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Large Population Cell Characterization Using Quantitative Phase Cytometer

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Abstract

A major challenge in cellular analysis is the phenotypic characterization of large cell populations within a short period of time. Among various parameters for cell characterization, the cell dry mass is often used to describe cell size but is difficult to be measured directly with traditional techniques. Here, we propose an interferometric approach based on line-focused beam illumination for high-content precision dry mass measurements of adherent cells in a non-invasive fashion—we call it quantitative phase cytometry (QPC). Besides dry mass, abundant cellular morphological features such as projected area, sphericity, and phase skewness can be readily extracted from the QPC interferometric data. To validate the utility of our technique, we demonstrate characterizing a large population of \(~10^4\) HeLa cells. Our reported QPC system is envisioned as a promising quantitative tool for label-free characterization of a large cell count at single cell resolution.

Key terms

image cytometry; quantitative phase imaging; high content; cell dry mass

Introduction

Flow cytometry is a technique that successfully combines many advantages of microscopy and those of various biochemical analysis techniques to achieve high throughput and high precision phenotyping and sorting of individual cells, microorganisms and organelles \((1)\). Over the last half century, it has evolved from custom-made apparatuses designed for
specific research needs to commercial instruments made available to clinical laboratories and hospitals for diagnostics and monitoring of diseases (2). As a cell analysis technique, the most significant advantage of flow cytometry is its unprecedented throughput with a typical processing speed of 10,000 cells/s; an extreme sample rate up to ~1 million cells/s has also been demonstrated (3). While flow cytometry is good for extracting biochemical information from cells via fluorometric assays, it mostly cannot quantify the morphological characteristics and molecular localizations due to its low spatial resolution (4). We also note that flow cytometry inherently requires cells to be suspended and dissociated for flow purposes; this requires the adherent cells to be trypsinized before each measurement (5).

Fortunately, both aforementioned problems can be addressed by another class of cytometry, known as image cytometry, which relies on microscopic imaging techniques and makes use of computer aided image processing methods. Indeed, image cytometers can accommodate most staining techniques developed for flow cytometry (5), thus maintaining the biochemical analysis capability. Although image cytometry has relatively lower processing throughput than flow cytometry (6), it is regarded as a high content screening technique (7), and has found wide applications in a variety of biological and clinical fields, such as drug discovery (8,9), cancer diagnostics (10,11), cell signaling (5,12), and genomics (13,14). Image flow cytometry, which combines the advantages of high speed in flow cytometry and high resolution in image cytometry, captures two-dimensional images of floating cells as they are transported through a microfluidic channel similar to the flow cytometry. Currently, commercialized system named ImageStream (Amnis Corp.) can simultaneously obtain bright field, dark field and fluorescence images of cells at a throughput up to 5,000 cells/s (15). In academia, an interesting and powerful technique termed serial-time-encoded amplified microscopy (STEAM) demonstrated a frame rate of 6.1 MHz for 2D gray scale imaging (16). Moreover, by mapping the image into the radiofrequency spectrum, fluorescence imaging using radiofrequency-tagged emission demonstrated fluorescence microscopy at a frame rate up to 4.4 kHz, which corresponds to a throughput of approximately 50,000 cells/s (17).

