# Field Label-Free
# Water Inspection using
# Interferometric Phase Microscopy

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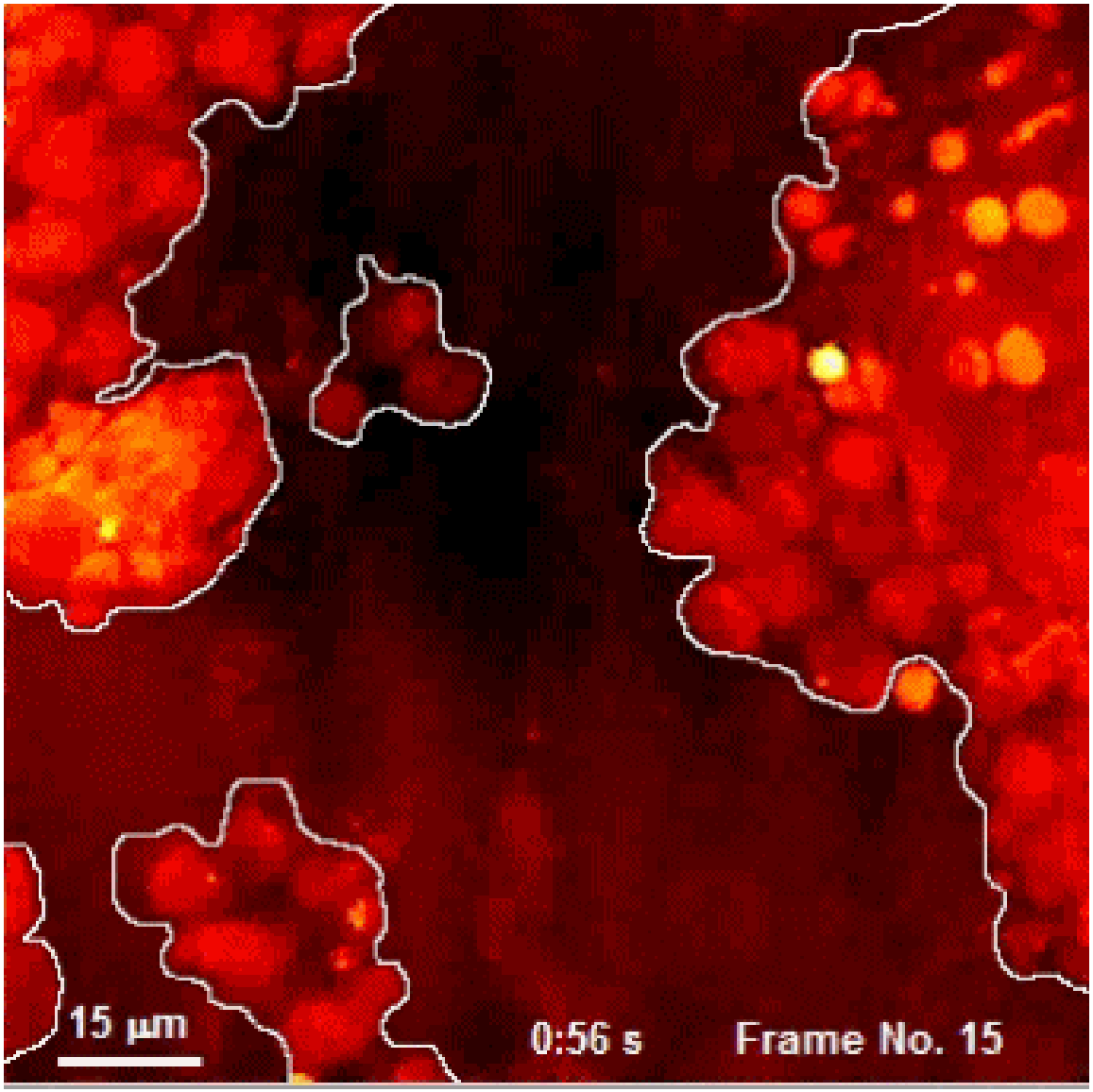
## Real-time (>video rate) interferometry of blood flowing rapidly in a micro-channel




**Potential:**
**Integration into cell sorters and water inspection devices**

**Throughput:**
**>10,000 fps**

*Optics Letters* **39** (2014)
*Optics Express* **23** (2015)

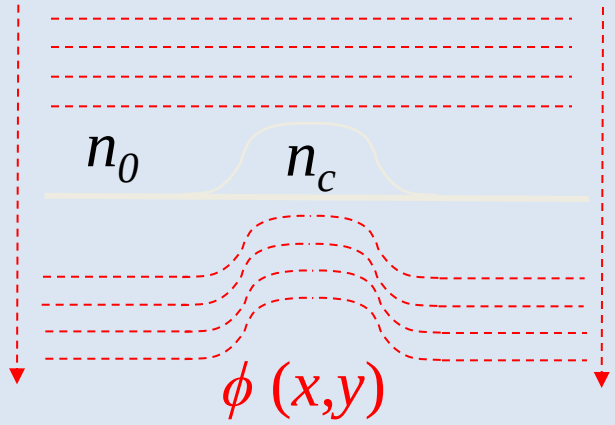
# Interferometric Phase Microscopy (IPM)

- IPM, also called **digital holographic microscopy (DHM)**, is able to **image transparent and semi-transparent samples** and **track sub-nanometric optical thickness** changes **quantitatively** with:
  - **Thousands of frames per second in wide field** (no scanning).
  - **Non-contact** manner.
  - **Label-free** (no sample preparation is needed),
    or **labeled-based, using plasmonic nanoparticles**.
  - **Minimal level of noise**.
  - **Ambient conditions** (good for live samples).

- These techniques provide a **unique and powerful tool** for**:**
  - **Biological research** and **medical diagnosis**.
  - **Material science – characterizing micro- and nano-structures.**

# Quantitative Imaging

- Wide-field IPM can capture the entire wave front (amplitude + phase) → Possible to reshape the captured complex wavefront and:
  - Compensate for optical aberrations.
  - Semi-axial scanning by digital propagation (no mechanical movement).
- Excellent contrast between the entire cell and its surrounding.



