**Imaging properties of extended depth of field microscopy through single-shot focus scanning**

Sheng-Huei Lu and Hong Hua*

College of Optical Sciences, University of Arizona, 1630 East University Boulevard, Tucson, Arizona 85721, USA

hhua@optics.arizona.edu

Abstract: Although the single-shot focus scanning technique (SSFS) has been experimentally demonstrated for extended depth of field (EDOF) imaging, few work has been performed to characterize its imaging properties and limitations. In this paper, based on an analytical model of a SSFS system, we examined the properties of the system response and the restored image quality in relation to the axial position of the object, scan range, and signal-to-noise ratio, and demonstrated the properties via a prototype of 10 × 0.25 NA microscope system. We quantified the full range of the achievable EDOF is equivalent to the focus scan range. We further demonstrated that the restored image quality can be improved by extending the focus scan range by a distance equivalent to twice of the standard DOF. For example, in a focus-scanning microscope with a ± 15 μm standard DOF, a 120 μm focus scan range can obtain a ± 60 μm EDOF, but a 150 μm scan range affords noticeably better EDOF images for the same EDOF range. These results provide guidelines for designing and implementing EDOF systems using SSFS technique.

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1. Introduction

Standard optical techniques for microscopy typically are limited to a small depth of field (DOF). For instance, a microscope objective with a numerical aperture (NA) of 0.25 and the working wavelength of 0.5 μm has about ± 8 μm DOF given by $n \lambda / NA^2$, which is the half width of the full depth of field range theoretically. Such a shallow DOF limits the capability
of imaging thick specimens. Extended depth of field (EDOF) microscopy has been pursued as a technique for capturing microscopic images of samples at a much greater depth range than those of standard microscopes while maintaining high image quality. Via an EDOF method, the information across the entire depth range being captured is displayed sharply, without necessarily retaining the depth information of the sample. Several different approaches have been explored for EDOF microscopy, such as wavefront coding [1], optical sectioning [2], and single-shot focus scanning [3].

Wavefront coding is a technique where a specially designed phase mask, such as a cubic phase mask described in [1], is placed at the aperture stop of a microscope objective to encode the wavefront of the optical system and generate a serial of point spread functions (PSF) that are insensitive to defocus. Because of the insensitivity of the system PSFs to defocus, though the acquired image of a sample with an extended depth range is blurry, a sharp EDOF image can be restored via a post image processing. By considering a number of trade-offs in design and performance, Cathey and Dowski successfully demonstrated the application of the wavefront coding technique for microscopy [4].

Optical sectioning, also known as focus stacking, is a technique where multiple images of a thick specimen taken at different focal depths are combined to reconstruct an EDOF image with a greater DOF than any of the individual source images. Typically this technique requires a method to suppress the out-of-focus signals when capturing the individual source images [2] or post-process the images via deconvolution before they are fused [2,5]. For high NA objectives, the depth scan typically is achieved by changing the physical distance between the objective lens and the specimen mechanically, while in low NA objectives it is also feasible to move the detector plane [2]. Murali et al. demonstrated the capability of depth scanning using a liquid lens [6]. Speed is one of the key limitations to the sectioning technique. The maximum frame rate for obtaining an EDOF image is F/N, where F is the frame rate of the system for capturing a single image, and N is the number of sampled image slices in depth. F typically is limited by the speed of the image detector and the speed of refocusing at different focal depths. Botcherby et al. developed a remote focusing technique to enable rapid change of focal setting without moving the objective lens or specimen and demonstrated the capability of acquiring EDOF images in real time by combining their remote focusing technique with a confocal microscope [7]. Because of the confocal nature, the system, through a rapid scan of the focal depth, naturally integrates infinite slices of in-focus images and obtain an integrated EDOF image without requiring post-processing. Like a typical confocal microscope, the acquired field of view (FOV) is very limited without field scanning.

Single-shot focus scanning (SSFS) is a wide field optical imaging technique where a thick specimen is imaged in a single exposure while the focal plane of the imaging system is scanned through the depth of interest. Unlike the optical sectioning technique in [2,6] where out-of-focus signals are suppressed at each focusing depth through a confocal microscope, the acquired image through SSFS technique is naturally blurred due to the integration of both the out-of-focus and in-focus signals across a wide field. A sharp EDOF image can then be restored by applying a single deconvolution step when the PSF of a focus scanning system is optimized to be invariant to depth within the extended depth range. Hausler [8] and Conchello and Dresser [9] have demonstrated the SSFS method in microscopy via mechanically moving the specimen through the focus plane of the objective lens. The advantage is that no modification is required for the optical system of a standard microscope, but the movement of the specimen can disturb the biological sample. Recently alternative approaches were proposed to achieve focus scanning. Hajime et al. suggest using a micro-actuator to translate the detector axially for the application in photography [10]. Our group demonstrated the use of an electrically-controlled variable focus lens (e.g. a liquid lens) integrated with a microscope objective to achieve focus scanning without involving mechanical movements [3]. By changing the power of the variable focus lens, the focus plane scans "optically" over the...
depth of interest in the object space. This method no longer requires the mechanical movement and retains the flexibility of focus scanning.

