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Dynamic 4-dimensional microscope system with automated background leveling

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Abstract

This paper describes recent advances in developing an automatic background leveling algorithm for a new, novel interference microscope system and presents images and data of live biological samples. The specially designed optical system enables instantaneous 4-dimensional video measurements of dynamic motions within and among live cells without the need for contrast agents. “Label-free” measurements of biological objects in reflection using harmless light levels are possible without the need for scanning and vibration isolation. This instrument utilizes a pixelated phase mask enabling simultaneous measurement of multiple interference patterns taking advantage of the polarization properties of light enabling phase image movies in real time at video rates to track dynamic motions and volumetric changes. Optical thickness data are derived from phase images. This data is processed with an automatic background leveling routine which separates the objects from the background by thresholding the calculated gradient magnitude of the optical thickness data. Low-order Zernike surfaces are fit to the unmasked background pixels and the resulting background shape is removed. This method effectively eliminates background shape for datasets containing both large and small objects. By applying this method to many sequential frames, it results in all the frames having the same mean background value across all frames which is essential for quantitatively monitoring time-dependent processes.

Keywords

phase imaging; interference microscopy; polarization interferometry; cellular imaging; cell dynamics; optical thickness measurement

1. INTRODUCTION

The ability to instantaneously measure live cells, and follow motions and processes over time, provides valuable information to researchers studying cellular dynamics, motility, and cell and tissue morphology. Quantitative phase imaging can measure structures from interference images analogous to those viewed with phase-contrast imaging and differential interference contrast imaging. Phase images can reveal features and quantitative data that are

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not available through conventional imaging. Phase imaging quantifies optical thickness variations due to small variations in refractive index relating to variations in density of different structures and materials within cells and tissues. Very small refractive index variations can manifest as large variations in optical thickness. Phase image data enable quantitative measurements that aren't possible with standard microscopy techniques. They can be directly combined and correlated with any other type of microscopic imaging such as fluorescence imaging. To measure optical thickness, harmless light levels are used, and samples do not need to be stained, labeled or marked. By taking short snapshots in rapid succession, the dimension of time opens up the ability to track motions of cells, see how cells interact with one another, and follow small motions within cells, tissues and structures. This type of imaging with quantitative analysis enables new types of studies involving cell tracking and process monitoring.

This paper presents an update on research in developing an automated background leveling technique used with a novel interference microscope. This instrument is designed for the measurement of living and moving biological samples in liquids, and slight piston changes and general background shape need to be removed such that the temporally varying optical thickness data reflects only the changes in the object being observed. The resultant processing routine will be presented as well as several different kinds of biological objects that have been processed with this routine.

2. BACKGROUND

Techniques developed for full-field phase-imaging interference microscopes have historically relied upon temporal phase-measurement methods, which obtain interferograms sequentially, and therefore require good vibrational damping, and static specimens so that high-quality data can be obtained. [1–3] These techniques have been used for biological measurements but have not been utilized as much as they could because most of them are sensitive to motion and vibration. [4–14] Most interferometric microscopes have utilized narrowband illumination with short coherence lengths of tens of microns. The reason for narrowband “low coherence” illumination (short temporal coherence length) is to reduce effects of reflections off of nearby surfaces and to help reduce effects of speckle in the imaging systems. Further improvement in imaging quality can be obtained by incorporating low spatial coherence extended sources. With biological samples, low spatial coherence illumination enables the samples to be isolated in space without getting spurious interference patterns from other surfaces (mainly the coverslip). This section briefly outlines the techniques used in this system. More details can be found in the references [15–17].

2.1 Dynamic interference microscope system

The interference microscope used for this work is based upon a Linnik configuration.[3] It is comprised of a Köhler-type illumination system utilizing a low coherence extended source, [18] and a simple imaging system as shown in Figure 1. An aperture stop enables controlling the size of the source in the entrance pupil of the microscope objectives, while a field stop enables easier alignment of the system.

The object and reference beams have orthogonal polarizations with the incoming illumination split into orthogonal polarizations before the microscope objectives using a polarization beamsplitter. The relative irradiances of the test and object beam are balanced for maximum contrast by changing the angle of the polarizer. A quarter-wave plate (QWP) before the camera combines the two polarized beams so that the two beams can interfere at the pixelated phase mask. For the measurements in this paper, samples in water or cell media are viewed in reflection through a cover slip. Sources with wavelengths of 660 nm and 785 nm were used with a 50X NA 0.8 or 20X NA 0.45 microscope objective. The imaging tube lens magnification is 1X with a flip-in 2.25X field of view (FOV) lens.

