# Democratization of Next-Generation Imaging, Diagnostics and Measurement Tools through Computational Photonics

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# Digital Diagnosis

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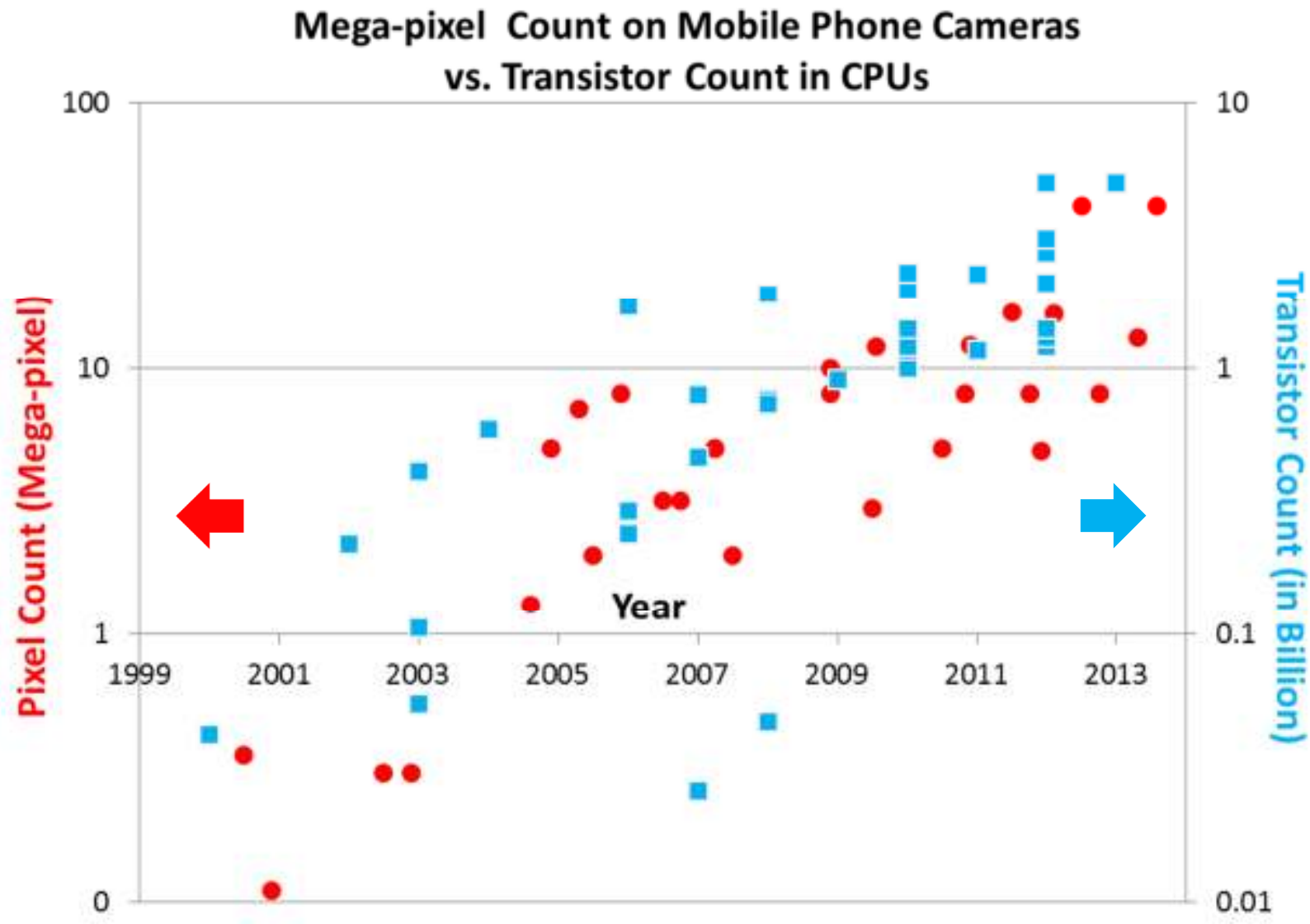
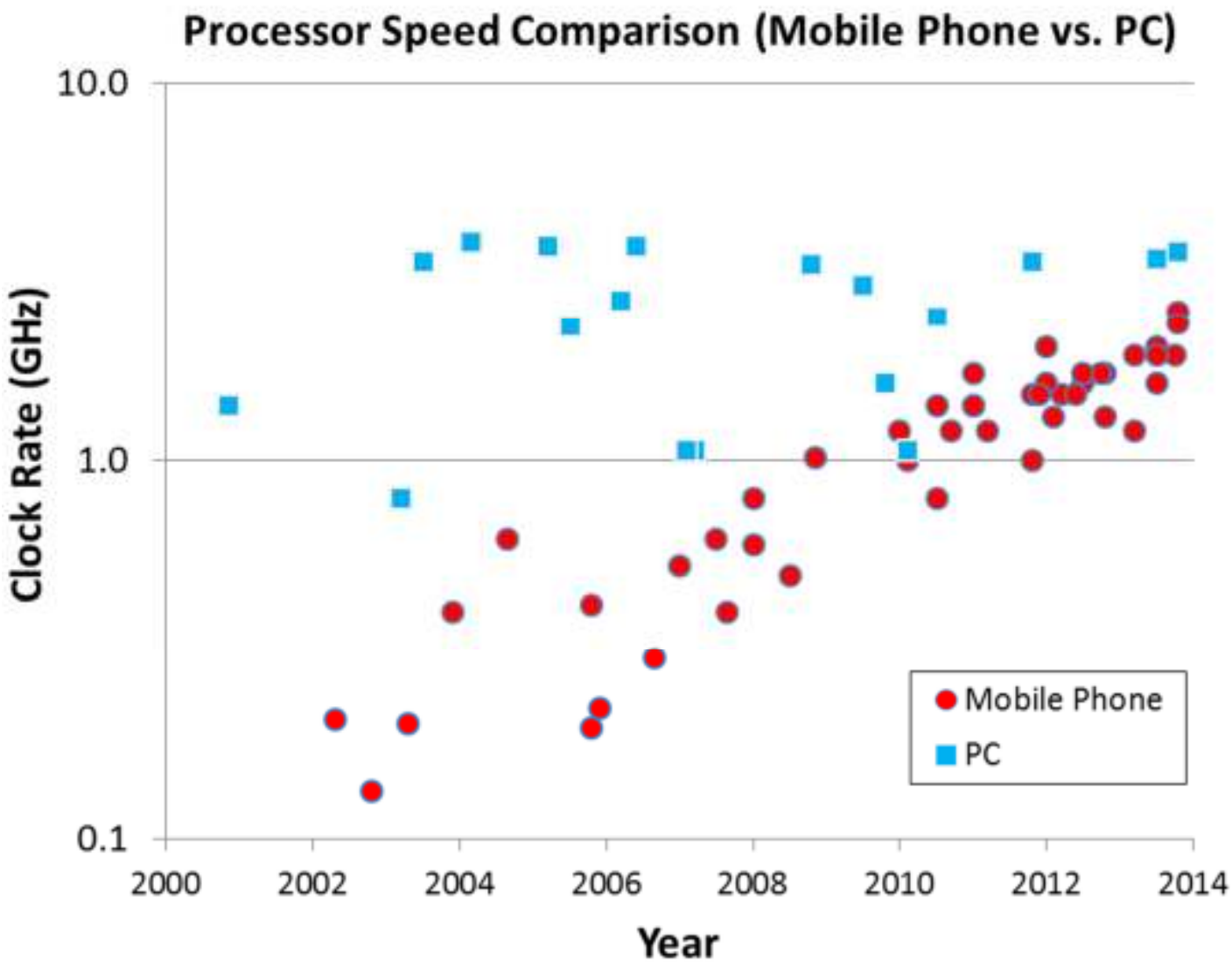
2015

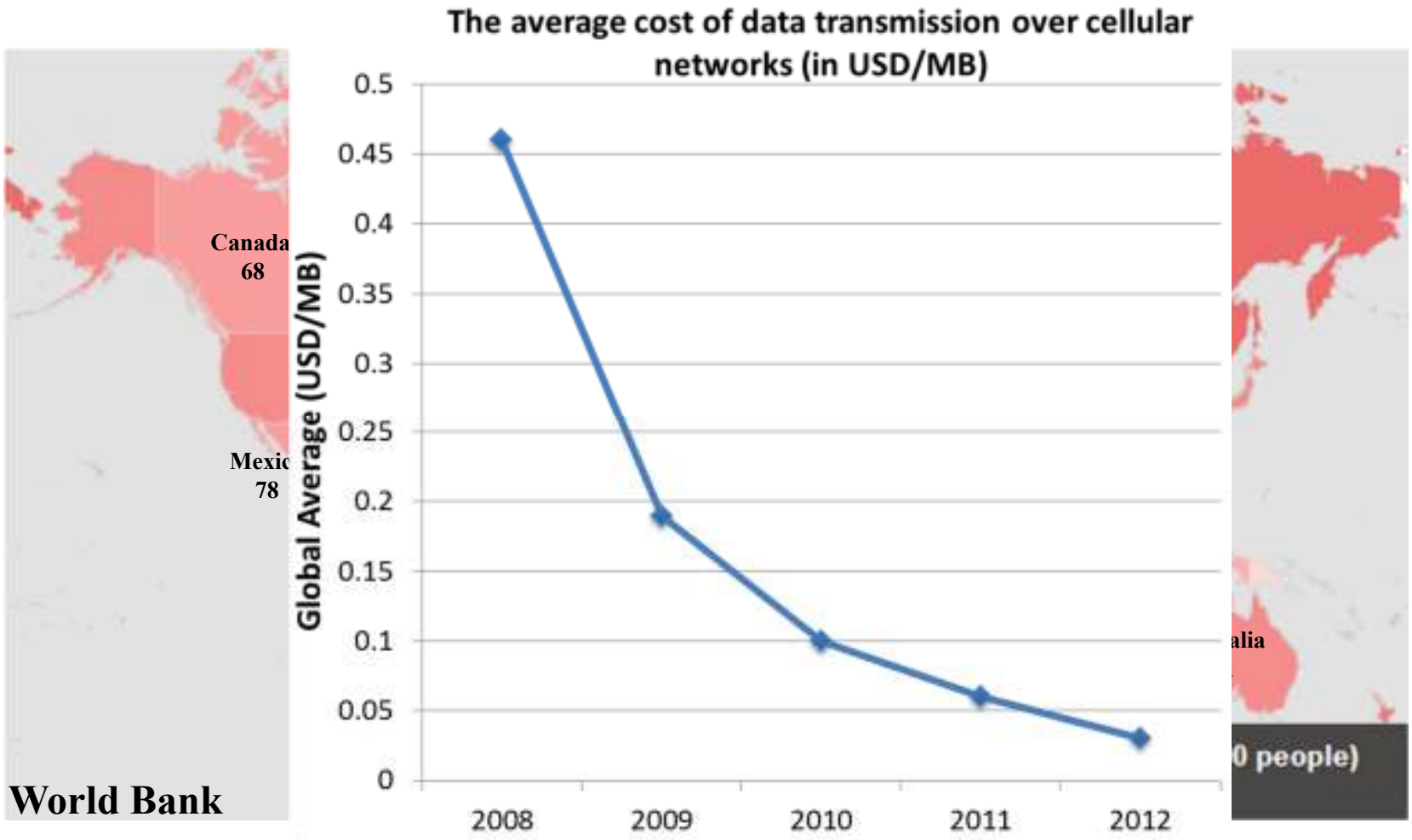
# *A different view of the Moore's Law*



# *Smart-phones as super-computers*

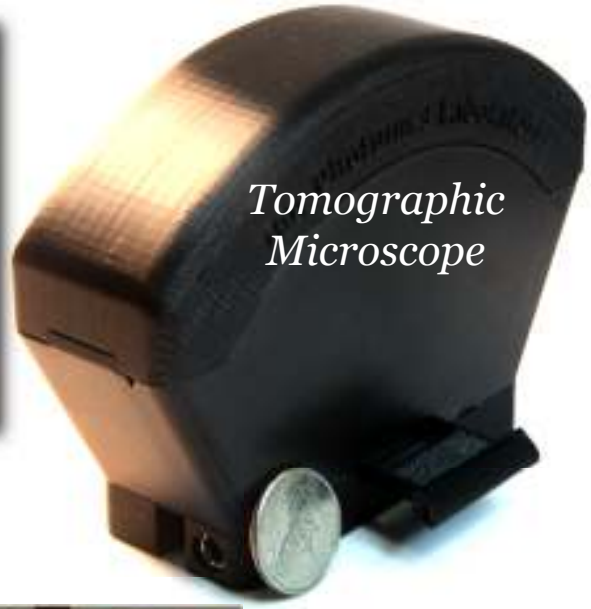


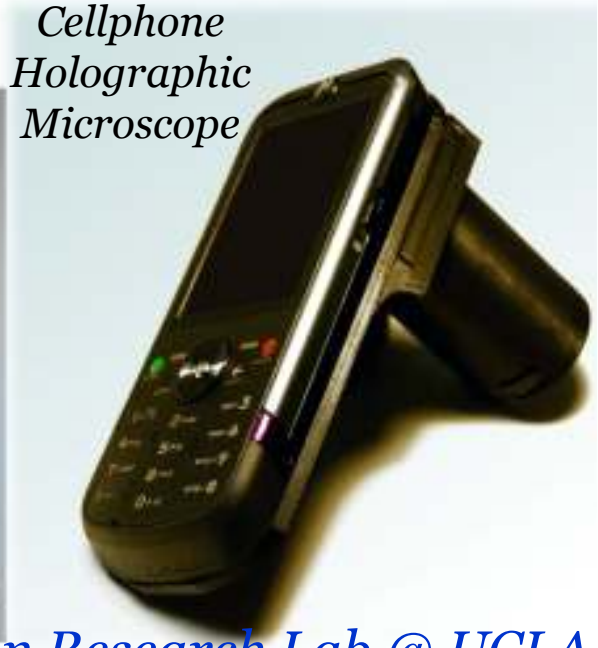
# *Cell phones are now everywhere: A great potential for Telemedicine needs*




- ~7 billion cell phones are being used worldwide.
- ~15 billion cell phones have been sold so far.
- > 75% are in developing countries. (International Telecommunication Union)

# *Providing a new platform for telemedicine*






*Ozcan Research Lab @ UCLA*

# *Providing a new platform for telemedicine*

*E. coli* sensor

*Allergen detector*

*Heavy metal detector*

*Google Glass based Diagnostics*

*Diagnostic test reader (HIV, malaria, etc.)*

*Blood Analyzer*

*Blood Analyzer*

# *Providing a new platform for telemedicine*

*E. coli* sensor

*Allergen detector*

*Heavy metal detector*

*Chlorophyll Measurement*

*Diagnostic test reader (HIV, malaria, etc.)*

*Blood Analyzer*

*Blood Analyzer*

# *The μ-Internet*

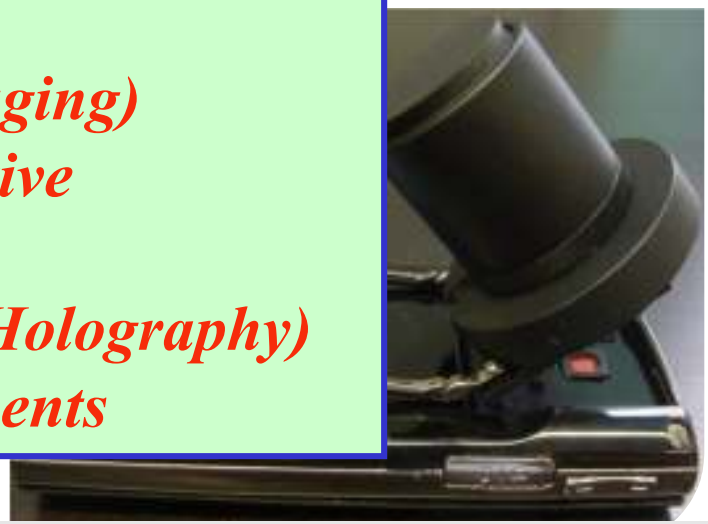
## ***Big Data***

*New opportunities in micro-analysis, medical diagnostics and epidemiology*

# *Providing a new platform for telemedicine*

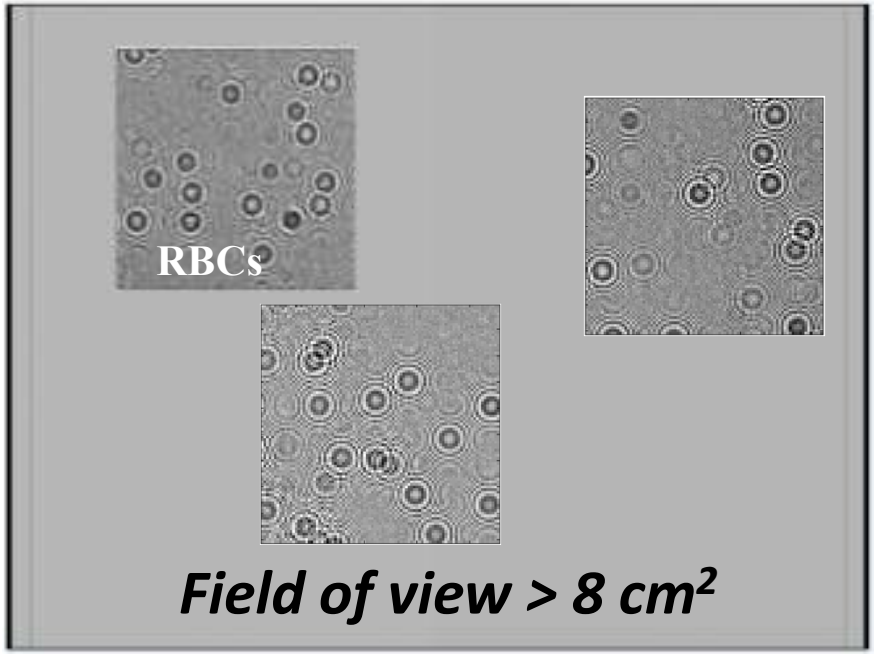
***LUCAS:***

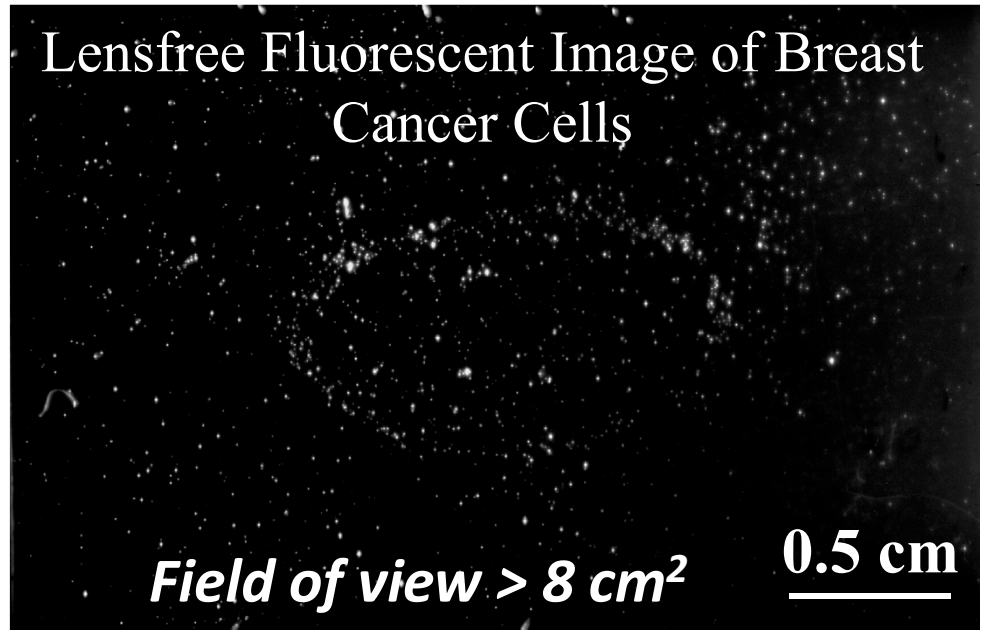
***Lensfree (On-Chip Imaging)***
***Compact, Cost-effective***
***High-throughput***
***Highly sensitive (Incoherent Holography)***
***Tolerant to Misalignments***



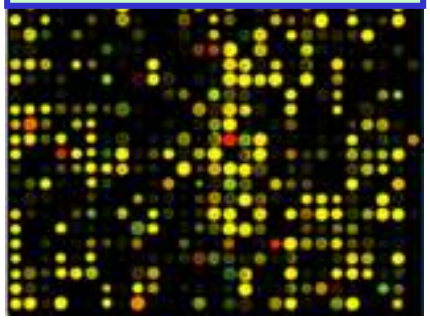
***LUCAS:*** ***L***ensless, ***U***ltra wide-field ***C***ell monitoring ***A***rray platform based on ***S***hadow imaging

# *Ultra-high throughput lensless imaging and cytometry*

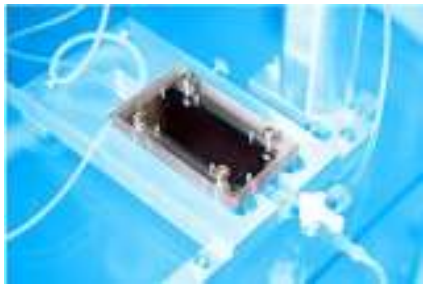


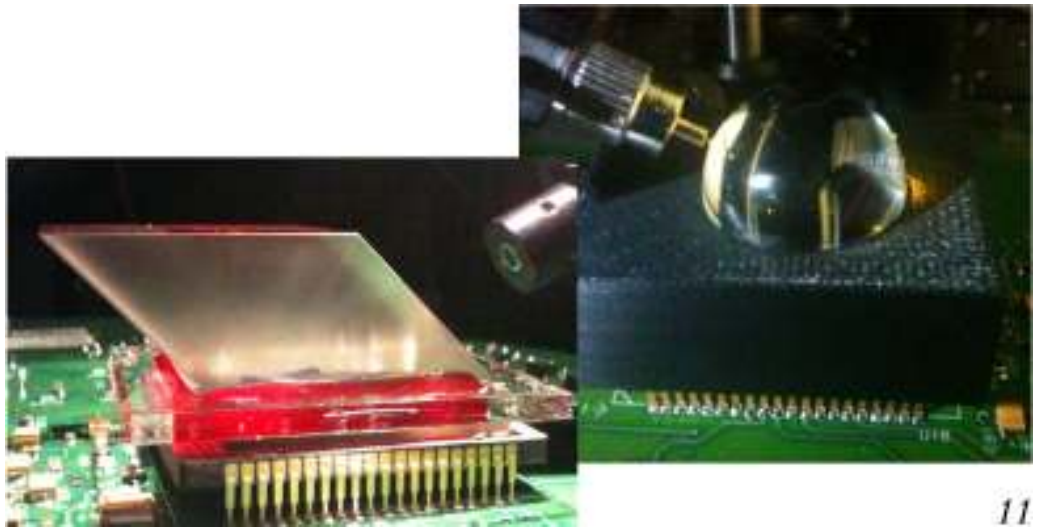




***High-throughput micro-array imaging***

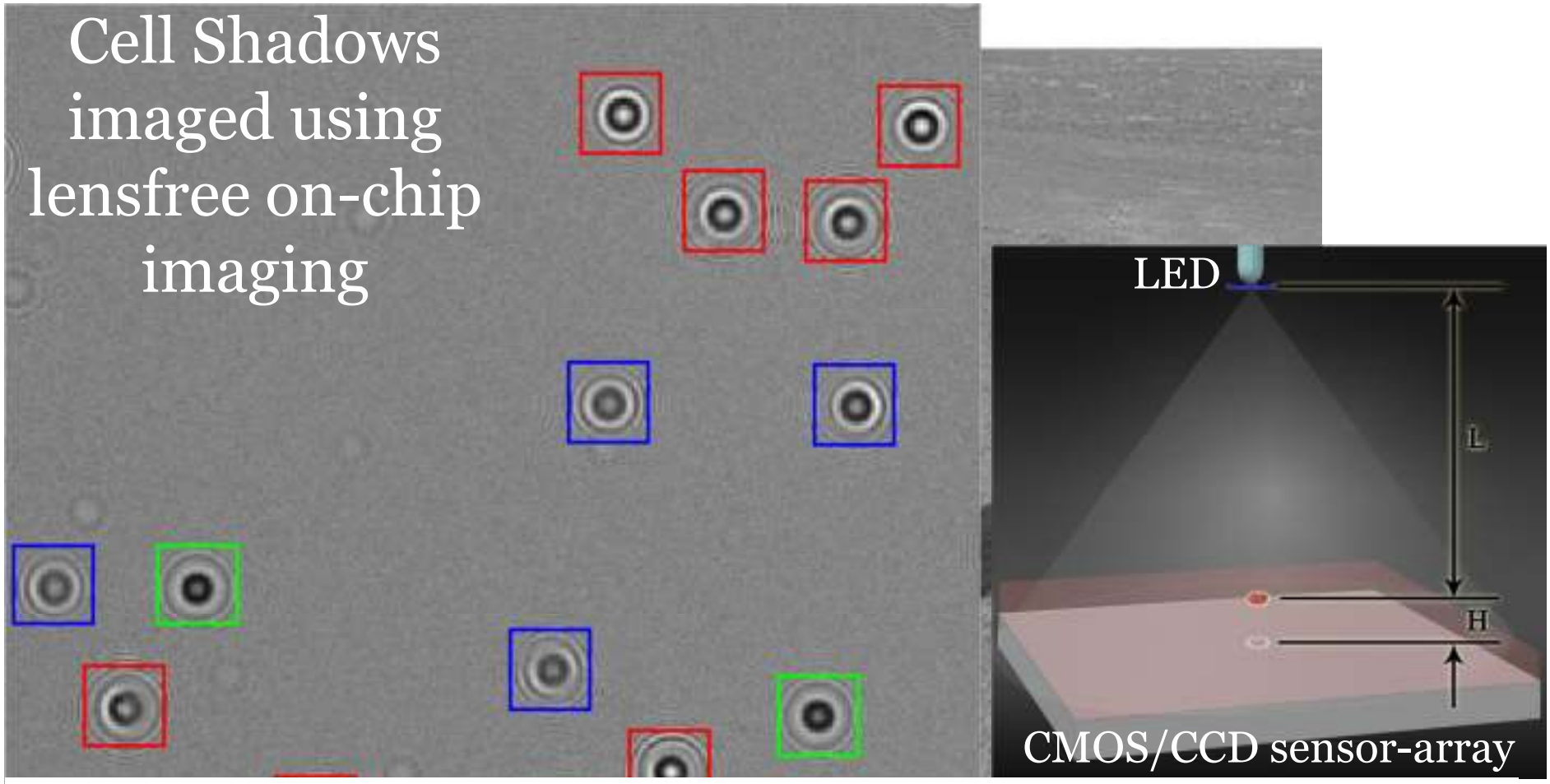


***Circulating tumor cell detection***



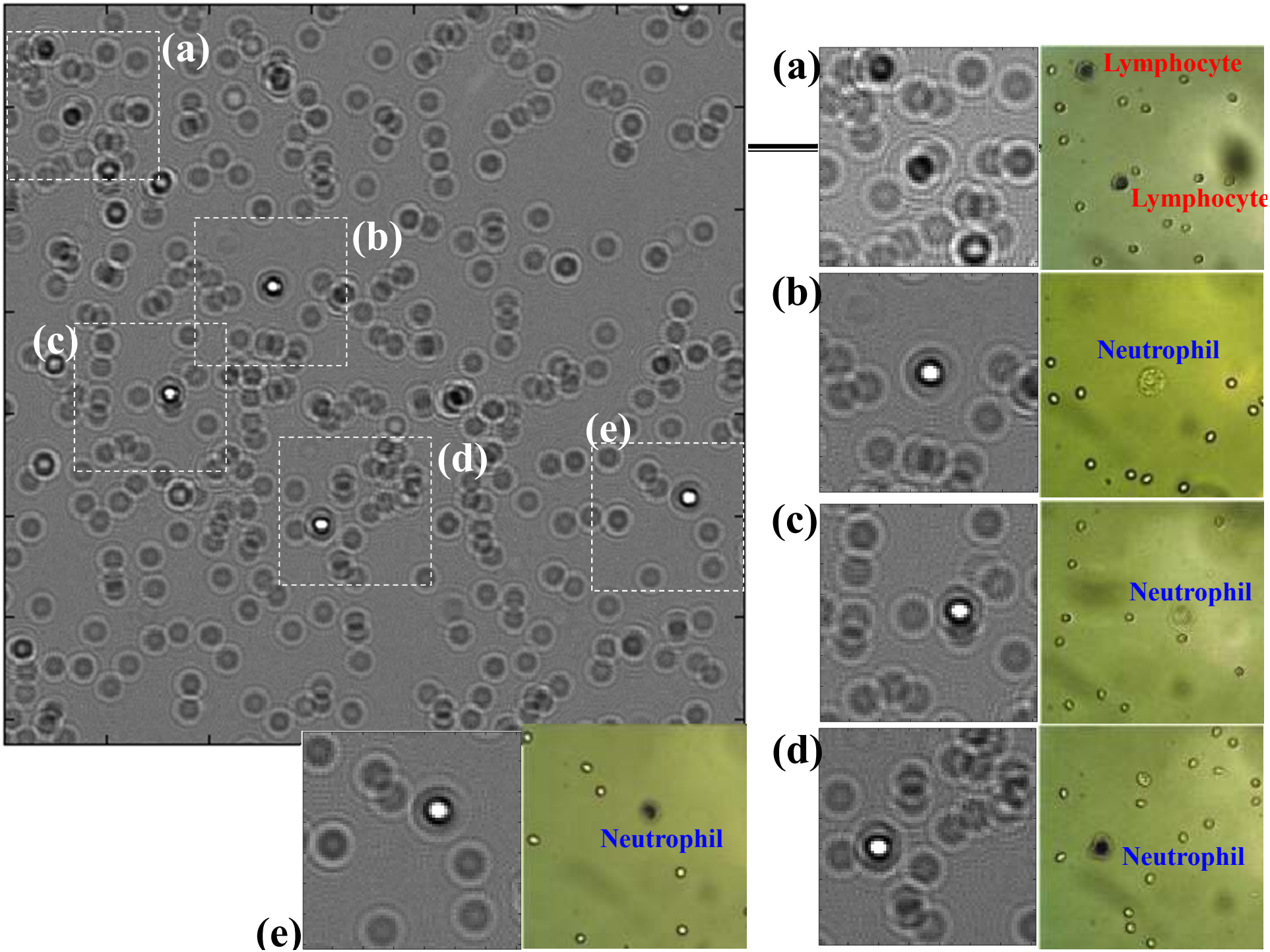


# *Imaging of shadows*

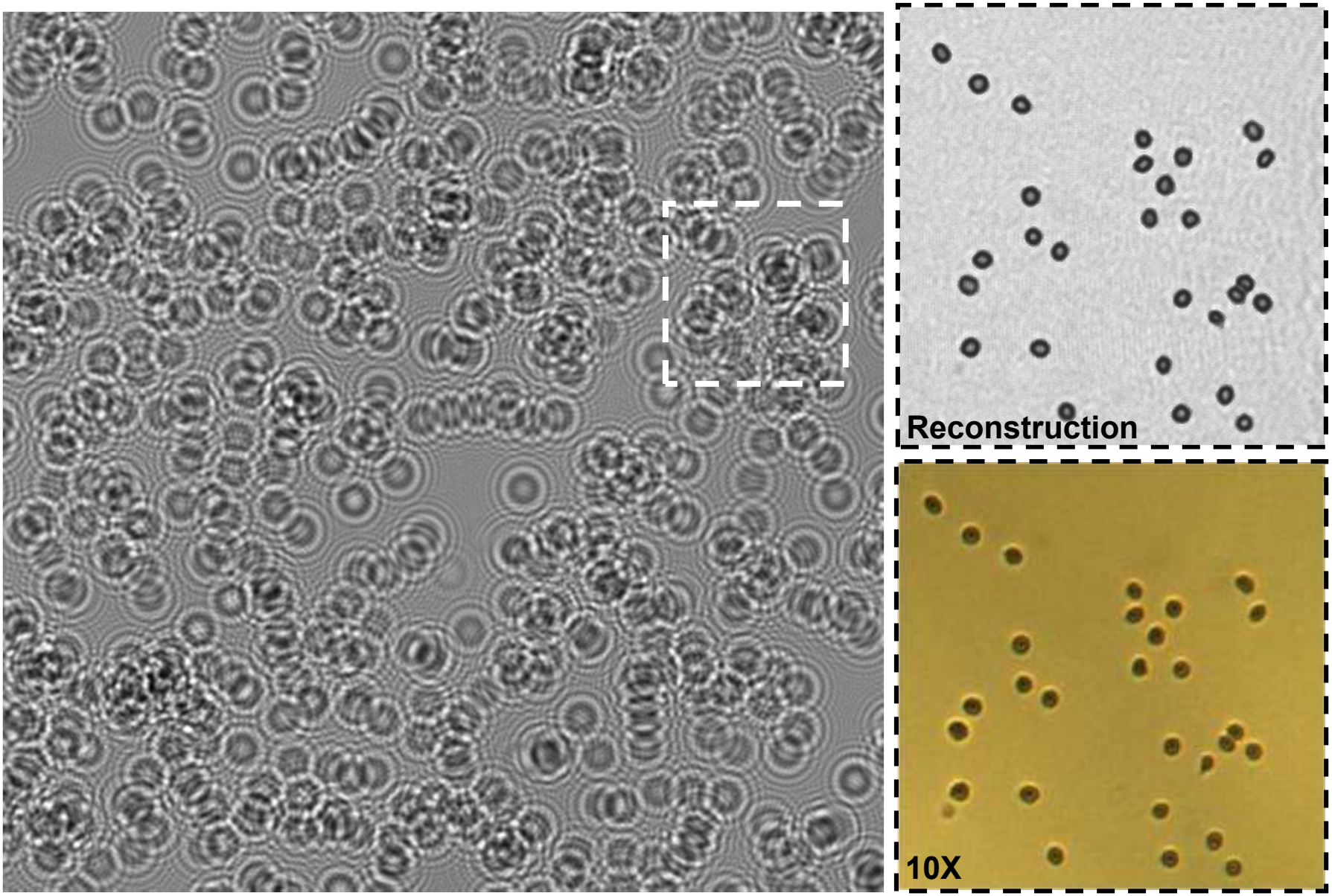


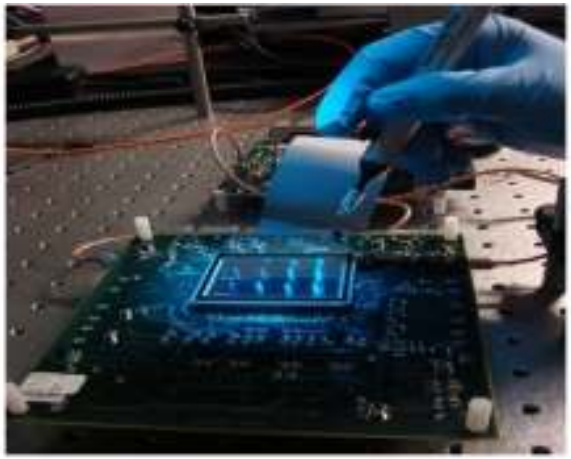

H<1-2 μm → contact on-chip imaging

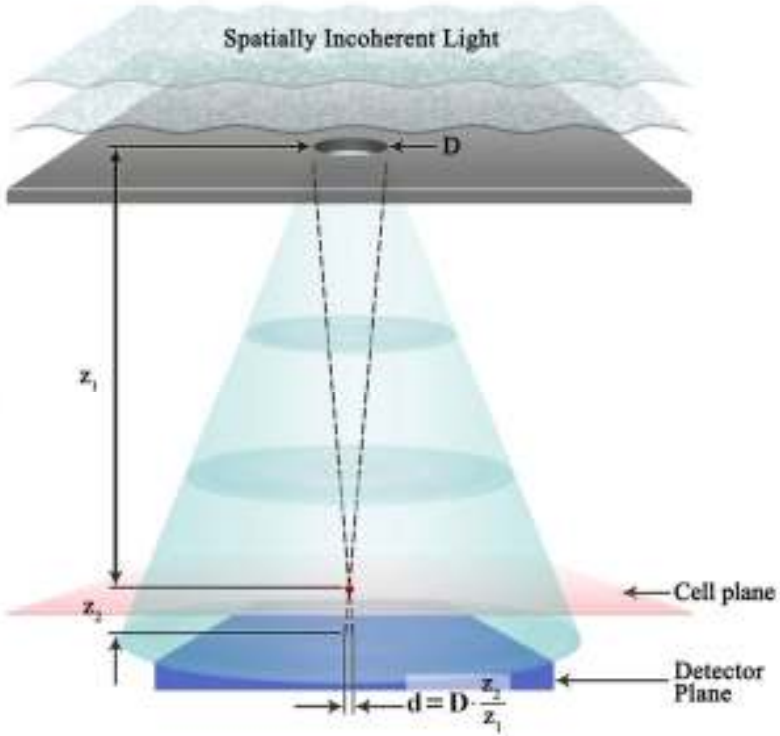
H ~ 10 - 1,000 μm → holographic on-chip imaging


(a)
(b)
(c)
(d)
(e)
(a)
Lymphocyte
Lymphocyte
(b)
Neutrophil
(c)
Neutrophil
(d)
Neutrophil
(e)
Neutrophil

# Cell Density: 102K [Cells/uL]







***Incoherent Lensfree Holography Using Large Apertures:***

*-Reduced speckle noise and multiple interference noise*

*-Reduced cross-talk among cells*

*-Tolerant to misalignments; No need for coupling optics*

*-No need for lasers; LEDs would be sufficient*

*-Significantly larger field of view*

# *Iterative reconstruction of microscopic images*

**Object (e.g.. cell):** $s(x_D, y_D)$

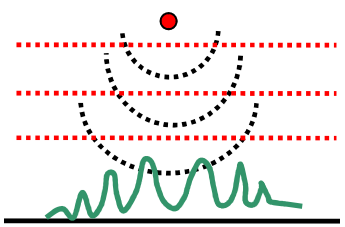
**Aperture (Diameter ~ D << M x resolution):** $p(x,y) \leftrightarrow \tilde{P}(f_x, f_y)$

**Source (Spatially Incoherent):** $S(\gamma) \cong S_0 \delta(\gamma - \gamma_0)$

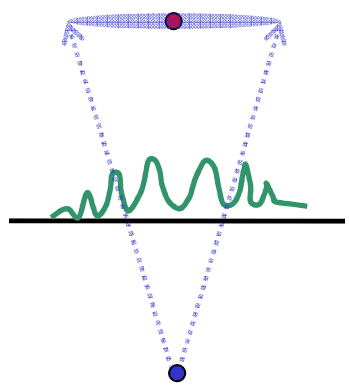
$$H(x_D, y_D) \propto \frac{S_0}{(\lambda_0 z_1)^2} \cdot \left(\frac{z_2}{z_1}\right)^2 \left[\left\{s\left(\frac{x_D}{F}, \frac{y_D}{F}\right) * h_c(x_D, y_D)\right\} + c.c.\right]$$

**Twin-image Problem**

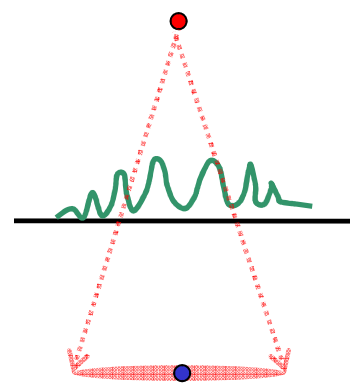
**Real Image**



**Virtual Image**



**Forward propagate the hologram**



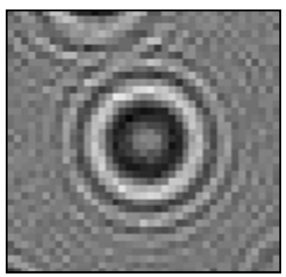
**Backward propagate the hologram**

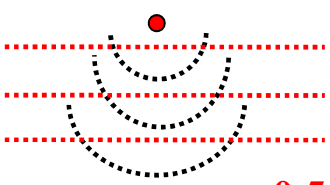
# *Iterative phase recovery eliminates the twin image: Exact transfer function enables convergence with <20 iterations*

$$U^{i+1}_{-z_2}(x,y) = \begin{cases} m \cdot D_{-z_2}(x,y), & x,y \in S \\ U^{i}_{-z_2}(x,y)\ , & x,y \notin S \end{cases}$$

$$m = mean(U^{i}_{-z_2}(x,y)/mean(D_{-z_2}(x,y)$$

**Measured Hologram (red blood cell)**





$[i(x_D,y_D)]^{0.5}$

**Support constraint**

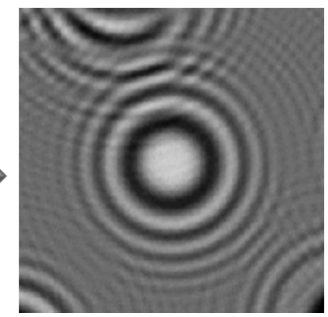
**Digital propagation to object plane**

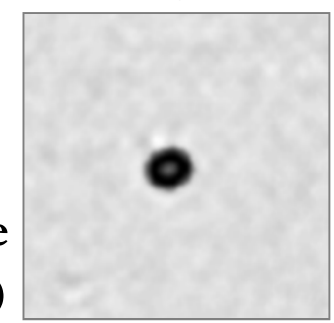
**Digital propagation to hologram plane**

