EXTRACTING A FOCUSED IMAGE FROM SEVERAL OUT OF FOCUS MICROMECHANICAL STRUCTURE IMAGES

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ABSTRACT

An all-focused image of a micromechanical structure can be extracted from several microscopic images with different focal planes. Spreading of the out-of-focus regions over the background, which shows the out-offocus object bigger than its actual size, causes errors when a sharpness function is applied. The focused area with low contrast is also one of the difficulties faced in order to easily distinguish them from the out-of-focus regions. We propose a method to estimate the focused section of each image and create a final focused image by concatenating these areas. We let the size of the tested areas vary in order to obtain a more accurate result. The method finds focused pixels among the images using a voting method and pastes them to the final image. This approach does not rely on any information of the microscope. No a priori knowledge of an optical system is needed.

1. INTRODUCTION

There are several focal planes in a microscopic image for objects that are non-planar. Focusing an image considering a particular plane results in an out-of-focus image in the areas located at other planes. Therefore, it is difficult to obtain an image with all planes in-focus using microscopes. Image processing techniques are required to restore an all-focused image from differently focused images.

The select and merge method has been proposed in order to generate a focus image from two differently focused ones [1]. This approach does not give good results when applied to a number of images [2]. The method proposed in [2], extends the select and merge method to handle more than two differently focused images. The method compares each image to its neighboring images with the estimation method of focused regions. This method is based on blurring an image using a Point Spread Function (PSF) which is approximated by Gaussian functions. Some auto focusing systems [3,4] require PSF estimation in their analysis to determine whether the input image is focused or not. ²Department of Electrical & Computer Engineering McMaster University, Hamilton, Ontario, Canada ²shoaah@mcmaster.ca

However, they cannot provide acceptable performance due to difficulties encounter when estimating the blur parameters accurately.

Another proposed method for generating a focus image is based on image fusion [5]. This method must be optimized for the fusion algorithm parameters which are expected to affect the contrast and stability of the image. In [6], a focus image is obtained out of two images using image fusion, the wavelet transform, and an area-based feature selection scheme. This method is a multi resolution analysis that estimates the focused regions using derivatives. It then merges the focused regions to obtain the all focused image. In general, these methods do not perform well around boundary regions [1].

In this work, we acquired several images of a micromechanical structure using a light microscope equipped with a CCD camera with different focus settings as shown in Figure 1. We propose a novel method for restoring the in-focus image out of these images without relying on any knowledge of the microscope camera.

2. CHARACTERISTICS OF THE IMAGES

Figure 1 shows a copper membrane, which is attached to the substrate through supported springs. The membrane is located 300 µm below the substrate and as shown in Figure 1, spring bars are bended in angle and attached to the membrane. The copper membrane is a reflective surface. It reflects the light illuminated by the light microscope and causes the planar surfaces to shine very brightly and the bended beams to look dark. A defocused object under a microscope is generally blurred and in some cases is even completely invisible. It appears also larger than the actual size. The focused areas in each image in Figure 1 are marked with circles. We can see that Figure 1a is focused on the highest level of the structure that contains attached bars. Figure 1f is focused on the lowest level which is the membrane. These images were taken using a microscope with a magnification of 50x and focus steps of 50µm.



Figure 1: (a)–(f) six differently focused images of a micromechanical structure. The focused regions are marked with a circle in each image and the image size is 300 by 400 pixels.

3. DETECTING FOCUSED PIXELS USING THE IMAGE GRADIENT

The focused areas are sharper in the image than the defocused areas which appear blurred or sometimes may not show at all. In a row that contains both, focused and out-of-focused regions, we expect a sharp transition from background to the focused part, while this transition is smoother for defocused area and yields to a sloped line as can be seen in Figure 2.

There are several techniques that can be used to determine the sharpness of an image. One of these techniques for example uses the Brenner function [7]. For our approach, a threshold is applied to the derivative of the image to obtain the edges of this derivative. The derivative of an image will have strong edges for focused objects. Considering that the out-of-focus regions can be modeled using a PSF estimated by a Gaussian function, the edges of the derivative of these regions will not have high intensity values. By counting the number of pixels in the edges of an out-of-focus area, it can be seen that the more blurring i.e. large standard deviation, this count will more likely be less than the count of edges in a focused area. This counting is the metric used in order to assess the amount of blurring. In this paper, the derivative is approximated using the Roberts operator [8].

