Three-Dimensional Tracking at Micro-scale Using a Single Optical Microscope

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Abstract. 3D tracking is of fundamental importance to the development of practical visual applications to micromanipulation and microassembly. In this paper, a new 3D tracking method based on CAMSHIFT and Depth-from-Defocus is developed for micromanipulation and microassembly task. CAMSHIFT algorithm is used to find the size and location of moving object even the micro-objects is highly blurred. And Depth-from-defocus method is used to recover the depth information of the object from a measure of the level of defocus. The experimental results of blurring tracking and depth recovery of micro gear validate the feasibility of the proposed 3D tracking method.

Keywords: 3D tracking, autofocus, microscope, micromanipulation, microassembly.

1 Introduction

Visual tracking of microscopic images is of fundamental importance to micromanipulation and microassembly in providing noncontact dynamic measurement of poses of the objects of interest and their spatial relations. It also increases the flexibility of micromanipulation and microassembly by reducing the dependence on precise calibration. In a typical 3D microassembly situation, the micro object should be tracked in the 3D space in real time, while general optical microscope can not get the depth information for its limited depth of field. Confocal laser scanning microscopy is much too slow for real time tracking. The limited optical working distance between a microscope lens and a device to be manipulated indicates that stereo vision is not feasible. Therefore, how to get the depth information in 3D tracking has become a major roadblock to the development of practical visual applications to micromanipulation and microassembly.

In prior research efforts, many methods were attempted to accomplish the 3D tracking using a single optical microscope. However, most work employs 2-D recognition methods [1-5] sometimes in combination with an auto-focusing system, which ensures that the object to be recognized always stays in focus [6][7]. So the object can just be tracked but can not obtain 3D trajectories which are essential to visual servoing. Geometric Hashing and the Bounded Hough Transform have been used to recognise with up to 4 degrees-of-freedom for micromanipulation and microassembly[8-10]. However, as both recognition and tracking are purely combinatorial

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approaches, the memory requirements for the algorithms are high and image features can not be extracted when the level of defocus is significant. Because of the unique properties of microscope optics, microscopic computer vision differs significantly from macroscale computer vision. The small depth-of-field of microscope optics is a fundamental constraint that must be considered in developing microscopic computer vision techniques for micromanipulation and microassembly. Small amount of displacement error of working distance causes significantly blurred of target object in sight. Therefore, how to track the blurred object and get the 3D trajectories is totally important. Using a quantitative defocusing method to obtain 3D trajectories of cells using a fluorescent microscope successfully [11][12].

In this paper, a novel imaging technique that tracks (x,y,z,t)coordinates of micro-object for micromanipulation and microassembly using a single optical microscope is proposed. A widely used macroscale 2D track method called camshaft is used for microscopy images to track defocusing miro-object. And a depth from defocus based method is used to obtain the depth information in z direction. The integration of these two techniques is implemented successfully for obtaining 3D trajectories of micro-object.

The paper is organized as follows. Section II briefly introduces the 3D tracking technique based on CAMSHIFT and DFD and gives the corresponding theoritical analysis which is feasible for microscopy images. In section Ⅲ, Experiments are performed to test the effect of this tracking method. Conclusions and future work are discussed in Section Ⅳ.

2 3D Tracking Technique for Microscopy Image

2.1 CAMSHIFT Based Blurred Image Tracking for Microscopy Images

CAMSHIFT algorithm is widely used in face tracking about apperceiving user interface now [12-16]. It makes use of color information in region to track object and adopts a non-parameter technique and searches moving target by clustering method.

The CAMSHIFT algorithm is a modification of the meanshift algorithm which is a robust statistical method of finding the mode (top) of a probability distribution. Typically the probability distribution is derived from color via a histogram, although it could be produced from correlation, recognition scores or bolstered by frame differencing or motion detection schemes, or joint probabilities of different colors/motions etc. To be brief, CAMSHIFT algorithm applies target's hue character to find the size and location of the moving object in video image. Initial value of search window is set as current position and size before search [13]. In the next frame video image, search window is initialized by current position and size of moving object. The process is repeated to realize continuous object tracking. The theoretical analysis can be found in [14-16].