**Update Phase**

N: number of iterations (e.g., <20)

**Recovered Hologram Phase**

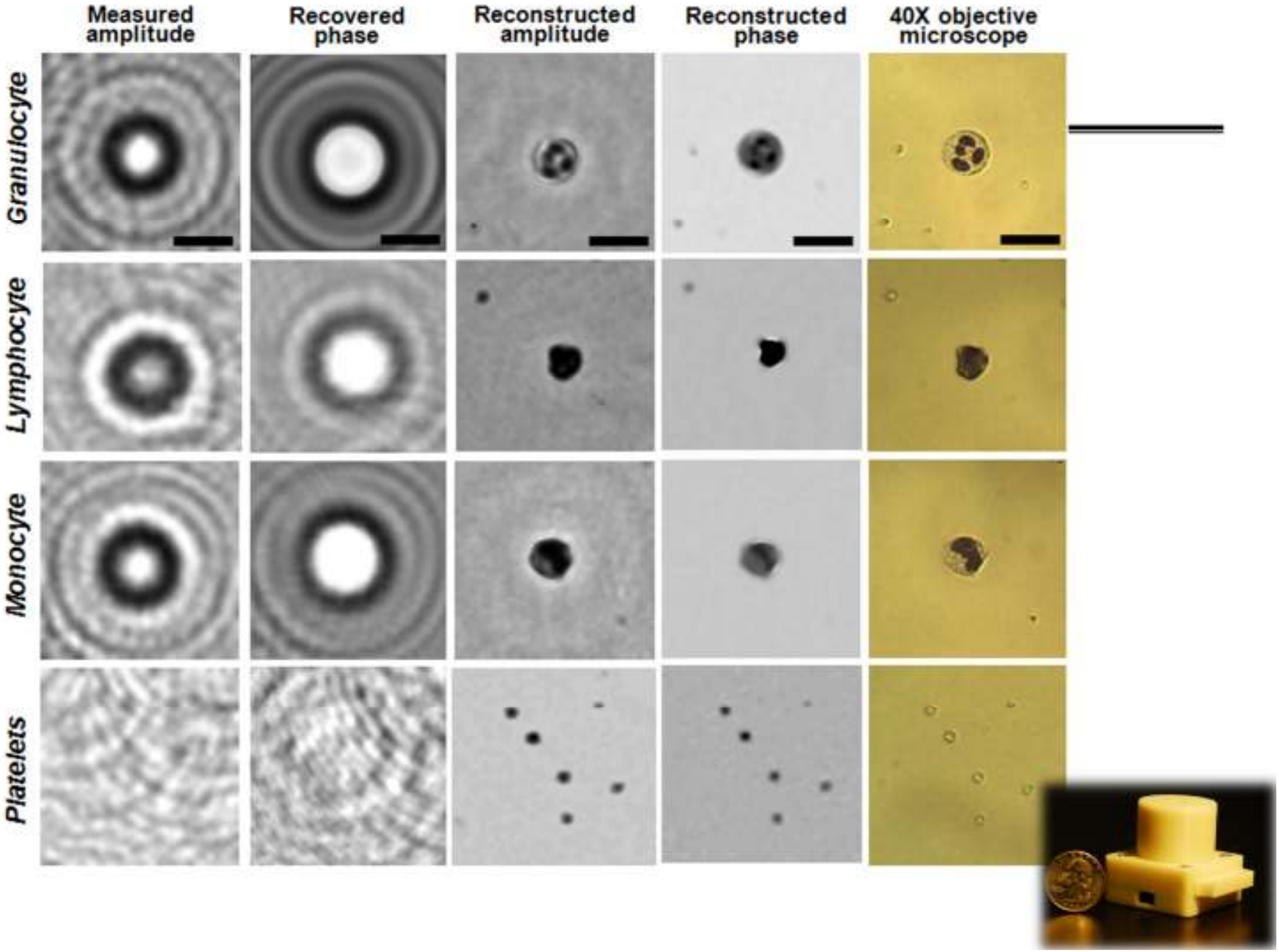




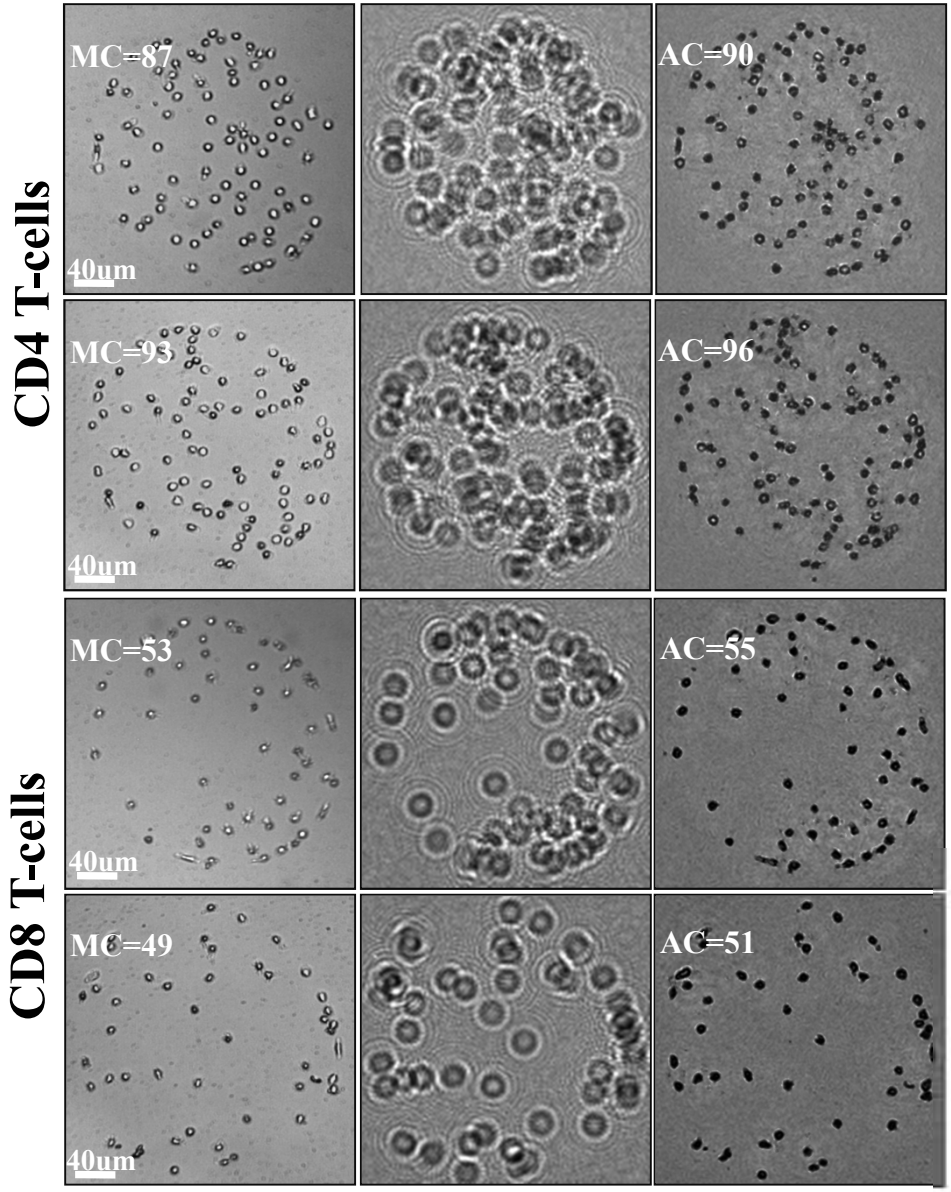
**Lensfree image (red blood cell)**

$$H_z(f_x,f_y) = \exp\left(j\frac{2\pi}{\lambda}z\right)\sqrt{1-(\lambda f_x)^2-(\lambda f_y)^2}$$

$$where\ \sqrt{f_x^2+f_y^2} < \frac{1}{\lambda}$$


Measured amplitude
Recovered phase
Reconstructed amplitude
Reconstructed phase
40X objective microscope
Granulocyte
Lymphocyte
Monocyte
Platelets

# *Cell counting in microfluidic channels based on LUCAS*



Measured Intensity | Reconstructed Amplitude | Recovered Phase | 10X objective microscope | 40X objective microscope

Test feature 1

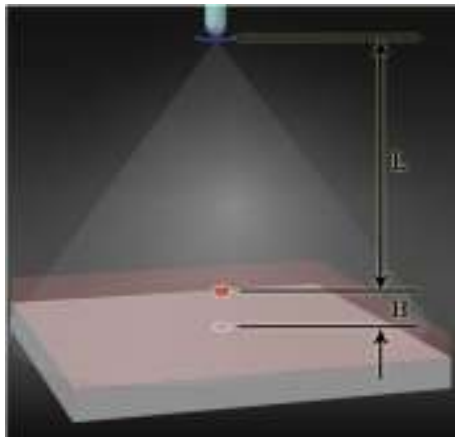
Test feature 2

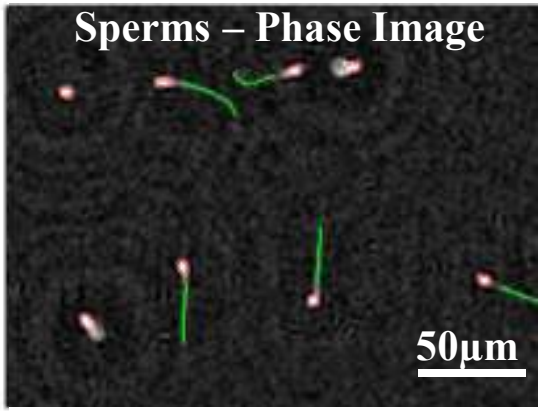
Test feature 3

Scale bar - holograms: 50um
Scale bar - all others: 5um

***Lensfree Incoherent Holography on a Chip***

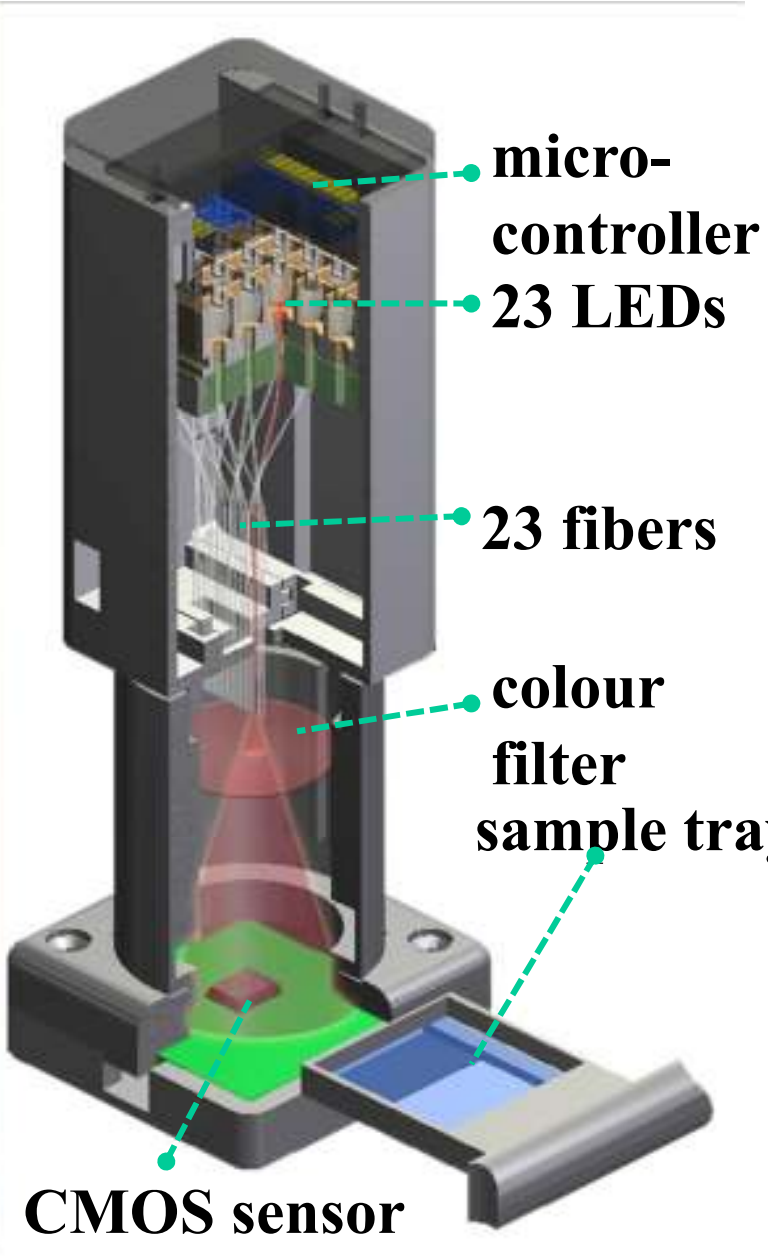
- ***Field of view: 24 mm$^2$***
- ***Numerical Aperture: ~0.15-0.2***
- ***Weight: <45 grams (<1.6 ounces)***
- ***Source: LED***





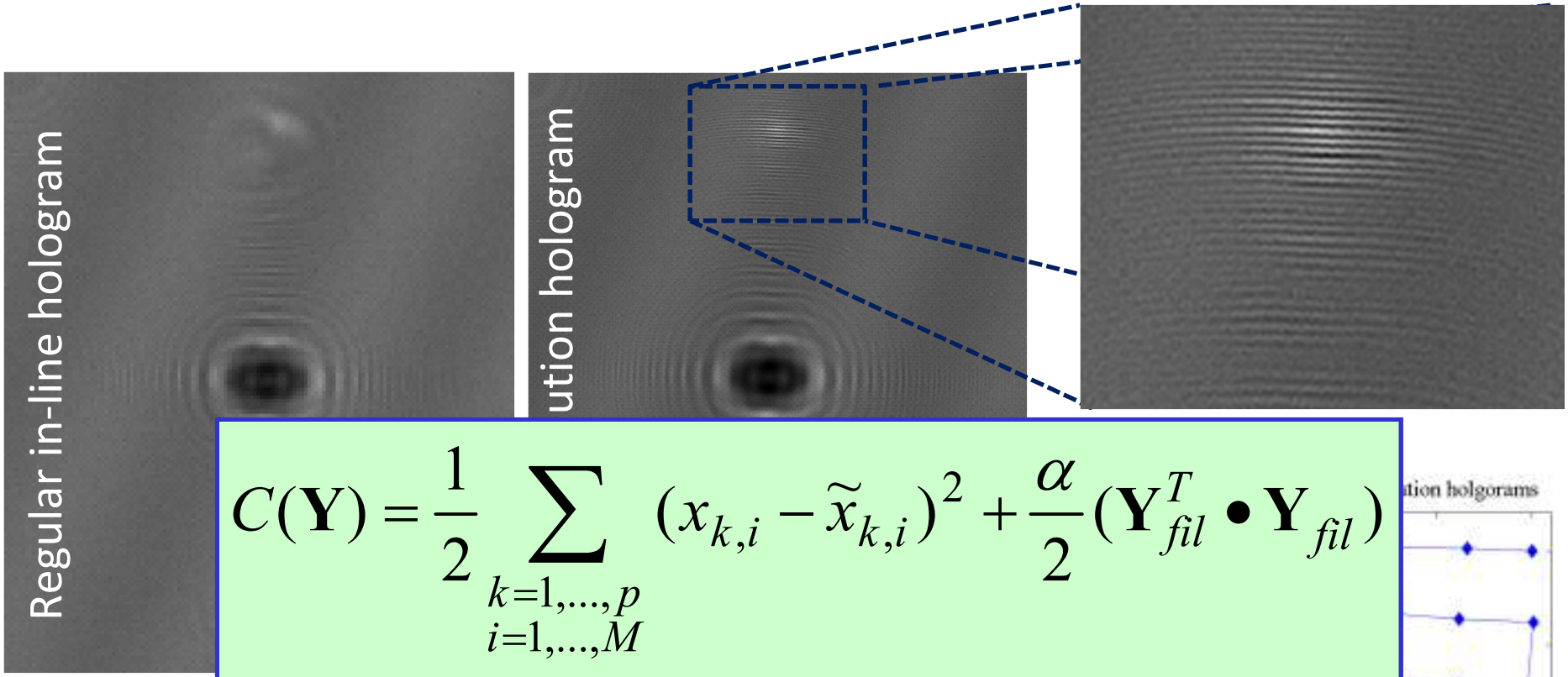
# *Super-resolution in lensfree on-chip imaging*

*Sub-micron resolution over 30 mm² field-of-view*


micro-
controller
23 LEDs
23 fibers
colour
filter
sample tray
CMOS sensor

ro-
troller
LEDs
ibers
our
er
ple tray
CMOS sensor

# *Improving resolution to sub-micron*

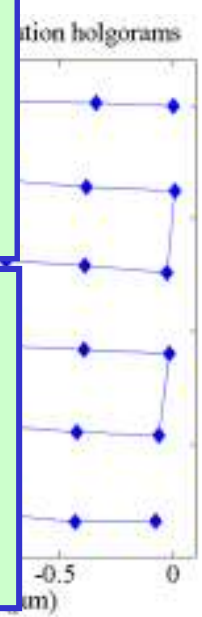



$$C(\mathbf{Y}) = \frac{1}{2} \sum_{\substack{k=1,\ldots,p \\ i=1,\ldots,M}} (x_{k,i} - \tilde{x}_{k,i})^2 + \frac{\alpha}{2} (\mathbf{Y}_{fil}^T \bullet \mathbf{Y}_{fil})$$

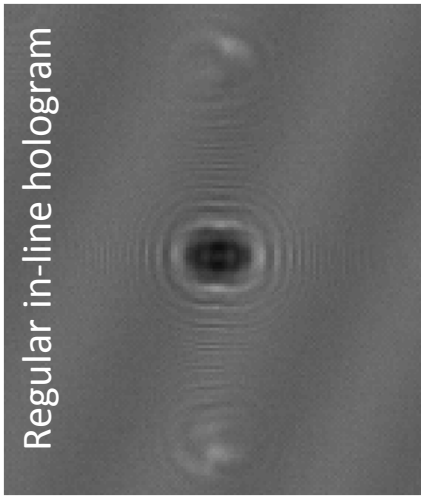
*Pixelsi*

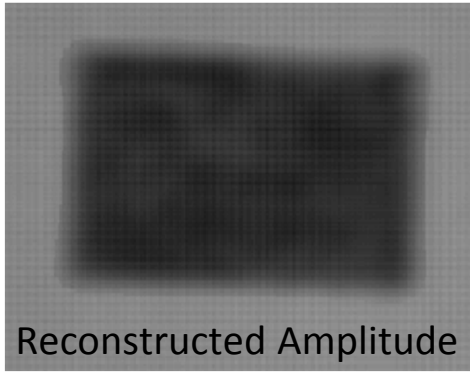
*5M*

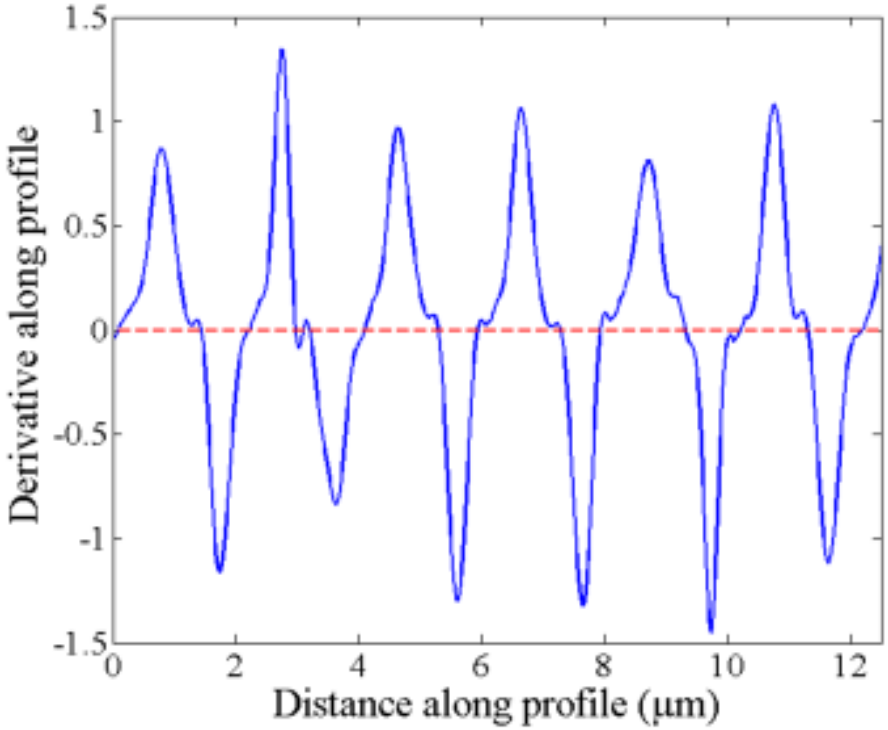
- *k=1:p --- Sub-pixel shifts*
- *i=1:M --- Pixels of the lower resolution hologram*
- *Y : High resolution hologram to be estimated*
- $Y_{fil}$ *: High-pass version of Y*

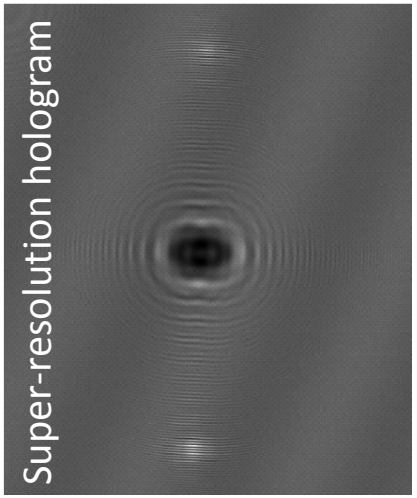


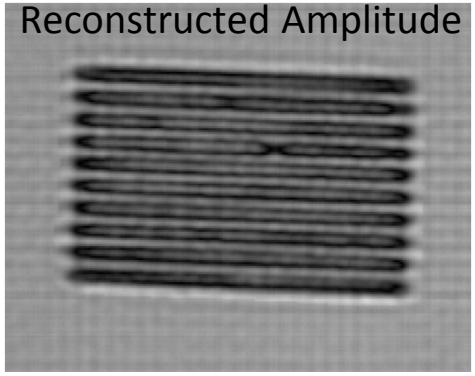
# *A numerical aperture of ~0.9 and a half-pitch resolution of <250 nm is confirmed*

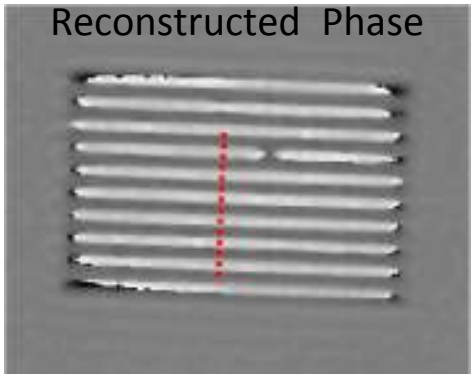


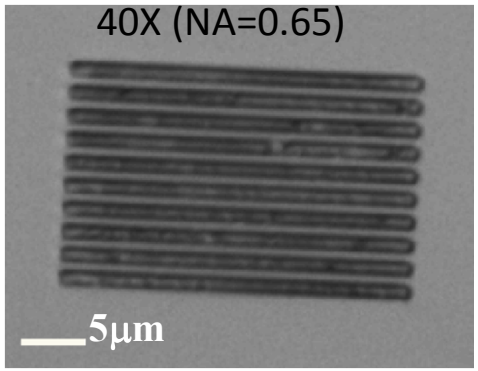



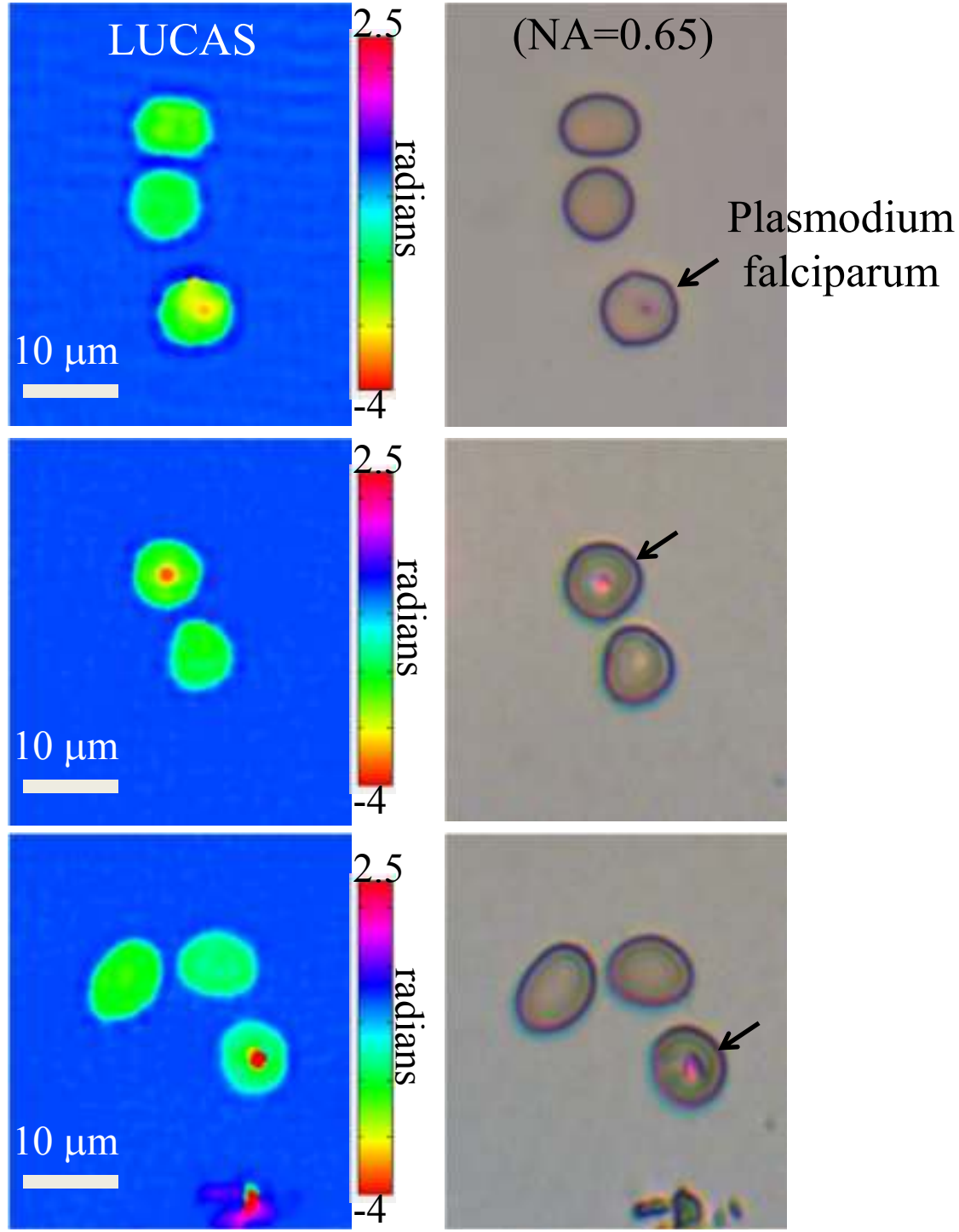



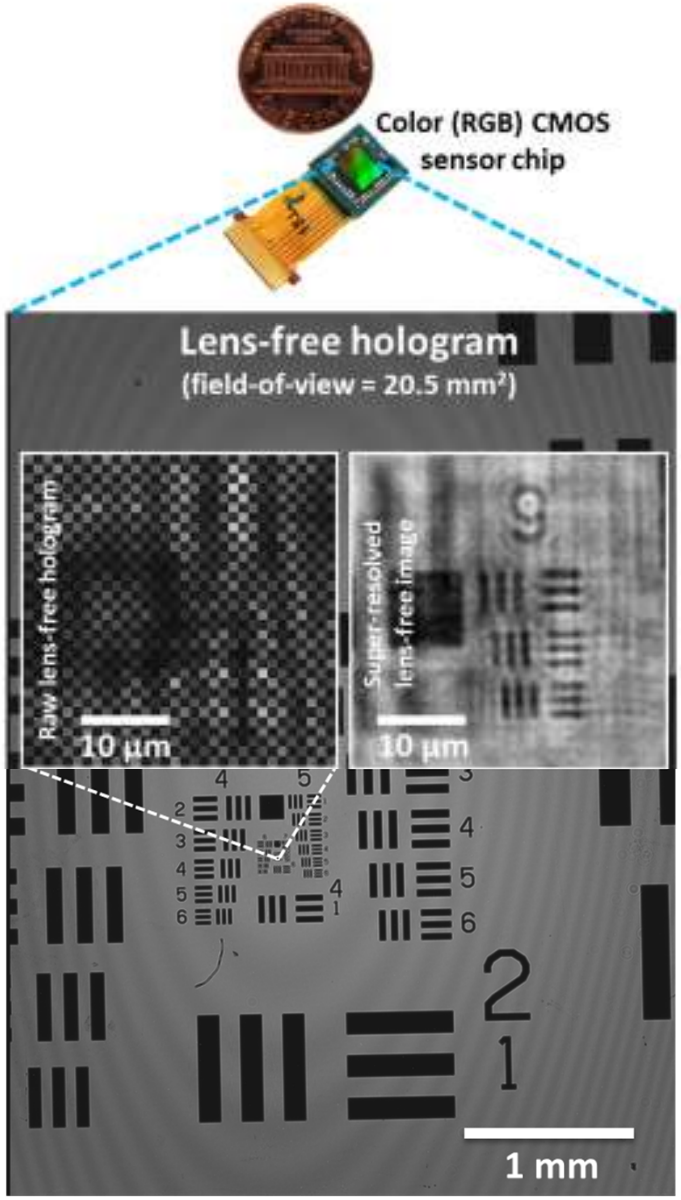



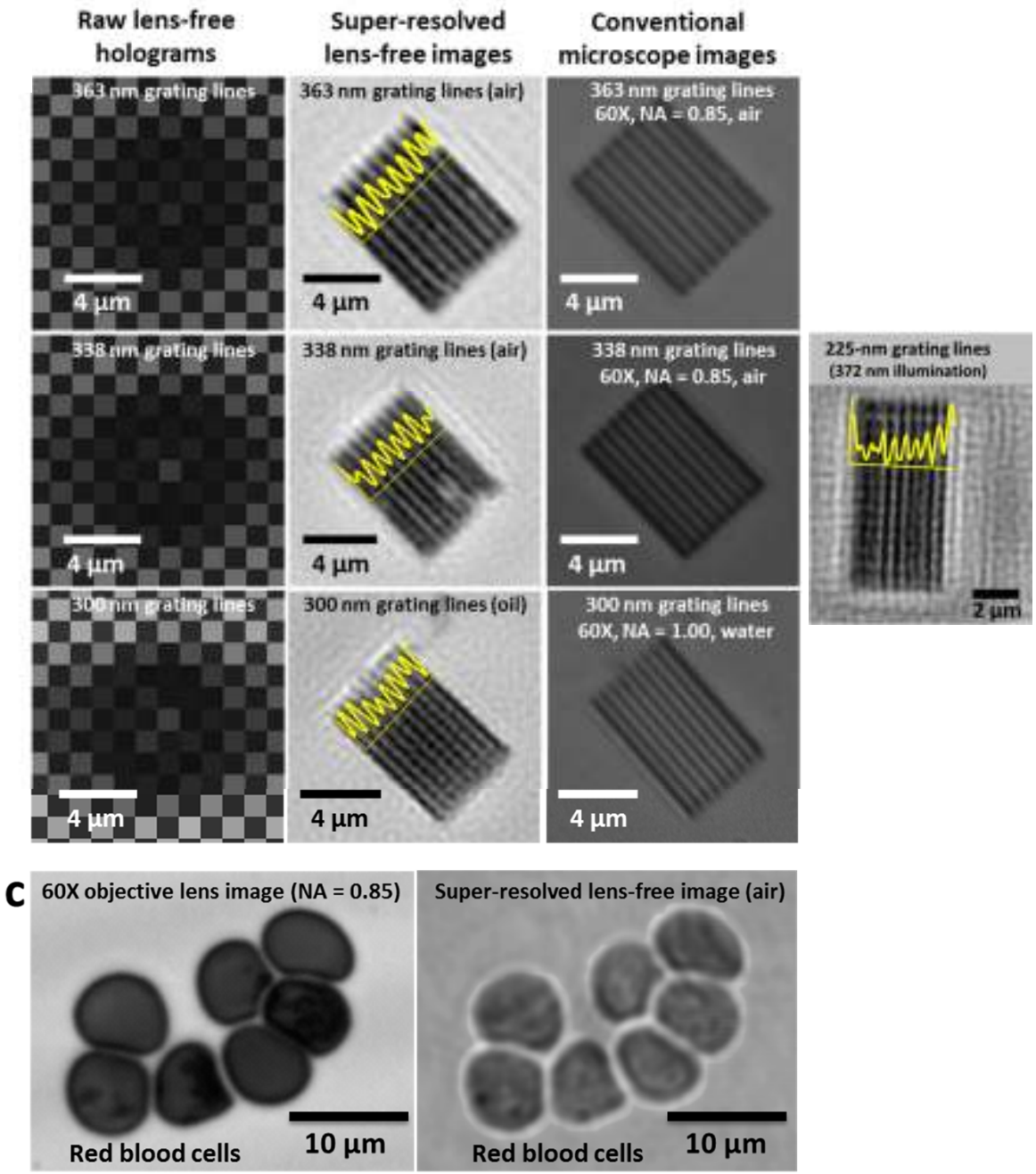



5 Megapixel
CMOS imager



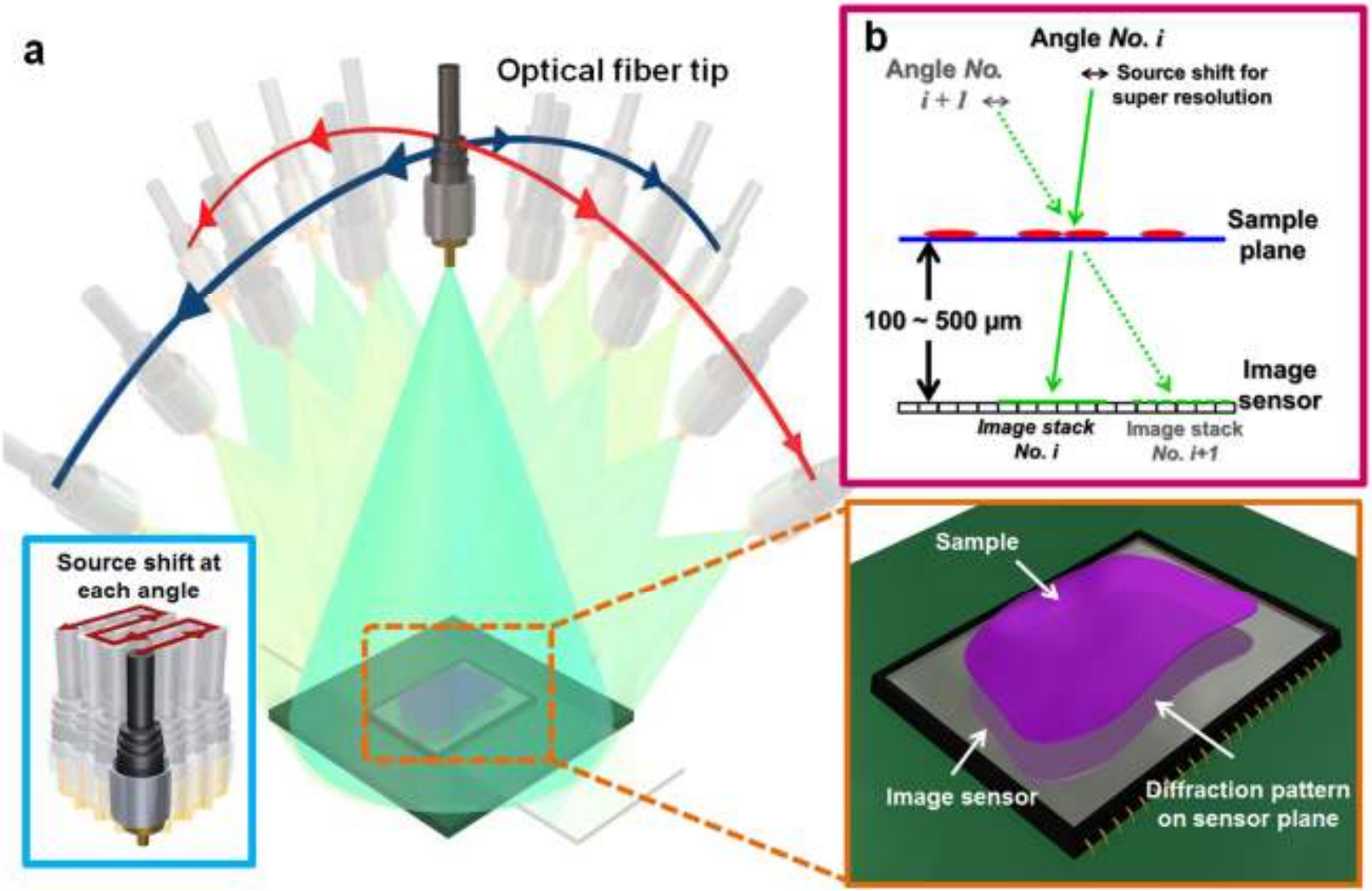
NA=0.9-1.0  FOV>20 mm$^2$



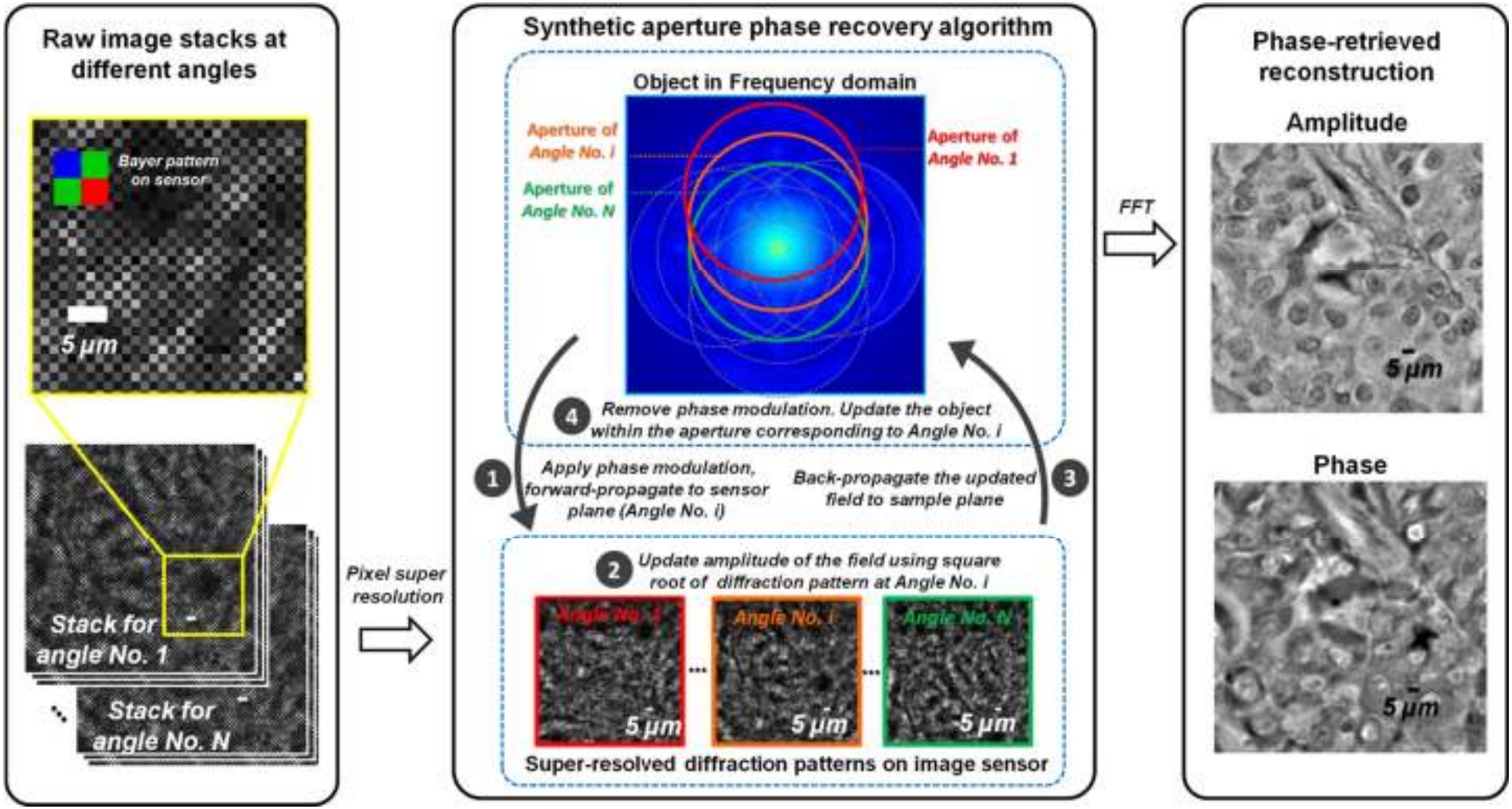

Nature Methods (2012)