The SSFS technique shares similarity to the well-known wavefront coding as a hybrid imaging approach. In both methods, the optical system acquires a blurred image of a specimen with an extended depth range through a well-defined pre-blur imaging mechanism, and a digital image processing is applied to remove the blur effect and restore a clear EDOF image. Although both methods achieve EDOF by compromising the acquired image quality, SSFS imaging provides a rotationally symmetric modulation transfer function (MTF) distribution, whereas the MTF of wavefront coding systems is not uniformly distributed. In a typical pure cubic phase mask design the MTF curves corresponding to the diagonal orientation (± 45°) is much more degraded than the principal directions (vertical and horizontal) [11]. Furthermore, the phase mask in wavefront coding is typically designed for optimizing the image quality within a fixed depth range. When exceeding the designed depth range, the non-negligible phase aberration will result in noticeable image distortion [4]. In contrast, a SSFS system can be more flexibly configured to adapt different depth ranges to some extent.

Although the SSFS technique has been experimentally demonstrated for its capability of substantially extending the DOF of various imaging systems, few works has been performed to analytically characterize the various imaging properties and limitations of this EDOF method and establish guidelines for system design and optimization. For instance, a fundamental assumption of the single-shot focus scanning approach lies in the invariance of the system PSF response across a substantially large depth range. The validation of this assumption, however, apparently depends on the optical system design, the focus scan range, as well as aberrations. In this paper, we analytically modeled the image formation process of a single-shot focus scanning EDOF microscopy system (Sec. 2). The theoretical system impulse response was explicitly described for the examination and simulation of the kernel used in the computational processing. Based on this model, we examined the properties of the system response, quantified the achievable EDOF and the variance of the deconvolution kernel, suggested the operated scan range for a desired EDOF, and examined the limitations of the EDOF range via using an 8-bit 48 dB detector as an example (Sec. 3). With assumed 8-bit resolution of the acquired images, we demonstrated the restored image quality in relation to the axial position of the object, scan range, and signal-to-noise ratio (Sec. 4). Though the predicted imaging properties are based on the analytical model under the condition of being aberration free except defocus, we experimentally verified them via a slightly aberrated prototype system (Sec. 5). The main aim is to provide valuable guidelines in designing and implementing an EDOF imaging system through single-shot focus scanning technique.

2. Analytical model of a single-shot focus scanning system

A SSFS system uses a volumetric optical imaging method that captures the image of a specimen in a single exposure while linearly scanning its focus plane through the depth of interest. Figure 1 illustrates the schematic layout of a SSFS microscope objective, where a variable focus lens and the system stop were placed at the back focal plane of a standard microscope objective. This configuration, similar to the optical layout in [3], forms an object-space telecentric objective design that is able to maintain a constant image magnification while performing focus scanning. By linearly changing the optical power of the variable focus lens during the exposure period of the detector, the focus plane of the imaging system is scanned from the far plane \( z_f \) to the near plane \( z_n \) or vice versa at a constant speed. The focus scan range, denoted as \( S \), is defined by the distance between the far and near planes \( z_f \) and \( z_n \). For convenience, the midpoint of the scan range, denoted as \( z_m \), is defined as the origin of the z-axis. In the rest of the paper, the axial positions were referred by the signed distance from the midpoint, and a position to the right side of the midpoint has a positive distance. For instance, the far and near edges of the scan range correspond to the axial positions of -\( S/2 \) and
Based on this convention, most of the physical quantities were plotted as functions of axial position only along the positive direction due to symmetry.

Considering when the focus plane is located at \( z \) as illustrated in Fig. 1, the instantaneous incoherent PSF of an object located at an arbitrary axial position \( z_0 \) is given by [2]

\[
PSF(r, z - z_0) = 2J_0 \left( \frac{2\pi}{\lambda} NA \cdot r \cdot \rho \right) \exp \left\{ i \frac{2\pi}{\lambda} \left[ \sigma \rho^2 + W(\rho) \right] \right\} \rho d\rho 
\]

where \( r \) is the radial distance from the center of the diffraction, \( z - z_0 \) is the instantaneous defocus distance, \( J_0 \) is the first order Bessel function, \( \lambda \) is the working wavelength, \( NA \) is the numerical aperture of the objective which is constant while scanning focus in such a high magnification object-space telecentric system, \( \sigma \) is the phase aberration related to defocus, \( W \) is the additional wavefront error except defocus, \( n \) is the refractive index in object space which is assumed to be unity here for simplicity, and \( \alpha \) is the half cone angle calculated from \( NA \). Under the assumption that the system is shift-invariant across the field, \( W \) is zero if the system is aberration free except defocus. When the focus plane \( z \) coincides with the axial position of the object, \( z_0 \), Eq. (1) expresses a standard in-focused PSF. For a SSFS system, the focus plane \( z \) varies between the axial positions of \( \pm S/2 \) while it is fixed at the midpoint for a standard microscope system.