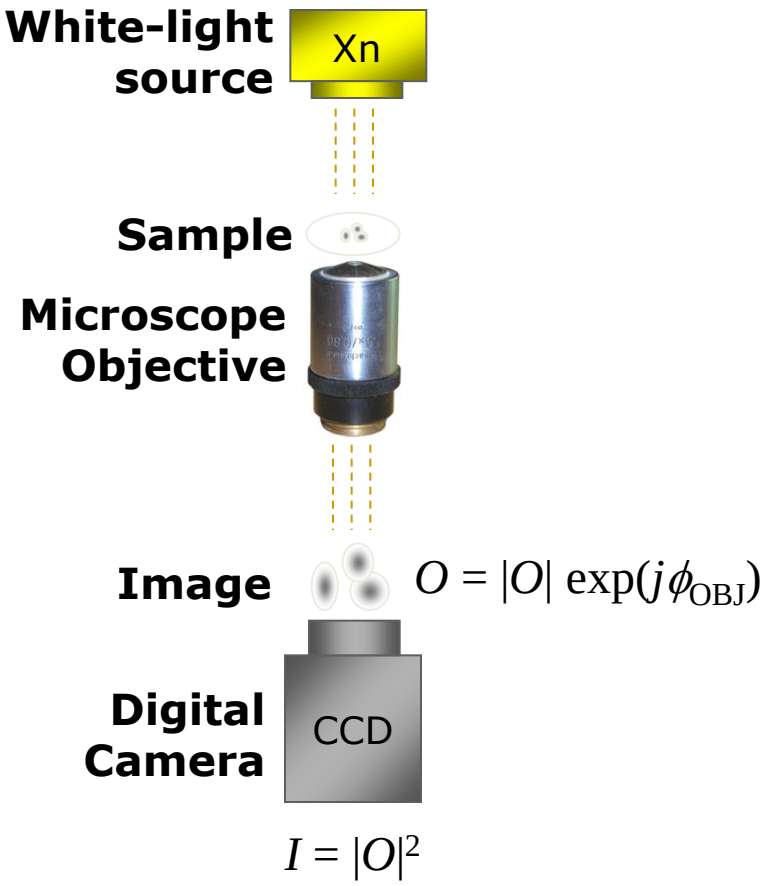

$$\phi(x,y) = 2\pi / \lambda \times \underbrace{h_c(x,y) \times (n_c(x,y) - n_0)}$$

OPD (optical path delay) profile = optical thickness
~ Physical thickness × Integral refractive index

- Sub-nanometer OPD quantitative measurement tracked overtime.

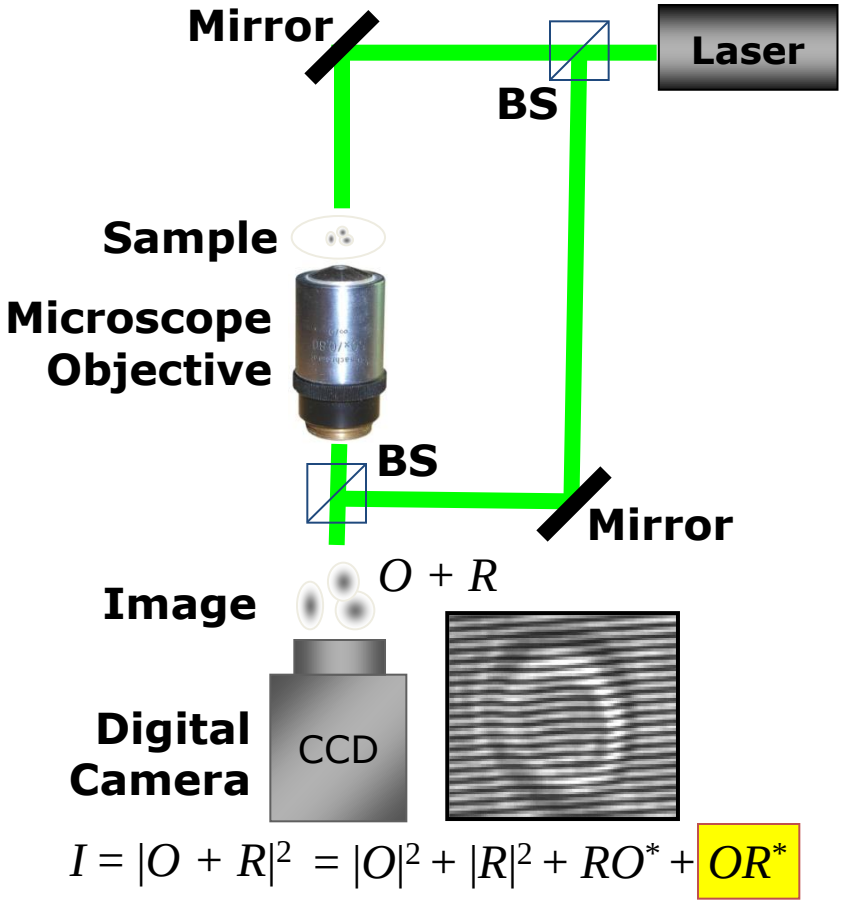
**Shaked *et al.*, *Optics Letters* 34, 767, 2009**
**Shaked *et al.*, *Optics Express* 17, 15585, 2009**

# Conventional Transmission Microscopy




**Phase information is lost.**

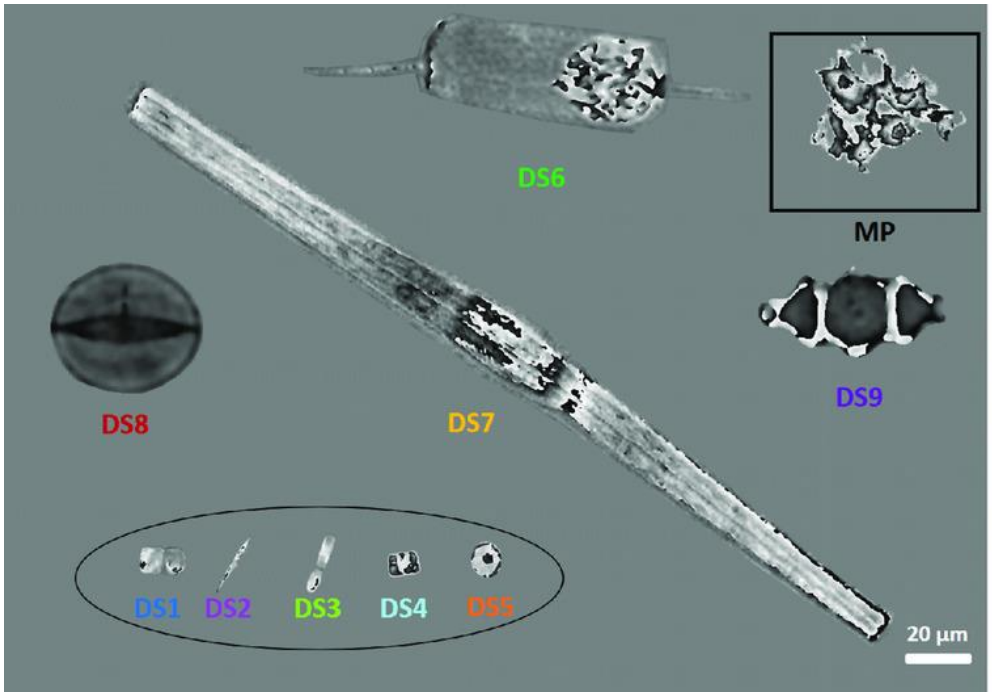
# Wide-Field Interferometric Phase Microscopy