# *Synthetic aperture based on-chip microscopy*

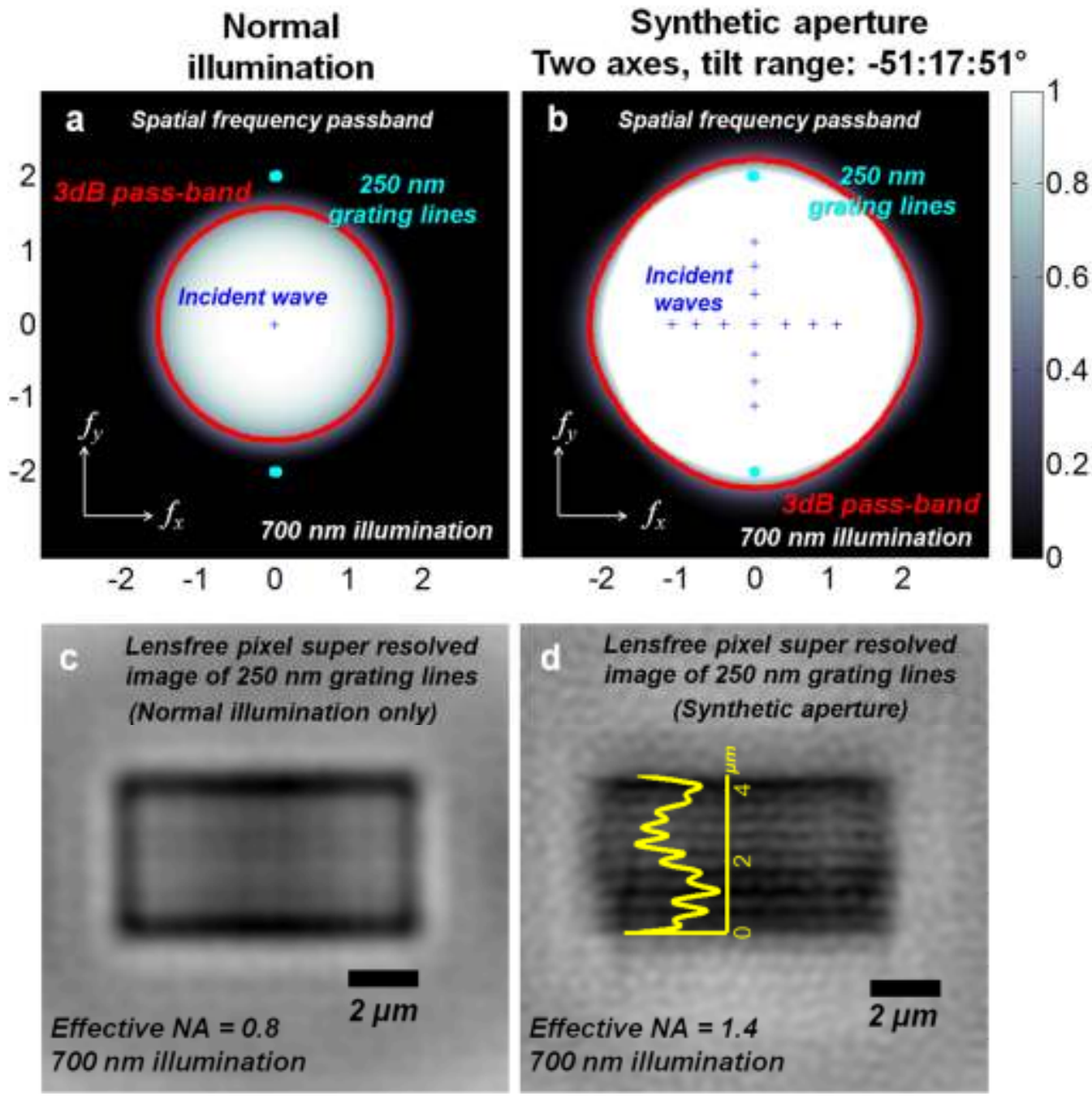



Light: Science & Applications (2014)

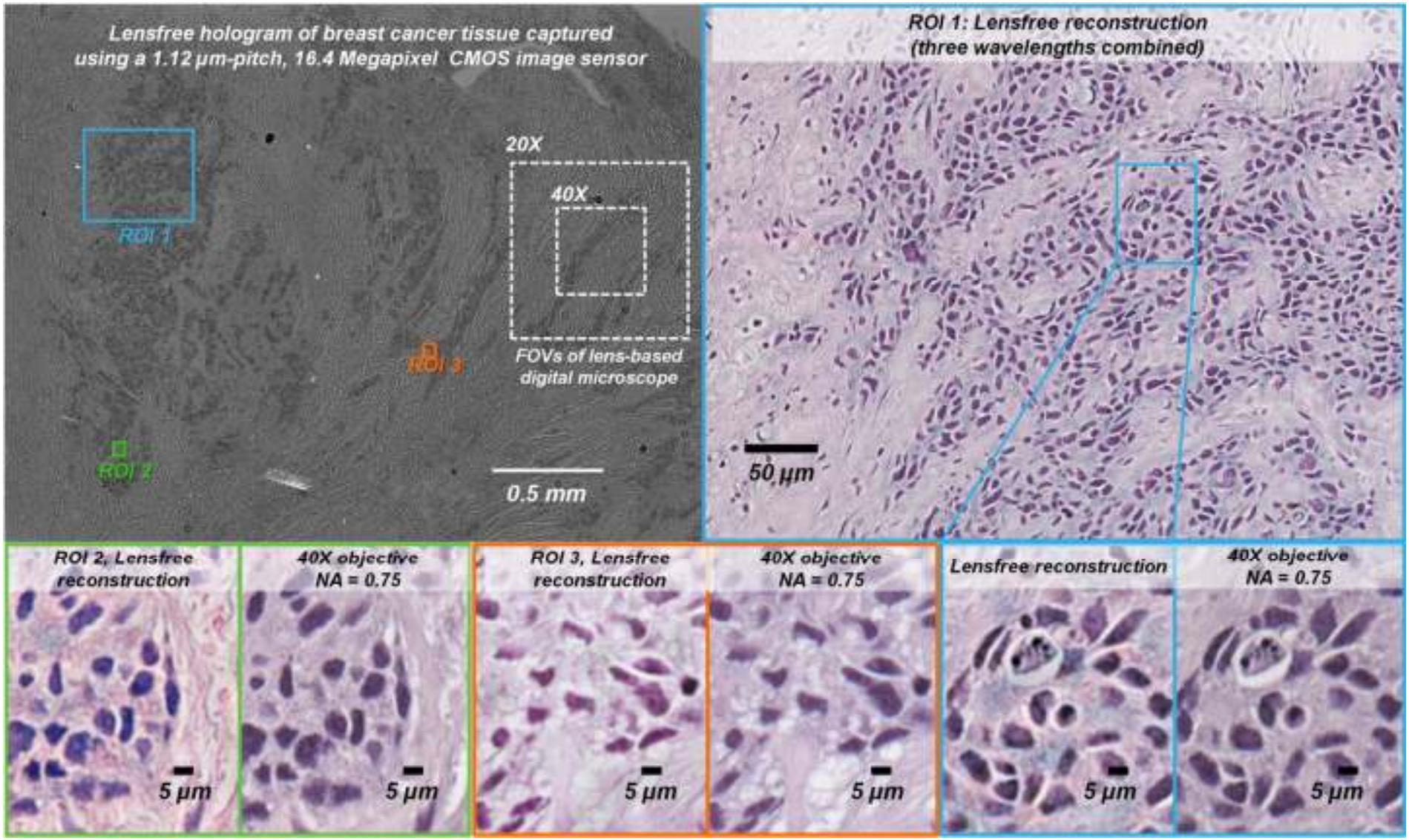
# *Synthetic aperture based on-chip microscopy*

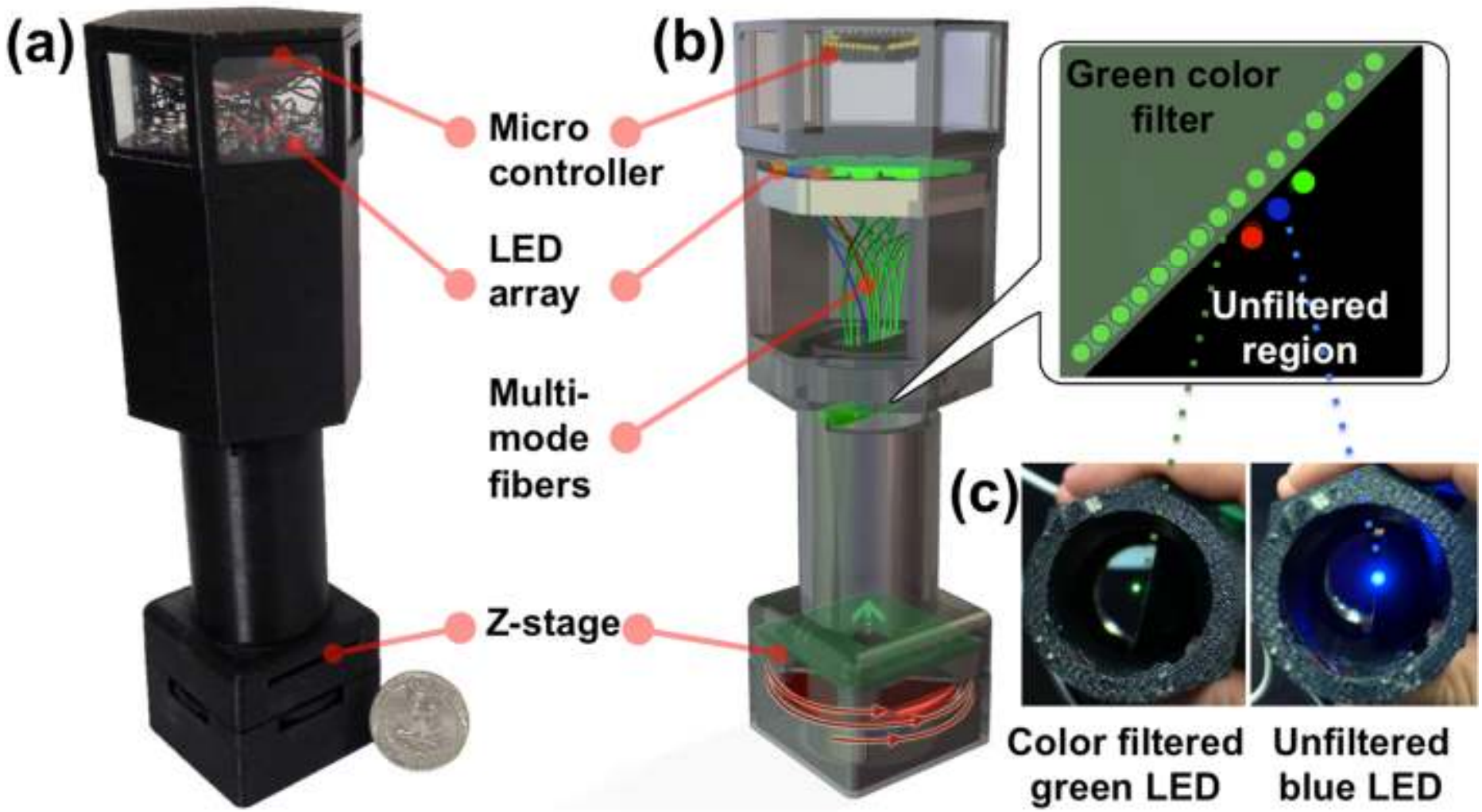


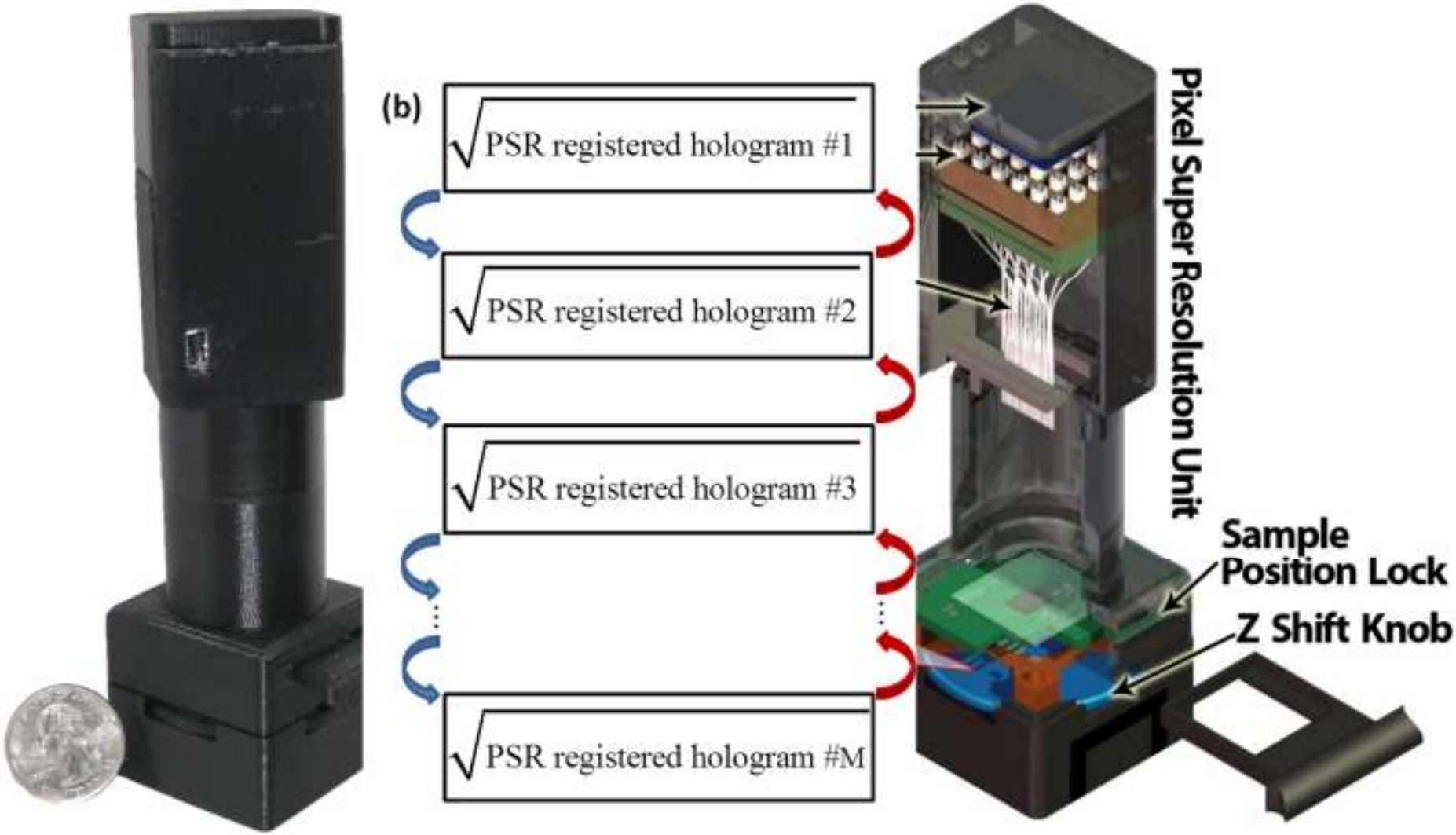

Light: Science & Applications (2014)



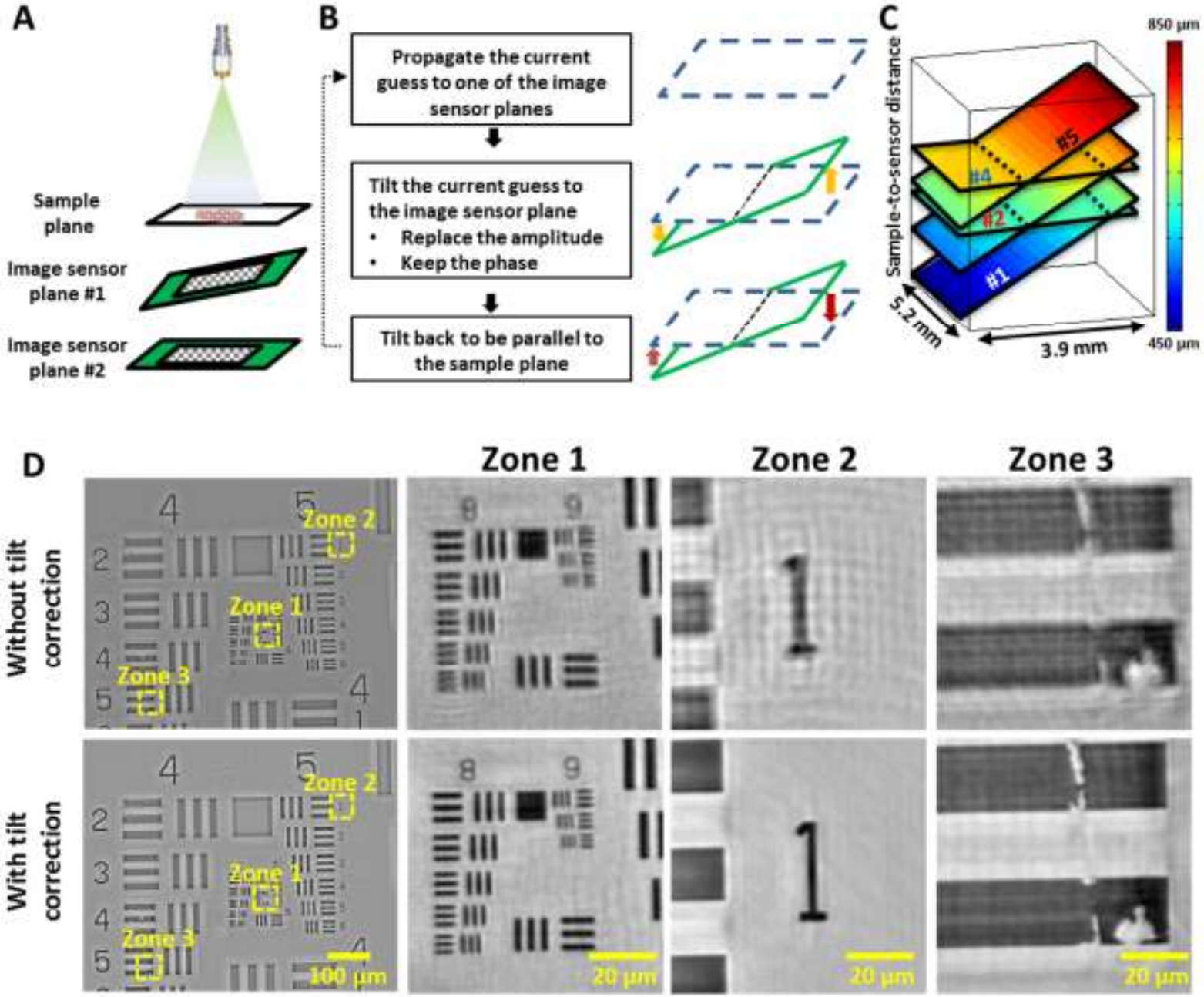

Light: Science & Applications (2014)


Lensfree hologram of breast cancer tissue captured using a 1.12 µm-pitch, 16.4 Megapixel CMOS image sensor
ROI 1
ROI 2
ROI 3
20X
40X
FOVs of lens-based digital microscope
0.5 mm
ROI 1: Lensfree reconstruction (three wavelengths combined)
50 µm
ROI 2, Lensfree reconstruction
40X objective NA = 0.75
ROI 3, Lensfree reconstruction
40X objective NA = 0.75
Lensfree reconstruction
40X objective NA = 0.75
5 µm
5 µm
5 µm
5 µm
5 µm
5 µm

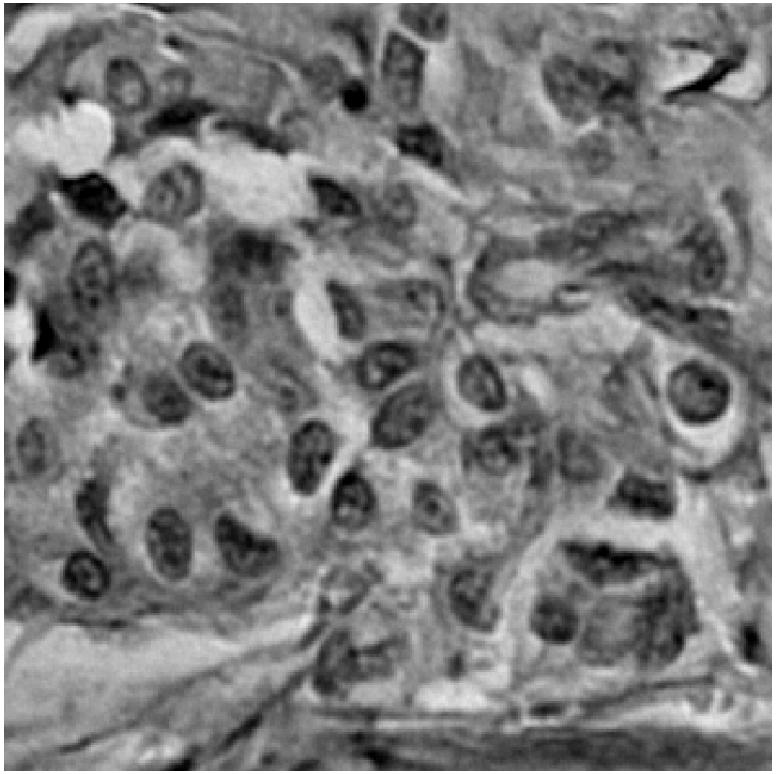

(a)
(b)
(c)
Micro controller
LED array
Multi-mode fibers
Z-stage
Green color filter
Unfiltered region
Color filtered green LED
Unfiltered blue LED

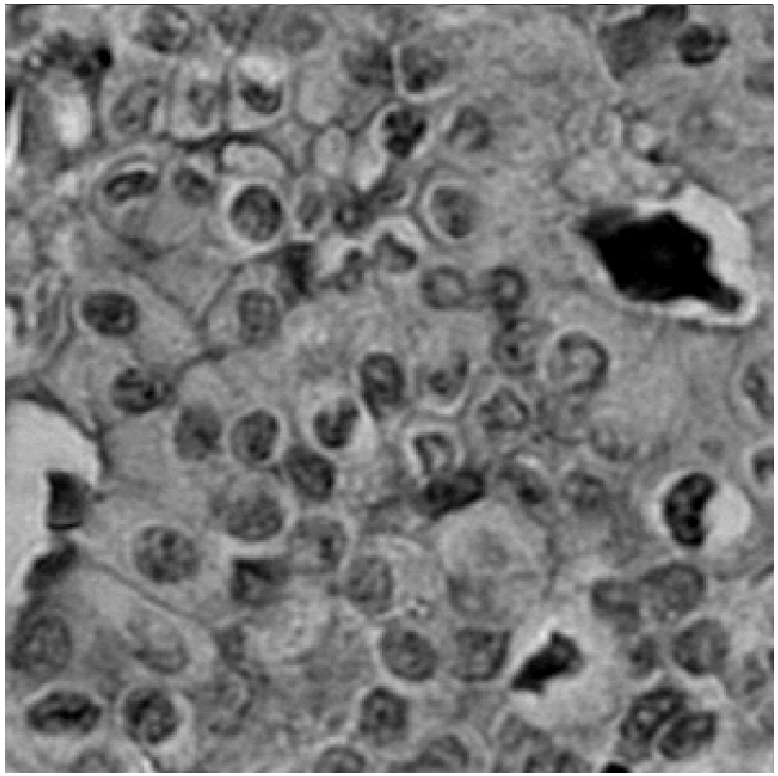

(b)
√PSR registered hologram #1
√PSR registered hologram #2
√PSR registered hologram #3
√PSR registered hologram #M
Pixel Super Resolution Unit
Sample Position Lock
Z Shift Knob

# *Tilt corrections through field rotations*



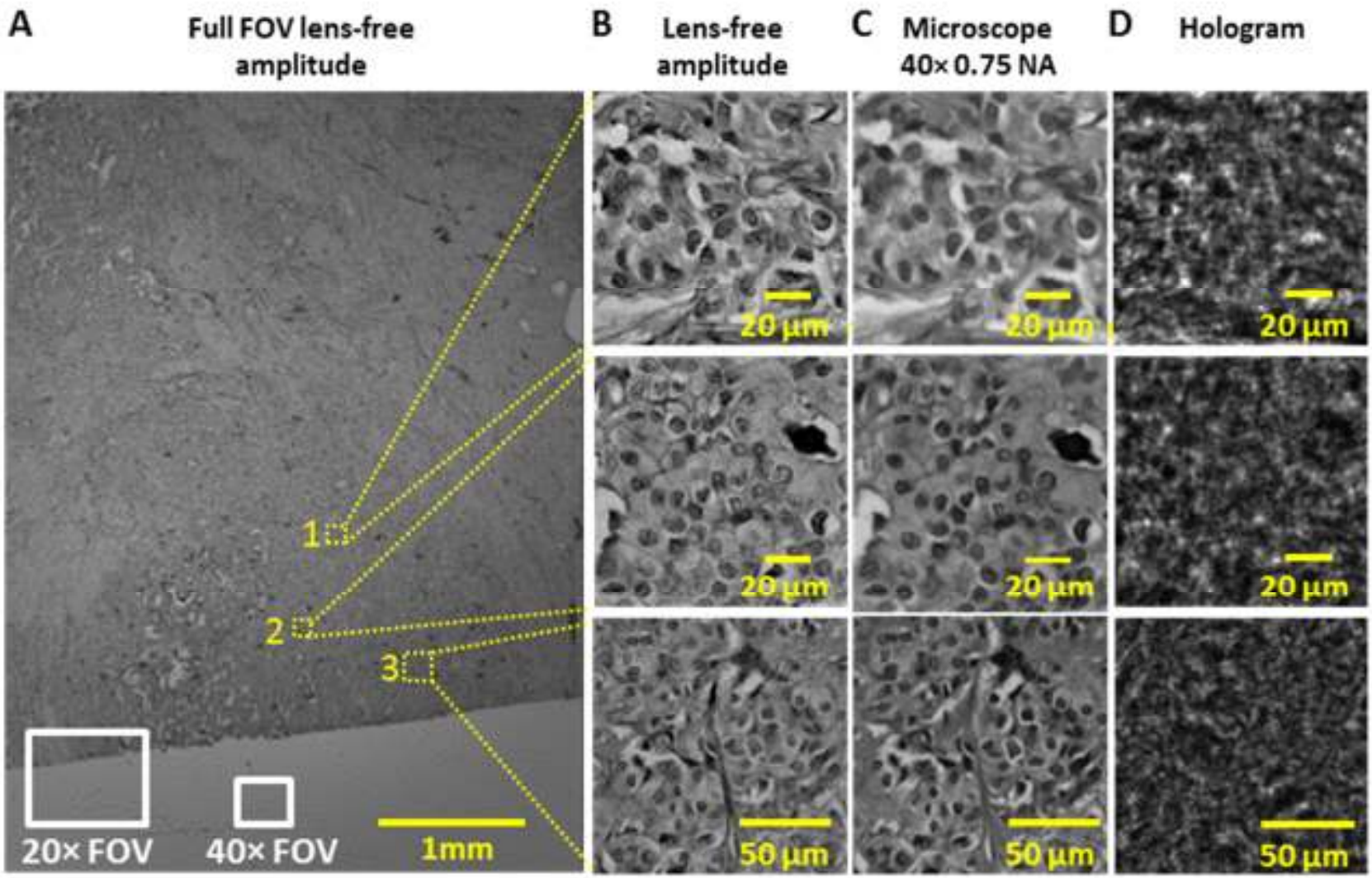
# *Lensfree imaging of histopathology slides*



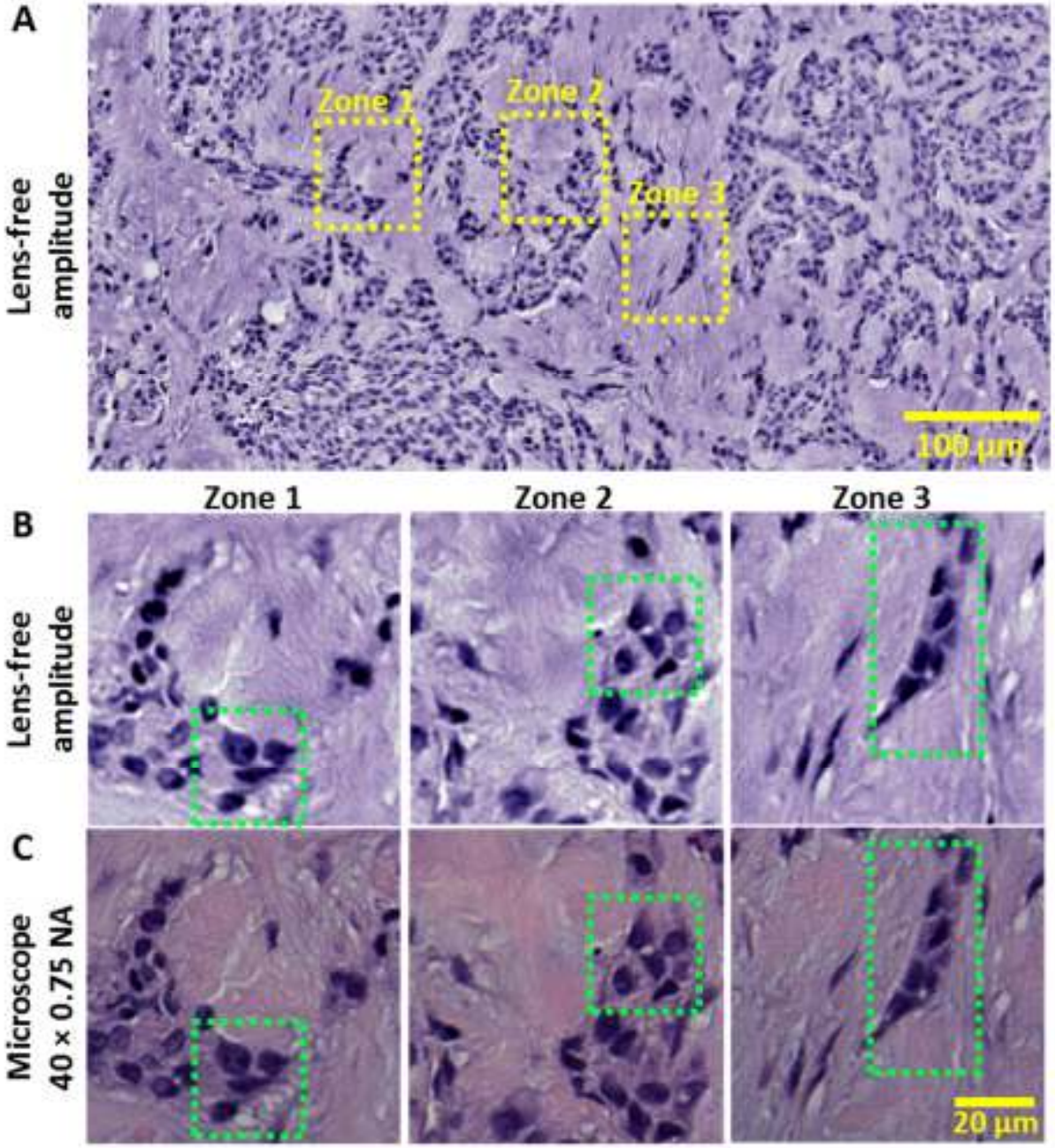


**Breast Cancer Tissue**

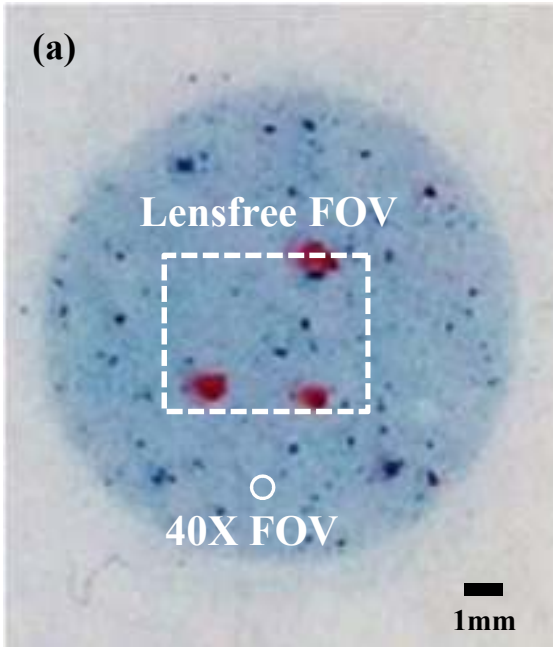
# *Lensfree imaging of histopathology slides*



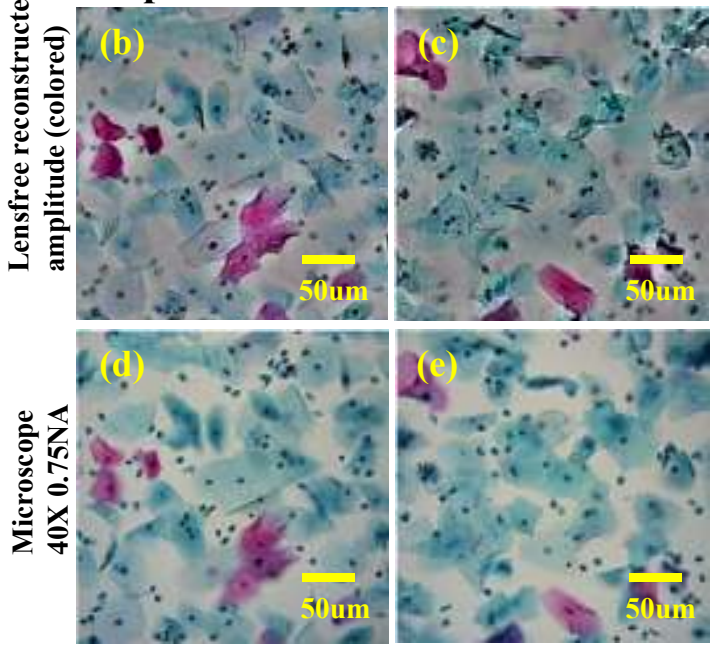

Science Translational Medicine - AAAS (2014)



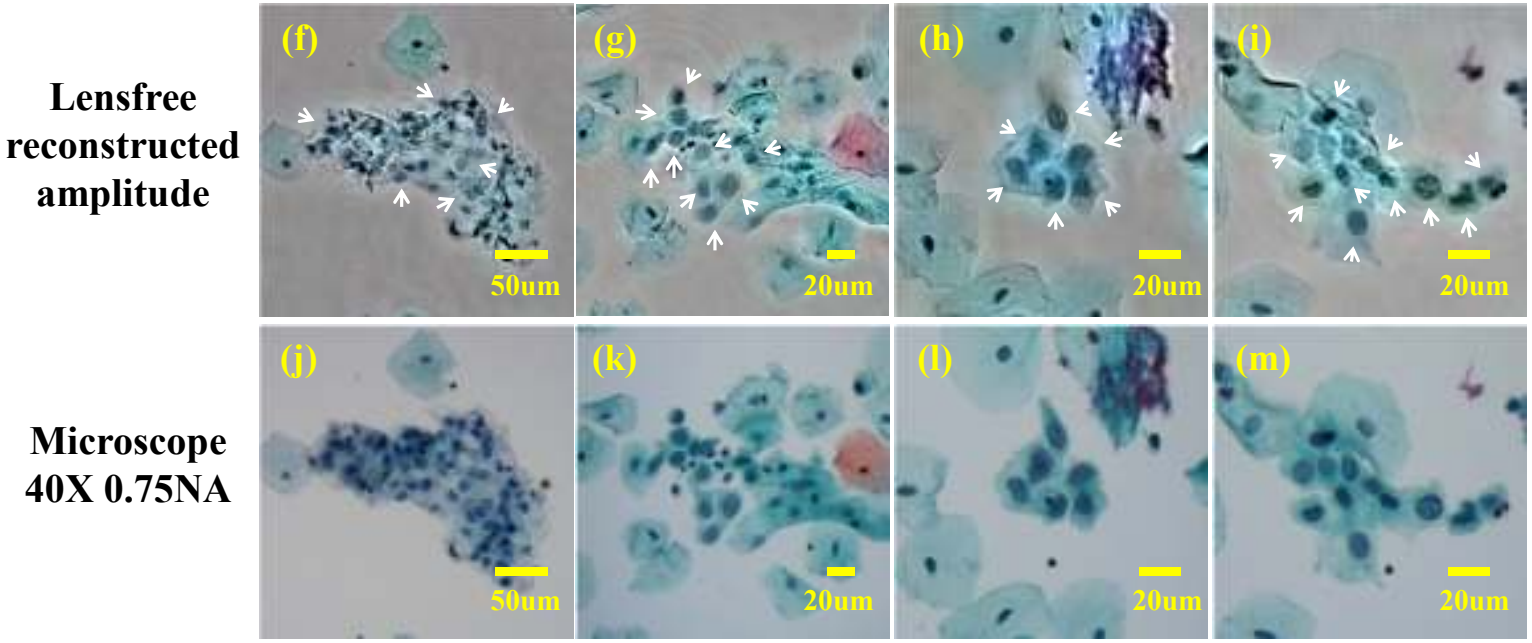
Science Translational Medicine - AAAS (2014)

