# **3D** Microscopic Image Construction using High Dynamic Range Imaging

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#### Abstract

Shape From Focus (SFF) is the most effective technique for recovering 3D object shape in optical microscopic scenes. Although numerous methods have been recently proposed, less attention has been paid to the quality of source images, which directly affects the accuracy of 3D shape recovery. One of the critical factors impacting the source image quality is the high dynamic range issue, which is caused by the gap between the high dynamic ranges of the real world scenes and the low dynamic range images that the cameras capture. We now present a microscopic 3D shape recovery system based on high dynamic range (HDR) imaging technique. We have conducted experiments on constructing the 3D shapes of difficult-to-image materials such as metal and shiny plastic surfaces, where conventional imaging techniques will have difficulty capturing detail, and will thus result in poor 3D reconstruction. We present experimental results to show that the proposed HDR-based SFF 3D method yields more accurate and robust results than traditional non-HDR techniques for a variety of materials.

#### Introduction

Shape from focus (SFF) is a key technique that is widely used in the field of microscopic systems to obtain the depth map of the observed samples[1]. This technique has generated considerable recent research interest, since depth can be recovered by means of only one off-the-shelf camera rather than a stereo camera system [2]. The main drawback of this method is that performances are affected by factors such as insufficient dynamic range of the captured images [3]. In light microscopy, when the specimens' surfaces reflect light unevenly or when the materials such as metal contain specular reflective surfaces, photomicrography will encounter the high dynamic range issue resulting in images that lack details in some areas, appearing either underexposed or overexposed [4]. Much research in recent years has focused on the accuracy of focus measure. Unfortunately, less attention has been paid to the challenge of how to capture satisfactory images for 3D shape recovery.

To overcome this limitation, many workers have developed high dynamic range (HDR) imaging algorithms based on a set of differently exposed images of the same scene. After calibration, these differently exposed images are combined into one HDR image, often called the radiance map, which is usually represented with 32 bits per pixels or even higher bit depth. Such an HDR imaging technique can effectively overcome the HDR problem [5]. However, due to the high computational complexity of the HDR imaging process, current common microscopy 3D construction systems are limited to SFF to HDR images, which involves capturing several differently exposed still photographs, generating a high dynamic range radiance map, and finally tone-mapping the radiance map for subsequent processing. With the rapid development and much more extensive applications of GPU on computer vision tasks, it is possible to construct a considerably robust 3D system based on HDR images in order to improve the accuracy of 3D construction for difficult-to-image specimen samples.

In SFF, for each pixel of each image in the image stack, a focus measure is calculated in order to determine the best candidate pixel for the construction [6]. In the literature, the focus measure strategies have been grouped into two families according to their application prospects, in terms of 3D shape recovery and multifocus image fusion, respectively [7]. The first family aims at generating accurate 3D shapes from the focus measure, whereas the goal of the second family is to generate one single image which is focused everywhere. Specifically, several comparative studies have been carried out for both two applications. Pertuz has presented numerous experimental analyses of the focus measure operators for SFF techniques [8] and experimental results showed how different imaging conditions such as saturation, contrast, noise level and window size can affect the accuracy of 3D construction. Tian has presented a bilateral focus measure criterion to exploit both the strength and the phase coherence that are evaluated using the gradient information of the source images [9].

In this paper, we present a 3D microscopic image construction system based on high dynamic range imaging. The system consists of two main stages: HDR images acquisition and 3D scene reconstruction. The first step aims at obtaining high quality source images whereas the 3D scene reconstruction aims at generating an accurate and real 3D shapes from the source images.

#### Shape from focus

A series of images is captured by a monocular microscope by means of varying the object to lens distance Z (see Fig. 1). In addition, the depth information is recovered from the image stack through the estimation of focus measure to every pixel of every image. During the analysis of the focus measure, the candidate pixel with the maximal focus measure is found and marked for subsequent 3D shape reconstruction. The depth map is constructed based on the Z value associated to the marked image [10].

When a series of images  $I_0...I_n$  is obtained under varying object to lens distances  $z_0...z_n$ , the focus measure is calculated for each image by applying a focus measure operator FM(). The expression can be written for every pixel  $I_i(x, y)$  as

$$fm_i(x, y) = FM(I_i(x, y))$$
(1)

Afterwards, for each pixel (x, y) in these images, the image index with the maximum fm is identified:

$$\hat{i}(x, y) = \operatorname{argmax}_{i} fm_{i}(x, y)$$
<sup>(2)</sup>

The depth map of the scene is then simply associated with Z-value

$$Z(x, y) = z_i(x, y)$$
(3)



Fig.1. Microscopic Shape from Focus.

