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Optical microscope illumination analysis using through-focus scanning optical microscopy

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Abstract

Misalignment of the aperture diaphragm present in optical microscopes results in angular illumination asymmetry (ANILAS) at the sample plane. Here we show that through-focus propagation of ANILAS results in lateral image shift with focus position. This could lead to substantial errors in quantitative results for optical methods that use through-focus images such as three-dimensional nanoparticle tracking, confocal microscopy, and through-focus scanning optical microscopy (TSOM). A correlation exists between ANILAS and the slant in TSOM images. Hence, the slant in the TSOM image can be used to detect, analyze and rectify the presence of ANILAS.

Industrial nanomanufacturing requires high throughput and low-cost three-dimensional (3D) shape metrology [1-4]. Through-focus scanning optical microscopy (TSOM) is one of the tools that meets these requirements with a sensitivity to dimensional differences down to the sub-nanometer level using a conventional optical microscope [2, 5-11]. TSOM used to be considered a low throughput technique, as several images are required to construct a TSOM image. However, developments in image acquisition techniques have significantly reduced the acquisition time. For example, a high-speed piezo scanner was used to capture 100 through-focus images in 200 ms [12]. Recently, using adaptive optics, image acquisition at a kilohertz (1 ms) speeds was successfully reported [9]. Multi-focus imaging microscopy produced through-focus images from a single measurement, and hence as fast as a conventional optical microscopy that captures a single image [13]. As a result, the TSOM method can be implemented in a high-throughput manner, satisfying the two most important requirements of measurements for industrial nanomanufacturing: sub-nanometer level sensitivity to three dimensional changes and high-throughput measurement.

In the past, we presented ways to optimize noise [14, 15] and enhance the sensitivity of TSOM measurements [16]. Even though TSOM is robust to optical aberrations due to its dependence on the differential method [5], it is desirable to obtain high fidelity TSOM images to enhance its sensitivity and also to enable comparison with simulated images and with other optical tools. In our experience, for moderately well-aligned optical microscopes (i.e., for most commercially available microscopes), one major source of distortion in TSOM images is the aberration in the illumination. Hence in the current paper, we concentrate on the effect of illumination quality on TSOM images.

Optical microscope images are sensitive to the alignment of the aperture diaphragm at a conjugate back focal plane. The main purpose of this aperture is to determine the illumination numerical aperture (INA). However, lateral deviation of the aperture diaphragm by few tens of micrometers from the optical axis can easily introduce substantial distortions in the illumination [17], especially with low INA as preferred for the TSOM method [16]. Unfortunately, it is common to find microscopes having aperture diaphragms misaligned from the optical axis. Misalignment is often observed even for microscopes with a fixed (or non-adjustable) aperture diaphragm location. Even if the microscope design satisfies the Kohler illumination condition (i.e., uniform spatial intensity), the illumination aperture misalignment may still cause considerable distortion in the image.

Distortions in illumination can be investigated by evaluating the angular illumination asymmetry (ANILAS) maps [17, 18]. Here we show that such aberrations in the illumination also produce distorted TSOM images, namely lateral image shift with focus height. Henceforth, such distorted TSOM images will be referred to as slanted TSOM images. We demonstrate here that the slant in TSOM images caused by illumination aberrations of this type can nearly be eliminated by simply re-aligning the aperture diaphragm to the optical axis. Conversely, the slanted TSOM images could provide an alternative, more convenient way of measuring ANILAS.

Illumination distortion can lead to quantitative misinterpretation of measurements that are based on through-focus images, such as confocal microscopy [19], 3D super-resolution imaging of nanoparticles [20], optical nanoparticle and bacteria tracking methods [12, 13, 21-23], and the TSOM method [6, 9, 24]. Similar to TSOM, the optical tracking methods also use a low illumination NA [12, 20], making the current study even more relevant to them also. We speculate that the slanted TSOM image reported in [9] could be due to distorted illumination, probably caused by illumination aperture misalignment. Hence, it is advisable to measure and correct the distortions in the illumination to make a proper analysis for these methods.