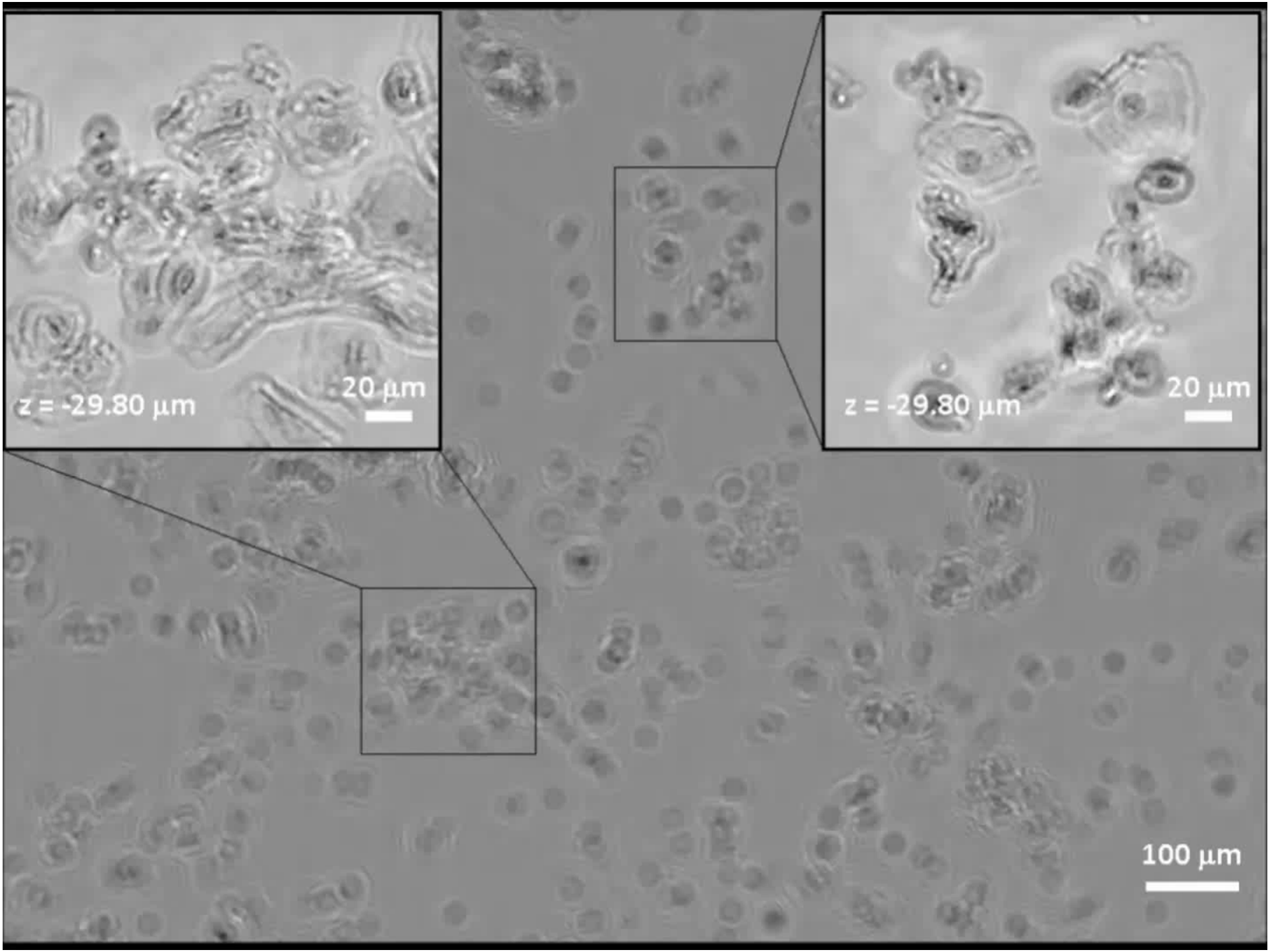



## Pap Smear

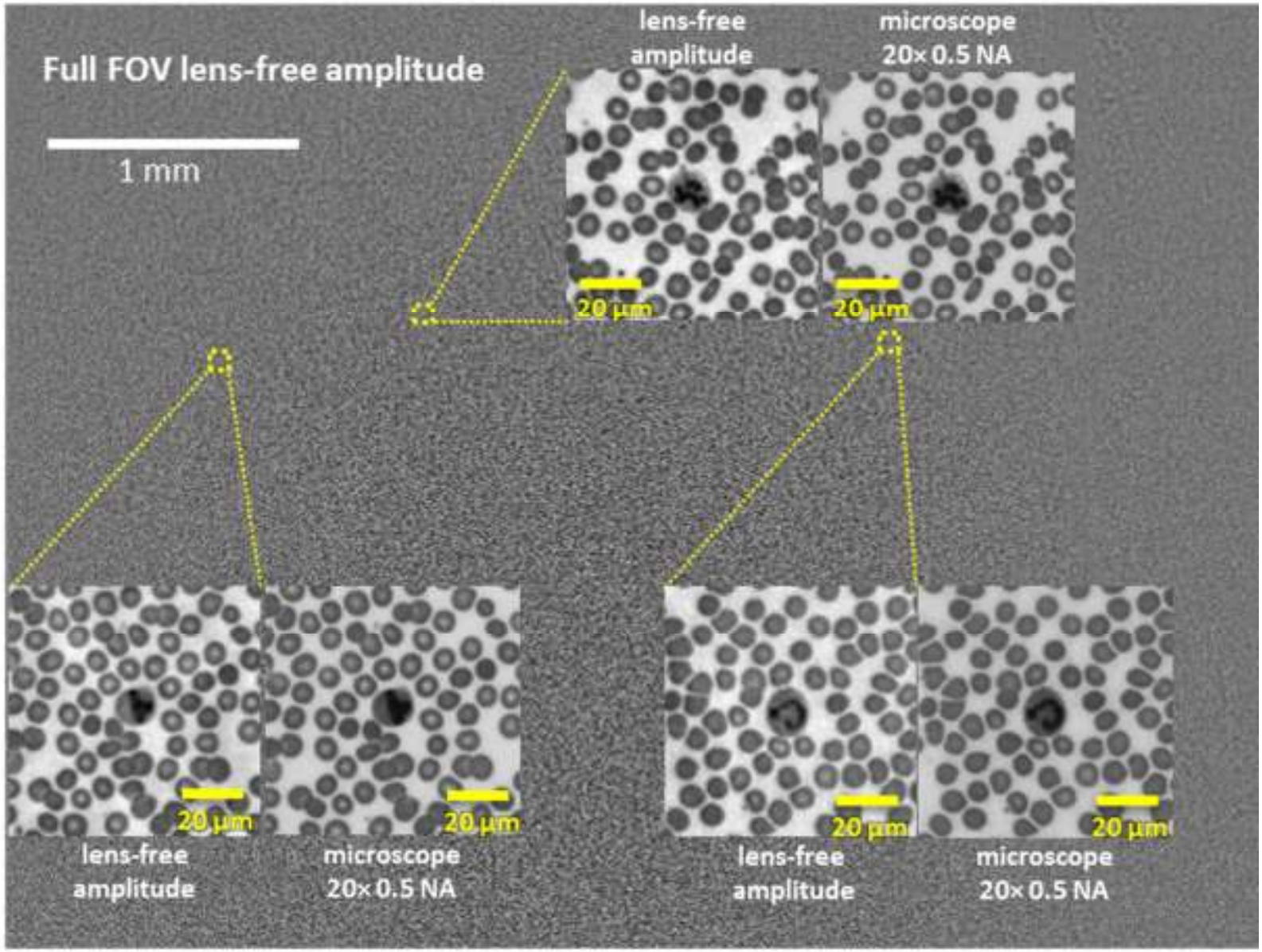



## Abnormal Pap Smear

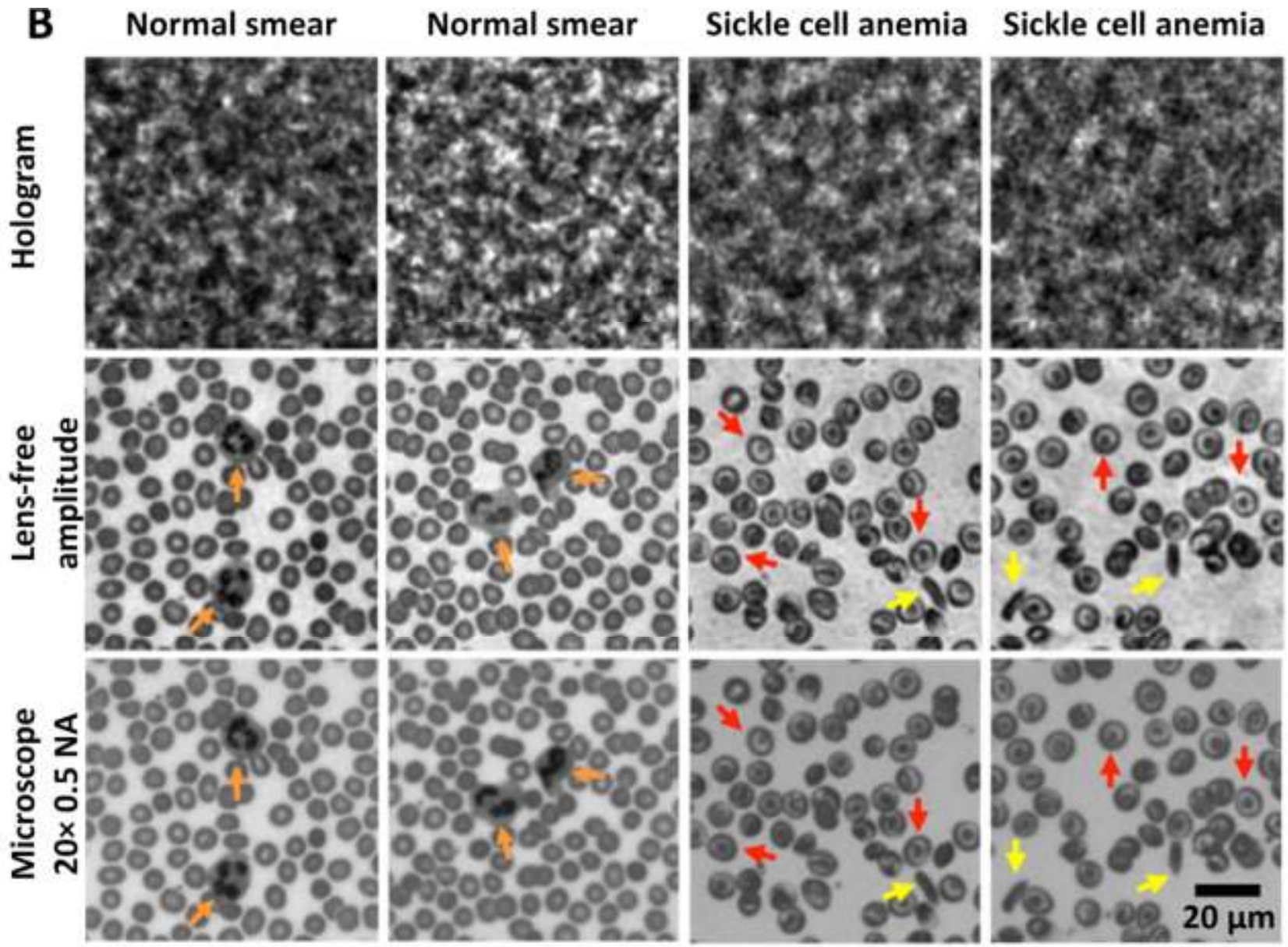


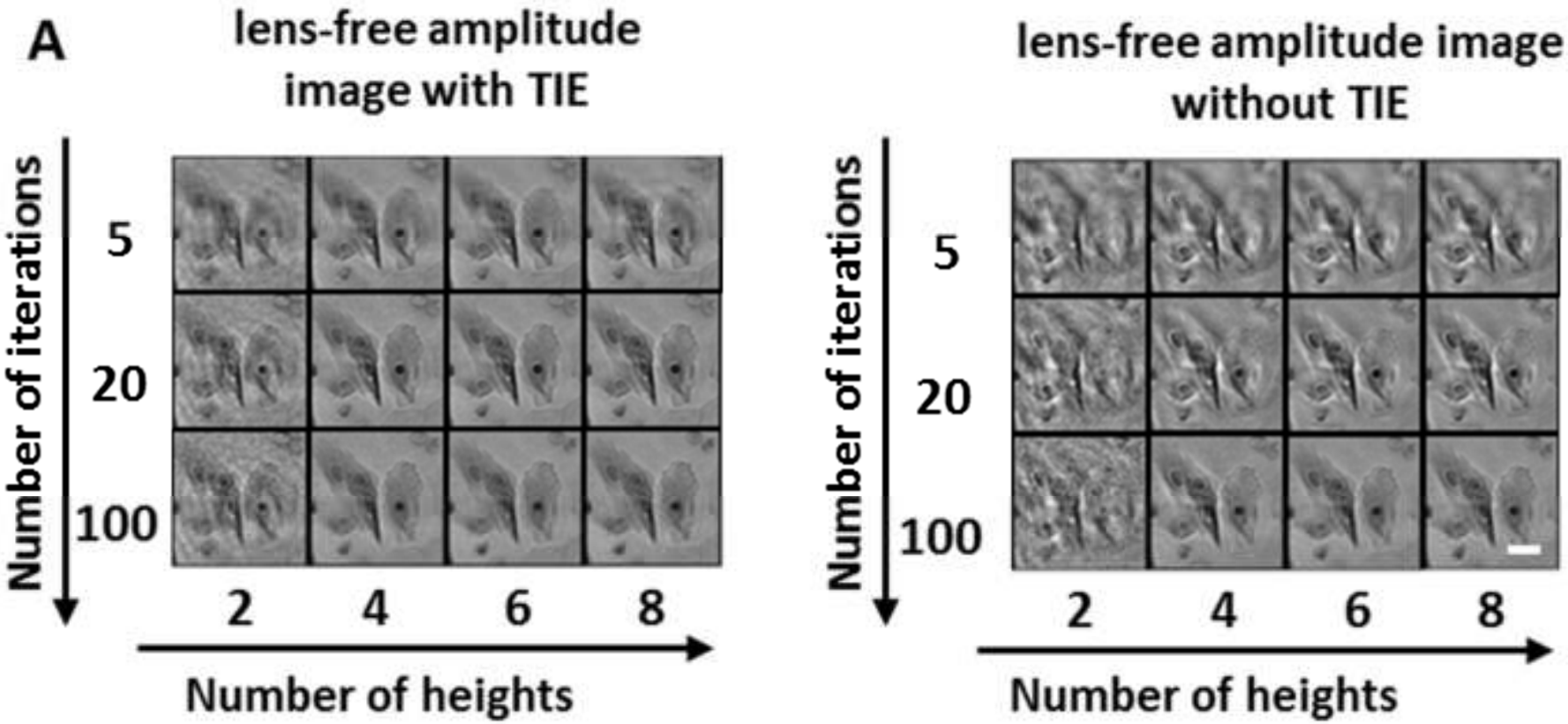

z = -29.80 µm
20 µm
z = -29.80 µm
20 µm
100 µm

# *Lensfree imaging of a blood smear*



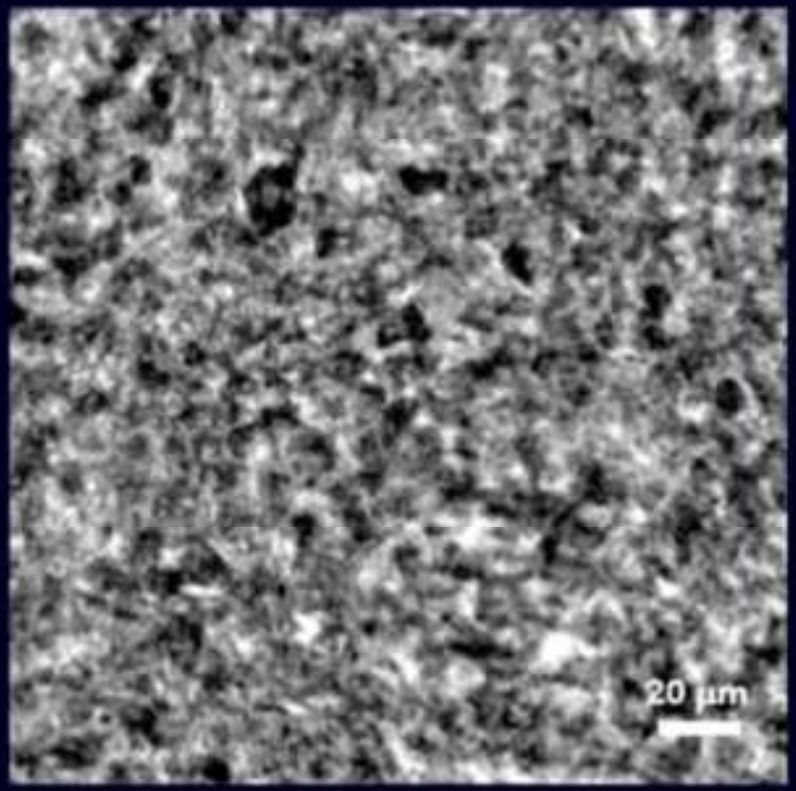
# *Lensfree imaging of sickle cells*



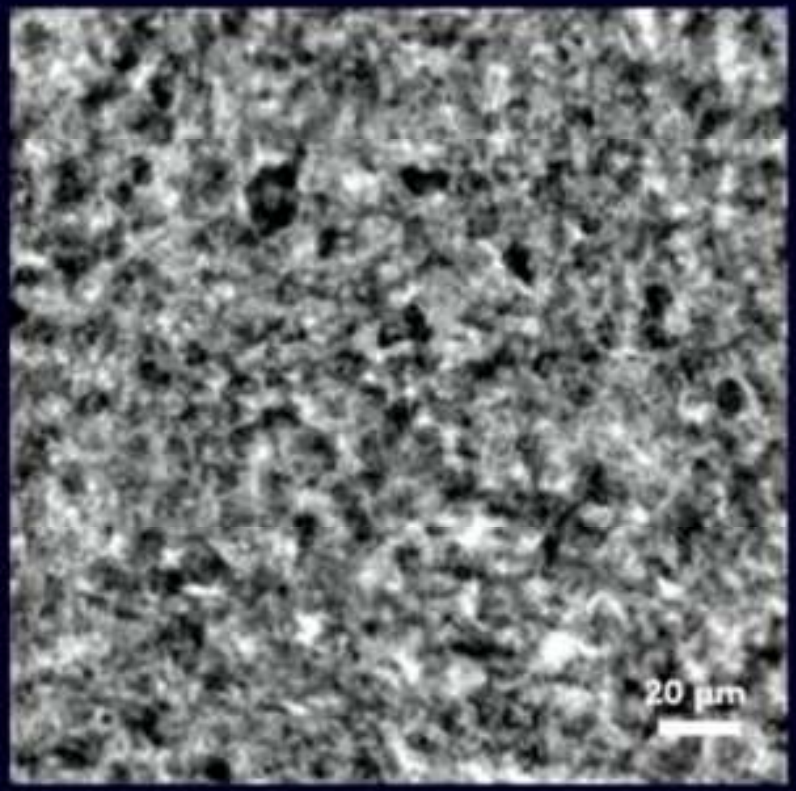


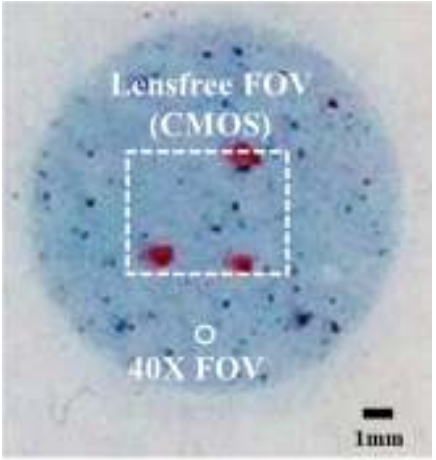

Science Translational Medicine - AAAS (2014)

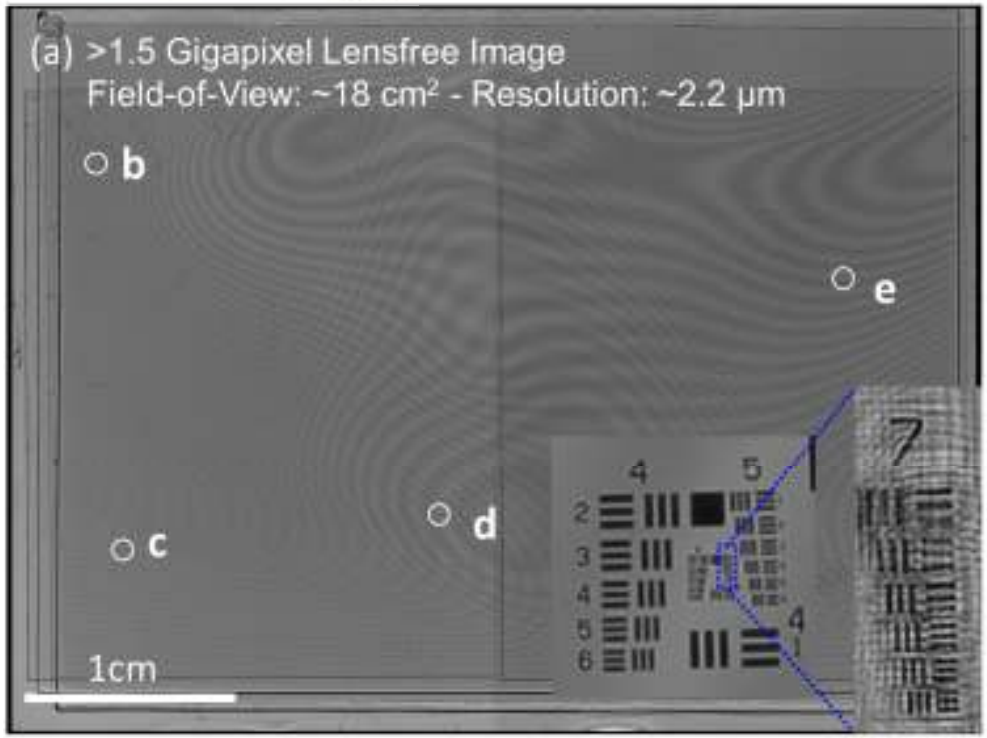
Backpropagated Pixel Super-Resolved Hologram

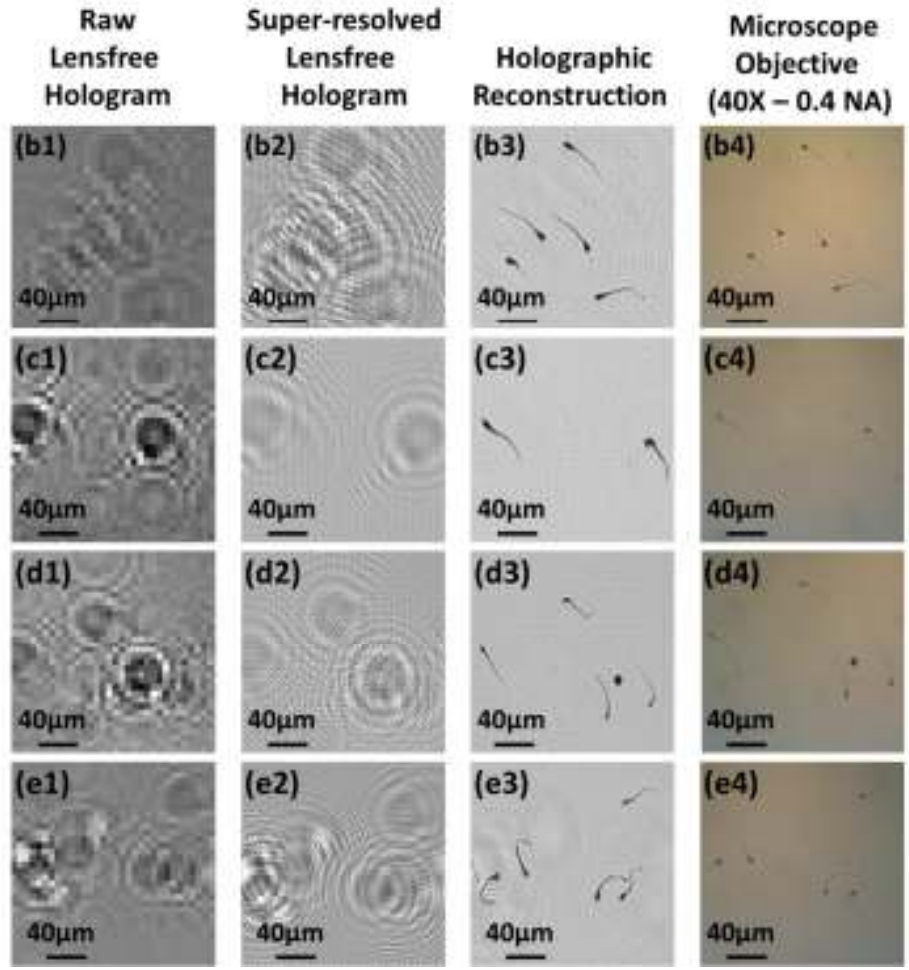



Backpropagated Pixel Super-Resolved Hologram





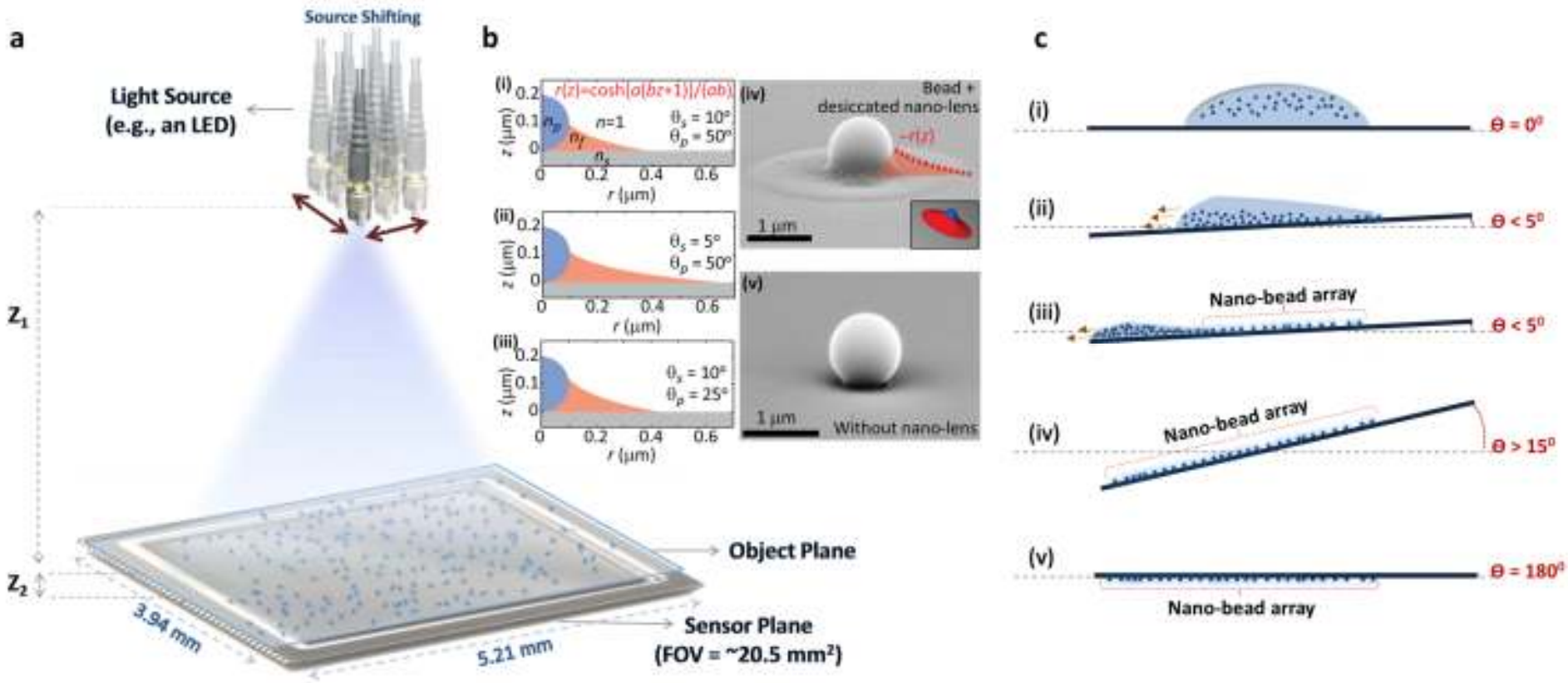




**Field-of-view Sample Volume**

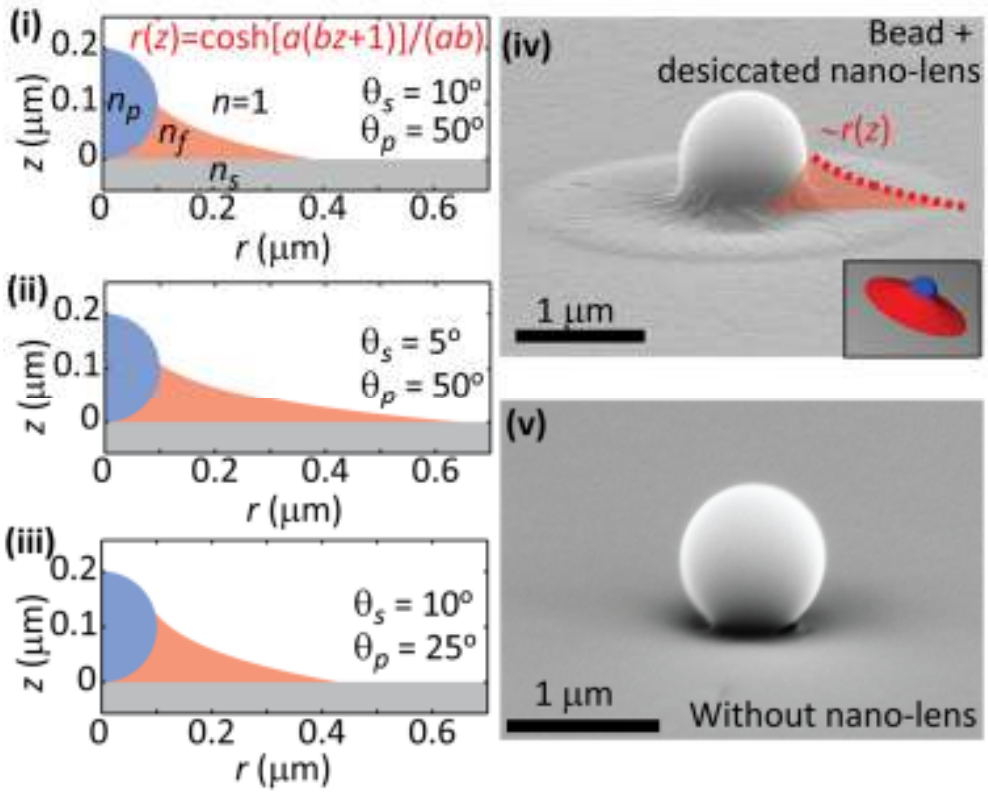
**Resolution**

# *Wide-field on-chip imaging of single viruses and nano-particles using self-assembled nano-lenses*

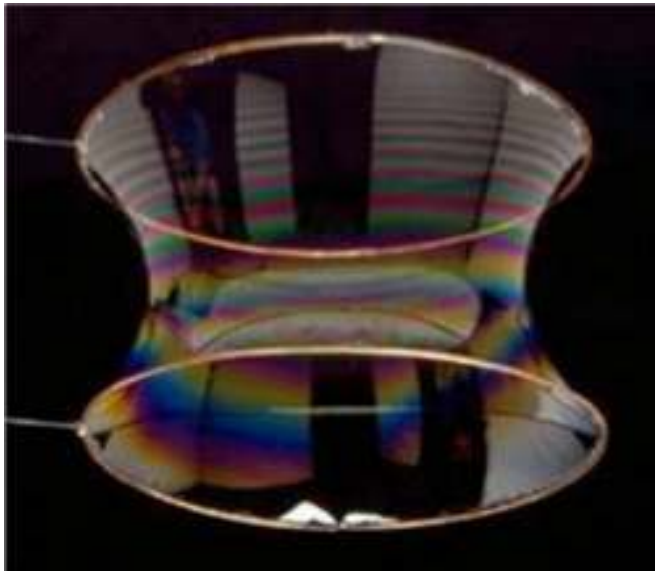



Nature Photonics (2013)

# *Nano-Catenoid Lenses for Imaging of Single Nano-particles*



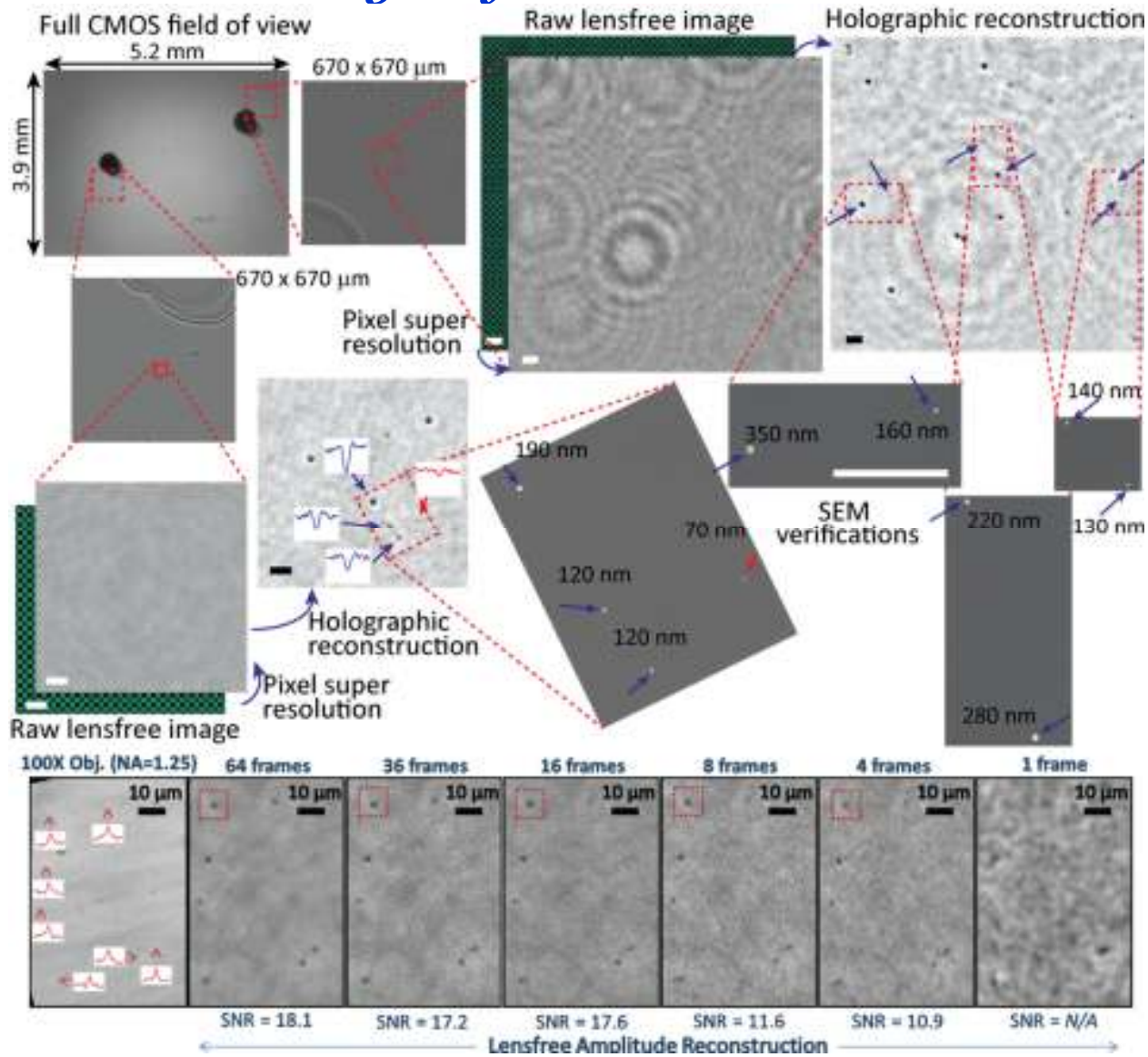

**Nano-catenoid lenses**



Two Rings in Soap Solution Form a ***Catenoid*** – i.e., a ***Minimal Surface*** that is self-assembled to have Zero Mean Curvature.
(L. Euler - 1744)

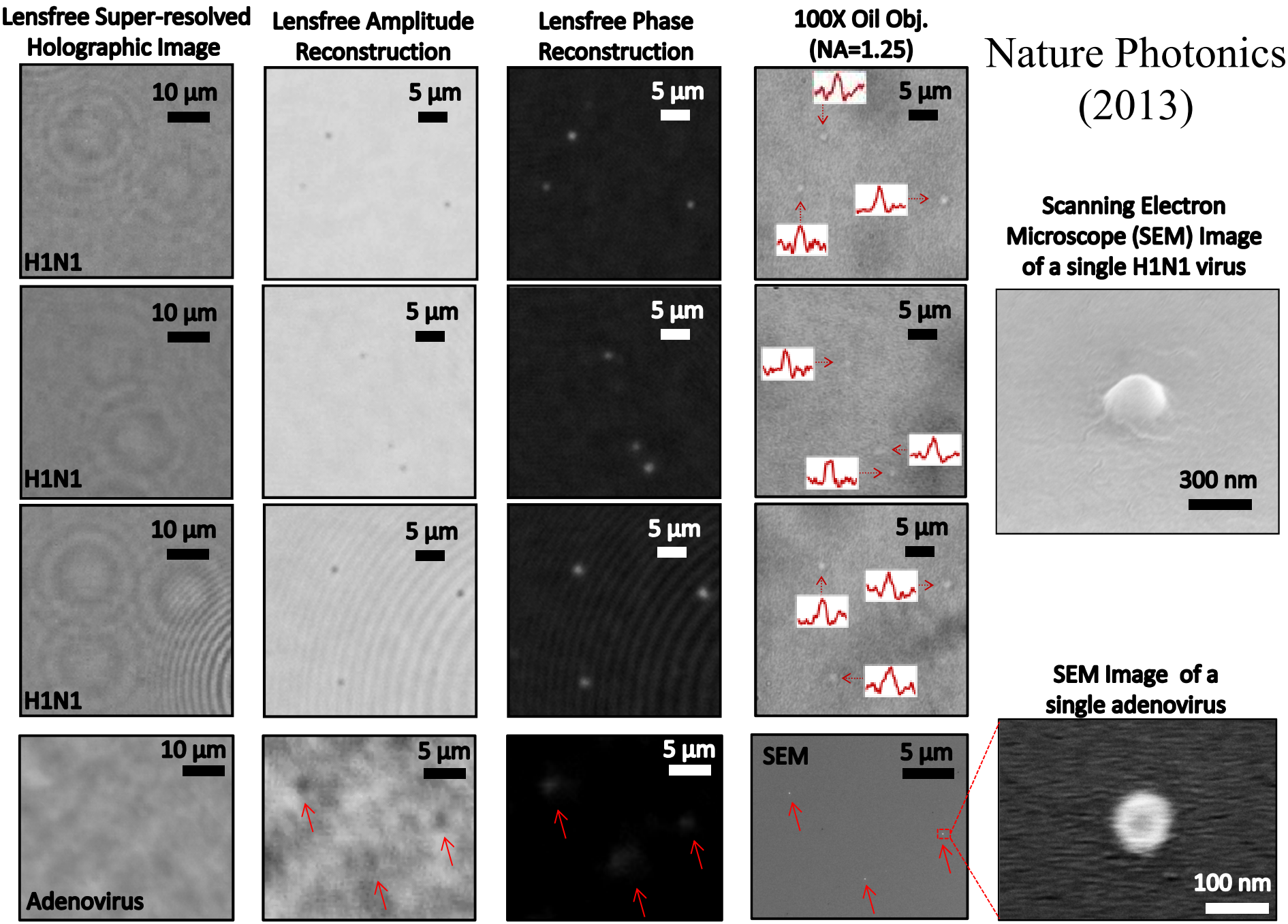
Nature Photonics (2013)

# *Wide-field on-chip imaging of single viruses and nano-particles using self-assembled nano-lenses*

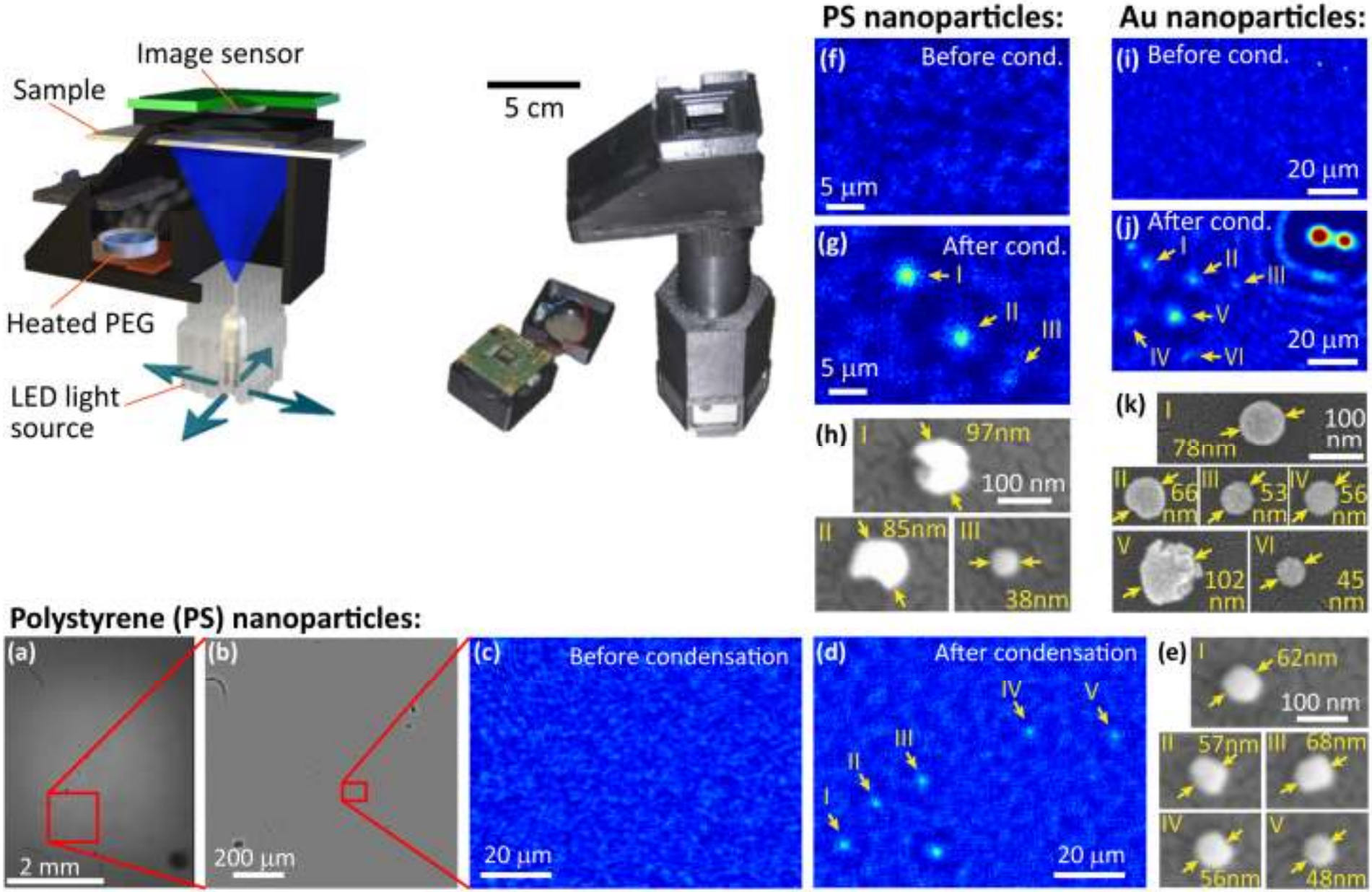



Nature Photonics (2013)

