Titles

1- High-precision microscopic phase imaging without phase unwrapping for cancer cell identification, OL (2013)

2- Label-free imaging of intracellular motility by low-coherent quantitative phase microscopy, OE (2011)

3- Wide-field optical detection of nanoparticles using on-chip microscopy and self-assembled nanolenses, Nature (2013)

By

D.G.Abdelsalam

1- High-precision microscopic phase imaging without phase unwrapping for cancer cell identification, OL (2013), Japan

Eriko Watanabe,[1,*] Takashi Hoshiba,[2] and Bahram Javidi[3]

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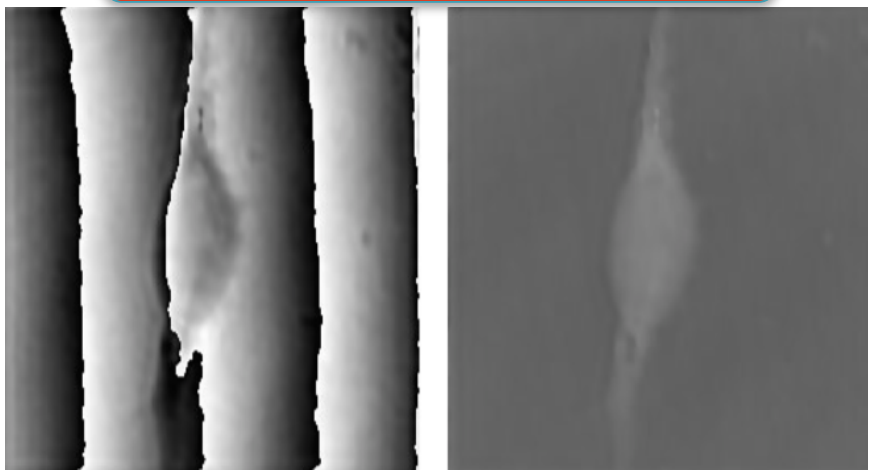
Objective

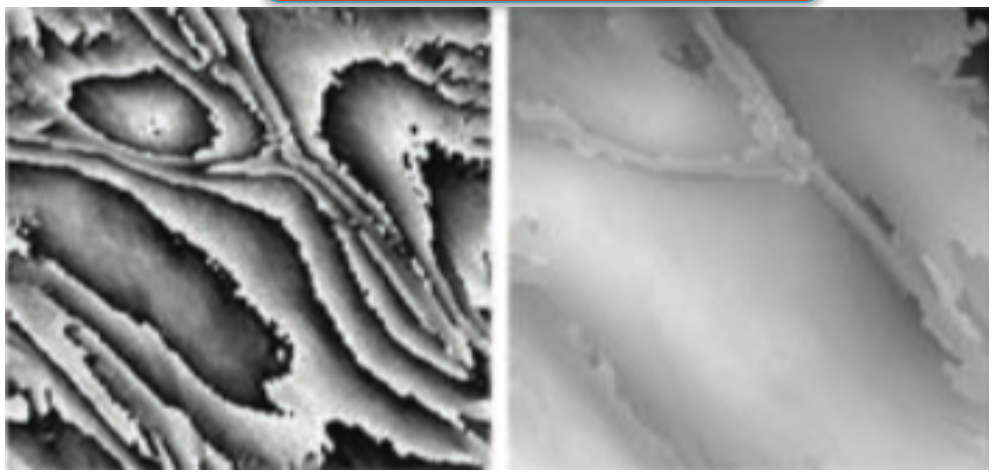
- ✓ Cancer cell identification using high-precision optical path length with no phase unwrapping

Brief Introduction

embryo fibroblast cell



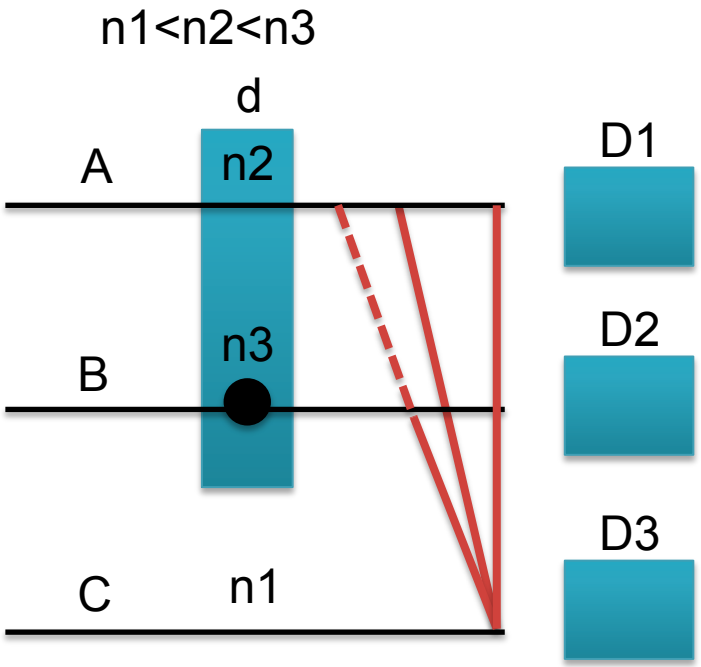
Onion cells

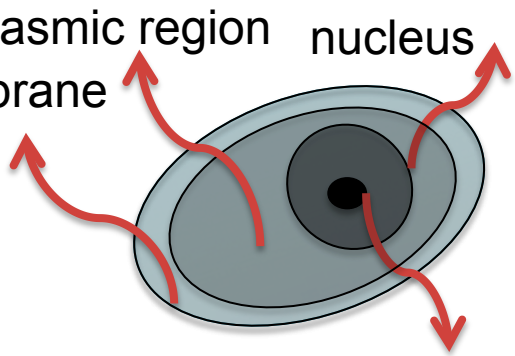


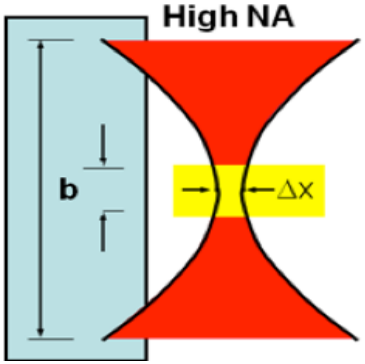
Brief Introduction

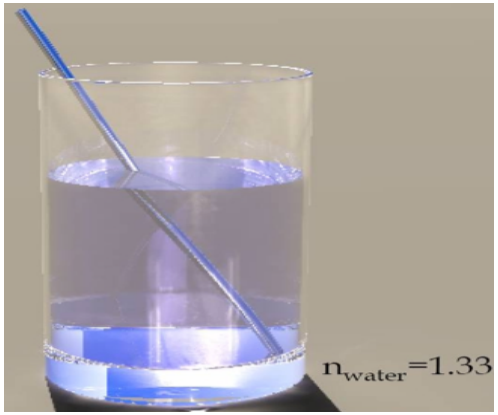
- ✓ The potential of this approach for cancer cell Identification by distinguishing normal cells from cancerous cells through comparison of their optical path length differences.

- ✓ Index of refraction of cancer cells is relatively higher than normal cells.









Axial Resolution

$$\Delta z = \frac{2\ln 2}{\pi}\left(\frac{\lambda^2}{\Delta\lambda}\right)$$

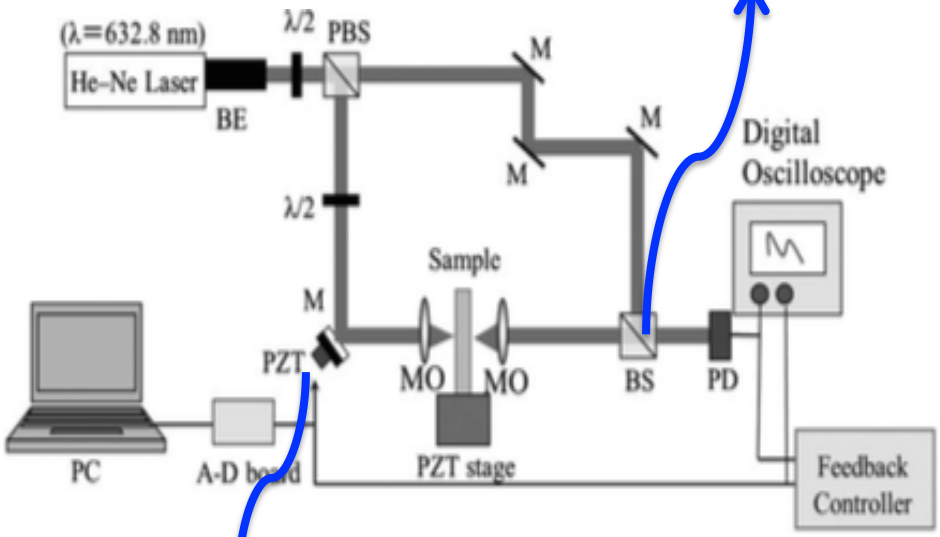
Transverse Resolution

$$\Delta x = \frac{4\lambda}{\pi}\left(\frac{f}{d}\right)$$

Depth of Focus

$$b = \frac{\pi \Delta x^2}{2\lambda}$$

Setup



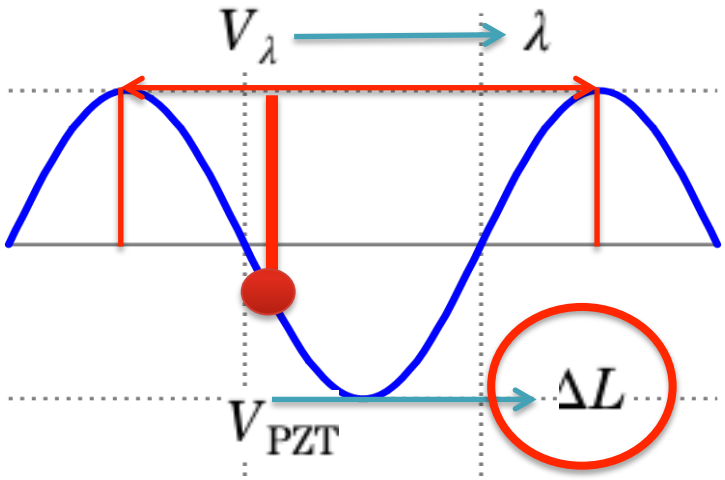
$$I(x,y) = I_0 \frac{1+\cos\{\Delta\varphi(x,y)\}}{2},$$

$$\Delta\varphi(x,y) = \frac{2\pi}{\lambda}\Delta L(x,y),$$

Optical path length distr.

