Experimental demonstration of an Optical-Sectioning Compressive Sensing Microscope (CSM)

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Abstract: In this paper we present the design and implementation of a Compressive Sensing Microscopy (CSM) imaging system, which uses the Compressive Sensing (CS) method to realize optical-sectioning imaging. The theoretical aspect of the proposed system is investigated using the mathematical model of the CS method and an experimental prototype is constructed to verify the CSM design. Compared to conventional optical-sectioning microscopes (such as Laser Scanning Confocal Microscopes (LSCMs) or Programmable Array Microscopes (PAMs)), the CSM system realizes optical-sectioning imaging using a single-pixel photo detector and without any mechanical scanning process. The complete information of the imaging scene is reconstructed from the CS measurements numerically.

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1. Introduction

Optical-sectioning microscopy is widely used in the fields of cellular imaging, bio-medical analysis, and semiconductor inspection. This process eliminates the “out-of-focus” light from the imaging scene and thus generates images of higher contrast and better resolution than wide-field microscopes [1]. Conventional optical-sectioning microscopy tools employ an
exhaustive sampling strategy. To capture an image, they record the information from every resolution-limited point in the imaging scene. For instance, in laser-scanning confocal microscopes (LSCMs), this sampling strategy is realized by spatial/temporal scanning instruments (such as galvo-mirrors or spinning disks) and single-pixel detectors, whereas in PAM systems, dynamic pinhole-masks and CCD cameras are used [2–4]. This exhaustive sampling strategy guarantees the completeness of the image-acquisition process, but it generates a certain amount of redundant information [5–7].

An alternative sampling strategy is to filter out the redundant information prior to image acquisition by the detector. Therefore, valuable detector resources can be dedicated to the collection of the more useful information. The compressive sensing (CS) method provides a feasible solution for realizing such an “efficient” sampling strategy [5–9]. This method has been used in building macro-field imaging systems [8,9]. In such systems, incoherent measurement patterns (such as random patterns) are used to modulate the intensity of optical images and the original image is reconstructed from the modulation results (CS measurements) by solving a minimization problem. In our previous work, we also reported the fabrication of a CS-based microscopy (CSM) imaging system and presented image reconstruction results based on reflective semiconductor samples [10].

Like PAM systems, CSM systems also use a digital micromirror device (DMD) to implement pinhole-patterns, which modulate the illumination/detection of the imaging scene. The most apparent difference between the PAM system and the CSM system is the design of the pinhole-patterns. PAM systems usually use raster-scanning patterns, such as line-patterns or dot-lattices, to collect information from every resolution-limited point in the imaging scene [2–4], whereas in CSM systems, CS measurement patterns are used. The implementation of the raster-scanning patterns puts high demand on the optical/digital design of the microscope system. For instance, PAM systems use a relay-lens to transfer the “confocal” information from the DMD mirror-plane to a CCD camera. To optimize the image quality, the relay-lens needs some special optical treatments such that the 24° relative tilting between the DMD mirror-plane and the CCD camera can be taken into consideration. Also, depending on the sparsity of the implemented raster-scanning patterns, multiple CCD images are needed to generate one “confocal” image. This means the majority of the data collected by the CCD camera is not utilized. In CSM systems, pinhole-patterns are designed according to the CS method. In this case, we are only interested in collecting the CS measurements, which are the summed intensities of the image-light modulated by different pinhole-patterns [7–9]. A simple focusing lens and a single-pixel detector can be used to collect the summed intensity. Also, CSM systems have a higher efficiency in utilizing the detector data, because a certain amount of the redundant information has been filtered out prior to the image acquisition by the detector [5–10].

The challenge of building a workable CSM system lies mostly in the design of the CS measurement pattern. In this work, we introduce the implementation of a special CS measurement pattern, called modified scrambled-block Hadamard ensemble (MSBHE) [11], with our prototype CSM system. Compared to other CS measurement patterns (such as random patterns or Hadamard patterns), MSBHE patterns can more effectively eliminate the “out-of-focus” light, and in the meantime collect the significant frequency components of the imaging scene in the Hadamard domain. The original image is reconstructed from the significant frequency components. We also present the application of the CSM system in the field of fluorescent optical-sectioning microscopy. The optical-sectioning performance of the CSM system is demonstrated in both simulation and experimental settings.

