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2223-Presentations

Winter College on Optics in Imaging Science

31 January - 11 February, 2011

Digital Holographic Microscopy Workshop

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Universidad Nacional de Colombia Sede Medellín
A.A. 3840
Medellín
Colombia

Digital Holographic Microscopy Workshop (Digital In-line Holographic Microscopy)

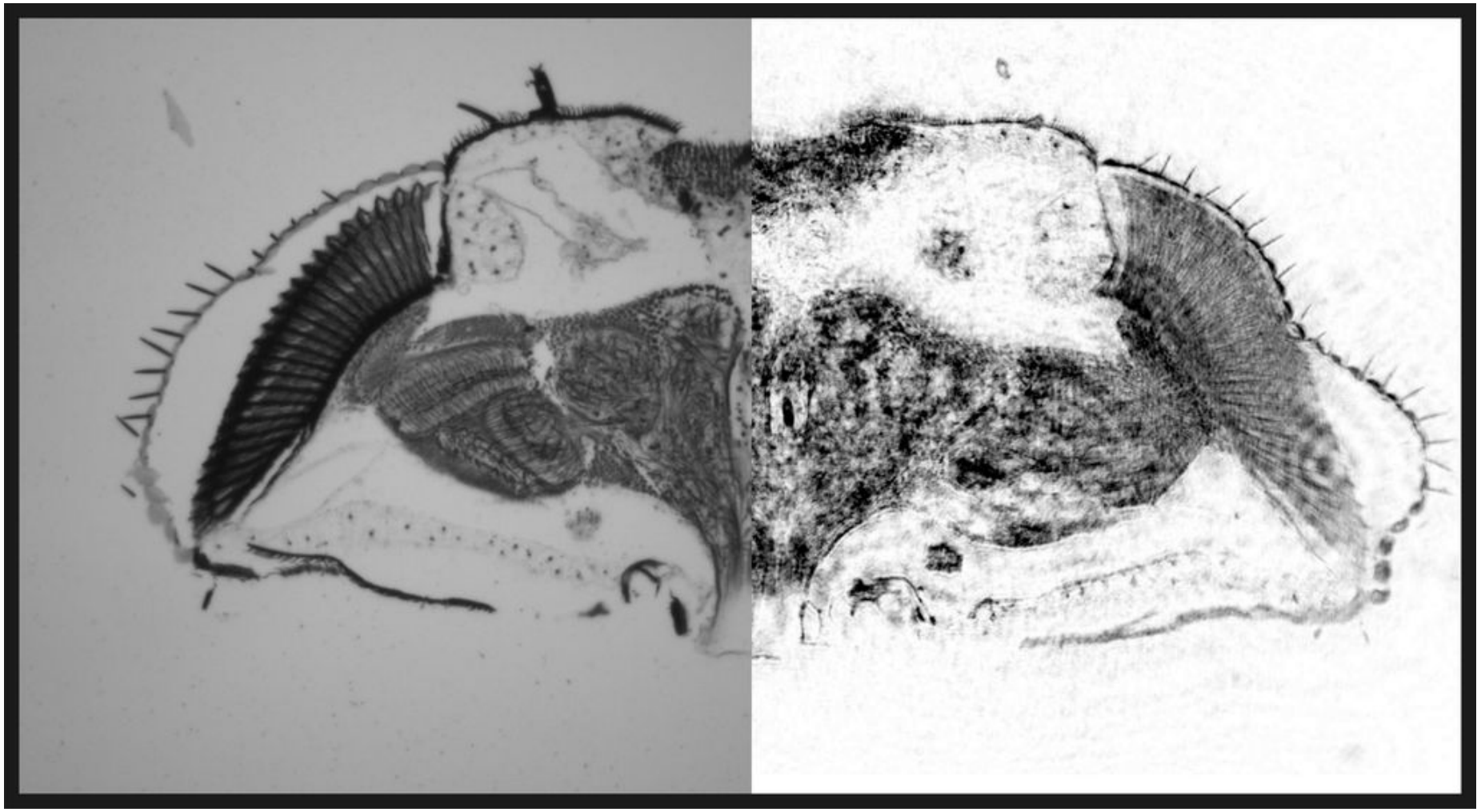
Jorge Garcia-Sucerquia

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

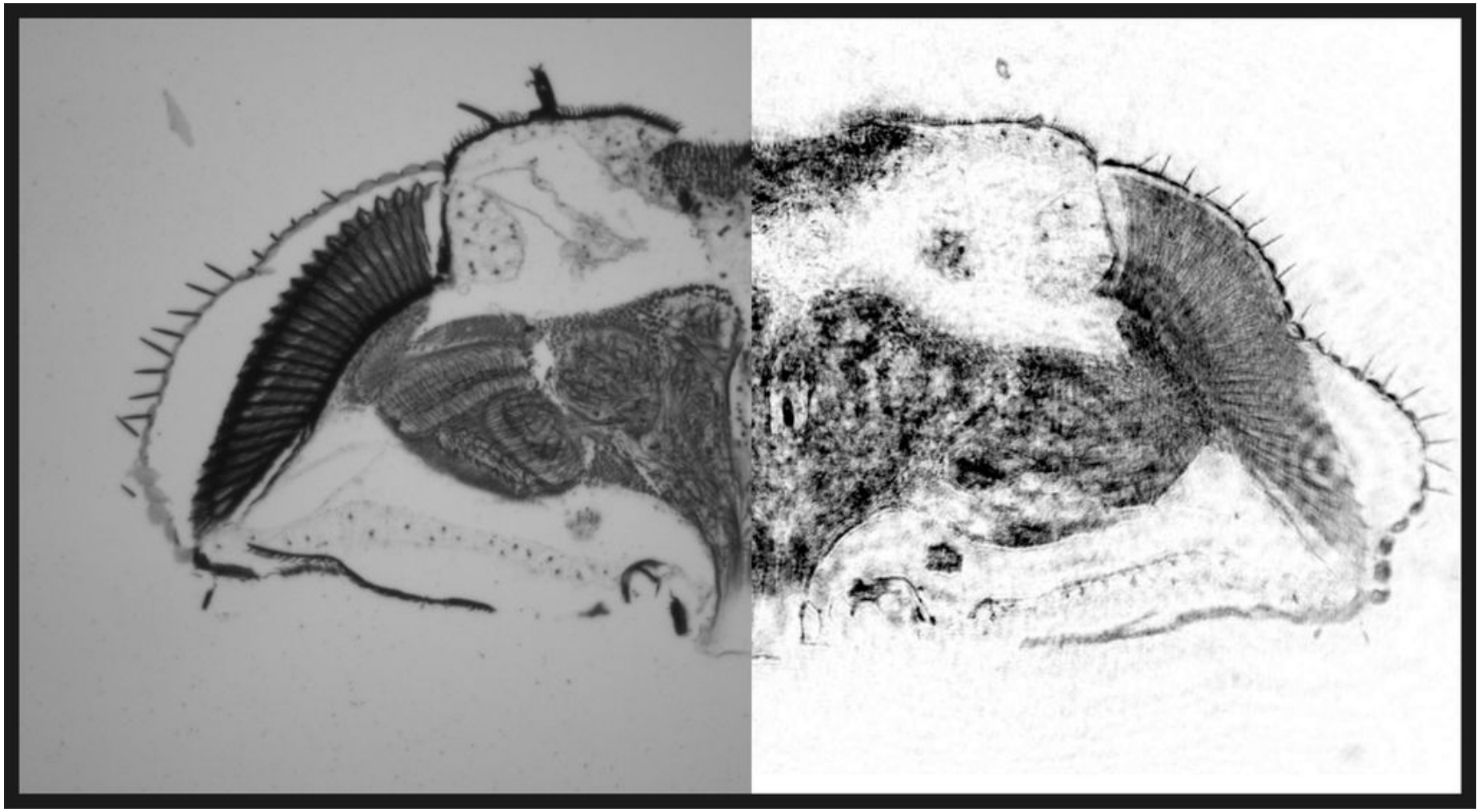
The Abdus Salam
International Centre for Theoretical Physics

Winter College on Optics in Imaging Science, Trieste 2011

Why should you do digital in-line holographic microscopy?