Figure 2 shows a photograph of the prototype system. The Linnik objective is seen below the microscope (blue and white box) with a 5-axis translation stage below for adjusting position and tip/tilt of the sample. The compact design of this system enables it to be used on a variety of stands so that it can be interfaced with different types of staging and cell handling systems.

2.1.1 Extended low coherence source—Standard practice in interference microscopes would be to use a temporally low coherence source like an LED. However, this reduces the temporal coherence length drastically and would require equal optical paths in both arms of the Linnik so that the temporal coherence function overlaps with the spatial coherence function. [19, 20] Essentially this means that there would also need to be an equivalent amount of glass and liquid in the reference arm as in the test arm in order to get equal optical path lengths.

To get around this limitation, it has been shown that by tailoring the temporal and spatial coherence, dispersive media can be unmatched in both arms and still have the positive effects of low coherence. [19, 20] This is done by designing the source so that the temporal coherence is long enough to enable path length differences generated by the dispersive media (the cover slip and liquid), while reducing the spatial coherence to localize the interference fringes in space so that spurious reflections and coherent noise are not present.

To reduce the spatial coherence to 25–35 μm , diode laser beams having temporal coherence lengths of 250–300 μm were focused onto a rotating diffuser and then coupled into an NA 0.2 multi-mode optical fiber with a 1000 μm core. The laser has sufficient temporal coherence over path length differences that take into account the cover glass, while the spatial size of the source given by the multi-mode optical fiber will limit the spatial coherence.

2.1.2 Obtaining high quality interference fringes—For a Linnik interference microscope objective, the reference objective and the reference mirror are moved together to match the path lengths in the reference and test arms to maximize fringe contrast. The reference mirror remains in a fixed position relative to the reference objective. When imaging an object, focus is first adjusted on the sample. The entire reference arm (reference microscope objective and reference mirror) is moved to maximize fringe contrast. As long as there is the same coverslip and liquid thickness, the reference pathlength should not need to be readjusted.

Further optimization of interference fringe visibility is accomplished by balancing the amount of light in the reference and test beams by rotating the polarizer in the illumination.

Because the objectives used are not designed for coverslips, the coverslip and liquid thickness will generate some spherical aberration in the measurements. However, because it is possible to subtract out small amounts of spherical aberration from measurements, and to also subtract a reference measurement file, there is no noticeable image degradation with magnifications of 50X or lower. At 20X and ~200–300 μm total thickness, very good fringe contrast is easily obtainable.

2.2 Obtaining phase images and determining optical thickness

For phase imaging applications, pixelated phase mask sensor technology uniquely provides a single frame phase measurement in a compact, robust format that is compatible with conventional microscope imaging systems, and permits the use of a wide variety of wavelengths and source bandwidths without the need for vibration isolation or scanning. [21–23] All necessary information to determine phase is recorded in a single snapshot. It enables the creation of a versatile and compact microscope interferometer for biological applications.

2.2.1 Wire-grid polarizers—The polarization-mask is constructed from an array of micropolarizers that are constructed from wire grid polarizers as shown in Figure 3. Wire-grid polarizers are made of tiny metal wires that are deposited on a transparent substrate (typically aluminum wires on a glass substrate). The linewidth, thickness and period of the wires are approximately 100nm, 120nm and 240nm respectively. These sub-wavelength structures have the property of reflecting light polarized parallel to the wires and transmitting light polarized perpendicular to them. They function as efficient polarizers over a wide range of wavelengths and angles.

2.2.2 Phase imaging with a pixelated phase-mask—At the phase mask, the reference and test beams have orthogonal circular polarizations (i.e., right-hand circular and left-hand circular). When combined, the measured irradiance at each pixel of the mask is given by Equation (1). [24]

$$I(x, y) = \frac{1}{2} \left\{ I_r + I_s + 2 \sqrt{I_r I_s} \cos [\Delta\varphi(x, y) + 2\alpha_p] \right\}, \quad (1)$$

where α_p is the angle of the polarizer with respect to the x, y plane, I_r and I_s are the intensity of reference and signal beams respectively, and $\varphi(x, y)$ is the optical path difference between the beams. When this equation is applied to each of the 4 pixels in the unit cell, phase differences of 0° , 90° , 180° , and 270° are encoded into a single image when a single interferogram is recorded. For each of the four pixel types we can write Equations (2)–(5) as,