In the remainder of this paper, we present the details of this work. This paper is organized as follows: Section 2 gives a short introduction to the CS method; Section 3 introduces the light propagation model of the CSM system; Section 4 examines the performance of the CSM system in the simulation environment; Section 5 presents the experimental results and Section 6 concludes the work.
2. Compressive sampling

In CS-based imaging (CI) systems, incoherent measurement patterns (such as random patterns) are used to modulate the intensity of optical images. Mathematically, the CS measurement process can be described as [5–9]:

\[ y_k = \langle \phi_k, x \rangle + v, \quad k = 1, 2, \ldots, K \quad \text{or} \quad Y = \Phi x + v = \Phi \phi \theta + v, \]

where \( x \) is the original image and \( x = \phi \theta \). Here, we assume \( x \) has a pixel dimension of \( N \times N \), \( \phi \) is the basis under which \( x \) has a sparse representation, and \( \theta \) is the corresponding coefficient vector. \( K \) is the number of measurements. \( \phi_k \) is the \( k \)-th measurement pattern and \( y_k \) is the \( k \)-th measurement result. The inner product \( \langle \phi_k, x \rangle \) represents the weighted measurement between \( \phi_k \) and the image \( x \). \( \Phi = [\phi_1, \phi_2, \ldots, \phi_K]^T \) and \( Y = [y_1, y_2, \ldots, y_K]^T \). \( v \) is the system noise. To reconstruct the original image from the \( K \) measurements, we solve the following minimization problem [5–9,12]:

\[ \min_{\phi \theta} \| \Phi \phi \theta - Y \|_2^2 + \lambda \| \theta \|, \]

where \( \lambda \) is a regularization parameter, whose value is dependent on the noise level. In CI systems, the measurement number \( K \) can be much smaller than the pixel number of the reconstructed image \( (N^2) \). We call the ratio between \( K \) and \( N^2 \) the down-sampling ratio of the CS measurement process.

3. Mathematical model of the CSM system

Like PAM systems, the CSM system can also be considered as a DMD-based microscopy imaging system, which uses a DMD to implement pinhole patterns. DMD patterns are used to eliminate the "out-of-focus" light such that the optical-sectioning imaging performance can be realized. The DMD is a reflective Spatial Light Modulator (SLM), whose optical sensitive area consists of an aluminum micromirror-array, with a pixel-pitch of 13.68 µm. Each mirror-pixel in the mirror-array can be electrically driven to be turned on (tilted about the diagonal hinge at an angle of +12°) or off (tilted at an angle of −12°) [13]. Prior to image reconstruction experiments, we upload the binary CS measurement patterns into the DMD memory and assign the “on” condition to mirrors responding to the binary value “1” and “off” to “0”.

The light-propagation process in DMD-based microscopes is similar to conventional optical-sectioning microscopes (such as LSCM systems), in the sense that it can be described as a double-stage information modulation process, consisting of an illumination-modulation stage and an image-modulation stage. The illumination-modulation stage uses pinhole patterns to modulate the intensity-distribution of the illumination light before the light interacts with the fluorescent specimen, and the image-modulation stage uses pinhole patterns to modulate the intensity of the fluorescent emission from the specimen.

Although the light-propagation processes in different optical-sectioning microscopes are similar, the specific optical realizations and the information acquired from those systems are different. In this section, we elaborate on the double-stage light-propagation process in the CSM system and derive a mathematical model, which can be used to evaluate the image reconstruction quality and the optical-sectioning imaging performance of the CSM system.

