Super-resolution microscopy via ptychographic structured modulation of a diffuser

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We report a new coherent imaging technique, termed ptychographic structured modulation (PSM), for quantitative super-resolution microscopy. In this technique, we place a thin diffuser (i.e., a scattering lens) in between the sample and the objective lens to modulate the complex light waves from the object. The otherwise inaccessible high-resolution object information can thus be encoded into the captured images. We scan the diffuser to different positions and acquire the corresponding images. A ptychographic phase retrieval process is then used to jointly recover the complex object, the unknown diffuser profile, and the defocus coherent transfer function of the system. Unlike the illumination-based super-resolution approach, the recovered image of our approach depends upon how the complex wavefront exits the sample – not enters it. Therefore, the sample thickness becomes irrelevant during reconstruction. After recovery, we can propagate the super-resolution complex wavefront to any plane along the optical axis. We validate our approach using a resolution target, a quantitative phase target, and biological samples. We demonstrate a 4.5-fold resolution gain over the diffraction limit. We also show that a 4-fold resolution gain can be achieved with as few as 30 images. The reported approach may provide a quantitative super-resolution strategy for coherent light, X-ray, and electron imaging.

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In structured illumination microscopy, non-uniform illumination patterns are used to modulate the otherwise inaccessible object information into the passband of the optical system [1-4]. Instead of using illumination patterns, modulation of the object information can also be performed at the detection path using disordered media [5-12]. In the past years, it has been shown that the disordered media can serve as a scattering lens for coherent light wave modulation. Resolution beyond the diffraction limit can be achieved via wavefront shaping or transmission matrix characterization [5-12].

Inspired by the concept of the scattering lens, we report a new coherent super-resolution imaging technique, termed ptychographic structured modulation (PSM), in this letter. In the PSM technique, we place a thin diffuser (i.e., a scattering lens) in between the sample and the objective lens to modulate the complex light waves. We then scan the diffuser to different lateral positions and acquire the modulated images through the objective lens. The acquired images are used to jointly recover the super-resolution complex object, the complex diffuser profile, and the defocus coherent transfer function (CTF) using a ptychographic phase retrieval process [13-16].

Fig. 1. Comparison between the regular coherent microscope platform (a) and the proposed PSM concept (b). In the PSM approach, we use a thin diffuser to modulate the light waves from the object. The diffuser is then scanned to different lateral positions and the captured images are used to recover the super-resolution complex object, the complex diffuser profile, and the defocus CTF of the imaging system. The final achievable resolution is limited by the smallest feature size of the diffuser, which modulates the otherwise inaccessible object information into the passband of the objective.
Figure 1 shows the comparison between a regular coherent microscopy imaging setup and the reported ptychographic structured modulation concept. As shown in Fig. 1(b), the high-resolution object information otherwise inaccessible is now encoded in the captured images in the reported approach. The final achievable resolution is not limited by the numerical aperture (NA) of the objective lens. Instead, it is limited by the feature size of the thin diffuser. In our proof-of-concept experiment, we demonstrate a 4.5-fold resolution gain beyond the diffraction limit of the employed objective lens. We also show that, a 4-fold resolution gain can be achieved with as few as ~30 images.

Drawing connections and distinctions between the proposed approach and its related imaging modalities helps to clarify its operation. In the turbid lens imaging technique developed by Choi et al. [7-9], the transmission matrix of the diffuser is measured and then used to recover the super-resolution complex object. In our approach, we use a thin diffuser for light wave modulation. The transmission matrix of our diffuser can be approximated via a diagonal matrix [17]. As such, we can model the interaction between the light waves and the diffuser using point-wise multiplication. Instead of directly measuring the transmission matrix in PSM, we employ the ptychographic phase retrieval process to jointly recover the super-resolution complex object, the complex diffuser profile, and the defocus CTF of the system, as shown in Fig. 1(b).

The reported PSM approach also shares its roots with super-resolution ptychography [13], near-field ptychography [14-16, 18, 19], and Fourier ptychography (FP) [20]. The joint recovery of the object and the diffuser profiles in PSM is similar to the joint recovery of the object and the probe (or pupil) function in a ptychography or FP implementation.

