



**The Abdus Salam  
International Centre for Theoretical Physics**



**IAEA**  
International Atomic Energy Agency

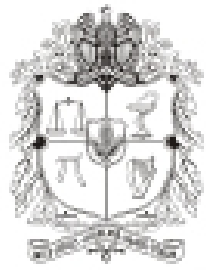
**2037-7**

## **Introduction to Optofluidics**

*1 - 5 June 2009*

**Digital in-line holographic microscopy: an alternative tool to visualization of microfluidics**

J. Garcia-Sucerquia  
*Universidad Nacional de Colombia  
Colombia*



UNIVERSIDAD  
**NACIONAL**  
DE COLOMBIA  
SEDE MEDELLÍN

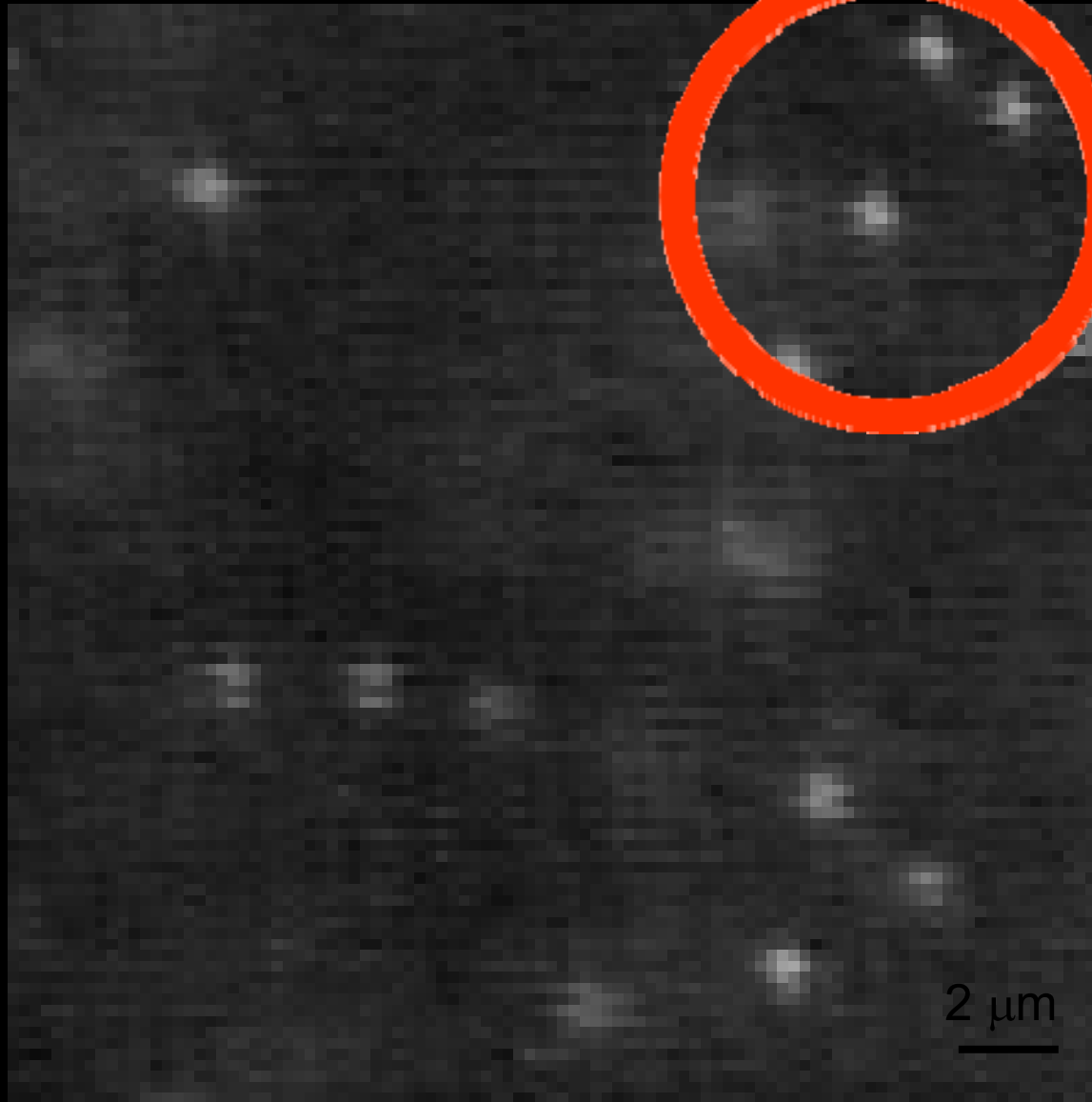


# Digital In-line Holographic Microscopy: an alternative tool to visualization of microfluidics

**Jorge Garcia-Sucerquia**

School of Physics  
Universidad Nacional de Colombia Sede Medellín.  
A.A. 3840, Medellín – Colombia

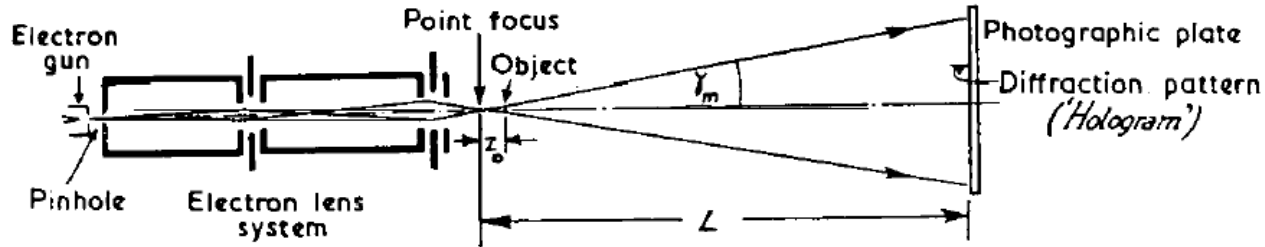
Introduction to Optofluidics  
ICTP, Trieste 2009



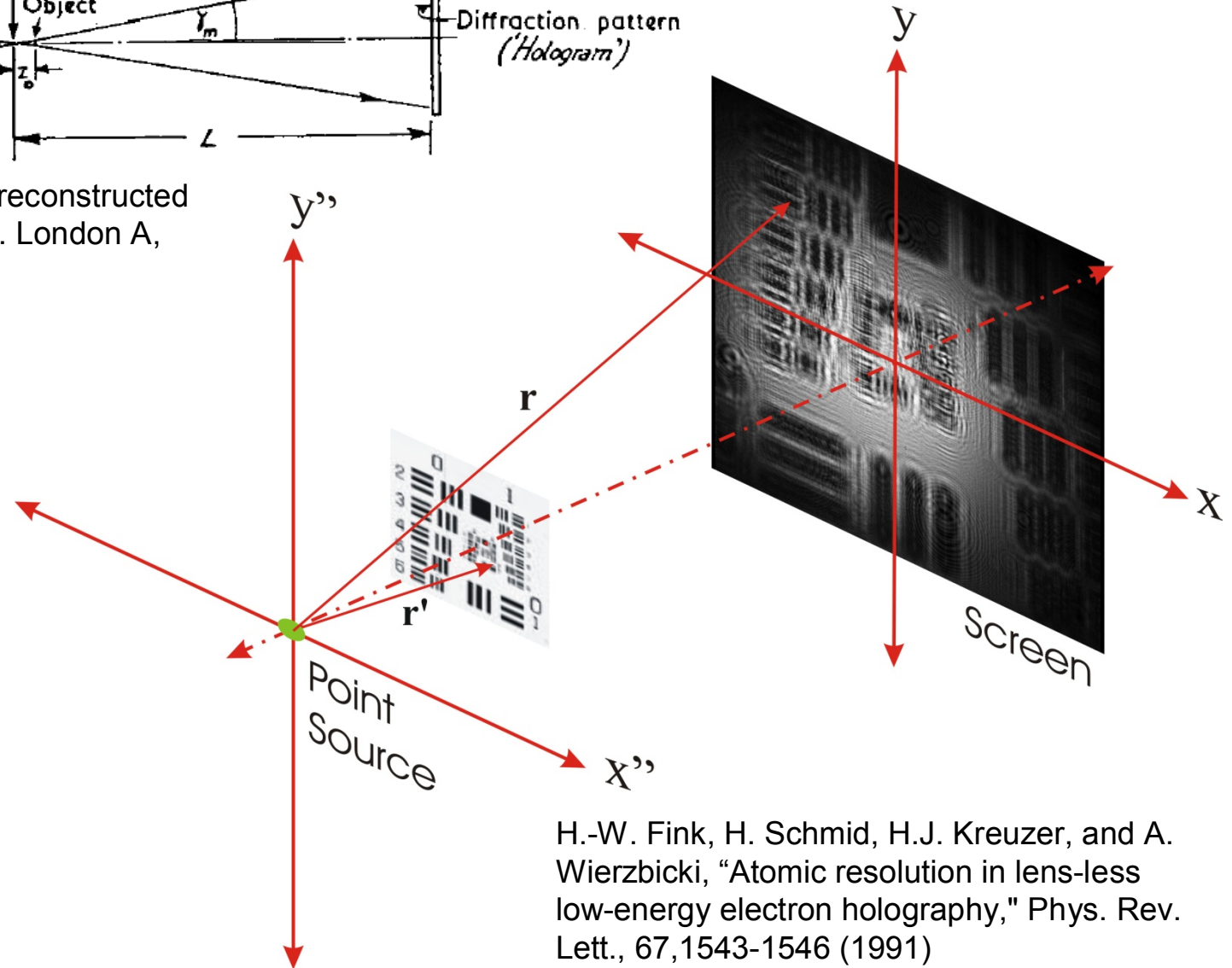
Optical microscopy.  
60X microscope objective. Depth of field  $0.4 \mu\text{m}$

# DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Recording



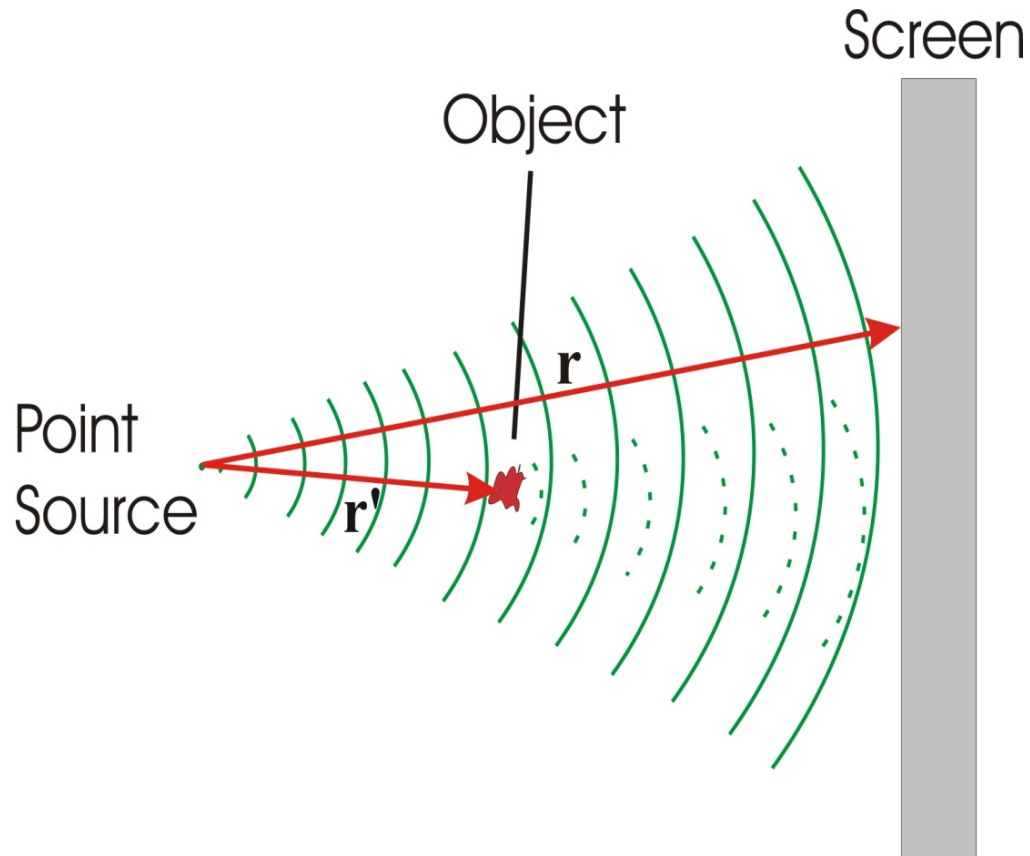
D. Gabor, "Microscopy by reconstructed wave-fronts," Proc. R. Soc. London A, 197, 454, (1949)