3.1 The illumination-modulation stage of the CSM system

The first stage of the double-stage information-modulation scheme is the illumination-modulation stage. In this stage, we use the DMD to define illumination patterns in the specimen. To achieve that, we first use a collimated beam to illuminate the DMD mirror-array at an angle of +24° away from the normal of the mirror-plane. In the reflection direction of the “on” mirror-pixels, we used an objective lens to collect the DMD reflection. In the focal-
plane of the objective lens (conjugate-position of the DMD), illumination patterns are formed as de-magnified optical images of the corresponding DMD mirror-patterns. Figure 1 uses red dotted-lines to demonstrate the light propagation in the illumination-modulation stage. In this figure, a dichroic mirror is used to reflect the illumination light from the source to the DMD. The dichroic mirror is designed to reflect the illumination light but transmit the fluorescent emission generated from the specimen.

**Fig. 1.** Schematic drawing of the CSM system.

According to the classic imaging theory, the intensity of the \(k_{th}\) illumination pattern \(P_k(u,v)\) can be expressed as [14]:

\[
P_k(u,v) = \begin{cases} 
|h_{ex}(u,v)|^2 \otimes |u_k^e(u,v)|^2 
& \text{(incoherent illumination)} \\
|h_{ex}(u,v) \otimes u_k^e(u,v)|^2 
& \text{(coherent illumination)} 
\end{cases}
\]  

(3)

In Eq. (3), \((\xi, \eta)\) and \((u,v)\) are used as coordinate systems for the image and object-plane of the objective lens respectively, as shown in Fig. 1. \(h_{ex}(u,v)\) is the excitation impulse-response-function of the objective lens. \(u_k^e(u,v)\) is the geometric image of the \(k_{th}\) DMD mirror pattern \((u_k(\xi,\eta))\). \(u_k^e(u,v)\) and \(u_k(\xi,\eta)\) are related to each other through the following geometric transformation: \(u_k^e(u,v) = M \cdot u_k(\xi,\eta) = M \cdot u_k(Mu, Mv)\) [13], where \(M\) is the magnification factor of the objective lens. The \(\phi_k\) term in Eq. (1) represents the reflection intensity of the \(k_{th}\) DMD mirror-pattern, and \(\phi_k(\xi, \eta) = |u_k(\xi, \eta)|^2\). The intensity of the fluorescent emission caused by the \(k_{th}\) illumination pattern is expressed as:
\[ E_k(u,v) = P_k(u,v) \cdot O(u,v) \]

\[
= \left[ \left[ h_{ex_k}(u,v) \right]^T \ast [u_k^* (u,v)] \right] \cdot O(u,v) \quad \text{(incoherent illumination)}
\]

\[
= \left[ \left[ h_{ex_k}(u,v) \otimes u_k^* (u,v) \right] \right] \cdot O(u,v) \quad \text{(coherent illumination)},
\]

where \( E_k(u,v) \) represents the fluorescent emission from the specimen at the location of \((u,v)\). \( P_k(u,v) \) is the \( k \)th illumination pattern. \( O(u,v) \) represents the intensity response of the specimen to the excitation light. The \( E_k(u,v) \) term in Eq. (4) is the outcome of the illumination-modulation stage, which describes the intensity-distribution of the fluorescent emission from the specimen caused by the \( k \)th illumination pattern. Fluorescent specimens always have thicknesses. Therefore, the \( E_k(u,v) \) term contains both the “in-focus” information, which is the fluorescent light generated from the focal-plane of the objective lens, and the “out-of-focus” information, which is the fluorescent light generated from specimen regions above and below the focal-plane. In Fig. 1, the “in-focus” information is represented by green lines and the “out-of-focus” information is represented by grey lines. The \( E_k(u,v) \) term is the input to the second stage of the double-stage information modulation scheme, and in that stage, the “out-of-focus” light can be effectively eliminated by implementing suitable CS measurement patterns with the DMD.