In super-resolution ptychography, speckle patterns are used to improve the achievable resolution. The illumination probe, however, is confined to a limited region in the object space, leading to a large number of image acquisitions. Our approach, on the other hand, employs a scattering layer to modulate the light waves for the entire field of view (instead of a confined region). In our demonstration, as few as 30 images can be used to recover the super-resolution object with 4-fold resolution gain beyond the diffraction limit.

Near-field ptychography uses a translated speckle pattern to modulate the object over the entire field of view. The key difference between our approach and the near-field ptychography is the use of a scattering layer for super-resolution imaging. By placing the diffruser in between the object and the detection optics, the otherwise-lost high-resolution object information can be now encoded into the captured images [7-9]. We can, therefore, substantially improve the resolution beyond the diffraction limit of the employed objective lens.

FP illuminates the object with angle-varied plane waves and recovers the super-resolution complex object profile. Thin object assumption is needed in FP as well as in super-resolution ptychography. Only under this assumption, the interaction between the illumination wave and the object can be approximated via point-wise multiplication. Unlike the illumination-based implementations in FP and ptychography, the reported PSM approach modulates the object light waves at the detection path. The recovered image of PSM depends upon how the complex wavefront exits the sample – not entering it. Therefore, the sample thickness becomes irrelevant during reconstruction. After recovery, we can propagate the super-resolution complex wavefront to any plane for 3D holographic refocusing.

The reported PSM approach can also be used in coherent X-ray and electron microscope. A thin diffusing layer can be added in between the object and the objective lens to modulate the X-ray photons and electrons otherwise inaccessible by the systems. It can improve the imaging resolution and recover quantitative object phase in current transmission X-ray and electron microscopes without major hardware modifications.

The forward imaging model of the PSM approach shown in Fig. 1(b) can be expressed as

\[ I_j(x, y) = \left| \left( O(x, y) * PSF_{\text{free}}(d) \right) \cdot D(x-x_j,y-y_j) \right|^2, \]

where \( I_j(x, y) \) is the \( j \)th intensity measurement (\( j = 1, 2, \ldots, J \)), \( O(x, y) \) is the complex object, \( D(x, y) \) is the complex profile of the diffruser, \( (x_j, y_j) \) is the \( j \)th positional shift of the diffruser, \( PSF_{\text{free}}(d) \) is the point spread function (PSF) for free-space propagation over a distance \( d \), \( CTF(k_x, k_y) \) is the defocus CTF with a defocus distance \( -d \), and \( * \) stands for inverse Fourier transform, ‘\(^2\)’ stands for point-wise multiplication, and ‘\(^\cdot\)’ stands for convolution operation.

**Recovery process of the PSM approach**

**Input:** Raw image sequence \( I_j(j = 1, 2, \ldots, J) \)

**Output:** Super-resolution object profile \( O(x, y) \), diffuser profile \( D(x, y) \), and CTF

1. Initialize \( O(x, y), D(x, y), \) defocus CTF; \( d \) is the defocus distance of the diffruser
2. for \( n = 1: N \) (different iteration loops)
3. for \( j = 1: J \) (different captured images by scanning the diffruser)
4. \( O'(x, y) = O(x, y) * PSF_{\text{free}}(d) \) % Propagate the object to the diffruser
5. \( D_j(x, y) = D(x - x_j, y - y_j) \) % Shift the diffruser to different \( xy \)-positions
6. \( \Phi_j(x, y) = O'(x, y) * D_j(x, y) \)
7. \( \Phi_j(k_x, k_y) = \text{IFT} \left( \Phi_j(x, y) \right) \) % FFT Spectrum filtering via the defocus CTF
8. \( \psi_j(k_x, k_y) = \text{IFFT} \left( \Phi_j(k_x, k_y) \right) \) % Amplitude replacement
9. Update the spectrum \( \Phi_j(k_x, k_y) \) using \( \Psi_j = \Phi_j * \text{IFFT} \left( \frac{\text{conjugate}(CFT(\Psi_j))}{\text{max}(|\Psi_j|)} \right) \)
10. Update the defocus CTF using \( CTF = CTF * \text{IFFT} \left( \frac{\text{conjugate}(CFT(\Psi_j))}{\text{max}(|\Psi_j|)} \right) \)
11. Update \( O'(x, y) \) using \( O' = O' + \text{conjugate} \left( \frac{\text{conv}(D_j(k_x, k_y))}{\text{max}(|\text{conv}(D_j)|)} \right) \)
12. Update \( D_j(x, y) \) using \( D_j = D_j + \text{conjugate} \left( \frac{\text{conv}(D_j)}{\text{max}(|\text{conv}(D_j)|)} \right) \)
13. Shift back \( D_j(x, y) \) % Shift back the updated diffruser
14. \( O(x, y) = O'(x, y) * PSF_{\text{free}}(-d) \) % Propagate the object to the object plane
15. Add Nesterov momentum
16. end
17. end