Here we briefly explain the process of constructing a TSOM image as we make use of it for illumination analysis. A typical TSOM image is a vertical cross-section of the three-dimensional optical data (3D space filled with optical data) [7] acquired using a conventional optical microscope as the focus height is scanned relative to the target [6, 8, 25]. A multimedia figure depicting the method for constructing a TSOM image is presented in Ref. [26]. In the TSOM image the X (horizontal), Y (vertical), and color scale axes represent the spatial position across the target, the focus position, and the optical intensity, respectively. A differential TSOM image (D-TSOM) is produced by subtracting two TSOM images, usually obtained from two similar targets that are being compared. D-TSOM images highlight three-dimensional shape differences down to a sub-nanometer scale [7, 8]. In addition, the D-TSOM image patterns are distinct for different types of parameter changes, but qualitatively similar for different magnitude changes in the same parameter [2, 5, 10], except with different intensity amplitude. TSOM has been successfully applied to the metrology of critical dimension (linewidth), overlay, patterned defect detection and analysis, fin field effect transistors (FinFETs), nanoparticles, photo-mask linewidth, thin-film thickness, through-silicon vias (TSVs), high-aspect-ratio (HAR) targets [2, 5-8, 10, 26-32]

and the like, with several three-dimensional-shape process-monitoring applications possible such as micro/nano electro mechanical system (MEMS/NEMS) devices, micro/nanofluidic channels, flexible electronics, self-assembled nanostructures, and waveguides.

A commercially available, research-grade optical microscope has been used for the experiment to compare the ANILAS measured by the grating method with the effects it has on the TSOM image [17]: illumination source = 520 nm, objective magnification = 50 $\times$, numerical aperture (NA) = 0.55. A 400 μm diameter aperture diaphragm is used to establish an illumination NA of 0.15. The aperture diaphragm is mounted on a X-Y-Z translational stage so that its position can be aligned relative to the optical axis using micrometers. To evaluate ANILAS, a grating target is used, which is an array of SiO_2 trenches with a nominal width of 100 nm having a pitch of 1000 nm over a Si substrate. Details of the analysis of illumination aberrations using the ANILAS maps are reported in our recent publications [17, 18]. The resulting ANILAS map shows the asymmetry of the angular illumination across the field-of-view (FOV). The illumination error is proportional to the ANILAS magnitude in an ANILAS map. For the TSOM measurements, isolated, amorphous silicon lines on a Si substrate (height = 97.3 nm) were used as targets. All the ANILAS data and the TSOM data reported here are the averages of five and three repeats, respectively.

In the current paper, we present the effect of four types of illumination conditions on the ANILAS map and on the TSOM image slant. The conditions include ideal alignment and three intentionally-created distortion conditions caused by moving the location of the aperture diaphragm by a known amount from the optical axis. Two such illumination conditions are shown in Fig. 1(a1) and (b1). For both of these, the aperture diaphragm is at the correct axial location ($Z = 0$). In Fig. 1(a1), the center of the aperture diaphragm aligns with the optical axis resulting in a symmetric angular illumination condition at the sample plane. This condition produces uniformly low ANILAS values across the entire FOV, creating a relatively flat, low-magnitude ANILAS map (Fig. 1(a2)). This is the most ideal (practically achievable) angular illumination condition for optical microscopes. Such an illumination condition produces an upright TSOM image (Fig. 1(a3)). A dotted line fit to the symmetric centers of the through-focus image profiles is almost normal to the X-axis, i.e., there is no lateral image shift with focus. This illumination condition produces no-slant TSOM images independent of the location and the orientation of the line target in the FOV.

Laterally shifted aperture diaphragm (in this case toward the right) produces asymmetric illumination as shown in Fig. 1(b1) ($X = 40 \mu\text{m} \pm 2 \mu\text{m}$, $Y = 0$, $Z = 0$), resulting in an ANILAS map as shown in Fig. 1(b2). In this case also the ANILAS map is still relatively flat, but is shifted up with some finite, positive mean ANILAS value. This indicates that the illumination distortions are relatively uniform across the FOV. An important observation with such a distorted illumination condition is that the TSOM image is slanted (Fig. 1(b3)), i.e., the center of the image shifts laterally with focus, giving an impression that the TSOM image is slanted left (tilted counter clockwise). Shifting the aperture diaphragm left ($X = -x$, $Y = 0$, $Z = 0$) produces a right-slanted TSOM image (tilted clockwise).