3.2 The image-modulation stage of the CSM system

The second stage of the double-stage information-modulation scheme is the image-modulation stage, which will eventually lead to the optical-sectioning imaging as mentioned before. In this stage, the fluorescent emission (represented by green lines in Fig. 1) caused by illumination patterns are collected into the objective lens and the corresponding fluorescent images are formed on the DMD mirror-plane. The \( k \)th fluorescent image is expressed as:

\[ I_k(\xi,\eta) = \left| h_{em_k}(\xi,\eta) \right|^2 \ast \left| E_k^e(\xi,\eta) \right|^2, \quad (5) \]

where \( h_{em}(\xi,\eta) \) is the emission impulse-response-function of the objective lens. \( E_k^e(\xi,\eta) \) is the geometric image of \( E_k(u,v) \). \( E_k^e(\xi,\eta) \) and \( E_k(u,v) \) have the following geometric relation:

\[ E_k^e(\xi,\eta) = \frac{1}{|M|} E_k(u,v) = \frac{1}{|M|} E\left(\frac{\xi}{M}, \frac{\eta}{M}\right). \]

Once formed on the DMD mirror-plane, the intensity of the \( k \)th fluorescent image is modulated by the \( k \)th DMD mirror-pattern, and the modulation result is the \( k \)th CS measurement for that imaging scene. The \( k \)th modulation result is expressed as:

\[ y_k(\xi,\eta) = \langle \phi(\xi,\eta), I_k(\xi,\eta) \rangle = +v. \quad (6) \]

As discussed previously, the fluorescent emission from the specimen contains both the “in-focus” and “out-of-focus” information. The image of the “in-focus” information matches the distribution of the “on” mirror-pixels of the DMD pattern. Those “on” mirror-pixels reflect the “in-focus” information to the direction of \(+ 24^\circ\) away from the DMD normal. In that direction, we have a focusing lens installed to focus the DMD reflection into a single pixel detector. The detector readings are the CS measurements. The image of the “out-of-focus” information falls onto the “off” mirror-pixels and is reflected away from the focusing lens. If suitable measurement patterns are implemented, the “out-of-focus” light can be effectively eliminated from the CS measurements, and thus optical-sectioning imaging can be realized with the CSM system.
4 Simulations

To verify the CSM design, we used Eq. (6) to generate CS measurements in the simulation environment and we performed image reconstruction experiments based on the simulated measurements (we used Matlab as the simulation tool). In this simulation, we need to provide Eq. (6) with three inputs: (a) the impulse-response-function of the objective lens, (b) the CS measurement patterns, and (c) the spatial information of the imaging target.

The first simulation input needed by Eq. (6), the impulse-response-function of the objective lens, is generated using a Zemax model of a 40x objective lens (Zemax, ZEBASE example number K_014). The numeric-aperture (NA) of the lens is 0.53. In this lens-model, we used a 550-nm incoherent excitation source and we only considered the 570-nm wavelength-component of the fluorescent emission. The impulse-response-functions and other optical parameters of the lens can be easily obtained from the Zemax model.

The second simulation input is the CS measurement patterns. Here we used MSBHE patterns as measurement patterns [11]. We did not use conventional CS measurement patterns (such as random patterns or Hadamard patterns) because those patterns contain clusters of mirror-pixels that are in the “on” condition at the same time. In the clusters of “on” pixels, there are no “off” mirror-pixels to eliminate the “out-of-focus” light and thus optical-sectioning imaging cannot be realized in those regions. In the MSBHE patterns, all the “on” mirror-pixels are surrounded by “off” pixels. The sparsity of the “on” pixels in the MSBHE patterns are determined by a parameter called block-size (BS), whose value is the ratio between the pixel-size of the measurement pattern in one direction and the smallest distance (in pixel) between two adjacent “on” pixels. Therefore, a larger BS value means the MSBHE pattern has a more dense distribution of “on” pixels and vice-versa. Figure 2 compares different CS measurement patterns. The white spots in these patterns represent “on” mirror-pixels. Figures 2(a) and 2(b) show examples of a random pattern and a Hadamard pattern, respectively. In these two patterns, we can easily find clusters of “on” mirror-pixels, which do not help to realize optical-sectioning imaging. Figures 2(c) and 2(d) show two examples of MSBHE patterns (64 × 64) with different BS values. The pattern shown in Fig. 2(c) has a BS value of 32 and the one shown in Fig. 2(d) has a BS value of 16. In these two MSBHE patterns, all the “on” mirror-pixels are separated by “off” pixels and no clusters of “on” pixels can be found. In Fig. 2(c), the minimum distance between two adjacent “on” pixels is 2 pixels, which means there is at least one “off” pixels between two “on” pixels. In Fig. 2(d), the minimum distance between two adjacent “on” pixels is 4 pixels.