Controls the path length

If the voltage applied to PZT compensates $\Delta\varphi$,we have the condition of phase locking as



$$I(x,y) = I_0 \frac{1+\cos\left\{\Delta\varphi(x,y) - \frac{2\pi}{\lambda}\Delta L_{pzt}(x,y)\right\}}{2} = \text{const.}$$

$$\Delta L(x,y) = \frac{V_{PZT}(x,y)\times\lambda}{V_\lambda},$$

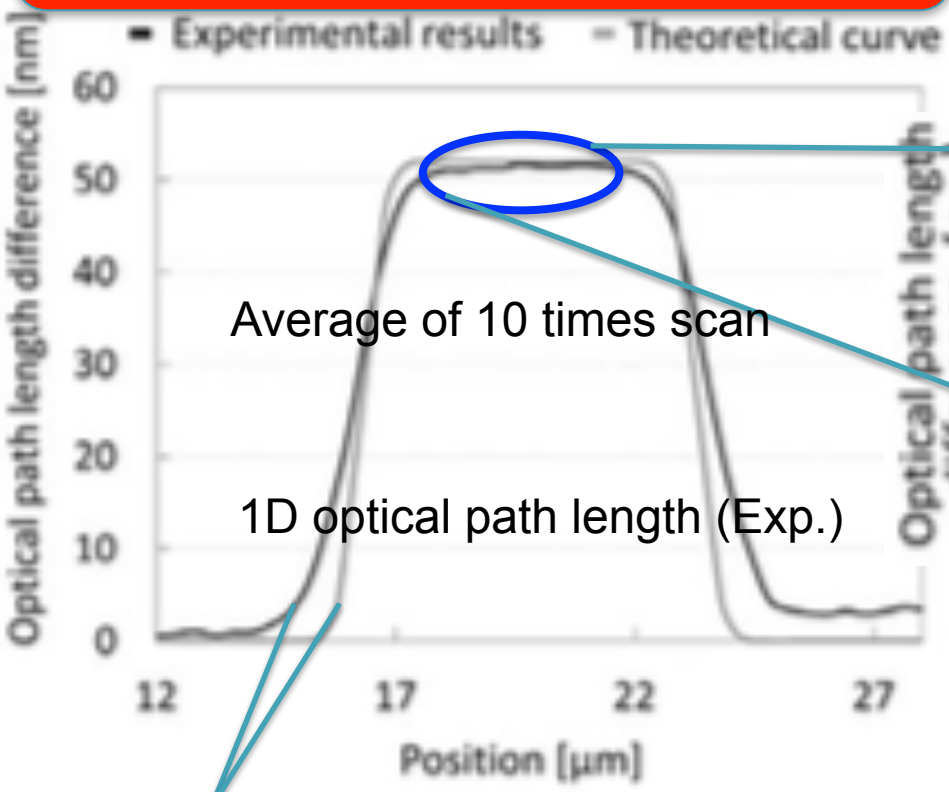
Phase sensitivity check

Example 1

Planar light wave circuit (PLC) with known refractive index

Measured optical path length 52.0 nm

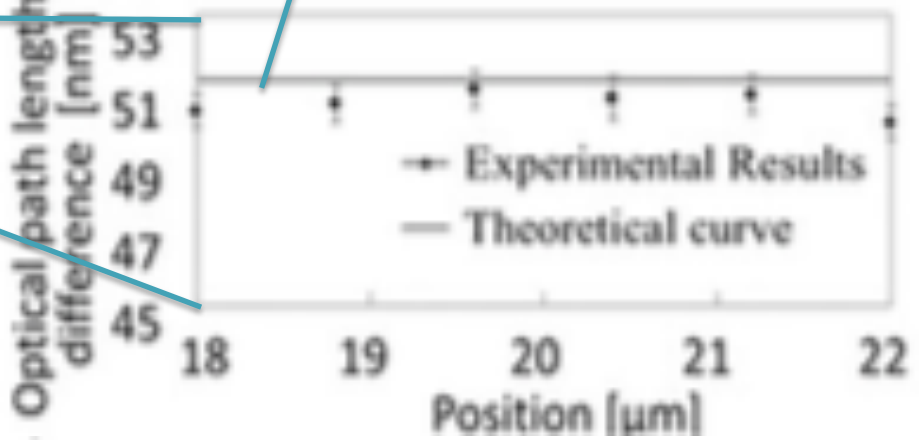
Theoretical (geometrical optics)



Average of 10 times scan

1D optical path length (Exp.)

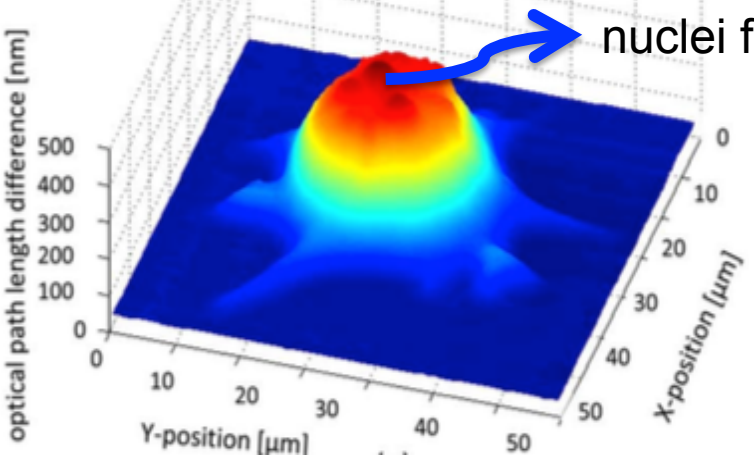
Spherical aberration



The sensitivity of optical path length is calculated by stv to be less than 2nm, n = 10, i.e. the error in measurement is 2nm

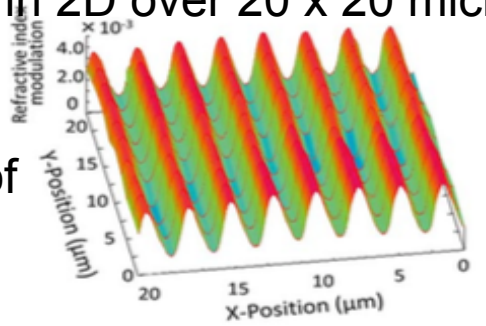
Example 2 (grating), 3 (thick cells), and 4 cancer

3D optical path length difference distribution of a breast cancer cell

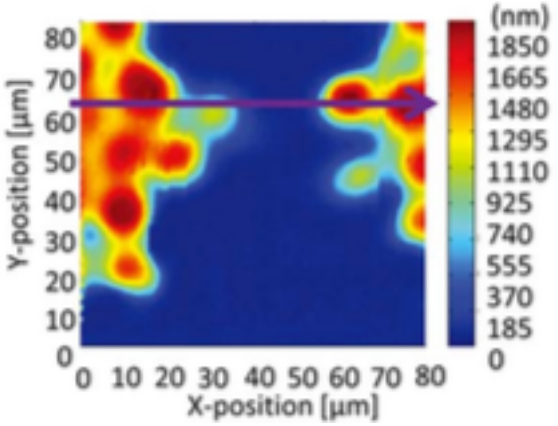


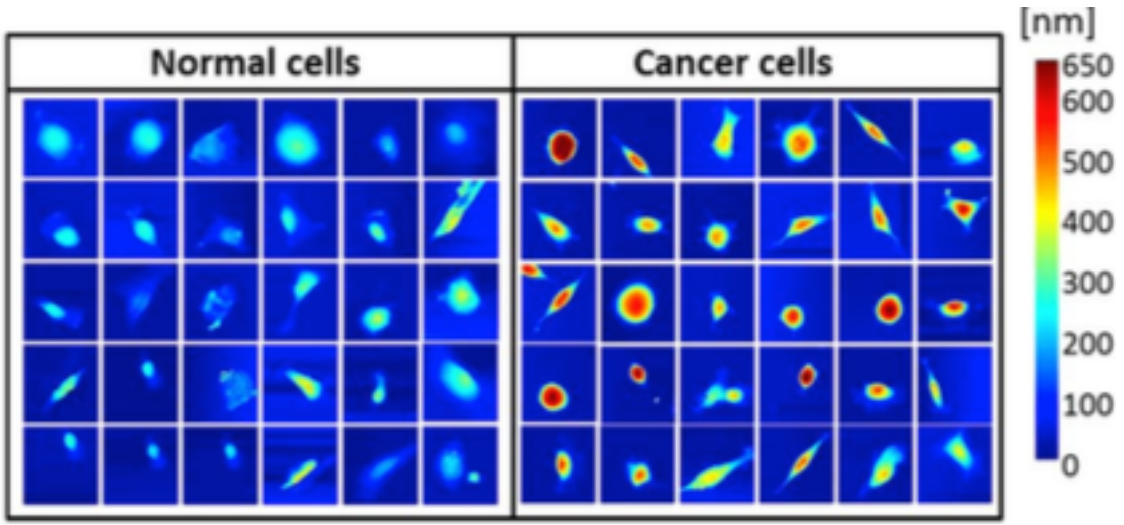
Scanned in 2D over 20 x 20 micron

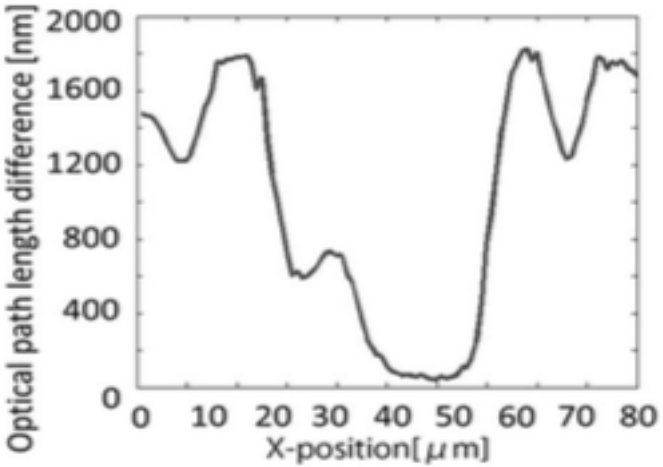
Nominal and measured pitch of 2.2 microns



Thick cells (about 3 lambda optical path length differences)







Summary and Conclusion

- Cancer cells associated with higher optical path length difference than that of the normal cells

- The system can measure transparent objects without using phase unwrapping process even if the thickness of the object is more than wavelength

Comment from the presenter

The calibration of voltage with displacement is not exactly linear, how did the authors calibrate the PZD for a high dynamic range?

