
optica

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,*]







100x/0.9NA, λ=460 nm

Overall ~ **20**-fold improvement in particle visibility

- ~**5**-fold with apodized illumination
- ~**4**-fold with pupil mask

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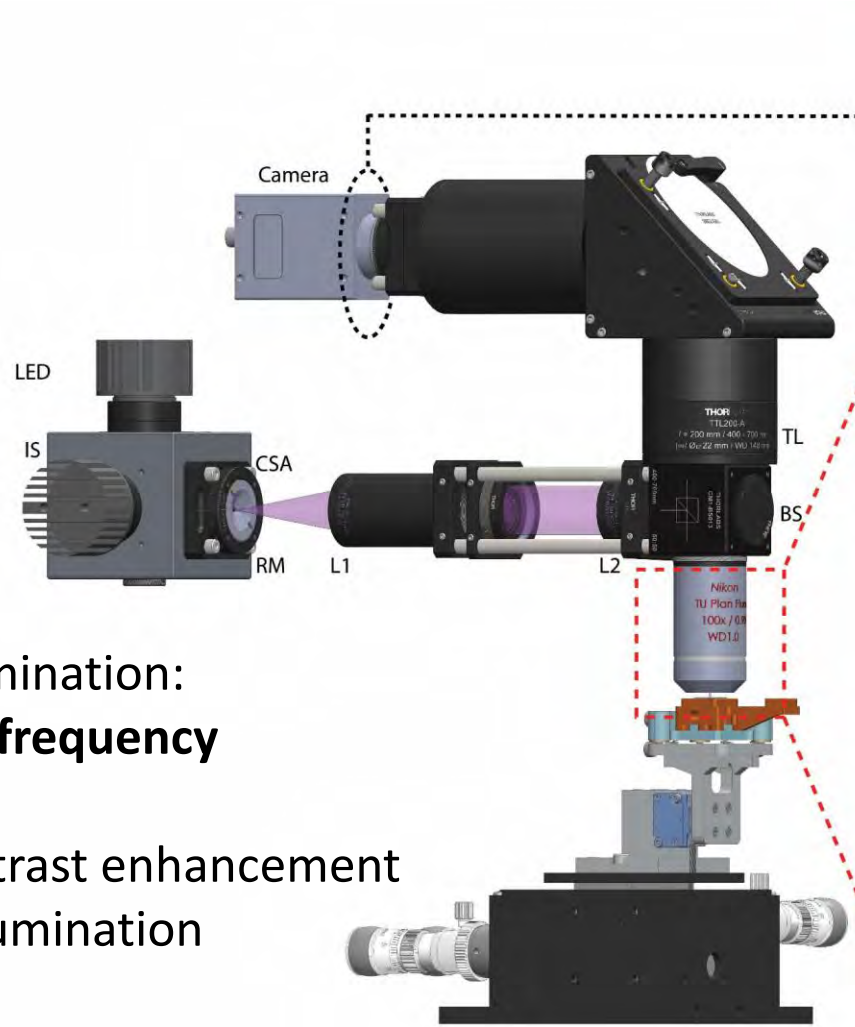
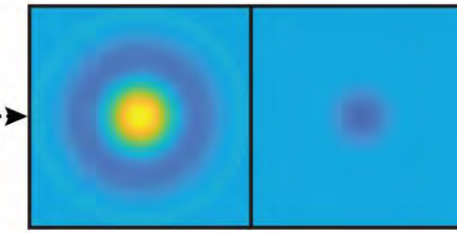
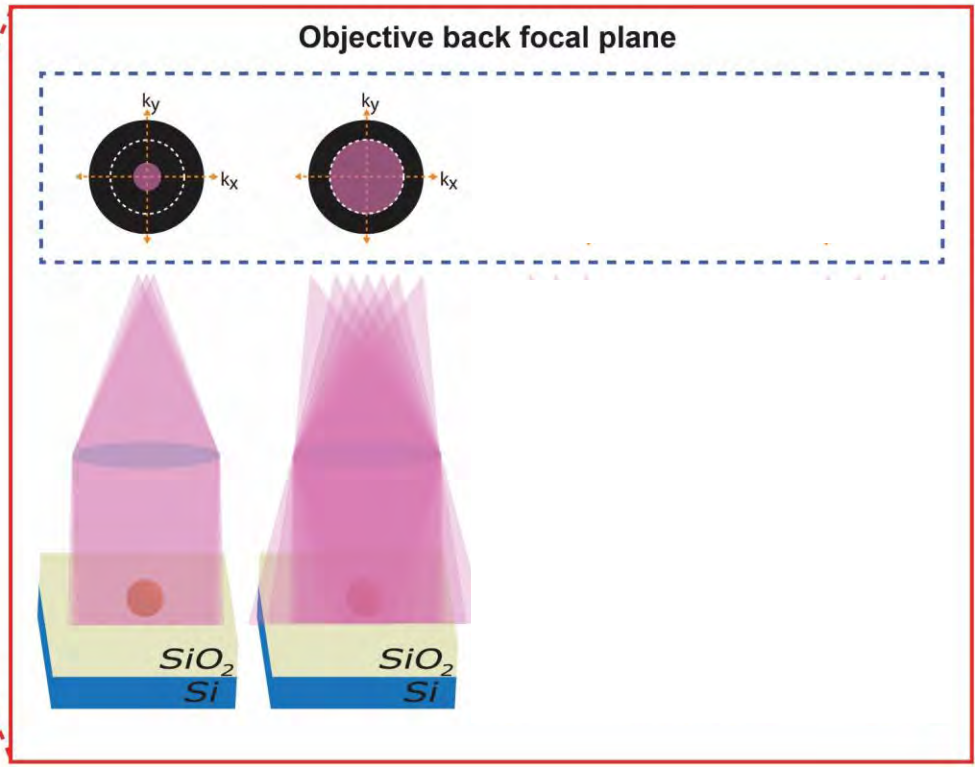
Computational asymmetric illumination SPIR (caSPIR)

Yurdakul '20

θ = 60 °







Asymmetrical illumination:

- Access to **high-frequency** information
- **Three-fold** contrast enhancement over **full-NA** illumination