In this application, we use only the most simplistic approach: A 1-D Hue histogram is sampled from the object in an HSV color space version of the image. In our experiments we find that even the micro-objects is highly blurred, the color information is still reserved which is shown in Fig.1. It indicates that CAMSHIFT method can also be used to track burred micro-objects.

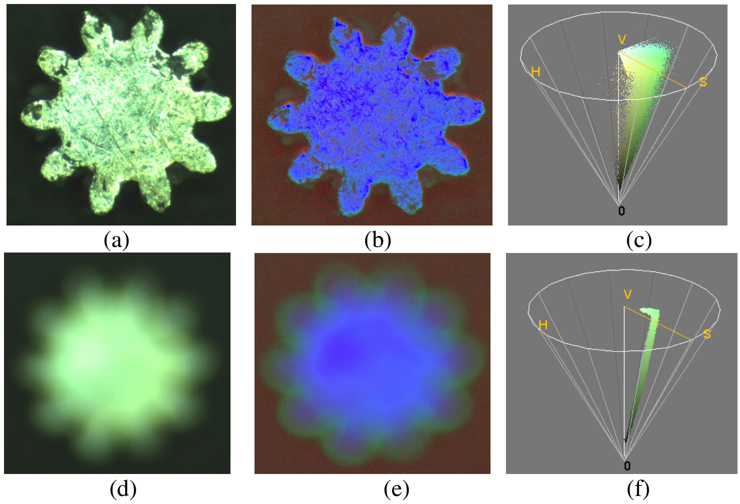

Fig. 1. Color information of focus image and blurred image of a micro gear: (a) Focus RGB image , (b)Focus HSVimage , (c) focus HSV model; (d)blurred RGB image (e) blurred HSVimage, (e) blurred HSV model

2.2 Depth Tracking Based on Depth from Defocus

Depth from defocus (DFD) was proposed by Pentland [16], it directly estimates the depth information from a measure of the level of defocus, which makes it suitable for real-time depth tracking. Blur is modeled as a point spread function (PSF). The sigma parameter of the PSF is proportional to the level of defocus and the depth information is related to the level of defocus, therefore, the depth information is obtained finally.

A spatial-domain Convolution/Deconvolution Transform (*S* Transform) has been developed for images and n-dimensional signals for the case of arbitrary order polynomials [13] [14]. If *f*(x, y) is an image that is a two-dimensional cubic polynomial defined by:

$$f(x,y)=\sum_{m=0}^{3}\sum_{n=0}^{3-m}a_{mn}x^{m}y^{n} \tag{1}$$

where a_{mn} is the polynomial coefficient; *m, n* are orders of the polynomial.

The restriction on the order of $f(x, y)$ is made to valid by applying a polynomial fitting least square smoothing filter to the image. The blurred or defocused image $g(x, y)$ can be described as the result of convolving the focused image $f(x, y)$ with the point spread function (PSF) $h(x, y)$:

$$g(x,y)=h(x,y)*f(x,y) \tag{2}$$

For any circularly symmetric PSF h(x, y), the spread parameter δ is defined:

$$\delta^{2}=\int_{+\infty}^{-\infty}\int_{+\infty}^{-\infty}(x^{2}+y^{2})h(x,y)dxdy \tag{3}$$

According to Equation (1), Equation (2) and Equation (3) can be rewritten as:

$$f(x,y)=g(x,y)-\frac{\delta^{2}}{4}\nabla^{2}g(x,y) \tag{4}$$

And obtain the following relations.

$$\delta^2 = \frac{4\iint_A (g(x,y) - f(x,y)dxdy}{\iint_A \nabla^2 g\ (x,y)dxdy} \quad (5)$$

where ∇^2 is the Laplacian operator.

Equation (5) is a very useful deconvolution formula and it provides a spatial-domain method to get the spread parameter.