H.-W. Fink, H. Schmid, H.J. Kreuzer, and A. Wierzbicki, "Atomic resolution in lens-less low-energy electron holography," Phys. Rev. Lett., 67, 1543-1546 (1991)

# DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Recording



$$I(\mathbf{r}) = \left| A_{ref}(\mathbf{r}) + A_{scat}(\mathbf{r}) \right|^2$$

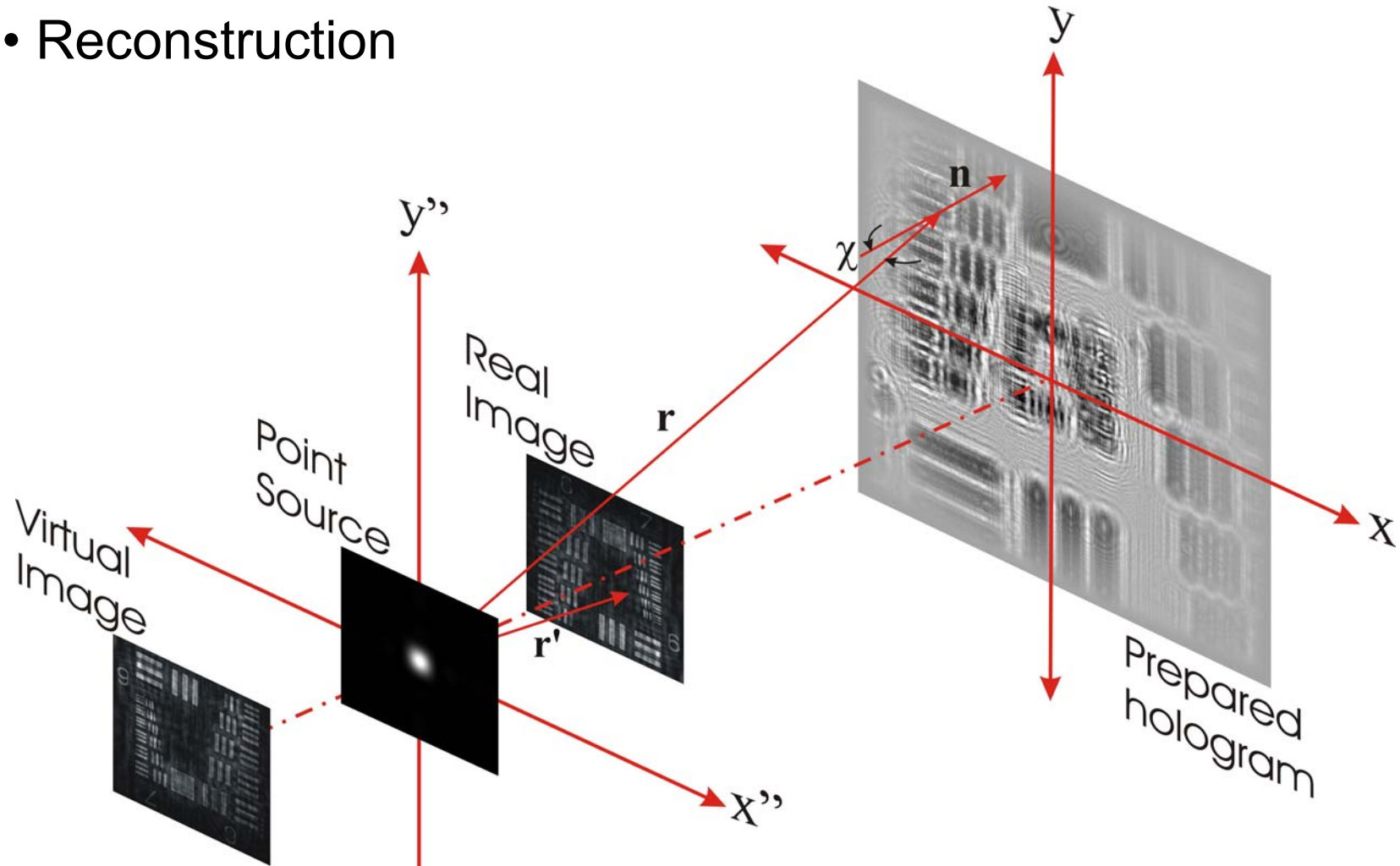
Prepared hologram

$$\tilde{I}(\mathbf{r}) = I(\mathbf{r}) - \left| A_{ref}(\mathbf{r}) \right|^2$$

$$\tilde{I}(\mathbf{r}) = [A_{ref}^*(\mathbf{r})A_{scat}(\mathbf{r}) + A_{ref}(\mathbf{r})A_{scat}^*(\mathbf{r})] + \left| A_{scat}(\mathbf{r}) \right|^2$$

# DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Reconstruction

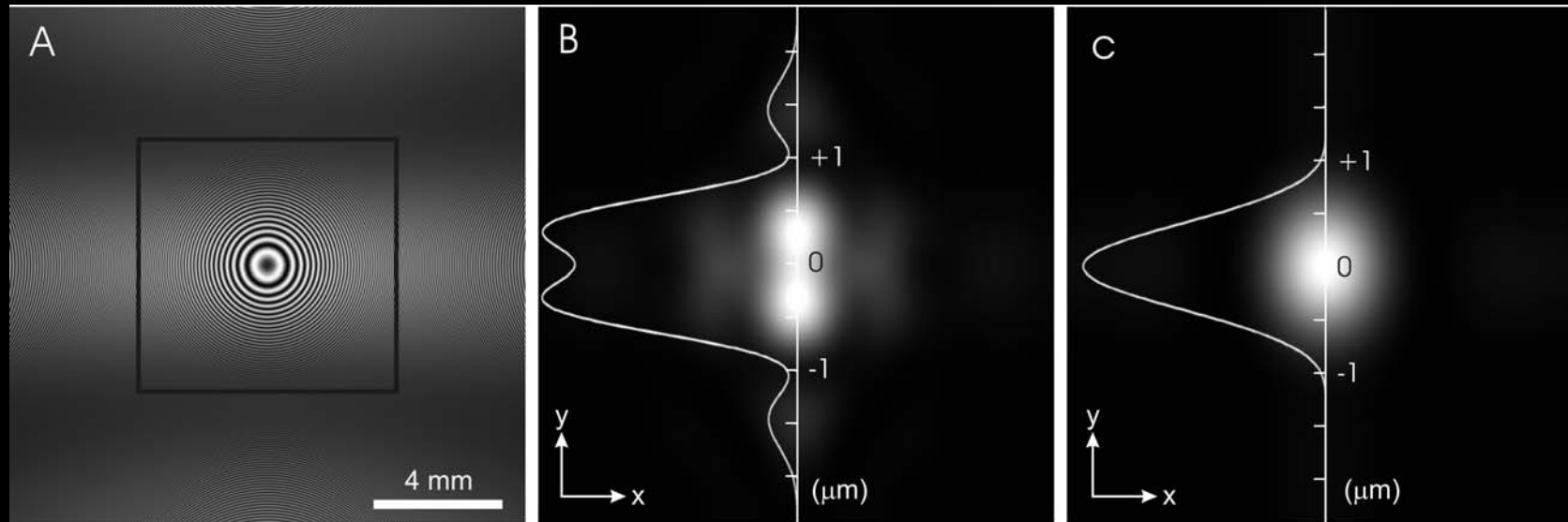


$$A_{scat}(\mathbf{r}') = -\frac{iA_{ref}}{r\lambda} \iint_{Screen} \tilde{I}(\mathbf{r}) \frac{\exp\left[ik \frac{|\mathbf{r} \cdot \mathbf{r}'|}{r}\right]}{|\mathbf{r} - \mathbf{r}'|} dS_r$$

Kirchhoff-Helmholtz transform.

# DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Lateral Resolution



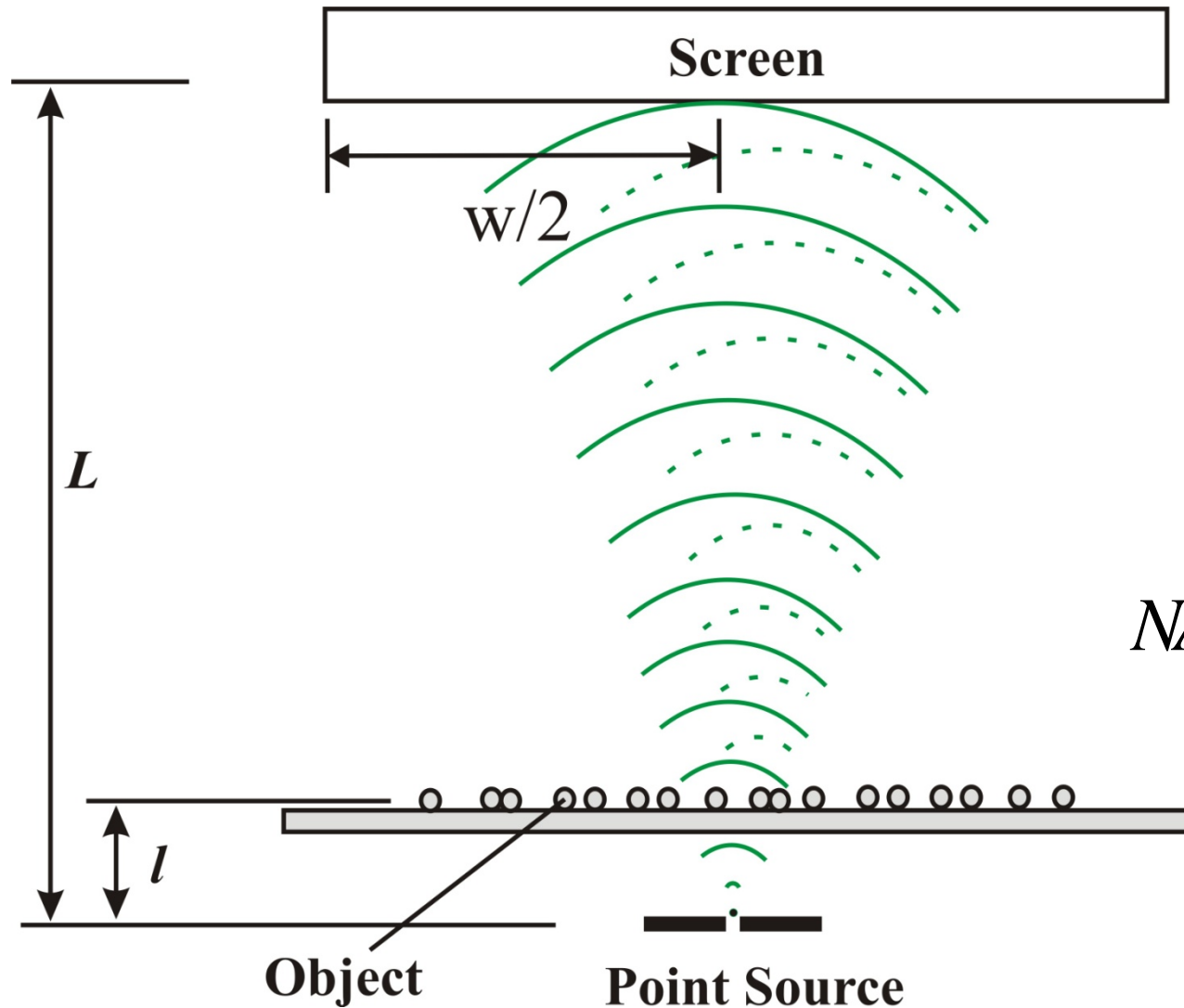
Test of lateral resolution. A: simulated hologram of two points close to the optical axis and  $0.8 \mu\text{m}$  apart, taken with a blue laser  $\lambda=4730\text{\AA}$  with an aperture of 0.5 (0.28 for the inner square). B: reconstruction from the full hologram with the insert showing submicron resolution. C: the same for the smaller hologram losing resolution.