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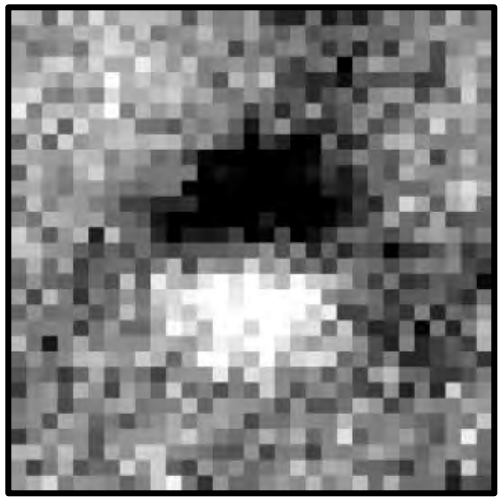
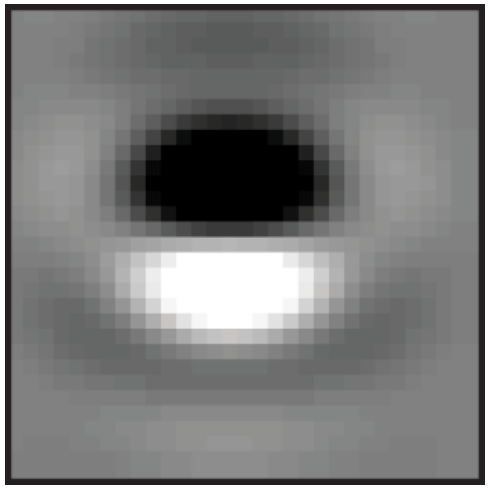
Experimental verification of caSPIR technique



$$y_j = A_j x + n_j$$

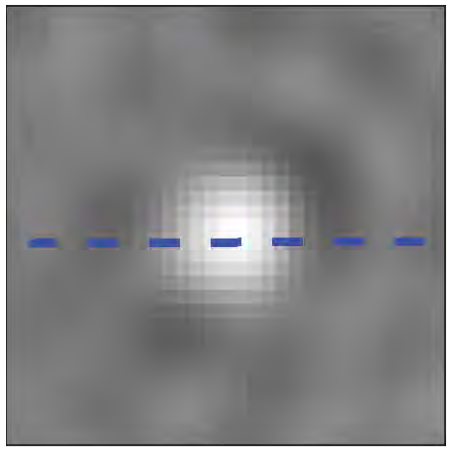


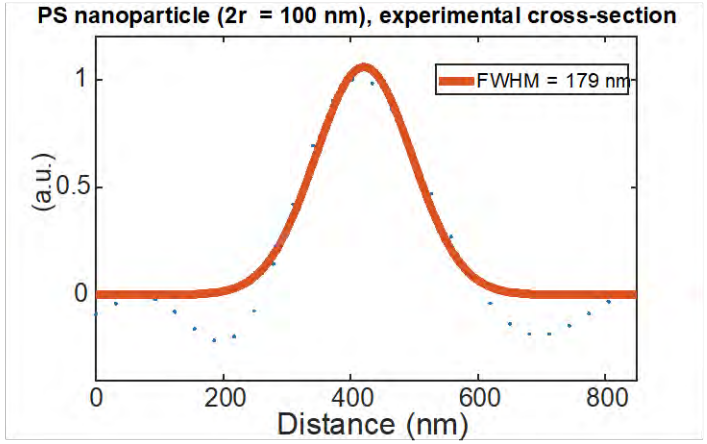
Solution for **x** using **computationally efficient Tikhonov regularization :**

$$\arg\min_x \sum_{j=1}^{N} \left[\| A_j x + n_j - y_j \|_2^2 \right] + \alpha \| x \|_2^2$$

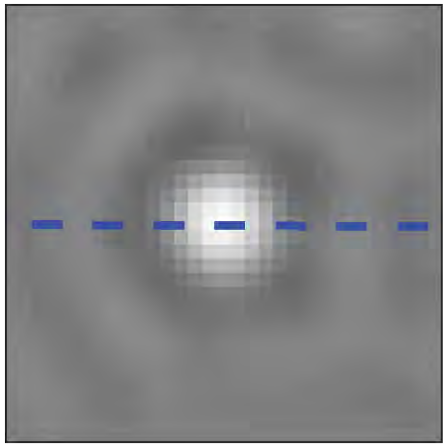
Improved lateral resolution by **2-fold** to **~150 nm**

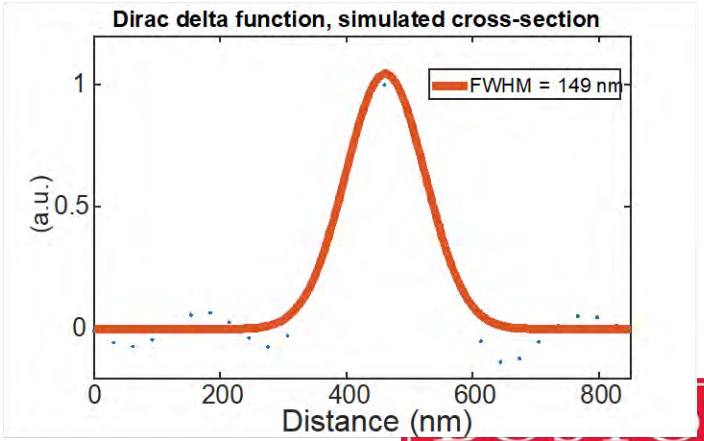
Experimental (FWHM = 148 nm)





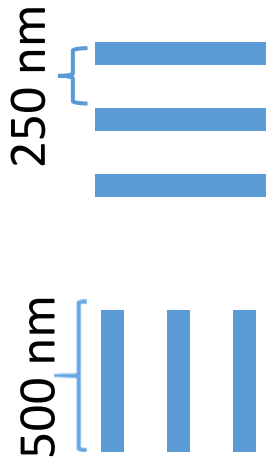
Theoretical (FWHM = 149 nm)

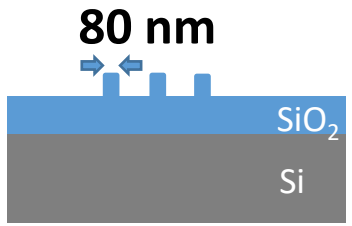




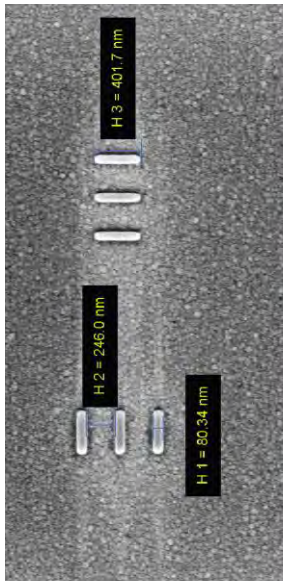
System performance on a resolution target fabricated by an e-beam lithography process

