Semiconductor Physics, Quantum Electronics & Optoelectronics. 2003. V. 6, N 4. P. 517-519.

PACS: 07.07.D.42.79.P.Q

Distortion compensation technique for high resolution microscopy

V.N. Borovytsky
Information Software Systems Ltd, 15 Bozhenko str., 03680 Kyiv, Ukraine

Abstract. The paper represents the technique for distortion compensation in digital images from high resolution optical microscopes. This technique is based on approximation of necessary pixel shifts as a power function that can be identified using small set of data from distorted digital images of diffraction gratings. The experimental studies confirmed efficiency of the proposed technique for decreasing distortion till a level of spatial discretization.

Keywords: distortion, optical microscope, digital image, pixel shift.

Paper received 20.11.03; accepted for publication 11.12.03.

1. Introduction

Optical microscopy has a wide field of applications including medicine, material science, semiconductor industry, machine building and *etc* [1,2]. Implementation of digital cameras and image processing software is one of the most perspective tendencies in optical microscopy [1]. Digital cameras installed on microscopes and attached to personal computers with image processing software make possible fast and effective capturing, saving, transmitting and processing of digital images [1].

Modern digital cameras have more than million photosensitive cells that are located with sub-micron accuracy in image plane of a microscope [3]. Thus small image corruptions caused by distortion of microscope optics become visible in digital images even a human observer can not see these small distortion. Good microscope optics has distortion in range 1–2 % and it can dramatically reduce precision of measuring locations and dimensions of large objects in digital images [2]. Thus the problem of distortion compensation becomes actual for optical microscopy.

2. Analysis of known techniques for distortion compensation

The known techniques for distortion compensation are based on operations like pixel shifts depending on pixel coordinates [4–6]. The necessary shifts are calculated using a distorted images of two dimensional periodical test-objects – grids, matrices of circles, boxes, points and etc. But direct application of these techniques for high resolution optical microscopy is difficult. First, two dimensional test-objects with micron dimensions of elements and sub-micron accuracy of their locations are extremely expensive and they are not widely used in microscopy laboratories. Second, digital images produced by optical microscopes have sufficient non-uniformity of intensity distribution. It introduces errors in binarization of a whole digital image and as a results pixel shift value can not be calculated for each pixel in a digital image. Third, for calculation of necessary pixel shifts and their application to digital images a user has to purchase the expensive software packages for microscopy such as Image Pro, Clemex, ASIS, Matrox Image Library and the others. Due to the mentioned reasons the distortion compensation using the known techniques becomes expensive and complicated procedure.

3. The proposed technique for distortion compensation

To overcome the disadvantages of the known techniques the new interesting approach has been found. The core idea of this approach is identification of pixel shift function using a small set of data from distorted images of a diffraction gratings. This pixel shift function in form of a displace mask for Adobe PhotoShop can be implemented to distorted images to reduce distortion [4,7]. The compensation procedure requires the following operations:

1. A set of digital images of vertical and horizontal diffraction gratings has to be prepared. The diffraction gratings with spatial periods of 300–1000 lines per mm are widely used in optical laboratories. They are not very expensive and they have sub-micron accuracy of line width, pitch and location.

2. The center zone in these images is considered as a reference one. In this zone an average value of spatial period of gratings in number of pixels can be calculated. It has to note that distortion appears in boundary zones of digital images [2, 4, 8]. The central zone of image can be considered as a zone without distortion.

3. For the boundary zone necessary pixels shifts should be identified for a number of pixels. These pixel shifts are equal to difference of pixel coordinated in a distorted digital image and in a non distorted one (Fig. 1):

$$\Delta x(x,y) = x_O - x = \frac{y}{y_O}\cdot x - x = \left(\frac{y}{n\cdot p_Y} - 1\right)\cdot x$$
$$\Delta y(x,y) = y_O - y = n\cdot p_Y - y, \qquad (1)$$

where $\Delta x(x,y)$, $\Delta y(x,y)$ – the necessary pixel shifst as a function of pixel coordinates in a distorted digital image,

x, y – the coordinates of the pixel in a distorted digital image,

x_O, y_O – the coordinates of the pixel in a non-distorted digital image,

n, p_Y – the number of lines from a center of the digital image till the point (x,y) and the average value of spatial period in pixel of grating (Fig. 1).

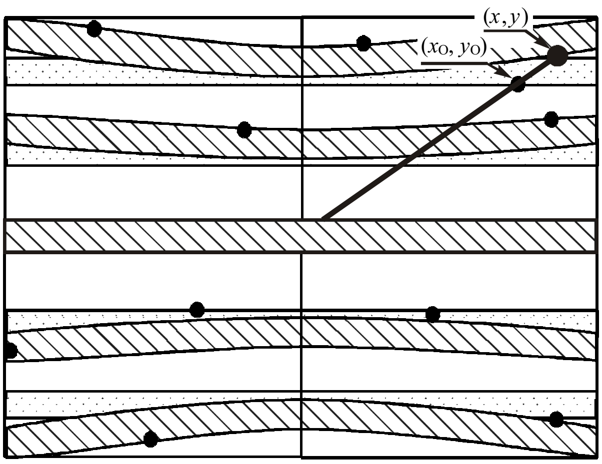

Fig. 1. Digital images of gratings with distortion (dark color) and without it (bright color). The pixel coordinates (x, y) in a distorted image correspond to the pixel coordinates (x_O, y_O) in a non-distorted one, data for distortion compensation is done by collection of this information from various pixels marked as black points in a distorted image.