Nature Photonics (2013)

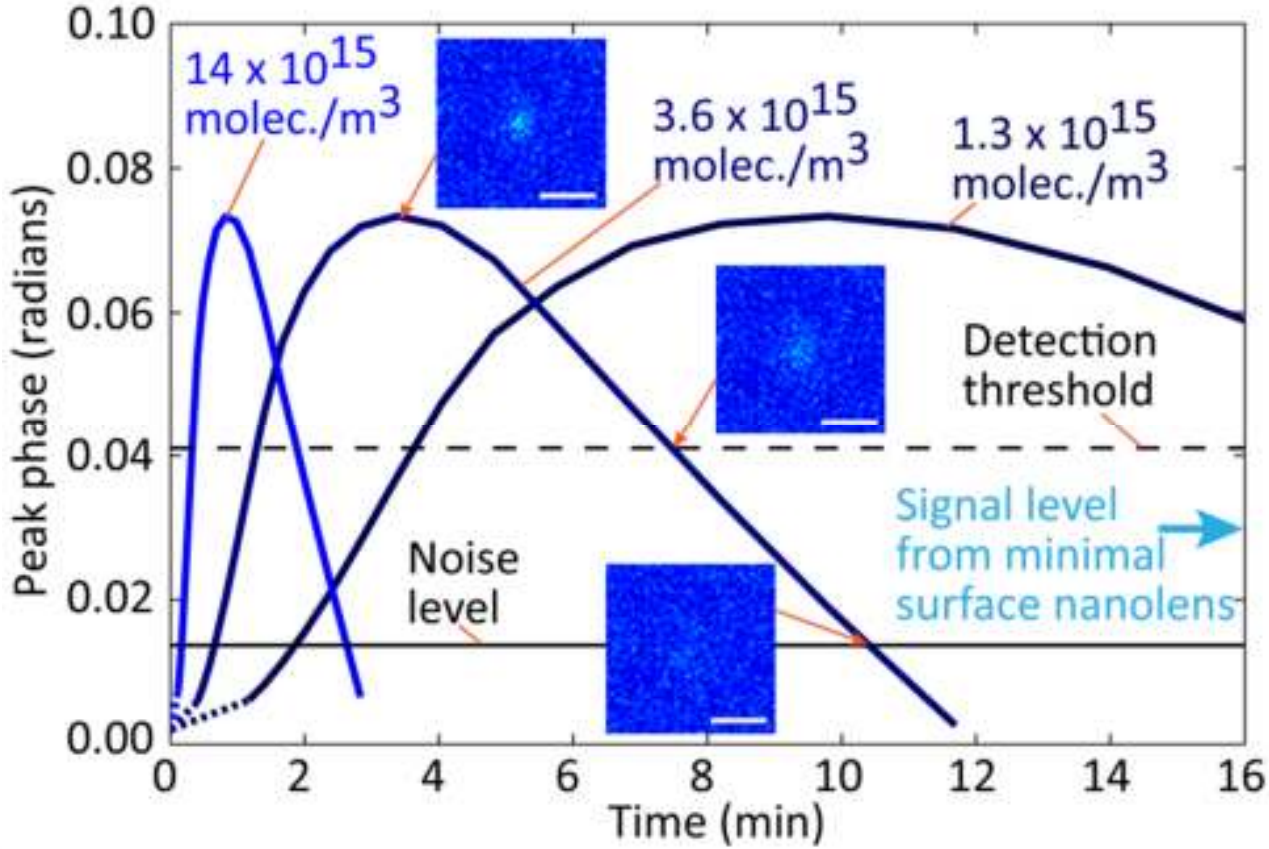


# *Vapor condensed Tunable Nano-lenses*

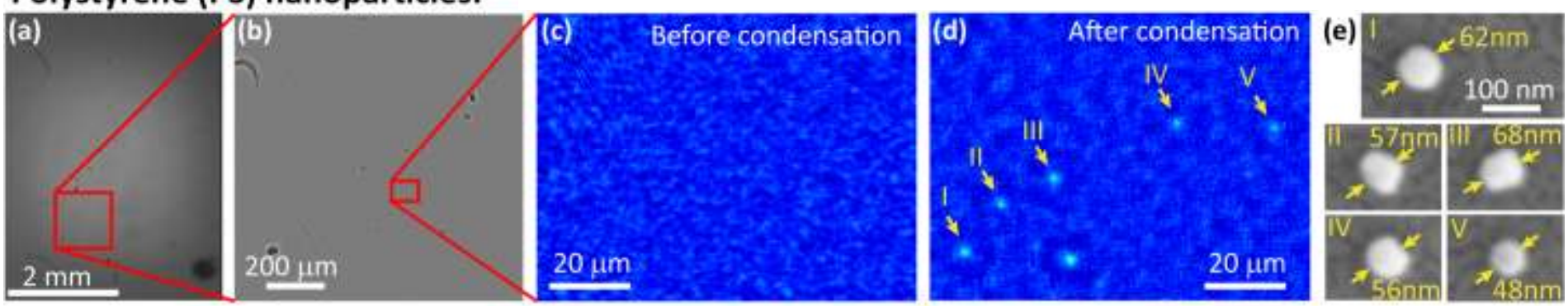



ACS Nano (2014)

# *Vapor condensed Tunable Nano-lenses*

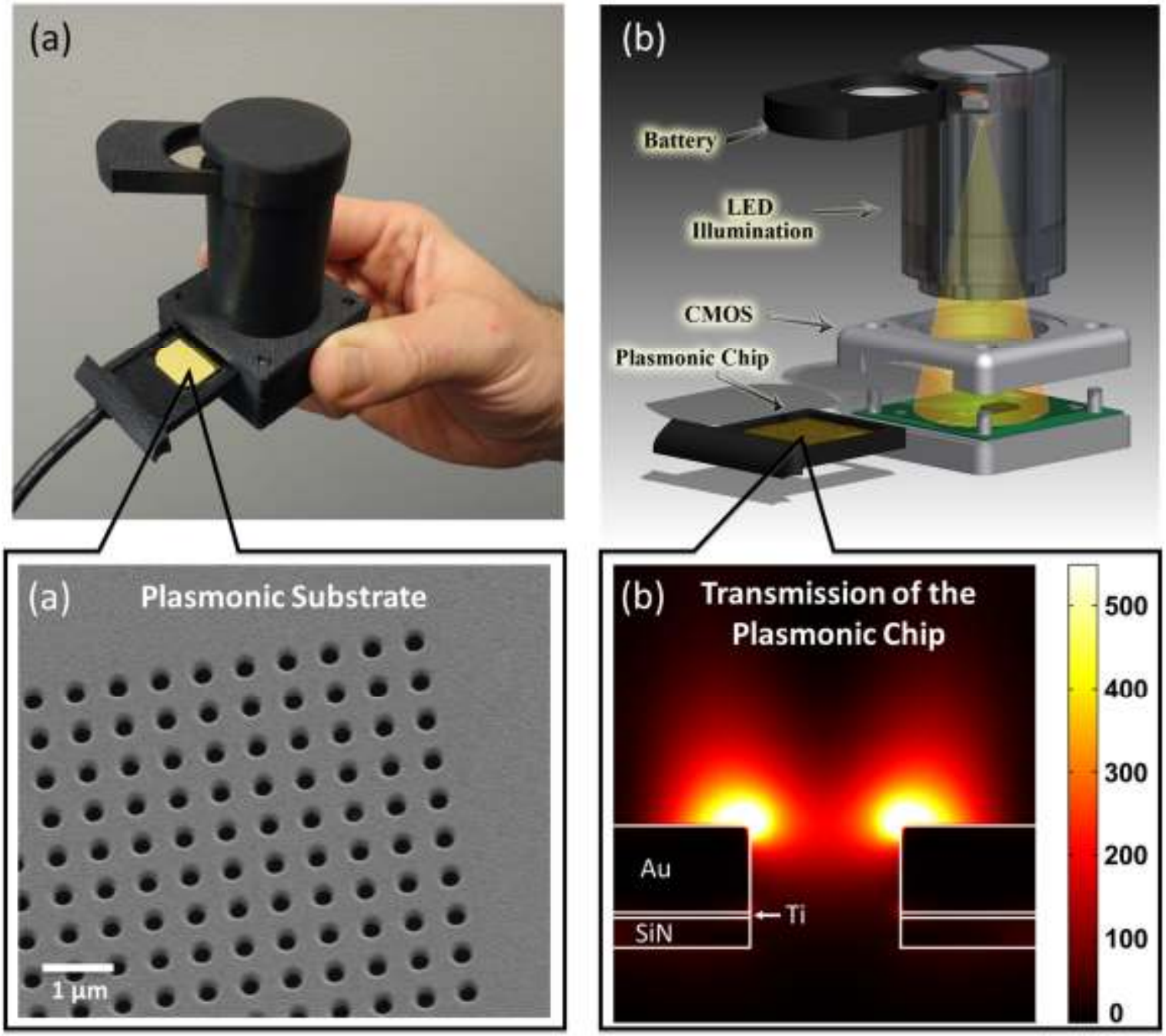



**Polystyrene (PS) nanoparticles:**

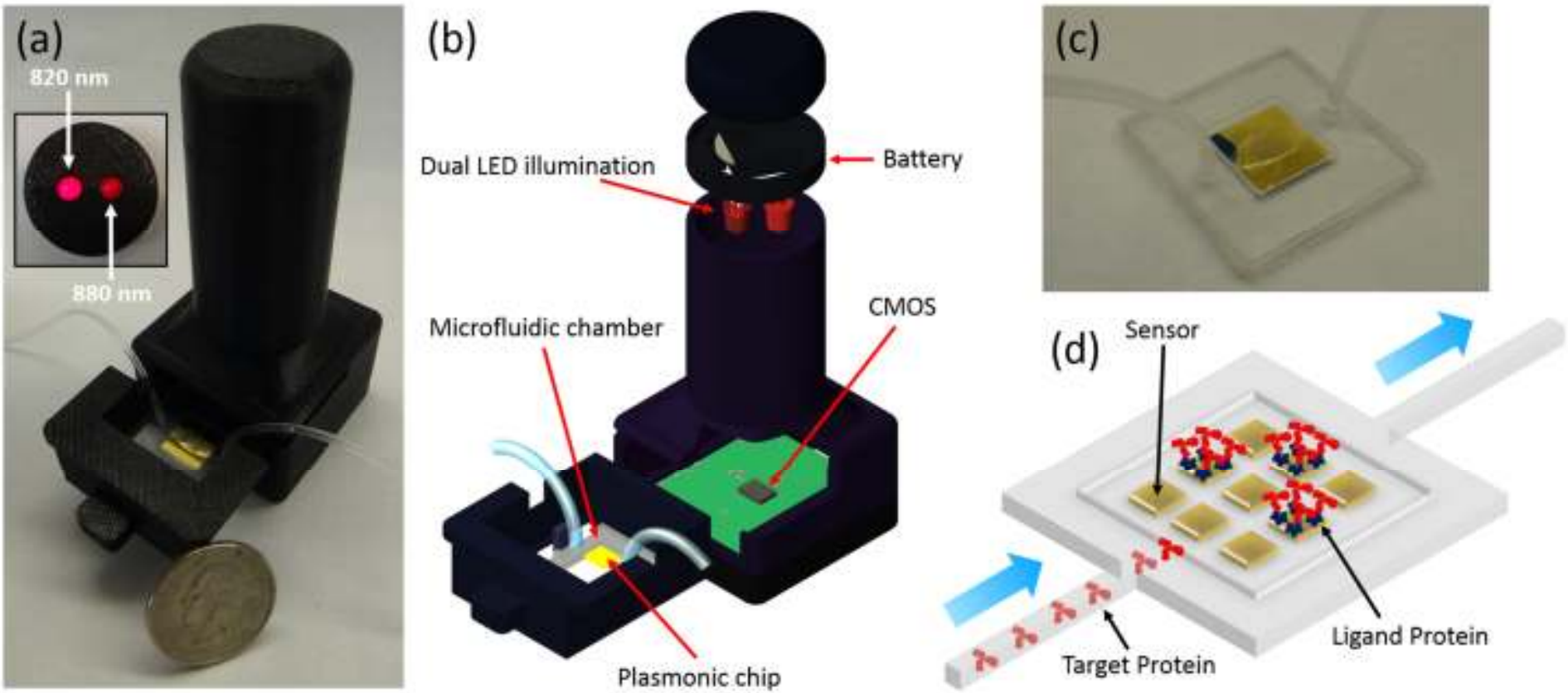



ACS Nano (2014)

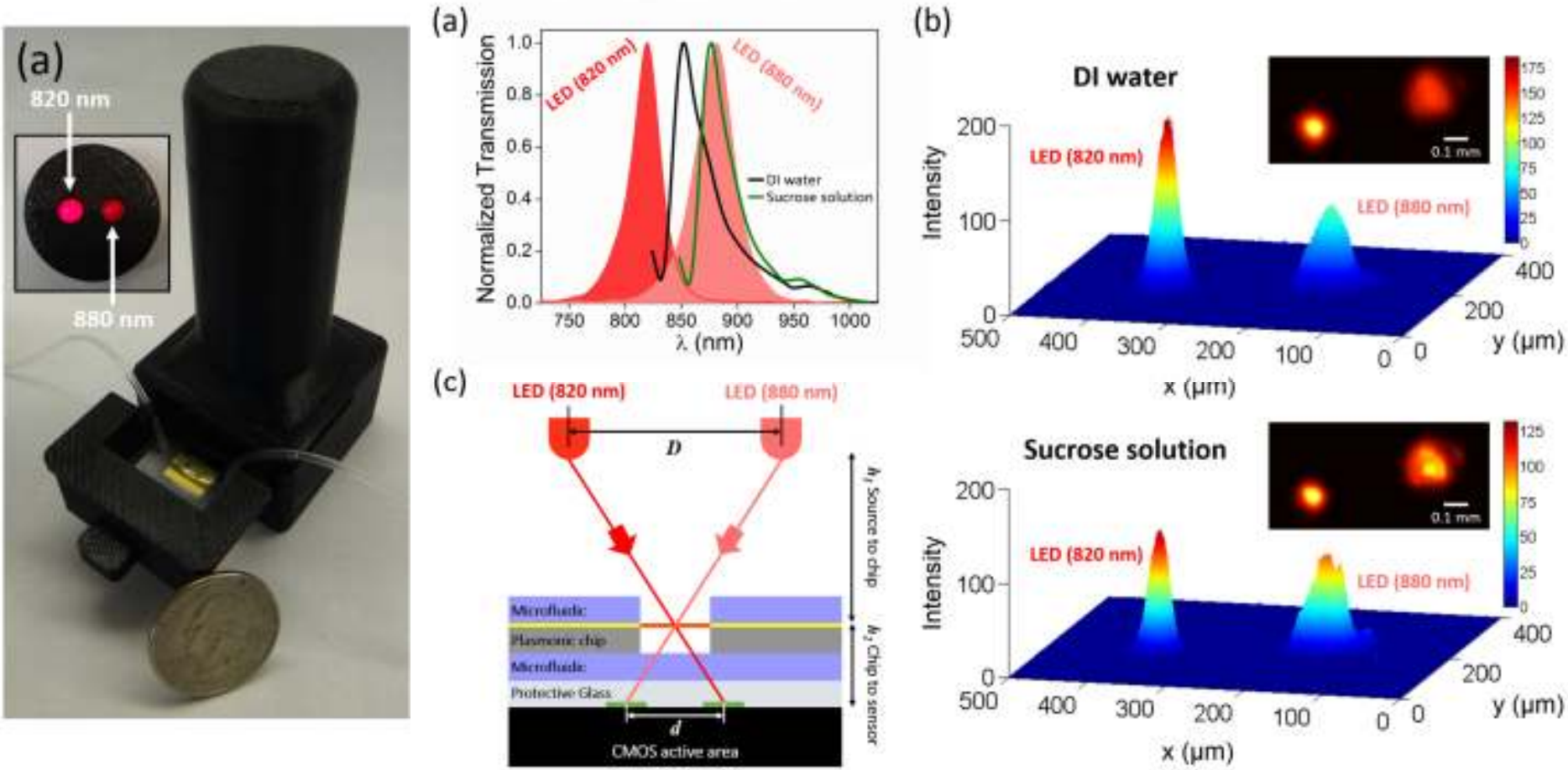
High-throughput and label-free single nanoparticle sizing based on time-resolved on-chip microscopy
Supplemental Video 1:
The Count-and-Clean Procedure
(a)
(b)
(c)
Particle counts
5 cm
RMSE = 11 nm



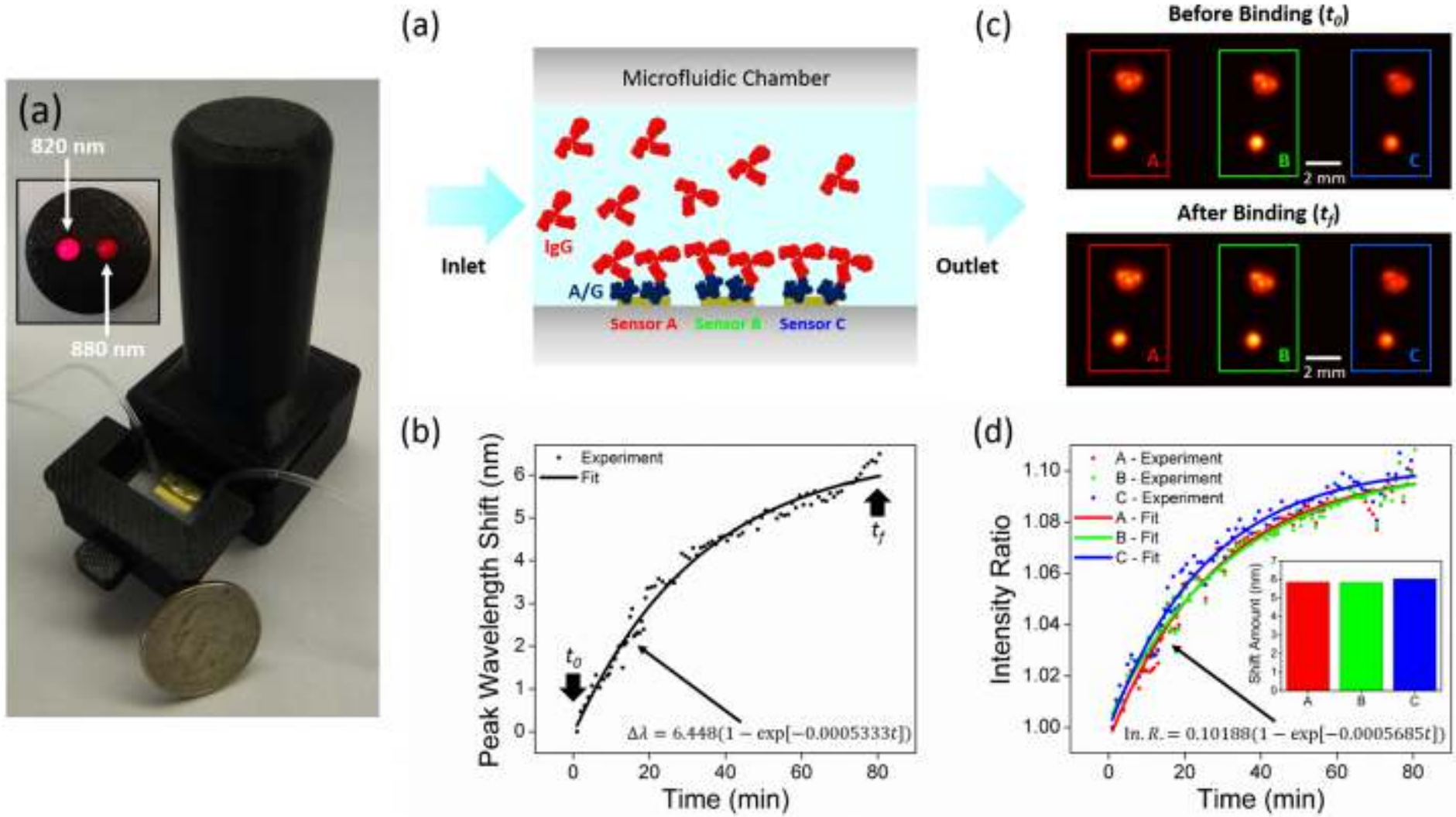

Light: Science and Applications – NPG (2014)




Sci. Reports – NPG (2014)

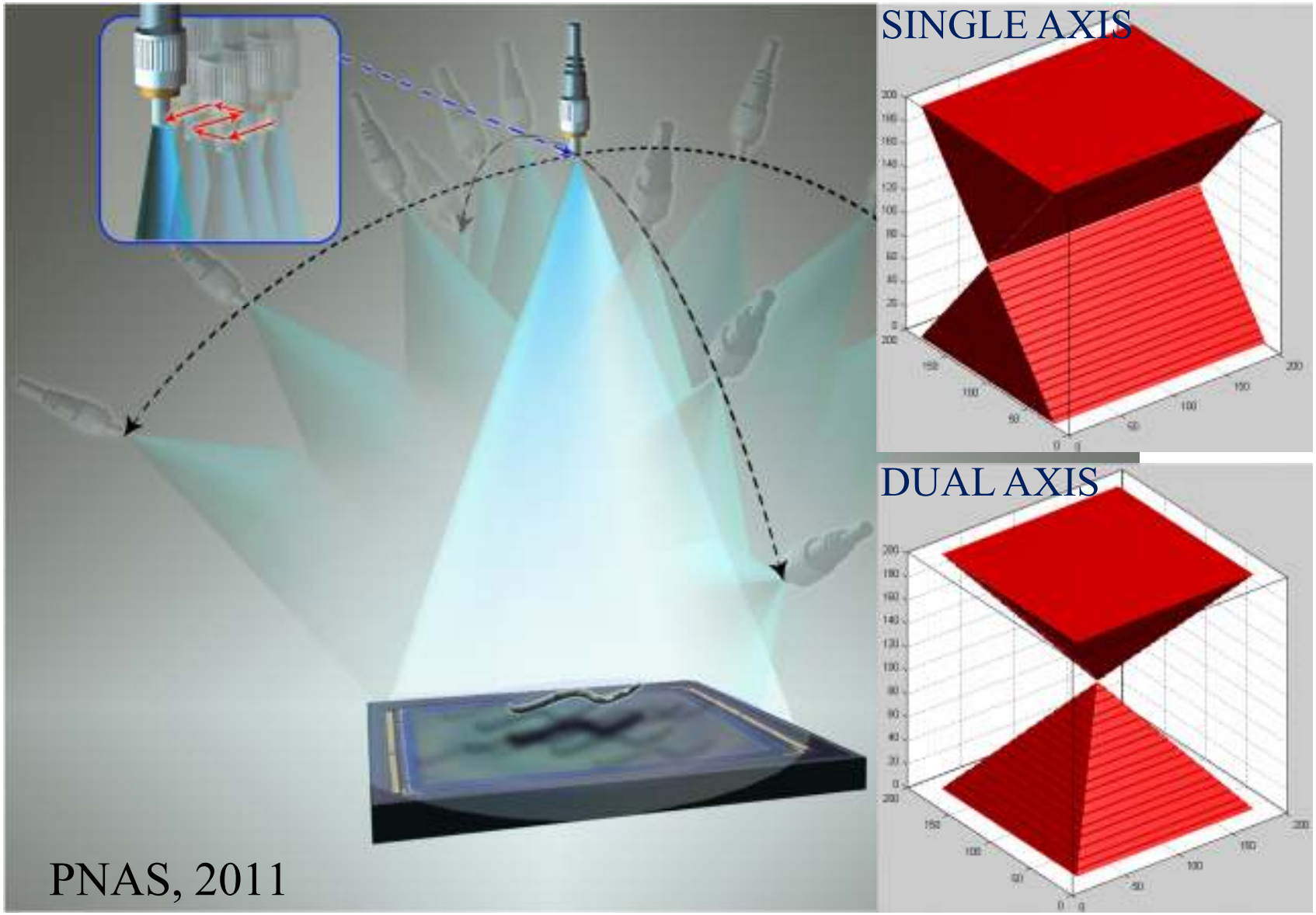


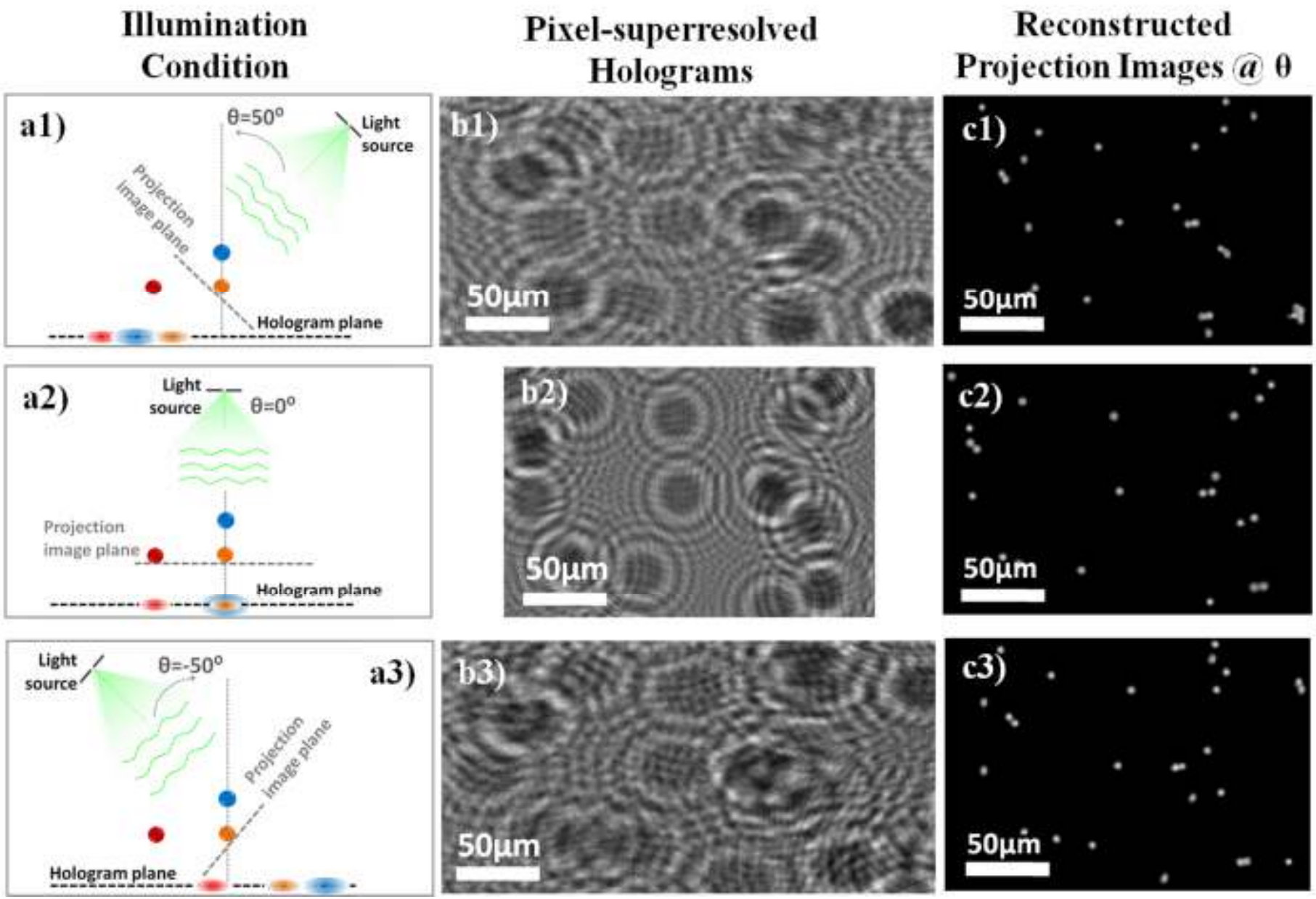

Sci. Reports – NPG (2014)

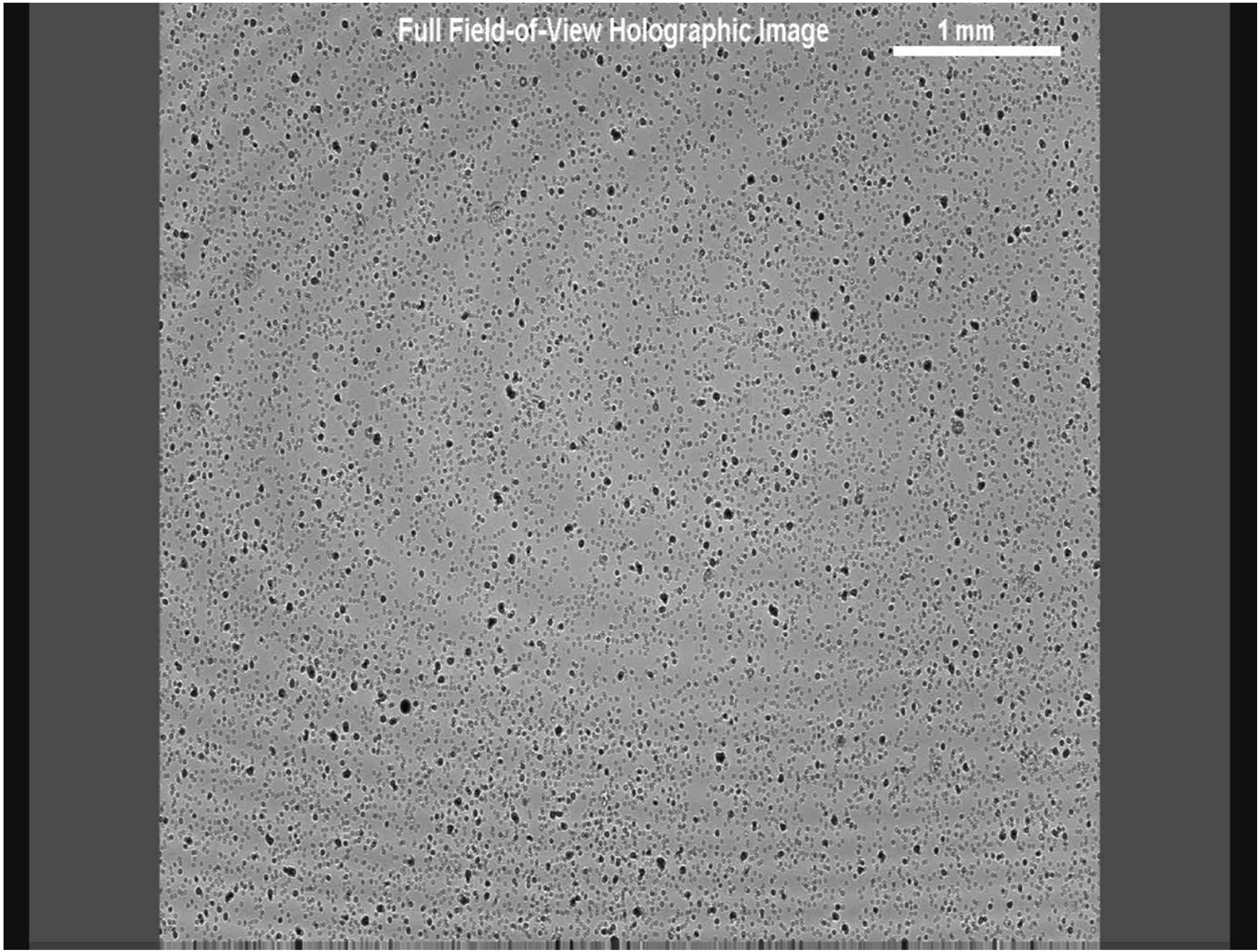


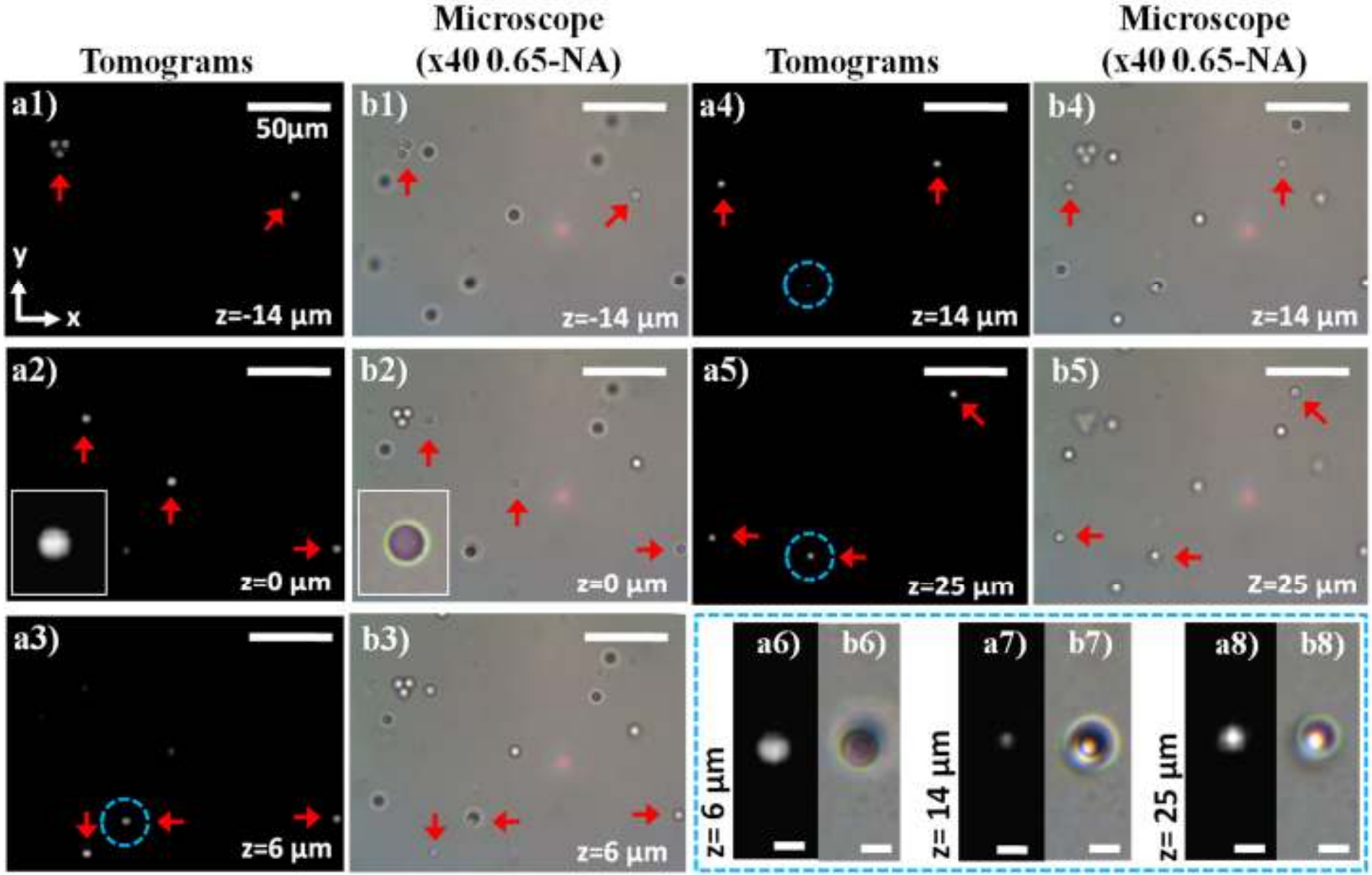

Sci. Reports – NPG (2014)

# *Lensfree Tomographic Imaging on a Chip*

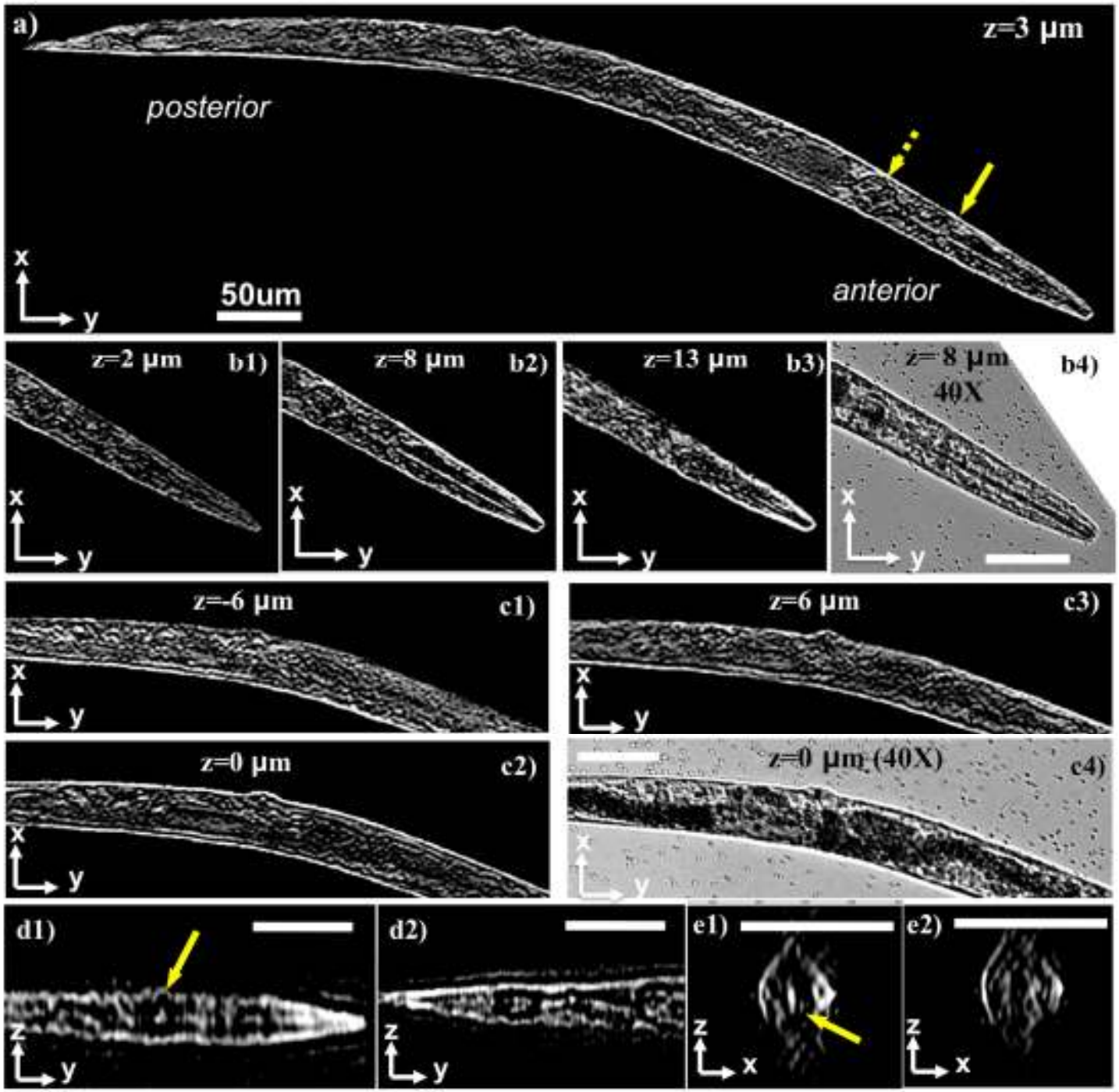


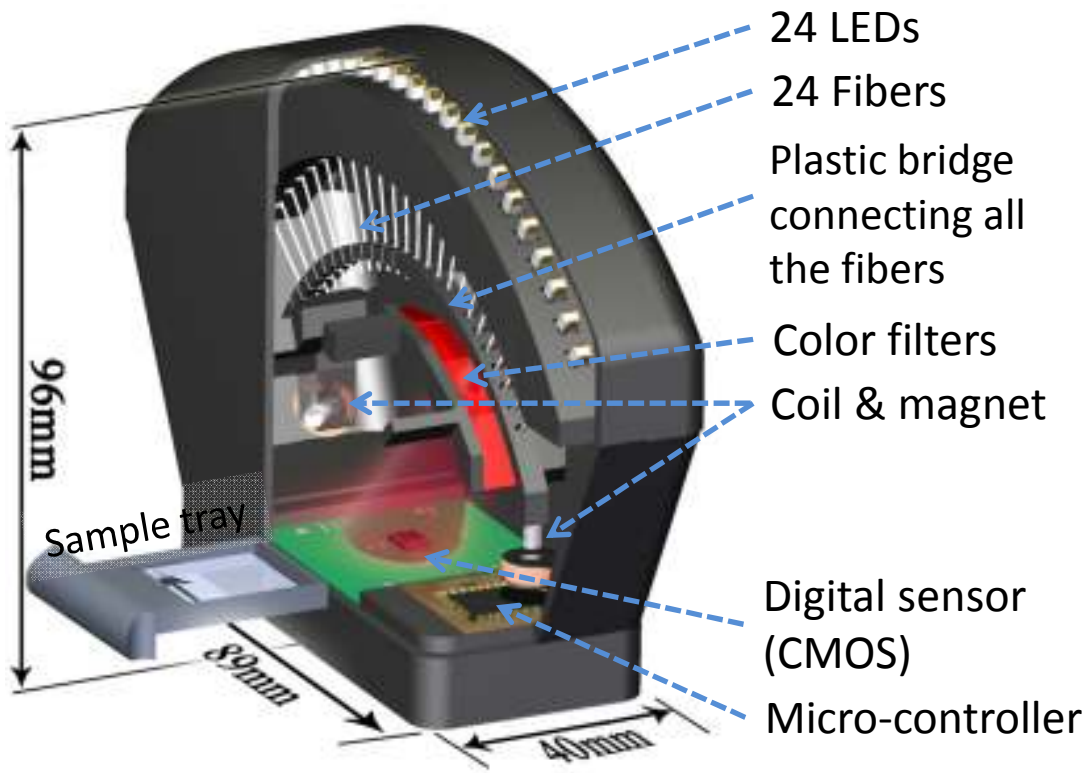

Illumination Condition
Pixel-superresolved Holograms
Reconstructed Projection Images @ θ
a1)
θ=50°
Light source
Projection image plane
Hologram plane
b1)
50μm
c1)
50μm
a2)
Light source
θ=0°
Projection image plane
Hologram plane
b2)
50μm
c2)
50μm
Light source
θ=-50°
a3)
Projection image plane
Hologram plane
b3)
50μm
c3)
50μm