$$A(x, y) = \frac{1}{2} \left\{ I_r + I_s + 2 \sqrt{I_r I_s} \cos [\Delta\varphi(x, y)] \right\} \quad (2)$$

$$B(x, y) = \frac{1}{2} \left\{ I_r + I_s + 2 \sqrt{I_r I_s} \cos \left[\Delta\varphi(x, y) + \frac{\pi}{2} \right] \right\} \quad (3)$$

$$C(x, y) = \frac{1}{2} \left\{ I_r + I_s + 2 \sqrt{I_r I_s} \cos [\Delta\varphi(x, y)] + \pi \right\} \quad (4)$$

$$D(x, y) = \frac{1}{2} \left\{ I_r + I_s + 2 \sqrt{I_r I_s} \cos \left[\Delta\varphi(x, y) + \frac{3\pi}{2} \right] \right\}. \quad (5)$$

Four simultaneous full-field interferograms can then be synthesized by combining pixels of each phase type. These four interferograms can be processed by a variety of algorithms that are well-known for calculating image phase.[25, 26] One well-known phase algorithm is the simple four-frame algorithm shown in Equation (6) as

$$\varphi(x, y) = \text{ATAN} \left\{ \frac{[C(x, y) - A(x, y)]}{[D(x, y) - B(x, y)]} \right\}, \quad (6)$$

where ATAN is the 2π arctangent function. This produces a modulo 2π (wrapped) phase map which then needs to be unwrapped using standard techniques.[27, 28] These phase image calculations can be done in real-time.

2.2.3 Determining optical thickness—Each interference fringe in reflection corresponds to one-half wave of optical path difference (OPD – the measured phase difference – or phase – for short). Typically, the raw units of this measure are in terms of wavelengths of the source light (waves). To make these data more useful we convert phase data to optical thickness $OT(x, y)$, given by Equation (7) as,

$$OT(x, y) = OPD \cdot \frac{\lambda}{2} = \varphi(x, y) \left[\frac{\lambda}{4\pi} \right] \quad (7)$$

Optical thickness (OT), seen in Equation (8), is an integrated measure of the overall optical path through the sample, which is the product of the localized index of refraction $n(x, y, z)$ and the physical thickness $t(x, y, z)$,

$$OT(x, y) = \int_0^T n(x, y, z) \cdot t(x, y, z) dz. \quad (8)$$

For viewing in reflection OT includes a double pass through the coverslip and liquid containing the objects. Denser areas of the object with higher indices of refraction will yield a larger OT as shown in Figure 4.

Typically, the index of refraction of the coverslip is ~1.5, the index of water is ~1.33, while cellular media is ~1.37, cytoplasm ~1.39 and cellular organelles like nuclei ~1.41–1.43. Subtle differences as small as the third decimal place in refractive index are detectable by this system.[15–17, 29, 30]

3. SAMPLE MEASUREMENTS

3.1 Brightfield, Dark field, DIC, interference and phase images

The pixelated phase mask sensor enables five different types of images to be obtained simultaneously as illustrated in images of a protozoa in Figure 5.

When the values of all 4 types of pixels are averaged a bright field image is obtained. When values from one type of pixel are displayed, an interferogram is obtained. Dark field images are simulated by computing the gradient magnitude of the phase as,

$$DF = \sqrt{\left(\frac{\partial \varphi}{\partial x}\right)^2 + \left(\frac{\partial \varphi}{\partial y}\right)^2}. \quad (9)$$

Differential interference contrast (DIC) images can be simulated by computing the lateral phase gradient. The only quantitative measurement that results is the calculated optical thickness.

This data was taken at 50X with a 660 nm source. The optical thickness of this sample is 354 nm. Optical thickness maxima are shown in red, minima in blue. Note that the internal cells are clearly visible.

3.2 Simple background removal

In order to retrieve quantitative optical thickness data to extract positional motion and/or volumetric changes, the objects of interest need to be isolated from the background. This process is meant to serve two functions. Besides removing the background shape so that objects can be quantified relative to a flat surface, the mean value can be kept constant from frame to frame to aid in tracking quantitative changes with time. This can be implemented using many different algorithms, many of which are highly dependent on the method by which the data is collected and the type of scene being analyzed.[31–33] The goal of this work was to determine a processing routine that works equally well on scenes with small and large objects and is automated with no need for user input. Because the intent of the processing routine is to ultimately create a movie of optical thickness data, there is the potential need to process a large number of frames of data. Requiring users to intervene and provide input during the algorithm for each frame would drastically increase the complexity, potential for failure and time required to complete the background leveling process.