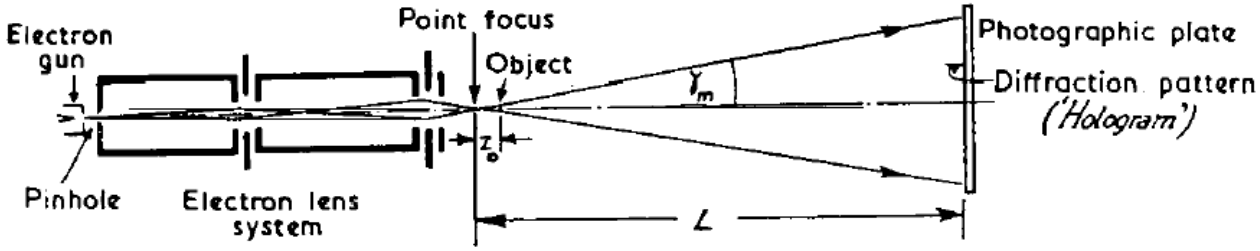


Why should you not do digital in-line holographic microscopy?

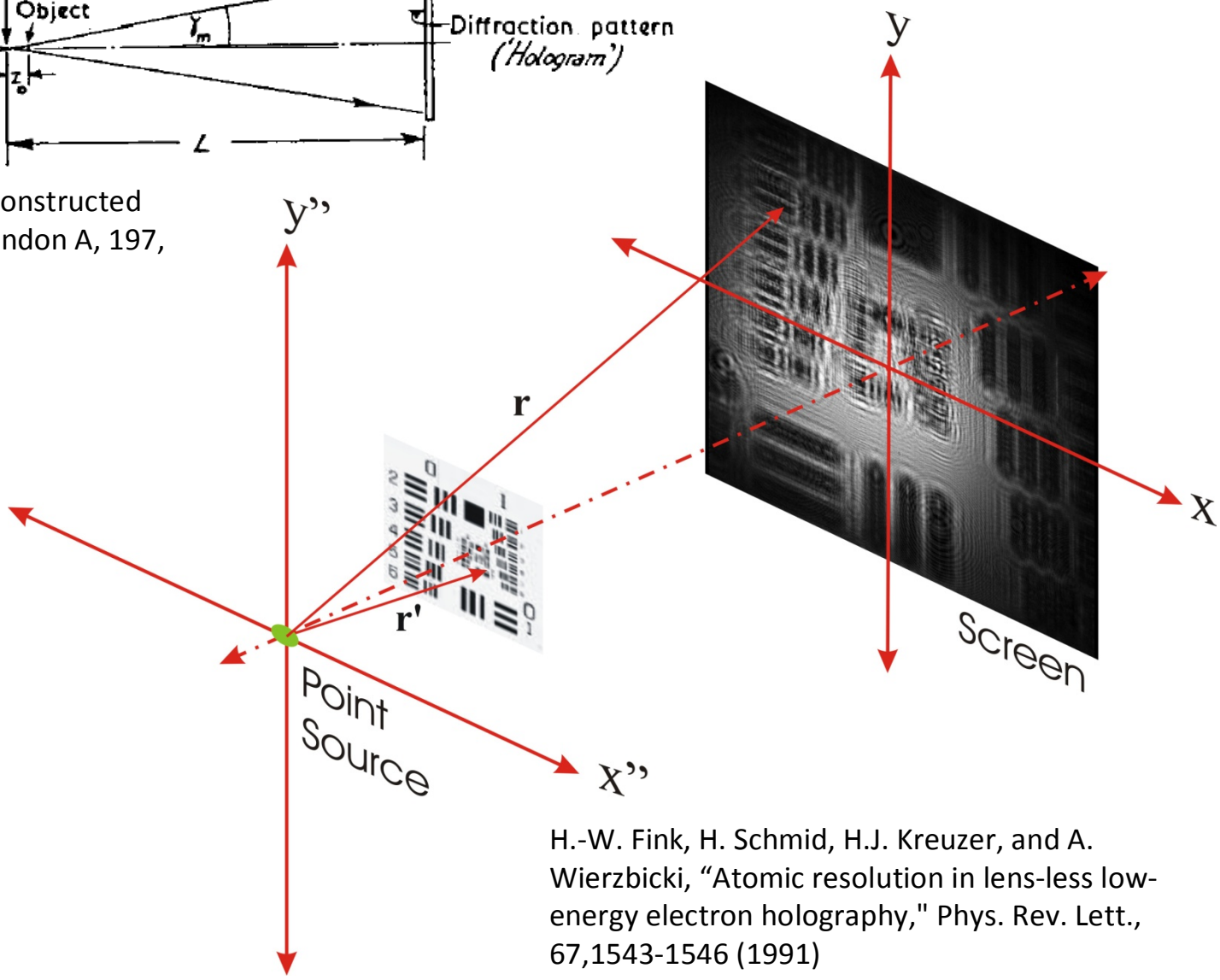


DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Recording**



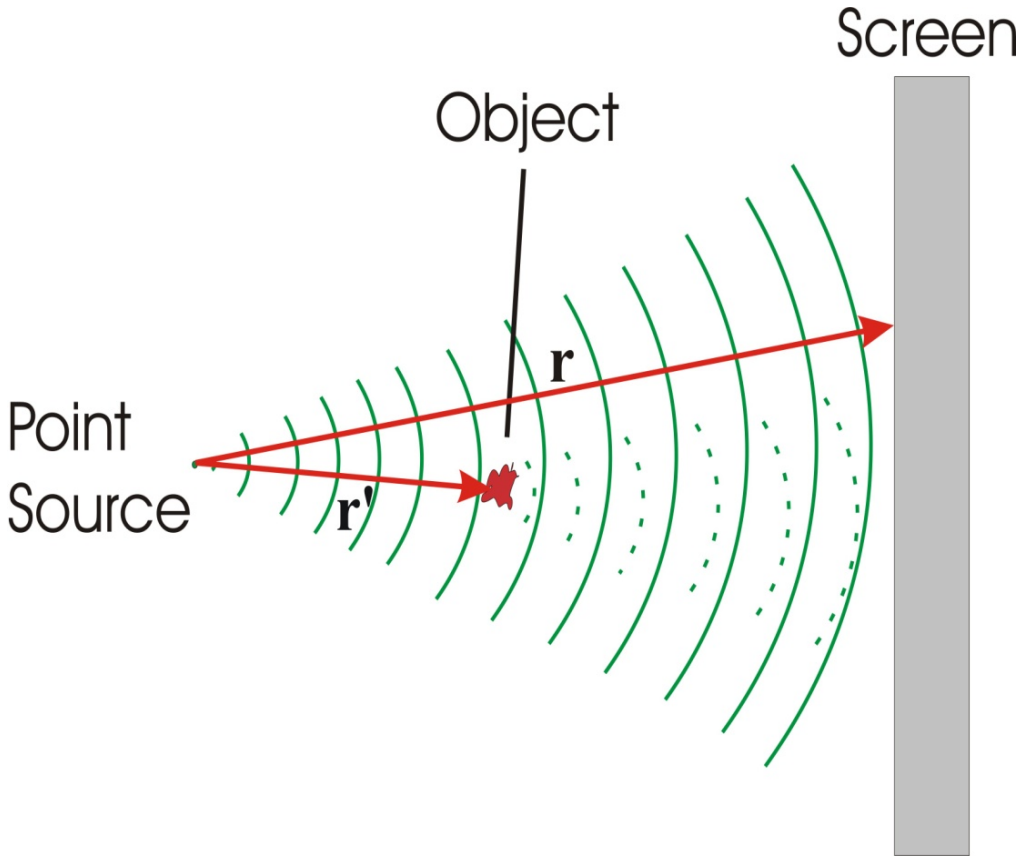
D. Gabor, "Microscopy by reconstructed wave-fronts," Proc. R. Soc. London A, 197, 454, (1949)