Independent of the location of the target in the FOV, the TSOM image will be slanted for the illumination condition shown in Fig. 1(b1), provided the orientation of the line target is

along the Y axis. For a line target oriented along the X axis, the TSOM image will continue to be upright (similar to Fig. 1(a3)), irrespective of the location in the FOV. However, if the aperture diaphragm is shifted both in the X and Y directions ($X = \pm x$, $Y = \pm y$, $Z = 0$), then the TSOM image will be slanted for the line target oriented along either the X or the Y axis, irrespective of the location in the FOV. (It would not be slanted for a line target aligned with the (x, y) misalignment vector.) The degree of the TSOM image slant is proportional to the amount of the lateral shift of the aperture diaphragm along the axis orthogonal to the orientation of the target line.

A primary application of TSOM is for 3D shape analysis of nanoscale targets using D-TSOM images. But this is expected to be done under optimal microscope conditions. Though it is not the main point of the current work, here we show that even under the sub-optimal illumination condition (Fig. 1(b1)) the TSOM method can still detect nanoscale dimensional differences as shown in Fig. 1(b4), similar to under the good illumination condition (Fig. 1(a1)) as shown in Fig. 1(a4), albeit with a bit lower sensitivity (optical intensity range (OIR) of 24.5 vs 20.5), confirming robustness of the TSOM method [5].

Moving the aperture diaphragm instead *along* the optical axis ($X = 0$, $Y = 0$, $Z = z$) toward the field diaphragm (Fig. 2(a1)) results in a non-flat ANILAS map (Figs. 2(a2) and 2(a3)). In this case, the degree of illumination distortion (based on the ANILAS maps) changes with the location within the FOV. The ANILAS value is lowest near the center of the FOV, increasing radially toward the edges. This indicates that the lowest illumination asymmetry exists near the center of the FOV and it increases gradually toward the edges. For this kind of illumination, the TSOM image of a Y-axis-aligned line target located away from the center region shows a slanted TSOM image (Fig. 2(a4)). However, if the target is located at the center region of the ANILAS map (with near-zero ANILAS value) the TSOM image is upright (Fig. 2(ab4)). As long as the line target is located in the center low-ANILAS region, it results in an upright TSOM image independent of its orientation.

Shifting the aperture diaphragm both axially and laterally ($X = -x$, $Y = 0$, $Z = z$) as shown in Fig. 2(b1) produces an off-center ANILAS map as shown in Figs. 2(b2) and 2(b3). The low-ANILAS region moves away from the center of the FOV. The amount of shift in the lowest ANILAS region from the center of the FOV is proportional to the amount of lateral shift of the aperture diaphragm. Shifting the aperture diaphragm in both the X and Y directions produces an ANILAS map where the lowest region is offset from the center of the FOV in both the X and Y directions in the FOV [17]. Even for such an off-center ANILAS map, the TSOM image at the center of the ANILAS map (lowest asymmetry) is upright (Fig. 2(ab4)), indicating that no matter where the “sweet spot” is located in the FOV, the through-focus data at that location provides a more undistorted result. This also shows the importance of determining the location of the “sweet spot” in the FOV. For such an asymmetric illumination condition, the TSOM image of a vertical line is slanted despite being at the center of the FOV (Fig. 2(b4)), similar to the illumination condition in Fig. 2(a4). However, the type of slant in the TSOM image (either left or right) depends on the location of the line target with respect to the center ANILAS region. The slant in the TSOM images are toward the left and the right, for the line targets located on the left and on the right sides of the center of the lowest-ANILAS region, respectively.

The slant in the TSOM images is equivalent to what would be seen if the microscope stage were moving laterally as a function of focus height. Hence precaution must be taken to identify the correct source for the TSOM image slant.

Based on the four conditions of illumination presented so far, a more generalized conclusion can be drawn as follows: the TSOM image of a line target located at the near-zero (lowest) ANILAS region produces an upright (or vertical) TSOM image, independent of its location and orientation within the FOV. Conversely, the TSOM image of a line target located anywhere in the FOV where there is a finite ANILAS value results in a slanted TSOM image. The presence of a slant in the TSOM image can be correlated with the illumination asymmetry, provided no physical lateral shift in the target occurs with focus position.

However, there is an exception. For a line target oriented parallel to a radial line drawn from its location in the FOV to the lowest-value center ANILAS region (e.g., as shown by the line identified by double arrows in Fig. 2(b3)), the TSOM image (taken perpendicular to the line target) will still be upright (no slant). Even though the location in the FOV has a finite ANILAS value, the illumination is still symmetric for a line oriented in this way. The slant in the TSOM image depends not only on the ANILAS value, but also on the line target orientation with respect to the ANILAS map [17].