2- Label-free imaging of intracellular motility by low-coherent quantitative phase microscopy, OE (2011), Japan

Toyohiko Yamauchi,* Hidenao Iwai, and Yutaka Yamashita

Hamamatsu Photonics K. K., 5000, Hirakuchi, Hamamatsu City, Shizuoka Pref., 434-8601, Japan

**t-yamauchi@crl.hpk.co.jp*

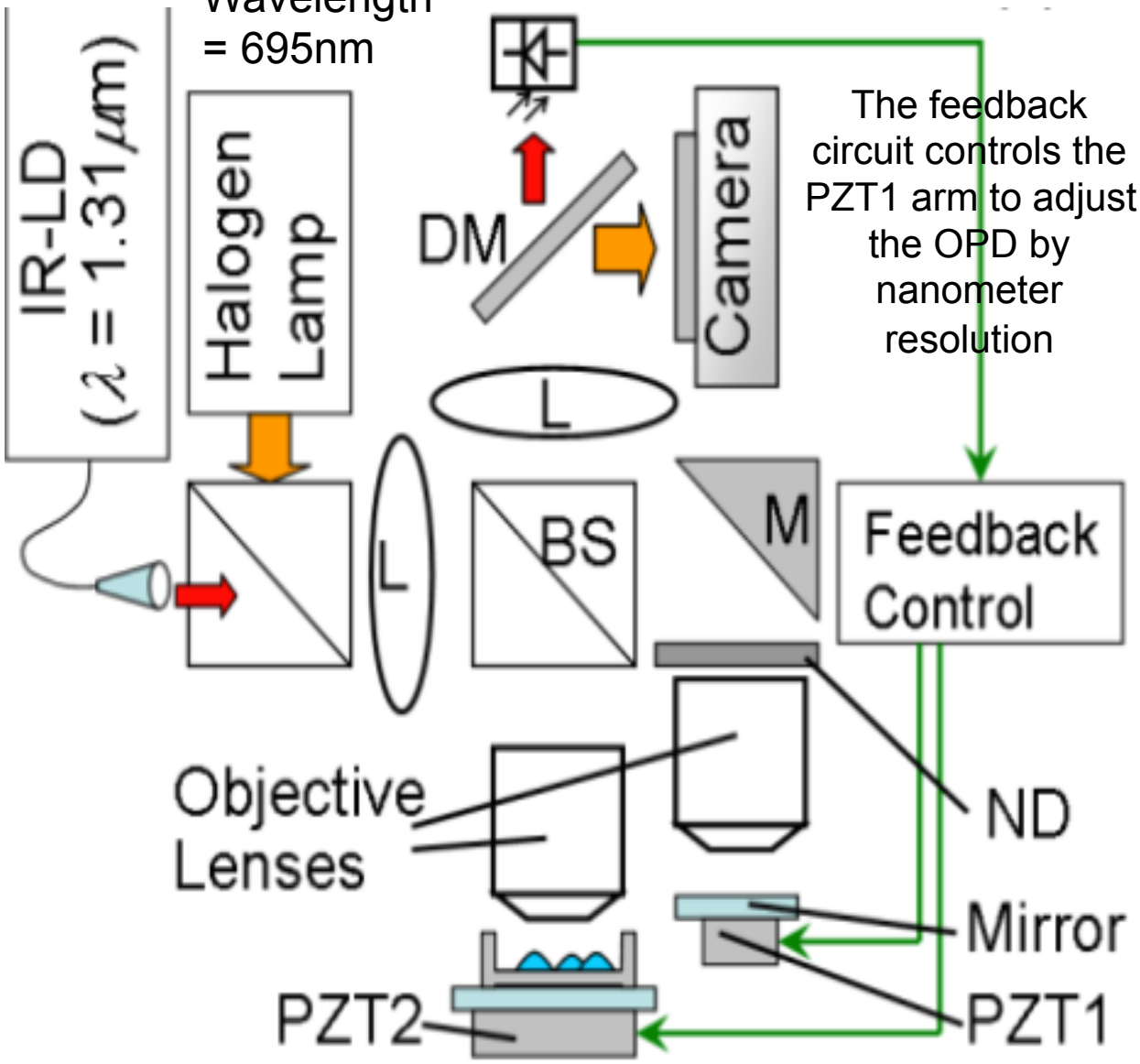
Objective

- ✓ Low-coherent interferometric technique and phase-referenced phase shifting technique were integrated to reveal the depth of intracellular motility without contrast agents.

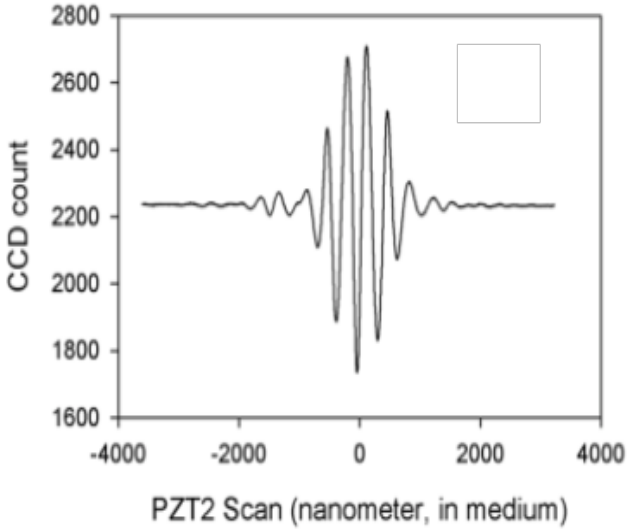
Notes

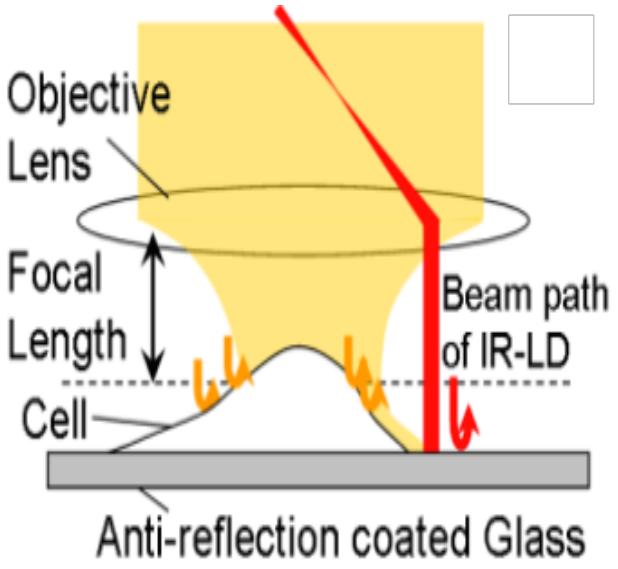
- ✓ This paper shows the 3D of quantitatively phase fluctuation caused by the motion of multiple-surfaces in cultured cells.
- ✓ The authors used high NA with low-coherence light source
- ✓ Axial and transversal resolutions were 0.93 and 0.56 microns
- ✓ results revealed the depth fluctuations of the intracellular surfaces such as plasma membrane, reflecting surfaces in cytoplasmic region and the surface of substrate independently.

Experimental setup based on Linnik configuration

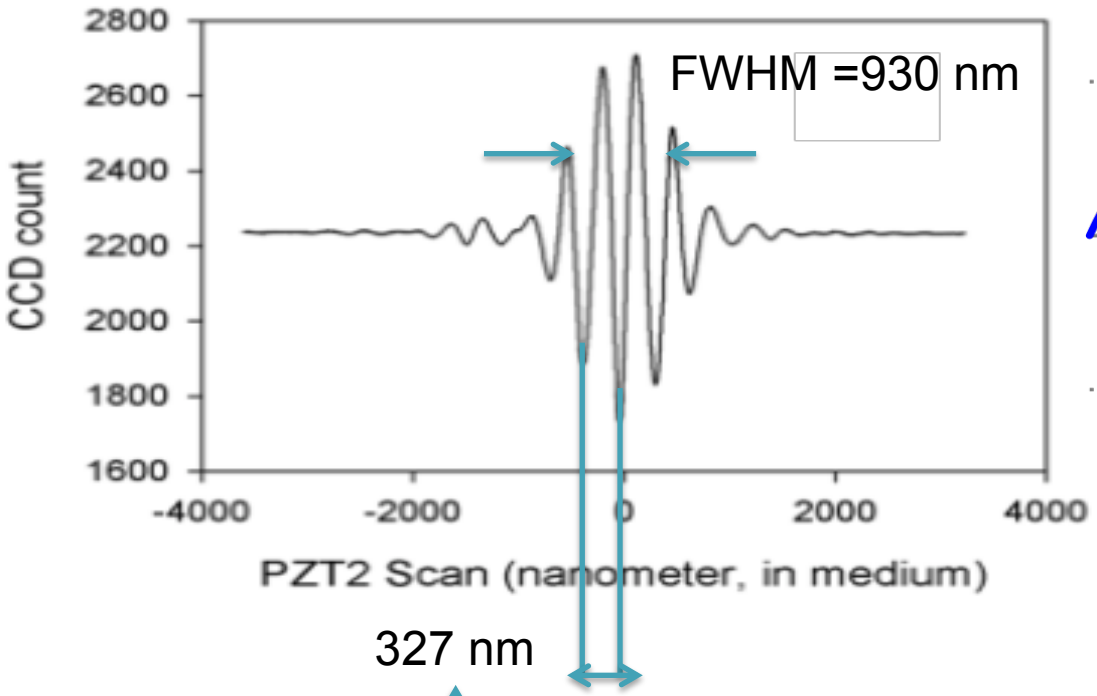


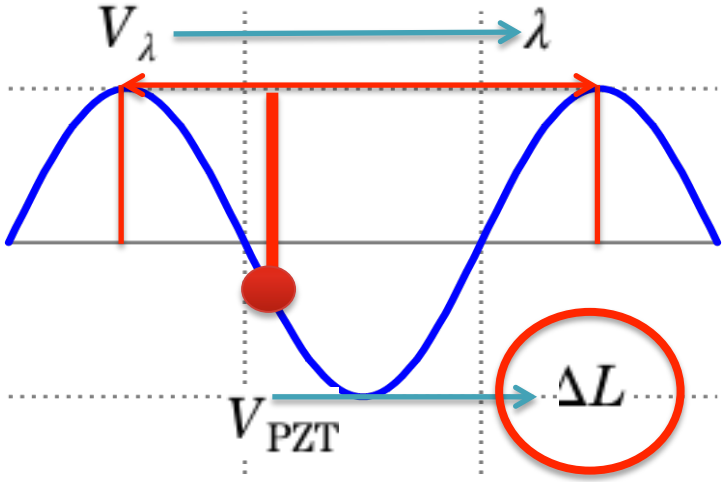
The feedback circuit controls the PZT1 arm to adjust the OPD by nanometer resolution