During the full period of focus scanning from one edge of the scan range to the other, the corresponding PSF of the object at \( z_0 \), referred to as the accumulated PSF (PSF\(_{acc}\)) [3], can be written by integrating all instantaneous PSFs within the scan range, as

\[
PSF_{acc}(r, z_0) = \frac{1}{S} \int_{-S/2}^{S/2} PSF(r, z_0 - z) dz, \tag{2}
\]

where \( 1/S \) is the normalization factor when compared to the standard in-focused PSF. For a given lateral position \( r \), Eq. (2) can be considered as the convolution of a normalized rectangular function with the same width as the scan range and the axial distribution of a standard PSF as described in Eq. (1) in the longitudinal direction, and is written as

\[
PSF_{acc}(r, z_0) = \frac{1}{S} \int_{-S/2}^{S/2} \text{rect}\left(\frac{z}{S}\right) PSF(r, z_0 - z) dz. \tag{3}
\]

When the scan range \( S \) is much larger than \( 4n\lambda/NA^2 \), which is four times of the DOF of a standard microscope, the factor \( 1/S \) dominates and the PSF\(_{acc}\) becomes less sensitive to the
axial position of the object, $z_0$. Because PSF_{acc} generally has a much wider spread than the standard in-focused PSF, the acquired image of a SSFS system is apparently blurred and has relatively low contrast. It is well understood that a sharp, high-contrast image of an object at $z_0$ can be restored through a deconvolution step with the PSF_{acc} described in Eq. (3) corresponding to the same axial position. As demonstrated in [3] under the conditions that (1) the PSF_{acc} is depth-invariant across the entire depth being imaged and (2) the PSF_{acc} is shift-invariant across the entire imaging field of a single exposure, a sharp EDOF image of the objects across the imaging volume can be restored by a single-step deconvolution process with a representative PSF_{acc}. In this context, the PSF_{acc} at the midpoint of the scan range, was chosen as the representative system response, PSF_{rep}, which is simplified as

$$PSF_{rep}(r) = \frac{1}{S} \int_{-S/2}^{S/2} PSF(r, z)dz.$$  \hspace{1cm} (4)

A restored image $\hat{o}$ is given by

$$\hat{o} = F^{-1}\{I \cdot G\},$$  \hspace{1cm} (5)

where $F^{-1}\{ \}$ denotes the inverse Fourier transformation, $I$ is the Fourier transform of the acquired image, and $G$ is the deconvolution filter defined in the frequency domain. One basic choice for $G$ is the Wiener filter given by

$$G = \frac{H_{rep}^*}{|H_{rep}|^2 + NSR},$$  \hspace{1cm} (6)

where $H_{rep}$ is the Fourier transform of the PSF_{rep}, the representative kernel for image restoration, * denotes a complex conjugate, and NSR is the noise-to-signal ratio of the acquired image power spectra.

The quality of the restored EDOF image in such a hybrid imaging system mainly depends on two factors. The first factor is the invariance of the system response, PSF_{acc}. Though the PSF_{acc} of a practical system may unlikely be absolutely invariant to depth and field positions, the kernel variance should be small enough through the depth and field of interests to ensure that the use of the representative kernel in the deconvolution filter can recover high-quality EDOF images across the entire imaging volume simultaneously. In a standard optical system with a fixed focal depth, it is well understood that nearly shift-invariant system response can be achieved by well correcting field-dependence aberrations across the field of interests in the specific object-image conjugate. In a SSFS system with variable focal depths, as demonstrated in [3], the optical system needs to be optimized to achieve shift-invariance for different object-image conjugates across the depth of interests. Because the various challenges of designing systems with shift-invariance performance are well understood and generally depend on the specifications of various systems, the rest of the paper will focus on analyzing the effects of kernel variance with axial positions and its relationship with the scan range, while assuming shift-invariance across the object-image conjugate planes.

The second factor is the signal-to-noise ratio (SNR) of the acquired image. In general, the acquired image signal should be sufficient to be distinguished from the noise level through the spatial frequencies of interest to prevent any loss of information. In addition, low SNR degrades image restoration and results the loss of contrast on restored image. Due to the wide spread nature of the PSF_{acc}, the acquired image of a SSFS system not only has lower contrast but also low SNR compared to a standard system. It is further expected the acquired image quality degrades as the scan range increases. The effect of low SNR on the SSFS image potentially limits the extension of the scan range.

Without loss of generality, in Sec. 3 through Sec. 4, we modeled a $10 \times 0.25$ NA SSFS system under the condition of being aberration free except defocus (i.e. $W = 0$ in Eq. (1)) as
an example to examine the properties of the system response and quantified the achievable EDOF range, the variance of the deconvolution kernel, and the restored image quality in relation to the axial position of the object, scan range, and signal-to-noise ratio. We also made comparison against a standard microscope system model with the same magnification and NA. We assumed incoherent illumination with a monochromatic wavelength of 0.5 μm and the refractive index of 1 in the object space. Using these specific examples shall not prevent from generalizing the results of the analysis to systems under different conditions, as we experimentally verified the predicted properties via a slightly aberrated prototype system in Sec. 5.