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**



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

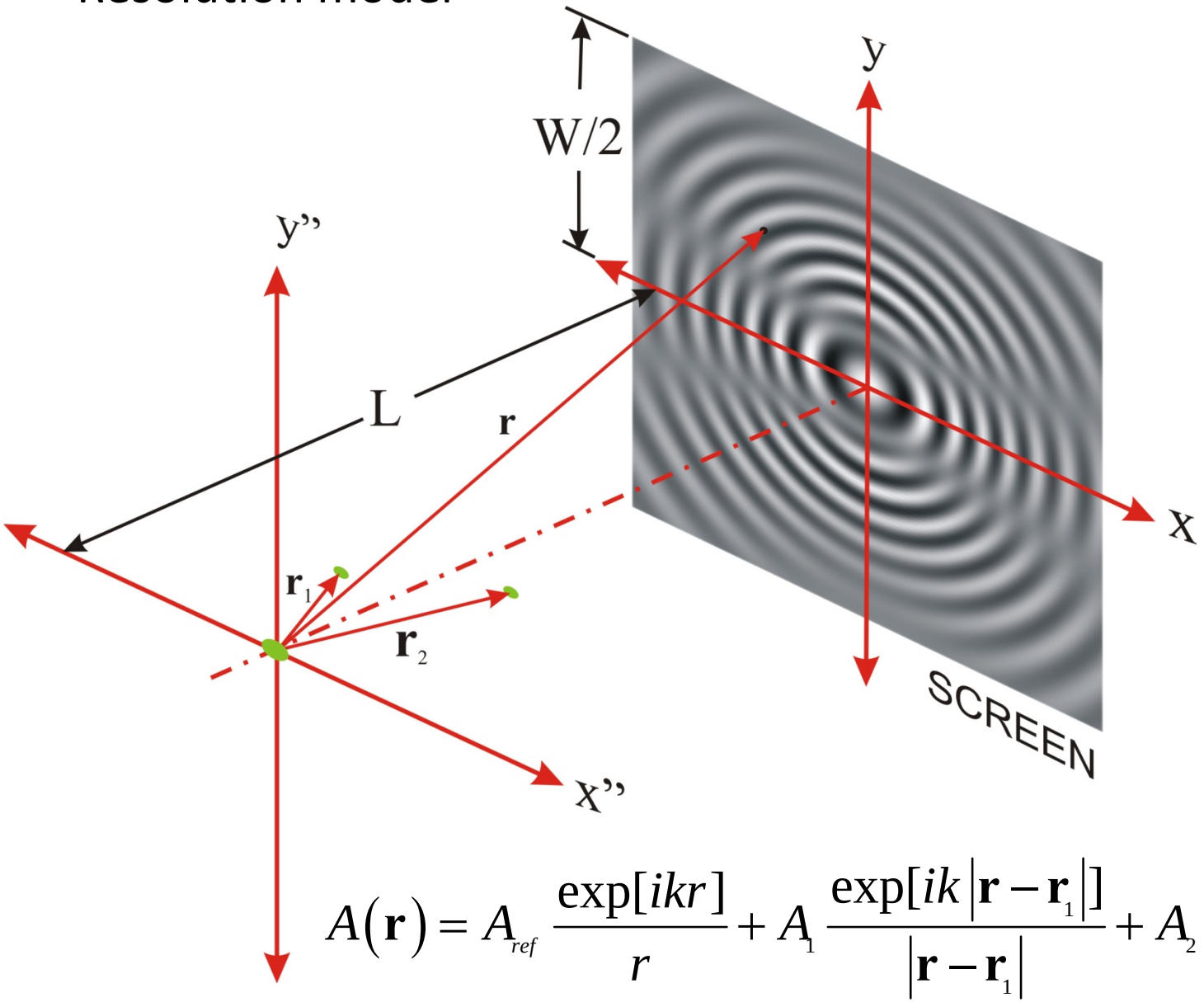
Prepared hologram

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

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

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Resolution model



$$A(\mathbf{r}) = A_{ref}\frac{\exp[ikr]}{r} + A_1\frac{\exp[ik|\mathbf{r}-\mathbf{r}_1|]}{|\mathbf{r}-\mathbf{r}_1|} + A_2\frac{\exp[ik|\mathbf{r}-\mathbf{r}_2|]}{|\mathbf{r}-\mathbf{r}_2|}$$

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Resolution model

$$I(\mathbf{r}) = \left| A_{ref} \frac{\exp[ikr]}{r} + A_1 \frac{\exp[ik|\mathbf{r}-\mathbf{r}_1|]}{|\mathbf{r}-\mathbf{r}_1|} + A_2 \frac{\exp[ik|\mathbf{r}-\mathbf{r}_2|]}{|\mathbf{r}-\mathbf{r}_2|} \right|^2$$

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

$$= \frac{A_1^2}{|\mathbf{r}-\mathbf{r}_1|^2} + \frac{A_2^2}{|\mathbf{r}-\mathbf{r}_2|^2} + 2\frac{A_1 A_2}{|\mathbf{r}_1-\mathbf{r}_2|^2} \cos\left[k\left(|\mathbf{r}-\mathbf{r}_1| - |\mathbf{r}-\mathbf{r}_2|\right)\right]$$