#### HDR images acquisition system

A simple approach to achieving HDR image using a single off-theshelf sensor is adopted [11], which has been published recently by the authors. As illustrated in Fig.2, our microscopic HDR images acquisition system involves four steps as follows: (1) calibrating a camera response curve; (2) image acquisition with varying exposure values; (3) HDR radiance map generation; (4) visualization and preservation of HDR images by tone-mapping. Fig.3 shows one example of tone mapped HDR image. From the HDR image we can see both the bright and dark areas clearly, so reducing the interference of both the shadows and the bright light. Once the HDR images have been acquired, a phase correction algorithm [12] and a foreground-background segmentation method are applied to keep all images aligned, which is important for the subsequent 3D reconstruction step.



Fig.2. Flowchart of HDR Image Generation Process.

#### 3D shape recovery

The goal of this paper aims at generating one 3D shape of object from multi-focus images. To achieve this goal, many focus measures have been proposed in the literature. Traditionally, the focus measure operator is applied to each pixel of the image which is only time-consuming but also induces much noise into the step of 3D recovery. To address this, a quad-tree based algorithm for multi-focus image fusion can be used [13]. In our application, we followed [13]'s quad-tree strategy to detect the maximum focused blocks with optimal sizes.



**Fig.3.** Comparison between normal auto-exposure images and HDR images. Left images are captured with auto exposure. Right images are captured in HDR mode.

In practice, the scenario in the microscopic field is not too complex, as the focused area of the scenario only occupies one limited and connected region rather than multiple separated and discrete areas like the scene in the macro-world. Therefore, it seems efficient and natural for us to apply the quad-tree strategy in order to decompose the source images into blocks with optimal sizes firstly.

At the beginning, the m source images are input as the root blockset at the first level of the quad-tree structure. For the root blockset, the maximum difference in focus-measures (MDFM) and the sum of the maximum difference (SMDG) in gradients are calculated respectively.

$$MDFM = FM_{max} - FM_{min}$$
(4)

$$SMDG = \sum \sum [grad_{max}(x, y) - (grad_{min}(x, y))]$$
$$= \sum \sum grad_{max}(x, y) - \sum \sum grad_{min}(x, y)$$
(5)

Where  $FM_{max}$  and  $FM_{min}$  are the maximum and minimum focusmeasures of each block. If  $MDFM \geq 0.98 \times SMDG$ , then this block-set includes the fully focused block which can be found by measuring the focus measure. Otherwise, this block-set should be further subdivided into four smaller blocks at the next level. This process is repeated on the smaller block-set, until all focused blocks is reached. Finally, the Z-value of the detected focused blocks will be selected as the candidates to be merged into 3D shape reconstruction.

### Experimental results

#### **Experimental setup**

In order to perform displacement of the optical lens, we use an automated microscope, which is composed of a conventional optical microscope, a displacement stage movable along x, y, z directions manually or motorized, a CMOS camera and a personal computer (see Fig.4). A first configuration uses the N-800D motorized microscope manufactured by Novel Optics which is fully automated and the precision of leading screw stage is 1 $\mu$ m. The CMOS camera is a 3 Mega pixel camera which can capture 20 frames per second with the resolution of 1024 × 768.

We carry out the accuracy evaluation on references specimens which include three different types of material: a standard metal screw with known geometry, a plastic English letter and a part of electronic chip.



Fig.4. Microscopic 3D Reconstruction System.

#### Accuracy

We have used two series of images to evaluate the performance of the proposed approach, SFF with HDR and normal SFF without HDR. A sequence of 14 images of a real screw is acquired and the scan range of this scene is 5.4mm. Fig. 5 shows the 3D visualization of the depth maps. It can be seen from the left column, the 3D shape recovered by HDR-SFF is better than the result of normal SFF by reference to the ground truth, which is created by us according to the source images and the associated depths. It is also noticeable that both in the shadow and highlight areas, the performance of HDR-SFF are more robust than the normal SFF.

In order to compare the performance of different techniques, a measure to evaluate the quality of the obtained depth-maps is utilized. In this case, we use the root mean square error (RMSE) between the measured depth of the object Z(i, j) and the ground truth depth  $G_T(i, j)$ .