Fig. 2. The recovery process of the PSM approach, where we acquire images by scanning the diffruser to different positions. The intensity measurements are then used to recover the complex object, the diffruser profile, and the CTF.

Based on all captured images \( I_j \) with the diffuser scanned to different lateral positions \( (x_j, y_j) \) s, we aim to recover the complex object \( O(x, y) \), the diffruser profile \( D(x, y) \), and defocus CTF. The recovery process is shown in Fig. 2. First, we initialize the amplitude of the object by averaging all measurements. The diffruser profile is initialized to an all-one matrix. The defocus CTF is initialized based on an estimate of the distance between the object and diffuser \( d \). In the reconstruction process, we first propagate the object to the diffuser plane and obtain \( O' \) in line 4. We then multiply \( O' \) with the shifted diffuser to obtain the exit wave \( \Phi_j \) in line 6. The exit wave is low pass filtered in the Fourier domain to get \( \psi_j \) in line 9. The amplitude of \( \psi_j \) is then replaced by the \( j \)th measurement \( \sqrt{I_j} \) while the phase is kept unchanged. The Fourier spectrum of exit wave \( \Phi_j \) and the CTF are updated in the Fourier domain in lines 11-12 using the rPIE approach [21]. Based on the updated exit wave \( \Phi_j \), we then update the object and the diffuser profiles in lines 14-15 using the rPIE approach [22]. We also add Nesterov momentum to accelerate the convergence speed in our implementation [22]. The processing time for 100 raw images with 1024
by 1024 pixels each is ~1 minute for 25 iterations using a Dell XPS 8930 desktop computer.

Figure 5 shows the recovered results using different numbers of acquired images. We can see that a 4-fold resolution gain can be achieved with as few as ~30 images (resolving the linewidth of group 8, element 6). If we further reduce the number of images to 20, the resolution gain becomes 3.2 in Fig. 5(d).

![Incoherent summation](image)

**Fig. 3.** Experimental validation of the super-resolution imaging capability of the PSM approach. (a) The raw image of PSM, which is modulated by the diffuser. (b) The incoherent summation of all captured images. (c) The recovered image based on 864 raw images. The NA of the employed objective lens is 0.055 and it can resolve the half-pitch line width of group 6, element 6 of the resolution target. In the recovered image, we can resolve the half-pitch line width of group 9, element 1, achieving ~4.5-fold resolution gain over the diffraction limit of the employed objective lens.

We first validate the super-resolution imaging capability of the PSM approach in Fig. 3. In this experiment, we use a microscope platform with a 2X, 0.055 NA objective lens (Mitutoyo Plan Apo) for image acquisition. The sample is a USAF resolution target for quantifying the resolution gain. The diffuser is made by coating 1-µm microspheres on a cover slip. The distance between the diffuser and the sample is about 0.5 mm. We use two mechanical stages (Applied Scientific Instrumentation LS-50) to scan the diffuser to different x-y positions and acquire the corresponding images. The positional shift of the diffuser is about 2-4 pixels in between adjacent acquisitions. Figure 3(a) shows the captured raw image of the resolution target, where the speckle feature comes from the diffuser modulation. Figures 3(b) shows the incoherent summation of all acquired images. The diffraction-limited resolution is 4.38 µm half-pitch linewidth, corresponding to group 6, element 6. Figure 3(c) shows our recovery, where we can resolve 0.98-µm linewidth from group 9, element 1 of the resolution target. The resolution gain is 4.5-fold over the diffraction limit. We also note that the current achievable is limited by the feature size (~1 µm) of the diffuser. One can, for example, use TiO₂ nanoparticles to make a diffuser with substantially stronger modulation capability [5-12].