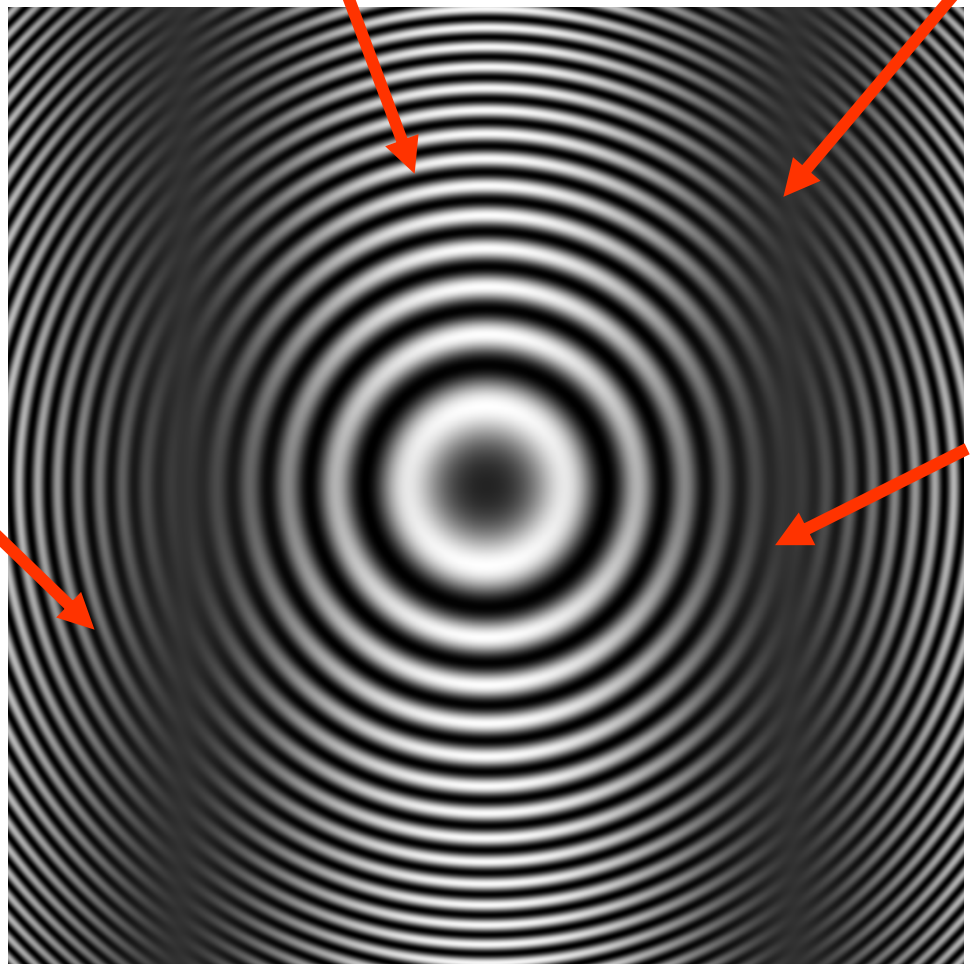
$$+ \left\{ 2\frac{A_{ref} A_1}{r|\mathbf{r}-\mathbf{r}_1|} \cos\left[k\left(r - |\mathbf{r}-\mathbf{r}_1|\right)\right] + 2\frac{A_{ref} A_2}{r|\mathbf{r}-\mathbf{r}_2|} \cos\left[k\left(r - |\mathbf{r}-\mathbf{r}_2|\right)\right] \right\}$$

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Lateral Resolution** $\left|\mathbf{r}_1\right| = \left|\mathbf{r}_2\right|$

$$\tilde{I}(\mathbf{r}) = 4\frac{A_0 A_1}{r\left|\mathbf{r}-\mathbf{r}_1\right|}\cos\left[k\left(2r-\left|\mathbf{r}-\mathbf{r}_1\right|-\left|\mathbf{r}-\mathbf{r}_2\right|\right)/2\right]\cos\left[k\left(\left|\mathbf{r}-\mathbf{r}_1\right|-\left|\mathbf{r}-\mathbf{r}_2\right|\right)/2\right]$$

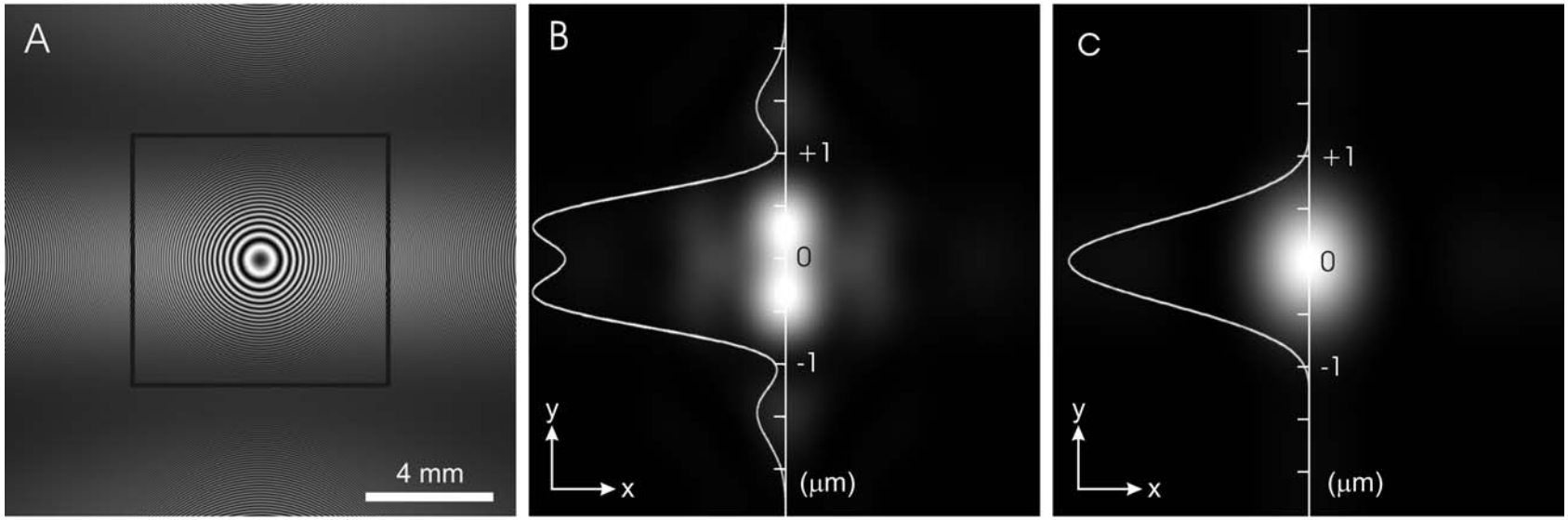
INFORMATION ABOUT POINT SOURCE TO PARTICLES DISTANCE



INFORMATION ABOUT THE PARTICLE SEPARATION

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 μm apart, taken with a blue laser λ=4730Å 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.

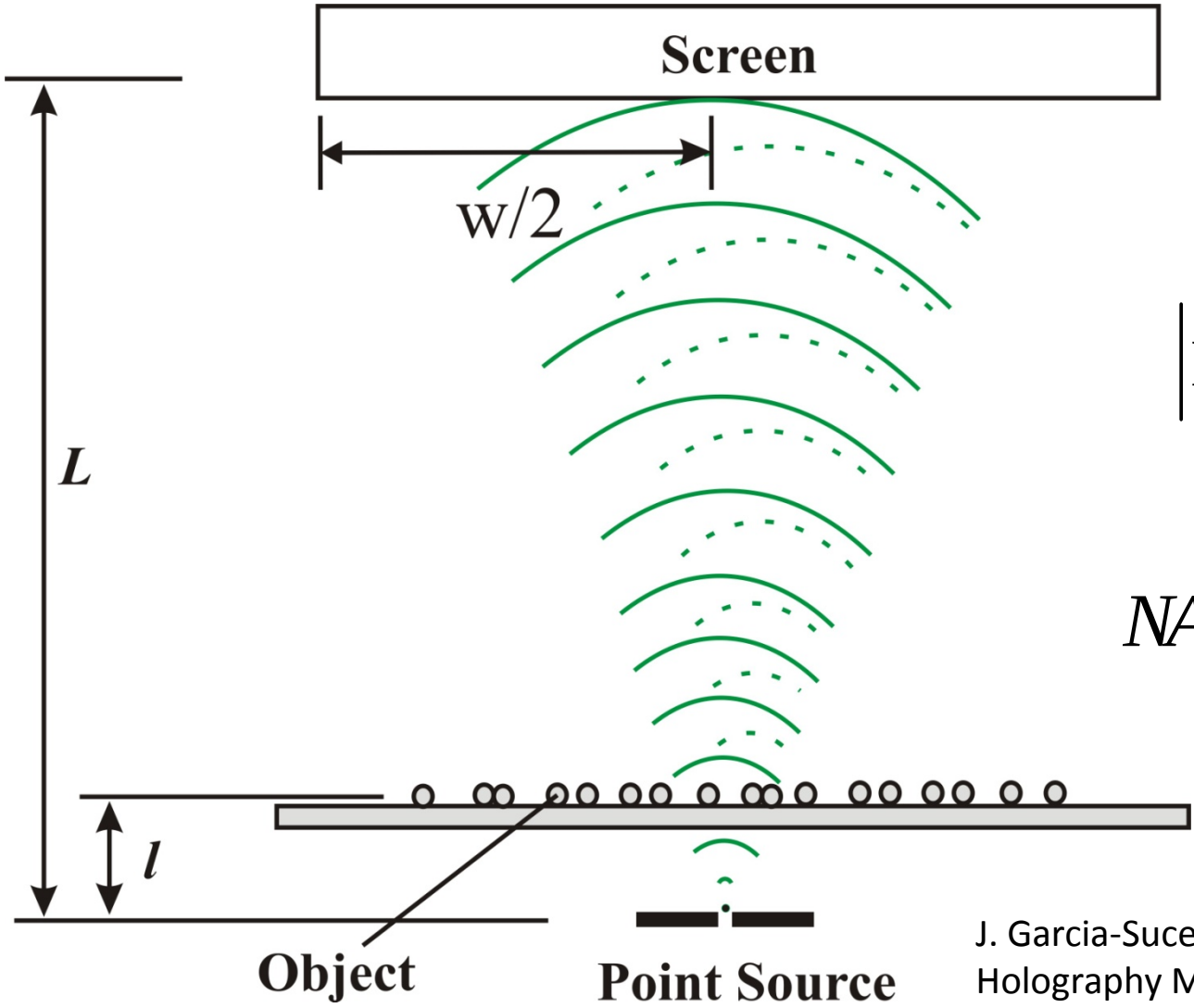
$$\left|\mathbf{r}_2 - \mathbf{r}_1\right| \geq \frac{\lambda}{2NA}$$

