Revista Bistua
ISSN 01204211
Universidad de Pamplona,
Pamplona-Colombia

Digital In-Line Holographic Microscopy of Static and Dynamic Colloidal Systems

D. C. Alvarez-Palacio[a], H.J. Kreuzer[b] and J. Garcia-Sucerquia[c*],

[a]Instituto de Química, Universidad de Antioquia, A.A.1226, Medellín – Colombia [b]Department of Physics and Atmospheric Science, Dalhousie University, Halifax, NS B3H 3J5 Canada, [c]Escuela de Física, Universidad Nacional de Colombia Sede Medellín.A.A. 3840, Medellín–Colombia, jigarcia@unal.edu.co

RESUMEN

Se presenta la aplicación de la Microscopia Holográfica Digital en Línea (MHDL) a la observación de sistemas coloidales estáticos y dinámicos de microesferas. MHDL se ha perfeccionado al punto resolución lateral sub-micrométrica con cientos de micrómetros de profundidad de campo es obtenible con radiación electromagnética visible; se muestra que la resolución lateral de MHDL es suficiente para resolver monocapas de sistemas coloidales auto-ensamblados construidos a partir de esferas de poliestireno con dimensiones sub-micrométricas. La resolución temporal de MHDL es del orden de 8 cuadros/s de 2048 x 2048 pixeles, lo que representa una mejora de 32 veces la resolución temporal de la microscopia confocal. Esta característica es aplicada a la visualización de procesos de nucleación y migración de frentes de secado en sistemas coloidales dinámicos.

Palabras Clave: Microscopia Holográfica Digital en Línea; sistemas coloidales; microscopia

ABSTRACT

We present the application of Digital In-line Holographic Microscopy (DIHM) to image dynamic and static colloidal systems of microspheres. DIHM has been perfected up to the point that sub-micrometer lateral resolution with hundred micrometers depth of field is achieved with visible light; it is shown that the lateral resolution of DIHM is enough to resolve self-assembled colloidal monolayers built-up from polystyrene spheres with sub-micron diameter. The temporal resolution of DIHM is of the order of 8 frames/s at 2048 x 2048 pixels, which represents an overall improvement of 32 times the time resolution of confocal scanning microscopy. This feature is applied to the visualization of nucleation process and dewetting fronts migrations in dynamic colloidal systems.

Keywords: Digital In-line Holographic Microscopy; colloidal systems; microscopy.

1. INTRODUCTION

Because of the technological and scientific interest in colloidal systems, different types of microscopy have been applied for their imaging to gain insight into their structure and dynamics[1]. For the study of colloidal systems that are formed by particles with sub-micron dimension, the application of optical microscopy is unfortunately limited to two-dimensional observation, due to the limited depth-of-field when sub-micron lateral resolution is required.

In this paper we use Digital In-line Holographic Microscopy (DIHM) as an alternative tool to image colloidal systems. DIHM has been perfected up to the point that sub-micron lateral resolution is customarily achieved[2,3]. In addition, with DIHM we can recover the three-dimensional structure of the self-assembled layers from a two-dimensional hologram without the need of any mechanical refocusing.

In DIHM, a highly coherent optical source (laser) of wavelength λ is focused onto a pinhole, of a diameter of order λ, to generate highly coherent spherical waves that illuminate a sample placed at a distance z from the pinhole. The weak scattered wave by the sample interferes with the strong unscattered reference on the surface of a CMOS or CCD camera, and the recorded intensity, called in-line hologram, is transferred to a PC for its processing (see Figure 1).