3. Properties and limitations of a SSFS system

3.1 System responses

By using Eqs. (1) and (3), Fig. 2 demonstrated PSF examples of a standard system and a SSFS system with the scan range of 160 μm under the condition of being aberration free except defocus. Figs. 2(a) and 2(b) showed the lateral distributions of the standard PSFs at the axial positions of zero (in-focus) and 20 μm (out-of-focus), respectively, while Figs. 2(c) and 2(d) were the lateral distributions of the accumulated PSFs of a SSFS system, PSFacc, at the same axial positions as those in Figs. 2(a) and 2(b), respectively. As expected, the spot size of a standard PSF at the out-of-focus position shown in Fig. 2(b) was much bigger than that at the in-focus axial position shown in Fig. 2(a). For the SSFS system, the PSFacc did not show visually appreciable differences at both axial positions.

Prior work by Liu and Hua [3] suggest that the PSFacc of a SSFS system has low variance to the axial position of an object. However, its relationship with the scan range and the extent of the invariance were not investigated. To characterize the PSFacc response in relation to the scan range and the axial position of object being imaged, let us consider the Strehl ratios (SR) of the standard PSF and PSFacc described by Eq. (1) and (3), respectively, under the condition of being aberration free except defocus. The SR of a system is defined as the ratio of the peak value of the PSF of a system to the theoretical maximum peak value of a diffraction-limited imaging system.

![Fig. 2. Examples of PSFs. (a) and (b) are the PSFs of a standard system at the axial positions of zero (in-focus) and 20 μm (defocus of 20 μm), respectively; (c) and (d) are the PSFaccs of a SSFS system with the scan range of 160 μm at the same axial positions corresponding to (a) and (b). Simulation parameters: λ = 0.5 μm and NA = 0.25.]

Figure 3(a) plotted the SR value corresponding to the origin of the axial positions (midpoint of the scan range), which represents the maximal SR value of a SSFS system with $W = 0$, as a function of the scan range. The curve was simulated by calculating the PSFacc at $r = 0$ with varied scan range using Eq. (4). As expected, the maximum value of the plot is unity corresponding to the SR of a standard microscope system (i.e. $S = 0$). As the scan range increases, the SR value drops rapidly. When the scan range is much larger than four times of the DOF of a standard microscope (32 μm in the example), the SR value of a SSFS system may be approximated by an inverse proportion to the scan range.

Figure 3(b) plotted the SRs of a standard microscope system and a SSFS system with the scan ranges of 80 μm, 120 μm, and 160 μm in relation to the axial positions of objects along
the positive direction. The SRs of a standard system and the SSFS system were simulated by calculating the PSFs at \( r = 0 \) with respect to the axial positions using Eqs. (1) and (3), respectively. In a standard system, the SR value drops rapidly from unity as the axial position of the object moves away from the origin (in-focus position). It drops to approximately half of the maximum value at its theoretical DOF position (8 \( \mu \)m in the example). On the other hand, in a SSFS system, the maximal SR value of a given scan range is much lower than unity due to the large magnitude of defocus. More noticeably, for a given scan range, the SR value remains nearly invariant as the axial position of the object increases until a transition point near the edge of the scan range is reached. Following the transition point the SR value drops rapidly. Interestingly, regardless of the scan range, the SR drops to the half of the maximum SR value when the object is at the axial positions of \( S/2 \) corresponding to the edge of the scan range. Though the absolute axial position of the transition point depends on the scan range, its relative position is approximately at a distance of a standard DOF from the edge of the scan range. These SR characteristics can be fully explained in Eq. (3) by considering the overlapping area of the rectangular function and the axial distribution of the standard PSF varied with respect to their relative separation. Figure 3 provided the whole picture of the acquired image quality of a SSFS system varied with respect to the scan range and the axial position. In general, the acquired image quality decreases as the scan range increases, and the image quality is almost consistent through the axial position within the central region of the scan range except a further degradation close to the edges.

![Image of SR plots](image-url)

Fig. 3. (a) Degradation of the SR at the origin of the axial position versus the increased scan range in a SSFS system; (b) SRs of a standard system and a SSFS system with the scan ranges of 80 \( \mu \)m, 120 \( \mu \)m, and 160 \( \mu \)m. Simulation parameters: \( \lambda = 0.5 \) \( \mu \)m and NA = 0.25.