$$|\mathbf{r}_2 - \mathbf{r}_1| \geq \frac{\lambda}{2NA}$$

J. Garcia-Sucerquia, et. al.  
"Digital In-line Holography  
Microscopy," *Appl. Opt.* **45**,  
836-850 (2006) .

# DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Lateral Resolution



$$|\mathbf{r}_2 - \mathbf{r}_1| \geq \frac{\lambda}{2NA}$$

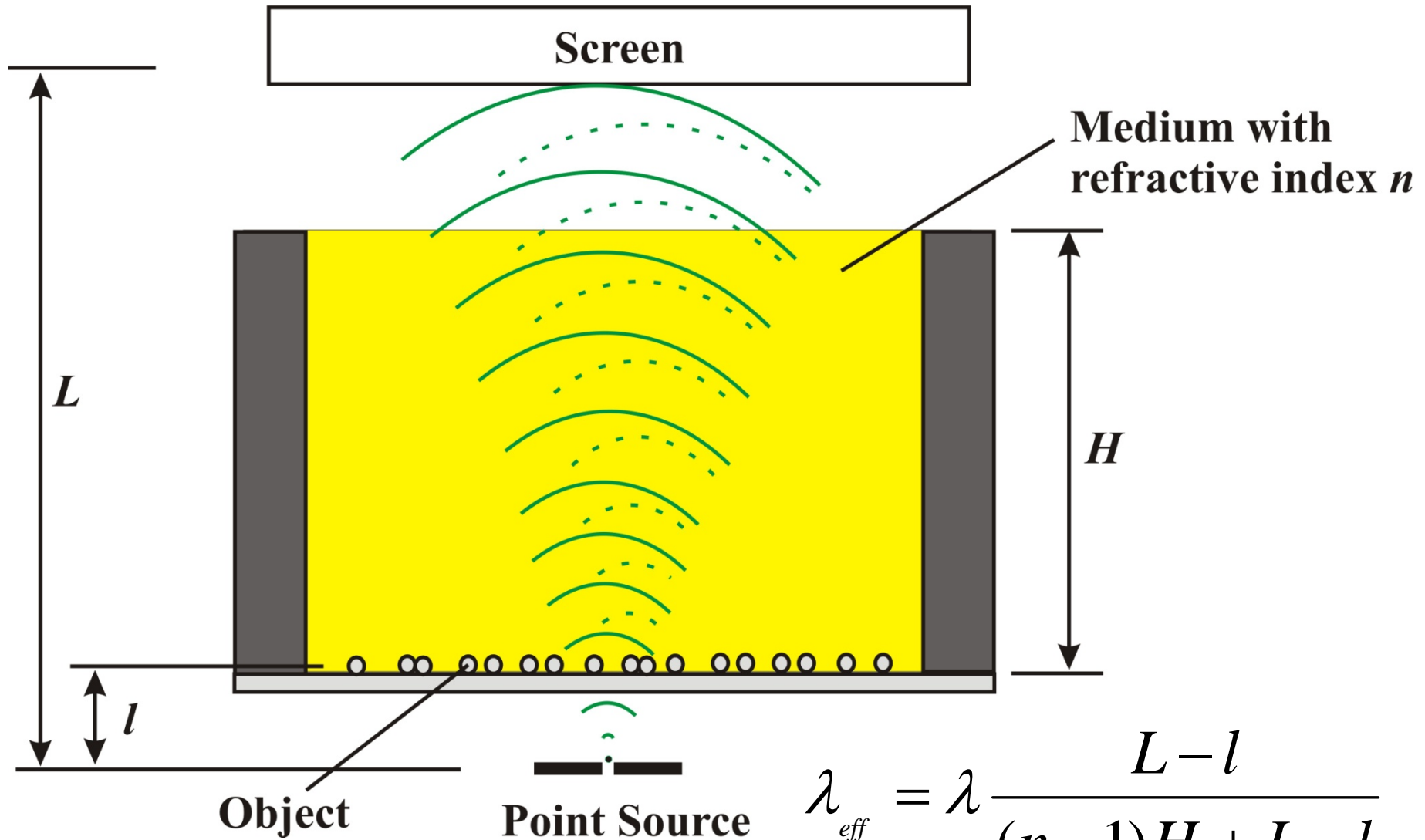
$$NA = \frac{W}{2\sqrt{(W/2)^2 + L^2}}$$

J. Garcia-Sucerquia, et. al.  
"Digital In-line Holography  
Microscopy," Appl. Opt. **45**,  
836-850 (2006)



# DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

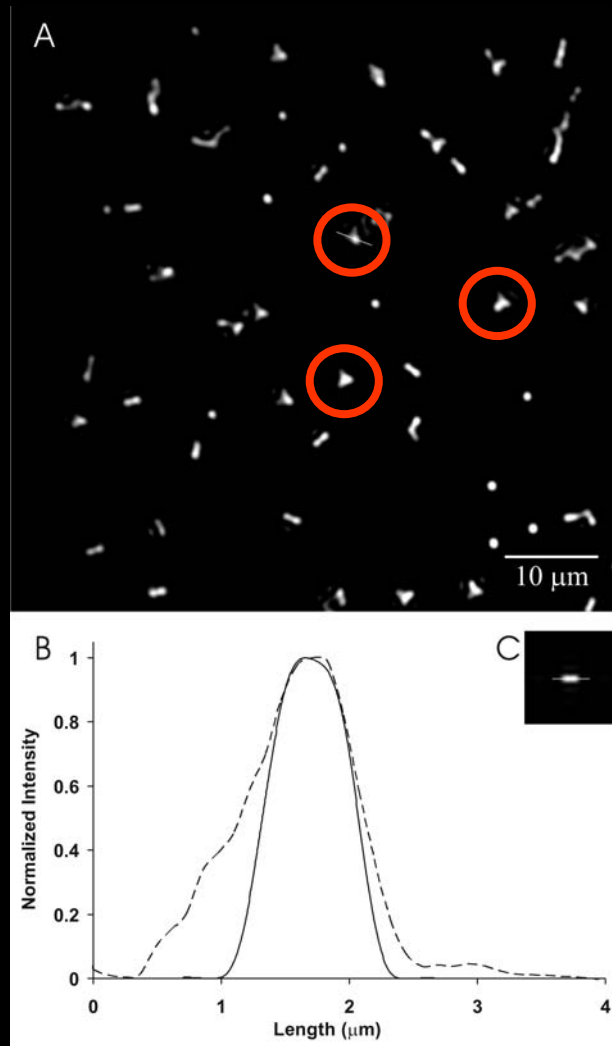
- Enhancement of Lateral Resolution



J. Garcia-Sucerquia, et. al. "Immersion Digital In-line Holography Microscopy," Opt. Lett. **31**, 1211-1213 (2006)

# DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

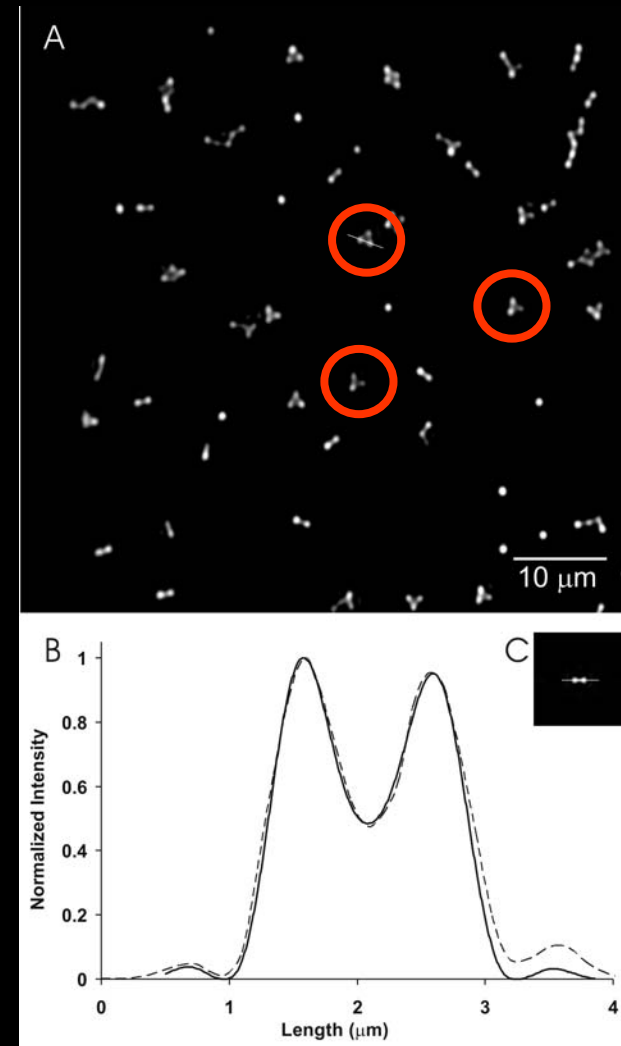
- Enhancement of Lateral Resolution



Empty chamber ( $n=1$ ,  
 $H=0$  mm,  $\lambda_{eff}=532$  nm)