Fig. 2. Example CS measurement patterns (64 × 64). (a) Random pattern (30% “on” pixels). (b) ODHE pattern (inverse Hadamard transform of a sampling impulse in the Hadamard space location (6,7)). (c) MSBHE pattern (BS = 32). (d) MSBHE pattern (BS = 16).

The third simulation input needed by Eq. (6) is the imaging target. Here, we used computer generated binary bar-patterns as the imaging targets. We considered three targets in this simulation, which have different thicknesses in the depth-direction. Using these targets, we can demonstrate that sparse patterns can more effectively eliminate the out-of-focus light and give a better reconstruction quality. Figure 3(a) shows a thin specimen used in the simulation, which has a pixel size of 128 × 128 × 1. In this target, the white bar areas have a
fluorescent response of intensity 1. The black regions do not have fluorescent response to the illumination light. The widths of the bars in Fig. 3(a) range from 2 to 6 pixels. The thin specimen has only one layer in its depth-direction and that layer is placed in the focal-plane of the objective lens. Figure 3(b) shows a thick specimen, whose pixel-size is 128 × 128 × 2. It has two layers in the depth-direction: one placed in the focal-plane of the objective lens (we call it the “in-focus” plane) and one placed +1.5-μm away from the focal-plane in the depth-direction (which we call an “out-of-focus” plane). The “in-focus” plane is represented by the binary bar-pattern shown in Fig. 3(a) and we use a uniform pattern (all the pixel-entries in the uniform pattern have a fluorescent response of intensity of 1) to represent the “out-of-focus” plane. Figure 3(c) shows a thick specimen with one “in-focus” plane and two “out-of-focus” planes, which are placed +1.5-μm and +3.0-μm away from the focal-plane of the objective lens. All the thin and thick specimens are used to generate CS measurements and an interior-point method is used to solve the reconstruction problem based on the simulated CS measurements [11]. The purpose of using thick specimens in this simulation is to introduce “out-of-focus” light to the simulated CS measurements. Thus, by examining the qualities of the reconstructed images, we can evaluate the influence of the “out-of-focus” light on the image reconstruction process. The reconstructed images are grouped into three columns in Fig. 3, labeled as columns (d), (e), and (f). MSBHE patterns (128 × 128) with BS values of 64, 32, and 16 are used in columns (d), (e), and (f), respectively. The top-row images in columns (d), (e), and (f) are reconstructed using the thin specimen as the imaging target, whereas the middle-row images are reconstructed using the 2-layer thick specimen as the imaging target. The bottom-row images are reconstructed using the 3-layer thick specimen as the imaging target. A 2-D median-filter with a window of 3 × 3 is used to remove noise-spikes in the reconstructed images before we show them in Fig. 3.

Fig. 3. Simulated image-reconstruction results. (a) A thin specimen, which is placed in the focal-plane of the objective lens (the “in-focus” plane). (b) A thick specimen, which contains the “in-focus” plane and one “out-of-focus” plane (+1.5-μm away from the focal-plane in the depth-direction). (c) A thick specimen, which contains the “in-focus” plane and two “out-of-focus” planes (+1.5-μm and +3.0-μm away from the focal-plane in the depth-direction respectively). Images in column (d) are reconstructed using 128 × 128 MSBHE patterns, with a BS value of 64. Images in column (e) are reconstructed using 128 × 128 MSBHE patterns, with a BS value of 32. Images in column (f) are reconstructed using 128 × 128 MSBHE patterns, with a BS value of 16. A 2-D median-filter with a window of 3 × 3 is used to remove noise-spikes in the reconstructed images.
From Fig. 3, we can visually judge that the quality of the reconstructed images degrades as thick specimens are used as imaging targets. This phenomenon indicates that the “out-of-focus” light affects the reconstruction quality of the CSM system. Also, when thick specimens are used, we notice that MSBHE patterns with smaller BS values (MSBHE patterns with sparser distributions of “on” pixels) can provide better reconstruction quality compared to MSBHE patterns with larger BS values (denser MSBHE patterns). This phenomenon indicates that sparser patterns can more effectively eliminate the “out-of-focus” light.