Quantitative phase imaging (QPI) is an emerging microscopic imaging technique dedicated to providing biophysical information about transparent micro-specimens by quantifying optical phase shift at diffraction limit (18–22). More specifically, this technique provides the phase maps, which can further yield quantitative information about the morphology and dynamics of the examined specimens with high spatial and temporal resolution (23,24). In addition to morphology, the measured phase maps can be converted to dry mass of the cells with accuracy that is of the order of femtograms per squared microns (25,26). It should be noted that, in contrast to various existing imaging modalities that rely on biochemical staining or tagging, QPI requires minimum preparation and is a label-free method that allows keeping the specimen viable in its near-natural state for an extended period of time. It is, therefore, ideal for biomedical imaging, especially when the living cells are rare and/or sensitive to subtle perturbations in their microenvironment (27,28). Above all, the sub-micron resolution, nanoscale sensitivity, quantitative morphological information and label-free operation have granted QPI an access to numerous biological applications including cell dynamics (29,30), cell growth (26,31,32), blood testing (33,34), cell and tissue diagnostics (35–37), and 3D cellular imaging (38,39).
The non-destructive and high throughput nature of QPI makes it a natural candidate for developing a QPI-based image cytometer. Quantitative phase cytometers (QPC) can promisingly provide both biophysical (e.g., whole cell morphology and intracellular structures) as well as biochemical (e.g., dry mass and dry mass density) information for a large number of cells so that a statistical analysis of the cell population is possible. The earlier reported modality for QPC in literature is based on diffraction phase microscopy, termed diffraction phase cytometry (DPC), which successfully implemented whole blood cytometry in a “lab-on-a-chip” instrument (a CD-ROM) for characterizing red blood cells (RBCs) (40). It was demonstrated that DPC can offer access to detailed 2D and 3D morphological parameters of RBCs such as volume, surface area, sphericity, diameter, minimum cylindrical diameter, and so forth. (40). Later on, Mir et al. (41) provided a convincing proof of comparable abilities of the DPC system for measuring and characterizing RBCs to a state-of-the-art clinical impedance counter, and further showed that DPC offered additional insight into the nature of the numerical abnormalities used to identify morphological blood disorders. Afterwards another QPC modality named phase imaging flow cytometer, using transport-of-intensity-based phase imaging, was proposed to measure the quantitative phase of human RBCs and leukemia cells flowing in microfluidic channels (42). More recently, Mahjoubfar et al. (43) introduced a label-free imaging flow cytometer based on STEAM, which can measure size (lateral resolution is ~2.5 μm) and total optical path difference of cells in flow rates as high as a few meters per second.

Although QPC has been reported previously, for systems with sub-micron resolution, the reported imaging volume (analyzed cell number) has been limited to ~10^3 (40–42). This article is dedicated to pushing this volume up to ~10^4 using the line-field illumination based QPI. Unlike conventional QPI methods that are based on wide-field illumination, line-field QPI focuses a collimated laser beam along one direction using a cylindrical lens. When a sample is translated across the focused line illumination, information equivalent to that acquired by varying the illumination angles of plane waves onto the sample becomes available (44,45)—an approach also known as synthetic-aperture QPI. The proposed method not only applies to imaging adherent cells by translating the sample stage but can also be used to study cells in suspension flowing across the field-of-view within a micro-fluidic channel, featuring the flexibility of this technology.

In summary, we hereby propose a QPC based on line-field illumination to acquire structural and functional information of continuously moving cells. Using this system, quantitative phase information of around ten thousand individual cells was acquired in about 13 min. Furthermore, we successfully converted the measured phase images of live cells into their dry mass density maps and obtained a dry mass histogram of ~8,000 cells. With enhanced imaging throughput, the proposed QPC holds promise as a powerful tool to monitor cell size as a label-free marker in various cellular studies (46,47).

**Materials and Methods**

**Sample Preparation**

HeLa cells were purchased from the American Tissue Culture Collection (ATCC), Manassas, VA, and cultured in Dulbecco modified Eagle medium (DMEM) supplemented...
with 10% FBS and 1% 100X penicillin-streptomycin solution. Several drops of media containing suspended cells were carefully placed on a coverslip (VWR, #48393–070, 22 × 60 mm) and placed in an incubator with humidified 5% CO\textsubscript{2} and 37°C environment. After around 12 h of incubation, the cells became flat and well adhered to the coverslip. Subsequently, another coverslip of the same kind was placed on top of the original one. The cells sandwiched between two coverslips were used for imaging. The whole experiment usually takes less than an hour, during which the cells stay in a healthy state.

**High Throughput Phase Cytometer**

Figure 1 shows the schematic layout of the optical system setup used for this study in the focal and planar axis, respectively. Briefly, the QPC system is based on the classic Mach-Zehnder interferometry. A HeNe laser with 633 nm wavelength is used as the illumination source. The illumination beam is split using a beam splitter (B1) into two parts, the sample and the reference beams. For the sample illumination arm, a cylindrical lens C1 (f=100 mm) and a high numerical aperture (NA) condenser lens L1 (Olympus, oil immersion, NA =1.2) are combined to convert the incident collimated beam into a light sheet, focused onto the sample plane (SP). The intensity profile of this light sheet has Gaussian distribution in both dimensions with ~0.24 μm thickness (diffraction limited) and ~130 μm width. The angular components of the scattered light after passing through the samples are collected by a high-NA 100X objective lens, L2 (Nikon, oil immersion, NA =1.25). The collected light is magnified after the tube lens, L3, and collimated using a cylindrical lens C2 (f=400 mm), which relays a sample image along the focused line beam. Afterwards, a combination of two other cylindrical lenses C3 and C4 are installed orthogonally to C2 focal axis as a 4-f configuration to deliver the image along the non-focusing axis. To record both the amplitude and phase of the angular spectrum in a single shot for each location of the samples, an off-axis holography setup is adopted. Specifically, the sample and reference beams are combined using a second beam splitter B2 for off-axis interferometry. The interferogram is recorded using a complementary metal-oxide semiconductor (CMOS) camera M1 (Optronis Inc., model # CP80-40-M-500) located at the back focal plane of C3. Note that only 2304 × 600 pixels are used for recording.