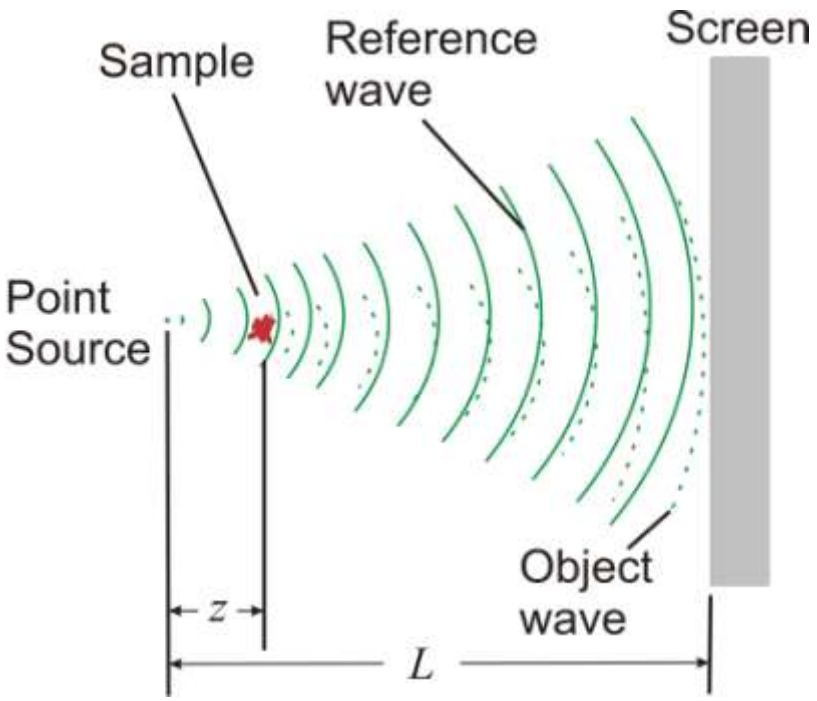

Figure 1. Schematic setup Digital In-line Holographic Microscopy

The scattered wave, which carries the information of the sample, is retrieved from the in-line hologram through the numerical diffraction of the conjugate unscattered reference wave when it illuminates the recorded intensity. Since in DIHM the reference wave is spherical, this process is mathematically described by the Kirchhoff-Helmholtz transform[4]

$$K(\mathbf{r}) = \int_{screen} d^2\xi \, \tilde{I}(\xi) \exp[ik\mathbf{r}\cdot\xi/|\xi|] . \quad (1)$$

In equation (2) the integration extends over the surface of the screen with coordinates $\xi=(X,Y,L)$ with L the distance from the pinhole to the center of the screen; $k = 2\pi/\lambda$ is the propagation number; and $\tilde{I}(\xi)$ is the contrast in-line hologram obtained by subtracting the images with and without the sample present. $K(\mathbf{r})$ is a complex quantity that can be calculated on a number of planes at various distances z_r from the point source (pinhole) in order to recover the three-dimensional information of the sample, from a single two-dimensional in-line hologram. This fact constitutes the main advantage of DIHM over optical microscopy, since it allows one to obtain information in three-dimensions without the need of any mechanical refocusing at sub-micron resolution, as it has been shown elsehere[5].

2. DIGITAL IN-LINE MICROSCOPY OF COLLOIDAL SYSTEMS

To validate DIHM as a tool to study colloidal systems we have deposit monodispersed suspensions of polystyrene beads (density of 1.05 gcm^{-3}) in deionised water (1% wt) over the prepared surface of a glass-slide. The microscope was setup up with a violet laser (λ = 405 nm) illuminating a 0.5 µm diameter pinhole and a CMOS camera of 4 Megapixels and 12.3x12.3 mm^2 area. After the solvent is completely dried off, the beads form self-assembled structures that exhibit hexagonal packing as seen in Figure 2 for polystyrene microspheres of 800 nm diameter, Obviously DIHM can easily resolve colloidal systems with particles of sub-micron dimension.

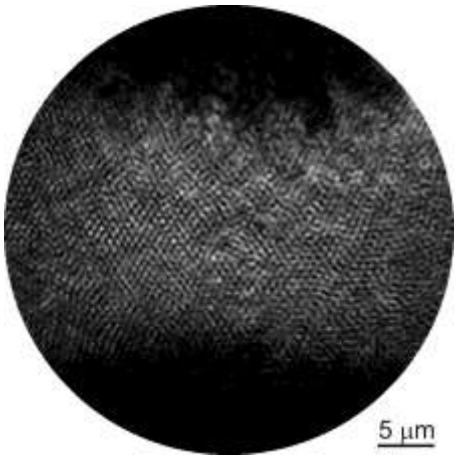

Figure 2. Self-assembled structure of polystyrene beads 800 nm diameter.

Note that some regions of the sel-assembled structure are fuzzy (or out of focus in the reconstruction), particularly close to the edges of the sample. This is due to differences in height at the sub-micron level of the sample positioning or to non-homogeneities of the glass-slide or the self-assembled structure itself as one can show by redoing the reconstruction at slightly different heights. It turns out that this can be used as an additional tool in the analysis of the layers.

The two-dimensional colloidal structures exhibit perfect hexagonal packing such that the ratio of the pitches along the coordinates x and y $P_x / P_y = \sqrt{1/3}$. It is well known that two-dimensional structures that fulfill the condition $P_x / P_y = \sqrt{n_1 / n_2}$ (n_1 and n_2 integers) exhibit the Talbot effect[6] when they are illuminated with spatially coherent light. This is shown in Figure 3 with a series of reconstructions obtained from the same hologram at different depths, namely that in the encircled regions in each panel the hexagonal packing structure for the leftmost panel is made of dark spots meanwhile the same structure is made of bright spot for the middle and right panels. Indeed, the theory of the Talbot effect predicts that two images separated by Talbot distance along the propagation axis will look alike and that there exist reversed contrast images separated by half this distance. The Talbot distance is given by $Z_T = \nu 2a^2 / \lambda$ [6] with ν an integer. This equation can obviously be used to measure the distances in colloidal structures in particular its pitch.