In order to quantitatively evaluate the quality of the reconstructed images, we calculated their peak-signal-noise-ratios (PSNRs). Here, the PSNR is defined as:

$$\text{PSNR} = 10 \times \log_{10} \left( \frac{10^2}{ \sum_{m,n} \left[ I_{\text{tar}}(m,n) - I_{\text{rec}}(m,n) \right]^2} \right)$$

where $M$ and $N$ indicate the pixel-numbers of the image in the vertical and the horizontal directions, respectively. $I_{\text{tar}}(m,n)$ represents the light-intensity of the imaging target in the pixel-position of $(m,n)$ and $I_{\text{rec}}(m,n)$ represents the intensity of the reconstructed image in that pixel-position. Both the intensities of the imaging target and the reconstructed images are normalized to the scale of 0-1. Figure 4 shows the PSNR values of the reconstructed images shown in Figs. 3(d)–3(f).

In Fig. 4, the $x$-axis indicates the thickness of the specimen and the $y$-axis indicates the PSNR value. The black curve shows the PSNR values of the images reconstructed using the BS = 64 MSBHE patterns, whereas the red curve shows the PSNR values of the images reconstructed using the BS = 32 MSBHE patterns. The blue curve shows the PSNR values of the images reconstructed using the BS = 16 MSBHE patterns. According to Fig. 4, when BS = 64 MSBHE patterns are used, the image-reconstruction quality drops 7.41dB from the case when a thin specimen is used as the imaging-target to the case when two “out-of-focus” planes are considered. When BS = 16 MSBHE patterns are used, this quality-drop is only 1.63dB. As expected, this result supports our previous discussion that sparser measurement patterns can more effectively eliminate the “out-of-focus” light and thus, sparser patterns are more desirable to achieve better reconstruction qualities in CSM systems.

We also used Eq. (6) to simulate the detector response of a thin specimen when it is placed at different optical planes of the CSM system, including the focal-plane of the objective lens, as well as optical planes that are in “out-of-focus” positions. Specifically in this simulation, we considered 10 “out-of-focus” planes above the focal-plane of the objective lens, and 10
below the focal-plane. All the optical planes considered here are parallel to each other and are perpendicular to the optical axis of the objective lens. The adjacent optical planes are 0.5-μm away from each other in the depth-direction. In the following discussion, we call this measurement process the depth-scanning process of the CSM system and the measurement results are represented as depth-scanning curves. The Zemax model of a 40x objective lens (Zemax, ZEBASE example number K_014) is used to generate numerical estimations of impulse-response-functions in those optical-planes. Figure 5 shows the depth-scanning curves of the CSM system when different measurement patterns are considered in Eq. (6), including an all “on” pattern, a single-pixel pinhole pattern, and all the patterns shown in Fig. 2. In the all “on” pattern, all the mirror-pixels are turned on. In the single-pixel pinhole pattern, all the mirror-pixels are turned off except the one in the center of the mirror-array, which effectively mimics a spatial pinhole. The x-axis in Fig. 5 indicates the relative position of optical planes with respect to the focal-plane of the objective lens and the y-axis indicates the normalized detector response calculated using Eq. (6).

![Detector response of the CSM system when an infinitely thin and uniform specimen is used as the imaging target. The specimen is placed in different optical planes of the objective lens. The x-axis in this figure indicates the position of different optical planes with respect to the focal-plane of the objective lens. The y-axis indicates the normalized detector response calculated using Eq. (6).](image)