4. The pixel shift function can be identified using the set of data about necessary pixel shifts in various pixels of digital images. The best fit power function is good formula for approximation of the pixel shift function because according to optical theory the geometrical distortion can be characterized by a power function with axial symmetry [8]. It is obvious that this axial symmetry remains only in case of square shape of photosensitive cells and equal spatial periods along axises OX and OY in a digital camera. The regression analysis of the data (1) helps to calculate the parameters of the pixel shift functions:

$$\Delta x(x,y) = A\cdot\left(2\cdot\frac{\sqrt{x^2+y^2}}{d}\right)^{PA}$$
$$\Delta y(x,y) = B\cdot\left(2\cdot\frac{\sqrt{x^2+y^2}}{d}\right)^{PB} \qquad (2)$$

where A, B, PA, PB – the parameters of the pixel shift functions that have to be calculated during search the best fit function for the data set (1), d – diagonal of a digital image:

$$d = \sqrt{x_M{}^2 + y_M{}^2}$$

x_M, y_M – the dimensions of a digital image in pixels.

5. The pixel shift functions (2) can be represented in form of a pixel displace mask for the widely used image processing software package Adobe PhotoShop [7]. This displace mask uses different color channels for coding necessary pixel shifts in horizontal and vertical directions:

$$r_{i,j} = \mathrm{int}(128 + k\cdot\Delta x(x - 0.5\cdot x_M, y - 0.5\cdot y_M))$$
$$g_{i,j} = \mathrm{int}(128 + k\cdot\Delta y(x - 0.5\cdot x_M, y - 0.5\cdot y_M)) \qquad (3)$$
$$b_{i,j} = 255$$

where $r_{i,j}$, $g_{i,j}$, $b_{i,j}$ – the color values of i, j – pixels of 24-bit color displace mask for distortion compensation [7], i, j – the pixel coordinates, k – the coefficient for getting values in range [–128, +128], int( ) – the function for conversion of real values into integer ones.

Generation of a pixel displace mask (3) can be done by any mathematical software packages such as MathCAD, MatLab only once for each combination of a microscope optics and a digital camera.

6. The generated displace mask (3) has to be applied to the distorted digital images. Adobe PhotoShop makes this operation quickly. This operation can be easily applied for a set of distorted digital images.

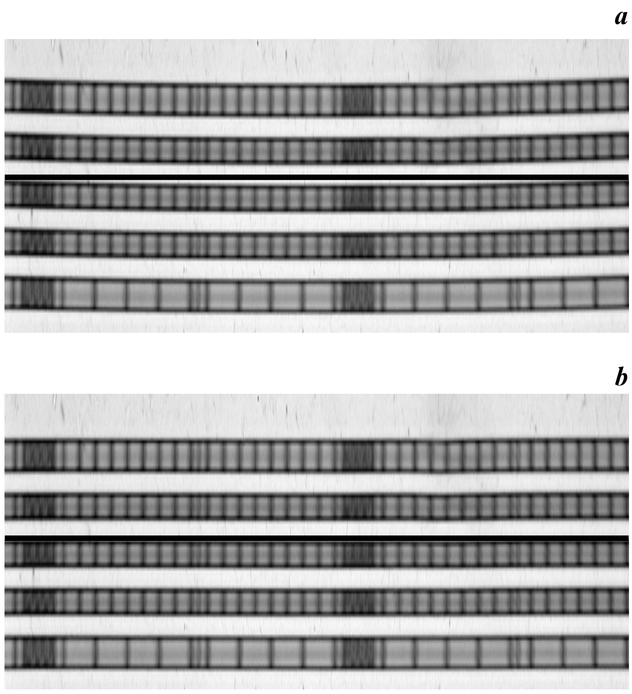

Fig. 2. Boundary fragments of digital images before (a) and after (b) distortion compensation (fragment dimensions in pixels – width 3040×height 230 – corresponds to a field in an object plane – 0.23×0.018 mm, image fragments are scaled in horizontal direction to make distortion 0.6 % visible, a black reference lines indicates how gratings images differs from straight lines before and after distortion compensation).

Conclusions

The proposed distortion compensation techniques is based on identification of pixel shift function using a small set of data from distorted digital images of diffraction gratings (1–3). The experimental studies have confirmed that this technique makes possible to reduce a geometrical distortion in high resolution microscopy from 0.6 % till 0.055 % (Fig. 2).

References

1. W. G. Hand, A practical guide to digital microscopy // *Photonics Spectra*, No10. p.100-104 (2001).
2. G.E. Skvortzov, V.A. Panov, N.I. Polakov, L.A. Fedin, *Microscopes.* – L.: Mashinostroenie, 1969.
3. B. L. Benamati, In search of ultimate Image Sensor // *Photonics Spectra*, No 9. p. 132–136 (2001).
4. Harri Ojanen, Automatic coorection of Lens distortion using digital image processing // *Preprint of the State University of New Jersey, Mathematical department, Rutgers. Preprint location is www.math.rutgers.edu/~ojanen/*
5. X. Wang, R. Ning, Accurate and efficient image intensifier distortion correction algorithm for volume tomo graphic angionaphy // Optical Engineering, **37**(3). p. 997-983 (1998).
6. V. Krzyzanek, Analysis of continiously distortted quasi-periodic images: two-dimensional reconstruction of S-layers of cyanobacteria // *Optical Engineering*, **39**(4), p. 872-878 (2000).
7. A.D.Greenberd, S.Greenberd, *Fundamental PhotoShop*, Berkeley: Osborne McGraw-Hill, 1997.
8. B.N. Begynov, N.P. Zakaznov, Theory of optical systems. – M.: Mashinostroenie, 1973.