A motorized X-Y stage (Marzhauser Wetzlar GmbH & Co. KG, SCAN 100 × 100 model) was installed to translate the samples across the line-field illumination. Figure 2a shows the scanning pattern of the X-Y stage. The stage first travels along X-axis for ~5.1 mm, subsequently shifts along Y-axis for 0.16 mm to avoid overlaps of acquired images (the actual field-of-view along Y-axis is 0.1325 mm), and then travels along X-axis again but in the reversed direction back to the original X coordinate. This zigzag scanning mode is repeated for 11 times at a rate of 0.07 mm/s; the corresponding scanned area is ~5.1 × 1.46 mm\textsuperscript{2}. As the stage travels along X-axis, the cells move across the line-focused beam. Meanwhile, the camera is triggered by the stage every 100 nm to capture the interferograms at a frame rate of 700 frames/s. Figure 2b shows the timing diagram for stage scanning. The total scanning time can be approximately calculated from the velocity of stage movement (here set as 0.07 mm/s) and the total distance of stage movement shown in Figure 2a, which yields ~817.50 s ((50,568 frames × 11 strips × 0.0001 mm + 0.16 mm × 10 steps)/0.07 mm/s).
Phase Reconstruction Process

Figure 3 illustrates the process of generating phase images from raw interferograms. Figure 3a shows a representative interferogram image with intensity profile expressed by the following equation:

\[
I(x, y) = I_S(x, y) + I_R + 2 \sqrt{I_S(x, y) I_R \cos \left[2\pi (px + qy) - \Delta \varphi(x, y)\right]},
\]

where \(I_S(x, y)\) and \(I_R\) are the intensity maps of the sample and reference beam, respectively; \(\Delta \varphi(x, y)\) is the phase difference between the two beams; \((p, q)\) vector indicates the relative angle of the sample beam with respect to the reference beam on the detector plane. The raw interferogram contains spatial information in one direction and Fourier component information in the other direction. Figure 3b shows the magnitude of Fourier Transform (FT) in the logarithmic scale. The region within the black circle, shown in Figure 3b, contains the information about the specimen. Furthermore, it is separated from the unscattered light located at the center (marked by the red circle) and can be described by

\[
\tilde{\Phi}(u, v) = \tilde{\Phi}(u-p, v-q),
\]

where \(\tilde{\Phi}(u, v)\) is the FT of \(\Phi(x, y) = \sqrt{I_S(x, y) I_R e^{-i\Delta \varphi(x, y)}}\). Therefore, \(\tilde{\Phi}(u, v)\) can be obtained by selecting the region within the black circle using a mask and moving it to the origin of the Fourier domain coordinate, as shown in Figure 3c. Then by taking inverse FT of Figure 3c, the complex angular spectra of a line scan sample \(\Phi(x, y)\) can be retrieved; Figure 3d shows the phase map associated with the measured interferogram.

Using the approach described above, all the raw interferograms shown in Figure 4a were processed to obtain a series of angular spectra \(E_{\text{samp}}(x, k_y; \eta)\) for different locations \(\eta\) of the specimen while it is scanned across the line-focused beam. The phase maps corresponding to this series of angular spectra can be assembled into a 3D array with coordinates \(x\) (dimension along the line focus beam), \(k_y\) (dimension along Fourier axis) and \(\eta\) (dimension along stage translation) as illustrated in Figure 4b. When viewing in the \(x-\eta\) plane, this 3D ensemble is actually comprised of a series of full field phase images of a sample in the real space corresponding to different angularly projected plane waves, as shown in Figure 4c. In the last step, all these phase images corresponding to varying incident angles of illumination were averaged to yield the final phase map as shown in Figure 4d.

Results and Discussion

To demonstrate the ability of the line-field QPC to characterize a large cell population, HeLa cells (a cervical cancer epithelial cell line) were cultured and imaged. Figure 5a is the quantitative phase map of \(\sim10^4\) HeLa cells obtained after data processing. It contains 11 strips, each of which covers an area of \(5.068 \times 0.1325\) mm\(^2\); the total field of view is \(5.068 \times\)
Figure 5b shows the magnified phase image from a small fraction of Figure 5a, where the range of phase values agrees well with previous reports (25,48).