In Fig. 5, we can see that when the thin specimen is placed in the focal-plane of the objective lens (the “in-focus” plane), the detector response reaches its peak intensity. The detector response drops as the specimen moves away from the focal-plane. The degradation rate of the detector response can be used as a measure to evaluate the effectiveness of different measurement patterns in eliminating the “out-of-focus” light. For instance, in Fig. 5, the black curve shows the depth-scanning result of the thin specimen when the all-“on” pattern is implemented. We note from this curve that when the thin specimen is placed + 5μm away from the focal-plane of the objective lens, the detector response is still quite significant, which is 83.1% of the “in-focus” detector response. In this case, the “out-of-focus” light is not effectively eliminated. The red curve in Fig. 5 plots the depth-scanning result when the single-pixel pinhole pattern is used. In the red curve, the detector response of the thin specimen from the + 5μm “out-of-focus” plane is only 2.3% of the “in-focus” detector response. In this case, we can say the “out-of-focus” light is effectively blocked by the single-pixel pinhole pattern.
From Fig. 5, we can see that sparse MSBHE patterns (small BS values) also provide a good optical-sectioning imaging performance. For instance, the pink curve shows the detector response when a BS = 16 MSBHE pattern (64 × 64) is considered in Eq. (6). When this pattern is used, the detector response of the thin specimen from the +5µm “out-of-focus” plane is 5.4% of the “in-focus” detector response. MSBHE patterns with larger BS values degrade the optical-sectioning imaging performance. For instance, when a BS = 32 MSBHE pattern (64 × 64) is considered in Eq. (6), the detector response from the +5µm “out-of-focus” plane is 18.2% of the “in-focus” detector response, which is more than 2 times larger compared to the BS = 16 MSBHE patterns. This result gives us two guidelines of selecting measurement patterns for the optical-sectioning CSM system: (a) the pattern should contain as few “on” pixels as possible; (b) the “on” pixels should be sparsely distributed.

5. Experimental results

Finally, we validated our simulation results by building a prototype CSM system using off-the-shelf products. The DMD used in the prototype system is a Texas Instruments (TI) Discovery 1100 series device, whose optical active area is an XGA format aluminum micromirror-array (768 × 1024). The pixel-pitch of the mirror-array is 13.68 μm. In this experiment, we group the DMD mirror-array into 2 × 2 mirror super-pixels. Each super-pixel is used to represent one pixel in the measurement pattern. Larger format super-pixels can be used to enhance the signal intensity in the image acquisition process, at the cost of reduced spatial resolution. The objective lens used in the prototype CSM system is an Olympus Plan-N lens (infinity-corrected, magnification = 40, \(NA = 0.65\)). A lens-tube (Edmundoptics, model number: NT56-125) is attached to the objective lens to facilitate the image formation on the DMD mirror-plane. A focusing-lens (Edmundoptics, model number: NT54-671) and a single-pixel detector (Hamamatsu, model number: H5784-20) is used in to collect CS measurements. Figure 6 shows the mechanical construction of the prototype CSM system.

![Fig. 6. Mechanical construction of the CSM system.](image-url)
We use a pollen-grain specimen (Carolina Biological Supply Company, item number: 304264) as the imaging target. Figure 7(a) shows the CCD image of the imaging target using white light illumination. Figure 7(b) shows the CCD image of the imaging target using a 532-nm laser illumination (B&W TEC, model number: BWN-532-100E). In Fig. 7(b), a set of fluorescent filters (Chroma, model number: 41007a Cy3) is used to isolate the fluorescent information. When capturing the CCD images, we control the DMD to display an all-“on” pattern and the single-pixel detector in the CSM system is replaced with a CCD camera (Edmundoptics, model number: NT59-919). Since an all-“on” pattern is used, the CCD images shown in Fig. 7 can be considered as wide-field microscopic images and we can see these wide-field images are blurred by “out-of-focus” light. We can see two pollen-grains in the imaging scene of Fig. 7, and in the following discussion, we call the larger pollen-grain Grain-1 and the smaller one Grain-2.