**Phase information is retained.**

# Water Inspection Uses for IPM

- Detecting micro-organisms (>1-300 µm) in water
- Detecting plastic particles (>1-300 µm) in water



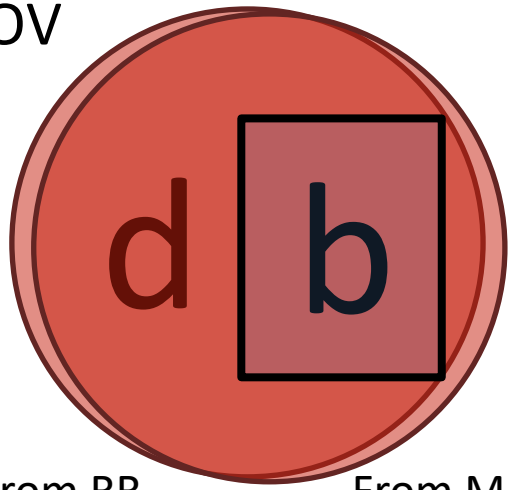

| | A [$\mu m^2$] |
|---|---|
| DS1: *Cyclotella cryptica* | [18.7, 149.8] |
| DS2: *Pseudonitschia arenysensis* | [33.7, 59.8] |
| DS3: *Skeletonema marinoi* | [33.8, 96.4] |
| DS4: *Thalassiosira rotula* | [58.9, 143.9] |
| DS5: *Thalassiosira weissflogii* | [85.2, 115.9] |
| DS6: *Dyticum brightwellii* | [374.0, 6792.4] |
| DS7: *Toxarium undulatum* | [1430.2, 10030.4] |
| DS8: *Cocconeis pseudomarginata* | [1281.8, 2094.6] |
| DS9: *Biddulphia tuomeyi* | [878.6, 1447.9] |
| MP : *Micro-Plastic (PP)* | [17.6, 6408.4] |

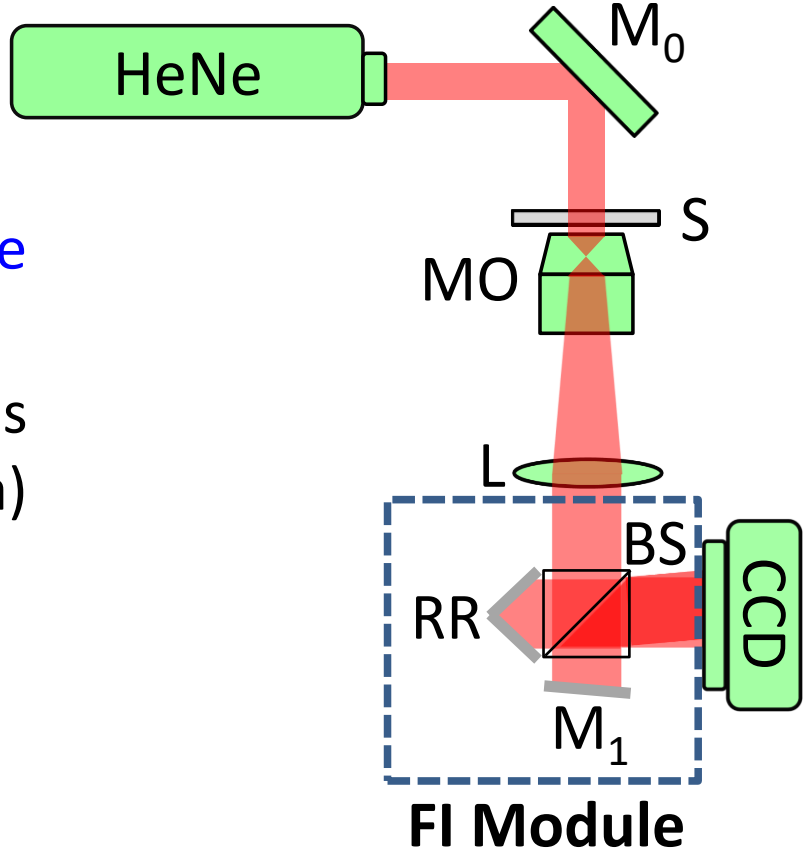
Bianco, et al., Ferraro, Adv. Intell. Syst. 1900153, 2020

- Detecting small metal nanoparticles (<100 nm) in water via the photothermal effect

# Flipping Interferometry (FI) Module

- $M_2$ ←→ Retro-reflector (RR)
  - Flips one FOV
  - FOV and off-axis angle uncoupled
- Allows dense samples in half of the optical FOV
- In microscopy, the optical FOV is much larger than the digital (camera) FOV