As the depth information has direct relation with the spread parameter, the depth of the object can be gain through calculation of the spread parameter of a defocus image. A look-up table based calculation method is adopted in this paper to get the depth of the object in images. And the look-up table itself is obtained through calibration as follow.

(1) First an object is placed in the center of field-of-view. The light microscope is moved to the focus location and a focused image *f*(x, y) is obtained.

(2) The light microscope is then moved to another position and the distance between this position and the focused position are recorded. Then a defocused image was gain and the corresponding spread parameter was calculated through Equation (5).

(3) Repeat the process of step (2), and a look-up table was gain which links the distance between the defocus image position and the focused image position through spread parameter.

Therefore, when a blurred image of the object is recorded, the corresponding spread parameter is calculated and its depth information can be gain through the look-up table. The depth tracking process can be shown in Fig.2.

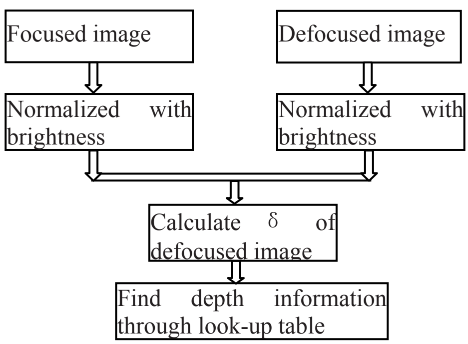

Fig. 2. Depth tracking process based on DFD method

2.3 3D Tracking Integrate CAMSHIFT and DFD Technique

As described above, the CAMSHIFT method can be used to track the X-Y position of the moving object, and the depth tracking can be realized based on DFD technique, therefore, the 3D tracking of moving object under microscope can be realized through integration of CAMSHIFT and DFD tracking method. The process of 3D tracking is shown in Fig.3.

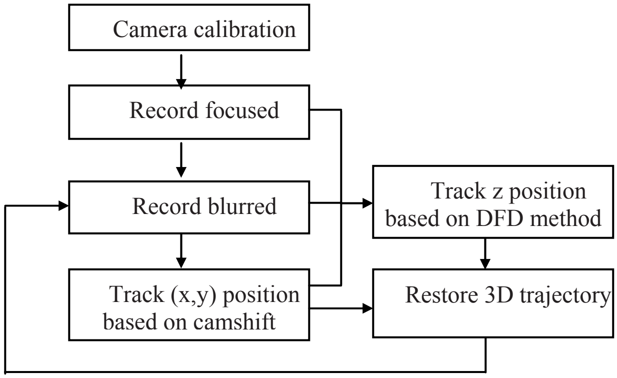

Fig. 3. 3D tracking process based on integration of CAMSHIFT and DFD

Firstly, the microscope system should be calibrated and a focused image is gain through autofocusing adjustment. Then, the object of interest is moved to a random position and a blurred image was recorded. The current (x,y) position of the object can be tracked through CAMSHIFT method. Accordingly, the calculation region in focused image and blurred image is obtained. Thirdly, the z position of the blurred object can be gain through look-up table calibrated beforehand based on DFD method. Finally, the 3D trajectory of the moving object can be gain by calculation at each position.

3 Experiment and Results

3.1 Microscope System

Experiment system is composed of a light zoom microscope, a CMOS camera and an auto focusing motor shown in Fig.4. The magnification of the microscope can be changed between 0.25×—1.8×. Because of the unique properties of zoom microscope, the depth-of-field is a little larger than the traditional microscope in the same magnification. The CMOS camera is capable of imaging a total of 800x600 pixels with each pixel 2.33μm.

The light microscope can be adjusted along the Z direction driven by a step motor. The positioning precision of the Z direction motor is about 0.05μm which corresponds to one step number of the motor. The speed of the step motor is 2000 steps per second which corresponds to 10μm per second. The 3D tracking algorithms can be programmed and executed on the computer.