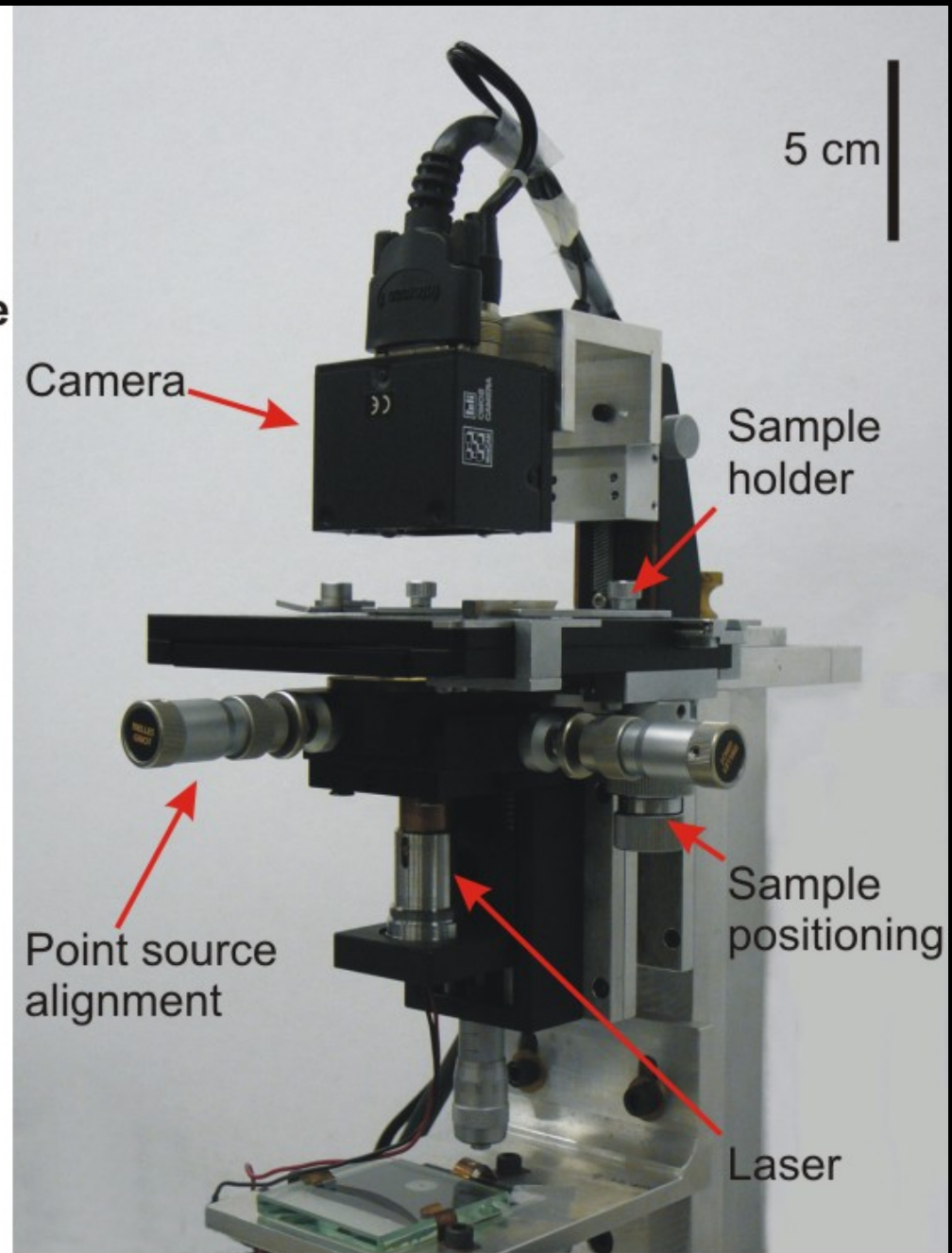
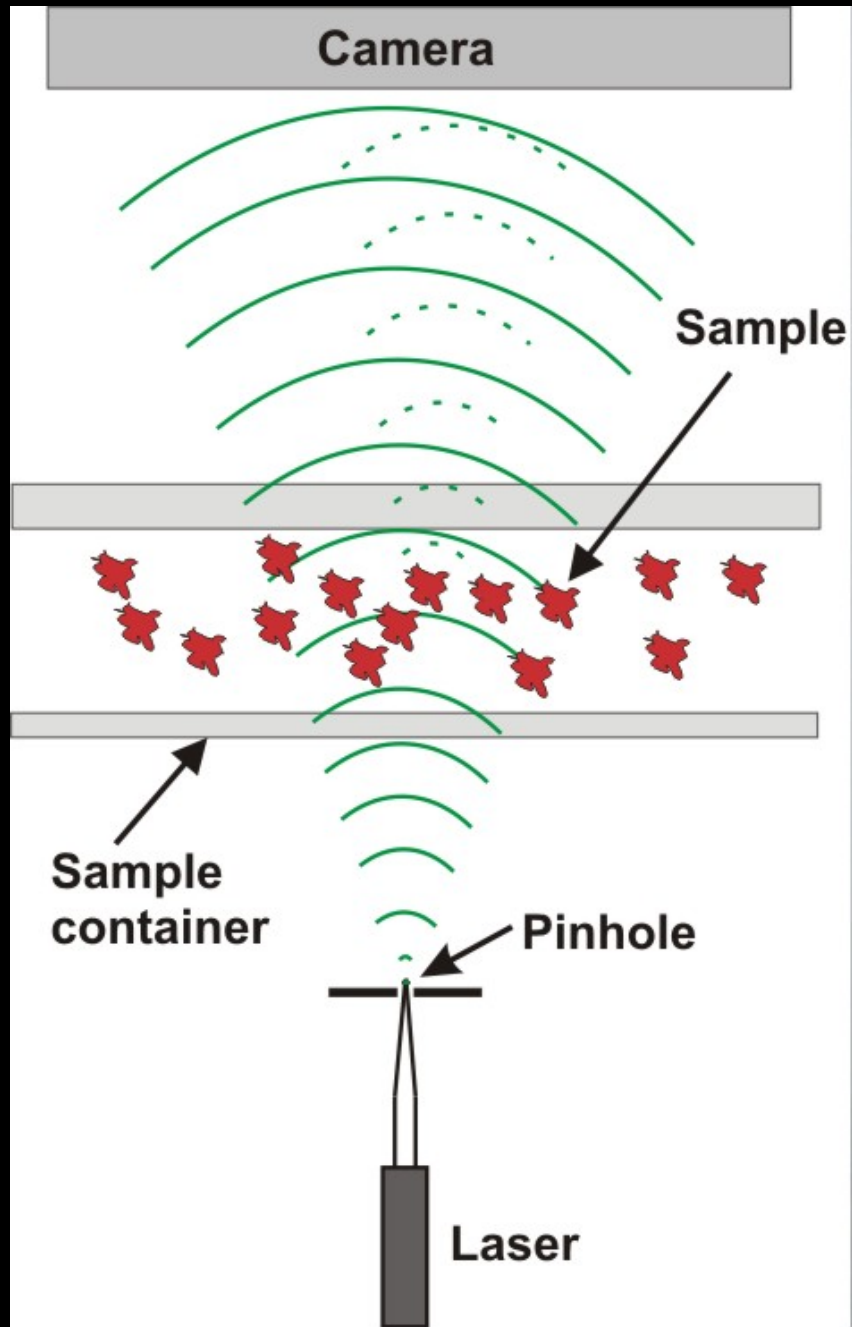
1.09  $\mu\text{m}$   
diameter latex  
beads on a  
cover slip.  
 $\lambda=532$  nm  
 $L=15$  mm  
 $l=0.3$  mm

J. Garcia-Sucerquia, et.  
al. "Immersion Digital In-  
line Holography  
Microscopy," Opt. Lett.  
31, 1211-1213 (2006)



Chamber with oil ( $n=1.515$ ,  
 $H=12$  mm,  $\lambda_{eff}=374$  nm)