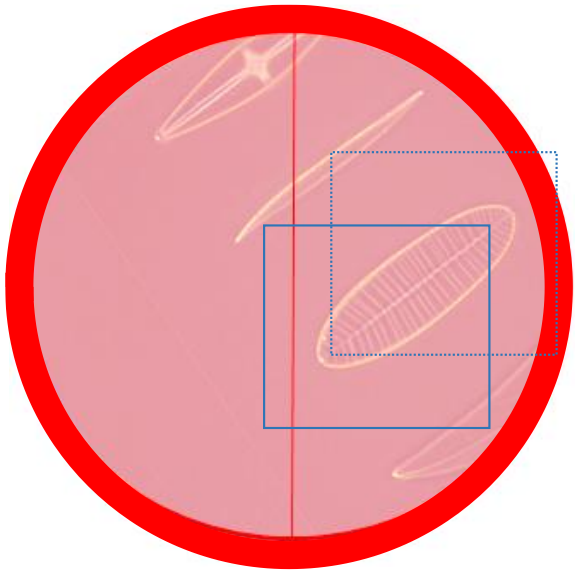


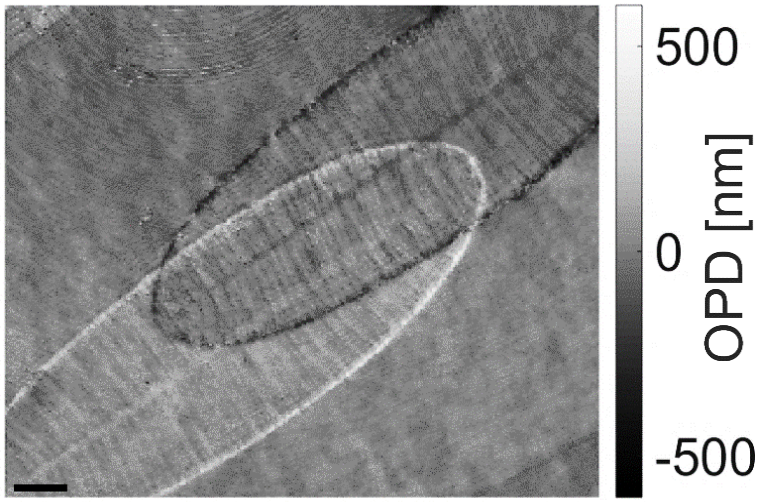




Roitshtain, Turko, Javidi, and Shaked
*Optics Letters* **41**, 2354 (2016)

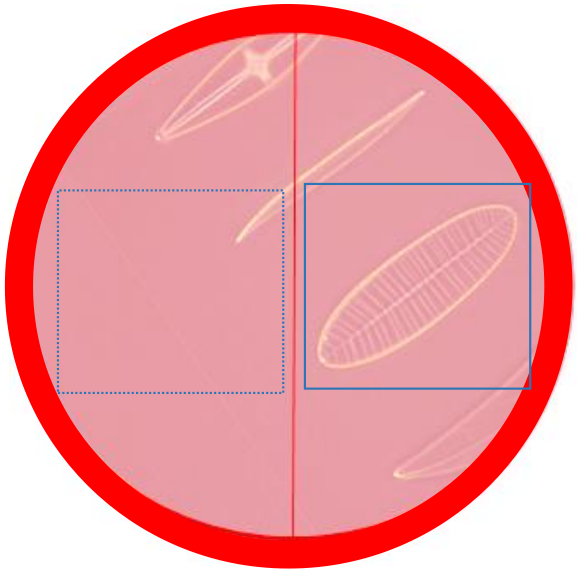
# Microcopy of Diatom Shells

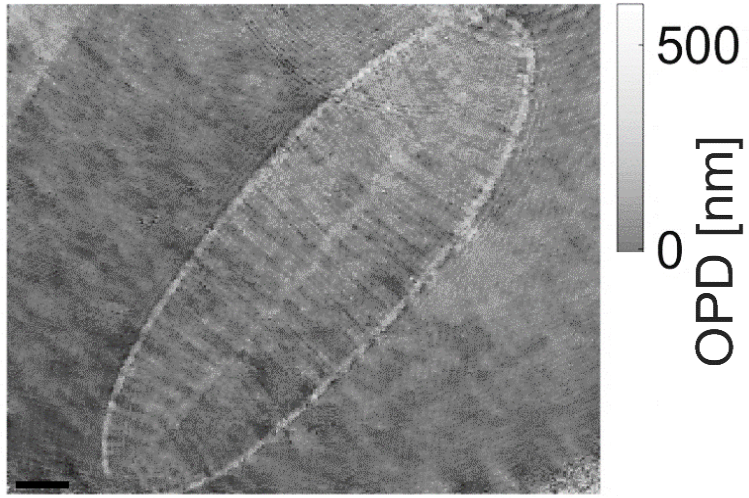
Conventional
shearing interferometry



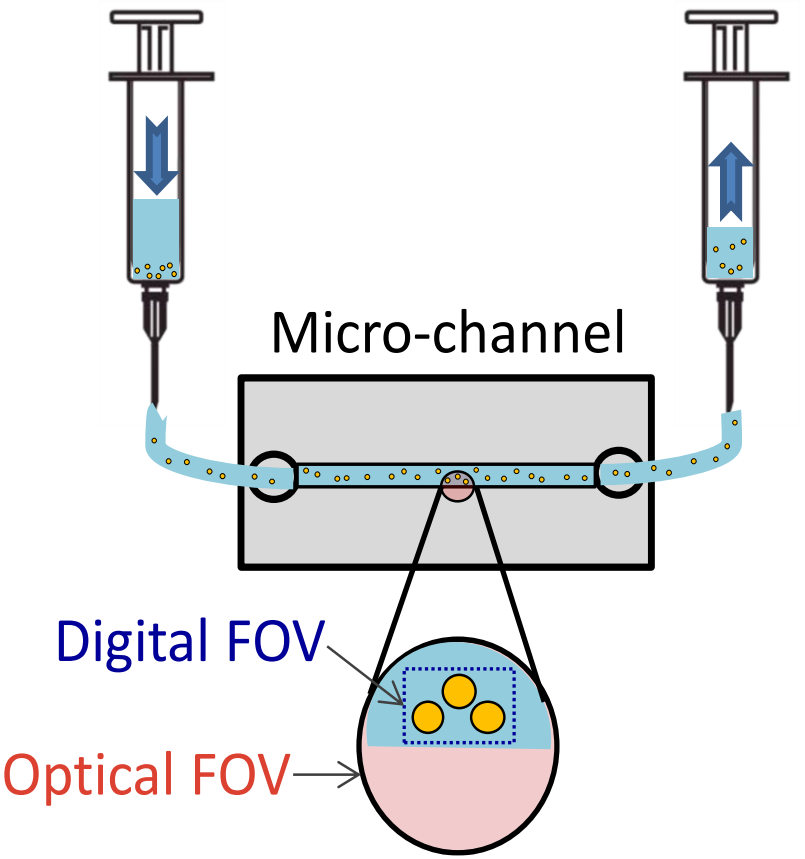



Flipping interferometry (FI)