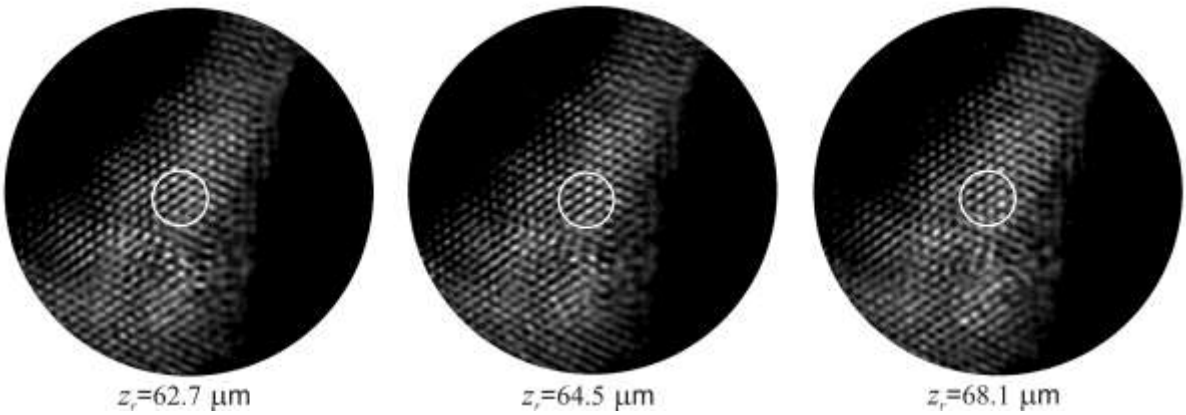

Figure 3. Talbot effect of self-assembled structure of polystyrene beads 800 nm diameter.

We note that the reversed contrast images (left and middle panels) are separated by 1.8 μm and that there are perfect self-images 3.6 μm apart. We therefore calculate that the Talbot distance is 3.6 μm and, with a wavelength of 405 nm, we find that the pitch of the colloid is about of 850 nm, in good agreement with the expected value for dense packing of polystyrene beads with a diameter of about 800 nm.