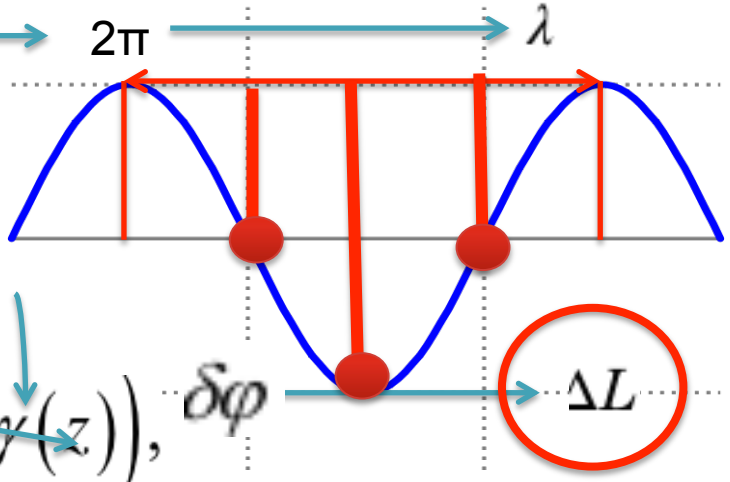
Method, similar to OL , phase shifting 7 frame algorithm





OL work

OE work



Seven raw images

$$I_{-3\pi/2}, I_{-\pi}, I_{-\pi/2}, I_0, I_{\pi/2}, I_\pi, I_{3\pi/2}$$

Optical path difference

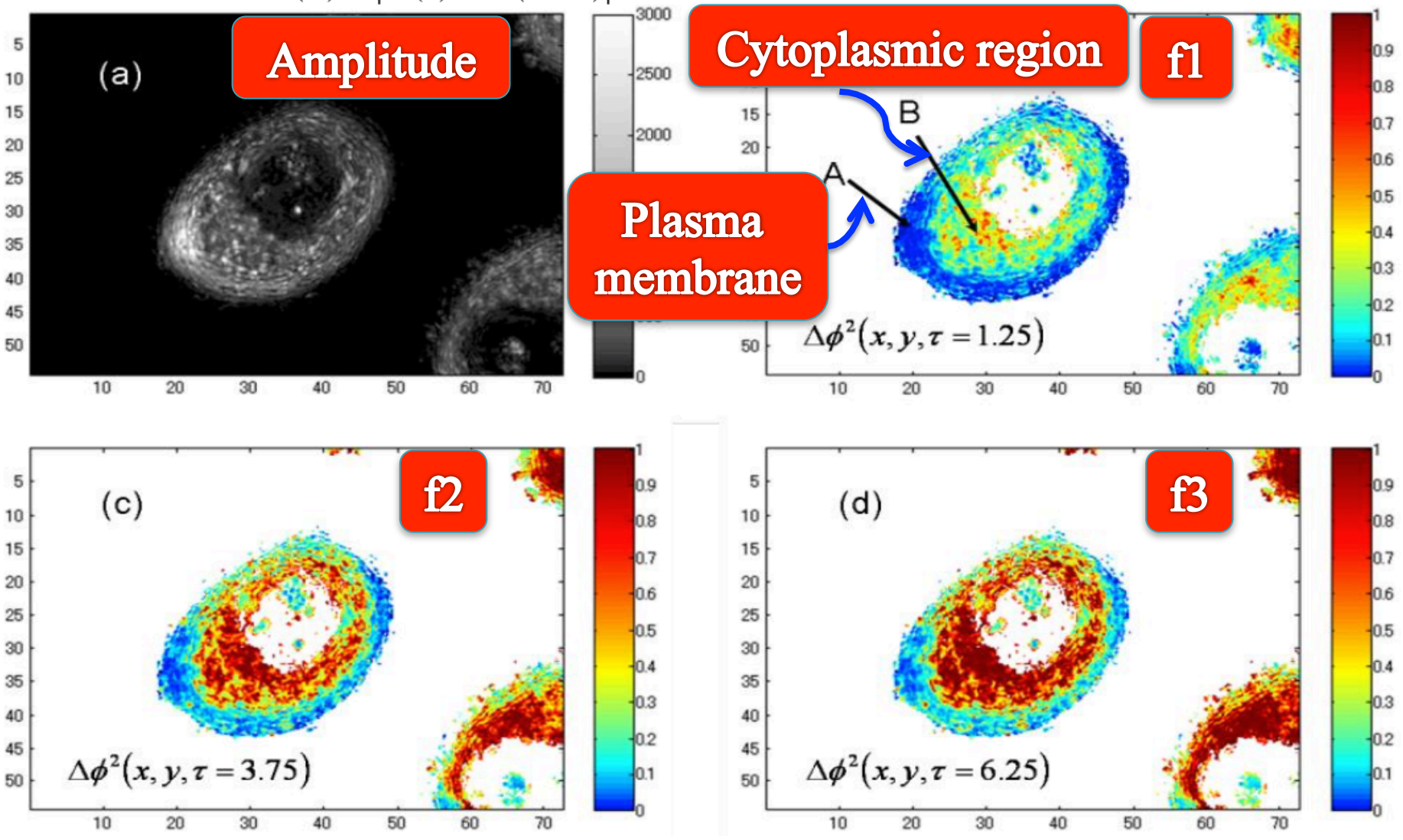
Optical Coherence Function

$$I_{\delta\varphi} = \eta\left(|E_r|^2 + |E_s|^2 + 2|E_r||E_s|\cos(\varphi - \delta\phi)\cdot\gamma(z)\right),$$

Results: human breast cancer cells (living)

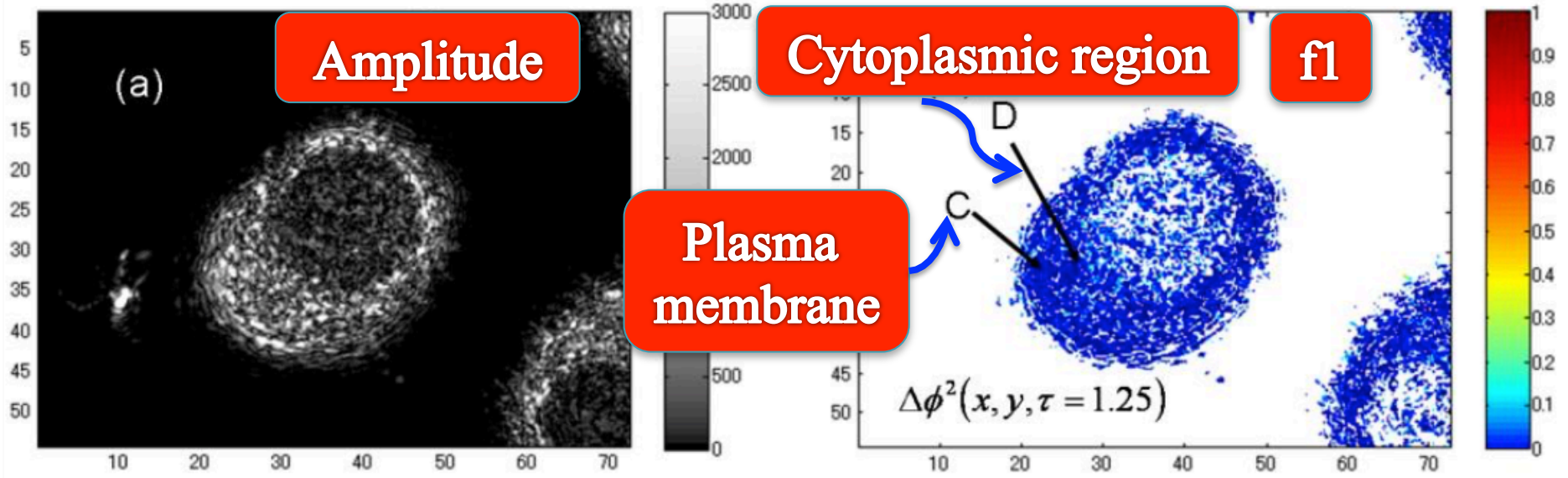
To quantify the phase fluctuation, the authors used mean squared displacement (MSD)