Besides the SR comparison, we further examined the MTFs of a SSFS system with various scan ranges. For the scan ranges of 80 \( \mu \)m and 160 \( \mu \)m, respectively, Figs. 4(a) and 4(b) compared the MTF curves corresponding to five axial positions along the positive direction, including the midpoint of the scan range (i.e. the origin) and axial positions corresponding to 1/4, 1/2, 3/4, and 1 of the half-scan range. On the same plots, we also included the diffraction-limited MTF of a standard microscope with equivalent NA. The MTFs were simulated by computing the absolute value of the Fourier transform of the PSF acc using Eq. (3). These curves demonstrated properties consistent with those characterized by the SR plots. As expected, the MTFs are nearly insensitive to the axial positions for over 75% of the scan range while a noticeable drop is observed close to the edge of the scan range. Similar to the SR value which drops to its half-maximum at the edge of the scan range, the MTF curve at the edge is approximately half of that at the other axial positions. All MTF values of the SSFS system are much lower than the diffraction-limited MTF values but still above zero till the diffraction cutoff frequency.
3.2 Extended depth of field and focus scan range

Based on the properties demonstrated by the SR and MTF performances, we concluded that the scan range is the dominant factor that determines the theoretically achievable extended DOF of a SSFS system. Given that the SR of a standard system drops to its half-maximum at the far and near edges of its DOF, the theoretically achievable EDOF of a SSFS system is therefore defined by using the same criterion as a standard microscope, that is the axial depth at the half maximum of the SR, and is given as

$$ EDOF = \pm S / 2. $$

For example, the EDOF of a 0.25 NA SSFS system with the scan range of 160 μm is 160 μm for the full width, or 80 μm for the half width, which is about 10 times of the DOF of a standard microscope with equivalent NA. The EDOF of a SSFS system, to some extent, can be further extended by increasing the focus scan range.

As described by Eqs. (4)-(6), the post-processing step replaced the kernel \( H_{acc} \) corresponding to a particular axial position with the representative kernel \( H_{rep} \) of the origin to form the deconvolution filter in Eq. (6). The kernels are the optical transfer functions (OTFs) of the imaging system. Any mismatch between the representative kernel and the actual kernel corresponding to a given position is expected to cause quality degradation and artifacts on the restored EDOF images. To evaluate this kernel invariance with respect to axial position, the normalized root mean square deviation (NRMSD) of the kernel, as the quantification of the kernel mismatch, is given by

$$ NRMSD(z_0) = \left[ \frac{1}{N} \sum_{i=1}^{N} \left| \frac{H_{acc}^i(z_0) - H_{rep}^i}{H_{rep}^i} \right|^2 \right]^{1/2}, $$

where \( N \) is the total number of samples in frequency domain, \( i \) denotes the numerical sampling for the 2D distribution of OTF within the diffraction cutoff frequency, and \( H_{acc}^i \) and \( H_{rep}^i \) are the OTF values corresponding to the axial position \( z_0 \) and the origin, respectively.

Under aberration free except defocus condition, Fig. 5 plotted the \( NRMSD \) of the kernels in a SSFS system with scan ranges of 80 μm, 120 μm, and 160 μm. Figure 5 also plotted the \( NRMSD \) of a standard microscope for evaluating the kernel mismatch in a standard system. The \( H_{acc} \) and \( H_{rep} \) in the SSFS system were simulated by calculating the Fourier transforms of the PSF \( H_{acc} \) using Eq. (3) and PSF \( H_{rep} \) using Eq. (4), respectively, while the \( H_{acc} \) and \( H_{rep} \) for the standard system were simulated by calculating the corresponding PSF using Eq. (1), where the in-focused PSF was used for calculating the representative deconvolution kernel.
It was observed that the NRMSD value for kernel mismatch for a standard system increases rapidly with increasing axial positions and reaches its maximal value about 0.63 at the edges of its DOF (i.e. ± 8 μm in the example). In the SSFS system with a given scan range, the NRMSD value for kernel mismatch increases slowly from zero as the axial position of object is moved away from the origin until the transition points which are approximately at a distance of a DOF from the far and near edges of the scan range. The NRMSD value for kernel mismatch within the axial region between the two transition points on the positive and negative sides of the z-axis is less than 0.15, which is considerably smaller than the NRMSD value of a standard system within its DOF region. Outside the region marked by the transition points, the kernel mismatch starts to increase rapidly, and the NRMSD values at the edges of the scanning range are 0.5. Based on these observations, we therefore concluded that the kernel invariance of a SSFS system is negligible within the central region between the two transition points.

![NRMSD of the representative kernels](image)

Fig. 5. NRMSD of the representative kernels in a standard microscope system and a single-shot focus scanning system with the scan ranges of 80 μm, 120 μm, and 160 μm. Simulation parameters: \( \lambda = 0.5 \) μm and NA = 0.25.