# THE ACTUAL IMPLEMENTATION

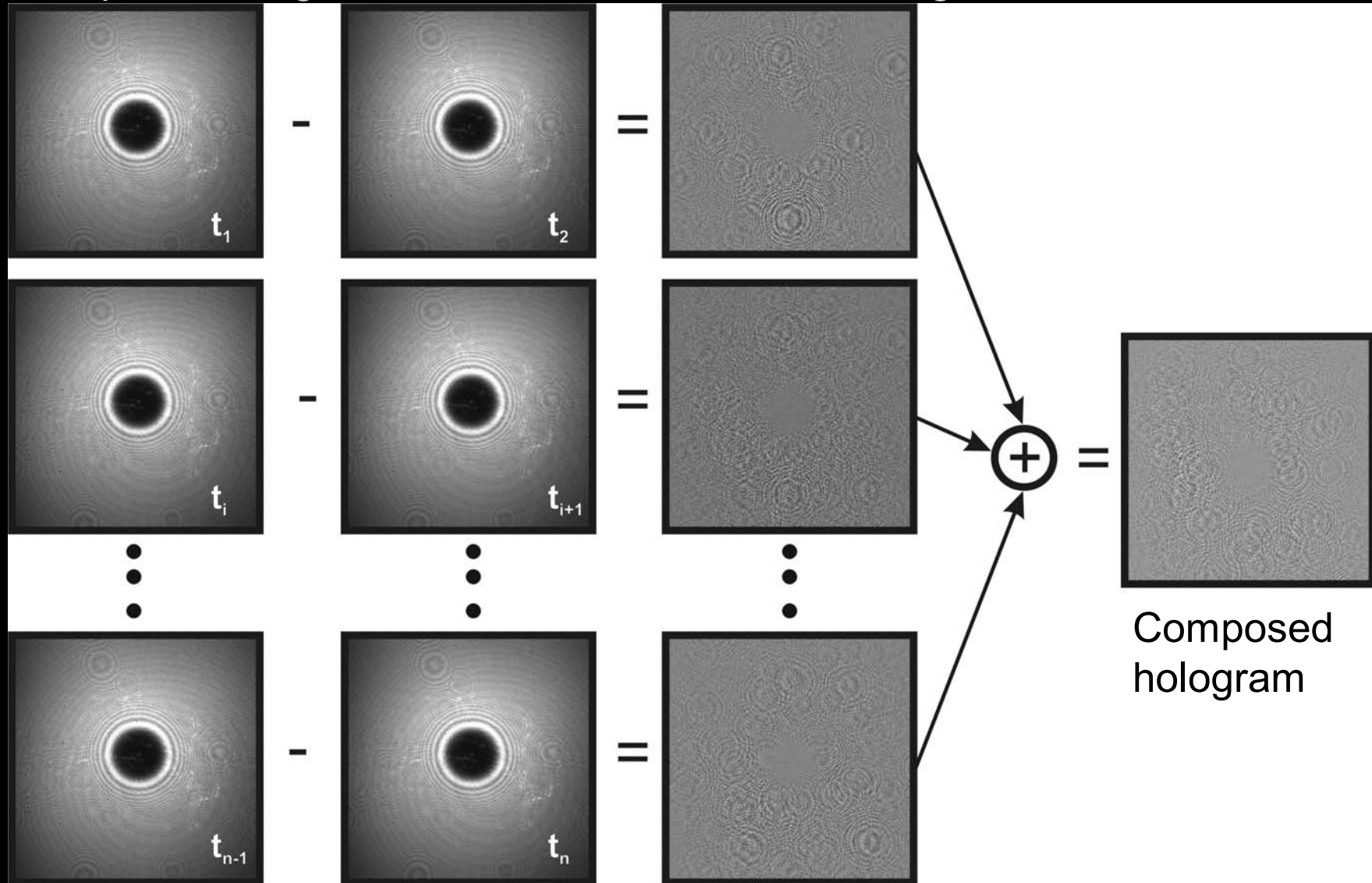


# APPLICATIONS

- Four dimensional tracking in micro-channels

Acquired holograms

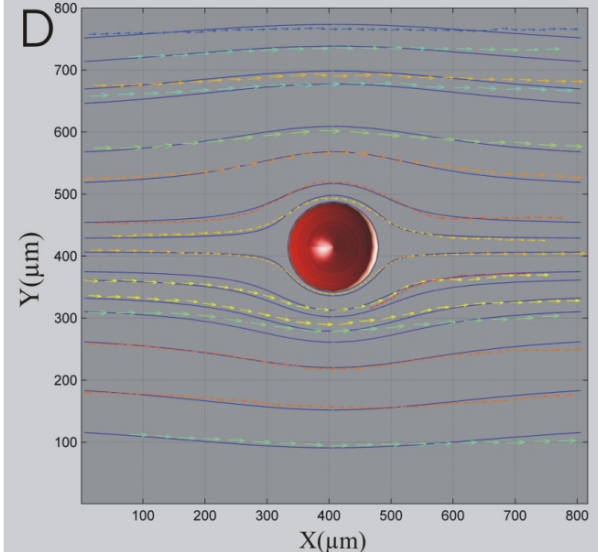
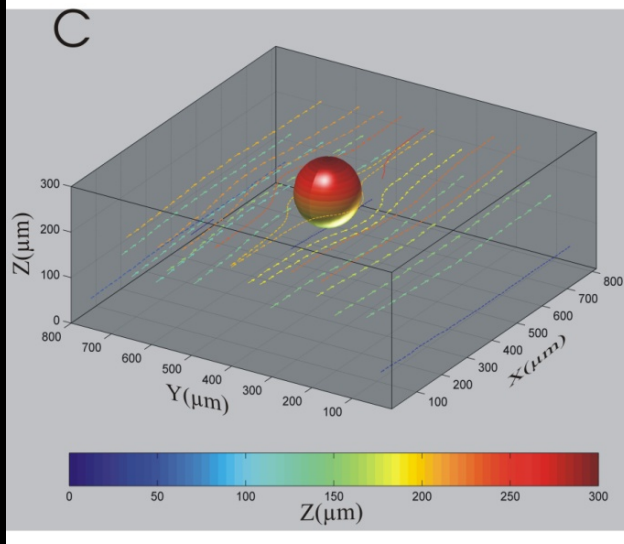
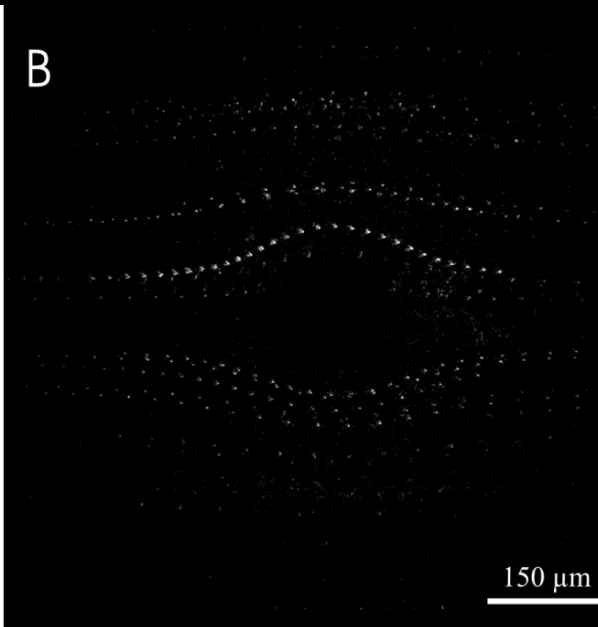
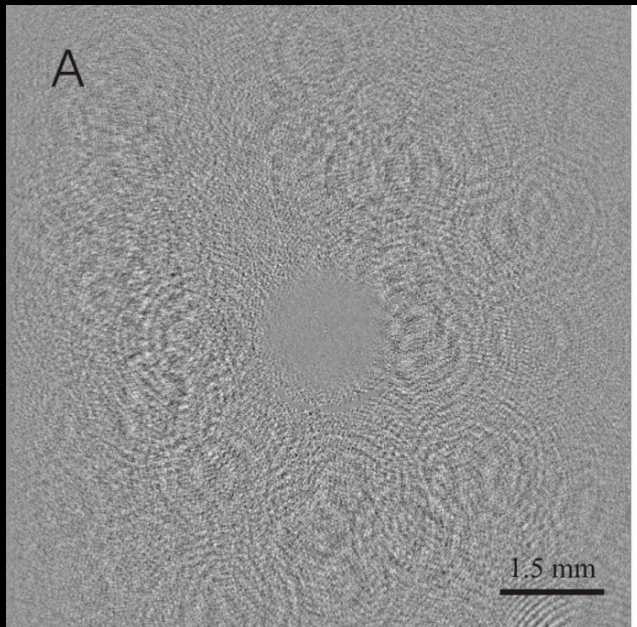
Difference holograms





# APPLICATIONS

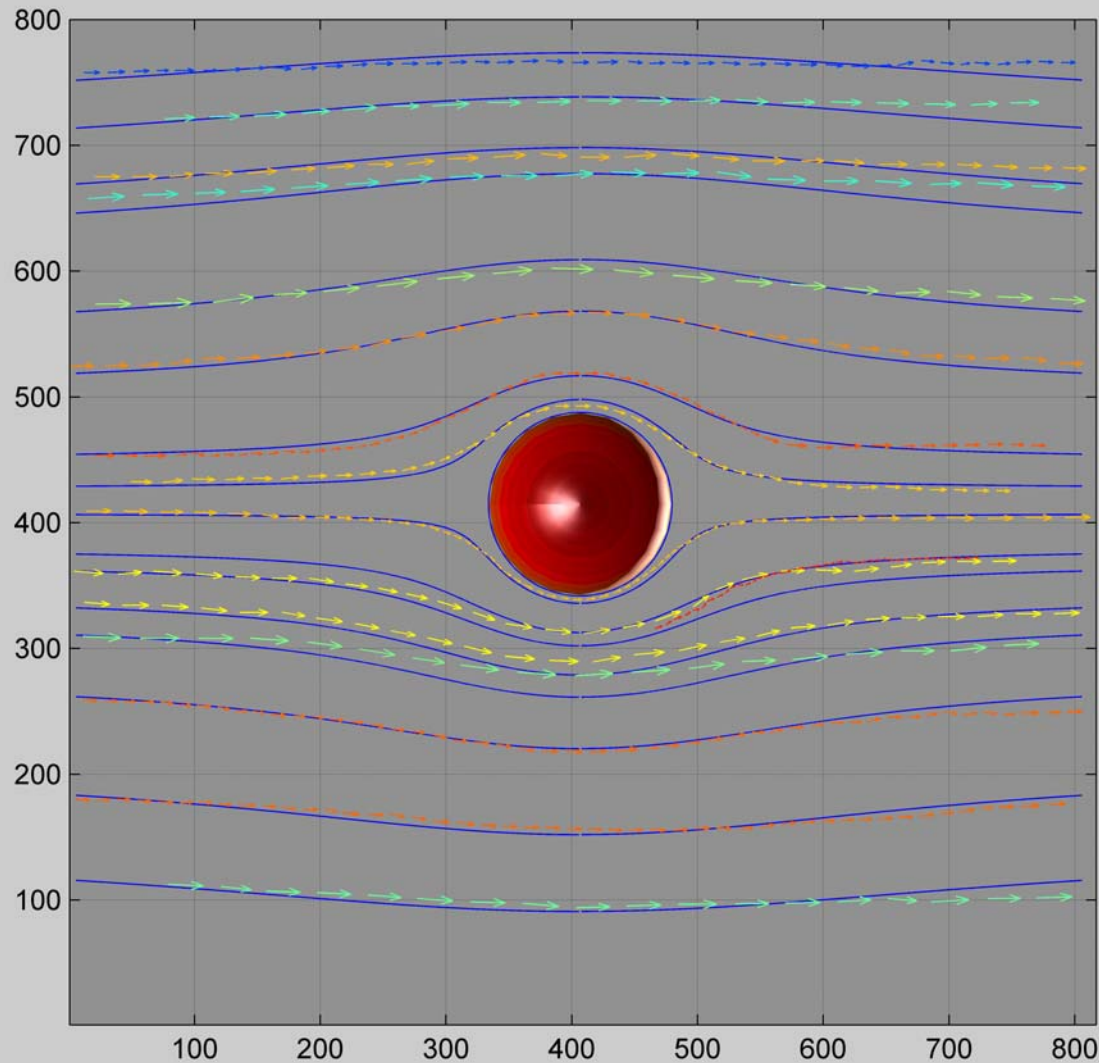
## •Microfluidic Studies



Stokes' flow around a fixed sphere. 60 holograms were taken at intervals of 0.17 s to generate a difference hologram, panel A. Panel B shows one of 60 reconstructions made to render the field velocity shown in panel C. In panel D the solid blue lines represent the solution to the Navier-Stokes equation for our experiment and the arrows correspond to the measured velocity field.

# APPLICATIONS

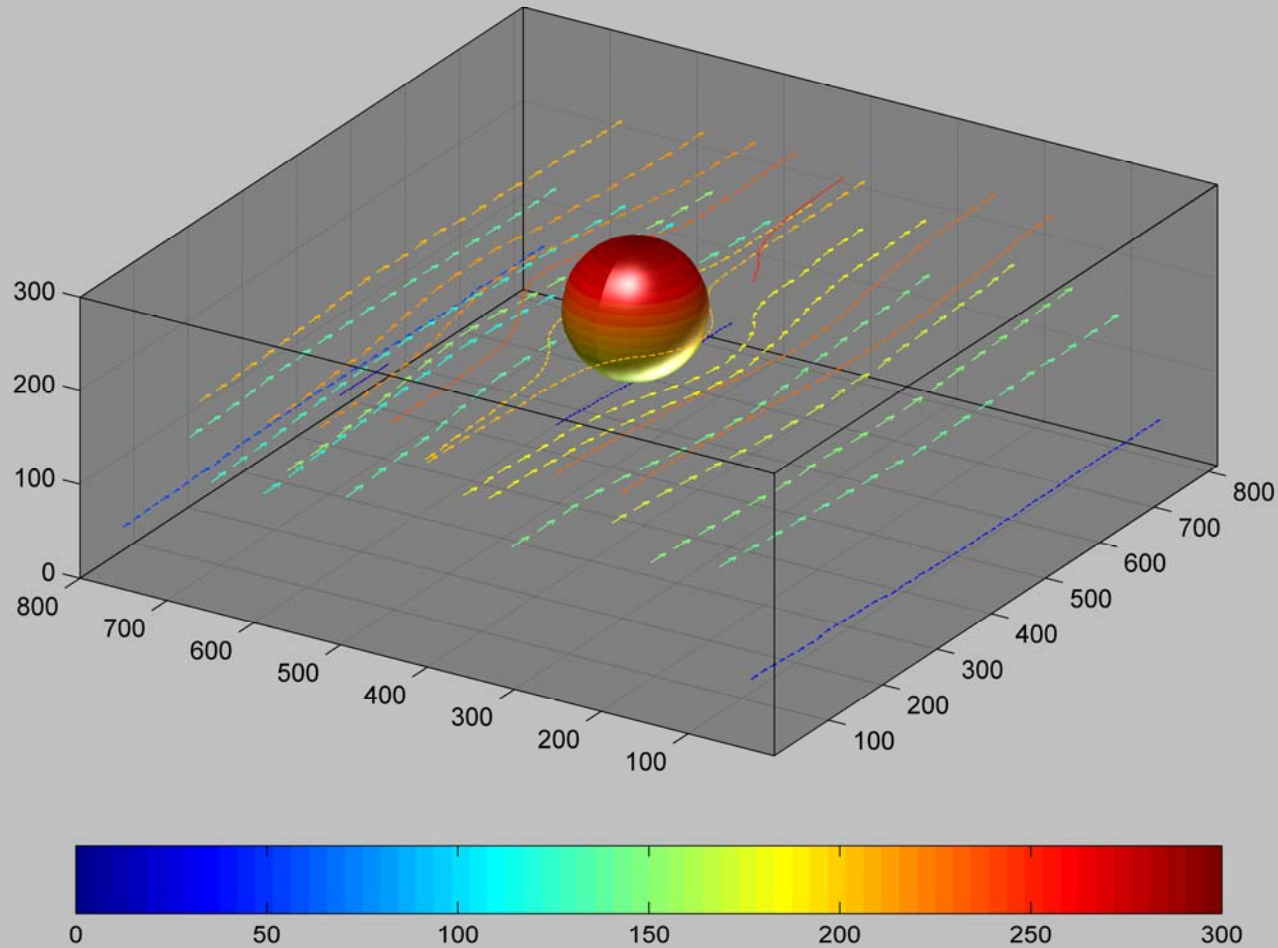
- Microfluidic Studies



The solid blue lines represent the solution to the Navier-Stokes equation for our experiment and the arrows correspond to the measured velocity field.