3.2 Tracking Blurred Object

CAMSHIFT method has been proved to be effective for tracking an object in focus. This experiment aims to test its tracking character when an object is blurring under

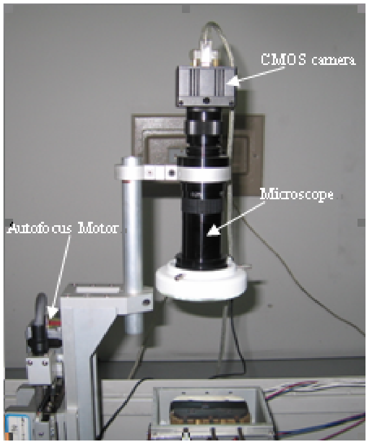

Fig. 4. Microscopic System

microscope. A micro gear with 1mm diameter was used to perform the tracking experiment. Firstly, the micro gear is positioned at the center of field of view of microscope, and the microscope is adjusted to gain in focus image of micro gear and the initials position of micro gear is recorded as (390,328) in pixels. Then, the microscope was adjust along Z direction with step of 100μm, and the defocus level of the current position is described with the distance between current position and the focus position. Finally, the center of micro gear was tracking with CAMSHIFT method, and the tracking error of this position was calculated. The tracking result was shown in Table 1, and the tracking error was shown in Fig.5, each pixel is 4.76μm.

Experiment results show that when the defocus level is less than 600μm, the average tracking error is under 10μm. Further increasing of the level of defocus will gradually increase in its tracking error, but do not exceed 50μm which is still acceptable to micromanipulation and microassembly. Therefore CAMSHIFT method gives a good result to track blurred microscopy images. Fig.6 shows the in focus image and a blur image of micro gear.

Table 1. Tracking result based on CAMSHIFT method

defocus Level (μm)	100	200	300	400	500	600	700	800	900	1000
X center (pixels)	390.0	390.0	389.5	389.5	389.5	389.5	387.5	386.5	385.5	384
Y Center (pixels)	327.0	327.0	328.0	327.5	327.0	327.5	327.0	327.0	327.0	327.5
Tracking error(μm)	4.76	4.76	2.38	3.37	5.32	3.37	8.58	12.82	17.33	28.66

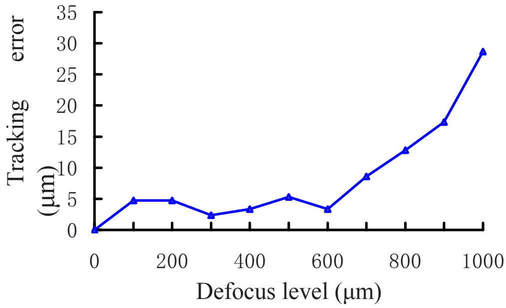

Fig. 5. Tracking error

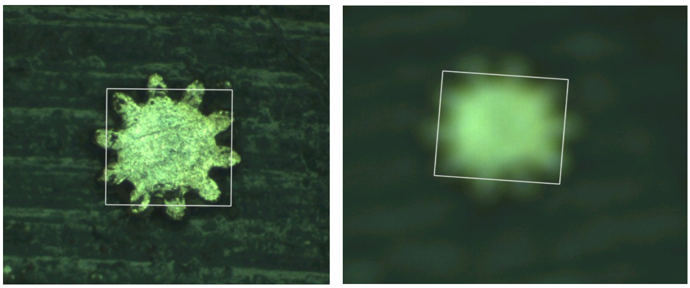

Fig. 6. Images of micro gear in different position during tracking based on camshift (a) In focus image; (b) a blur image of micro gear

3.3 Depth Recovery

As described in Section II, depth information of a defocused object can be gain through look-up table. Therefore, the look-up table must be calibrated beforehand.