RMSE = 
$$\sqrt{\frac{1}{A} \sum_{(i,j \in A)} (G_{T}(i,j) - Z(i,j)^{2})}$$
. (6)

Table 1 summarizes the RMSE of 3D shape reconstruction achieved under different focus measure operators with two image sequences for the metal specimen. For comparison purposes, all focus operators have been implemented for two image sequences in equal terms. Comparing the RMSE (mm/pixel) under different operators, it is evident that the performance of SFF with HDR images is superior to the performance of SFF without HDR images. To demonstrate the effectiveness of our method, we test the algorithm on another two different materials: plastics and electronic chip. As for the plastic material, we selected an English letter d from a bank card, as shown in Fig.6. As described in previous section, the accuracy of the two methods has been assessed by performing 3D reconstructions with different source images (see Fig.7).



**Fig.5.** 3D visualizations of the depth map constructed with HDR images (a, b) and without HDR (c, d) associated the ground truth (e, f).

 Table 1. 3D reconstruction accuracies of HDR SFF and Normal SFF for a metal screw.

	Reconstruction Errors(RMSE)	
Focus Measure Operators	HDR SFF	Normal SFF
SML	0.1368	0.1608
TENG	0.1485	0.3252
WAVS	0.1486	0.1672
LAPM	0.1530	0.1723
LAP3	0.1530	0.1723
SWML	0.1538	0.1759
GDER	0.1542	0.3318
SFRQ	0.1543	0.2638
LAPD	0.1553	0.1697
GLVM	0.1560	0.1670
GLVA	0.1574	0.3384
TENV	0.1596	0.2627
GRAI	0.1641	0.3310
WAVV	0.1649	0.1824
BREN	0.1712	0.1960
LAPE	0.1720	0.2183
LAPV	0.1723	0.2162
GRAS	0.1746	0.2531
HISR	0.1756	0.3577
GRAE	0.1762	0.1959
SFIL	0.2167	0.4028
GRA3	0.2193	0.3829



**Fig.6.** 3D visualizations of the depth map constructed with HDR images (a, b) and without HDR (c, d) associated the ground truth (e, f).



**Fig.7.** Comparison of 3D reconstruction accuracy (RMSE) of plastic material with different focus measure operators.

Similarly, we have done experiments on an electronic chip, as shown in Fig.9. And the accuracy evaluation can be found in Fig.8. It is noticeable that HDR–SFF clearly outperforms traditional SFF schemes. The depth maps obtained with HDR SFF are smooth, contain fewer discontinuities and closely resemble the actual structures of the observed sample.



**Fig.8.** Comparison of 3D reconstruction accuracy (RMSE) of electronic chip with different focus measure operators.



**Fig.9.** 3D visualizations of the depth map constructed with HDR images (a, b) and without HDR (c, d) associated the ground truth (e, f).

## Effectiveness of the local video tone-mapping operator

In order to compare the performance of our tone mapping method to other methods, another set of experiments is carried out, which involves tone-mapping the raw 16-bit images into normal 8-bit images with different tone-mappers. Then using those low dynamic range images obtained by different tone mapping methods, the same process of 3D construction explained in section 4.4 was repeated. Fig.10. shows the 3D visualization for shapes constructed by LDR images with different tone mappers. The list of tone mapping operators we used for comparison is shown in Table 2.

 Table 2. The evaluation is based on equation 6 and the root mean square error (RMSE) is shown in right column.

	Tono monning Operators	RMSE (mm/nival)
	Tone mapping Operators	(mm/pixer)
а	OURs	0.1368
b	AshikhminTMO	0.2000
с	DragoTMO	0.2225
d	DurandTMO	0.1822
e	FerwerdaTMO	0.1917
f	KrawczykTMO	0.1853
g	LogarithmicTMO	0.1781
h	MertensTMO	0.1789
i	GlobalSGB	0.2377
j	GlobalPow SGB	0.2064
k	NormalizeTMO	0.1787
1	ReinhardBilTMO	0.1841
m	ReinhardTMO	0.1816
n	SchlickTMO	0.2311
0	TumblinRushmeierTMO	0.1822
р	WardGlobalTMO	0.1831
q	WardHistAdjTMO	0.1789



Fig.10. 3D shape construction results using low dynamic range images of different tone mapping methods.

# **Concluding Remarks**

We have presented a 3D microscopic image construction using high dynamic range imaging. We have demonstrated that it is possible to employ a conventional optical microscope and an off the shelf camera without any physical modification to build a 3D system based on shape form focus technique. The use of HDR images is the key enabling technology. Regarding the reconstruction accuracy, a root mean square error (RMSE) is calculated for two different source images (HDR images and common images) under various focus measure operators. We also tested the algorithm on three different materials and 17 types of tone-mapping operators, including ours, were implemented in the same condition to demonstrate the effectiveness of our video tone mapping method. The experimental results have demonstrated that the use of HDR images can achieve better results than a method implemented with common images, especially in shadow and highlight areas.

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