S.K. Jericho, et. al, "Submersible Digital In-line Holographic Microscope", *Rev Sci Instrum*, **77**, 043706-1, 043706-10, (2006)

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

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Lateral Resolution**



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

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

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

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

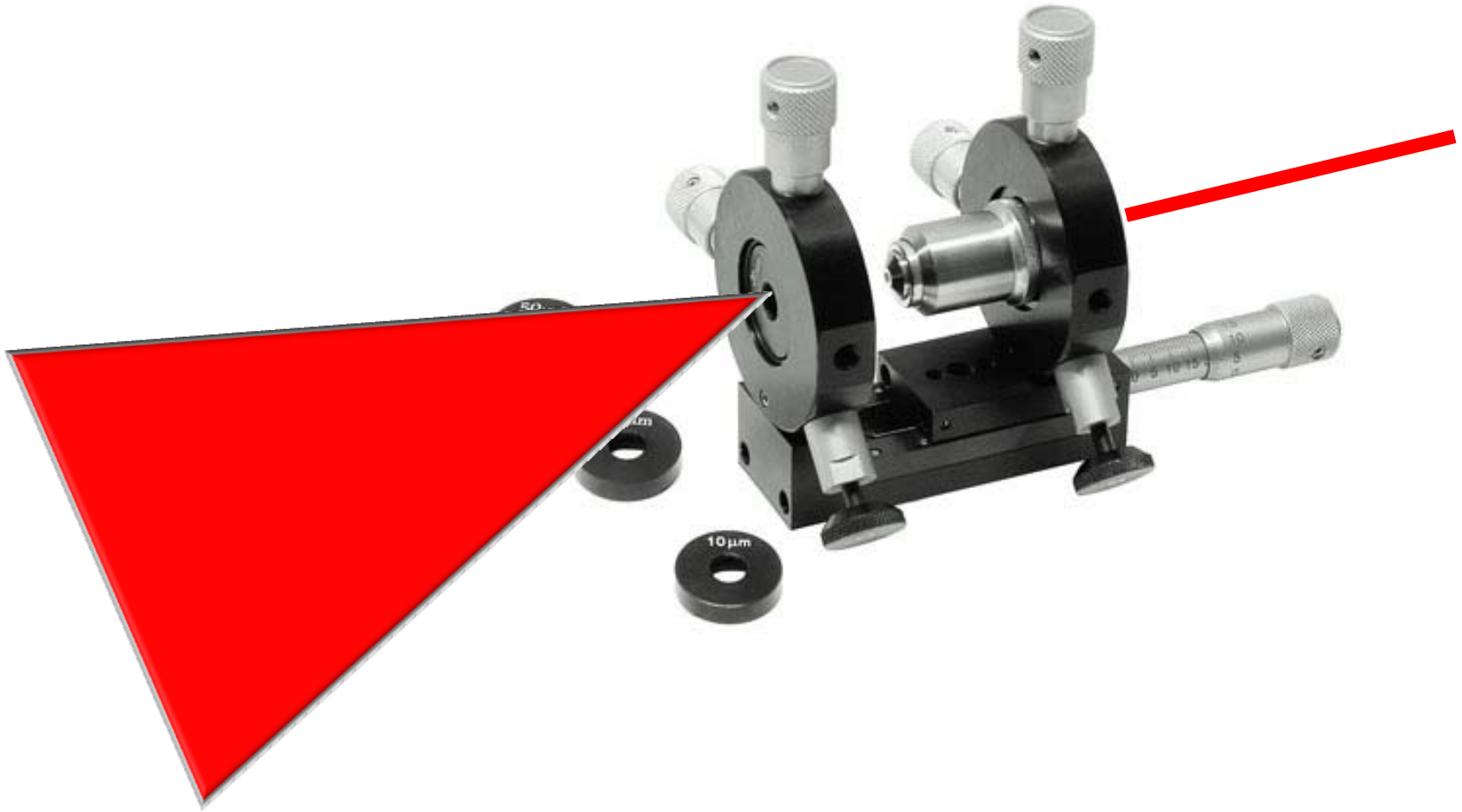
- **Practical recording of in-line holograms**

1. Point source
2. Recording device
   - ✓ Number of pixels
   - ✓ Pixel dimensions
   - ✓ Width-Height
   - ✓ Spectral responsivity
   - ✓ Acquisition frame rate
   - ✓ Communication protocol
   - ✓ ……
3. Point-source to recording device distance
   - ✓ Numerical aperture
4. Point-source to sample distance
   - ✓ High spatial frequency fringes

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Practical recording of in-line holograms**