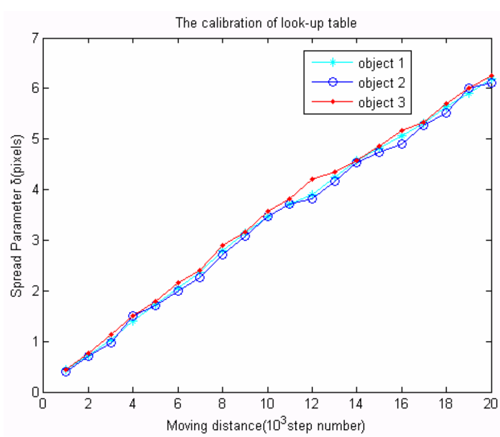

Fig. 7. Relationship between the distance away from the in focus position and the spread parameter which calibrated with three different objects

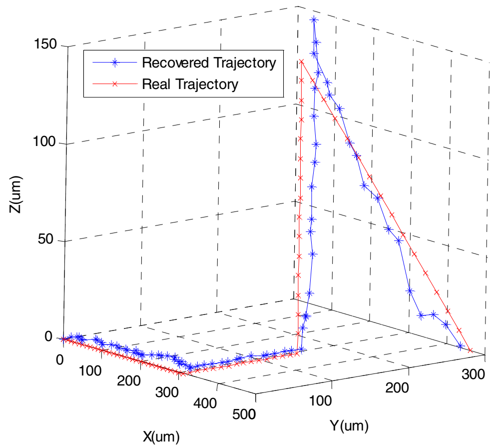

Fig. 8. Recovered 3D trajectory of micro gear in X, Y, Z direction

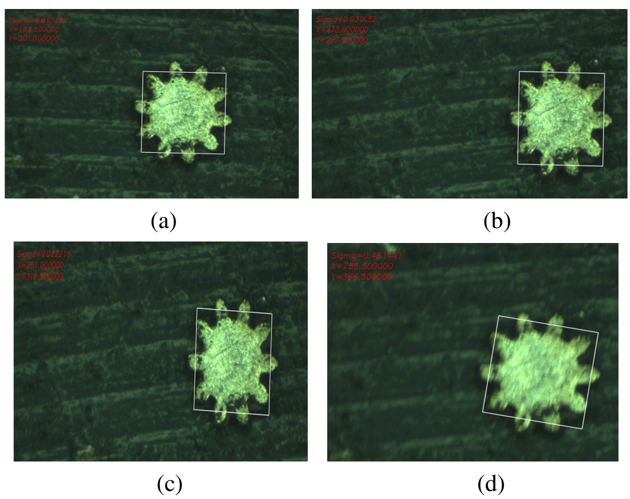

Fig. 9. Images in different tracking stages (a) Initial position; (b) Moving along X axis; (c) Moving along Y axis; (d) Moving in three direction at the same time

In this paper, three different objects are used to calibrate the look-up table. The calibrated result is given in Fig.7. As for the large depth of field of zoom microscope, the distance away from the in focus position and the spread parameter is approximate linear relationship. At the same time, the relationship is indispensable to the content of image.

Then a micro gear depth tracking experiment is performed, and the tracking error is about 8μm.

3.4 Trajectory Recovery

In this section, a 3D trajectory of micro-gear is recovered through integration of CAMSHIFT and DFD method. The micro gear is moved respectively along X axis, Y axis and Z axis, and then move in three direction at the same time, the travel line is shown in Fig.8 with red line. The recovered trajectory of the micro gear is shown with blue line. The tracking images at each stage are shown in Fig.9.

4 Conclusion

In this paper, a new 3D tracking method based on CAMSHIFT and DFD using a single camera is constructed. The CAMSHIFT based tracking method is proved to be effective even when the tracking object is blurring under microscope. Depth-from-defocus method based on look-up table is used to recover the depth information of an object in different focus plane. Through integrating the two methods, three dimensional information of micro object can be gain. The tracking experiments of micro gear under zoom microscope are performed and the experimental result validates the effectiveness of proposed tracking method.

Zoom microscope based vision system is usually used in micromanipulation and microassembly system, the tracking method presented in this paper is experimentally demonstrated to be efficient and effective for common optical microscope to micromanipulation and microassembly.

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