Full Field-of-View Holographic Image
1 mm

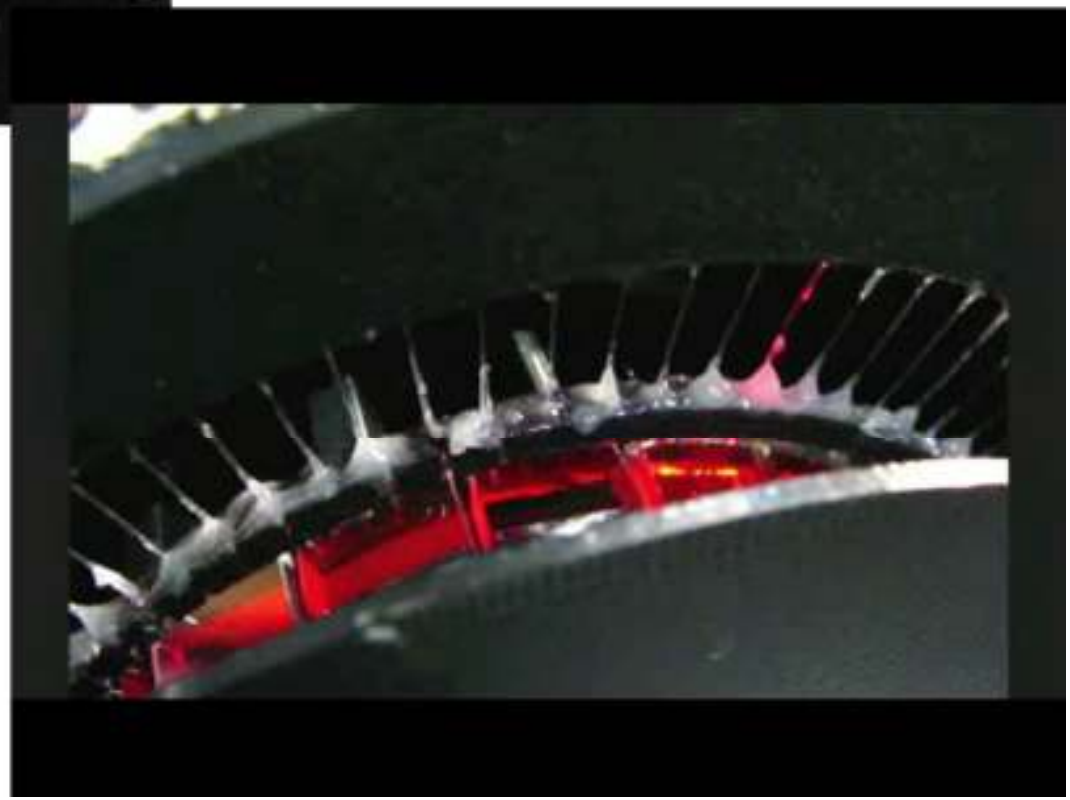

Tomograms
Microscope
(x40 0.65-NA)
Tomograms
Microscope
(x40 0.65-NA)
a1)
50µm
y
x
z=-14 µm
b1)
z=-14 µm
a4)
z=14 µm
b4)
z=14 µm
a2)
z=0 µm
b2)
z=0 µm
a5)
z=25 µm
b5)
z=25 µm
a3)
z=6 µm
b3)
z=6 µm
a6)
b6)
z= 6 µm
a7)
b7)
z= 14 µm
a8)
b8)
z= 25 µm

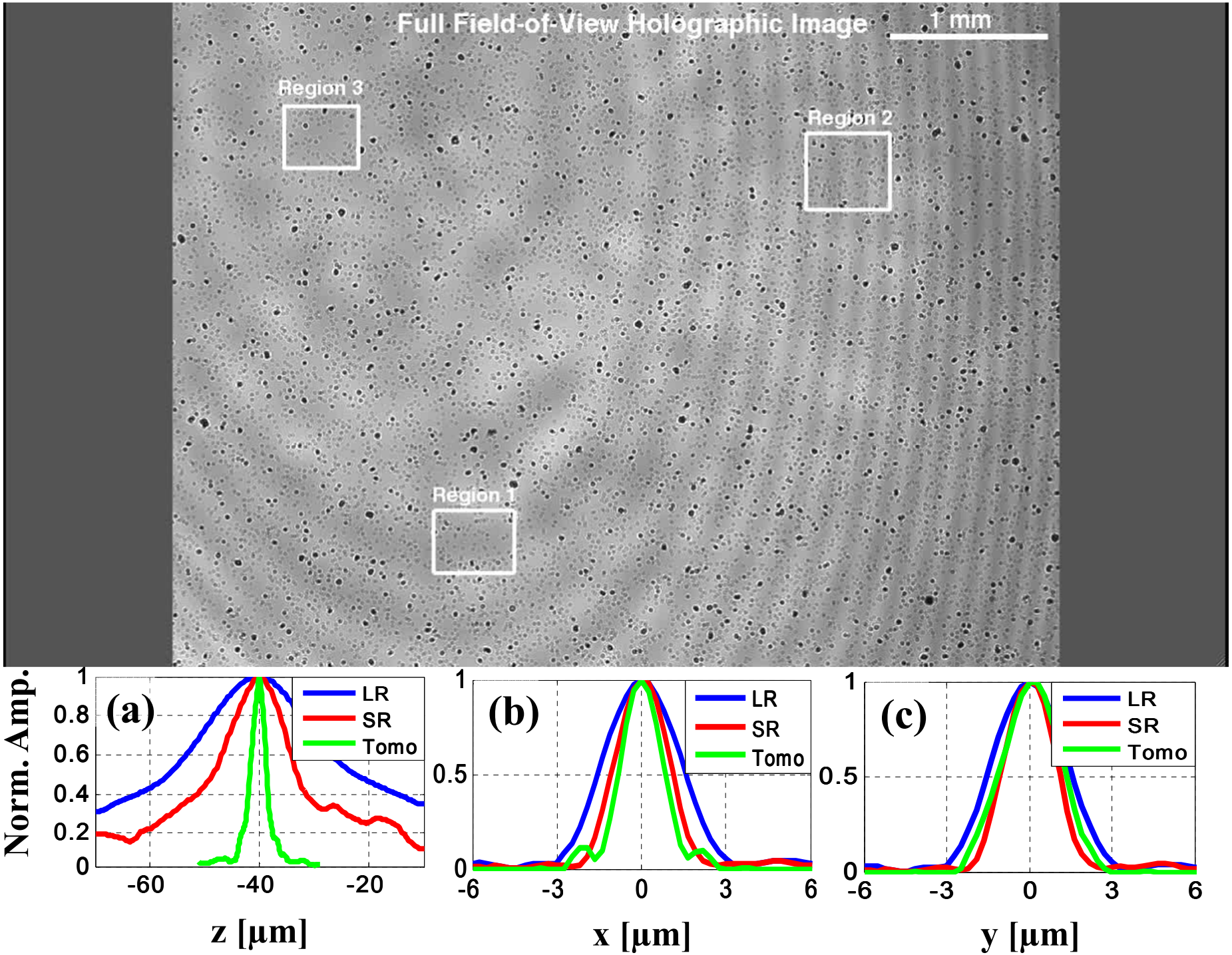
PNAS, 2011

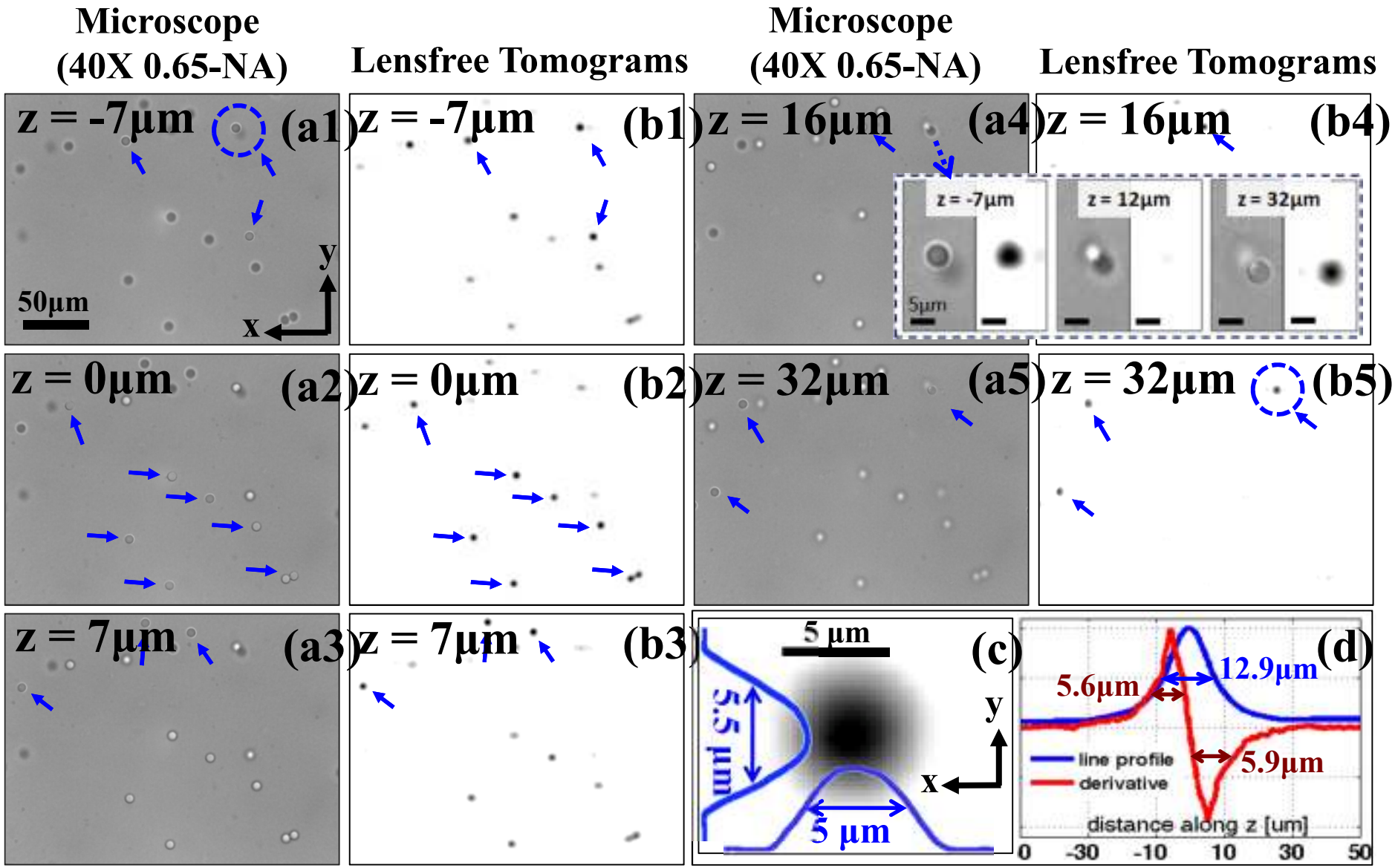

a)
z=3 µm
posterior
anterior
50um
x
y
z=2 µm
b1)
z=8 µm
b2)
z=13 µm
b3)
z=8 µm
40X
b4)
z=-6 µm
c1)
z=6 µm
c3)
z=0 µm
c2)
z=0 µm (40X)
c4)
d1)
d2)
e1)
e2)
z

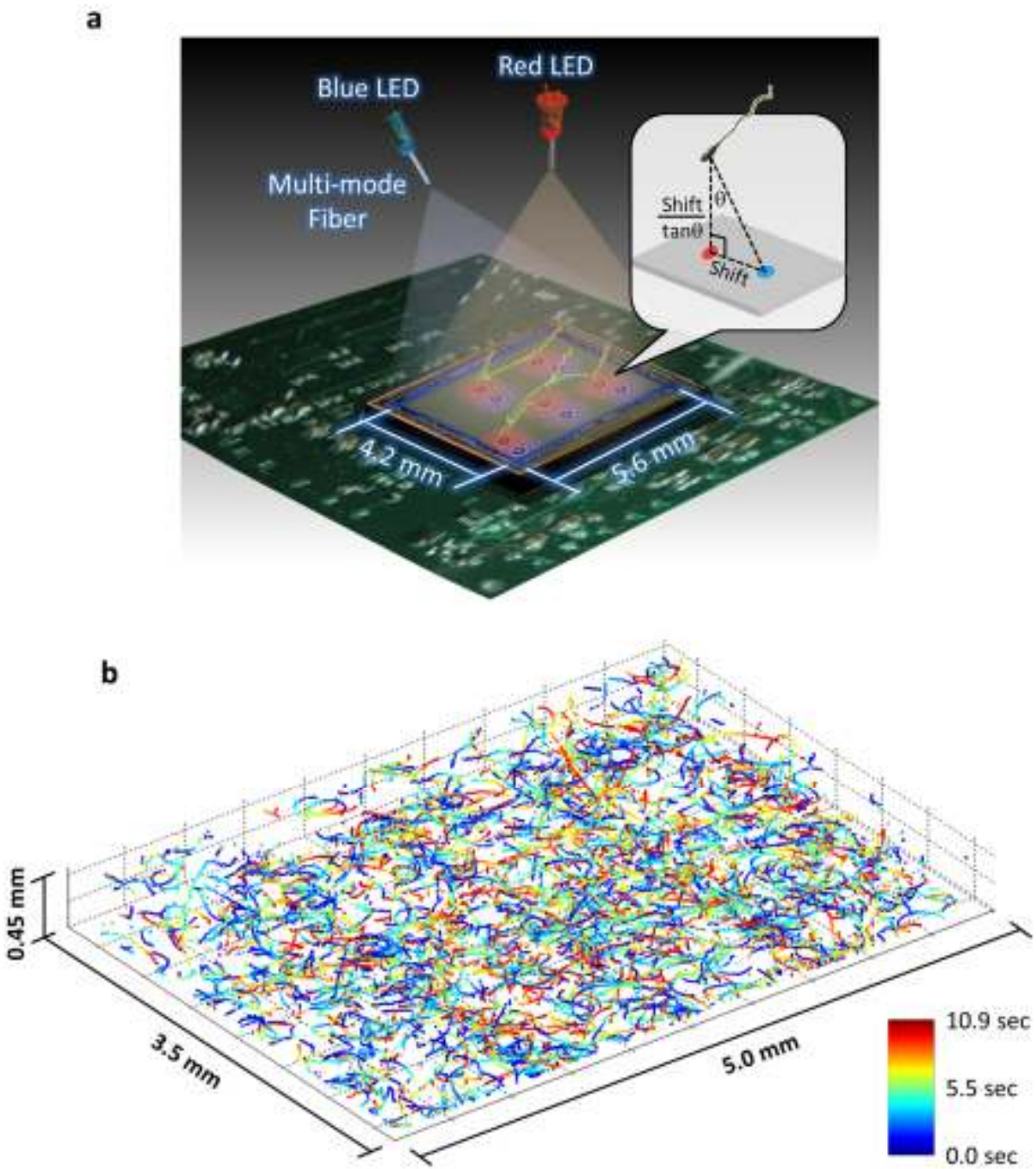

24 LEDs
24 Fibers
Plastic bridge connecting all the fibers
Color filters
Coil & magnet
Digital sensor (CMOS)
Micro-controller
96mm
89mm
40mm
Sample tray

BioPhotonics Laboratory

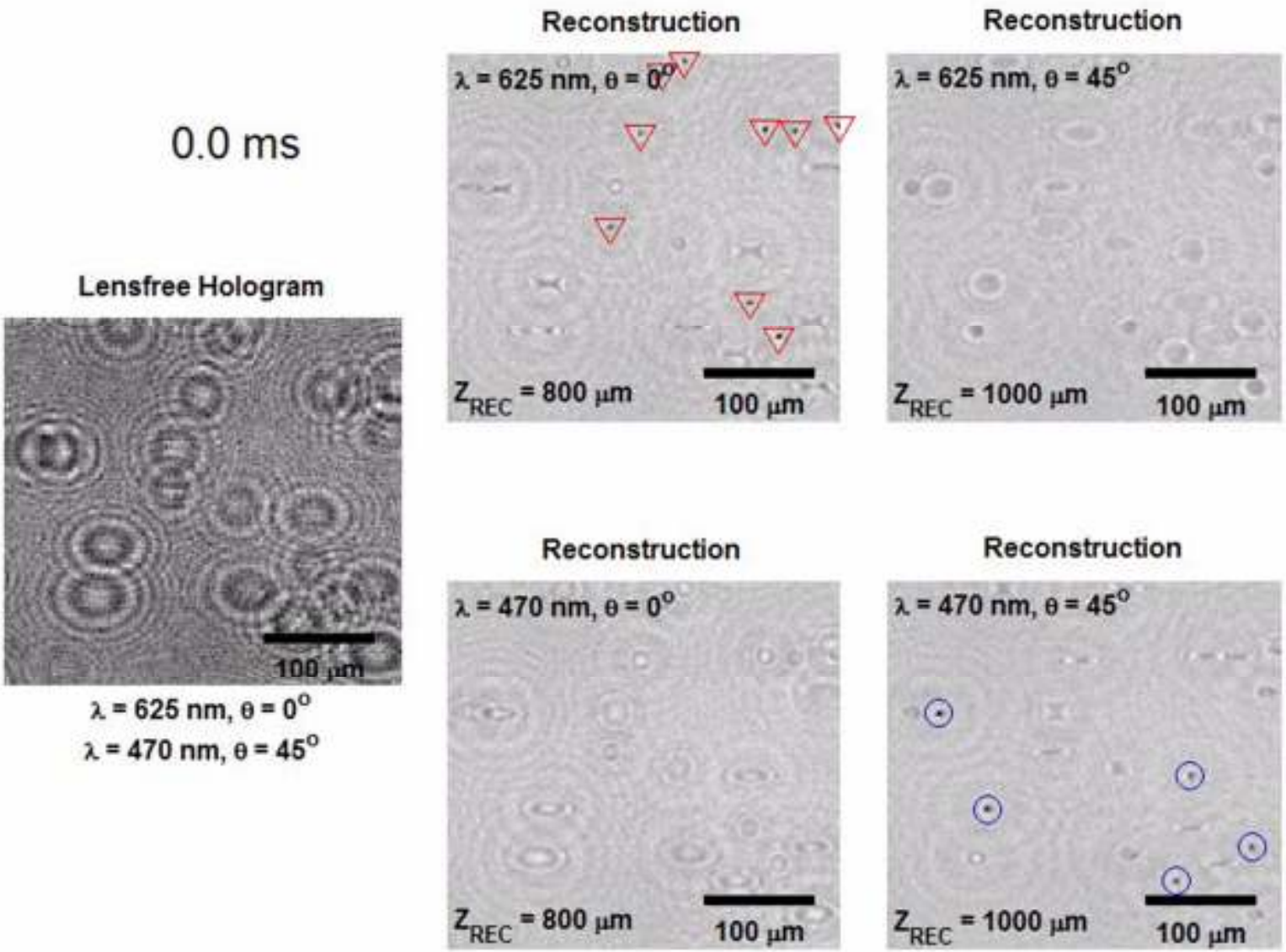


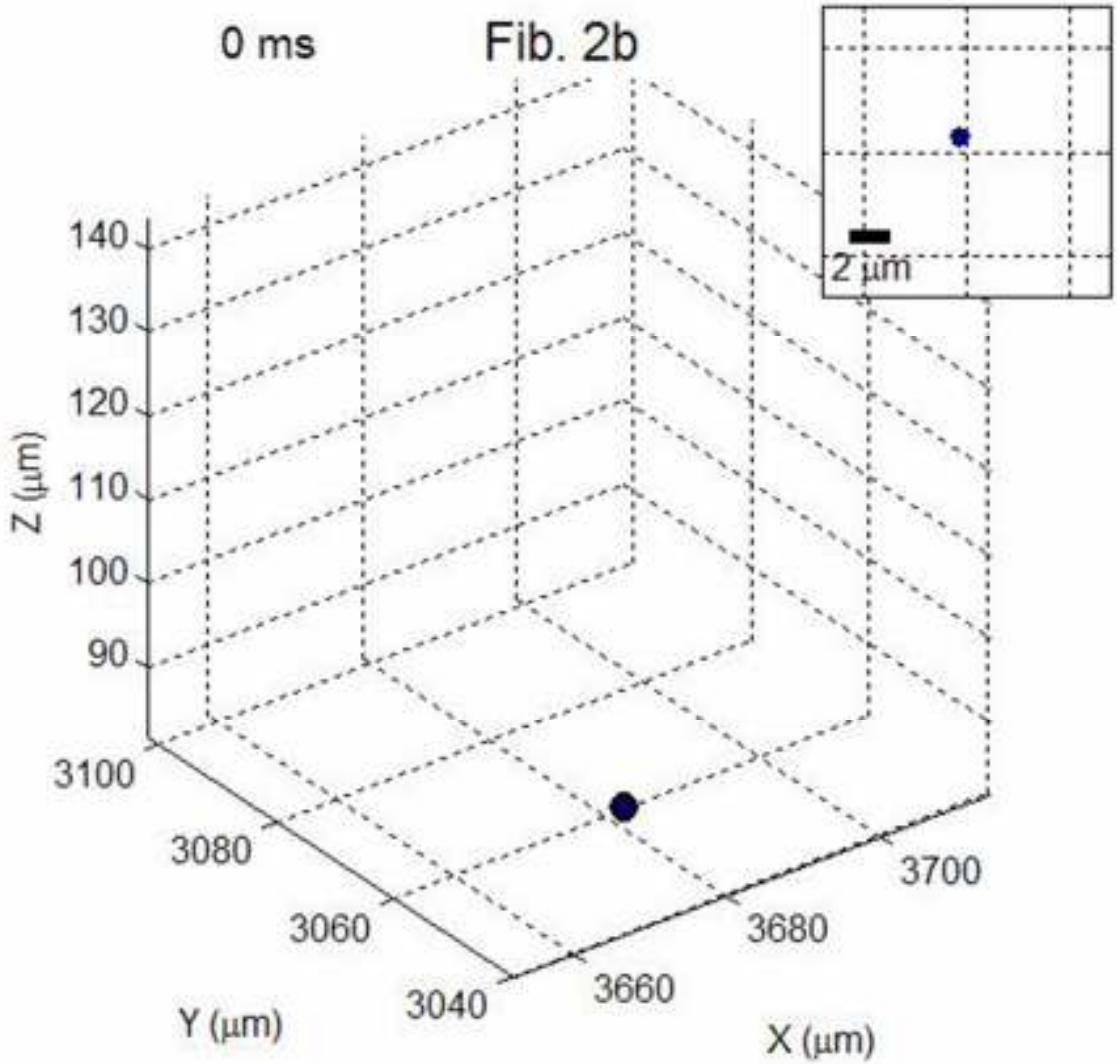

Full Field-of-View Holographic Image
1 mm
Region 3
Region 2
Region 1
Norm. Amp.
(a)
(b)
(c)
LR
SR
Tomo
z [µm]
x [µm]
y [µm]


Microscope
(40X 0.65-NA)
Lensfree Tomograms
Microscope
(40X 0.65-NA)
Lensfree Tomograms
z = -7µm
(a1)
z = -7µm
(b1)
z = 16µm
(a4)
z = 16µm
(b4)
z = -7µm
z = 12µm
z = 32µm
5µm
50µm
y
x
z = 0µm
(a2)
z = 0µm
(b2)
z = 32µm
(a5)
z = 32µm
(b5)
z = 7µm
(a3)
z = 7µm
(b3)
5 µm
(c)
5.5 µm
5 µm
y
x
(d)
5.6µm
12.9µm
5.9µm
line profile
derivative
distance along z [um]
0
-30
-10
10
30
50

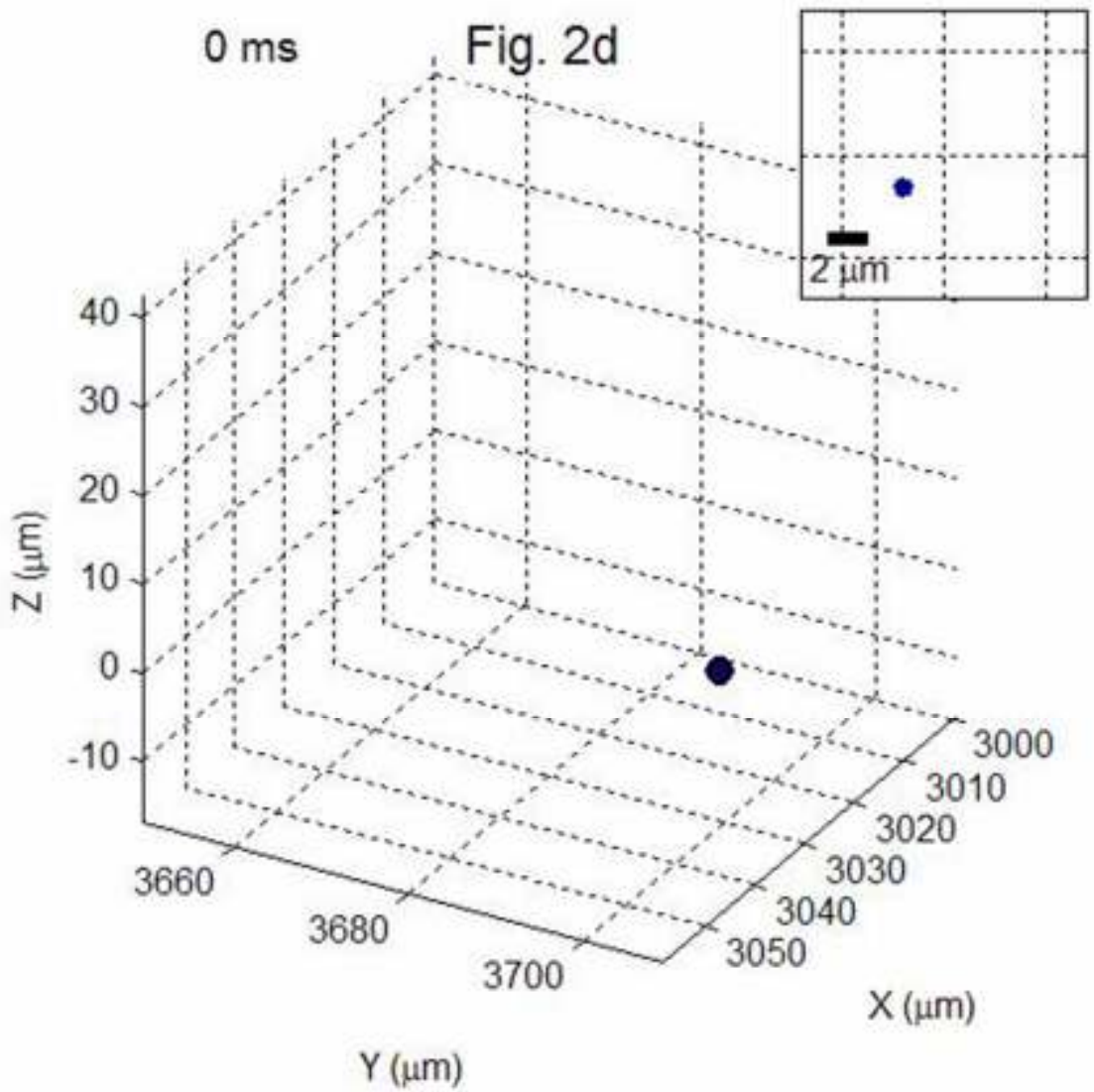

a
Blue LED
Red LED
Multi-mode
Fiber
Shift
tanθ
θ
Shift
4.2 mm
5.6 mm
b
0.45 mm
3.5 mm
5.0 mm
10.9 sec
5.5 sec
0.0 sec

PNAS, 2012

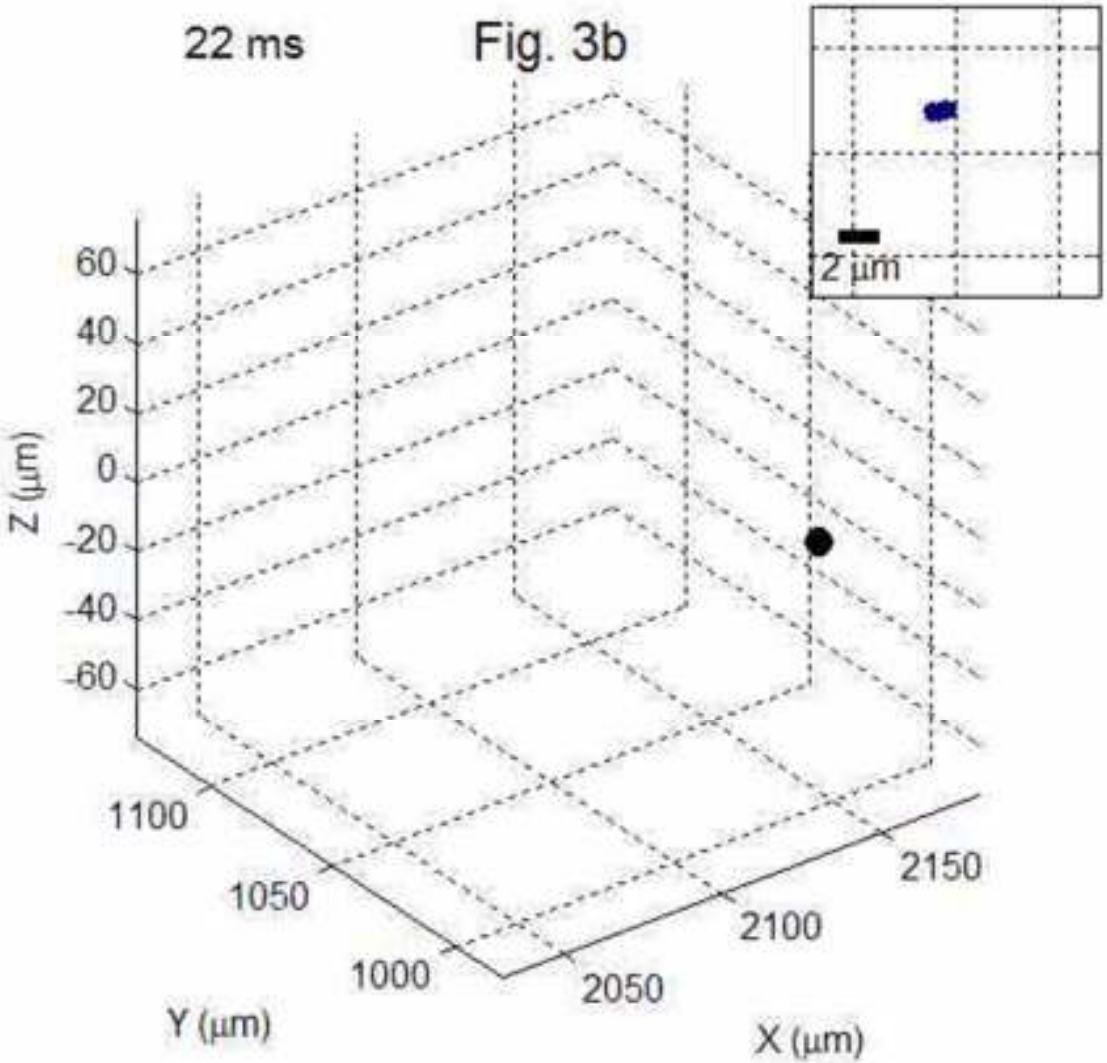

0.0 ms
Lensfree Hologram
100 μm
λ = 625 nm, θ = 0°
λ = 470 nm, θ = 45°
Reconstruction
λ = 625 nm, θ = 0°
Z_REC = 800 μm
100 μm
Reconstruction
λ = 625 nm, θ = 45°
Z_REC = 1000 μm
100 μm
Reconstruction
λ = 470 nm, θ = 0°
Z_REC = 800 μm
100 μm
Reconstruction
λ = 470 nm, θ = 45°
Z_REC = 1000 μm
100 μm


0 ms
Fib. 2b
2 µm
140
130
120
110
100
90
Z (µm)
3100
3080
3060
3040
Y (µm)
3660
3680
3700
X (µm)

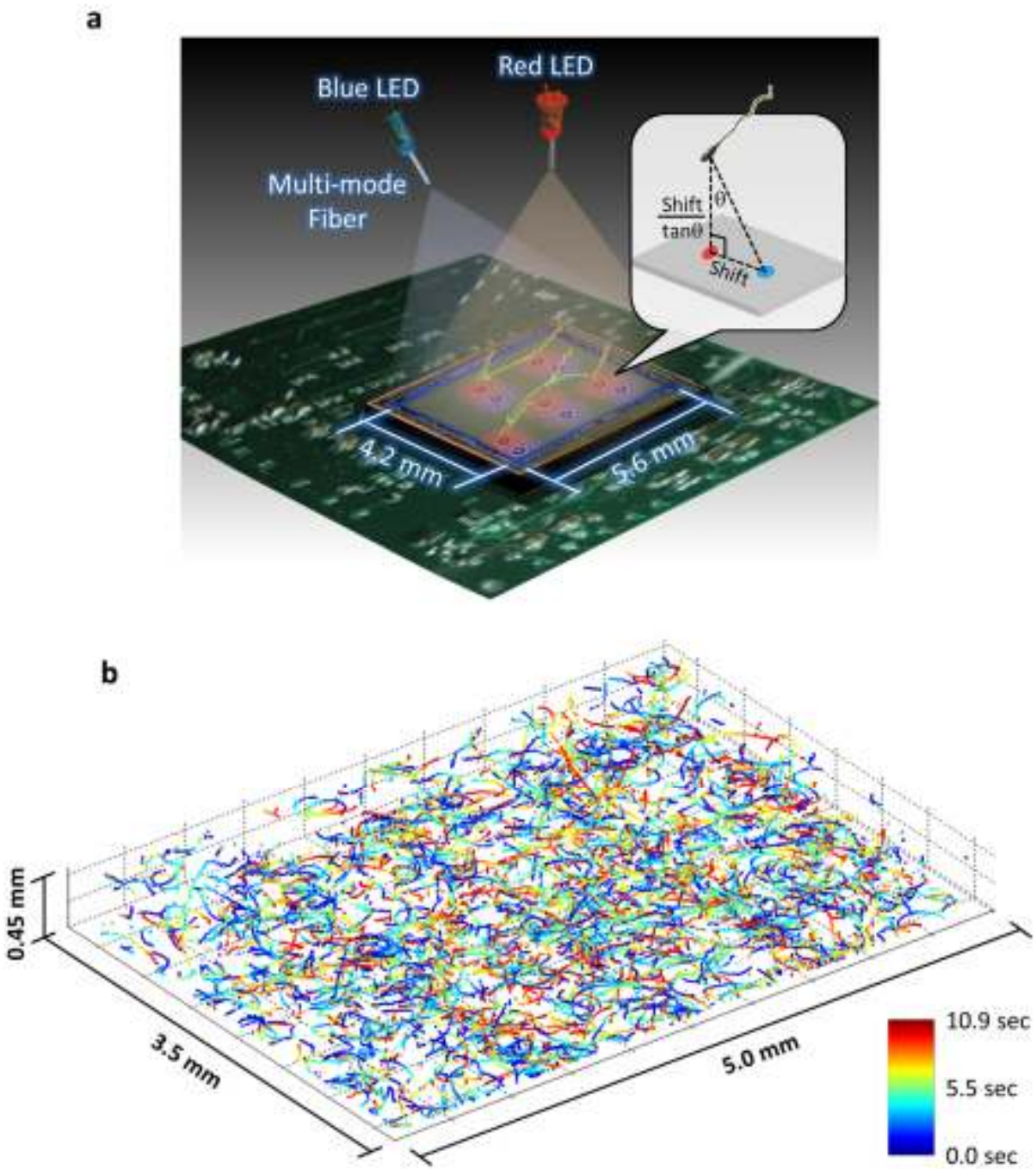
PNAS, 2012


0 ms
Fig. 2d
2 µm
40
30
20
10
0
-10
Z (µm)
3660
3680
3700
Y (µm)
3000
3010
3020
3030
3040
3050
X (µm)

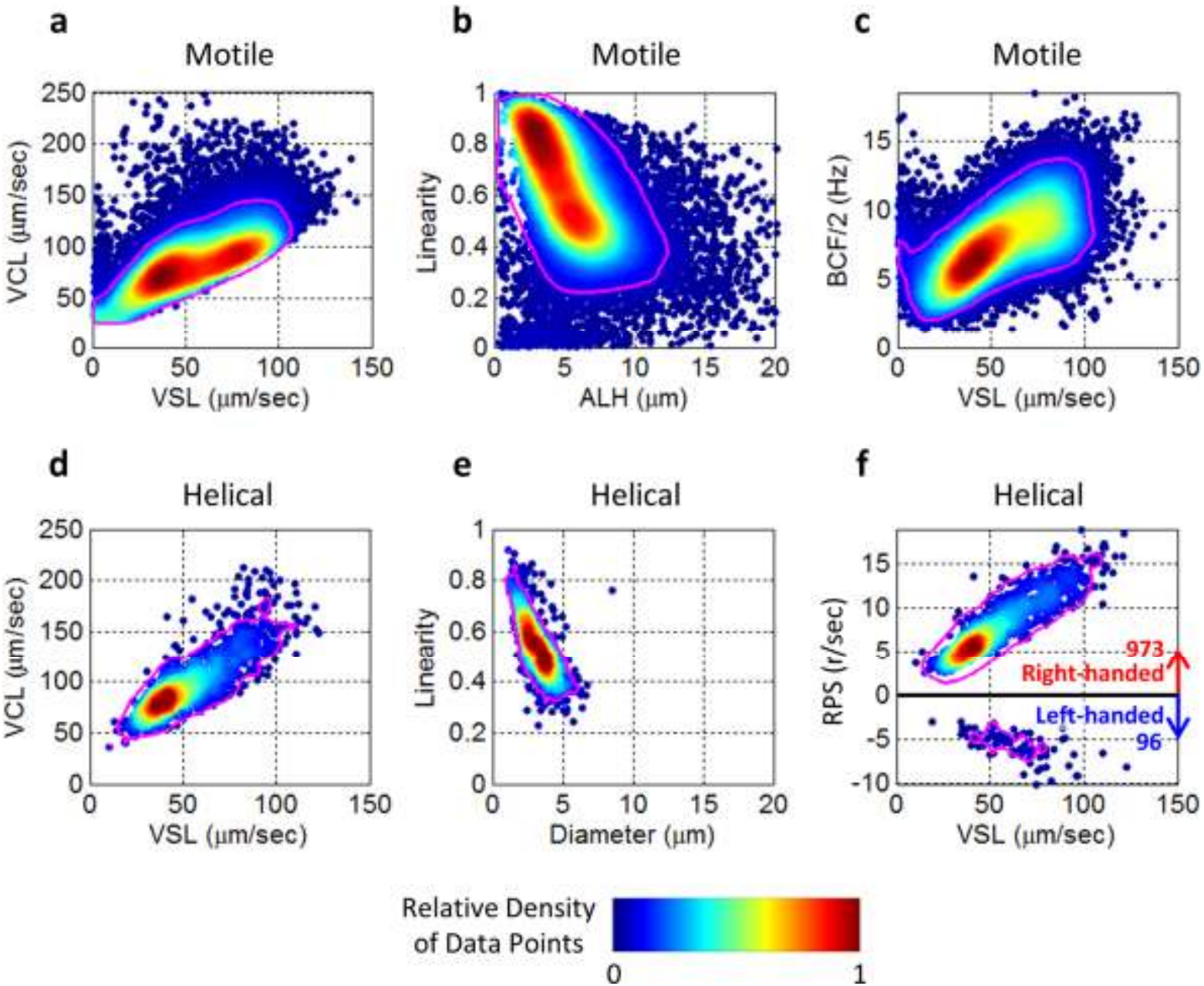
PNAS, 2012


22 ms
Fig. 3b
2 µm
60
40
20
0
-20
-40
-60
Z (µm)
1100
1050
1000
Y (µm)
2050
2100
2150
X (µm)

