High-Resolution Light-Field Microscopy

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Abstract: By combining a high-resolution image from a standard camera with a low-resolution wavefront measurement from a Shack-Hartmann sensor, we numerically reconstruct a high-resolution light field. We experimentally demonstrate the method with a commercially available microscope.

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The light field of an optical signal simultaneously stores spatial (x) and spatial frequency (k) information in a four-dimensional description. It accounts for the locally varying coherence of an optical beam and treats propagation in the full \{x,k\} phase space, allowing such features as numerical image refocusing [1]. A popular method to capture light field is using a lenslet array, which is able to obtain the four-dimensional information in a single shot. The lenslet configuration has been used in a variety of contexts, ranging from aberration correction in adaptive optics [2] to plenoptic photography [3] and microscopy [4]. However, the use of an array limits both spatial and angular sampling, resulting in poor resolution in both domains. Recently, a full resolution of the light-field measurement by using a spatial light modulator (SLM) has been proposed [5]. In this method, the SLM creates a scanning aperture for extracting spatial frequency from a spatially localized window position. However, the use of an SLM increases the complexity of the imaging system, while the requirement of point-by-point scanning decreases the acquisition speed. To date, there has been no method available for high-speed, high-resolution light-field imaging.

Here, we develop a computational method to overcome these practical issues. By combining two shots, one from a coarse-grained lenslet array and one from a charge-coupled device (CCD) camera, a high resolution light field can be reconstructed. The method involves an iterative process with a real-space image, \(I_R\), taken by a conventional CCD camera, and a light field image, \(I_{LF}\), taken by a Shack-Hartmann wave-front sensor. A schematic of the algorithm is shown in Fig. 1. In the first step, the algorithm expands \(I_{LF}\) by a factor of eight in spatial domain using linear interpolation, and then executes the fast Fourier transform (FFT). In the Fourier space, we put the constraint of \(I_R\) as a projection (by Fourier slice theorem) and then apply an inverse fast Fourier transform (IFFT) back. In the computed light field, we put constraints on the consistency of measured \(I_{LF}\), viz. that the light field is real and non-negative, and then apply a FFT again. We repeat the cycle of FFT, IFFT and constraints iteratively until convergence happens. The final output is a high-resolution light field, \(I^*_{LF}\).

Figure 1. Schematics of proposed algorithm.
A proof-of-principle experiment, shown in Fig. 2. A white light source is concentrated by a 10X/0.25 objective lens and illuminates a tiled air force resolution chart. Transmitted light is then collected by a 4f imaging system, with a 20X/0.4 objective as a front lens and a back lens with focal length 15cm. While a beamsplitter could be used to collect both $I_R$ and $I_LF$ simultaneously, we measure them sequentially here. We first record a real-space image using a conventional CCD camera with pixel size of 4.65 µm (Thorlabs DCU224) and then switch to a Shack-Hartmann wavefront sensor (Thorlabs WFS150-5C) for a (low resolution) light-field image. After these two measurements, we perform the iteration algorithm to reconstruct a high-resolution light field. Once the high-resolution light field is known, we are able to generate super-resolved images at different focal plans by a simple shearing transformation [2-4]. Fig. 3 shows the computed sequence of focusing of the tiled resolution chart from element 6 to element 3 of group 5 with improved resolution. Fig. 3(a-c) are obtained by using a lenslet array in a single shot, we can observe that the use of the lenslet array results in poor resolution and limited the depth of view. Fig. 3(d) is the measured image using the CCD camera (i.e. $I_R$). By combining $I_R$ and $I_LF$ in the proposed algorithm, the high-resolution light field is reconstructed using 5 iterations. A series of focused images can then be generated computationally by the reconstructed light field. Fig. 3(e) and (f) show a significant improvement on both resolution and depth of view, with the bars and numbers of the chart more clearly visible.

![Figure 2. Schematic of the proof-of-principle experiment](image)

![Figure 3. Computed sequence of focusing of the tiled resolution chart with improved resolution: different axial depths of view by (a-c) conventional lenslet array measurement. (d-f) by using our reconstructed high-resolution light-field (d) at starting focal plan with the element 6 (e) moving focal plan 100µm to the element 5 (d) moving focal plan 200µm to the element 4 and 3.](image)

We now extend the method to a commercial microscope by integrating the Shack-Hartmann wavefront sensor and the CCD camera with a digital reflected light microscope (Olympus BX60M). Fig. 4 shows the computed axial slices of the tissue paper using a 20X/0.4 objective by a single lenslet measurement and the proposed method. As before, the new method gives more details in both lateral and axial directions, including the width of a variety of fibers from 5µm to 50µm, the micro-holes among fibers, and the relative depth of these micro-structures (shown by white arrows).
Figure 4. Computed axial slices of the tissue paper using 20X/0.4 objective: different axial depths by (a-d) conventional lenslet array measurement. (e-h) by reconstructed light field (e) at starting focal plan, (f) moving focal plan 33µm, (g) moving focal plan 49µm, (h) moving focal plan 66µm.

In conclusion, we have demonstrated a high-resolution light-field imaging method by combining measurements from a coarse-grained Shack-Hartmann sensor and a high-resolution image from a conventional digital camera. With the proposed iterative algorithm, a super-resolved sequence of focusing, axial slices and refocusing can be reconstructed computationally. The images show a significant improvement in both lateral and axial resolution. The integration with an existing commercial microscope further proves useful of the proposed method. This paves a way for high-resolution three-dimensional microscopy without optical sectioning or manual scanning.

References