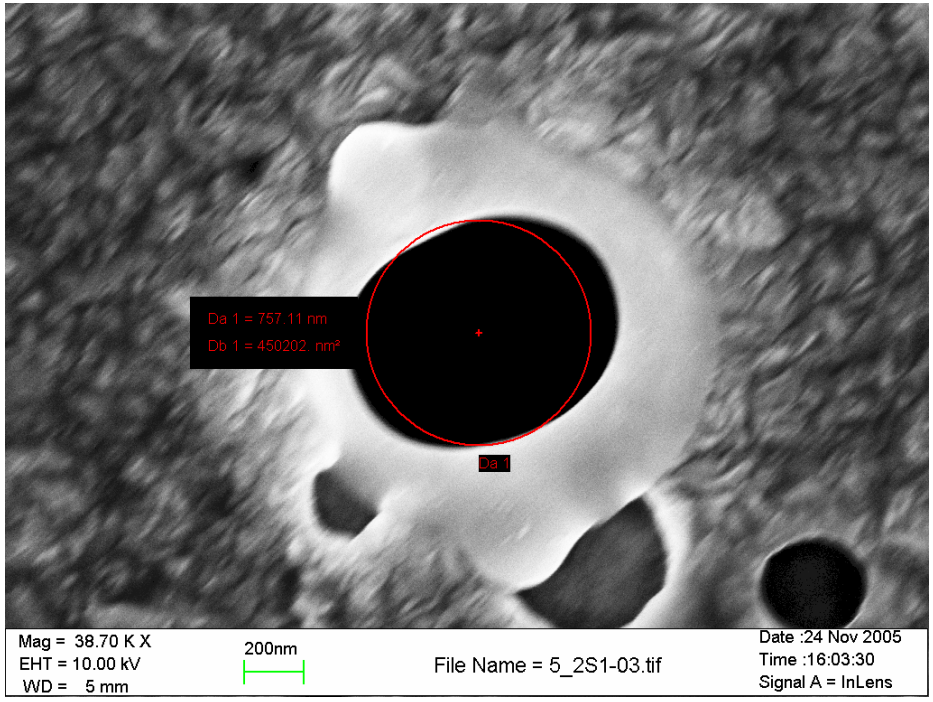
1. Point source

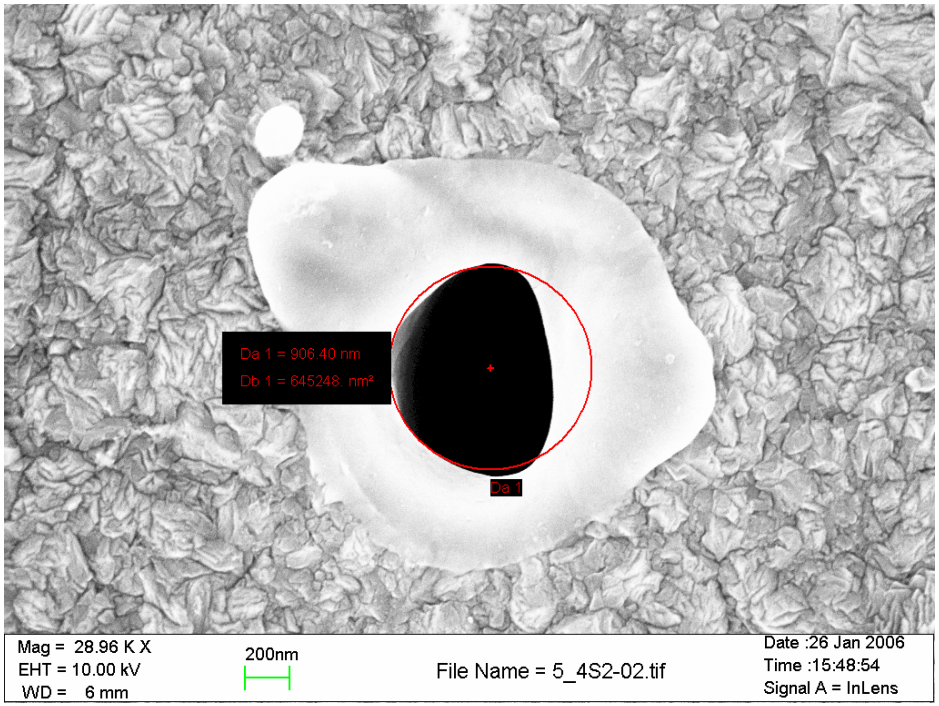


DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Practical recording of in-line holograms**

1. Point source

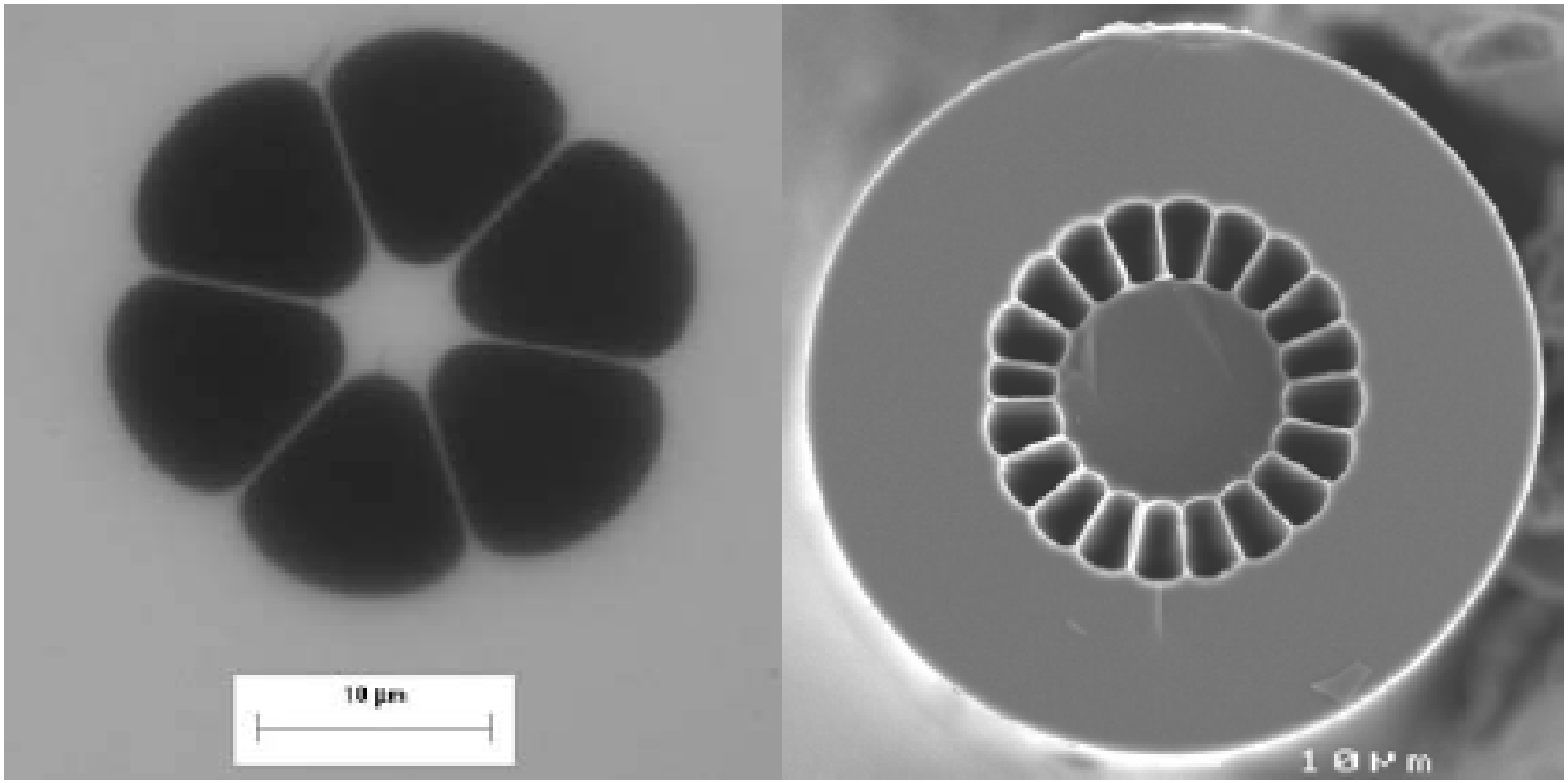




DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Practical recording of in-line holograms**