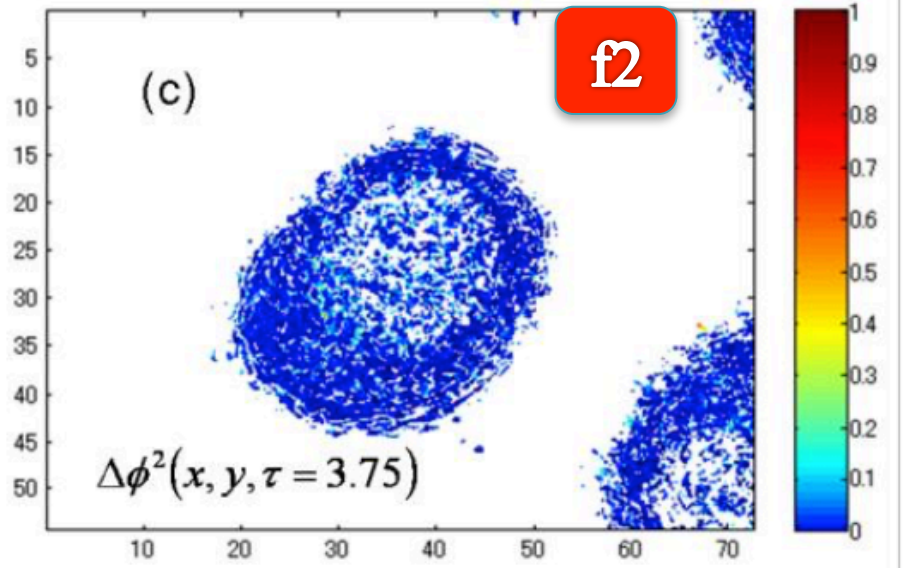
$\Delta\phi^2(\tau)$ $\Delta\varphi^2(\tau) = \left|\varphi(t) - \varphi(t-\tau)\right|^2$

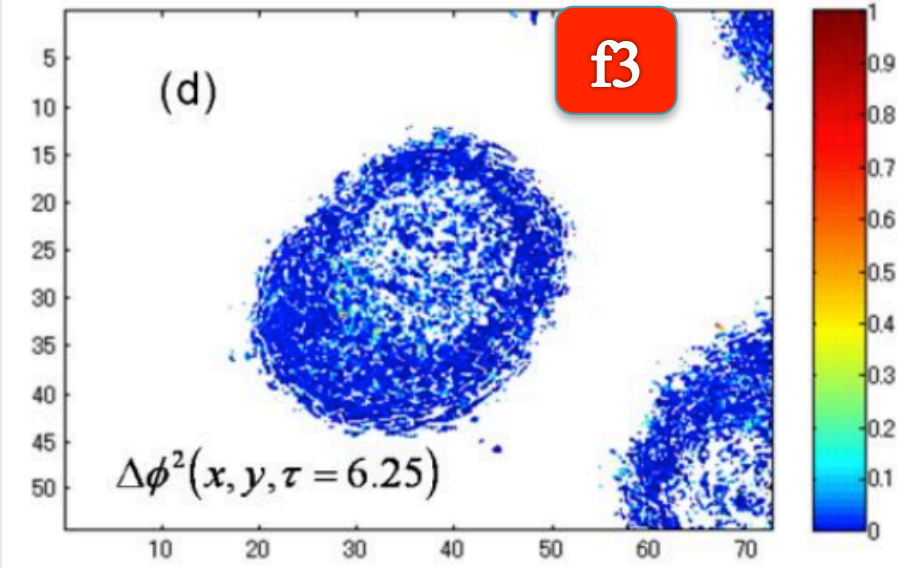


Results: human breast cancer cells (dead)

The samples were fixed with 2% paraformaldehyde, after treatment, the cells died.

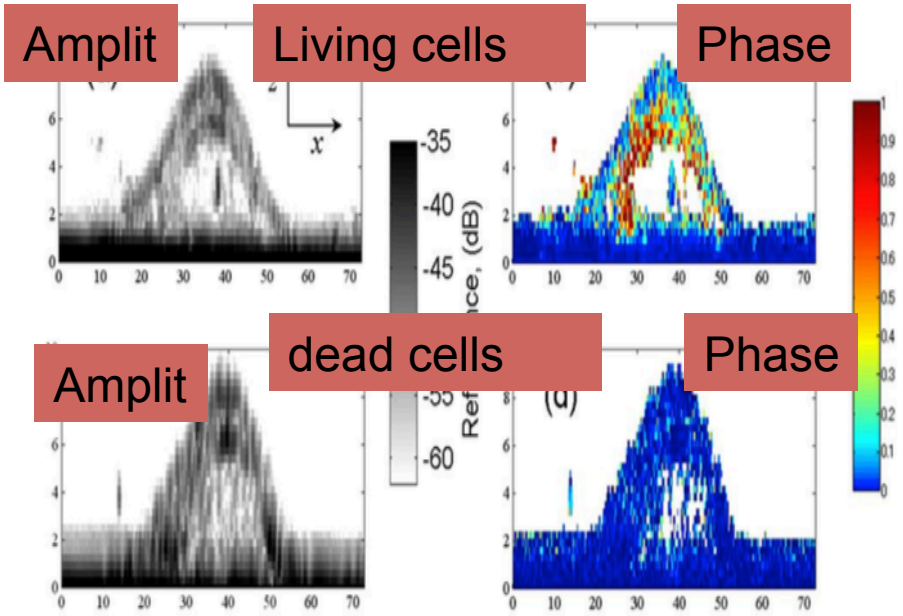


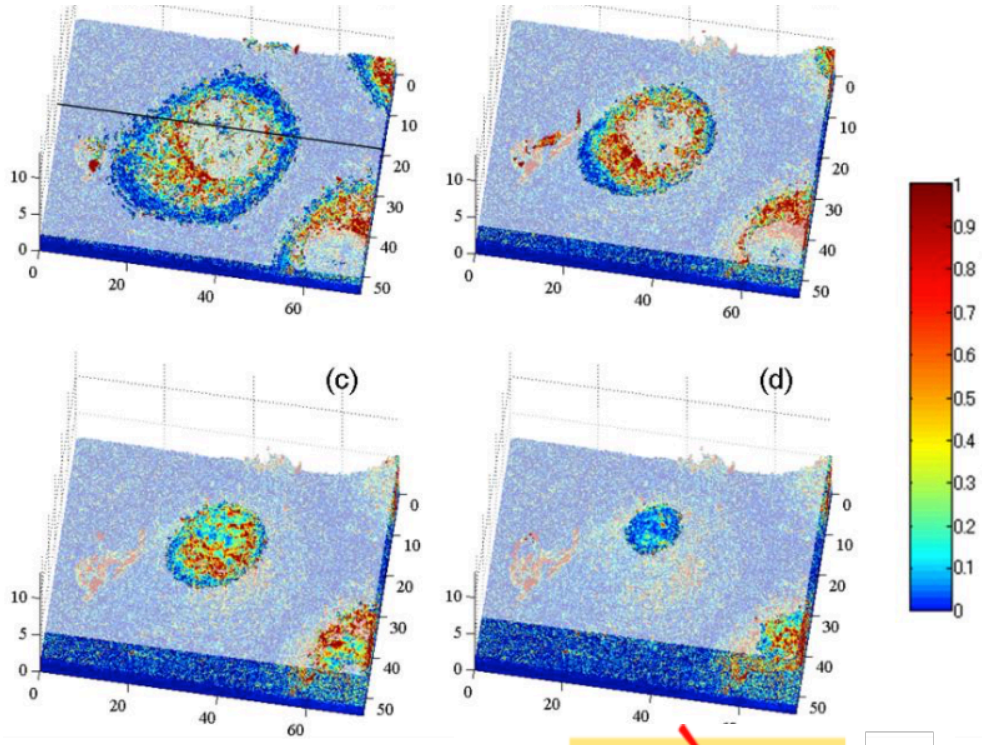




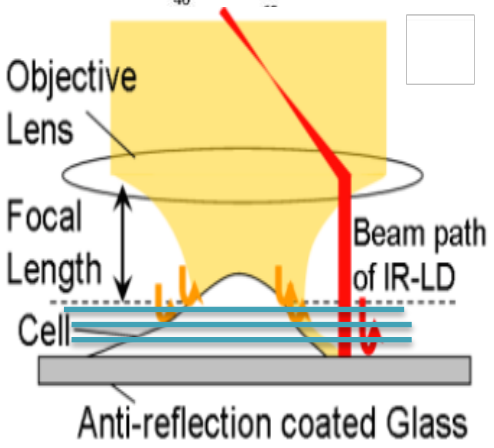
To see the nuclear membrane

To analyze the intracellular structure in detail. The authors captured the phase-fluctuation image at different heights





The authors claim that there is a spherical membrane inside the cell, which they believe is the nuclear membrane



Summary and Conclusion

- The authors used low coherent QPM to visualize the distribution of the fluctuations of the intracellelular surfaces and cell membrane.

- This was done in the paper by using low-coherent interferometric technique and phase-referenced phase shifting technique without contrast agents.

3- Wide-field optical detection of nanoparticles using on-chip microscopy and self-assembled nanolenses, Nature (2013)

Onur Mudanyali[1,2†], Euan McLeod[1,2†], Wei Luo[1,2], Alon Greenbaum[1,2], Ahmet F. Coskun[1,2], Yves Hennequin[3], Cédric P. Allier[3] and Aydogan Ozcan[1,2,4,5★]

University of California

Objective

Compact, cost-effective and high-throughput optical microscopy technique that can detect individual sub-100 nm particles and moderately sized viruses across and ultralarge FOV of 20.2 mm^2 is presented.