To quantify the phase measurement sensitivity of our system, we measured the standard deviation (SD) of each pixel within a region with no cells indicated by the white dash square boxes in Figures 6a and 6c. Figure 6a shows the phase image corresponding to a single (normal) incident illumination whereas Figure 6b is the 3D height map of background phase for each pixel within the square box in Figure 6a, which yields a SD of 0.14 rad. Correspondingly, Figures 6c and 6d illustrate the case where the phase image is reconstructed by averaging a series of phase images for various incident angles in the line-field illumination. We have used 10 × 10 median filtering since the optical resolution is about tenfold the digital resolution, which yields a SD of 0.016 rad. By comparison, it can be found that the median filtering and phase image averaging corresponding to multiple angles of illumination can provide around 10-fold phase sensitivity improvement. The SD of 0.016 rad in phase corresponds to 1.2 nm in path length when the surrounding medium is cell culture medium, DMEM (Dulbecco’s Modified Eagle Medium, refractive index is ~1.3371), which demonstrates the nanometer sensitivity to height changes in sample structure.

Since the cytometer is based on QPI, it can also readily provide images that are typically offered by other modalities in commercialized microscope systems such as the phase contrast microscopy and the differential interference contrast (DIC) microscopy. Conveniently, this digital simulation requires no physical modification to the imaging system. Figure 7a is the original QPI. Correspondingly, Figures 7b and 7c show the generated digital positive phase contrast image and the digital negative phase contrast image in dark field, respectively, which are similar to the measured images by phase contrast microscopes (49). Moreover, the phase gradient image is produced and converted into intensity differences map to simulate the DIC image as shown in Figure 7d, which has similar appearance to what we could see under a DIC microscope (50).

Cell dry mass is a measure of the non-aqueous content of the cell and can be calculated by the obtained QPI. Importantly, the relationship between dry mass and refractive index (RI) is used to convert cell phase shifts to dry mass density, which can be expressed as (51)

$$n_c(x, y) = n_0 + \alpha C(x, y), \quad (3)$$

where $\alpha$ is the refractive index increment (mL/g), $C(x, y)$ is the concentration (g/ml) of dry protein in the solution, $n_0$ and $n_c(x, y)$ are the RI of the surrounding medium and the dry protein, respectively. Refractive index increments for most biomolecules, especially proteins and nucleic acids that form the majority of a typical cell’s mass, fall within a very narrow range (0.18–0.21 mL/g) (52). Here, we use an average refractive index increment value of 0.2 mL/g to compute dry mass of HeLa cells from measured quantitative phase maps (51,53). Based on Eq. (3), the dry mass density $\sigma$ of the cellular matter can be obtained from the measured phase map, $\Delta \phi(x, y)$, as follows:
\[ \sigma(x, y) = \frac{\lambda}{2\pi a} \Delta \varphi(x, y), \]

where \( \lambda \) is the wavelength of the illumination beam. Figure 8a shows an example of the dry mass density distribution (pg/\( \mu \)m\(^2\)) obtained from a subpopulation of HeLa cells monolayer, which agrees well with Ref. 25 and may have more immediate biological interpretation. Furthermore, the integration of dry mass density across the entire projected cell area \( A \) can yield the dry mass \( M \) of whole cells, which can be formulized by

\[ M = \int \sigma dA = \frac{\lambda}{2\pi a} \int \Delta \varphi dA. \]

To determine the cell area \( A \) of each cell in a large population, the obtained phase images in Figure 5 were first converted to gray scale images and then segmented from the background and individualized from other cells in the field-of-view using a cell segmentation algorithm; Figure 8b shows the segmentation process. For details, we first segment the cells from the background by thresholding the image intensity. Second, we average the image using a Gaussian kernel (the size is determined according to the minimum cell diameter) and detect the local maxima, which would act as the seeds. Third, the contours of cells are deduced from the seeds obtained in the last step using a marker-controlled watershed segmentation algorithm. Finally, an edge-based active contour method described in Ref. 54 is implemented to expand and refine the obtained segmentation boundary according to the image gradient. To be noted, partial cells on the borders of images are not taken into account since they are incomplete. After cell segmentation, the calculation of dry mass based on Eq. (5) is performed for each segmented cell. Finally, a histogram of dry mass for 7,864 asynchronized HeLa cells is obtained as shown in Figure 8c. The whole data analysis procedure from segmentation of the raw phase images to the calculation of dry mass for each cell is performed in MATLAB software (MathWorks Inc.). Currently, the data analysis takes about half an hour. We note, however, that with the help of coding optimization such as vectorization and graphics processing unit computing, this speed can be augmented by at least one order of magnitude.