1. Point source

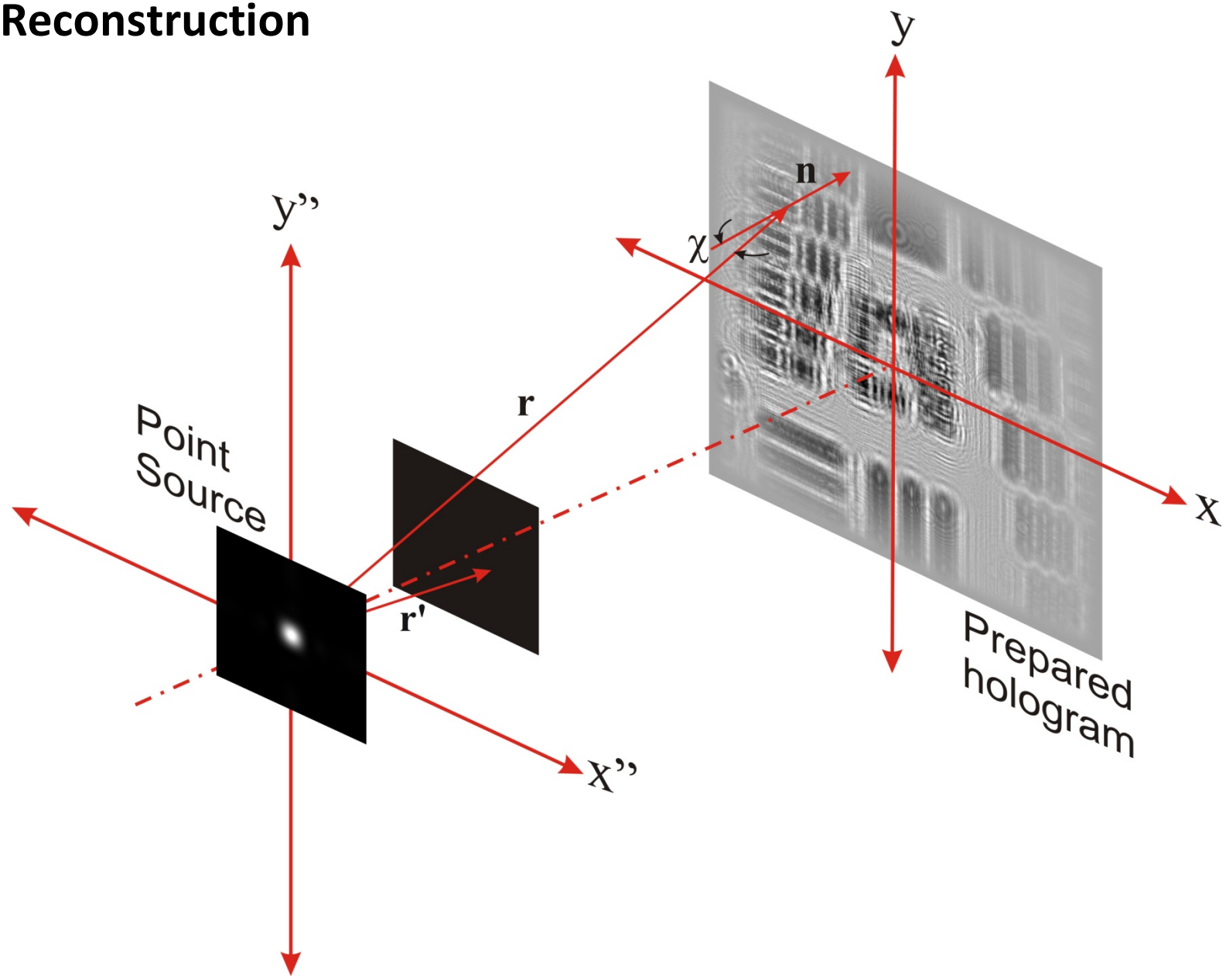


Highest NA 0.65
Wavelength =0.45 µm

⇨ Theoretical resolution = 0.36 µm

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Reconstruction**



DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Reconstruction**

$$A_{scat}(\mathbf{r}') = -\frac{i}{2\lambda}\iint_{Screen}\tilde{I}(\mathbf{r})A_{ref}(\mathbf{r})\frac{\exp\left[-ik\left|\mathbf{r}-\mathbf{r}'\right|\right]}{\left|\mathbf{r}-\mathbf{r}'\right|}\left(1+\cos\chi\right)dS_{\mathbf{r}}$$

Fresnel-Kirchhoff diffraction formula.

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

Fresnel-Kirchhoff in the Fraunhofer approximation.

$$A_{ref}(\mathbf{r}) = A_{ref}\frac{\exp\left[ikr\right]}{r}$$

Reference wave.

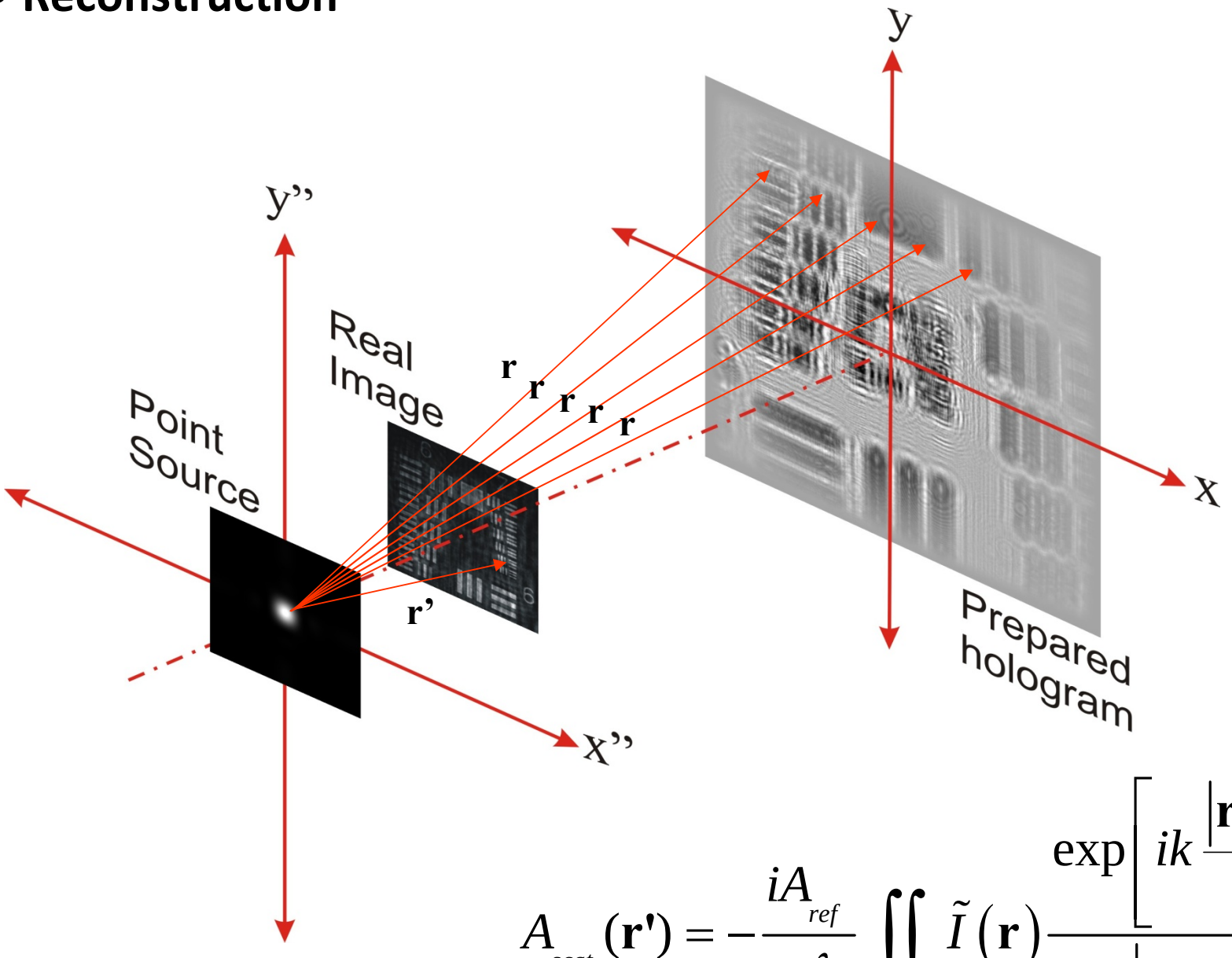
$$A_{scat}(\mathbf{r}') = -\frac{iA_{ref}}{r\lambda}\iint_{Screen}\tilde{I}(\mathbf{r})\frac{\exp\left[ik\frac{\left|\mathbf{r}\cdot\mathbf{r}'\right|}{r}\right]}{\left|\mathbf{r}-\mathbf{r}'\right|}dS_{\mathbf{r}}$$

Kirchhoff-Helmholtz transform.