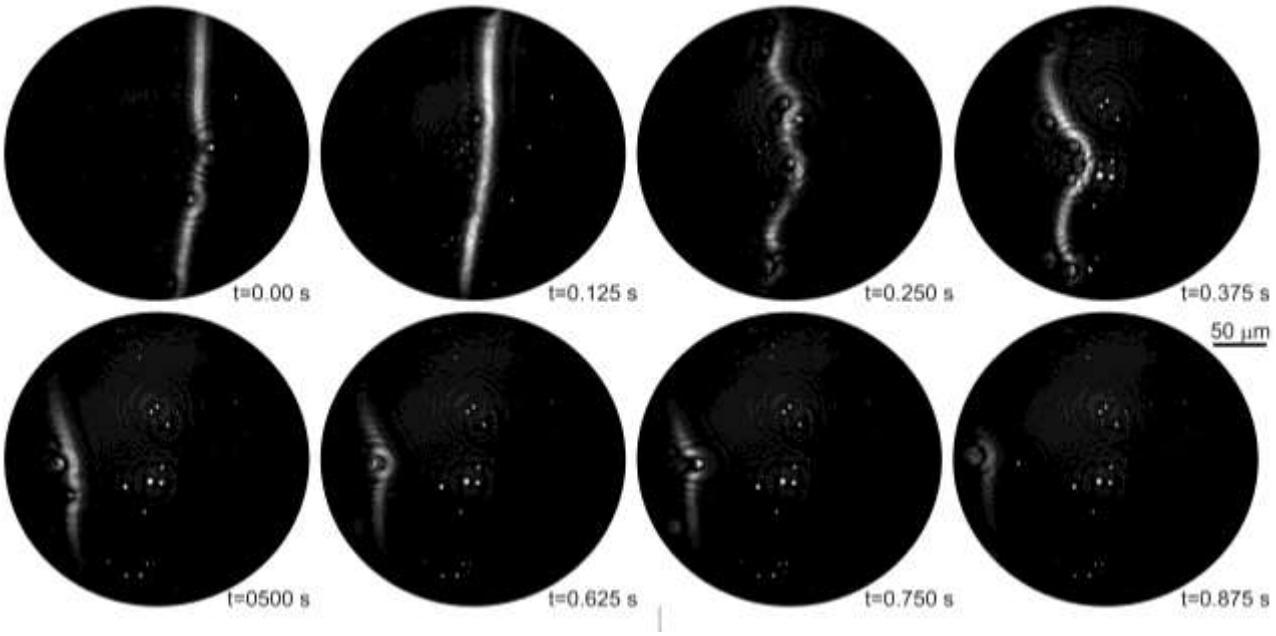

Figure 4. Reconstructions of temporal observation of dewetting wavefront on colloidal suspension with DIHM. Holograms were acquired at 405 nm and 0.46 numerical aperture.

As one example of the microscopy of dynamic colloids with DIHM, in Figure 4 are shown different frames of the temporal evolution of the deweeting front on a colloidal suspension. The temporal resolution of DIHM, 8 frames/s at 2048 x 2040 pixels, added to the sub-micrometer the lateral resolution allows for the study of the drying-off process on colloidal suspension of 1.10 μm polystyrene beads in distilled water. From Figure 4 one can learn that the speed of the dewetting front is highly perturbed by the presence of particles already glued to the substrate and or temperature gradients. While initial speed between t = 0.000 s and t =0.125 s is of the order of 300 μm/s where the surface is almost free of glued particles, the final speed between t = 0.750 s and t =0.875 s is of the order of 180 μm/s. This reduction of the speed of the drying-off front, might be due to the important perturbation introduced by the particles glued to the substrate and/or the existence of a temperature gradient that could be introduce by the spherical illumination of the DIHM microscope; one could think that center of the field of view must be hotter than the extremes. This latter affirmation must be closer investigated and the findings might give insight about thermal forces that driven the behaviour of dewetting process of paints, cosmetics products, and antibacterial clinic products, among others.

Figure 5 shows some early stages of the nucleation process of 1.5 μm diameter polystyrene beads in a colloidal suspension. According to those records, the initial stage of the nucleation process is the dimer formation; this entities travel around the colloidal suspension until they find a hydrophilic enough spot on the substrate surface. Initially one of beads of the dimer is glued to that spot; the loose sphere waves in three dimensions and interacts with others beads until finally both spheres are glued to the substrate. The encircled areas on the different panels of Figure 5 draw the attention of the reader to illustrate the described process in the above paragraphs. These analyses are possible because the power of DIHM on doing microscopy of dynamic colloids: i) its submicron lateral resolution allows for the observation of the dimers, trimers and larger structures as it has been shown and ii) its temporal resolution makes possible the tracking of the different elements of the dynamic colloid.