To validate the segmentation accuracy, we also manually segmented the same phase images and obtained the dry mass histogram of 8,143 cells as shown in Figure 8d. Therefore, it can be calculated that the cell counting accuracy of the automatic segmentation is \(-96.5\%\). The histogram profiles in Figures 8c and 8d look similar. Quantitatively, the mean value of dry mass distribution in Figure 8c is 426.7 pg with a SD of 157.6 pg, whereas the mean value in Figure 8d is 430.5 pg with SD of 149.9 pg. We note that the shift in mean dry mass is <1% using automatic segmentation, which indicates that our segmentation approach is accurate for retrieving dry mass distribution of large cell population. From Figure 8c, it can be seen that the dry mass distribution is approximately lognormal, which agrees well with the results for other cell lines in previous reports (55,56). Moreover, this dry mass profile is also very similar to the cell volume histogram measured by a commercial Coulter Counter (57).
Assuming that same cell line should possess the same density (or at least very close), this similarity is indeed expected.

The regulation of cell size is essential with significant consequences in tissue and organ architecture, but its detailed mechanism has been largely obscure, mainly because of the lack of effective and efficient measuring methods at the single-cell level (26). In literature, cell size has been reported as either cell volume or cell dry mass (58). One of the most popular methods to measure cell volume is the use of a Coulter Counter, which has been widely utilized to study how cells regulate growth (57,59). However, cell volume can be readily affected by osmotic pressure controlled by intracellular conditions and extracellular environment together. Furthermore, for most adherent mammalian cells, cell shape is highly variable, thus making it difficult for cell geometry to fully and accurately describe the cell size (60). In contrast, cell dry mass is the direct result of biosynthesis and degradation processes within a cell, so it is inherently much more closely linked to cell growth, granting it to be a more precise indicator of cell size (61). Among various methods, QPI is a suitable optical approach for measuring the dry mass of adherent cells with high accuracy and minimal perturbation, and has been extensively used in the studies of cell growth, drug response, cell cycle, and so forth, where cell size had been used as a key indicator (26,62,63).

Previous studies using QPI to measure live cell dry mass have mostly focused on phenotyping a few or tens of cells. However, large cell populations must be taken into account to fully comprehend most cellular processes such as response to a specific drug. Concisely, throughput is of primary importance when QPI will be applied in clinics. In this case, QPI needs to make most use of its inherent high-throughput nature to be evolved into QPC. With the help of ongoing progress and development in computer and imaging devices, the QPC system in this study has potential to process tens of thousands of cells within ten minutes, which fulfills the throughput requirement brought out by various cell-population based studies such as drug screening and cell toxicity assay. Conversely, thanks to the high precision single cell assay capability, the QPC system can take into account the cell heterogeneity, which plays an essential role in understanding complex biological processes that are often obscured by the average measurements of large populations.

As a preliminary demonstration, in this study we achieved a throughput of around 700 cells per minute. We recognize that this is still lower throughput (cells/s) than the state of the art imaging flow cytometry techniques. This was mainly due to limited data transfer rate of the RAID controller (<1 GByte/s) about twice slower than that of the camera (~2 GByte/s). This challenge, however, can be overcome in the future with the help of the rapid advances in automated image acquisition and processing technologies including both hardware (a pair of M.2 PCIe SSDs and software RAID function of the Windows OS itself can provide ~3 GByte/s writing speed these days) and algorithms. Moreover, the actual diffraction-limit resolution of this QPC system is ~0.6 μm but our sample stage movement along X-axis in experiments was set as 0.1 μm, which yields a threefold oversampling according to Nyquist sampling law. Overall, our system throughput can be improved by at least a factor of 5 by optimizing data acquisition and data transfer strategy.
We note that the proposed QPC system has its own unique advantages. First of all, it allows cell movement while imaging so that it can continuously image moving cells using either a translation stage or microfluidics. In this way, the number of cells that can be imaged is no longer limited by the field-of-view of the system. When using a translation stage to move cells, we note that the cells are not required to stop during data acquisition, so the translation speed is not constrained by the long settling time of the stage (typically ~ 100 ms). Conversely, this technique is also easy to be combined with existing microfluidic systems for implementing image flow cytometry. Second, this QPC system can image adherent cells and does not necessarily require the cells to be detached to suspend in the flowing solution, which often requires a pre-treatment process such as exposure to trypsin that has been documented to have negative influence on cell functioning and growth (64). Third, flow cytometry mostly relies on staining or tagging techniques to highlight the specimen from the background, which may cause toxicity to the cells under study. In contrast, QPC features itself as a label-free tool, which is extremely useful for rare and sensitive cells or the cells that may need to be implanted into humans for therapeutic purposes. Last but not the least, QPC is able to extract morphological information for each cell at the submicron lateral solution with nanoscale sensitivity, which provides quantitative insights into the morphology based cellular analysis. Based on the obtained QPI, various parameters relevant to morphological features can be calculated, which include optical phase delay (OPD) profile, phase volume, phase surface area, projected area, sphericity index, phase skewness, phase kurtosis, and so forth (48).