Sketch

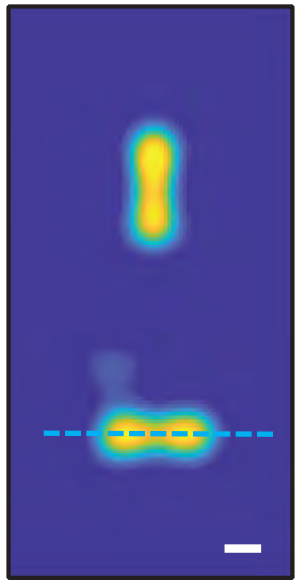




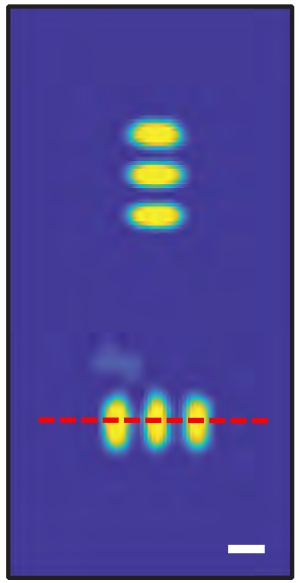
SEM



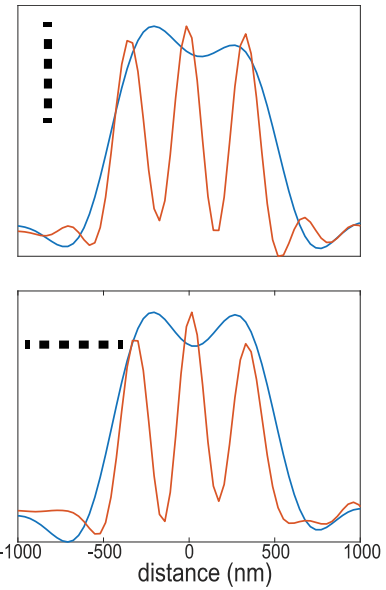
Conventional SPIR



caSPIR



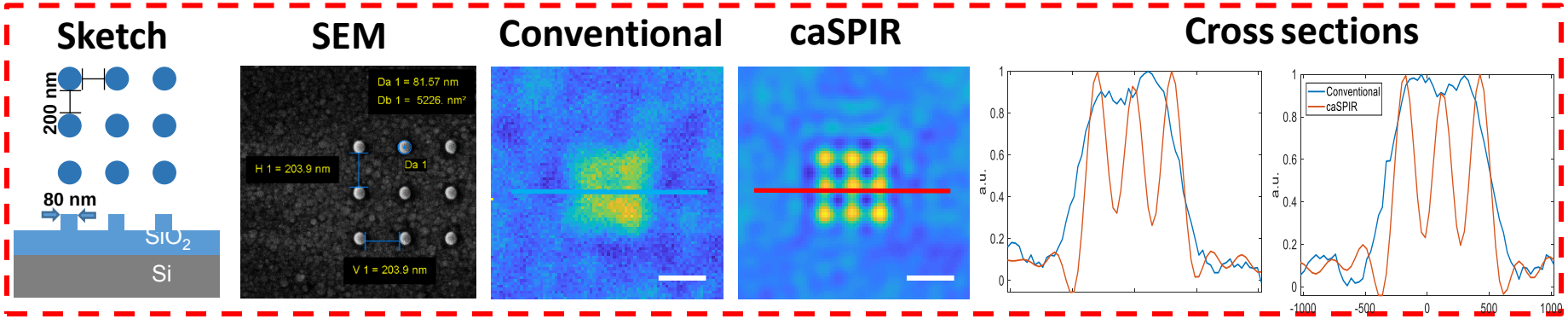
Cross-sections

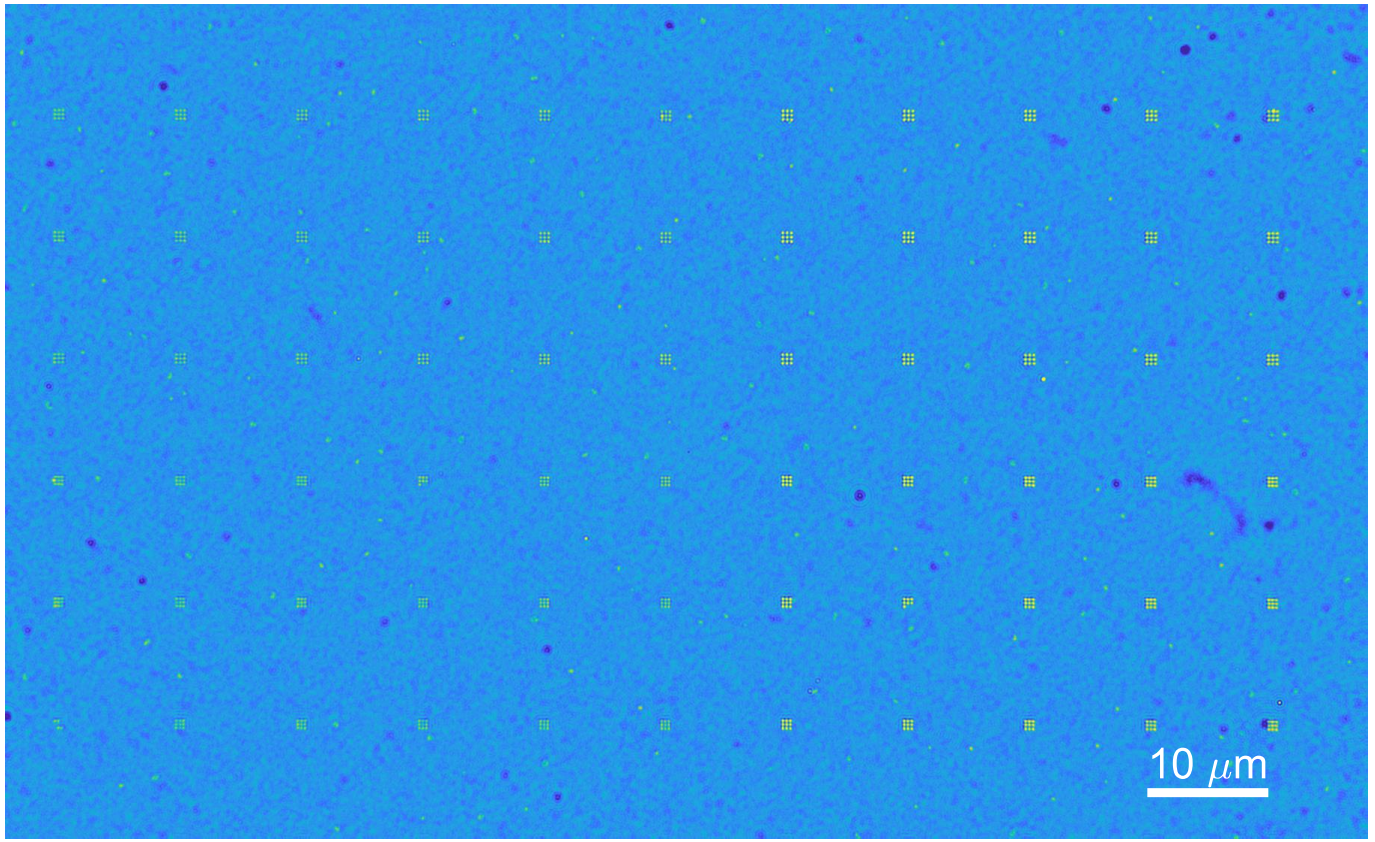