# Microfluidics Channel FI



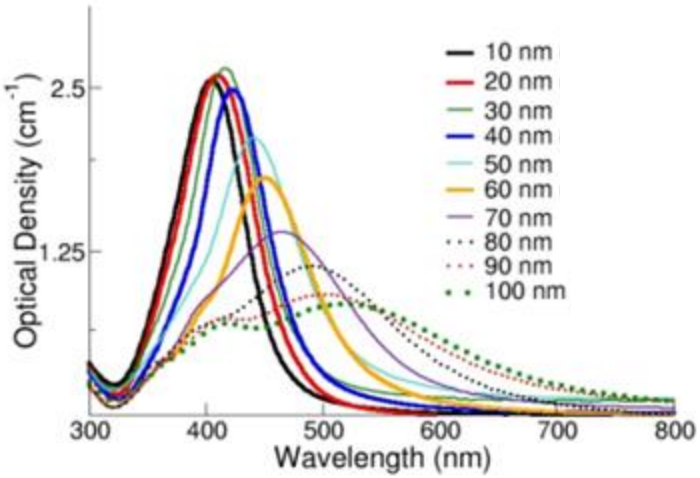

Microfluidic channels can easily satisfies the condition of half empty optical FOV

Roitshtain, Turko, Javidi, and Shaked
*Optics Letters* **41**, 2354 (2016)

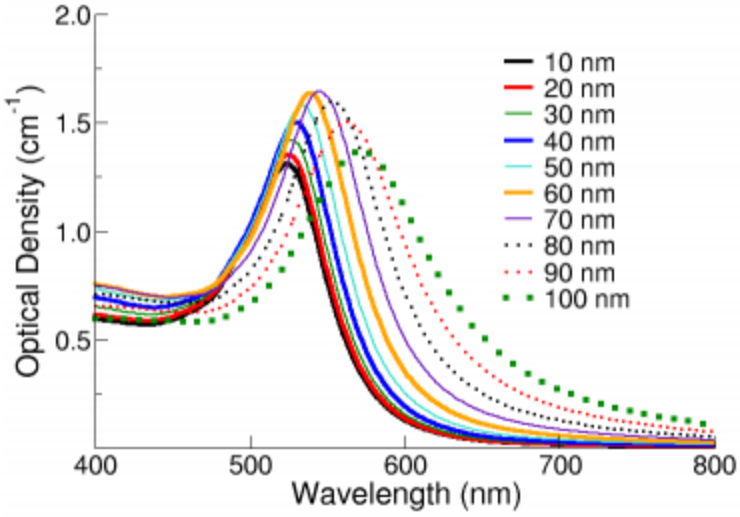
# Detection of gold and silver nanoparticles via their plasmonic peaks

- The plasmonic peak is specific to the nanoparticle size, shape, and type

Silver NPs

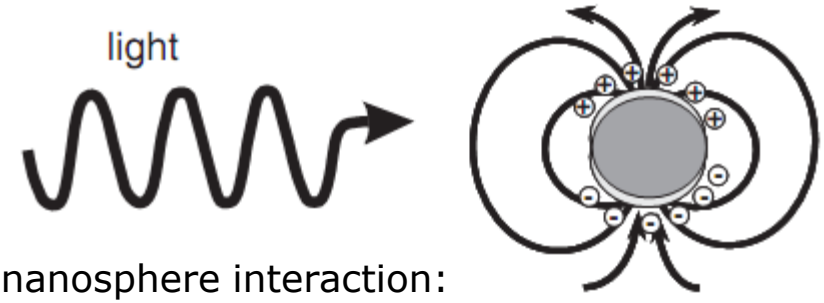



Gold NPs



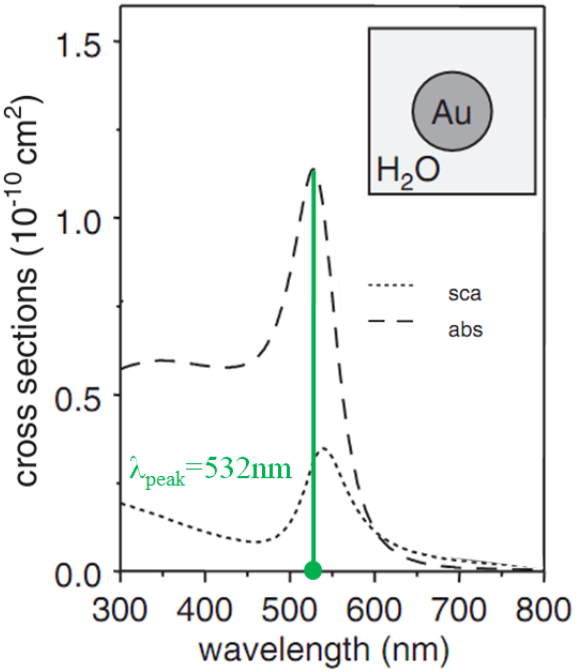
# Photothermal (PT) Effect

- Small metal particles have a large optical absorption.
- This strong absorption gives rise to a photothermal effect, i.e., an increase in temperature around the particle when it is illuminated by laser light.




Light-nanosphere interaction:
Electron cloud oscillation

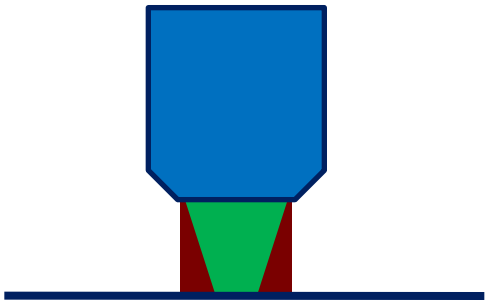
- **Example:** Temperature rise of ~15 K for 5nm NPs (with heating intensity of ~20 MW/cm$^2$).
- Temperature declines as the distance from the particle increases (~ 3 K at a distance of 13 nm from the center of the particle).