In addition to providing dry mass information at a certain time stamp, time-dependent evolution of quantitative phase maps can readily reveal the temporal distributions of cell mass as a function of time. Indeed, in many studies of cell cycle, mass quantification needs to be made continuously over the cell cycle by tracking many individual cells or cell clusters over multiple days or cell cycles (60). In such a scenario, the proposed line-field QPC together with a mini incubator (mounted on the microscope) can lead to precise and time-lapsed dry mass measurements. The non-invasive nature of proposed QPC allows long-term quantitative observation of live cells. Although we plan to improve the system throughput as discussed previously, the current time resolution (each measurement of the cell population takes around ten minute) is still enough for cell cycle studies that usually last from hours to days. In future, we plan to utilize the line-field QPC system to obtain time-lapsed investigation of fundamental cellular processes such as the heritability of cell size and the heterogeneity of cell growth in response to different stimuli.

Conclusion

In conclusion, we have presented a high-content image cytometry methodology based on line-field interferometric quantitative phase cytometer. We have successfully imaged and analyzed large cell population, on the order of $10^4$, for single cell analysis. Specifically, we report quantification of cell dry mass distribution for the whole population, which has long been viewed as an important property of cells but never studied for a large cell population due to the lack of readily accessible methods. With the QPC system reported here, both spatial distribution and temporal dynamics of dry mass for each cell can be measured. Furthermore, the QPC approach can also output cellular morphological features, such as...
surface area, sphericity and phase volume, which are valuable to studies based on cell shapes. We believe that, in the future, advancements in imaging processing algorithms, data acquisition facilities, and computation power can significantly augment the throughput of our QPC while still maintaining its current advantages including label free quantitative imaging, simplicity, flexibility and high measurement sensitivity.

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Literature Cited


Figure 1.
Schematic of the experimental setup. Left diagram shows the focal axis (Fourier) of the setup, whereas the right one shows the planar axis (image). C1–C4 are cylindrical lenses; L1 is an oil immersion condenser lens (1.2 NA); L2 is a 100× oil immersion objective lens (1.25 NA); L3 is a tube lens; B1 and B2 are 50/50 beam splitters; M1 is a CMOS camera; IP is the image plane; SP is the sample plane. [Color figure can be viewed at wileyonlinelibrary.com]
Figure 2.
(a) X-Y sample stage scanning map diagram; (b) X-Y sample stage scanning timing diagram. [Color figure can be viewed at wileyonlinelibrary.com]
Figure 3.
(a) A representative raw interferogram acquired using line-field QPC (the image is 8 bit); (b) Fourier transform of (a) (the color bar is in logarithmic scale); (c) Selected region within the black circle in (b) shifted to the origin of coordinate (the color bar is in logarithmic scale); (d) Phase map of inverse Fourier Transform of (c) (the color bar indicates the phase in radians). [Color figure can be viewed at wileyonlinelibrary.com]
Figure 4.
(a) Raw interferometric images for varying locations $\eta$ of the specimen when passing across the linefocused beam ($x$ is the image axis in real space; $y$ is the Fourier axis); (b) A series of phase maps of complex angular spectra; (c) Assembled QPI maps corresponding to different angles of plane wave illumination; (d) Final averaged phase image. [Color figure can be viewed at wileyonlinelibrary.com]
Figure 5.
(a) QPI maps of HeLa cells imaged by the line-illumination based QPC system (11 stripes of 5.068 × 0.1325 mm); (b) Magnified phase image from a selected region of (a) (the color bar indicates the phase in radians). [Color figure can be viewed at wileyonlinelibrary.com]
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Figure 7.
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