Scale bars: 200 nm

100x/0.9NA, λ=420 nm

caSPIR image of nanodots





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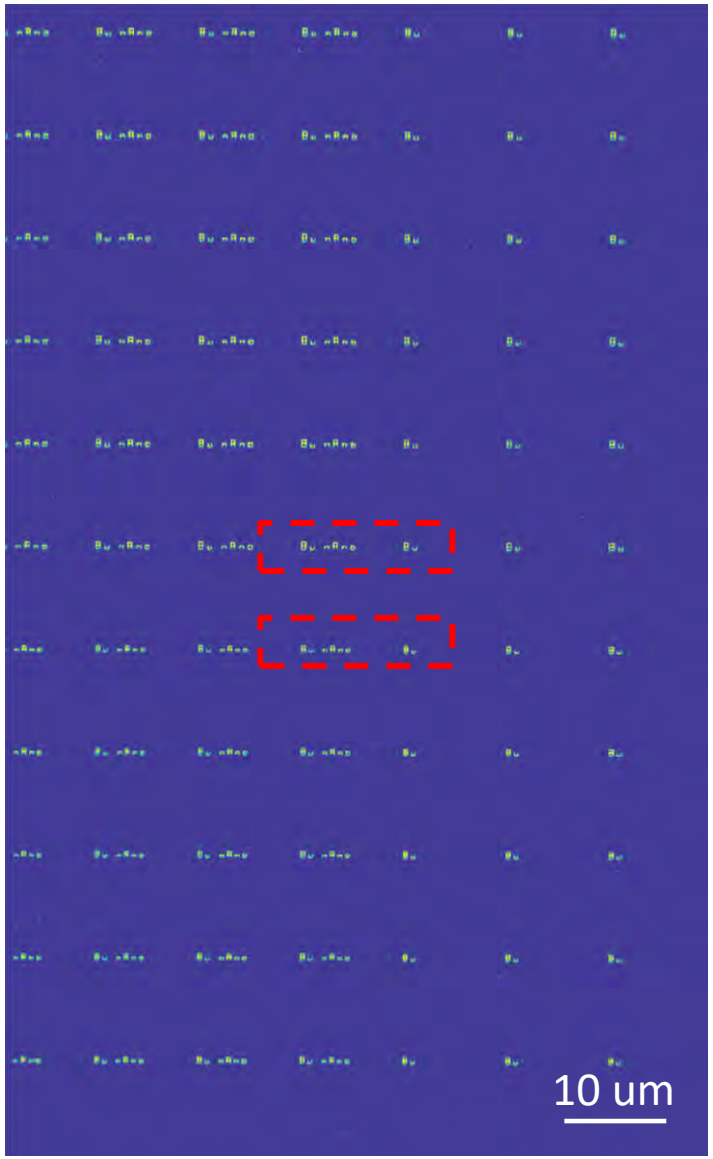
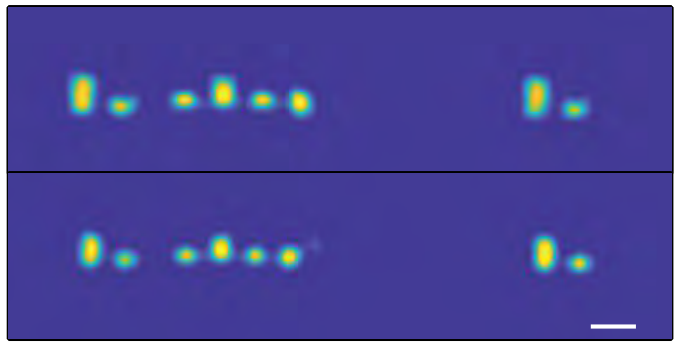
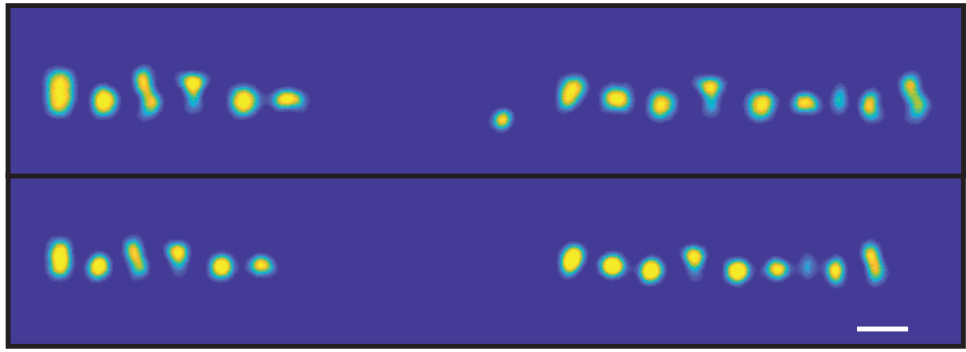
caSPIR image of nanowords