# APPLICATIONS

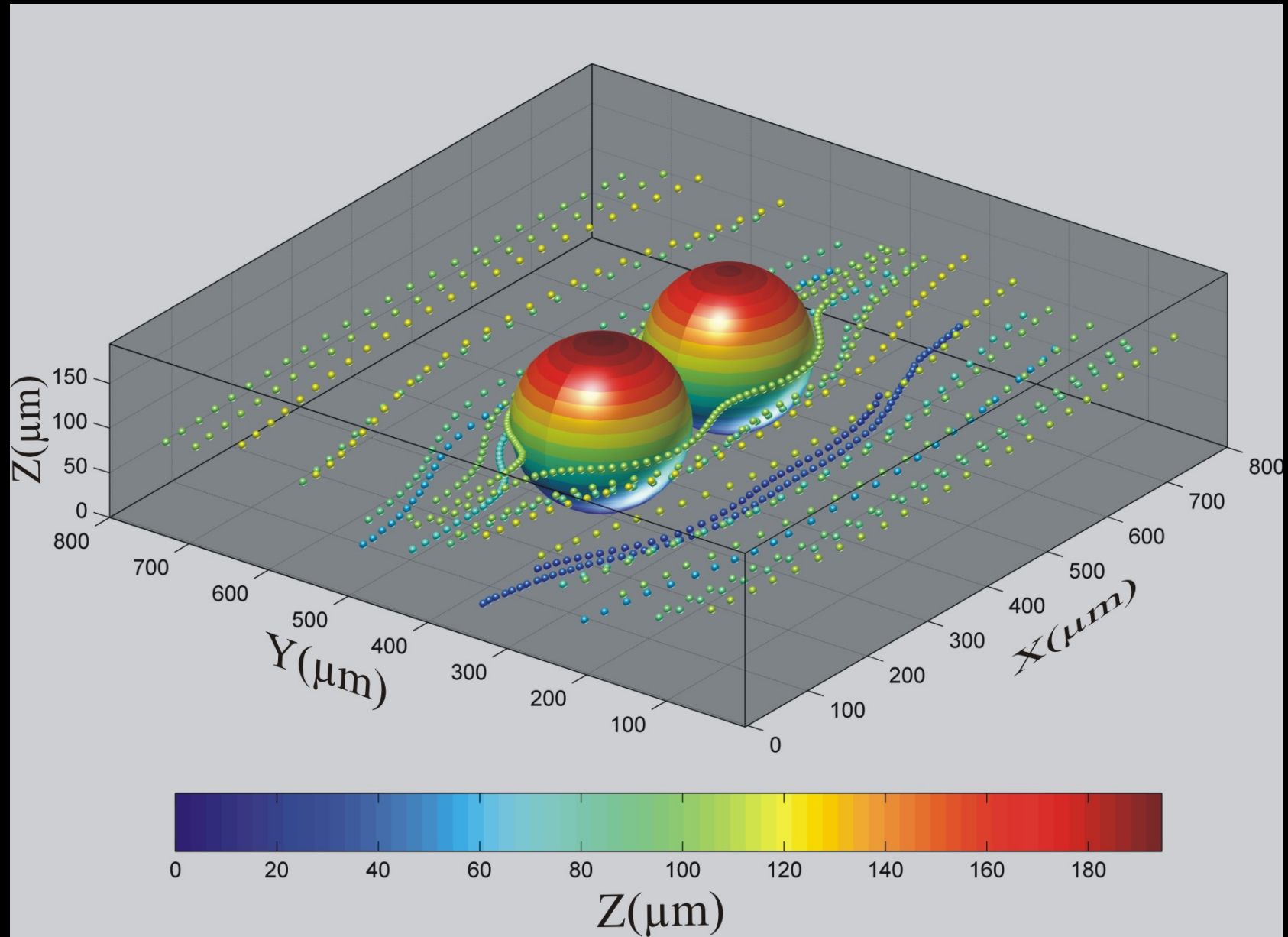
- Microfluidic Studies



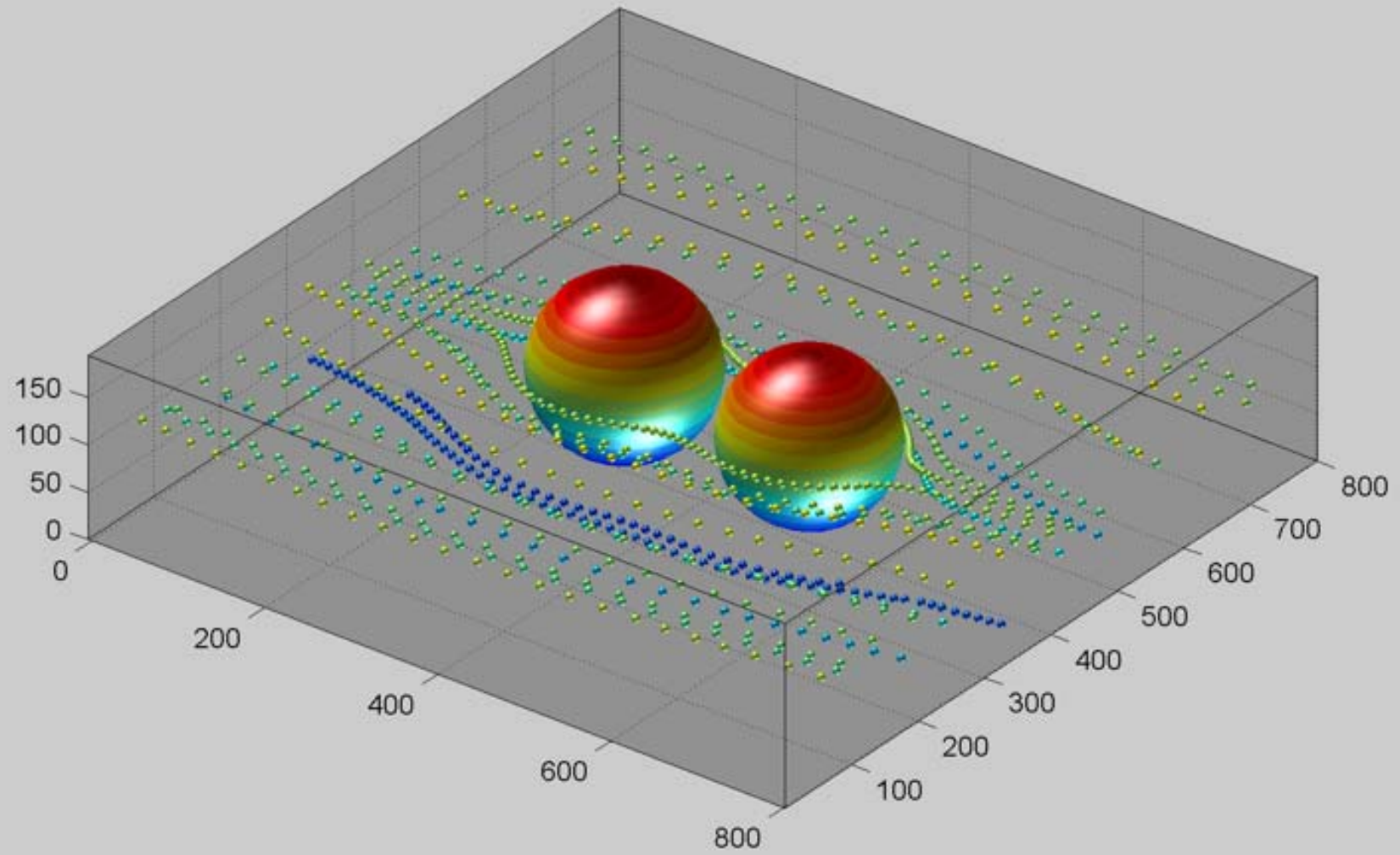
Velocity field rendered from the reconstruction of the 60 holograms.