Method

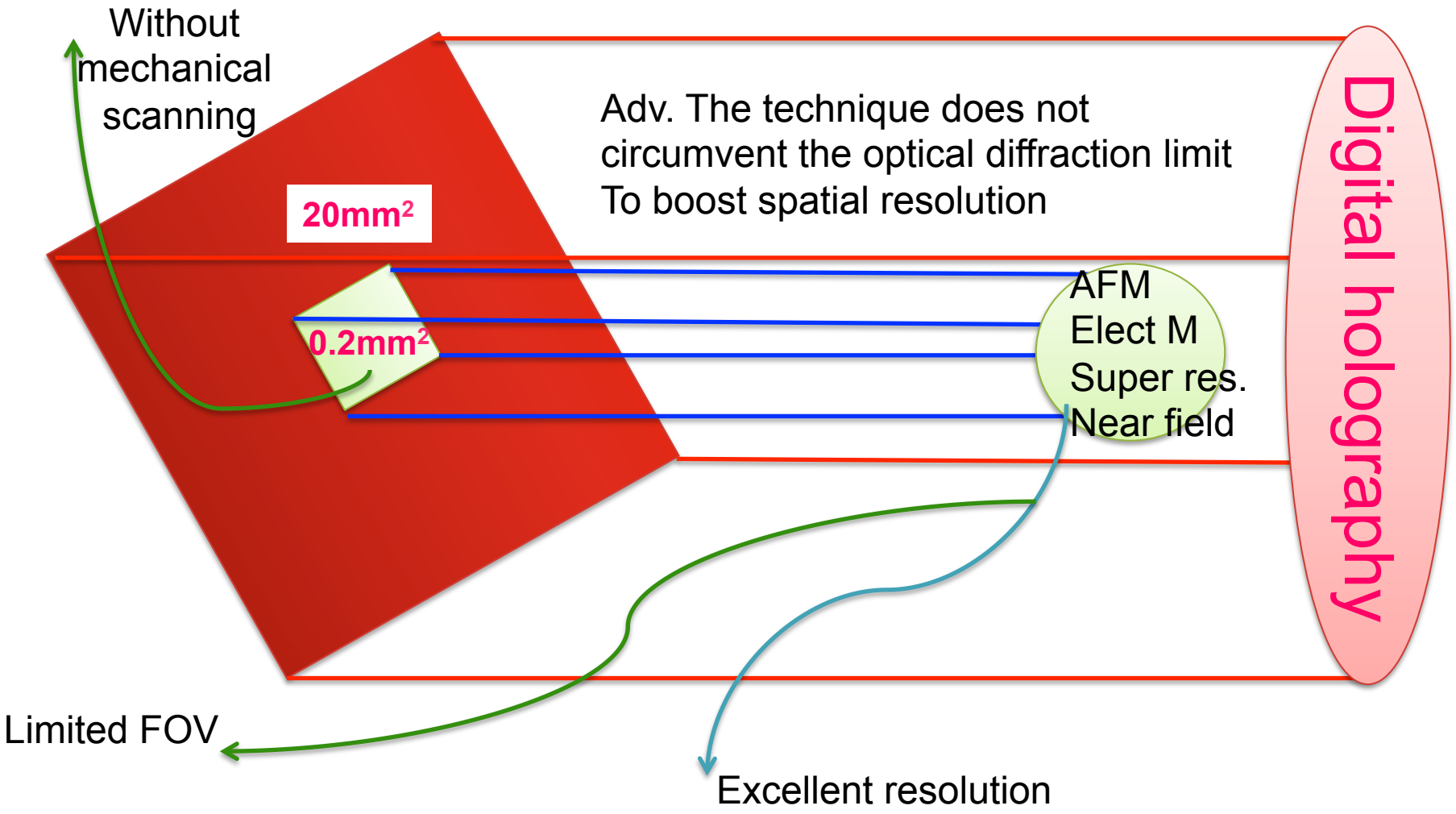
Digital holography – Angular spectrum method

Objects

Polystyrene nanoparticles, adenoviruses and influenza A (H1N1) viral particles

Introduction

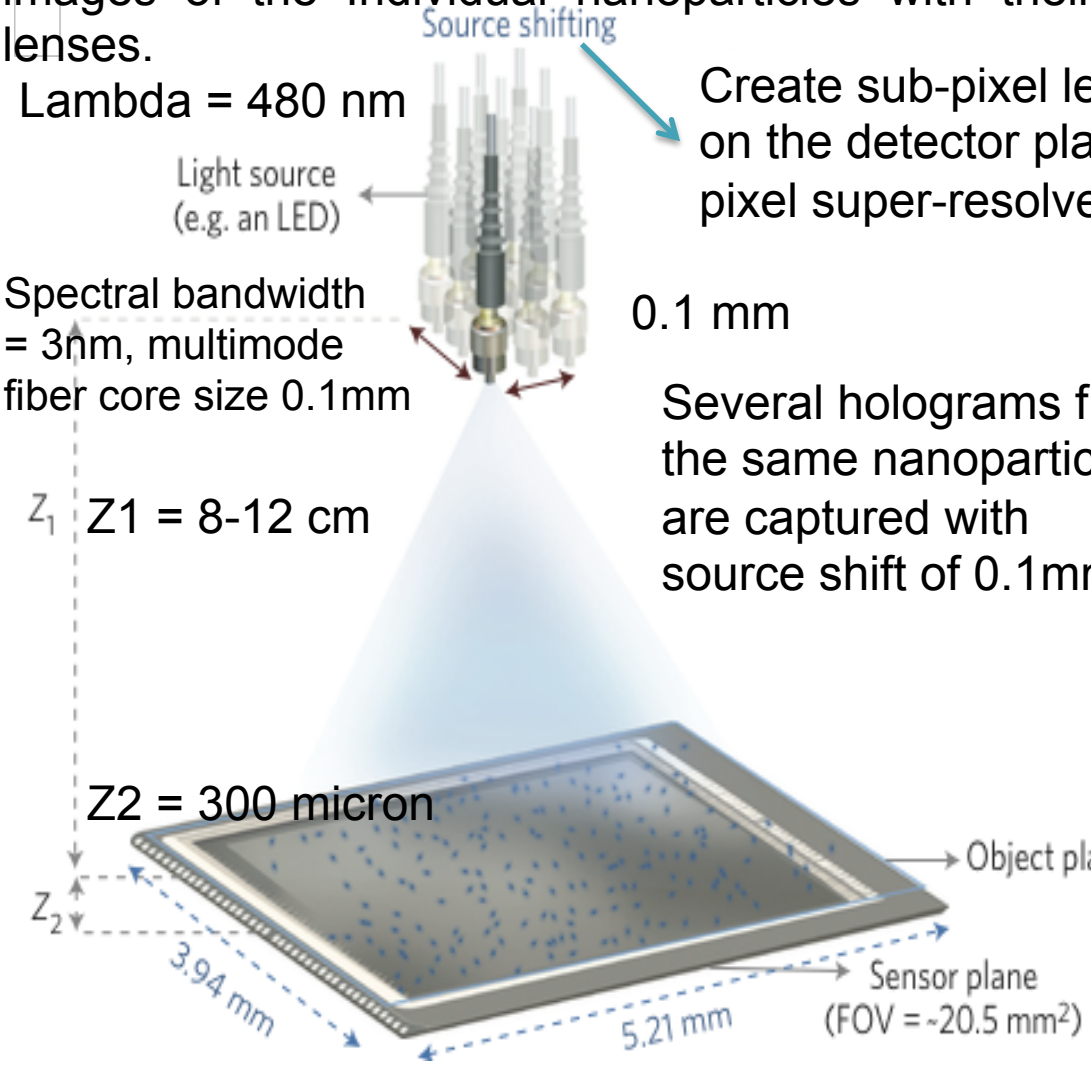
Nano scale objects are difficult to visualize using optical techniques (wavelength is greater than nano size)



Setup Lensfress pixel super-resolution holography schematic

A single pixel super-resolved holographic image is then synthesized from these sub-pixel shifted holograms, and is finally reconstructed to yield phase and amplitude images of the Individual nanoparticles with their surrounding self-assembled liquid lenses.

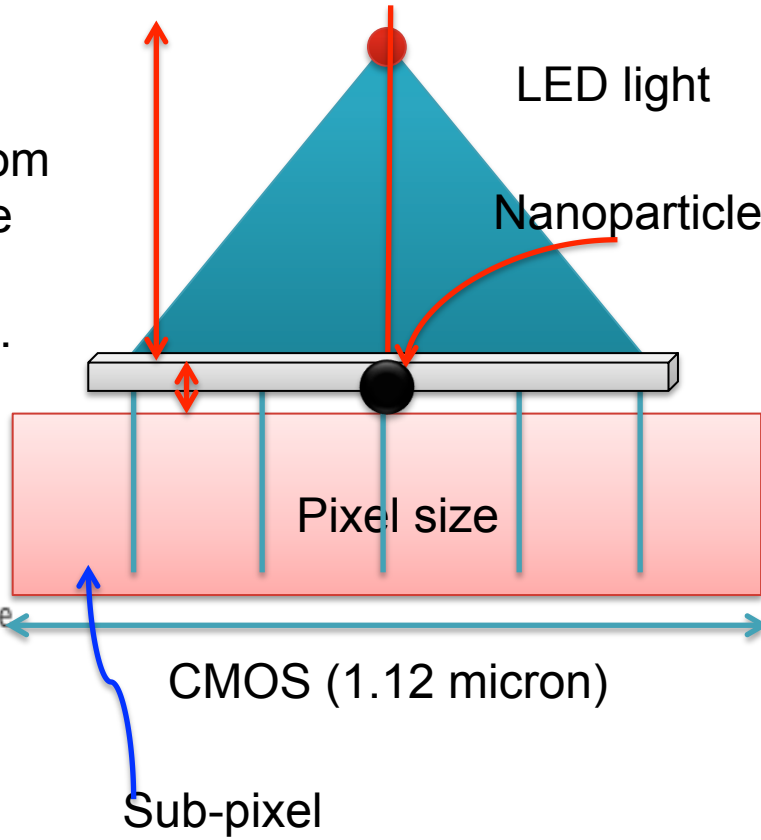
Lambda = 480 nm



Create sub-pixel less than 1.12 micron image shifts on the detector plane, which are used to generate a pixel super-resolved holographic image