Figure 8 shows three sets of experimental results obtained with our prototype CSM system when three different types of measurement patterns are implemented with the DMD, including BS = 16 MSBHE patterns, BS = 32 MSBHE patterns, and conventional Hadamard patterns. Figures 8(a)–8(d) are reconstructed using MSBHE patterns (128 × 128). In Figs. 8(a) and 8(b), BS = 16 MSBHE patterns are used and in Figs. 8(c) and 8(d), BS = 32 MSBHE patterns are used. Figures 8(e) and 8(f) are reconstructed using conventional Hadamard patterns. The down-sampling ratio is 40% (6554 measurements are used). The top-row images in Fig. 8 are reconstructed at first. To reconstruct the bottom-row images, we moved the objective lens 10μm further away from the specimen in the depth-direction. The moving mechanism is realized by a linear stage (Newport, model number: M-UMR8.25) equipped with a motorized actuator (Newport, model number: LTA-HL).
Comparing Fig. 8(a) with Fig. 8(b), we can see that the reconstructed spatial information changes significantly as the objective lens moves 10-μm in the depth-direction of the specimen. In Fig. 8(a), Grain-1 is dark and Grain-2 is bright, whereas in Fig. 8(b), Grain-1 is bright and Grain-2 is dark. According to the simulation results shown in Fig. 5, in CSM systems, the detector response from the “in-focus” plane is always much stronger than the detector response from “out-of-focus” planes. Therefore, in Fig. 8(a), we can say that Grain-1 is placed in some “out-of-focus” planes and Grain-2 is placed very close to the focal-plane of the objective lens. In the case of Fig. 8(b), the focal-plane of the objective lens moves 10μm closer to Grain-1 and thus, Grain-1 is reconstructed with stronger signal intensity and in the meantime, Grain-2 goes “out-of-focus” by 10μm and it is reconstructed with much weaker signal intensity. This is the expected optical-sectioning imaging performance from the CSM system. As discussed in Sec. 4, when MSBHE patterns with larger BS values are used, the optical-sectioning performance of the CSM system is compromised. Figures 8(a)–8(d) provide experimental supports to that discussion. In Figs. 8(c) and 8(d), BS = 32 MSBHE patterns are used. In these two figures, we can see that the contrast of the “in-focus” signal and the “out-of-focus” signal is much less apparent compared to the signal contrast in Figs. 8(a) and 8(b), which use BS = 16 MSBHE patterns. Also, in Sec. 4, we mentioned conventional Hadamard patterns are not suitable for the CSM system because they contain clusters of “on” mirror-pixels. Figures 8(e) and 8(f) provide experimental supports to that discussion. Figures 8(e) and 8(f) are reconstructed using conventional Hadamard patterns, which look quite similar to each other even though they are reconstructed with the objective lens moved by 10μm in the depth-direction.

To better evaluate the performance of the CSM system, we performed a more-detailed optical-sectioning imaging experiment with the pollen-grain specimen. Figure 9 shows the results. In this experiment, 30 optical-sections are captured and the adjacent optical-sections are 1μm away from each other in the depth-direction. The optical-sections are labeled sequentially from (1) to (30) in Fig. 9. To reconstruct these images, 6534 MSBHE patterns (128 × 128 patterns, BS = 16) are used and we control the objective lens to move 1-μm further away from the specimen each time when we capture one set of CS measurements.
Figure 9 demonstrates the depth-discrimination of the pollen-grain specimen with better details than Fig. 8. As we go from (1) to (30) in this figure, we can clearly see the reconstructed spatial information changes gradually as the objective lens scans across the specimen in the depth-direction.

6. Conclusion

In this paper, we present the design and fabrication of a CSM system, which utilizes the theoretical advantages of the CS theory to reduce the optical complexity and enhance the data-utilization efficiency of conventional PAM systems. Using this system, optical-sectioning imaging can be realized using a simple focusing lens and a single-pixel detector and no mechanical scanning device is needed to acquire the complete spatial information from the imaged scene. In this work, we investigated the behavior of a special type of CS measurement patterns, MSBHE patterns, in the CSM system. Through simulations and experimental work, we noted that MSBHE patterns help the CSM system to realize optical-sectioning imaging more effectively than conventional CS measurement patterns. Also, we tested MSBHE patterns of different sparsities and noted that sparser MSBHE patterns can more effectively eliminate the “out-of-focus” light and thus provide better image-reconstruction qualities.

In the future, we will consider using faster DMDs or other SLM products to improve the image acquisition speed/quality of the CSM system. Also, we are working on more efficient CS measurement patterns such that fewer measurements can be used to reconstruct an image.
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