J. J. Barton, “Photoelectron holography,” Phys. Rev. Lett. **61,** 1356–1359 (1988).

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

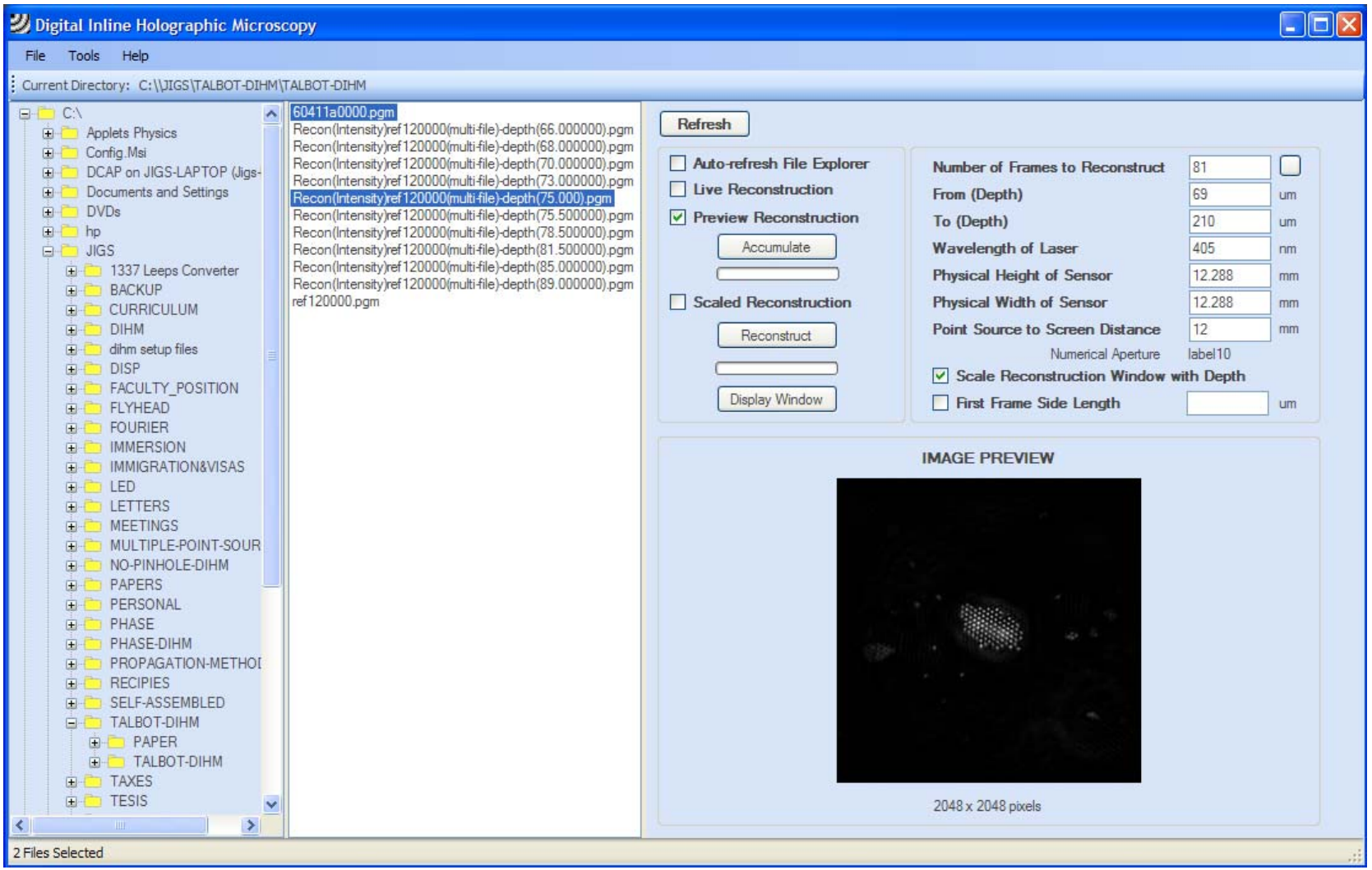
- **Reconstruction**



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

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Commercial reconstruction software



H.J. Kreuzer, US Patent 6,411,406 B1, June 25, 2002 “Holographic Microscope and Method of Hologram Reconstruction”

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DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- **Practical reconstruction of in-line holograms**

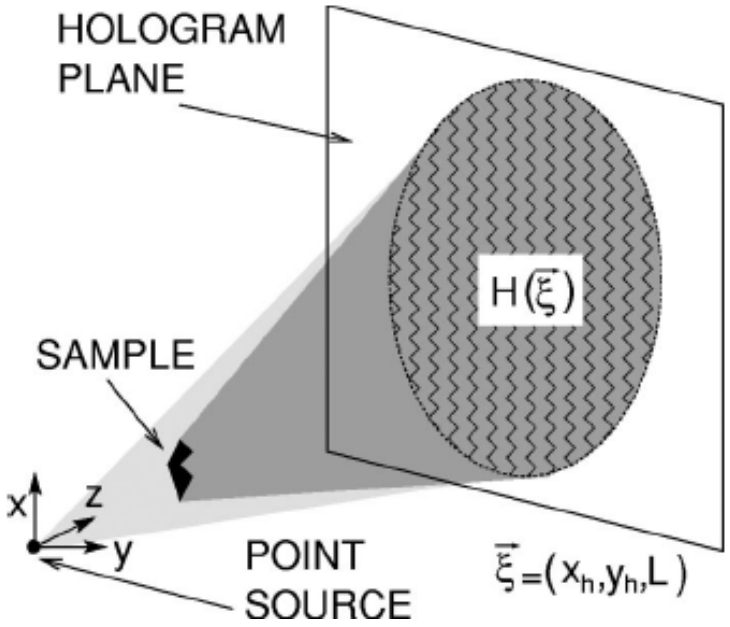
1. What is the need of using a prepared hologram?
2. Had Gabor the chance of removing the zeroth order of diffraction?
3. What is the role of the wavelength in the reconstruction process?
4. What parameters should be controlled to avoid aliasing?
5. Is the scaling trustable in DIMH?

DIGITAL IN-LINE HOLOGRAPHIC MICROSCOPY

- Playing with Gabor´s hologram

Gabor's hologram in a modern perspective

L. Repetto,[a] F. Pellistri,[b] E. Piano, and C. Pontiggia
Dipartimento di Fisica dell'Università di Genova, Via Dodecaneso, 33-16146 Genova, Italy



$$U(\mathbf{P}) = \int P_{\xi}(X - x_h, Y - y_y) H(x_h, y_y)\, dx_h\, dy_h,$$

$$\xi = \frac{L}{z_0}(L - z_0),$$

$$P_{\xi}(x_h, y_h) = e^{ik\sqrt{x_h^2 + y_h^2 + \xi^2}}.$$

L. Repetto, F. Pellistri, E. Piano, C. Pontiggia, "Gabor's Hologram in a Modern Perspective", AJP, Vol. **72**, 964- 967 (2004)