The initial background leveling technique attempted was to fit a set of low-order Zernike polynomials to the phase or optical thickness data [26, 34]. The difference between the data and the Zernike surface is filtered to isolate the objects and subtract the shape of the background surface.

Figure 6 illustrates this process on two frames of a sequence of images from a phase movie of a moving paramecium taken at 660nm with a 20X objective and 2.25X FOV lens. Tilt and subtle shape are noticeable in the top images where the left side of the images is higher than the right side. This is removed in the bottom set of images enabling the isolation of the paramecium by itself. Another byproduct of this procedure is removal of the mean value from frame to frame. This effectively eliminates jumps between frames caused by noisy pixels, small pieces of debris in the cell solution or scratches on the mirror.

As this simple processing technique was applied to a variety of phase or optical thickness data, it was found that it was not a universal solution. This technique would fail when applied to a scene where the phase object occupied roughly half of the image. Figure 7 shows this technique applied to a large phase object, the back half of a rotifer imaged at 50X with a 660nm source. The original data is shown on the left. A low-order Zernike surface is fit to the data, and the data is filtered and masked to eliminate outliers (masked pixels are shown in white). The resultant Zernike surface is then subtracted from the original data resulting in the residual surface furthest to the right. In this case, the Zernike surface fit to the data does not contain the expected amount and orientation of tilt. A more sophisticated algorithm needed to be developed that was more effective on this type of phase object.

3.3 Automated background leveling

A new background leveling algorithm has been developed that operates very well on scenes that have varied sizes of phase objects against a background. The algorithm developed is based on similar techniques that utilize thresholding with respect to gradients of the data. [35, 36] For this application, by considering the gradient magnitudes of the phase as opposed to the phase values, it is straightforward to locate the regions where phase is slowly varying. The resulting gradient magnitudes are then thresholded to a minimum value, resulting in any unmasked pixel being associated with only the background. This mask is then applied to the phase or optical thickness data and a low-order Zernike surface can be fit to the resultant background. This surface can then be subtracted from the original data resulting in a background-leveled optical thickness map.

The tail of a rotifer, shown in Figure 7 with the simple background removal, can have a greatly improved background removal by applying the new automated background leveling algorithm. Figure 8 shows the initial step in the algorithm, applying a mask to the gradient magnitudes and iterating until the rotifer is nearly completely masked, shown by white pixels, leaving only background pixels, after four iterations.

The remaining steps of the algorithm are shown diagrammatically in Figure 9. The final mask that is applied to the original optical thickness data prior to zernike fitting the surface is the same as the fourth iteration from Figure 8.

It is illustrative to compare the results of the automatic background leveling routine and manually selecting a region that is known by the user to be solely background. This is illustrated in Figure 10. The results for the rotifer are very consistent and there are no major differences in the resultant background removal aside from minor scaling of the color axis. The advantage of the automatic background removal routine is that it requires no user input.

This is particularly useful because the algorithm can be used to remove the background from a potentially large sequence of phase images. For phase objects whose position changes rapidly across a time-sequence of data, it would require a tremendous amount of user intervention to ensure that the background region chosen never contains an object across all frames in the sequence.

Other examples of the automatic background leveling algorithm applied to a moderately sized protozoa taken at 50X with 660nm source are shown in Figure 11. Notice the mask, shown with white pixels, is applied to pixels that are associated with camera noise.

Osmotically swollen cells, taken at 20X with 660 nm source are shown in Figure 12. The dominating background shape is more complicated for this example and requiring a larger-term Zernike surface to be fit to the surface. When larger-term Zernike fits are necessary, the need to ignore objects becomes more critical because the spherical shape of cells can confuse a simple background subtraction technique if those objects are not ignored with a mask. The mask in Figure 12 additionally shows that calculating the gradient magnitude of the optical thickness is an excellent method to detect edges of objects.