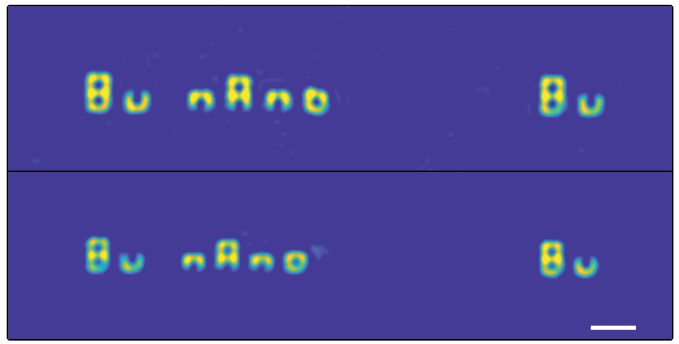


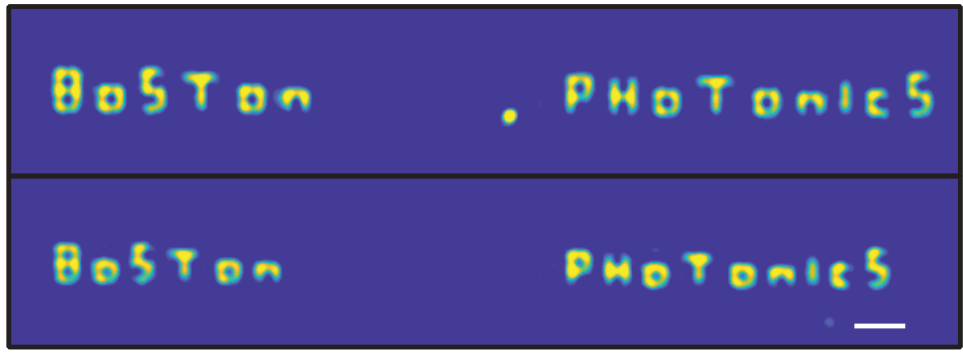
Conventional SPIR

caSPIR

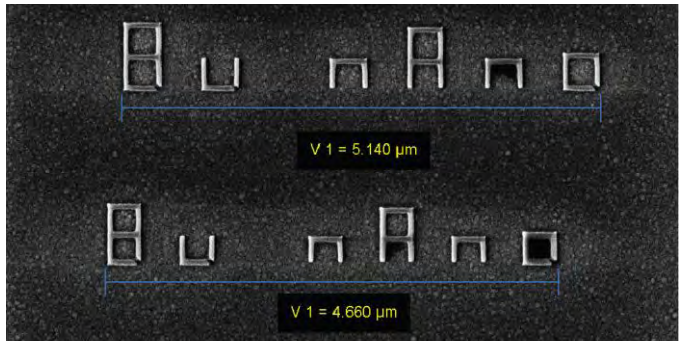








SEM



Scale bars: 1 um

100x/0.9NA, λ=420 nm

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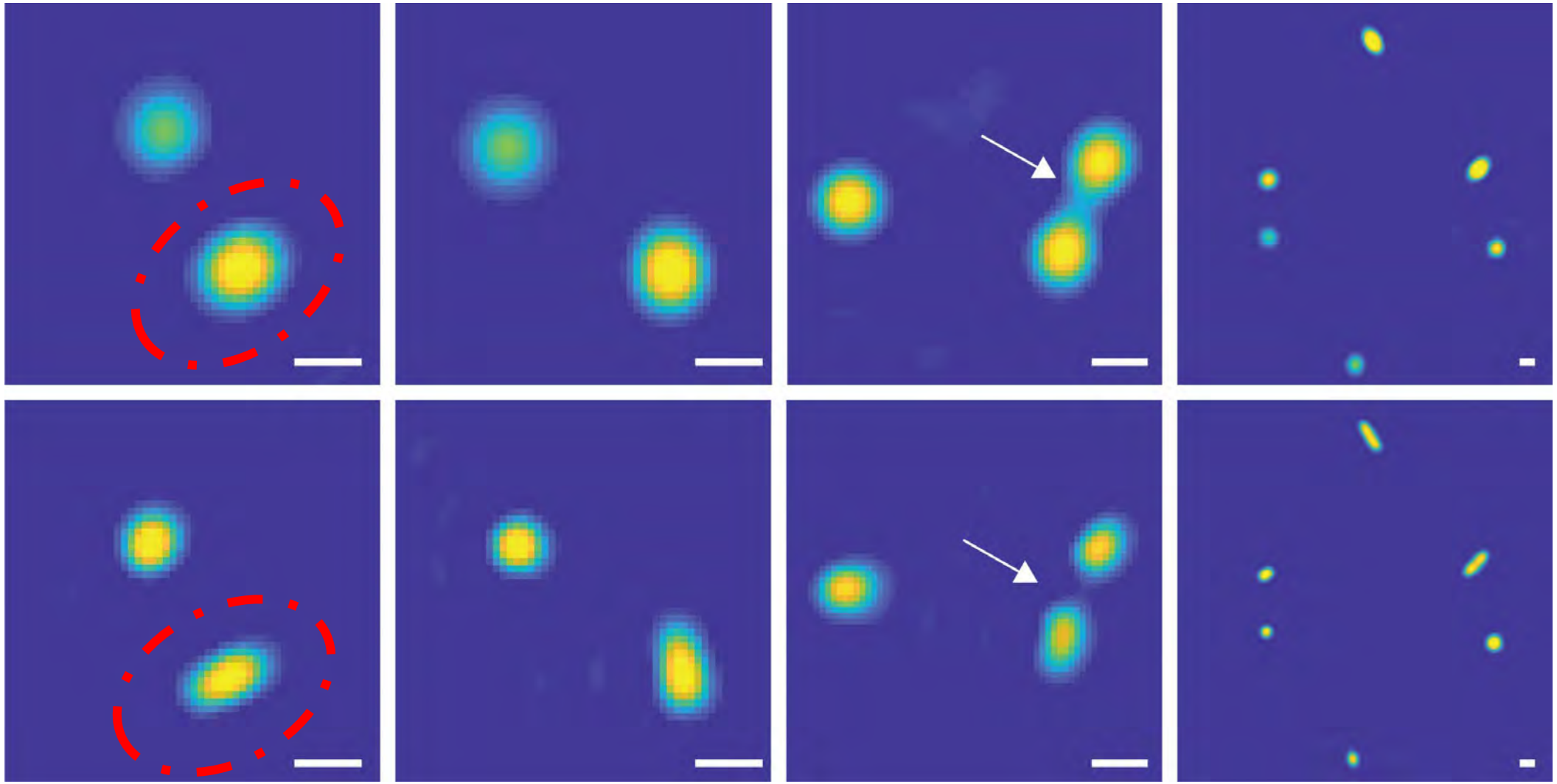
caSPIR image of nanocarriers