In the second experiment, we validate the quantitative imaging nature of the PSM approach. A quantitative phase target (Benchmark QPT) is used as the object. Figure 4(a) shows the captured raw image through diffuser modulation. Figure 4(b) shows the recovered phase using the PSM approach. The line profile across the red dash arc in Fig. 4(b) is plotted in Fig. 4(c). The recovered phase is in a good agreement with the ground-truth phase height of the phase target, validating the quantitative imaging nature of the PSM approach.

In the third experiment, we investigate the number of raw images needed for our recovery. Once we recover the complex diffuser profile, we can substantially reduce the number of images for reconstruction.

![Recovery image](image)

**Fig. 4.** Validating the quantitative imaging nature of the PSM approach. (a) The captured raw image through the diffuser. (b) The recovered phase image based on 864 raw images. (c) The line trace of the red arc in (b).

![Recovery with different number of images](image)

**Fig. 5.** Reconstruction with different numbers of raw images. (a) 100 images. (b) 50 images. (c) 30 images. (d) 20 images. We can achieve 4-fold resolution gain with as few as 30 images.

We also test the PSM approach with two biological samples in Figs. 6-7. In Fig. 6, we place a tilted microscope slide (Oesophagus cancer slide) at a defocus position. Figure 6(a) and 6(b) show the recovered amplitude and phase of the complex wavefront exiting the sample. Similar to holographic imaging techniques, we can digitally propagate the recovered complex wavefront to any plane along the optical axis. Figure 6(c) and 6(d) shows the recovered amplitude after digitally propagating to three different axial positions. Region (c) in Fig. 6(c) is in focus at z = 1.3 mm and region (d) is in focus at z = 1.4 mm.

In Fig. 7, we use an extended object, arycria, in the experiment. Figure 7(a) shows the captured raw image modulated by the diffuser. Figure 7(b) and 7(c) show the recovered amplitude and phase of the complex wavefront exiting the sample. We then digitally propagate the recovered complex wavefront to different positions along the optical axis. Figure 7(d) and 7(e) shows the recovered amplitude after digitally propagating.
to \( z = 0 \) \( \mu \text{m} \), 60 \( \mu \text{m} \), and 120 \( \mu \text{m} \). Region (d) in Fig. 7(a) is in focus at \( z = 0 \) \( \mu \text{m} \) and region (e) is in focus at \( z = 60 \) \( \mu \text{m} \).

Fig. 6. Digital propagation of the recovered complex wavefront. The recovered amplitude (a) and phase (b) of a defocused and tilted microscope slide. (c)-(d) The recovered amplitude of two different regions in (a) after digitally propagating to \( z = 1.3 \) mm, 1.36 mm, and 1.4 mm positions.

Fig. 7. (a) Captured raw PSM image of an extended object (arciaria). The recovered amplitude (b) and phase (c) of the complex wavefront exiting the object. (d)-(e) The recovered amplitude of two different regions in (b) after digitally propagating to \( z = 0 \) \( \mu \text{m} \), 60 \( \mu \text{m} \), and 120 \( \mu \text{m} \) positions.

In summary, we report a coherent imaging technique, termed ptychographic structured modulation, for quantitative super-resolution microscopy. The reported PSM technique has several advantages. First, it can bypass the diffraction limit of the employed objective lens. We demonstrate a 4.5-fold resolution gain over the diffraction limit. We also show that a 4-fold resolution gain can be achieved with as few as 30 images. Second, different from the structured illumination technique, the reported PSM modulates the wavefront at the detection path and it recovers the complex wavefront exiting the sample. The sample thickness becomes irrelevant during reconstruction. After recovery, we can propagate the super-resolution complex wavefront to any plane along the optical axis. Thin sample assumption plagued in regular ptychography and FP is no longer an issue in PSM. Third, the reported platform provides the true quantitative contrast of the complex object. The reported approach may provide a quantitative super-resolution strategy for coherent light, X-ray, and electron imaging. Finally, we also note that the PSM technique can also be implemented in a lensless microscopy platform \[18\] and the result will be presented elsewhere.

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