4. DISCUSSION AND CONCLUSIONS

This paper has described recent research into development of a dynamic phase imaging microscope and shown a number of examples of 4D phase measurements of living biological organisms with an emphasis on an automatic background leveling routine developed for this data. All the data to determine phase and optical thickness can be gathered in a single snapshot, so no scanning is necessary. Data from brightfield imaging, phase contrast (interference image), simulated images from dark field and DIC, are also obtained simultaneously along with phase and optical thickness. A new automated background leveling routine has been developed and applied to a wide range of biological samples. By thresholding the gradient magnitudes of the optical thickness data, objects can be successfully masked, leaving only pixels associated with the background that can then be characterized with a low-order Zernike surface. This method successfully removes background shape without the need for user input. This technique is easily scalable and allows a large number of frames to have background shape removed, and combine the resulting frames into a movie of optical thickness data where the mean value of the background stays constant through the entirety of a measurement cycle.

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References

1. Sommargren GE. Optical Heterodyne Profilometry. *Applied Optics*. 1981; 20:610–618. [PubMed: 20309165]

2. Wyant JC, Koliopoulos CL, Bhushan B, et al. An optical profilometer for surface characterization of magnetic media. *ASLE Trans.* 1984; 27:101–113.
3. Schmit, J.; Creath, K.; Wyant, JC. Ch. 15. Surface Profilers, Multiple Wavelength, and White Light Interferometry. Wiley-Interscience; Hoboken, N.J: 2007.
4. Dunn GA, Zicha D. Phase-Shifting Interference Microscopy Applied to the Analysis of Cell Behaviour. *Symposia of the Society for Experimental Biology.* 1993; 47:91–106. [PubMed: 8165581]
5. Zicha D, Dunn GA. An image-processing system for cell behavior studies in subconfluent cultures. *Journal of Microscopy-Oxford.* 1995; 179:11–21.
6. Eppich B, Beuthan J, Dressler C, et al. Optical phase measurements on biological cells. *Laser Physics.* 2000; 10(2):467–477.
7. Tychinsky VP, Kretushev AV, Klemyashov IV, et al. Quantitative real-time analysis of nucleolar stress by coherent phase microscopy. *Journal of Biomedical Optics.* 2008; 13(6):064032–6. [PubMed: 19123678]
8. Yang C, Wax A, Hahn MS, et al. Phase-referenced interferometer with subwavelength and subhertz sensitivity applied to the study of cell membrane dynamics. *Optics Letters.* 2001; 26(16):1271–1273. [PubMed: 18049583]
9. Popescu, G. [Quantitative Phase Imaging of Nanoscale Cell Structure and Dynamics]. Elsevier; San Diego: 2008.
10. Hogenboom DO, DiMarzio CA, Gaudette TJ, et al. Three-dimensional images generated by quadrature interferometry. *Optics Letters.* 1998; 23(10):783–785. [PubMed: 18087341]
11. Rockward WS, Thomas AL, Zhao B, et al. Quantitative phase measurements using optical quadrature microscopy. *Applied Optics.* 2008; 47(10):1684–1696. [PubMed: 18382601]
12. Reed J, Frank M, Troke JJ, et al. High throughput cell nanomechanics with mechanical imaging interferometry. *Nanotechnology.* 2008; 19(23)
13. Ross GJ, Bigelow AW, Randers-Pehrson G, et al. Phase-based cell imaging techniques for microbeam irradiations. *Nuclear Instruments & Methods in Physics Research Sec B.* 2005; 241(1–4):387–391.
14. Yu LF, Mohanty S, Liu GJ, et al. Quantitative phase evaluation of dynamic changes on cell membrane during laser microsurgery. *Journal of Biomedical Optics.* 2008; 13(5)
15. Creath, K. [Dynamic quantitative phase images of pond life, insect wings, and in vitro cell cultures]. SPIE; Bellingham, WA: 2010.
16. Creath K. Dynamic phase imaging utilizing a 4-dimensional microscope system. *Proc SPIE* 7904. 2011:79040O.
17. Creath K. Dynamic Phase Imaging for in Vitro Process Monitoring and Cell Tracking. *Proceedings of the 33rd Annual International Conference of the IEEE EMBS, Boston, Ma, August 29–September 3, 2011.* 2011; 33:5977–80.
18. Born, M.; Wolf, E. [Principles of Optics]. Pergamon Press; Oxford: 1975.
19. Abdulhalim I. Competence between spatial and temporal coherence in full field optical coherence tomography and interference microscopy. *J Opt A: Pure Appl Opt.* 2006; 8:952–958.
20. Ryabukho V, Lyakin D. Longitudinal pure spatial coherence of a light field with wide frequency and angular spectra. *Optics Letters.* 2005; 30(3):224–226. [PubMed: 15751866]
21. Brock, NJ.; Millerd, JE.; Wyant, JC., et al. [Pixelated phase-mask interferometer]. 4D Technology Corporation; United States: 2007.
22. Kimbrough BT. Pixelated mask spatial carrier phase shifting interferometry algorithms and associated errors. *Applied Optics.* 2006; 45(19):4554–4562. [PubMed: 16799664]
23. Novak M, Millerd J, Brock N, et al. Analysis of a micropolarizer array-based simultaneous phase-shifting interferometer. *Applied Optics.* 2005; 44(32):6861–6868. [PubMed: 16294959]
24. Kothiyal MP, Delisle C. Shearing interferometer for phase-shifting interferometry with polarization phase-shifter. *Applied Optics.* 1985; 24(24):4439–4442. [PubMed: 18224225]
25. Creath, K. [Phase-measurement interferometry techniques]. Elsevier Science Publishers; Amsterdam: 1988.