Figure 3 shows that the degree of slant in the TSOM images is proportional to the ANILAS magnitude in the map. The lowest slanted TSOM image provides the location of the best (symmetric) illumination in the FOV. In the absence of the ANILAS map for a microscope, it is possible to measure the illumination distortion by observing the slant in the TSOM images of a series of lines filling the FOV as shown in Fig. 3(b); the slant-TSOM images should be measured with the target lines oriented alternately along both the X axis and Y axis.

In summary, a slight misalignment of the aperture diaphragm with the optical axis introduces asymmetry (or distortion) in the illumination even for optical microscopes designed for Kohler illumination. The angular illumination asymmetry (ANILAS) map provides a convenient way to visualize the distortions in the illumination at the sample plane. We have shown that through-focus propagation of the illumination distortions results in lateral image shift with focus position. The lateral image shift can be easily visualized as slant in the axis of TSOM images. The degree of slant in the TSOM image increases with the magnitude of the angular distortion in the illumination. Therefore, the slant in the TSOM image can also be used as an illumination test. A well-aligned microscope that produces an upright TSOM image improves the dimensional sensitivity of the TSOM method and also improves tool matching and simulation matching. The illumination test using the TSOM image method could be utilized for optimizing confocal microscopy which also makes use of through-focus images. However, proper precaution must be taken to distinguish a slant in the TSOM image from a lateral drift during the through-focus scanning with improper optomechanics.

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Fig. 1.

ANILAS with the illumination aperture at the ideal axial location. (a1) and (b1) are ray traces showing angular illumination at the sample plane for (a1) no lateral shift ($X = 0$, $Y = 0$, $Z = 0$), and (b1) finite lateral shift in the X direction ($X = x$, $Y = 0$, $Z = 0$). (a2) (a3) and (a4) are the ANILAS map, TSOM image, and D-TSOM image, respectively, for the illumination condition shown in (a1). (b2), (b3) and (b4) are the ANILAS map, TSOM image, and D-TSOM image, respectively, for the illumination condition shown in (b1). For (b2) the aperture diaphragm was moved to the right ($X = (40 \pm 2) \mu\text{m}$, $Y = 0$, $Z = 0$). The TSOM images (a3) and (b3) were obtained using a 950 nm wide line target. The D-TSOM images in (a4) and (b4) were obtained by subtracting TSOM images obtained from 950 nm and 900 nm wide line targets. OIR indicates the residual optical strength in the D-TSOM images.

Fig. 2.

Schematics showing angular illuminations at the sample plane with the aperture diaphragm moved along the optical axis toward the field diaphragm for (a1) no lateral shift ($X = 0, Y = 0, Z = z$), and (b1) with finite lateral (left) shift in the X direction ($X = -x, Y = 0, Z = z$). (a2) (a3) and (a4) are the 3D ANILAS map, 2D ANILAS map and TSOM image, respectively, for the illumination condition shown in (a1) with no lateral shift ($X = 0, Y = 0, Z = (1250 \pm 5) \mu\text{m}$). (a4) was obtained by placing the line at the location and in the orientation as shown by the arrow mark in (a3). (b2), (b3) and (b4) are the 3D ANILAS map, 2D ANILAS map and TSOM image, respectively, for the illumination condition shown in (b1) with $(20 \pm 2) \mu\text{m}$ lateral left shift ($X = (-20 \pm 2) \mu\text{m}, Y = 0, Z = (1250 \pm 5) \mu\text{m}$). (b4) was obtained by placing the line vertically at the location as shown by the arrow mark in (b3). (ab4) represents a typical upright TSOM image obtained by placing the vertically oriented line at the locations as indicated in (a3) and (b3) by red arrow marks. All the TSOM images were obtained using a 950 nm wide line.

Fig. 3.

Comparison of ANILAS map and slant-TSOM images. (a) ANILAS map obtained by moving the aperture diaphragm axially toward the field diaphragm and laterally toward left ($X = (-50 \pm 2) \mu\text{m}$, $Y = 0$, $Z = (1250 \pm 5) \mu\text{m}$). (b) An optical image of several vertical line targets obtained under the illumination condition shown in (a). TSOM images (c), (d) and (e) are of line the targets located as shown by arrow marks in (b). These locations within the microscope field are also indicated by arrow marks in (a). (The nominal line widths in (c), (d) and (e) are 950 nm, 800 nm, and 650 nm, respectively.)