Sectioning and super-resolution using unknown random patterns

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ABSTRACT

Random structured illumination patterns are used to demonstrate effective sectioning as well as super-resolution images in conjunction with an incoherent light source. By projecting patterns of varied spatial frequencies and using blind deconvolution of an unknown point spread function, super-resolution is achieved. Random patterns produce more consistent sectioning and super-resolution given an unknown optical transfer function. Further, using a randomly distributed pattern provides a low cost solution to obtaining information similar to that produced in confocal microscopy and other methods of structured illumination, without the requirement of precise projection patterns, coherent light sources, or fluorescence.

Keywords: Structured Illumination, Sectioning, Super Resolution, Incoherent, Reflectance, Random

1. INTRODUCTION

Wide-field microscopy has proven to be an invaluable tool within the context of biological research and clinical practice, allowing for great insight into the inner workings of the extremely small. However, there are many restrictions which ultimately limit how much information can be obtained from conventional microscopy. Specifically, scattering from extraneous objects can occlude and distort information about specific layers of interest, making it difficult to extract any subsurface information. Confocal reflectance microscopy (CRM) has achieved clinical success for its ability to section the specimen, removing out of focus light and providing a clear image of only a single plane [1,2]. Structured illumination microscopy (SIM) has recently been providing an alternative to CRM, using patterned light to produce sectioning [3]. It may have the potential to encompass many of the advances in CRM such as providing real-time sectioned image, while also reducing much of the cost and complexity. After the introduction of SIM for sectioning, Gustaffson provided a method of encoding high resolution information into the patterned light, allowing for an increase in resolution [4]. The diffraction limit provides fundamental restriction to the smallest resolvable object, however there is much more information contained beyond this limit [5]. Through some novel processing adjustments to SIM, the total resolution of the system can be increased by a factor of two.

Specifically, SIM requires extremely precise modulation patterns, as well as precise adjustments to their positions to achieve both the sectioning and super resolution. Further, by adjusting the frequency of the modulation pattern, we find that there is a trade-off between the sectioning depth and the resolution improvement. At very low frequency, the modulation pattern will still be present in the image even when projected through very thick specimens, allowing for very deep sectioning. As the pattern frequency is increased, less information can be encoded in the pattern, reducing the total resolution that can be reconstructed. With these limitations in mind, it can be shown that random illumination patterns can provide both sectioning and resolution improvements, without the precision required of typical modulation patterns [4,5,6,8,9]. Beyond this, in an attempt to extend our work to clinical practice, only incoherent light is used in reflectance, without fluorescent tagging, making it a prime candidate for \textit{in-vivo} imaging.

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2. RANDOM PATTERN SIM

2.1 Frequency Adaptation

Among the advantages described above, one of the major motivators behind using a random pattern is the fact that it effectively adapts to the resolution limits of the system. Specifically, when using a discrete sine pattern, the best resolution improvement will come from a frequency as close to the Nyquist frequency as possible [10,11]. However, the possibility of aliasing becomes an issue if the pattern isn’t selected correctly. Looking at Figure 1, an aliased pattern is projected on the target, subverting any attempt to section or provide super-resolution. When considering a random pattern, because the pattern is inherently broadband, there are no aliasing effects and the highest frequency available will always be present. Reviewing Figure 2, it can be seen that in the frequency domain, the random pattern fills out the entire optical transfer function (OTF), but does not produce aliasing in the spatial domain. This means that the OTF does not have to be measured ahead of time to produce an optimal projection pattern. Further, if the OTF changes in the system, the pattern does not need to be updated to provide reasonable results.

![Figure 1. An aliased high frequency pattern projected on a resolution target (left) and the Fourier transform showing the pattern outside of the optical transfer function (right).](image)

2.2 Sectioning

To test the sectioning ability of the system, a small piece of ground glass is placed in the illumination path of the microscope conjugate to both specimen under investigation and the focal plane of the camera. In order to provide sectioning capabilities, there is a need to translate the modulation pattern such that all areas of the specimen are uniformly illuminated on average. Attached directly to the ground glass is a motor which can be used to rotate the orientation of the pattern. Ideally, we would be able to randomize the pattern perfectly from frame to frame to decorrelate each consecutive frame. While rotating the projection pattern is not actually random, it provides a simple method to sampling the specimen while exposed to various light intensities. Care has been taken to ensure that the fundamentals of this method are not dependent on rotation, thus this could be extended to any type of scheme for translating the pattern.