# APPLICATIONS

- Microfluidic studies

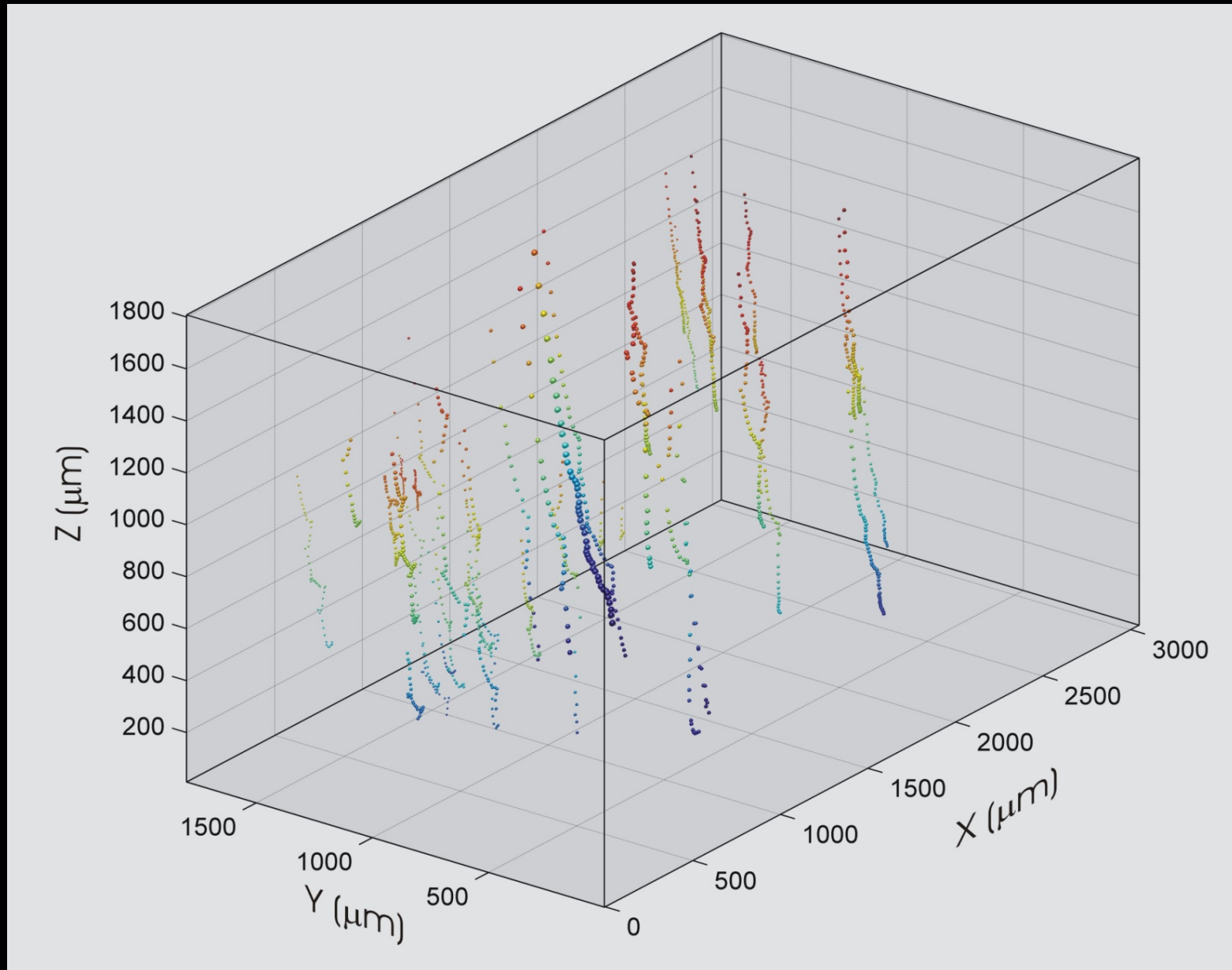






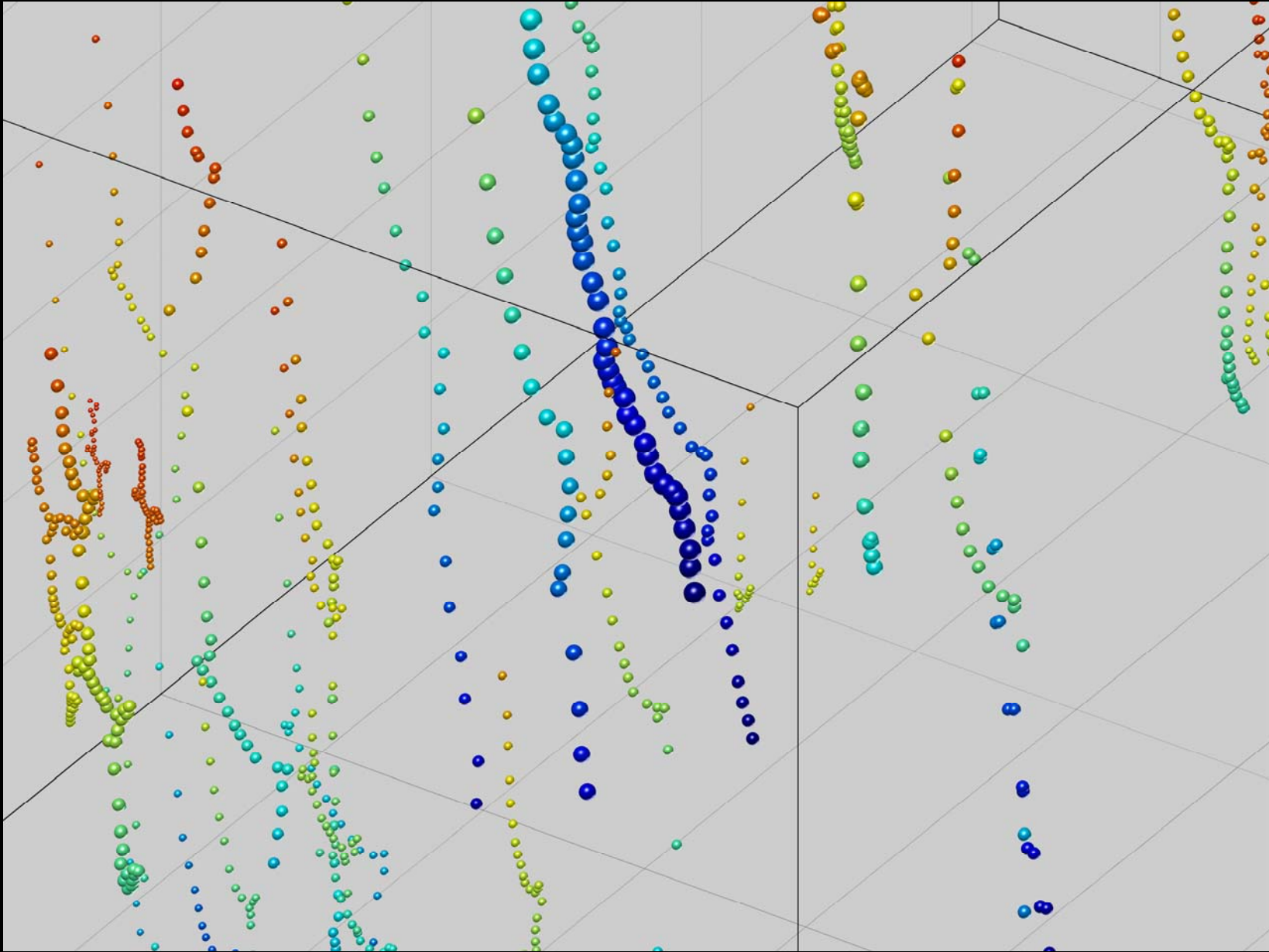
# APPLICATIONS

- Four dimensional bubble convection tracking



# APPLICATIONS

- Four dimensional bubble convection tracking



# APPLICATIONS

- Watching Triton® X-100 injection into water

**Triton® X-100 oil  
viscosity = 240 cP.  
270 times larger  
than water**



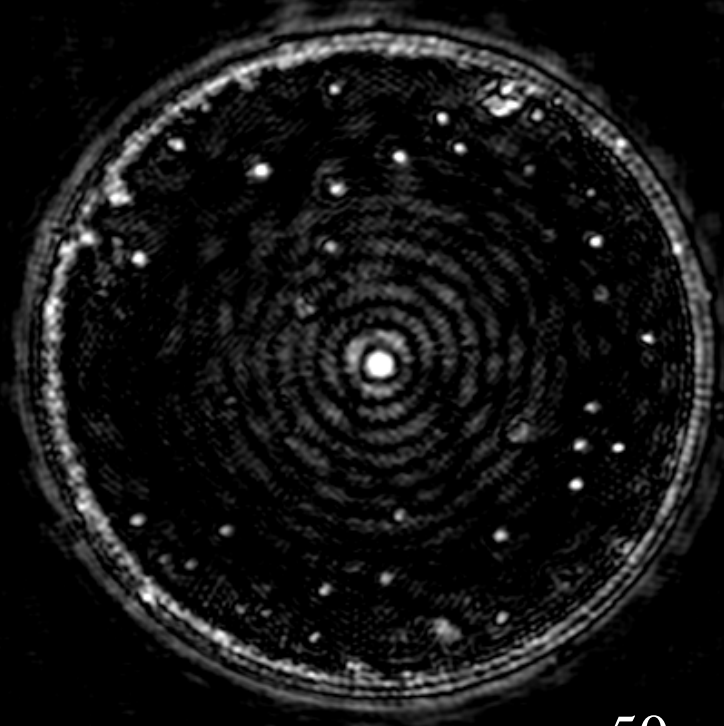
200µm



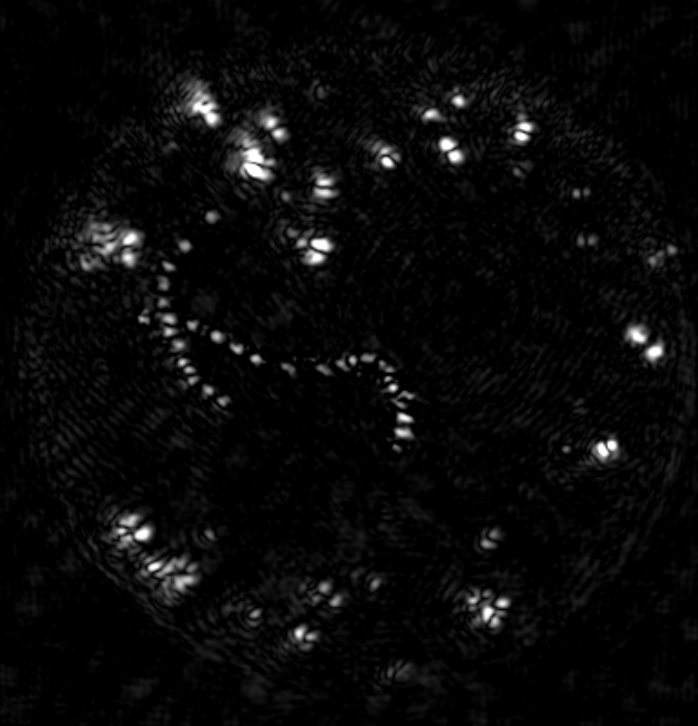
# APPLICATIONS

- Watching inside cells

A



B



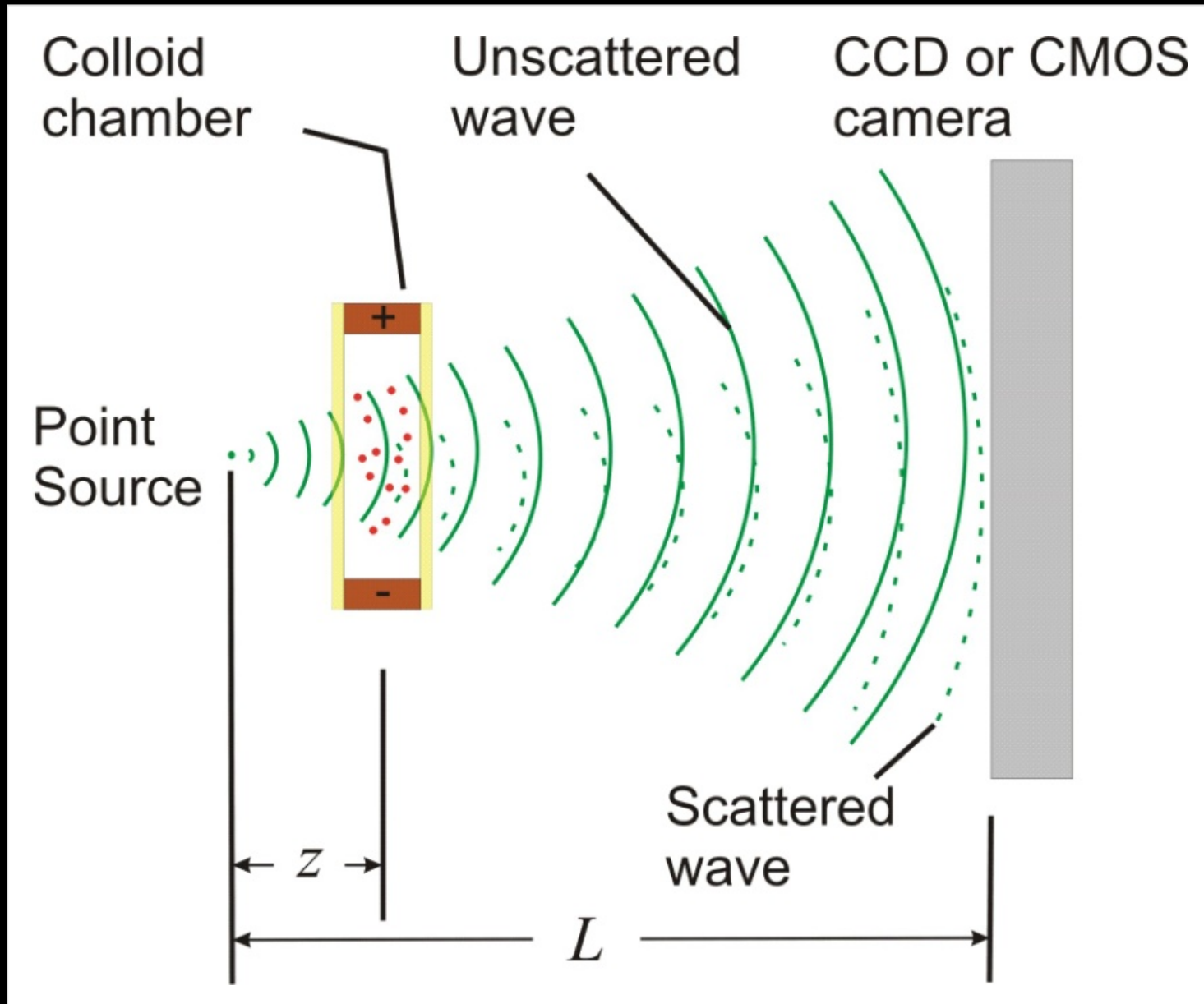
50  $\mu\text{m}$

Bacteria in a diatom (*coscinodiscus wailesii*). A: reconstruction from one hologram showing the siliceous outer shell with labiate processes (bright spots). B: reconstruction from a composed hologram. green laser, 0.5  $\mu\text{M}$  pinhole, pinhole to sample (diatom) 1 mm and numerical aperture of 0.22.