PNAS, 2012


a
Blue LED
Red LED
Multi-mode
Fiber
Shift
tanθ
θ
Shift
4.2 mm
5.6 mm
b
0.45 mm
3.5 mm
5.0 mm
10.9 sec
5.5 sec
0.0 sec

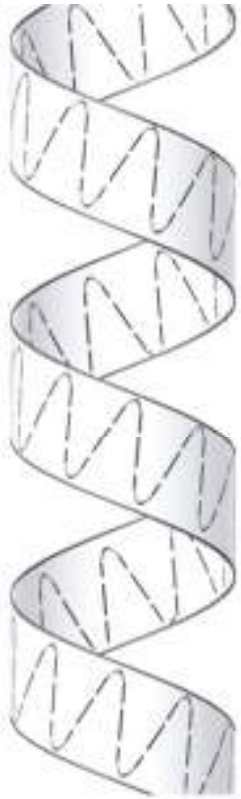
PNAS, 2012


a
Motile
VCL (μm/sec)
VSL (μm/sec)
b
Motile
Linearity
ALH (μm)
c
Motile
BCF/2 (Hz)
VSL (μm/sec)
d
Helical
VCL (μm/sec)
VSL (μm/sec)
e
Helical
Linearity
Diameter (μm)
f
Helical
RPS (r/sec)
973
Right-handed
Left-handed
96
VSL (μm/sec)
Relative Density
of Data Points
0
1

PNAS, 2012

# *Sperm Trajectories form Chiral Ribbons*

Helical Ribbon

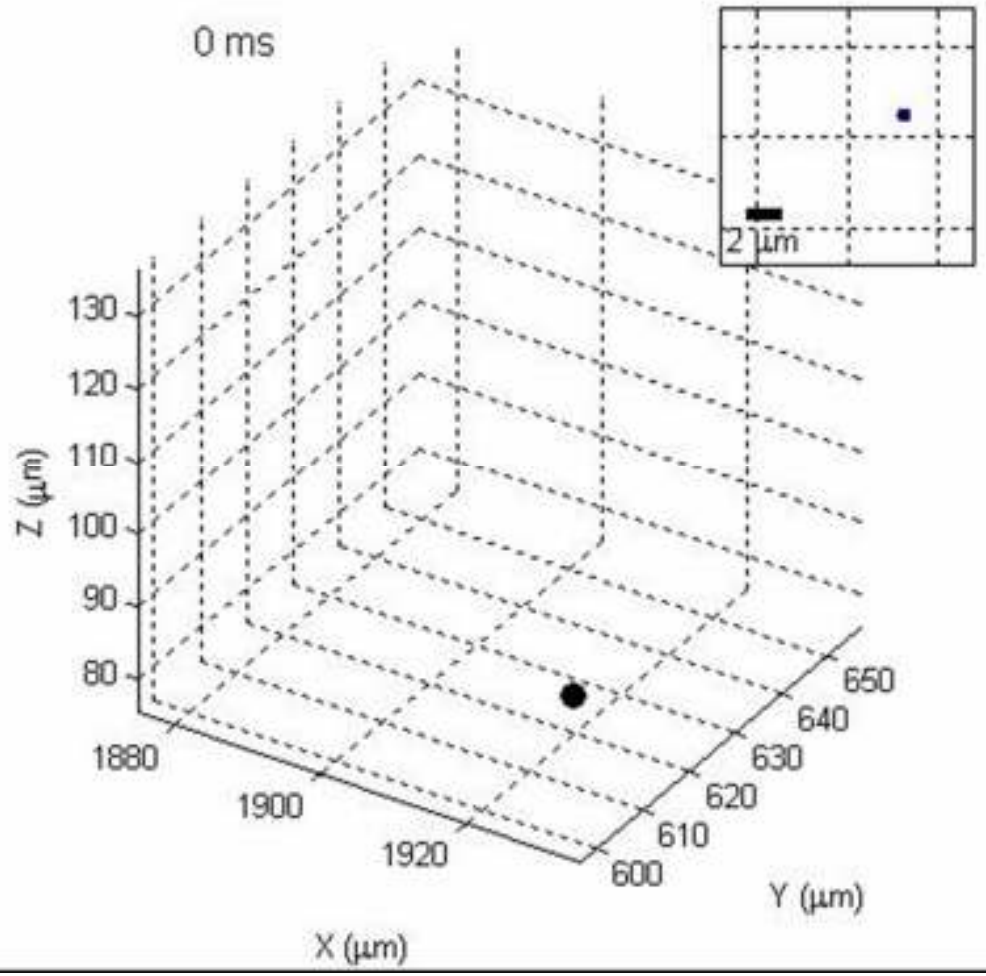


Twisted Ribbon

Scientific Reports, 2013

# *Sperm Trajectories form Chiral Ribbons*

Helical Ribbon




Scientific Reports, 2013

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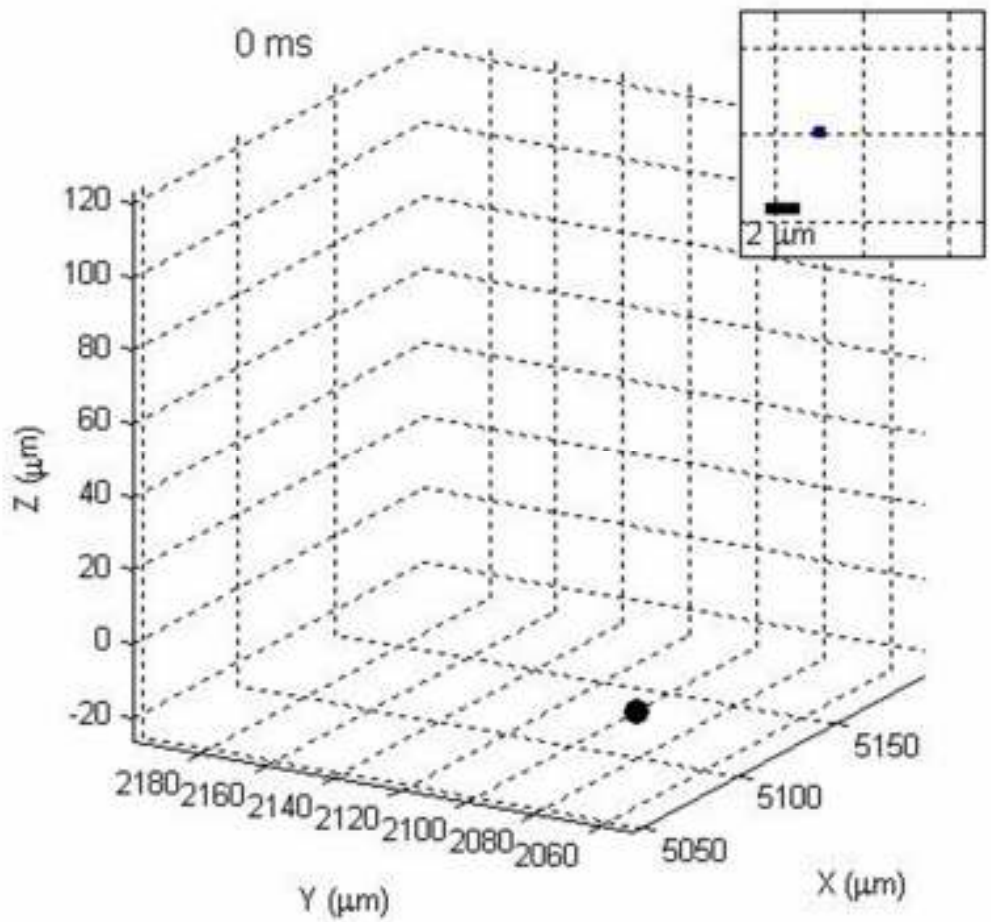
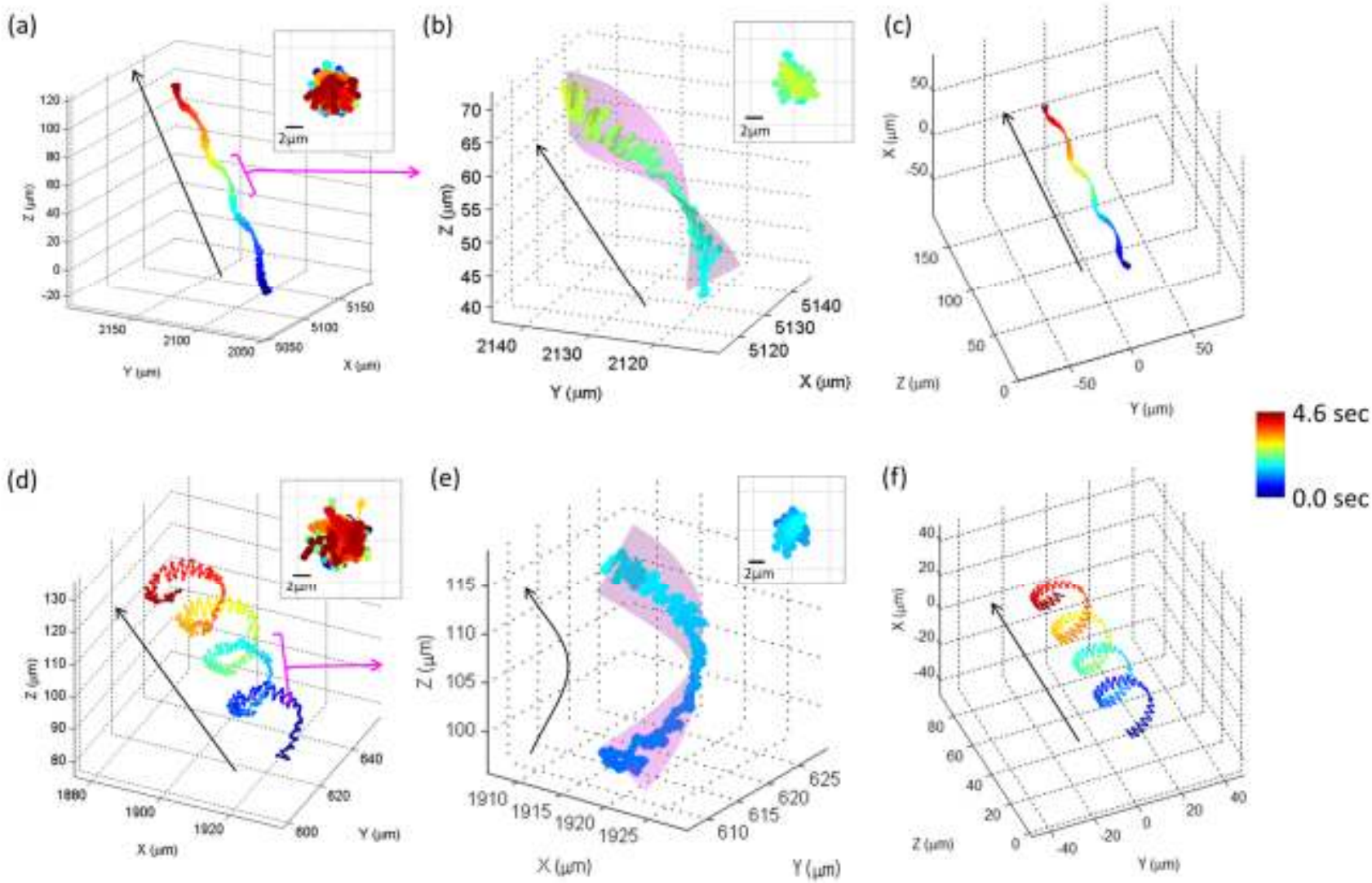


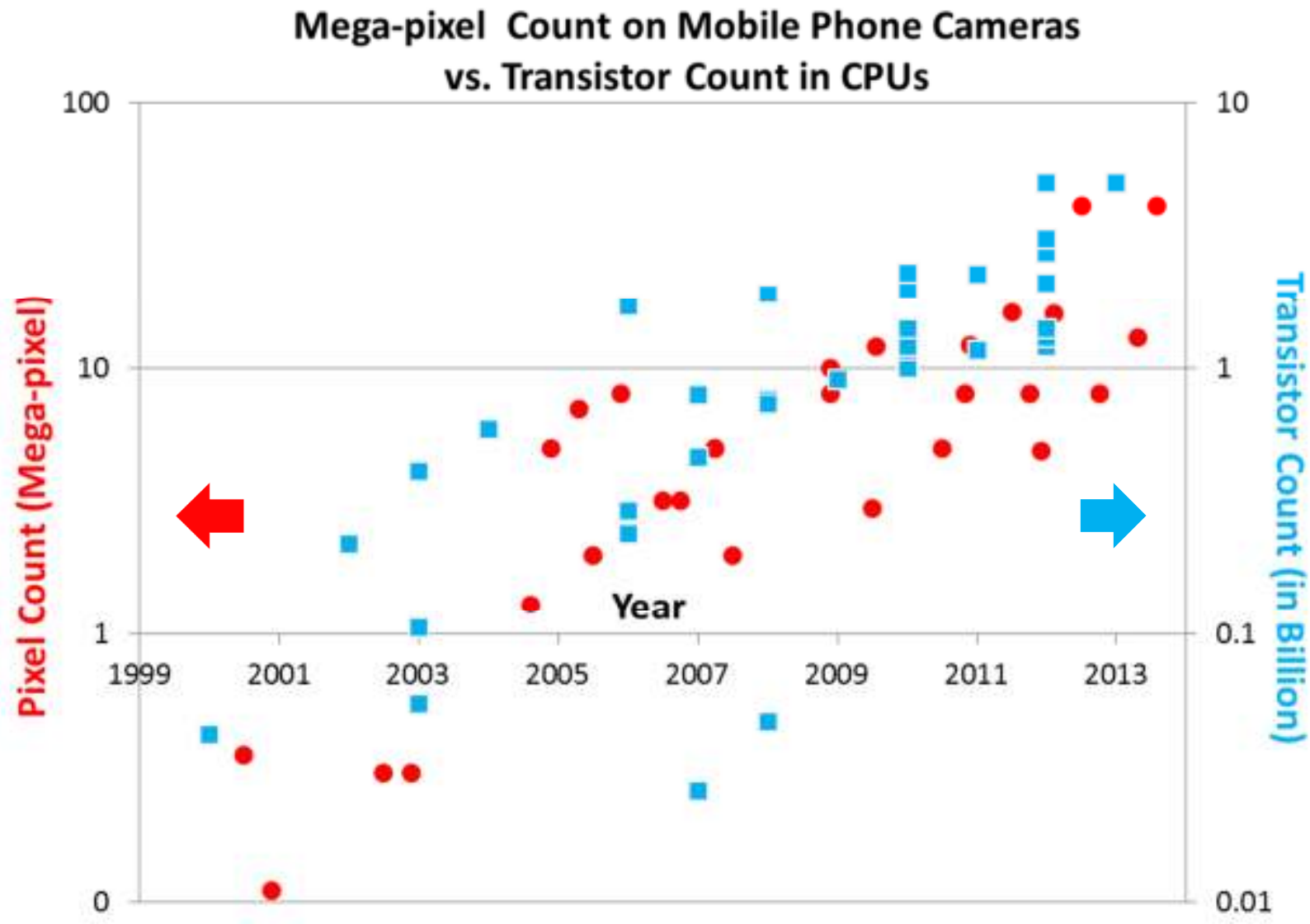

Scientific Reports, 2013

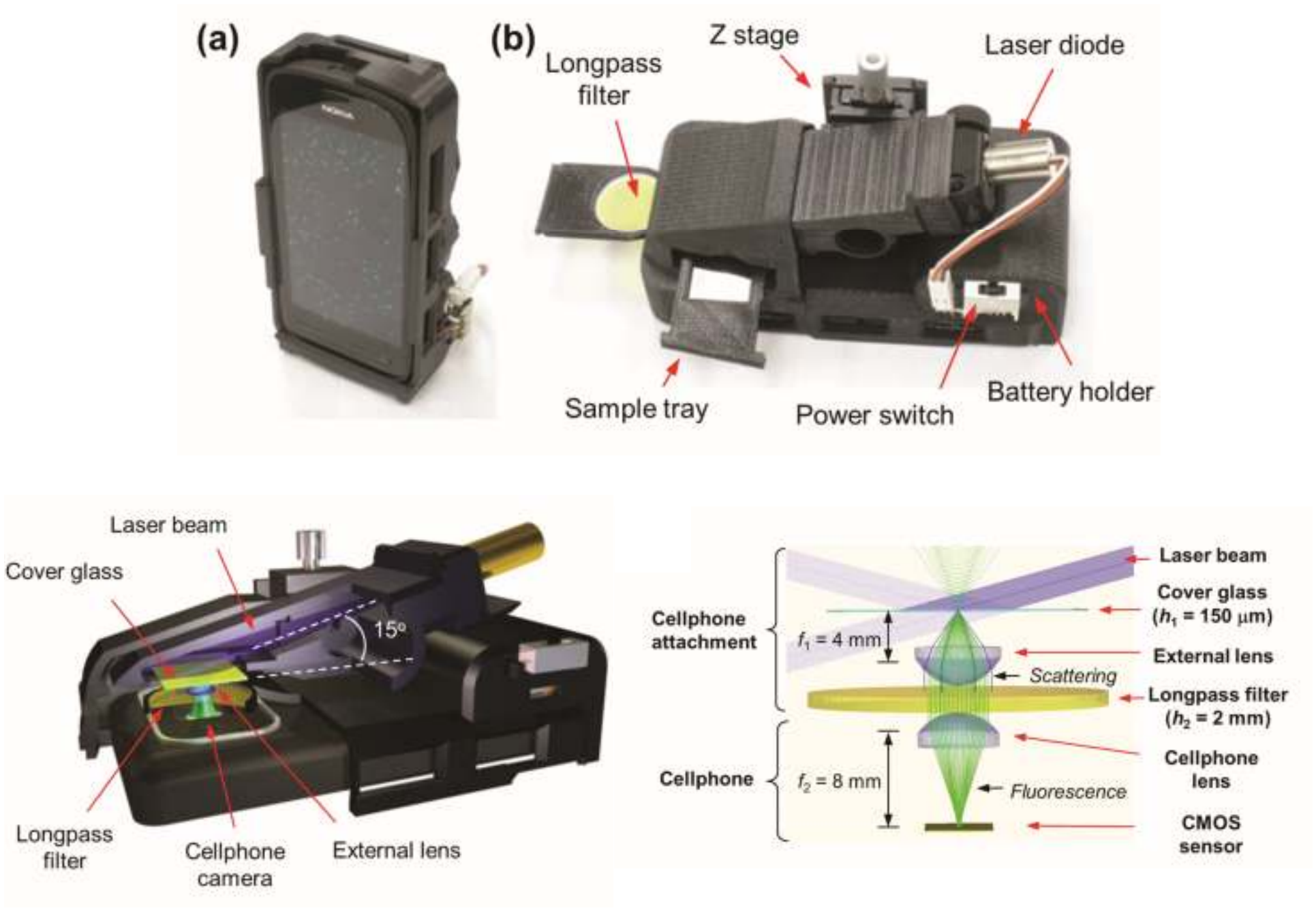
# *Sperm Trajectories form Helicoids (Also Minimal Surface)*



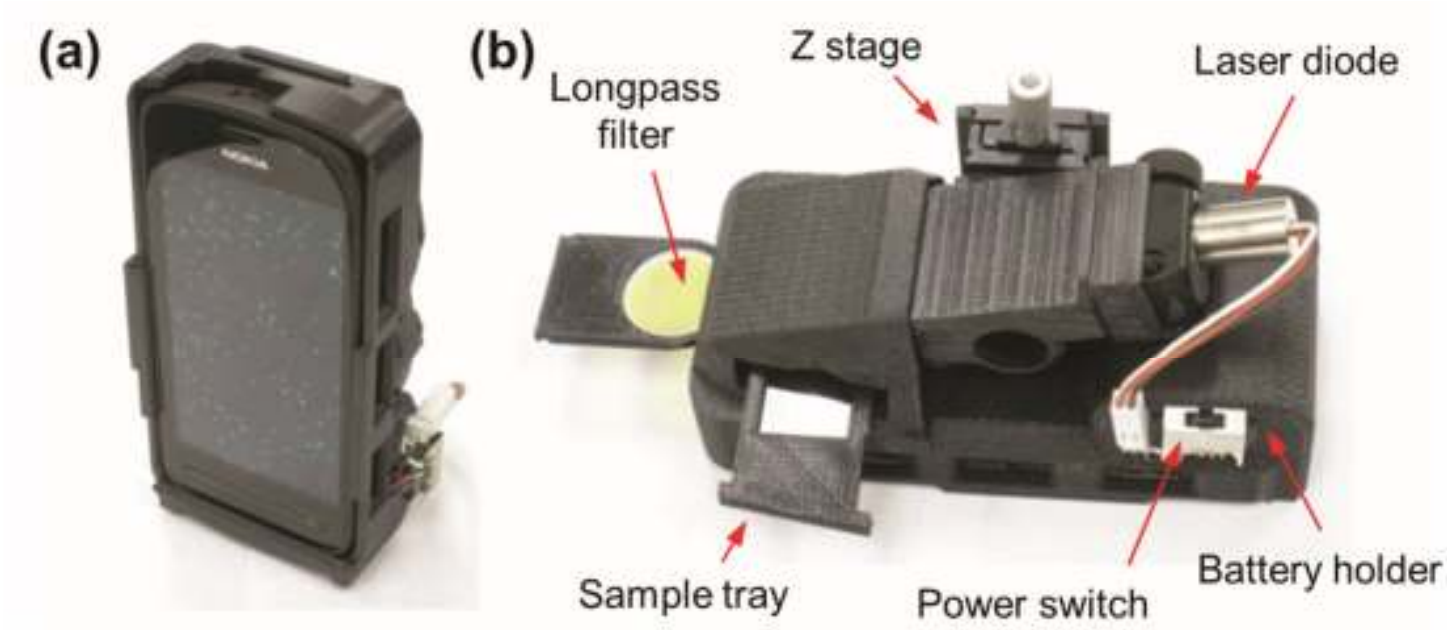

Scientific Reports, 2013

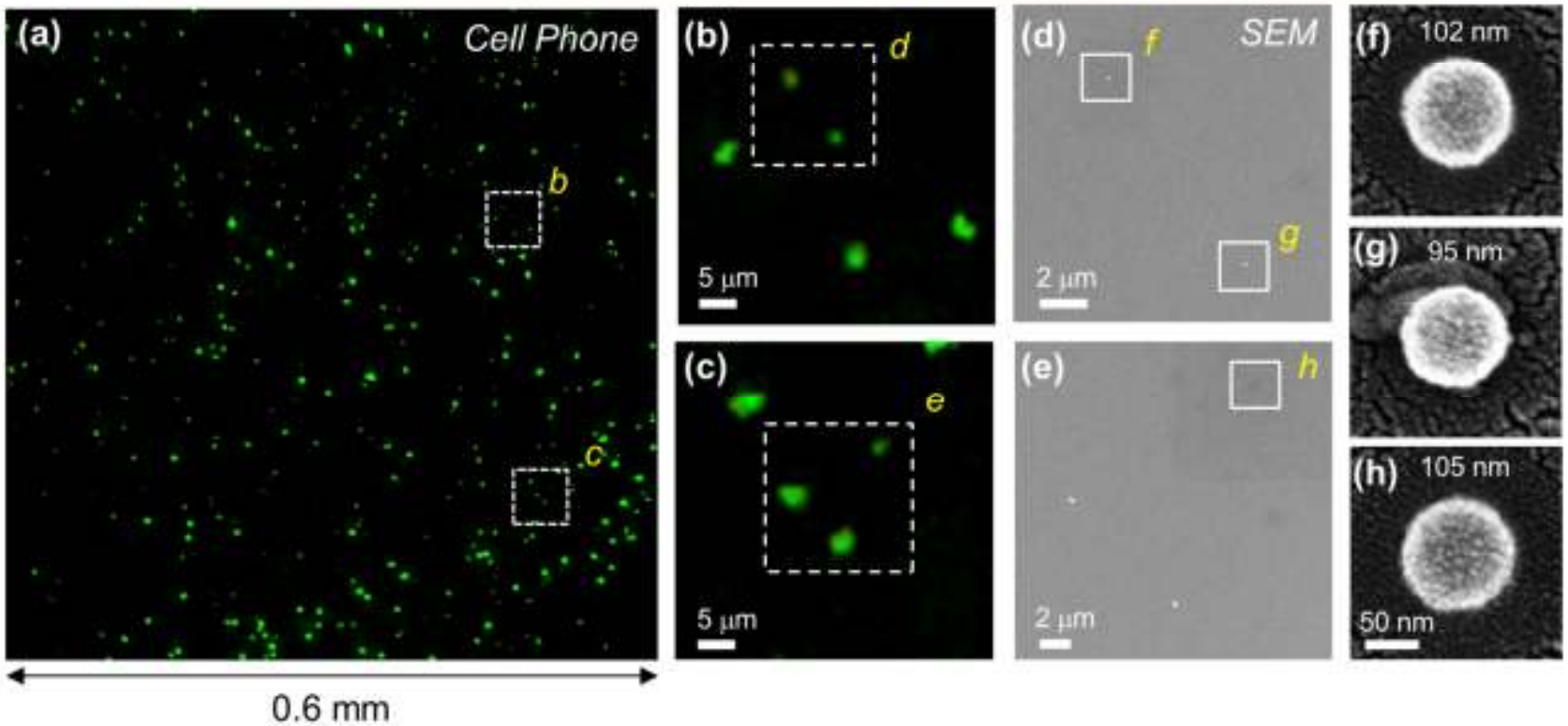
# *A different view of the Moore's Law*

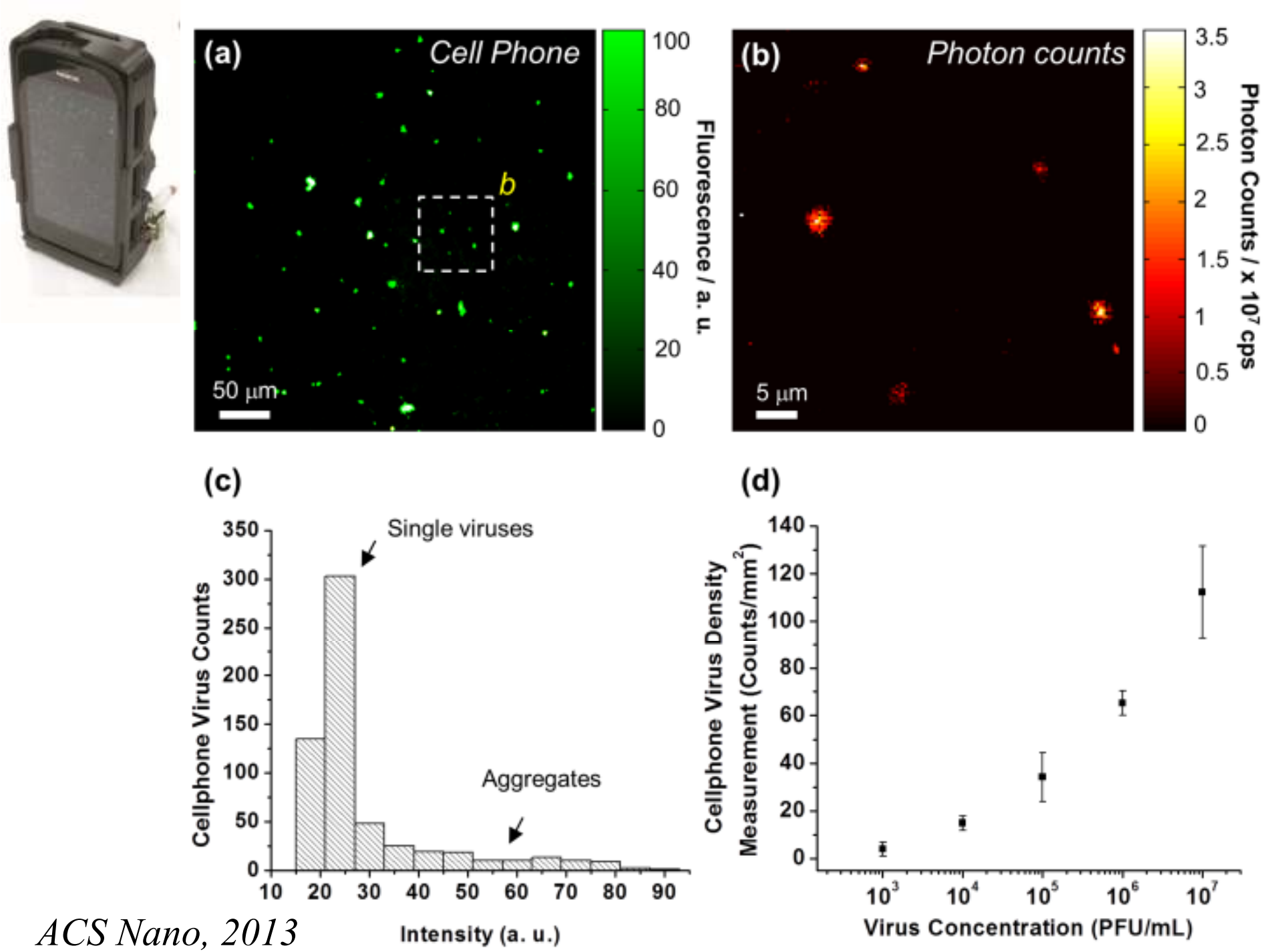





*ACS Nano, 2013*

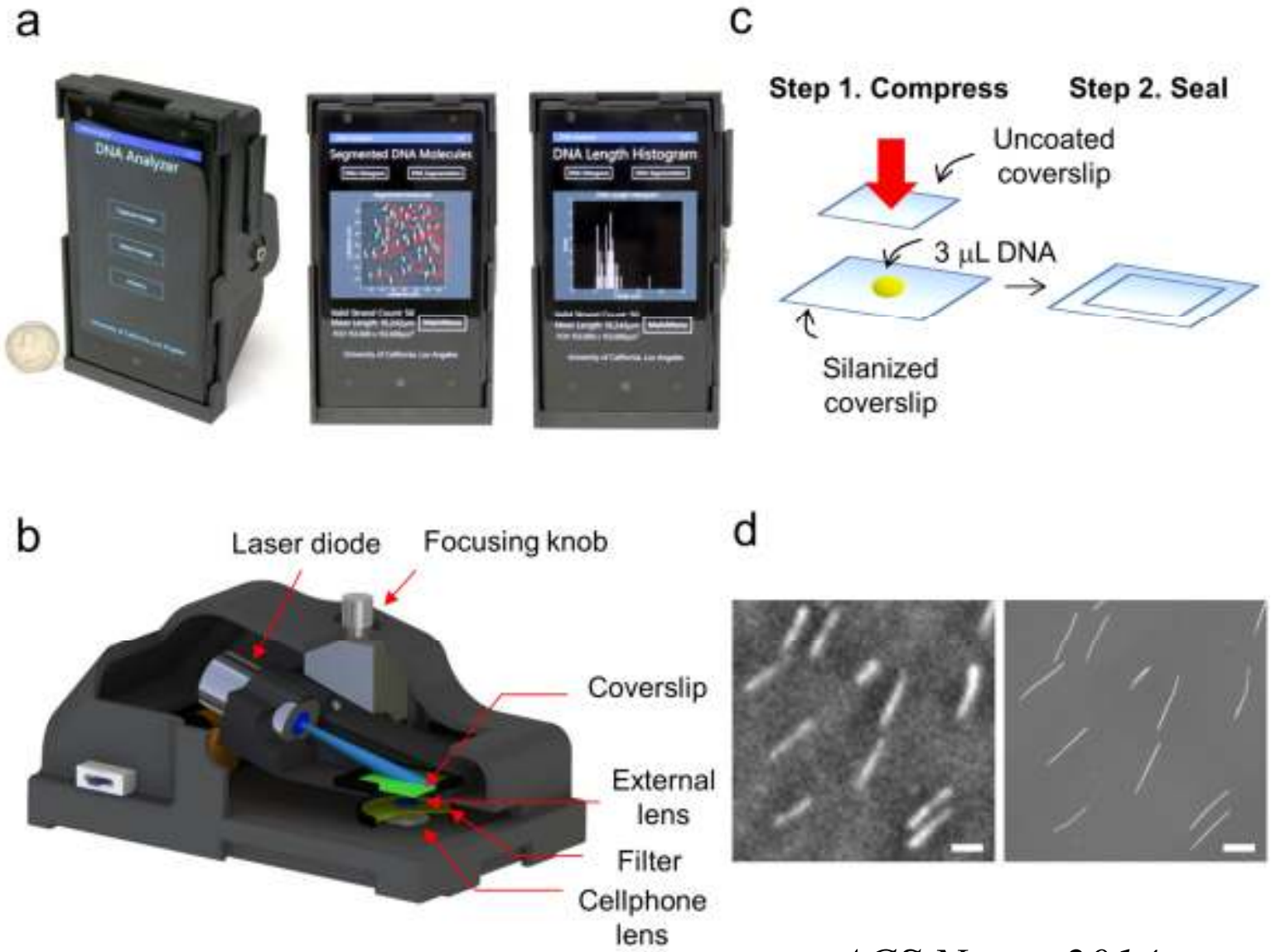

(a)
(b)
Longpass filter
Z stage
Laser diode
Sample tray
Power switch
Battery holder


(a)
Cell Phone
b
c
0.6 mm
(b)
d
5 μm
(c)
e
5 μm
(d)
f
SEM
g
2 μm
(e)
h
2 μm
(f)
102 nm
(g)
95 nm
(h)
105 nm
50 nm




*ACS Nano, 2013*

# *Imaging and Sizing of Single DNA Molecules on a Mobile-Phone*




*ACS Nano, 2014*

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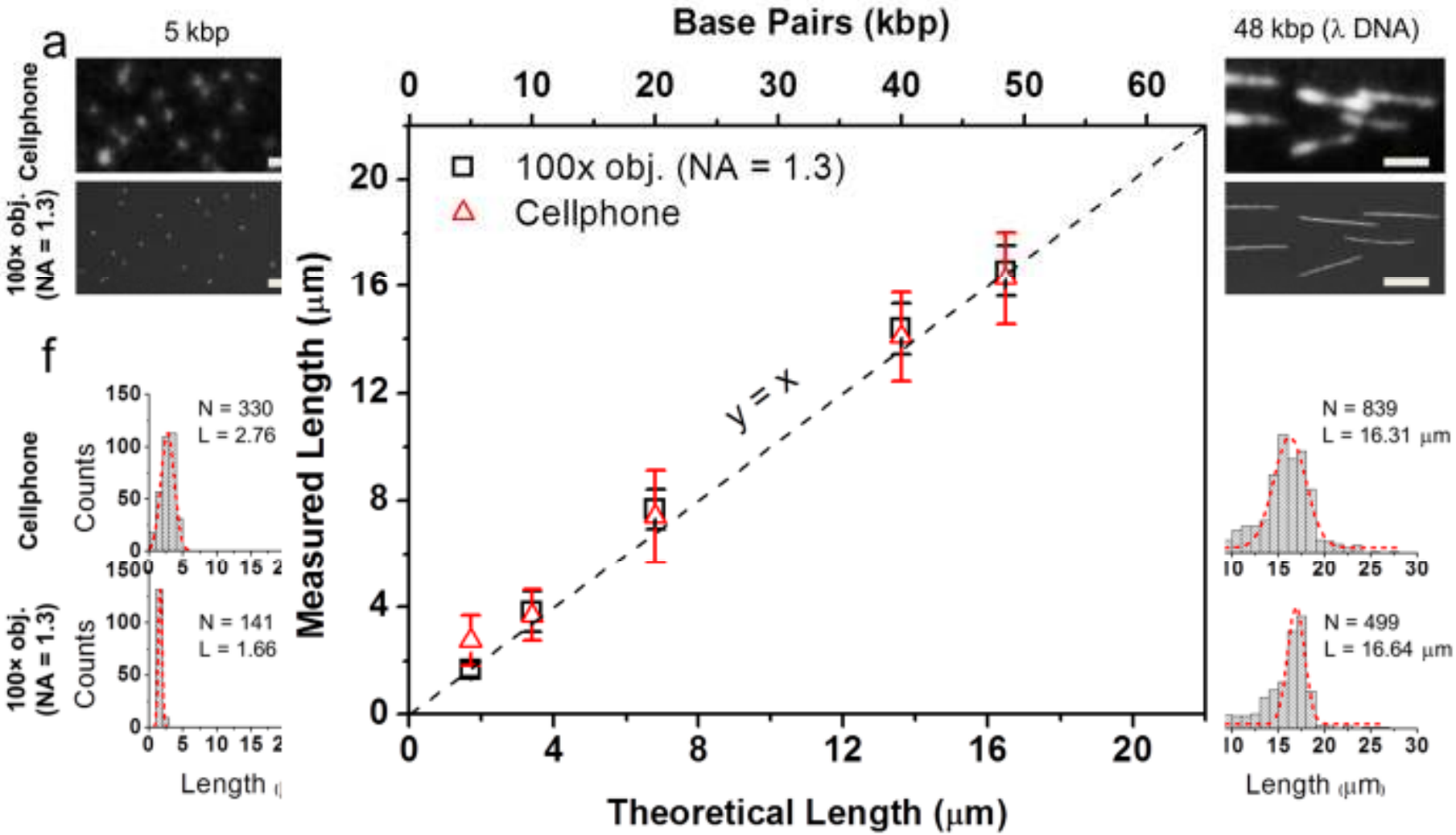
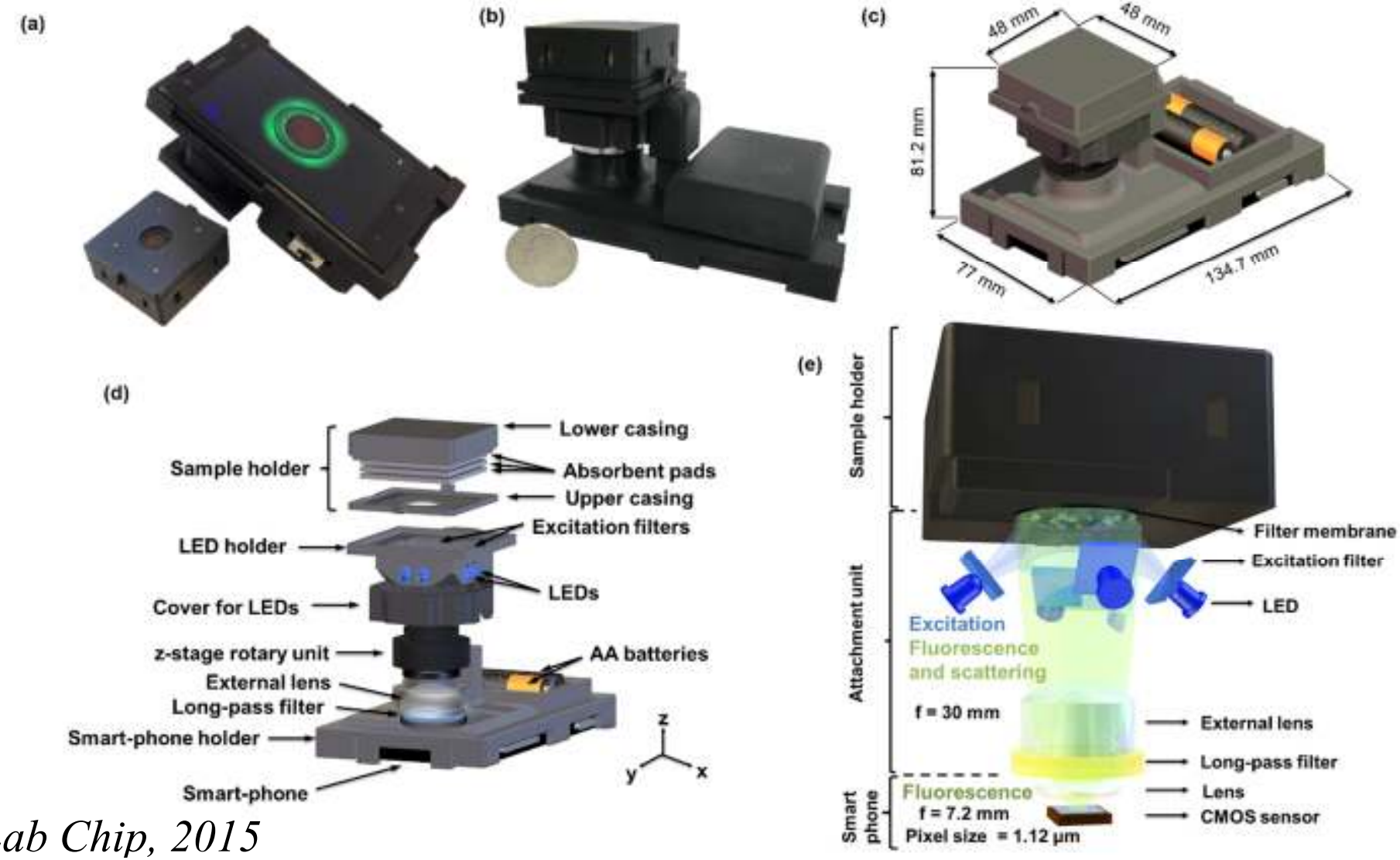
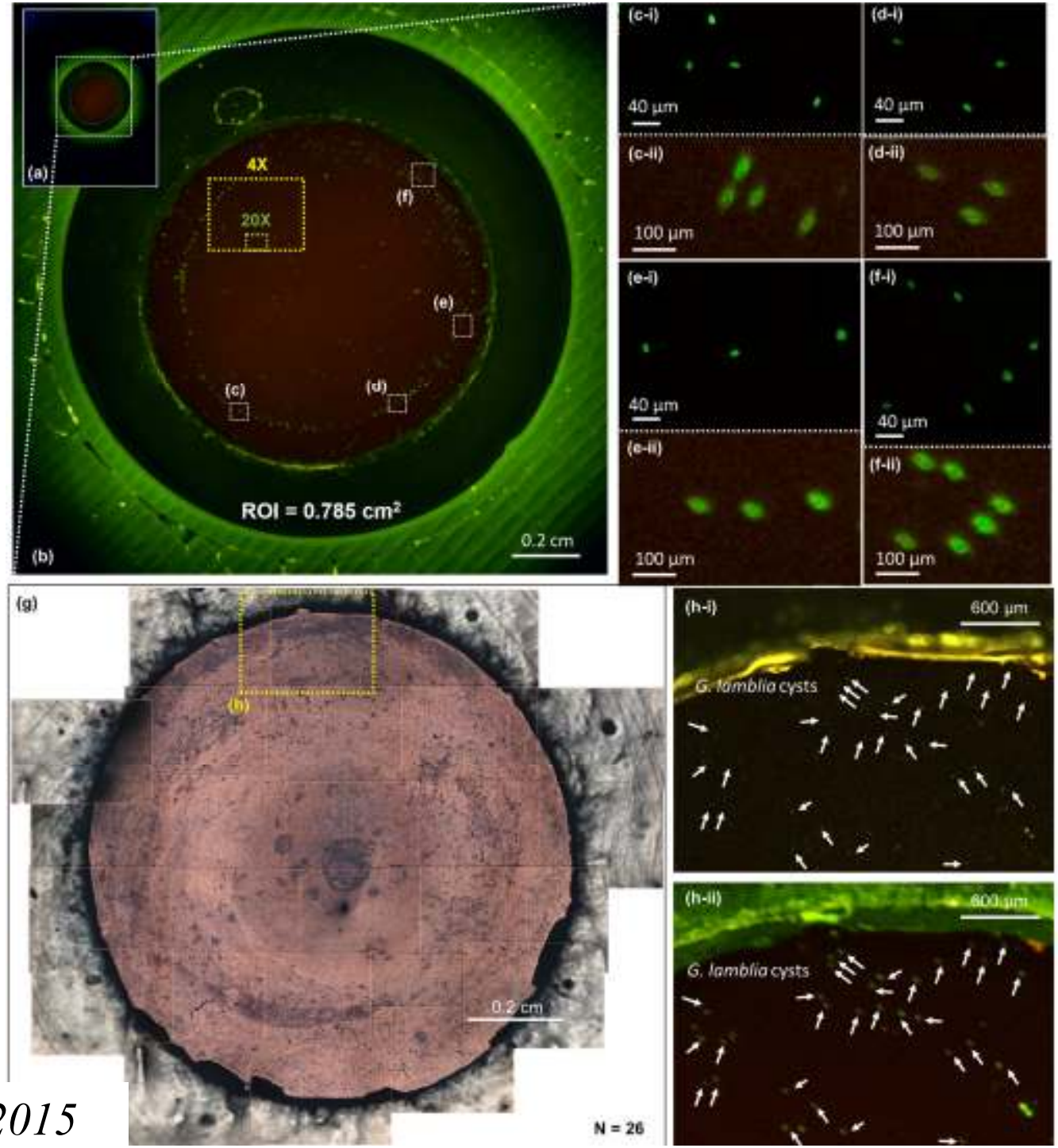