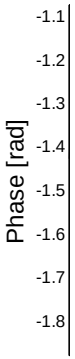



Scattering and **absorption** spectra of 60nm gold nanosphere

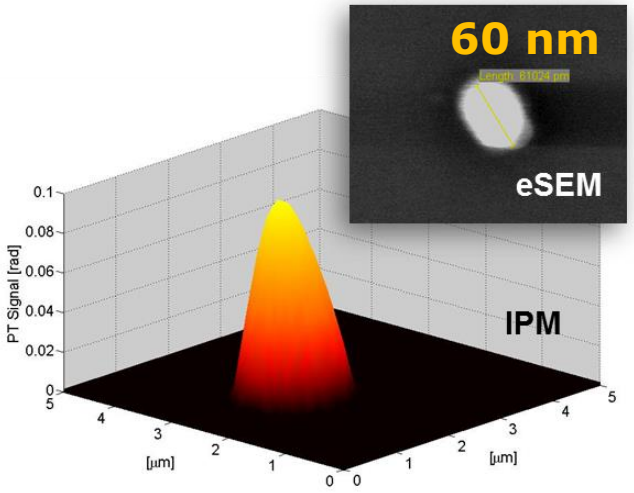
# Signal Processing

- AuNP Excitation:
  - 30 Hz
  - λ=532 nm
- Wide-field phase acquisition, rate > 60 fps





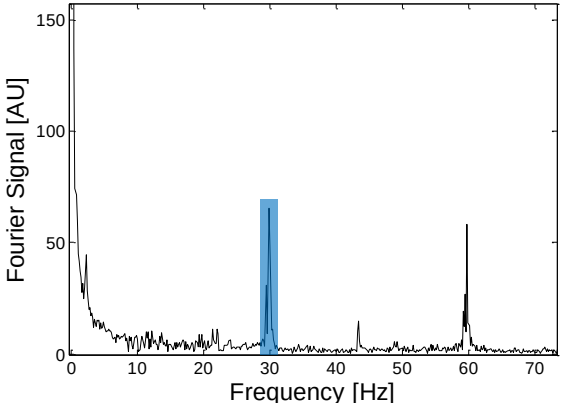

**Single Isolated AuNP Imaging:**



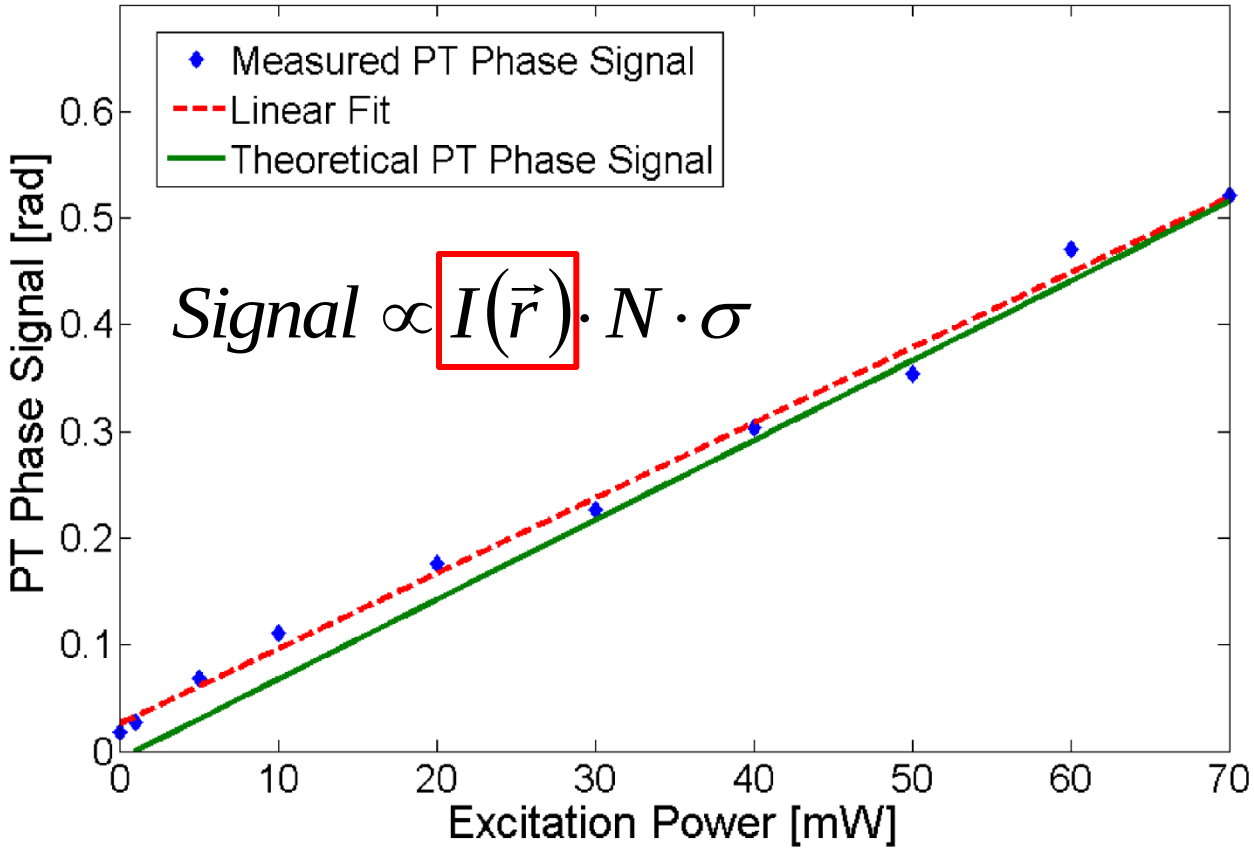

Digital Filter

Time [msec]