Next, the approach divides the input images into blocks and calculates their sharpness as indicated before. For a given position, the blocks that have the maximum sharpness among the corresponding blocks in other input images, are the ones used in the final image.



Figure 2: The 245th row of the image shown in Figure 1d.

4. IMAGE ENHACEMENT

One of the problems encountered when creating a complete focused image, is that the bended beams are dark and the sharpness function does not detect enough transition between these beams and the background in order to detect them as a sharp area. The marked area in Figure 3a refers to a focused area which is not detected in Figure 3b.



Figure 3: (a) Image showing focused areas marked with a circle, (b) the result of applying the gradient to (a).

In order to solve this problem image contrast enhancement is applied. This can be achieved by applying a gamma function [9] to each input image. As Figure 4 indicates, all focused areas in Figure 4a are now detected.

5. SIZE DISTORTION EFFECT

Spreading of the defocused regions over the background causes the proposed sharpness functions to detect a false

sharpness value in blocks that contain those areas. As Figure 5 shows, there are some errors in detecting the focused areas. The left arrows indicate the areas which are selected from Figure 1f. On the right side of Figure 5, we see that the out-of-focus areas at the right side of Figure 1a cause errors as those found in Figure 5. These false selections are due to the fact that the defocused areas in that image appear bigger than the actual size. The locations of these errors are also different for different block sizes.

In our proposed method the image is divided into blocks of different sizes. Then we determine which image is the most focused one for each of these blocks and assign that image to all the pixels in that block. This process is repeated for different block sizes to provide a solution which is independent of the block size.

The most focused pixel then is defined by taking a vote for each pixel. The idea is to find the maximum times each pixel is selected from a particular input image and assign that image to that pixel. It means that the pixel has to be selected from the image whose vote in this process has the largest count.



Figure 4: The result of applying the gradient to Figure 3a after enhancing the image using the gamma function.



Figure 5: The result of applying the gradient to detect the focused regions, the block size is 25. The arrows show some of the errors.

6. THE PROPOSED METHOD ALGORITHM

The final focused image is formed from the differently focused images using the following two part algorithm.

Part 1: Identifying the best focused location.

- 1. Load the differently focused images.
- 2. Apply the gamma function to each image.
- 3. Apply the gradient function to each image and obtain the binary image by applying a threshold.
- 4. Divide each binary image into blocks of size n x m.
- 5. In each binary image, count the pixels with value 1, detected edges, within the blocks.
- 6. For a given block, the image that has the best focus for that block region is the one which has the highest count for that block region.

However, the above procedure has a problem with outof-focus regions due to the size distortion of the out-offocus location. The error due to the out-of-focus size distortion is solved using part 2 of the algorithm.

Part 2: Removing the size distortion effect.

- 7. Repeat steps 4 to 6 for different arbitrary block sizes.
- 8. Now the final image is formed pixel by pixel by identifying the image which was selected as the most focused, the most often amongst the arbitrary block sizes, in step 6. This voting process, thus, identifies from which sub-image each pixel in the final image is sourced.

7. FINAL RESULT

Figure 6b shows the result of applying the gamma function followed by the gradient applied to Figure 1a. It can be seen that the focused parts are detected.

Figure 7 shows the result of dividing the image into blocks of sizes 10, 20, 25 and 50. The assigned input images to the pixels are shown in different gray scale level. Each level corresponds to one of the input images.

After repeating step 4 to 6 using different block sizes, it is time to find which pixels are focused. This is achieved by taking a vote for the assigned input images to each pixel. Figure 8 shows the final all-focused image. We can see that there is less error due to out-of-focus size distortion. Repeating the algorithm for more block sizes will result in a more accurate result.

8. CONCLUSIONS

An all focused image has been extracted from several micromechanical structure images with different focal planes. The proposed algorithm contains two parts. In the first part of the algorithm, the images are enhanced using the gamma function and the gradient function is used to detect the best focused blocks among the images. The focused image is generated by concatenating the best focused blocks to the final image. At the second part, the first part was repeated for different block sizes and the voting process was then performed to remove the out-offocus size distortion error.



(a)



(b)

Figure 6: (a) the image, (b) the gradient.



Figure 7: Assigned input images to the pixels in step 7 when the block size is (a) 10, (b) 20, (c) 25, (d) 50.



Fig.8: The final all-focused image.

9. REFERENCES

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