*ACS Nano, 2014*

# *Quantification of Giardia lamblia cysts using mobile-phone based fluorescent microscopy and machine learning*




*Lab Chip, 2015*




*Lab Chip, 2015*

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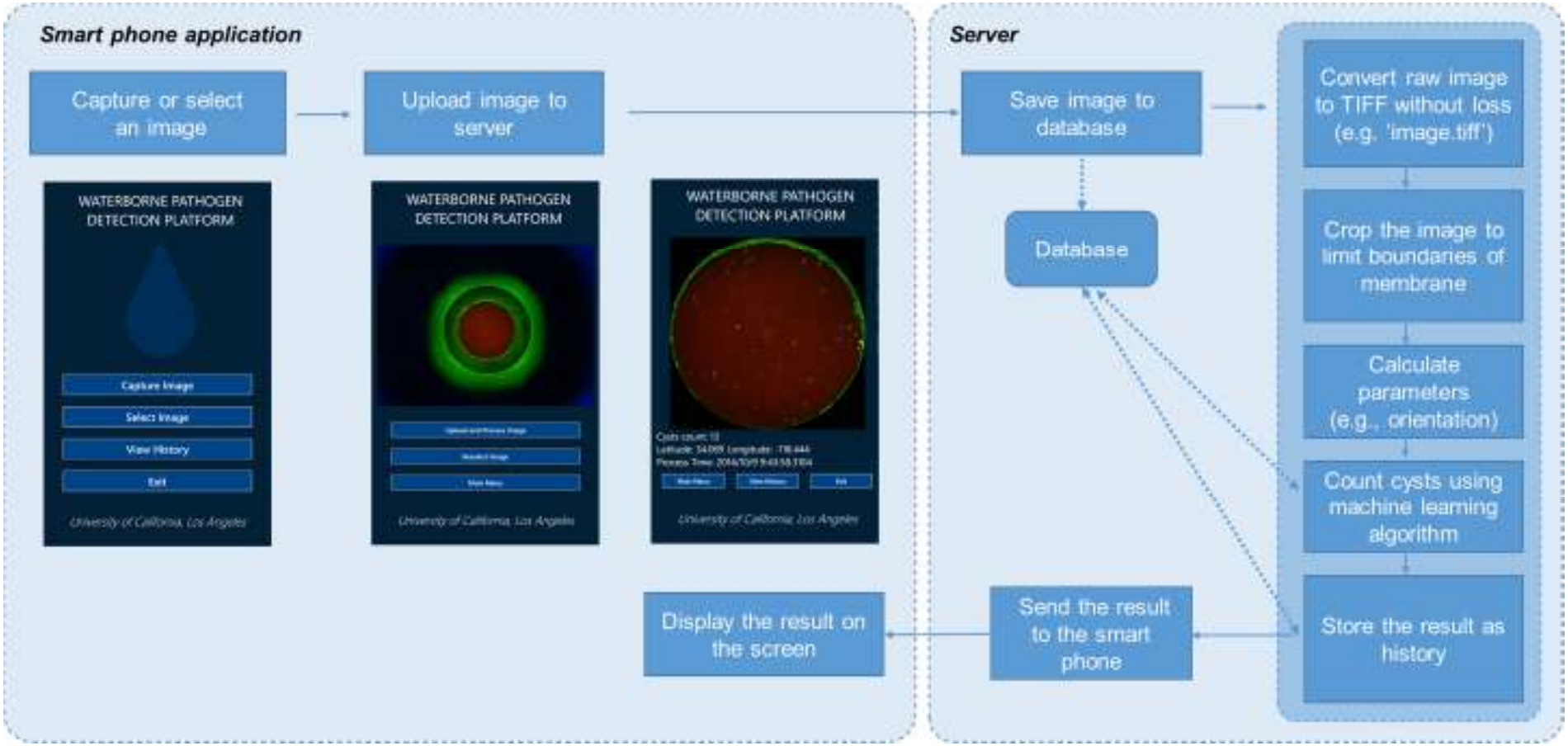



*Lab Chip, 2015*

# *An Analogy: From PCs to the Internet*

# *The μ-Internet*

## ***Big Data***

## *- An opportunity or a challenge?*

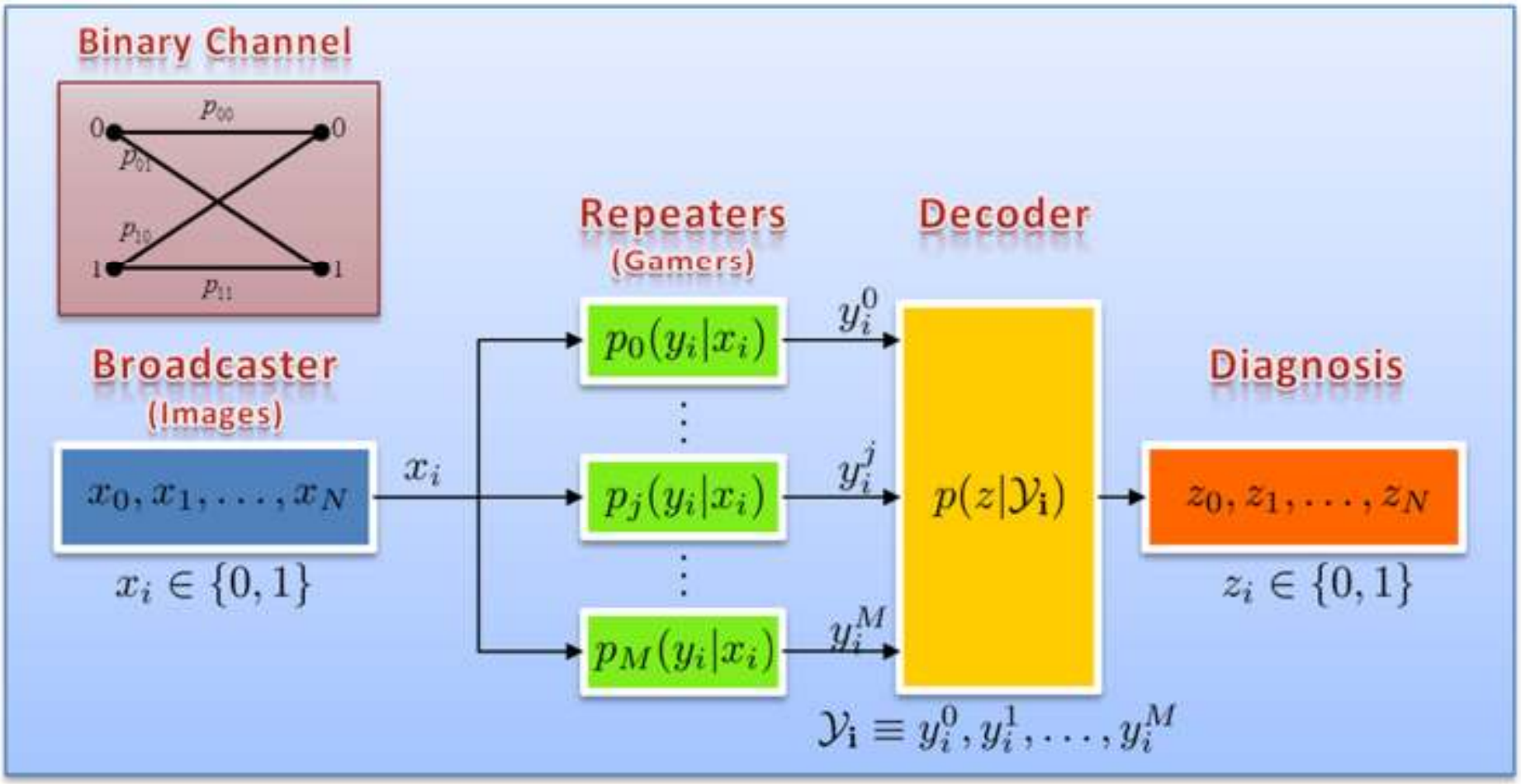
# *Diagnosis of Malaria using Crowd-Sourced Games*

**http://biogames.ee.ucla.edu/**

# *Diagnosis of Malaria using Crowd-Sourced Games*

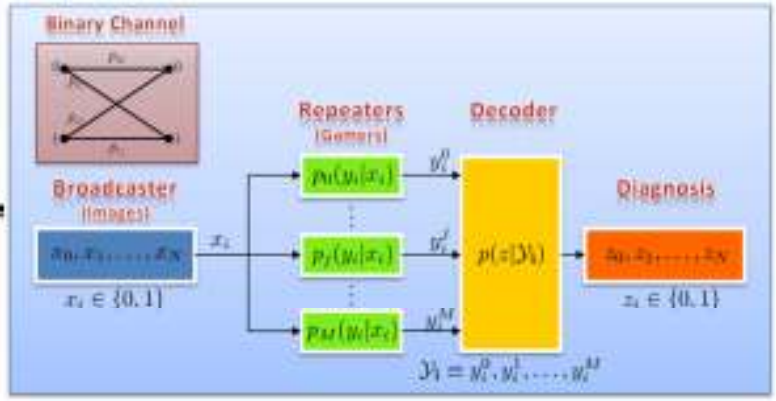
**More than 80 countries are playing BioGames, with >2.5 Million cell diagnoses so far.**

# *Each gamer is a "Repeater"*




**Decoder: Maximum a Posteriori Probability (MAP)**

# Decoding of Diagnosis




$$p(x_i = x^* | \mathcal{Y}_\mathbf{i}) = \frac{p(\mathcal{Y}_\mathbf{i} | x_i = x^*) \cdot p(x_i = x^*)}{p(\mathcal{Y}_\mathbf{i})}$$

$$= \prod_{j=0}^{M} p(y_i^j | x_i = x^*) \cdot \frac{p(x_i = x^*)}{\sum_{l=0}^{1} \left[ p(x_i = l) \prod_{k=0}^{M} p(y_i^k | x_i = l) \right]}$$

$$\log p(x_i = x^* | \mathcal{Y}_\mathbf{i}) = \sum_{j=0}^{M} \log p(y_i^j | x_i = x^*) + \log p(x_i = x^*)$$

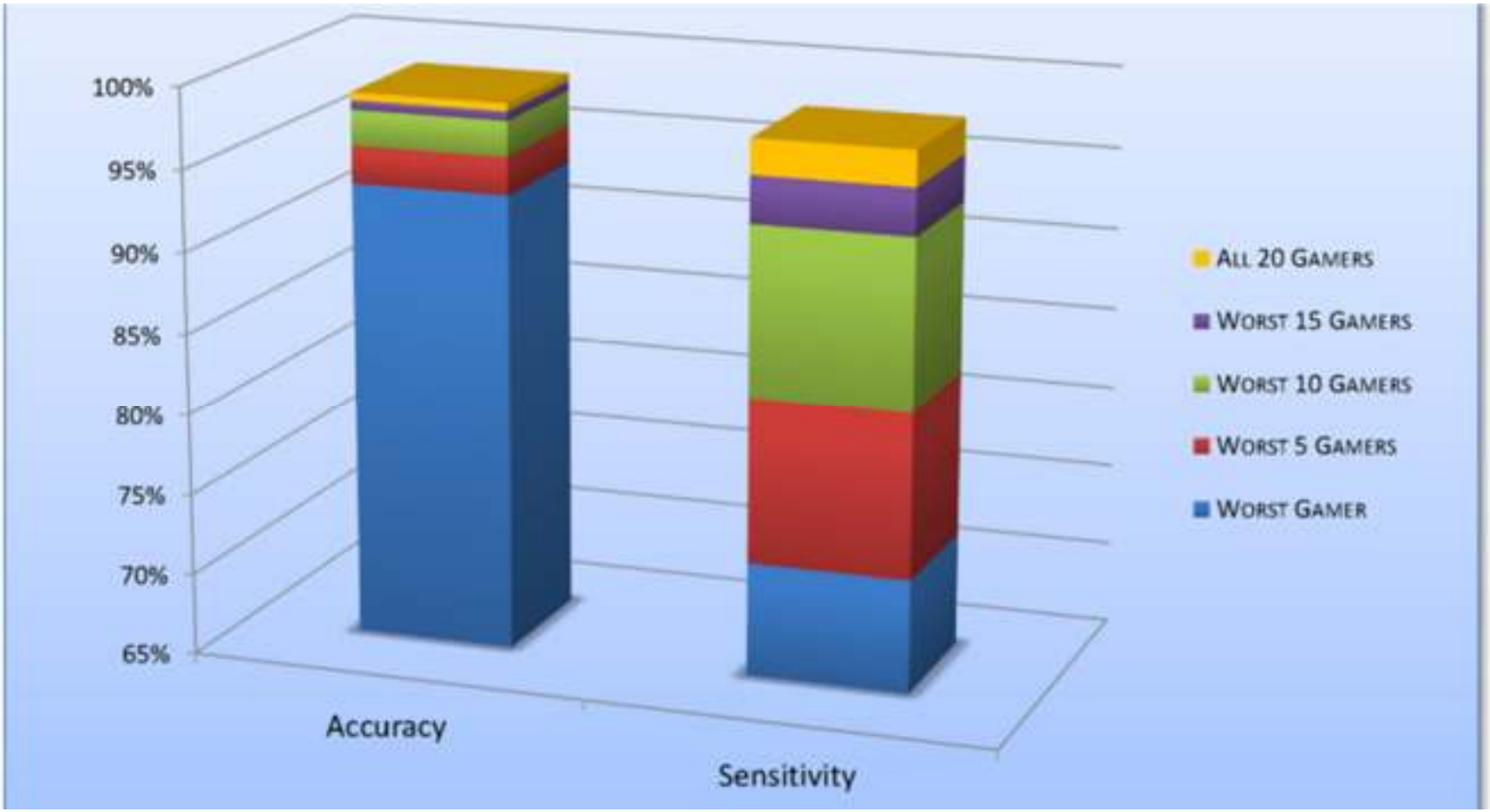
$$- \log \left[ \sum_{l=0}^{1} p(x_i = l) \prod_{k=0}^{M} p(y_i^k | x_i = l) \right]$$

$$z_i = \arg\max_{l \in \{0,1\}} \left[ \varphi_l + \sum_{j=0}^{M} \log p(y_i^j | x_i = l) \right] \ , \ \varphi \equiv \log p(x_i = l)$$

# *Non-expert human crowd can collectively diagnose malaria infected cells*

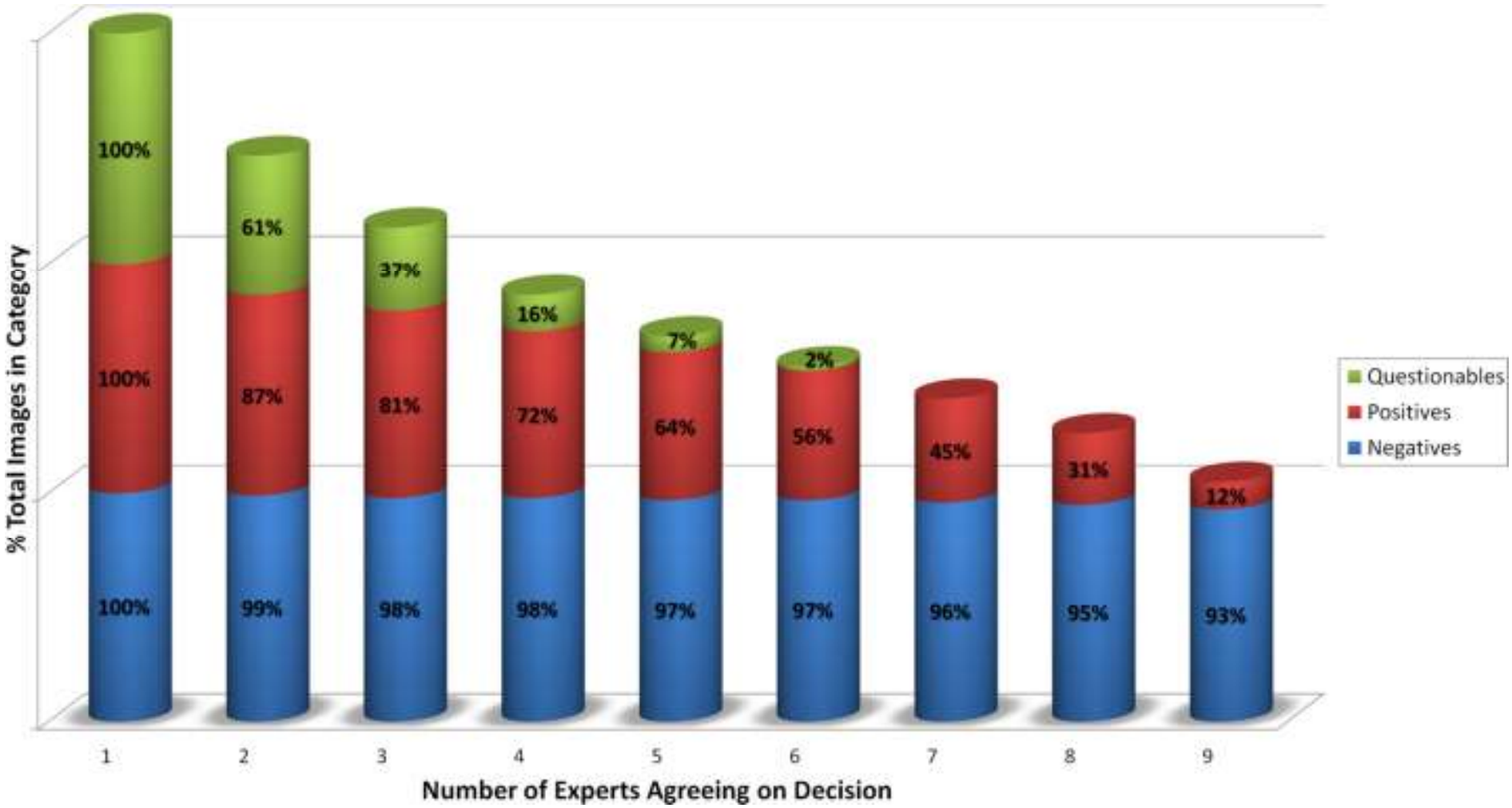
| Experiment | Description of Test Images | Gamers | Positive RBCs | Negative RBCs | Control Images | Accuracy | SE | SP | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5055 test RBC images crowd-sourced to human gamers | 19 | 471 | 4584 | 1266 | 99.01% | 95.12% | 99.41% | 94.32% | 99.50% |
| 2 | 5055 test RBC images presented to a boosted set of classifiers, trained on 1266 RBC images | NA | 471 | 4584 | 1266 (Training Images) | 96.26% | 69.64% | 99.00% | 87.70% | 96.95% |
| 3 | 459 low-confidence test images taken from the results of experiment 2 | 27 | 274 | 185 | 1266 | 95.42% | 97.81% | 91.89% | 94.70% | 96.59% |
| 4 | Hybrid diagnosis results using experiments 2 & 3 | 27 | 471 | 4584 | 1266 | 98.50% | 89.38% | 99.43% | 94.18% | 98.91% |
| 5 | 7045 test RBC images crowd-sourced to human gamers | 20 | 1549 | 5496 | 2349 | 98.78% | 97.81% | 99.05% | 96.68% | 99.38% |

# *Non-expert human crowd can collectively diagnose malaria infected cells*

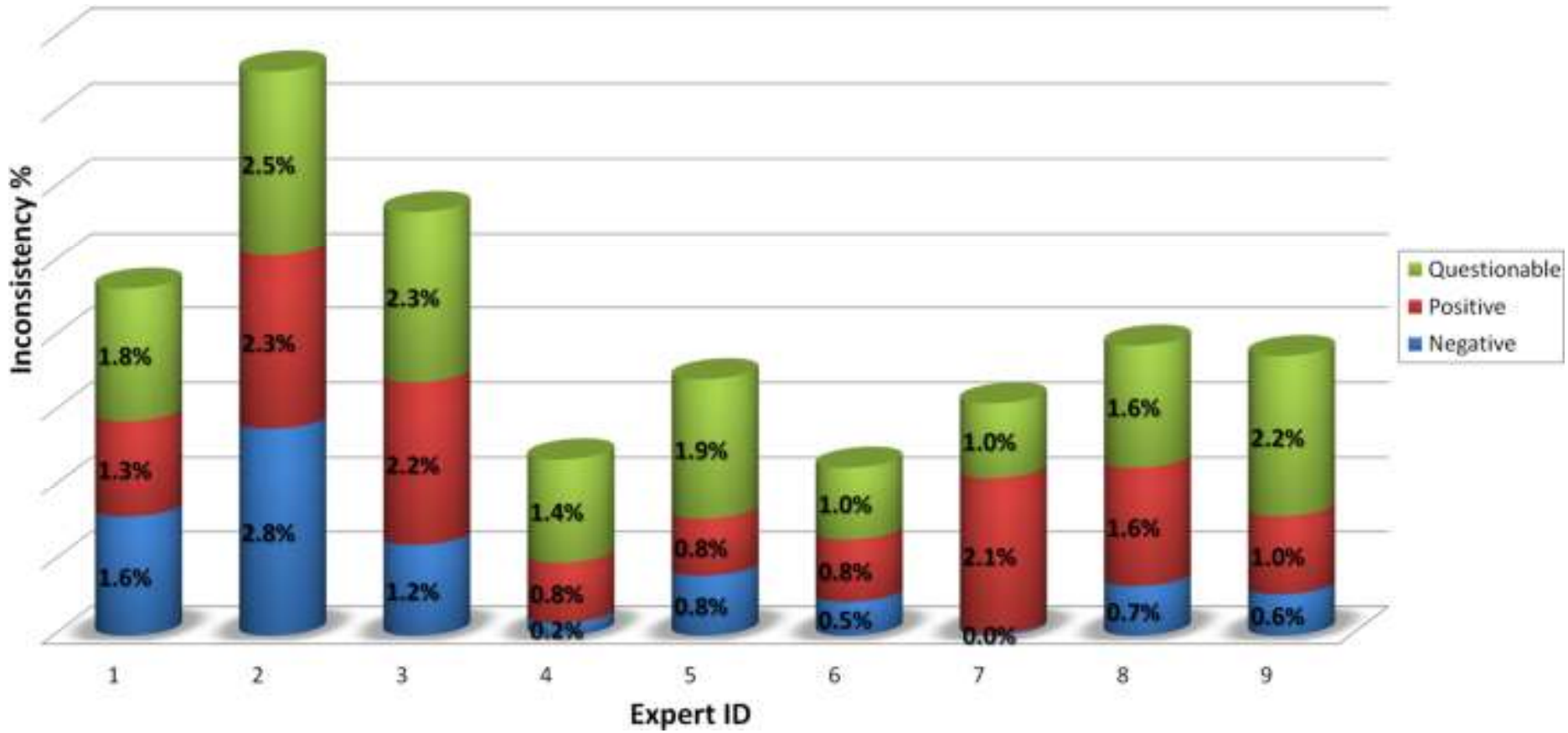



| Term | Acronym | Definition |
|---|---|---|
| **Accuracy** | ACC | $\frac{TP + TN}{TP + TN + FP + FN}$ |
| **Sensitivity or True Positive Rate** | SE or TPR | $\frac{TP}{TP + FN}$ |

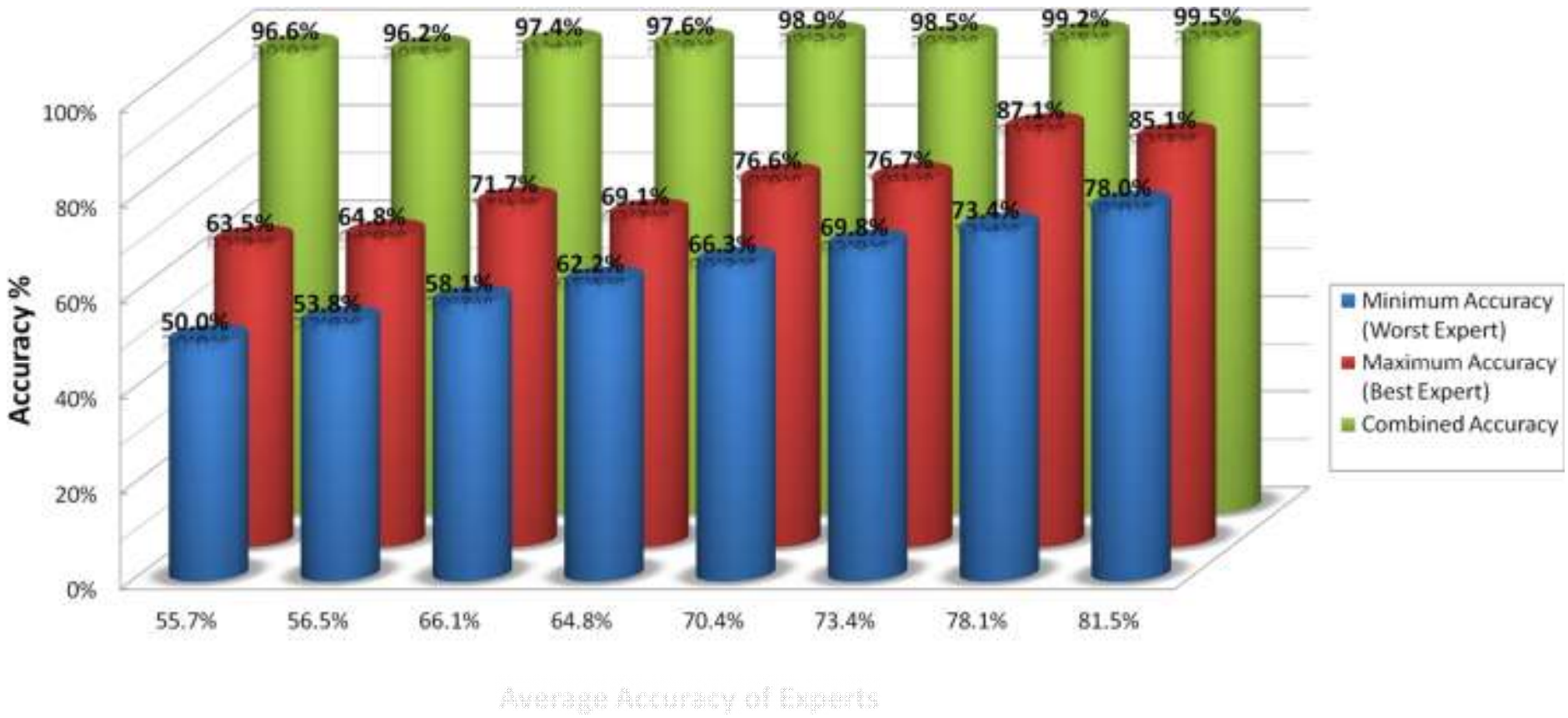
# *Experts disagree with each other*



# *Experts are 'not' self-consistent*

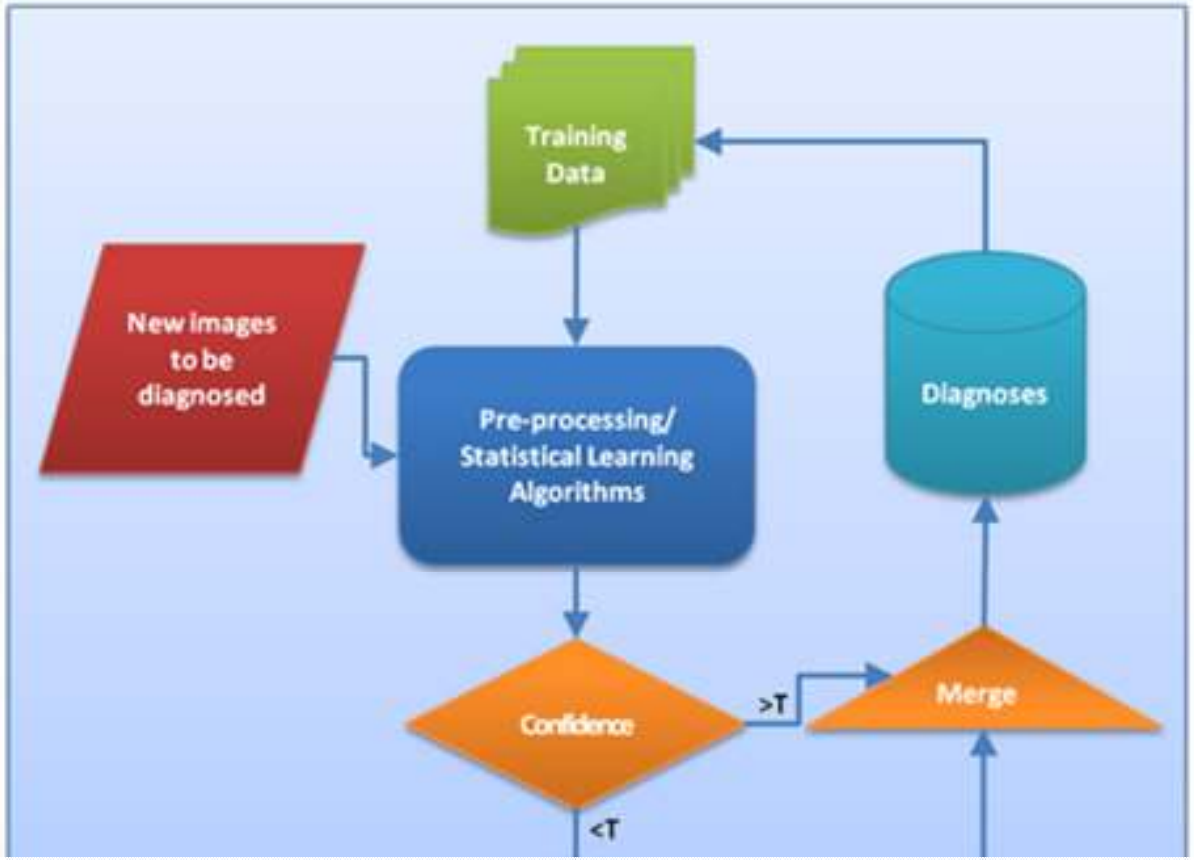


# *Digital 'Super' Pathologist & Training of experts*




- ***Digital 'Super' Pathologist***
- ***Training of Experts***

# *Big Data Management: Crowd + Machine*

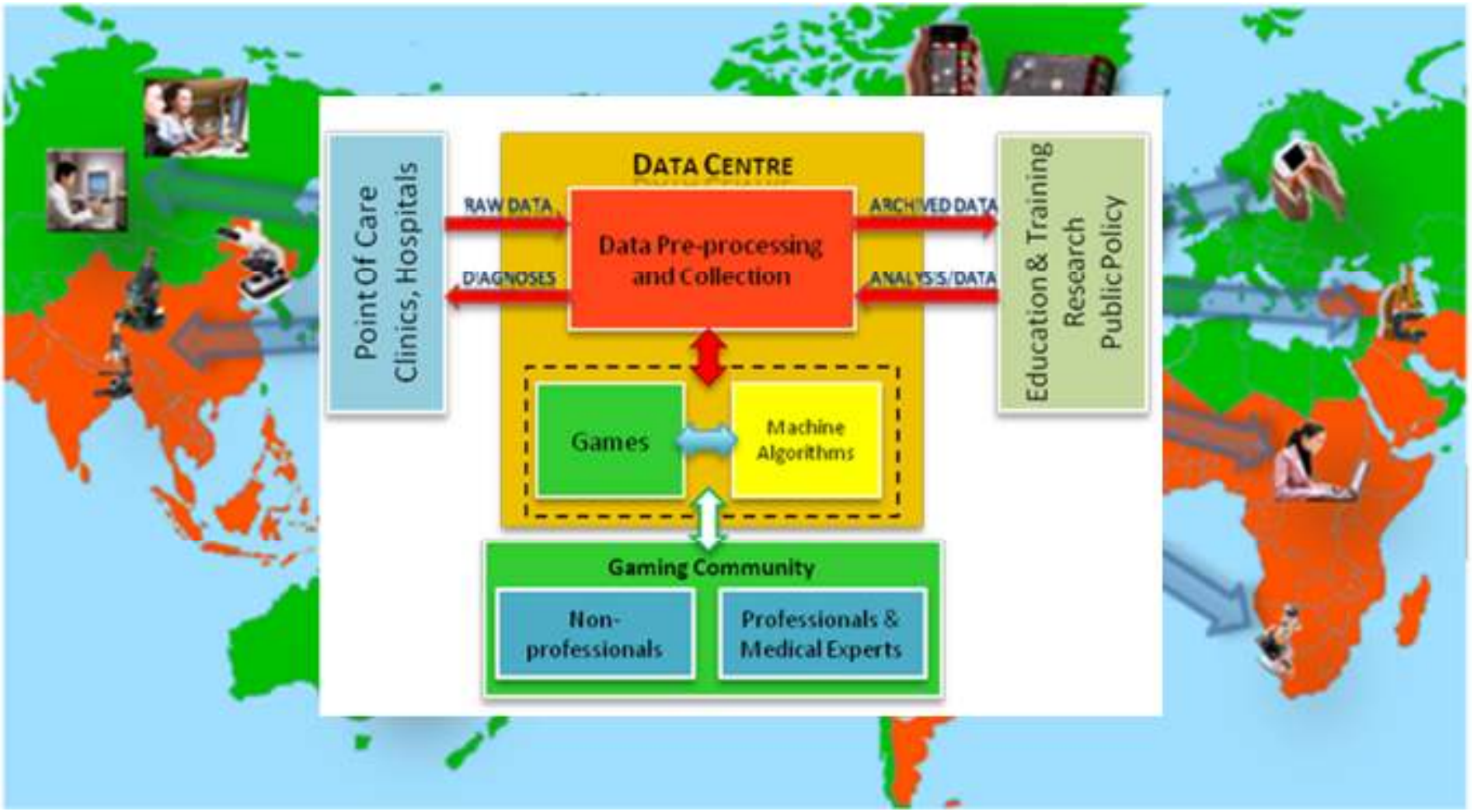



# ***Crowd:***

## ***Professionals + Non-experts***

*(Smart and Cost-effective Telemedicine)*

# *Distributed Medical Image Analysis and Diagnosis through Crowd-Sourced Games*



# *Training on Malaria Diagnosis*

| Rank | Username | Score |
|---|---|---|
| 1 | malariadoctor6666 | 72 |
| 2 | malariadoctor6666 | 72 |
| 3 | malariadoctor6666 | 70 |
| 4 | Rodrigo Medeiros | 70 |
| 5 | malariadoctor6666 | 69 |
| 6 | sf | 69 |
| 7 | ao | 69 |
| 8 | malariadoctor6666 | 66 |
| 9 | ao | 66 |
| 10 | ts | 65 |
| 11 | malariadoctor6666 | 64 |
| 12 | malariadoctor6666 | 64 |
| 13 | malariadoctor6666 | 63 |
| 14 | UCSF | 63 |
| 15 | aoo | 63 |
| 16 | AG | 62 |
| 17 | malariadoctor6666 | 62 |
| 18 | ao | 62 |
| 19 | ana | 61 |
| 20 | ao | 61 |
| 21 | sf | 61 |
| 22 | SD0004K | 59 |
| 23 | anonymous | 59 |
| 24 | ao | 59 |
| 25 | ao | 59 |

# *The μ-Internet*

## ***Big Data***

## *- An opportunity or a challenge?*

# *Providing a new platform for telemedicine*

*E. coli* sensor

*Allergen detector*

*Heavy metal detector*

*Google Glass based Diagnostics*

*Diagnostic test reader (HIV, malaria, etc.)*

*Blood Analyzer*

*Blood Analyzer*

# *Ozcan Research Group – Members & Funding*

NSF

NATIONAL INSTITUTES OF HEALTH

DEPARTMENT OF THE NAVY ONR Science & Technology

SCIENCE TO SHAPE THE FUTURE OF THE ARMY ARMY RESEARCH OFFICE 1951

DEPARTMENT OF DEFENSE UNITED STATES OF AMERICA

HHMI HOWARD HUGHES MEDICAL INSTITUTE

BILL & MELINDA GATES foundation

Vodafone Americas Foundation™ Wireless Innovation Project™

NATIONAL GEOGRAPHIC™

Google