caSPIR image of nanocarriers



100x/0.9NA, λ=420 nm

Scale bars are 300 nm

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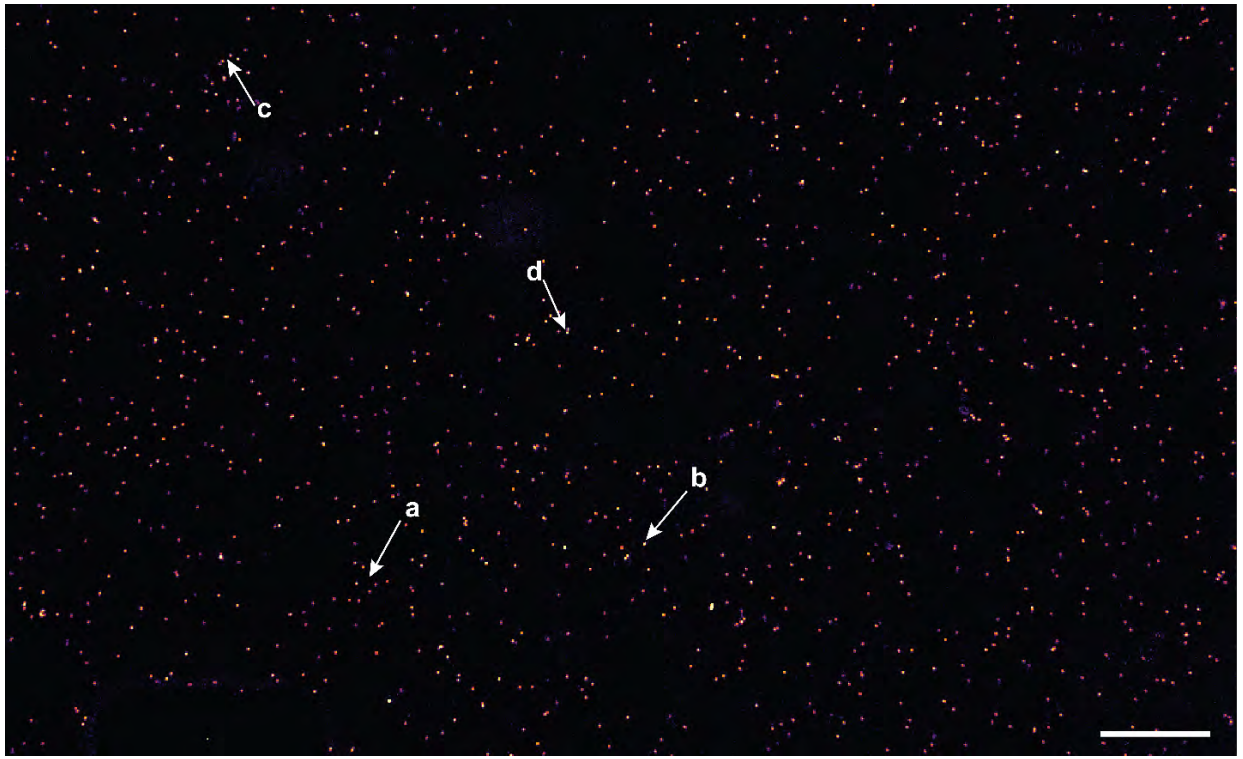
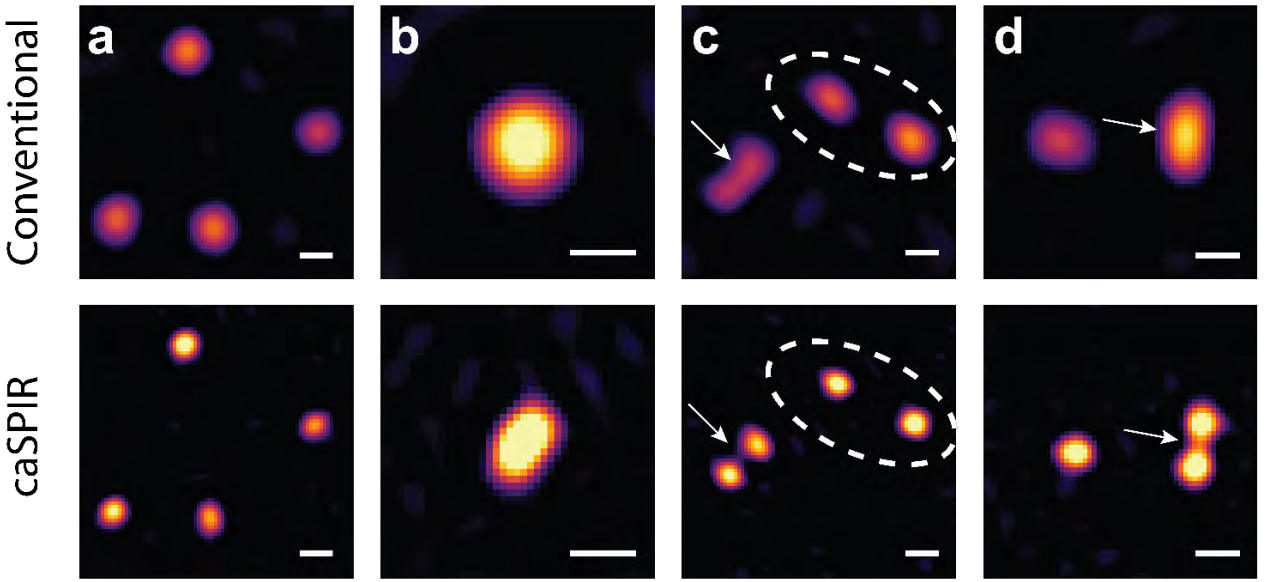
caSPIR image of Ebola vaccine candidate

- Bullet-like (80 nm x 180 nm) Ebola vesicular stomatitis virus
  - A vaccine candidate for deadly Ebola virus
- 1350 VSVs in a single FOV (120 um x 80 um)