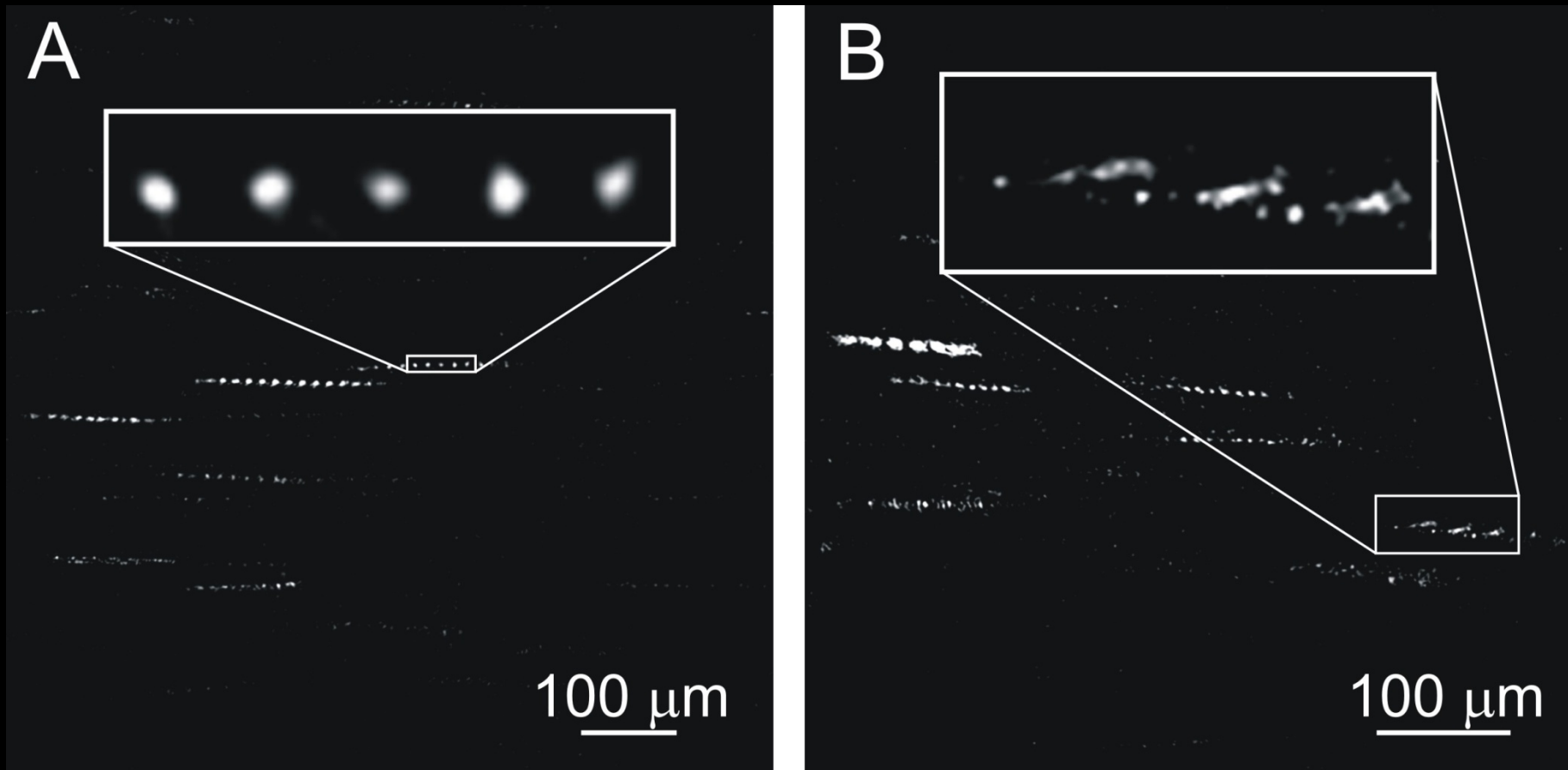
# APPLICATIONS

- Electrokinetic studies



# APPLICATIONS

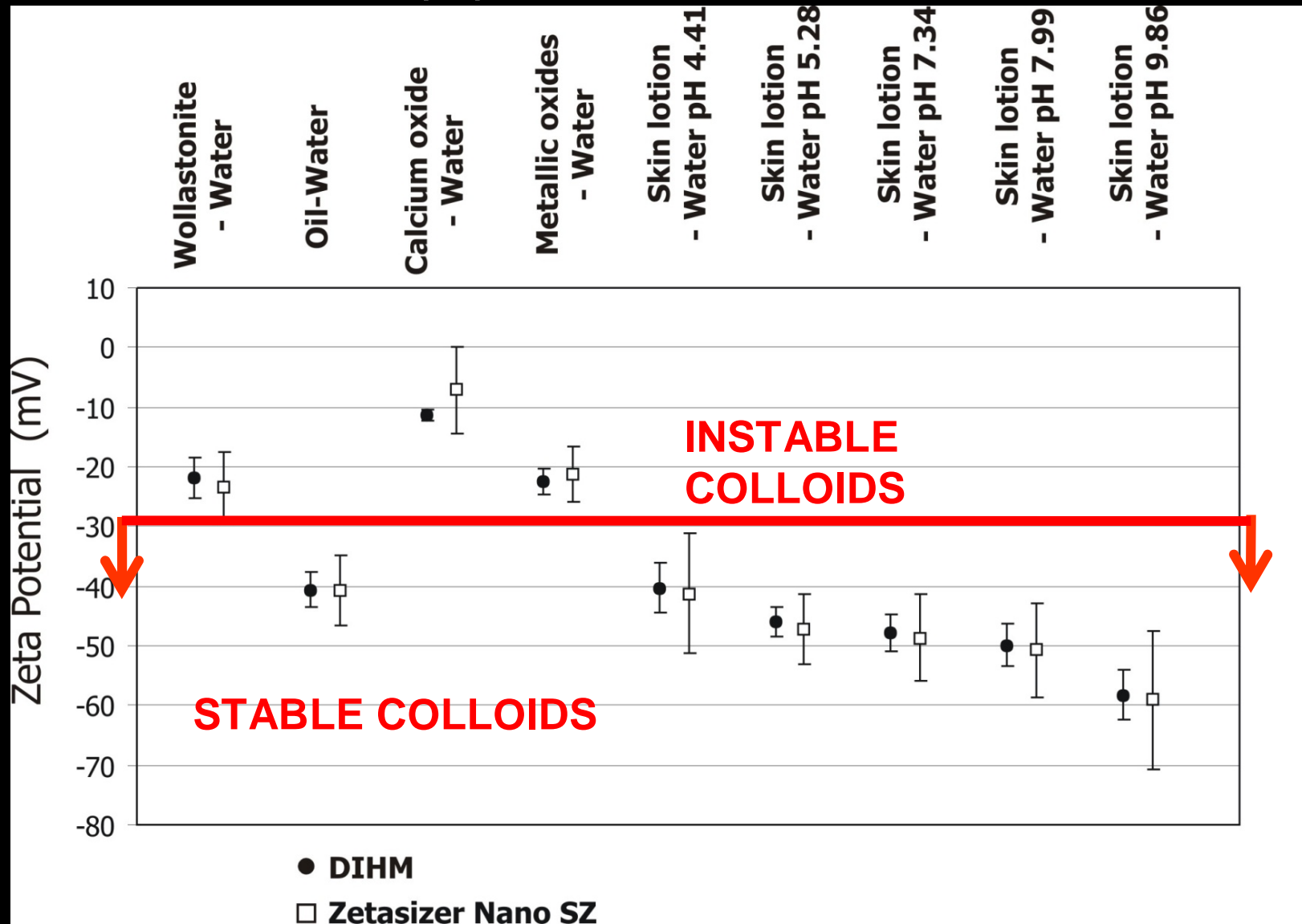
- Electrokinetic studies



Trajectories for particles of different shapes and sizes. Averaging over samples leads to an electrophoretic velocity of  $(10.1 \pm 0.3) \mu\text{m/s}$ . The individual tracking of particles allows one studying multi-dispersed colloids.

# APPLICATIONS

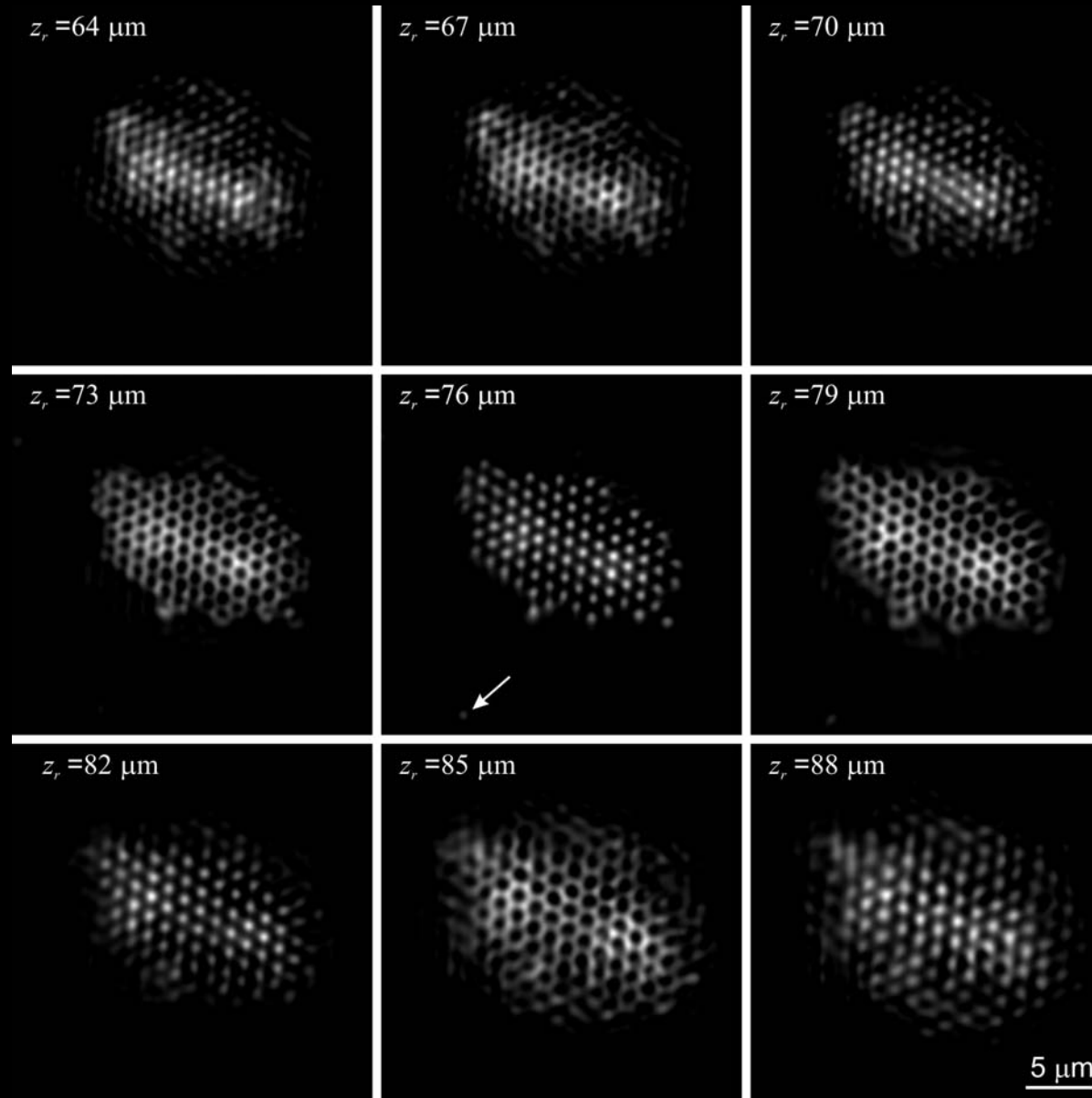
- Electrokinetic studies. Result comparison between DIHM and a commercial equipment





# APPLICATIONS

- Talbot effect of self-organised monolayers



For a two-dimensional array that follows:

$$P_x / P_y = \sqrt{n_1 / n_2}$$

the Talbot distance is

$$Z_T = \nu 2 a^2 / \lambda$$

J. Garcia-Sucerquia, et. al., "High resolution Talbot self-imaging applied to structural characterization of self-assembled monolayers of microspheres", *Appl. Opt.* **47**, 4723-4728 (2008)

# SUMMARY

- GABOR'S INVENTION OF HOLOGRAPHY HAS HELPED TO IMPROVE THE IMAGING CAPABILITIES IN DIFFERENT FIELDS.
- DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY (DIHM) IS THE SIMPLEST DIGITAL APPROACH TO GABOR'S IDEAS THAT ALLOWS NUMERICALLY RECOVERING THE COMPLEX WAVEFRONT OF SCATTERED WAVES; THE HARDWARE REQUIRED IS A LASER, A SPATIAL FILTER AND A CAMERA.
- THANKS TO THE LARGE DEPTH OF FIELD OF DIHM ONE STRIKING APPLICATION OF THIS MICROSCOPY TECHNIQUE IS THE VISUALIZATION OF MICROFLUIDICS.

# ACKNOWLEDGMENTS



M.Sc Diego A. Hincapie  
Grupo de cerámicos  
y vítreos



Cesar Restrepo  
Pedro Araque  
Diana Alvarez  
Dr. Herley Casanova

M.Sc Student  
M.Sc Student  
B.Sc Chemistry



Prof. Hans Jurgen Kreuzer  
Prof. Manfred Jericho  
Eng. Stefan Jericho  
Peter Klages

Ph.D Student