As to be demonstrated in Sections 4 and 5, the central axial region within the two transition points yields noticeable better quality EDOF images than the regions outside due to better kernel invariance. It is worth highlighting that the estimated points move relatively with respect to the edges of the scanning range by a distance of a standard DOF. Therefore, systems that can afford for a large scan range can take advantage of this property by increasing the scan range as

\[
S' = 2 \cdot (EDOF_D + DOF),
\]

where DOF is the depth of field for the standard image as the focus plane is fixed during the exposure, which is \( \frac{\lambda}{NA^2} \) for a diffraction-limited standard system. \( EDOF_D \) is the half width of the desired full depth of field range, and \( S' \) is the extended scan range. This approach allows extending the central region with low kernel variance to match the desired EDOF range and gain better-quality EDOF images across. On the other hand, the increased scan range leads to lower system SR and MTF responses as demonstrated in Figs. 3 and 4. Potentially the quality of the acquired images may be slightly compromised and degrade the restored image quality through deconvolution processing, which will be discussed further in the next section. Typically the additional scan range, \( 2 \times DOF \), is much smaller than the desired EDOF range and therefore shall not cause significant compromise on the acquired image quality or take a major toll on the hardware.

3.3 The limitations on EDOF range

Although the EDOF of a SSFS system can theoretically be unlimitedly extended by increasing the scan range, as demonstrated in Figs. 3 and 4, the acquired image quality of a SSFS system
is expected to degrade as the scan range increases. When considering practically image detectors with their inherent characteristics that set limits to their capabilities, it is important to select an appropriate focus scan range and a proper detector to ensure the contrast levels of the acquired raw images are sufficient at the frequencies of interest for the selected detector. It is unrealistic to provide a general model for determining the optimal scan range and detector choice because these factors are application driven. Instead we simulated a typical 8-bit detector with a dynamic range of 48 dB as an example to demonstrate the relationship between scan range and the limit of spatial resolution. As shown in Fig. 4, the MTF value of the SSFS system decreases as the scan range and the spatial frequency increase, and can eventually below the sensitivity of the detector.

Figure 6 plotted the theoretical limit of the spatial frequency as a function of the scan range for objects located at the midpoint and the edge of the scan range without considering additional noise. The limiting spatial frequencies were determined by finding the corresponding spatial frequencies where the MTFs of the scan ranges are at −48 dB. For example, with a 200 μm scan range, the theoretical limiting resolutions are about 925 lps/mm at the midpoint and about 820 lps/mm at the edge of the scan range for a diffraction-limited system with 0.25 NA. These curves provide the intrinsic limitation of spatial resolution, which should be aware while using camera dynamic range of 48 dB. Additional noise in the system naturally will further reduce these limits. Besides, the presence of noise can degrade the restored image quality. Since the raw image of a SSFS system generally provides low SNR when comparing to that of a in-focus standard microscope and results in contrast degradation on the restored image, which will be demonstrated in the next section, noise depression plays a role in order to pursuing high-quality restored image. Detectors with better dynamic range such as 12-bit 72 dB or 10-bit 60 dB are expected to have higher sensitivity and lower read-out noise, which may afford larger scan range or better image quality.

Moreover, for a selected detector, the acquired image of a SSFS system with a larger scan range can naturally lose its SNR. With the same illumination condition and exposure time but an increased scan range, a SSFS system captures smaller amount of captured photons from the object at certain axial position as the focus plane moves faster. Therefore, the SNR of a SSFS system with a larger scan range suffers further degradation in photon noise. As will be illustrated in Fig. 7, a serious SNR degradation makes it difficult to restore a high-quality EDOF image from the acquired image. Generally the SNR may be retained by increasing illumination, extending exposure time, or using detectors with larger pixels. However, increasing the exposure time comes at the cost of speed, which can be crucial for applications requiring fast imaging speed, while using detectors with larger pixels also comes at the cost of compromised spatial resolution. Overall, to satisfy the required quality criterion, the extension of the scan range of a SSFS system is limited by a series of trade-off to overcome the lower quality of the acquired images.

Fig. 6. Cutoff frequency as a function of the scan range while using camera dynamic range of 48 dB for objects located at the midpoint and the edge of the scan range. Simulation parameters: \( \lambda = 0.5 \mu m \) and NA = 0.25.
4. Restored image quality

The quality of the restored EDOF images, which generally depends on the kernel invariance, the quality of the acquired raw images, and the deconvolution filter, can be evaluated by the overall MTF of the hybrid imaging system. The overall MTF of the hybrid imaging system for the object at $z_0$ obtained through deconvolution post-processing can be calculated theoretically by taking the absolute value of the direct product of $H_{acc}$ and the deconvolution filter $G$, as

$$MTF_{z_0} = |H_{acc}(z_0) \cdot G|.$$  \hspace{1cm} (10)

Consider the Wiener filter $G$ described in Eq. (6) used in the deconvolution processing. The NSR contained in $G$, as the reciprocal of the SNR, may be considered to model the amount of read-out noise in the signal quantization process of a detector as well as the presence of other intrinsic noise. Under aberration-free except defocus condition, Fig. 7 plotted the overall MTFs for object at five different axial positions along the positive direction, including the origin and axial positions corresponding to $1/4$, $1/2$, $3/4$, and 1 of the half-scan range. Two scan ranges, 80 μm and 160 μm, were examined, and their results were shown in left and right columns of the figure, respectively. For each scan range, the effects of three different levels of NSR were evaluated, including $-48$ dB, $-30$ dB, and $-20$ dB, and the results were shown in each row of the figure. The NSR of $-48$ dB corresponds to the amount of read-out noise in the signal quantization process of an 8-bit detector, while the other two levels of NSRs account for additional noise.