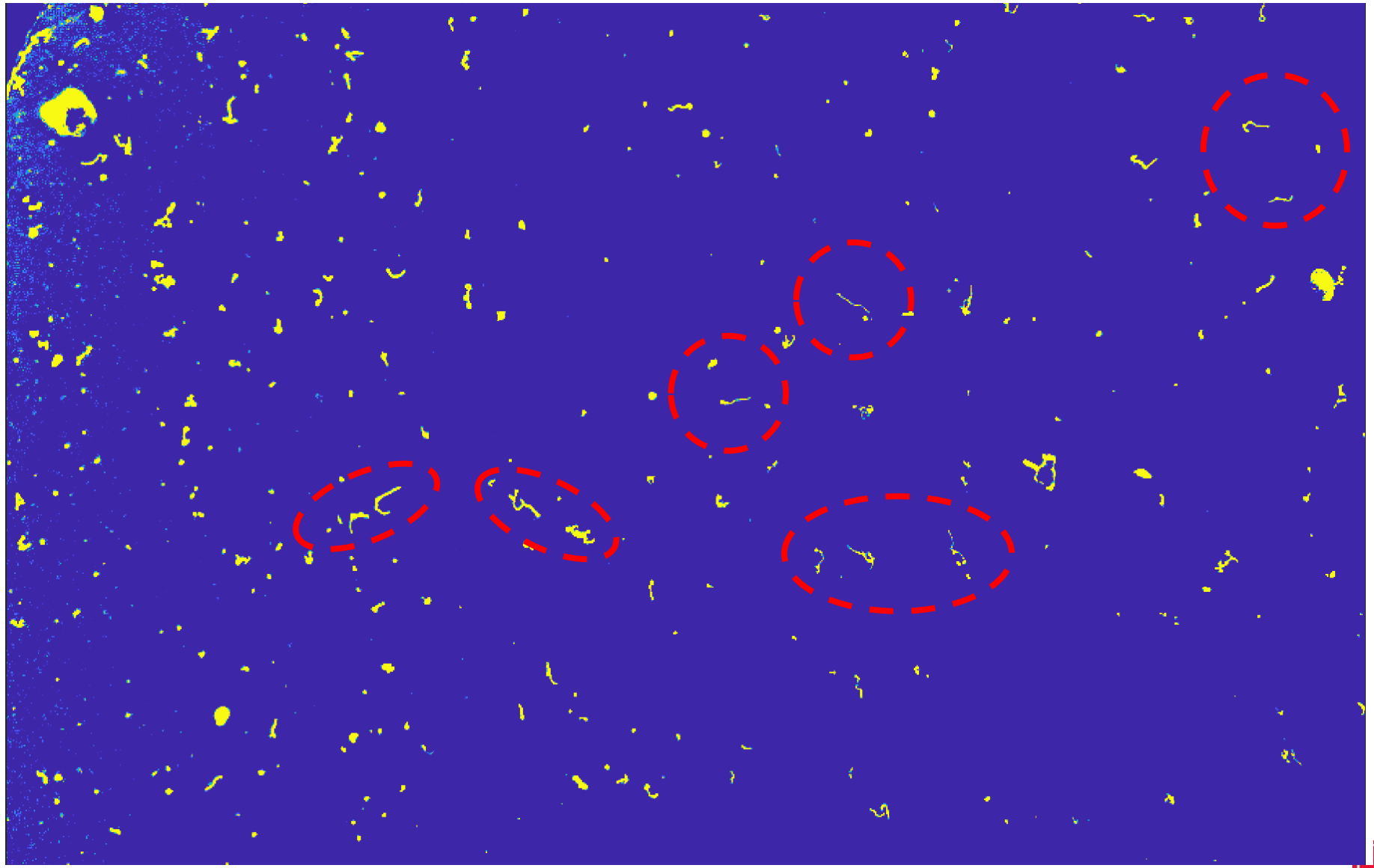




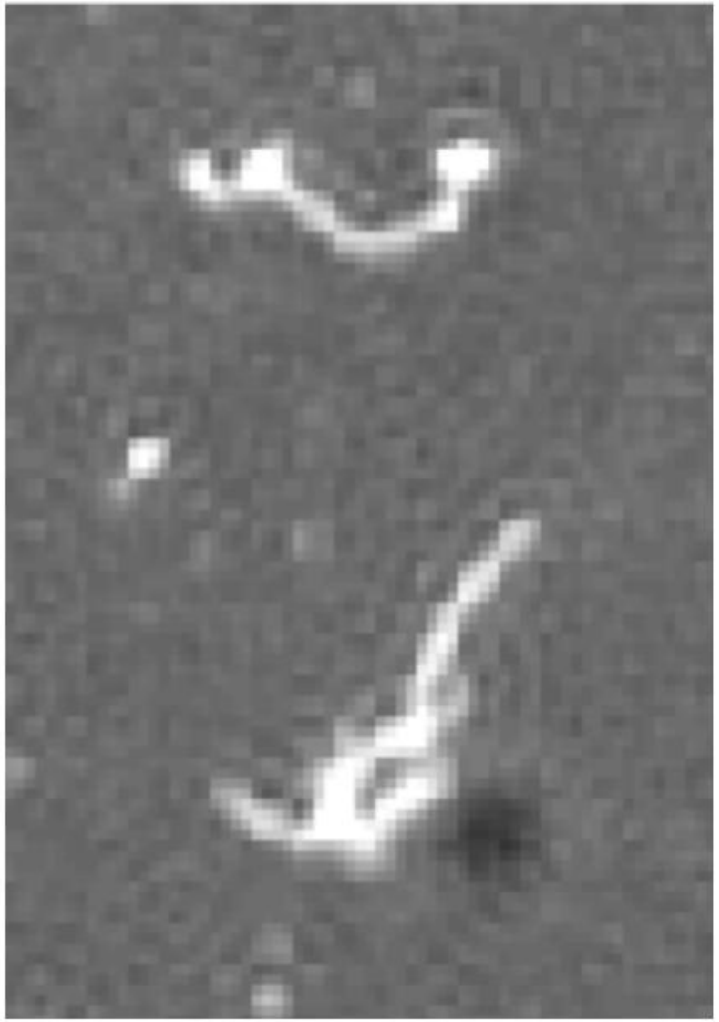
Scale bars: 300 nm

100x/0.9NA, λ=420 nm

caSPIR image of Ebola VLPs



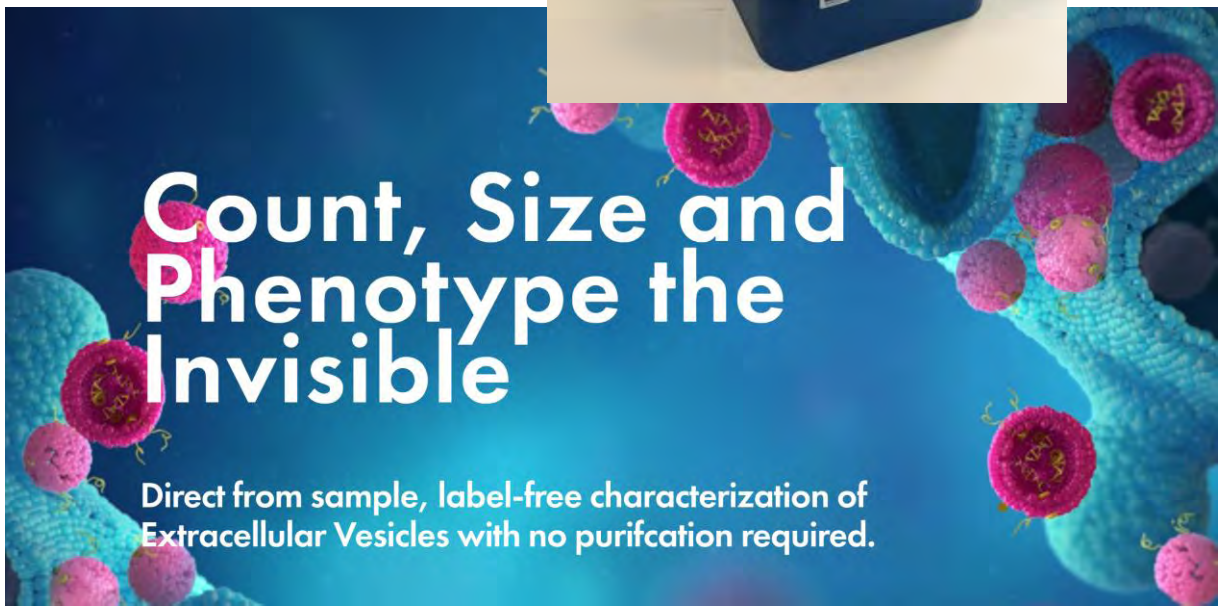
caSPIR image of Ebola VLPs



Commercialization

NanoView BIOSCIENCES

DAVID FREEDMAN
Chief Executive Officer and Founder

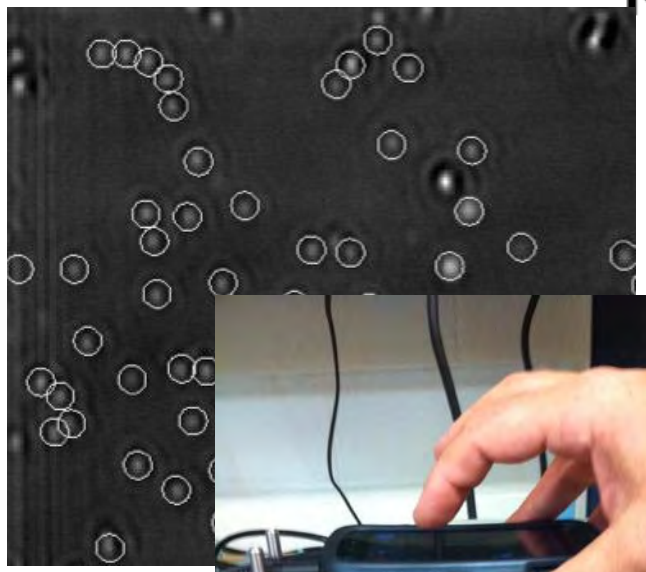


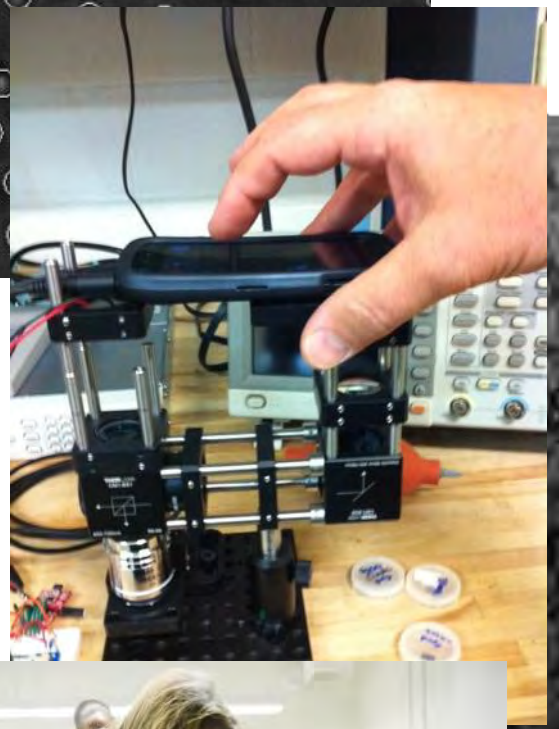
Interferometric Detection and Enumeration of Viral Particles using Si-based Microfluidics

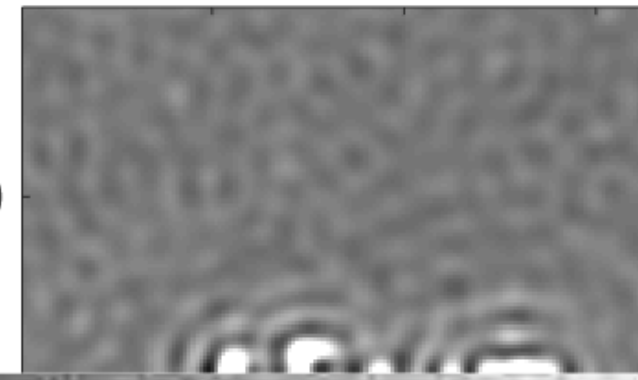
Ayca Yalcin Ozkumur, Fulya Ekiz Kanik, Jacob Trueb, Celalettin Yurdakul, and M. Selim Ünlü, *Fellow, IEEE*

CONCLUSIONS & FUTURE

- Optical interference is a very powerful measurement 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







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