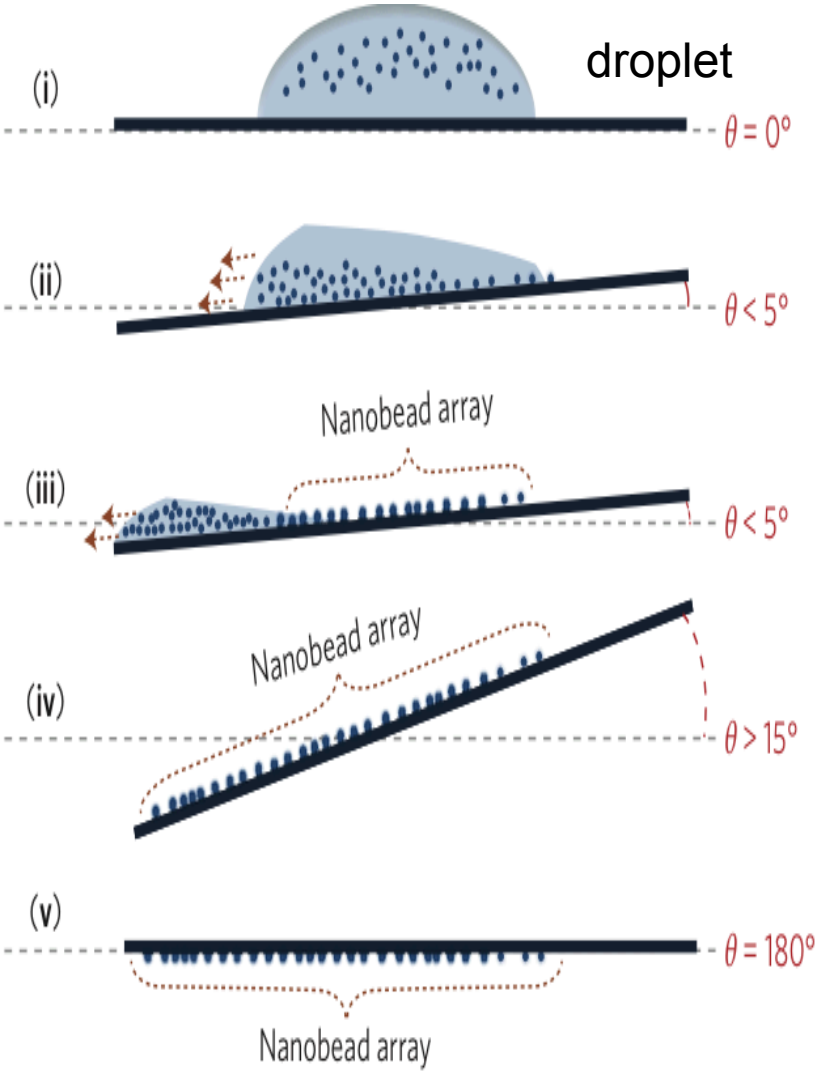
0.1 mm

Several holograms from the same nanoparticle are captured with source shift of 0.1mm.

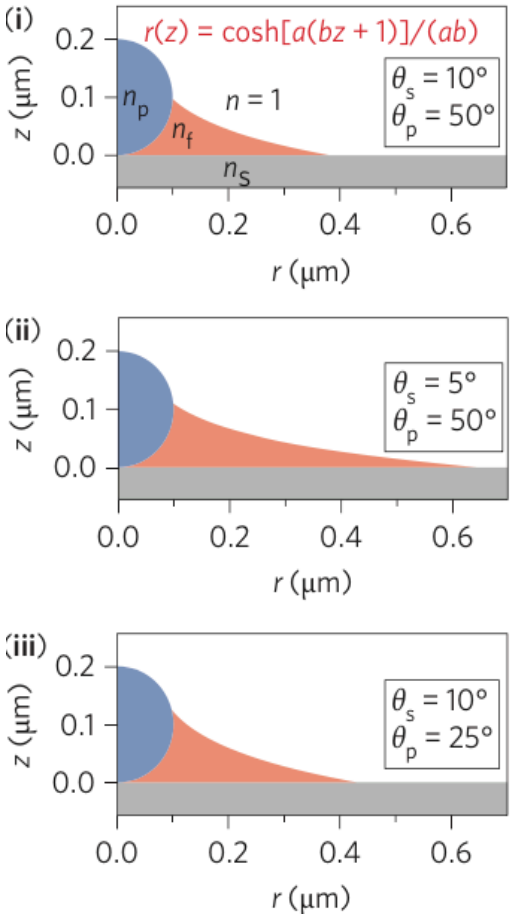


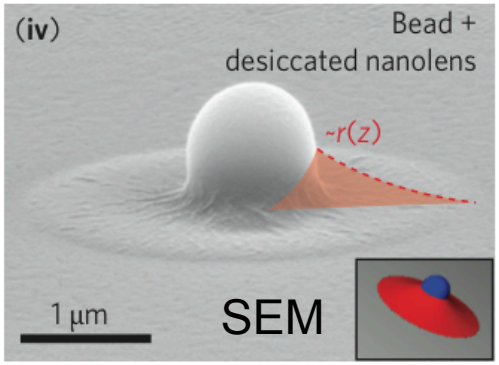
Sample preparation (nanoparticles)

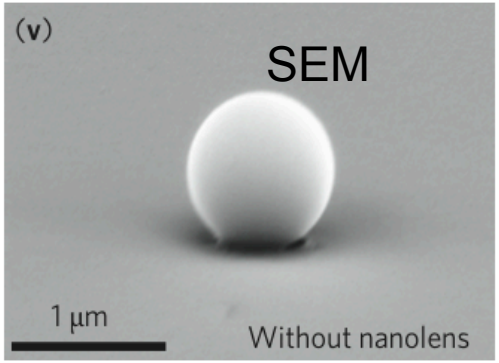
Liquid nanolenses are assembled around each nanoparticle seated on hydrophilic surface



These liquid lenses acting as a spatial phase mask that enhance the diffraction signature of the embedded nanoparticles/nanolense