![Figure 3. Sectioning ability of the system against a piece of hair.](image)

Figure 3 shows the sectioning ability of the system against a piece of hair. In the first image (A), we see that the many of the fine details are retained at the surface of the hair. As we increase the depth and begin to section our portions of the hair, we can see that new details are revealed in the image (B and C). Here we can see the sectioning working as anticipated. In Figure 4, we compare images of plant cells in CRM as compared to SIM with a random pattern, demonstrating similar capabilities between the two techniques. One particular
Figure 2. A random pattern projected onto the resolution target (left) and the Fourier transform showing the pattern fully within the optical transfer function (right).

An interesting aspect of this comparison is the difference between using an incoherent versus coherent light source. In the incoherent case (with random modulation), we find that the image is much smoother, as there is no speckling resulting from the laser.

Figure 3. Images of hair showing three different depths - A: 0µm, B: 100µm, C: 200µm.

To ensure that we can use the above techniques in-vivo application, we can test it against a human subject. Figure 5, shows the results of measuring the surface of the skin, while Figure 6, shows the results of measuring
Figure 4. Images of cell structure of a leaf using CRM (a) and random SIM (b). It can be seen how smooth the details are when using the incoherent light source in the random SIM.

the skin at the depth of a few 10s of microns.

Figure 5. Image taken of the surface of human skin in-vivo. On the left is the widefield image, showing a great deal of clutter from scattered light. On the right, the sectioning image greatly increases the contrast of the image and defines areas relevant to a given plane of interest.
2.3 Super Resolution

Beyond sectioning, we want to ensure that the introduction of a random pattern allows for super resolution. Many of the previous techniques rely on a known pattern with discrete phase and frequency to produce super-resolution. However, due to the fact that our random pattern contains neither a known frequency or phase, a new processing technique must be applied. Through a process of deblurring or deconvolution, the point spread function (PSF) can be removed and details which would have otherwise been lost can be reconstructed [12,13]. Unfortunately, this requires that the point spread function is known a priori, making it quite cumbersome to reconstruct properly. This constraint can be overcome by processing with Maximum a posteriori probability (MAP), which estimates the most likely high resolution image given the entire set of low resolution images [14]. During this process, the PSF is eliminated through blind deconvolution. Further, by making some assumptions about the image, the regularization function can be modified such that the high resolution image can be reconstructed with extremely high fidelity [15].

Below are the results from applying the super-resolution techniques. Figure 7 shows the improvement in resolution against a 1951 Air Force resolution chart. Image A shows the full resolution image, before the total resolution was artificially lowered. In B we have the widefield image displayed, where many of the lines are difficult to resolve. It can be see that both techniques greatly improve the resolution, as objects that were unavailable in the widefield image can now be seen (C,D). However, with additional assumptions in the regularization function, the resolution can be further improved. In order to show both sectioning and super-resolution in a biological target, onion cells were placed on a top of the resolution chart. In Figure 8, we can see the onion cells at the surface of the image. In the widefield image, the dark spots underneath are due to the black bars in the resolution chart. After the sectioning processing is complete, the dark spots are removed and the contrast between cells is greatly increased. In Figure 9, the resolution chart is imaged through the cells. Comparing the widefield to the processed images, we can see that regions of the targets have been enhanced. The target’s contrast has been enhanced and there is more resolution present in the image than in the widefield image. This clearly demonstrates the sectioning and super-resolution working in conjunction with one another.
3. DISCUSSION

The introduction of random patterns removes many constraints that can be expected when designing a typical structured illumination system. The use of random patterns requires new processing techniques to produce both sectioning and super-resolution. The above work shows that sectioning can still be achieved \textit{in-vivo}, making it a good candidate for clinical practice. We can see from these experiments that the sectioning and super-resolution work well even with random patterns and can be applied to biological samples. It has also been shown that
Figure 9. Resolution target imaged underneath the onion cells. (Left) the widefield image shows the target without any additional processing. (Center) The MAP processing shows the target and contains a slightly more resolvable target. (Right) The sparse processing further enhances the resolution, the '7' in the target is resolved with good clarity.

the super-resolution processing techniques continue to work, even at depth. Additionally, these two algorithms provide super-resolution even when the pattern and optical transfer function of the system are unknown. These techniques provide a low cost alternative to current SIM implementations, while also making for a much simpler implementation.

4. REFERENCES