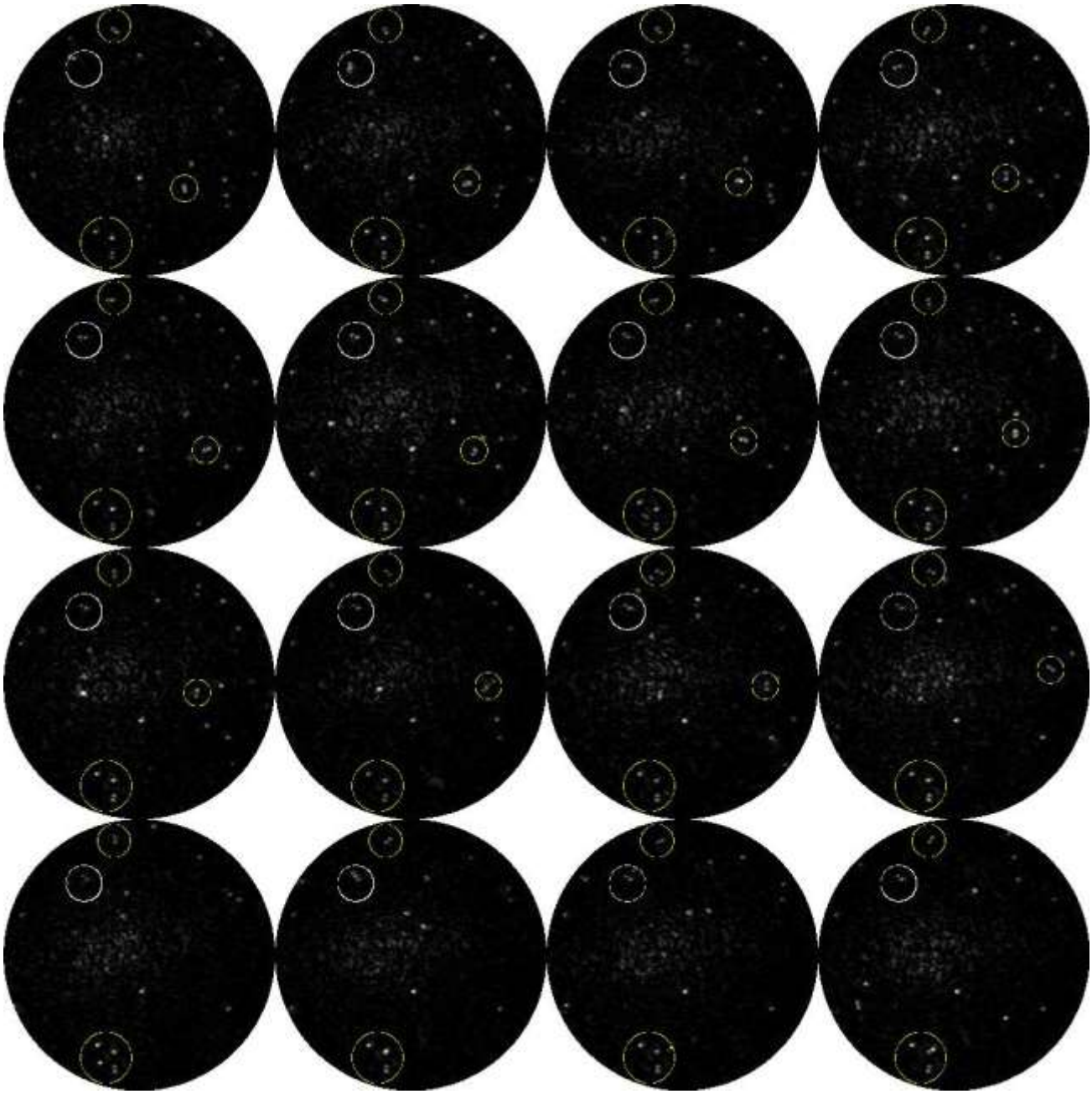

Figure 5. First stages of nucleation process of 1 μm diameter polystyrene beads. 200 holograms were recorded at 0.39 numerical aperture and 532 nm wavelength. The first frame is set at the upper left corner and the time sequence evolves along the respective rows, such that the lower right corner is the last frame of the observed window. Only some scattered frames have been shown; between consecutive frames there are 266 ms.

3. CONCLUSIONS

We have used Digital In-line Holographic Microscopy (DIHM) to image colloidal systems. In addition to being a tool with sub-micron lateral resolution, DIHM also contains three-dimensional information which can be retrieved from the same hologram by doing numerical reconstructions at different distances without the need of any mechanical refocusing. This feature can be used in combination with the Talbot imaging theory to determine the pitch of self-assembled

colloidal structures. The ability of tracking particles in four dimension of DIHM has been merged with its spatial resolution to follow the first stages of nucleation process as well as the migration of drying-off fronts. With this tool will be possible to gain new insight about forces and others physical parameters that driven the formation of monolayers, dewetting in colloidal suspension and a number of process in which the dynamic tracking of particles plays a key role.

REFERENCES

1. J.C. Crocker and D.G Grier, "Methods of Digital Video Microscopy for Colloidal Studies," J. Colloid Interf. Sci., **179**, 298-310 (1996)
2. J. Garcia-Sucerquia, W. Xu, M. H. Jericho, and H. J. Kreuzer, "Immersion digital in-line holographic microscopy," Opt. Lett. **31**, 1211-1213 (2006)
3. J. Garcia-Sucerquia, D. C. Alvarez-Palacio, M.H. Jericho, H.J. Kreuzer, "Comment on "Reconstruction algorithm for high-numerical aperture hologram with diffraction limited resolution"", Opt. Lett. **31**, 2845-2847 (2006).
4. H.J. Kreuzer, K. Nakamura, A. Wierzbicki, H.-W. Fink, H. Schmid, "Theory of the Point Source Electron Microscope," Ultramicroscopy **45**, 381-403 (1992).
5. W. Xu, M.H. Jericho, H.J. Kreuzer and I.A. Meinertzhagen, "Tracking particles in four dimensions with in-line holographic microscopy," Opt. Lett. **28**, 164-166 (2003).
6. J.M. Cowley and A.F. Moodie, "Fourier images: I-The point Source," Proc. Phys. Soc. B **70**,486-496 (1956); "Fourier images: II- The out-focus patterns," Proc. Phys. Soc. B **70**,497-504 (1956); "Fourier images: III- Finite sources," Proc. Phys. Soc. B **70**, 505-513 (1956)