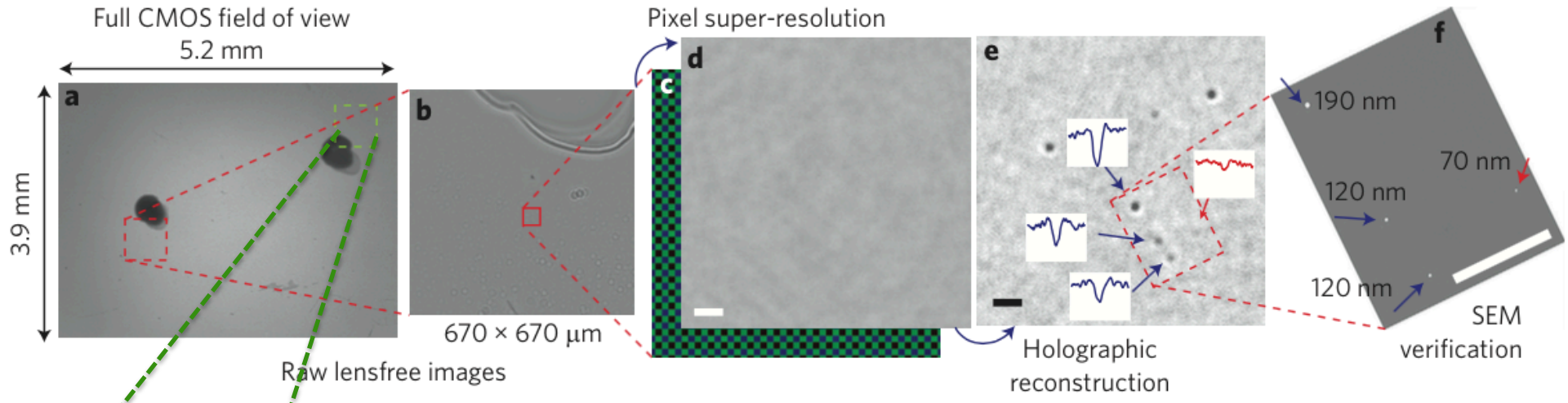


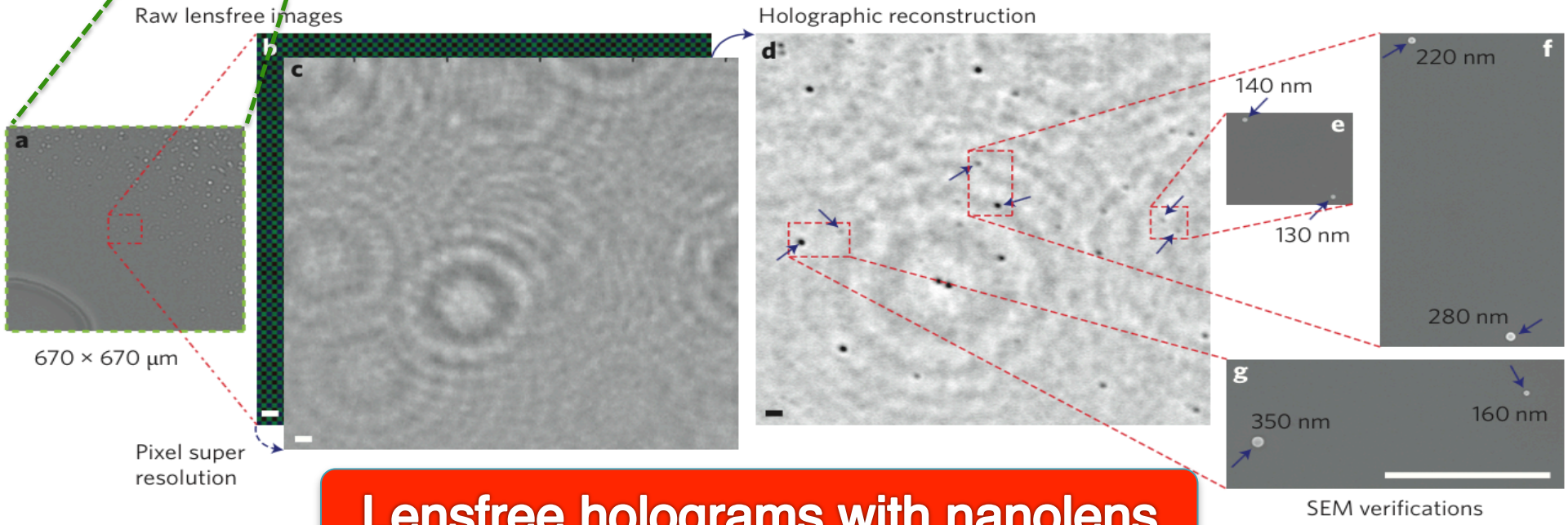




Results

Lensfree holograms without nanolens



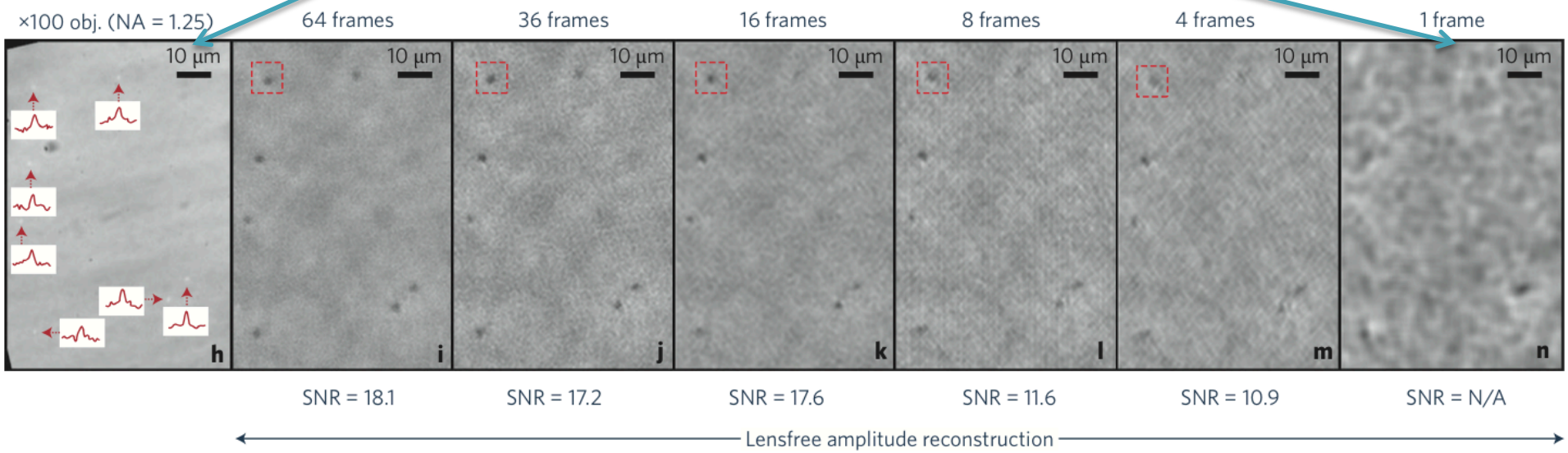


Lensfree holograms with nanolens

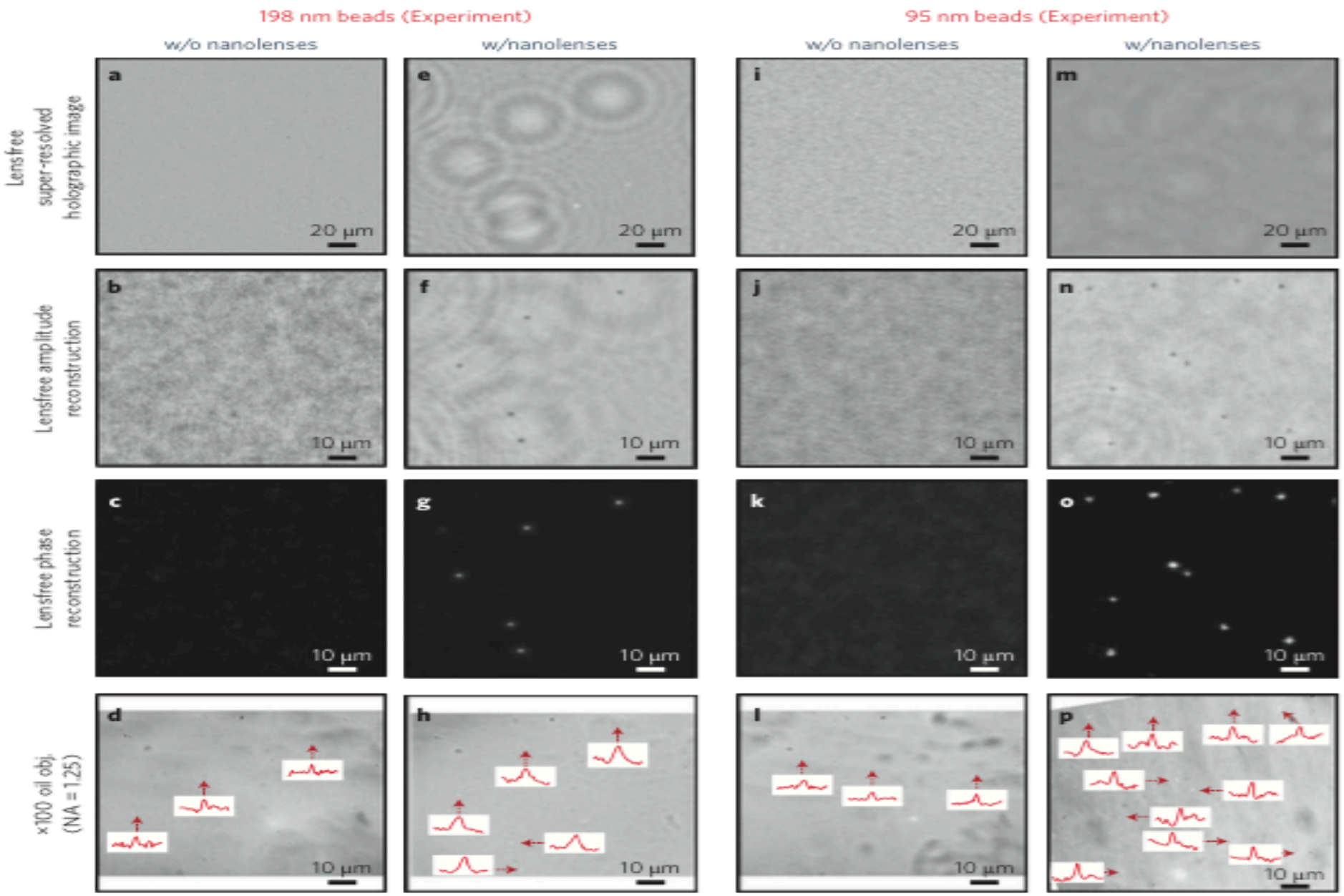
Effect of no.of holograms in super-resolution on contrast of 95 nm particles

- Increasing the number of holographic frames used in pixel super-resolution algorithm significantly enhance the contrast and the SNR of individual nanoparticles.

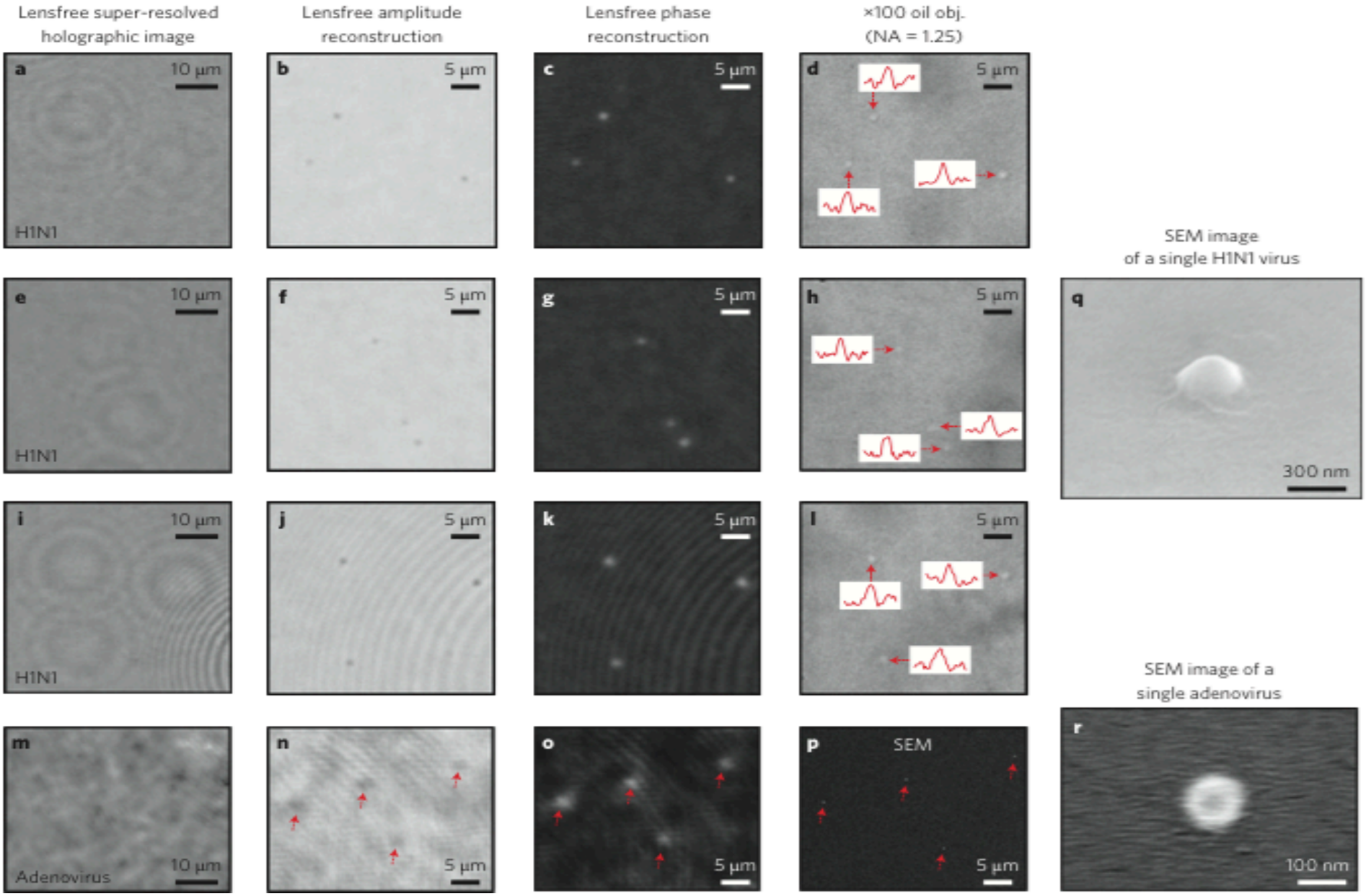
The combination of self-assembled liquid nanolenses and holographic computational on-chip microscopy enables the detection of individual sub-100 nm particles that are Not visible with holographic imaging alone and have low contrast with conventional oil immersion objective lenses NA of (1.25).



Particles of 95 nm and 198 nm with nanolenses



Individual influenza A (H1N1) viruses and adenoviruses



Summary and Conclusion

- The authors introduced a compact, cost-effective and high throughput computational on-chip microscopy technique that can detect individual sub-100 nm particles and viruses across an ultralarge FOV of 20.5 mm^2.

- Through a wetting film-based method that induces self-assembled liquid nanolenses around individual particles, the authors reconstructed both amplitude and phase images of single nanoparticles that are otherwise undetectable with conventional microscopy.

Thank you for listening.
Any questions or suggestions?