Fourier Transform

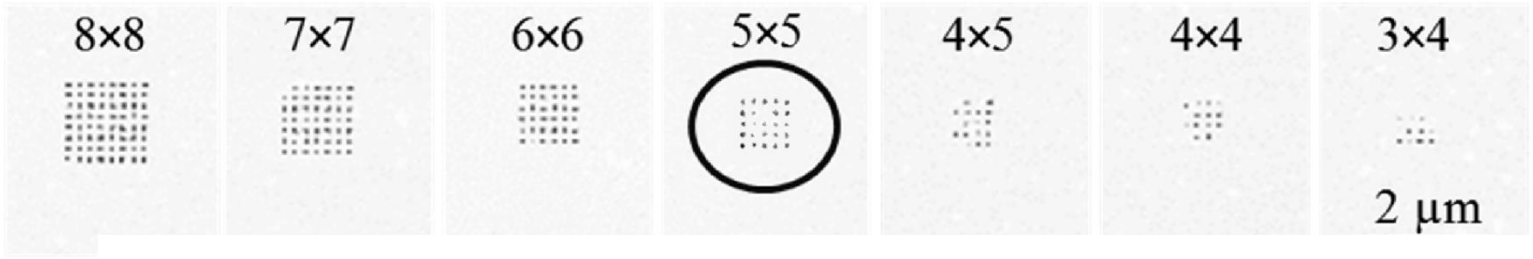


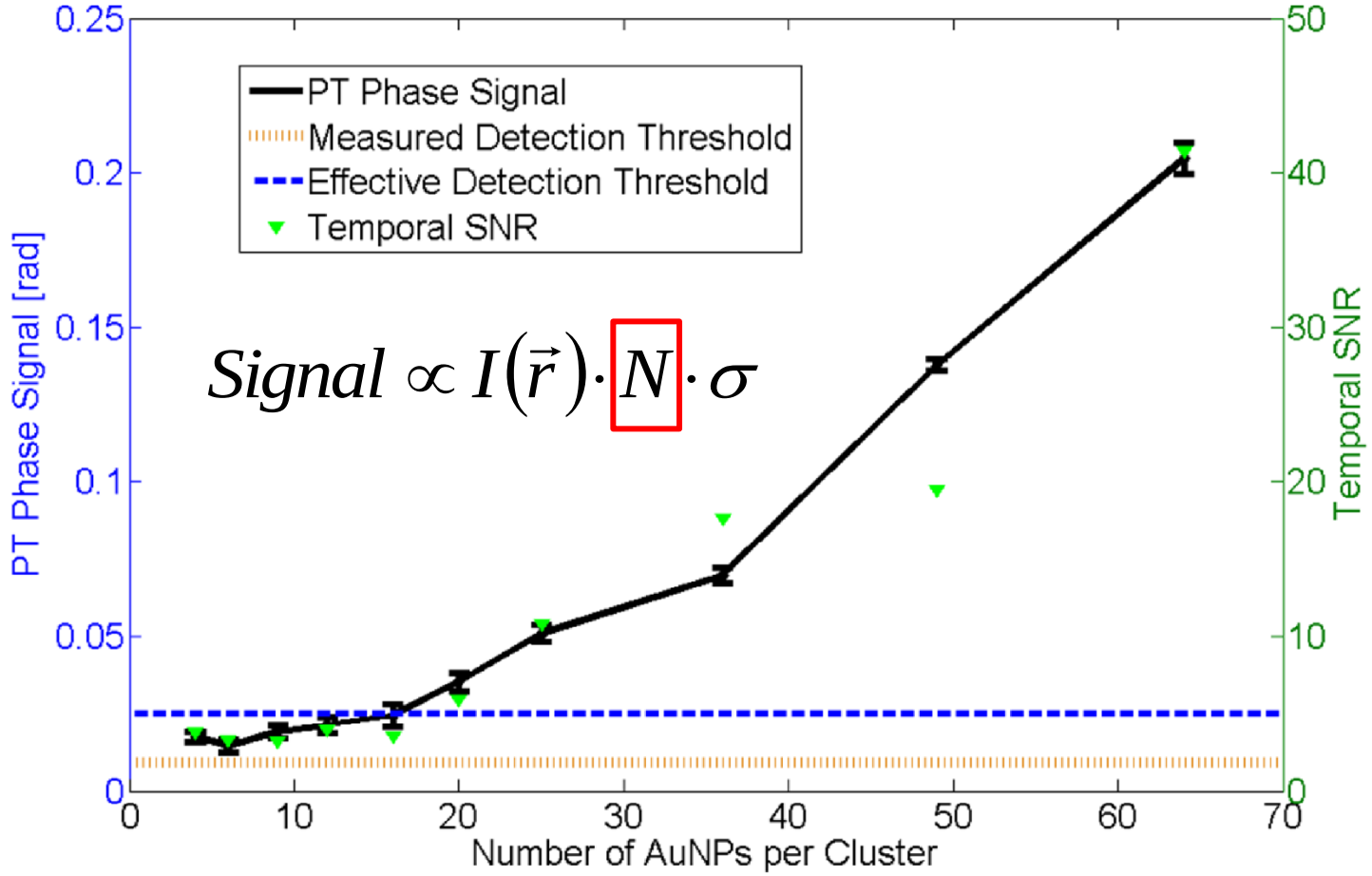
# IPM Imaging (5×5 μm² area) of Solution of 70-nm AuNPs in Water




- PT phase signal rises linearly with an increase in the excitation beam power.
- This gives us an indication that we measure a PT signal originated from the AuNPs.