Each of the sub-figures in Fig. 7 demonstrated the effects of kernel mismatch on the restored EDOF image quality for a given scan range and NSR level. In each sub-figure, the MTF curve (in Blue) for the origin of the axial position is smooth and indicates the possibly best restored image quality of the system at the given scan range and noise level because there is no kernel mismatch for the midpoint of the scan range. Among the other four axial positions corresponding to $1/4$, $1/2$, $3/4$, and 1 of the scan range, due to the presence of the kernel mismatch, the MTF curves in all subfigures showed noticeable ripples bouncing around the blue curves, and the departures of these curves from their corresponding MTF curve for the origin become significant as the axial position increases, especially in the low-frequency regions. Some ripples even exceed the unity and cause the overshooting of the transfer function, which will result in artifacts on the restored images. However, in each sub-figure, the MTF curves for the axial positions corresponding to $1/4$, $1/2$, and $3/4$ of the scan range are on average the same as the corresponding MTF curve for the origin except the ripple artifacts. This indicates that the restored image will retain uniform quality for objects within these regions except artifacts for some spatial frequencies. For the axial positions corresponding to the edges of the scan ranges, the MTF curves (in Magenta) drop to approximately halves of MTF values for the origin position (the blue curves) at corresponding frequencies. Since the MTFs across the entire scan range are above zero throughout the diffraction cutoff frequency, the deconvolution post-processing shall be able to retain details for objects located within the scan range, as long as the sensitivity of a selected detector is high enough to capture the low-contrast details throughout the frequencies of interest in the acquired raw images.

In each column of Fig. 7 the effects of SNR degradation in the acquired raw images on the restored EDOF image quality were demonstrated. Apparently the MTF curves for all axial positions degrade as the NSR increases. This indicates that as the noise level increases in the acquired raw images, the contrast of the restored EDOF images decreases. Moreover, maintaining low system noise level is crucial for systems with a wide scan range because, as discussed in Sec. 3.3, photon noises will naturally raise as the scan range increases with a fixed exposure time, which fundamentally limits the extension of scan range.
Fig. 7. The overall MTFs of sampled axial positions (0, 1/4, 1/2, 3/4, and 1 of the half-scan range) in a SSFS system with the scan ranges of (a, c, and e) 80 μm and (b, d, and f) 160 μm. Three different levels of NSRs, −48 dB, −30 dB, and −20 dB, were applied in the simulations for the figures (a, b), (c, d), and (e, f), respectively. Simulation parameters: \( \lambda = 0.5 \) μm; NA = 0.25.

The sub-figures in each row of Fig. 7 demonstrated the effects of varied scan range on the restored EDOF image. For a given NSR level, the MTF curves of the system with a larger scan range (e.g. 160 μm) present less ripples but showed more noticeable degradation at middle-high spatial frequency region than a smaller scan range (e.g. 80 μm). For instance, when the NSR level is low as shown in Figs. 7(a) and 7(b), the overall MTFs of the restored image are nearly identical in the mid-low (< 600 lps/mm) frequency region for both scan ranges, and show noticeable differences in the high frequency region. As the NSR level increases as shown in Figs. 7(c) through 7(f), the overall MTFs of the restored images for the scan range of 160 μm are noticeably lower than those for the scan range of 80 μm at the same NSR level. This suggests that the dynamic range and sensitivity of the selected detector is inadequate for capturing the low-contrast signals at the given scan range and a better detector can be used to improve the restored image quality.

Finally, Eq. (9) suggested that the scan range could be further extended beyond the desired EDOF range in order to minimize the effect of kernel mismatch and substantially improve the quality of the restored image. This was further confirmed by the results shown in Fig. 7. In each row of the subfigures in Fig. 7, which have the same NSR level, the MTF values for the
axial positions of 40 μm in the 160 μm scan range system (right column) are generally higher than those for the axial position of 40 μm position in the 80 μm scan range system (left column), especially in the mid-low frequency region. The amount of further SNR degradation due to the increased photon noise when the scan range was doubled, corresponding to +1 dB NSR in the simulation, should not make significant difference on the simulated MTF curves in Figs. 7(b, d, and f) for a given noise level. As the system noise level increases, where the acquired image quality may dominate the effect on the restored image quality, the advantage of using the larger scan range (e.g. 160 μm) starts diminishing in the mid-high frequency region. However, as the suggested extension of scan range, 2DOF, is typically a small fraction of the desired EDOF, the effect of low acquired image quality resulting degraded restored image quality is not as noticeable as the example in Fig. 7 where the scan range in the right column was doubled.