26. Malacara, D.; Servin, M.; Malacara, Z. [Interferogram analysis for optical testing]. Taylor & Francis; Boca Raton, FL: 2005.
27. Robinson, DW. [Phase unwrapping methods]. IOP Publishing; Bristol: 1993.
28. Ghiglia, DC.; Pritt, MD. [Two-Dimensional Phase Unwrapping: Theory, Algorithms and Software]. Wiley-Interscience; New York: 1998.
29. Creath, K.; Schwartz, GE. [Dynamic visible interferometric measurement of thermal fields around living biological objects]. SPIE; Bellingham, WA: 2004.
30. Creath, K.; Goldstein, G. Proc SPIE 8227. 2012. Dynamic phase imaging and processing of moving biological organisms; p. 82270M-10
31. Khmaladze A, Matz RL, Jasensky J, et al. Dual-wavelength digital holographic imaging with phase background subtraction. Optical Engineering. 2012; 51(5):055801–8.
32. Walker PG, Cranney GB, Scheidegger MB, et al. Semiautomated method for noise reduction and background phase error correction in MR phase velocity data. Journal of Magnetic Resonance Imaging. 1993; 3(3):521–530. [PubMed: 8324312]
33. Lieber CA, Mahadevan-Jansen A. Automated Method for Subtraction of Fluorescence from Biological Raman Spectra. Appl Spectrosc. 2003; 57(11):1363–1367. [PubMed: 14658149]
34. Malacara, D. [Optical shop testing]. Wiley-Interscience; Hoboken, N.J: 2007.
35. Weszka JS. A survey of threshold selection techniques. Computer Graphics and Image Processing. 1978; 7(2):259–265.
36. Yanowitz SD, Bruckstein AM. A new method for image segmentation. Computer Vision, Graphics, and Image Processing. 1989; 46(1):82–95.

Figure 1.
Optical schematic of Linnik interference microscope

Figure 2.
Prototype interference microscope.

Figure 3.

The pixelated phase mask has 4 different polarizations comprising a unit cell of 2x2 different relative phase values.

Figure 4.

Optical path length through test sample includes the coverslip, liquid and objects. Graph (bottom) shows optical thickness profile for the section within the highlighted area.

Figure 5.

Images of a protozoa. Left to right, Brightfield, Interferogram, DIC simulated image, Dark field simulated image and phase image showing optical thickness. All images are 129 by 125 microns. For optimal viewing, please see color version of paper online.

Figure 6.
(top) Raw optical thickness data. (bottom) After background leveling.

Figure 7. Simple background leveling applied to large phase object, a rotifer, where simple background subtraction technique breaks down. Notice the mask (masked pixels shown in white) does not include a large portion of the rotifer's body. All images are 129 by 125 microns.

Figure 8.

Automated background leveling masking routine being applied to rotifer tail. All images are 129 by 125 microns.

Figure 9.
Automatic background leveling technique applied to rotifer tail

Figure 10. Manual selection of background region and resultant residual optical thickness. All images are 129 by 125 microns.

Figure 11. Automatic background leveling applied to protozoa. Masked pixels are shown in white. All images are 129 by 125 microns.

Figure 12.

Automatic background leveling routine applied to osmotically swollen cells. All images are 344 by 347 microns.