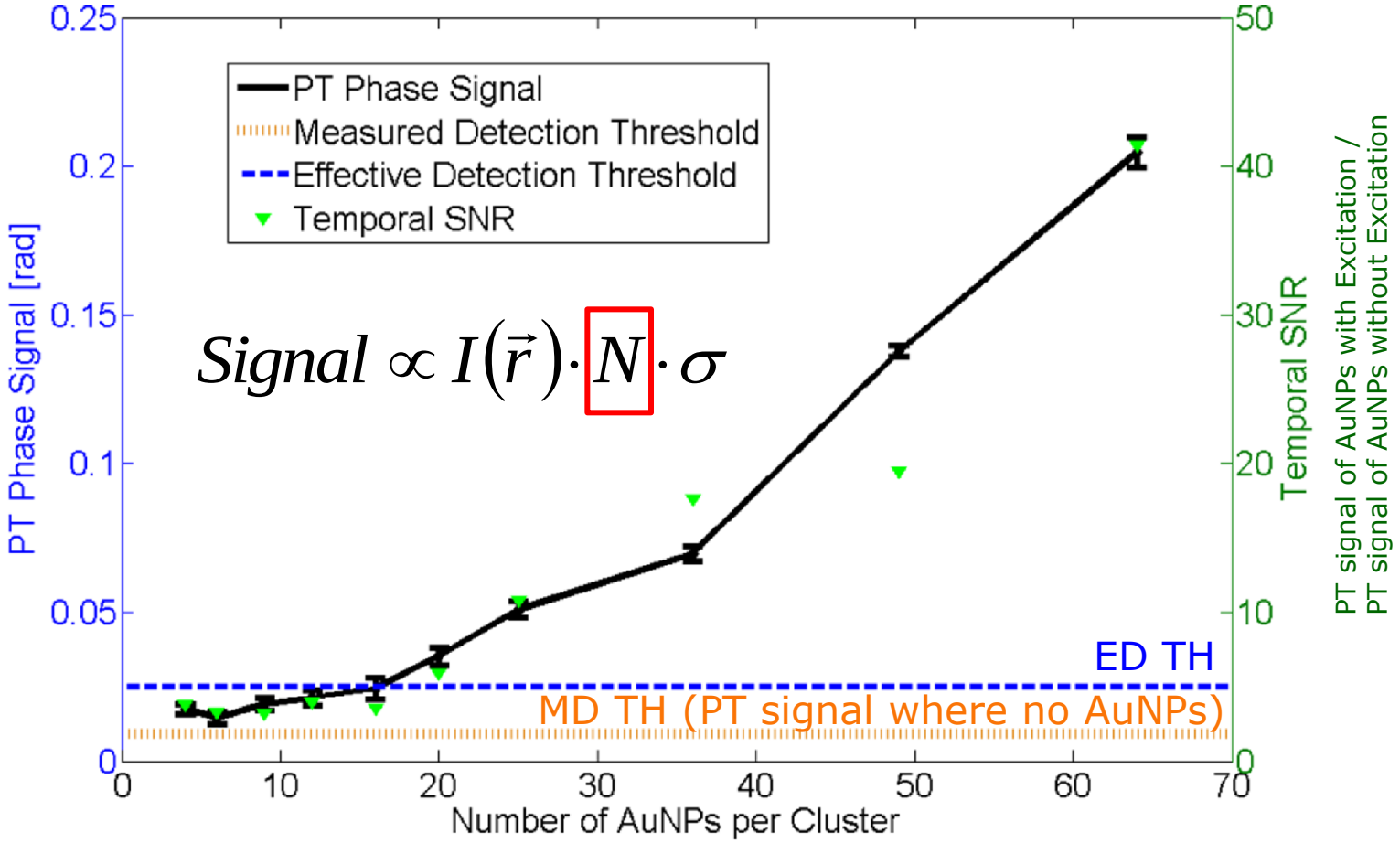
# IPM Imaging of Groups of Isolated 40-nm AuNPs

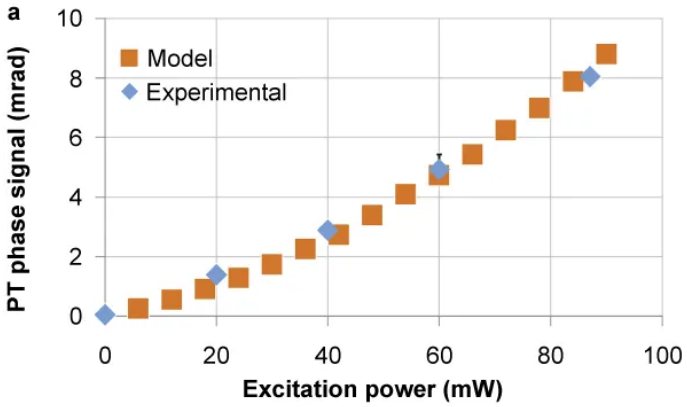


# IPM Imaging of Groups of Isolated 40-nm AuNPs

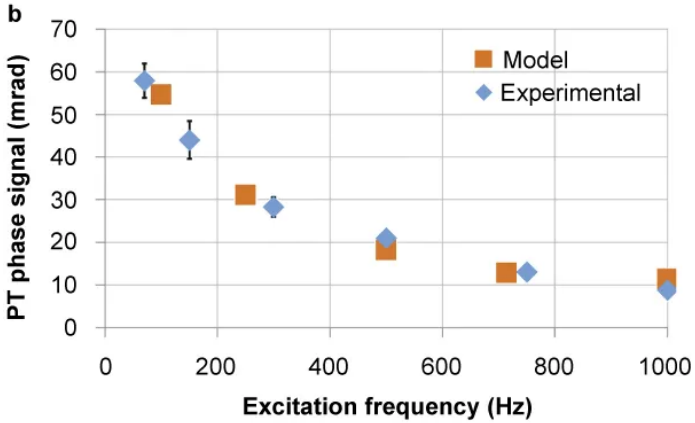
- Above *N*=16 (ED TH), PT phase signal rises linearly with an increase in *N*.
- Again, This gives us an indication that we measure a PT signal originated from the AuNPs.



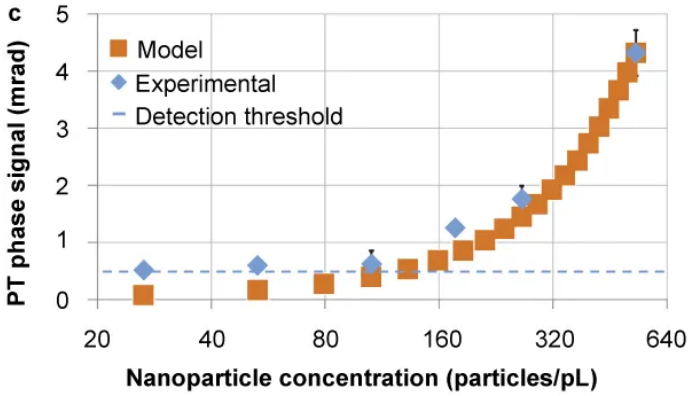



Gold nanorods in water

(**a**) Spatially averaged PT phase signal as a function of the excitation power




(**b**) Spatially averaged PT phase signal as a function of the excitation laser frequency




(**c**) Spatially averaged PT phase signal as a function of the nanoparticle concentration in logarithmic scale

Blum and Shaked
Nature LSA **4**, e322 (2015)

# Conclusions

- **IPM** can capture the quantitative thickness of via refractive index imaging in water in 10,000 fps.
- We proposed a simple portable IPM modules that connect to existing imaging systems
- Can detect via quantitative phase imaging:
  - Micro-organisms (diatom typing, etc.)
  - Plastic particles
  - Plasmonic nanoparticles (gold, silver, etc.)

# Biomedical OMNI Group

**Biomedical Optical Microscopy, Nanoscopy and Interferometry Research Group**

*Prof. Natan T. Shaked*

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- **Dr. Mattan Levy**, PhD Biology
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