5. Experimental demonstration

While the analyses in Sections 3 through 4 were based on the simulated results of the theoretical model (Sec. 2) under the condition of being aberration-free except defocus with simulated NSR, we further verified the predicted properties via the experimental results under a practical condition where the system is not diffraction-limited and an aberration-free deconvolution kernel was used instead of the experimental kernel in the deconvolution processing. We built a prototype system using a liquid lens integrated with a microscope objective composed of two stocked doublets that provides a fair imaging quality. As illustrated in Fig. 1, the liquid lens was placed at the rear focal plane of the objective, and its clear aperture served as the system stop for accomplishing an object-space telecentric configuration that maintains image magnification while performing focus scanning. The system provides a constant 10 × magnification with an approximate 0.25 NA, and its focus plane can be fixed for capturing a standard image or scanned through an extended depth range up to 160 μm during a single exposure for obtaining a SSFS image.

A 1 μm pinhole mounted on a z-direction translation stage with a 5 μm graduation was imaged by the system through both standard and SSFS imaging methods. The PSF of the standard microscopy and PSFacc of the SSFS method, and their properties in relation to the axial positions were investigated with a limited axial resolution of 5 μm. Figures 8(a) and 8(b) showed the axial distributions of the peak values of the PSF of the standard image and the PSFacc of the SSFS image with 160 μm scan range, respectively. The values were normalized by the maximum peak value of the standard PSF across the depth to compare with the SRs in Fig. 3. As the criterion used for the SRs, by examining the half width at half of the maximum of the standard PSF values in Fig. 8(a), we found the DOF of the standard image in our prototype system is about ±15 μm, which is larger than the diffraction-limited DOF, ±8 μm, and the system is considered to be slightly aberrated by spherical aberration. However, the half width at half of the maximum of the aberrated PSFacc peak values in Fig. 8(b) is approximately the same as the half of the scan range, 80 μm. It implies that the achievable EDOF range of a SSFS system with small aberration can still be comparable to that of an aberration-free system with the same scan range described in Eq. (7). In Fig. 8(b) we observed two transition points that separate two dropping regions near the edges of the scan range from the relatively flat region at center. The relative positions of the transition points are approximately at a distance of 15 μm from the edges of the scan range. It is worth noticing under the slightly aberrated condition, the relative distance between a transition point and the corresponding edge of the scan range is approximate to the practical DOF of the system instead of the theoretical DOF of an aberration-free system with equivalent NA. On the other hand, comparing to the invariant region of the SRs of the SSFS system in Fig. 3(b), the aberrated PSFacc peak values in Fig. 8(b) showed noticeable variance within the central region. The variation of the PSFacc indicates the raise of kernel mismatch in a SSFS system with small aberration, which is expected to result in quality degradation on the restored image.
Interestingly, as will be demonstrated later in this section, the slightly aberrated SSFS system is able to tolerate small kernel mismatch and perform comparable restored image quality within EDOF to a standard in-focused image.

To quantify restored image quality and demonstrate the predicted imaging properties, a series of images of a USAF resolution target placed at different axial positions across the depth of interest were taken by a Point Grey camera with a 8-bit depth and approximate Nyquist frequency of 500 lp/mm. Images were captured at a fixed exposure time for both standard and SSFS imaging methods. To restore the EDOF image from the SSFS raw images, instead of calculating the Fourier transform of the experimentally measured PSF acc, the representative kernel was calculated by the ideal PSF acc using Eq. (4) for a given scan range with \( W = 0 \) under aberration free except defocus condition. Regardless of kernel mismatch, the sharp EDOF SSFS images were restored via a single-step deconvolution process as described in Eq. (5) with the Wiener filter described in Eq. (6) using a constant NSR. To compare the image quality of the restored EDOF images with a standard image and create a standard format image, a normalization process was performed to the intensity values of the restored image at each pixel position fit the same range of 0 to 255 by an universal scaling factor for a given scan range. Based on the convention in this paper, the following evaluations were performed for the axial positions only along the positive direction due to symmetry.

![Fig. 8. Experimental results of the axial distributions of the peak value of the PSFs in the prototype system taking from (a) PSF of the standard image captured by fixing focus plane during exposure; (b) PSF acc of the SSFS image captured by scanning focus plane within the scan range of 160 \( \mu \text{m} \) during a single exposure.](image)

To visually demonstrate the fair quality of the restored SSFS images, Fig. 9(a) showed the standard image of the target placed within the standard DOF region, while Figs. 9(b) through 9(f) showed the restored images of the prototype SSFS system with the scan range of 160 \( \mu \text{m} \) for the targets placed at the midpoint of the scan range and the axial positions corresponding to 1/4, 1/2, 3/4, and 1 of the half-scan range from the midpoint, respectively. Due to the effect of kernel mismatch, all restored images showed noticeable artifacts at blank regions which contain low spatial frequency contents. Because the presence of artifacts lowered the minimum image value and hence raised the averaged background level through the normalization process, the background of the restored images appeared lighter than that of the standard image shown in Fig. 9(a). On the other hand, the restored image quality was visually consistent for the axial positions from the midpoint to 3/4 of the half-scan range as shown in Figs. 9(b) through 9(e). The restored image quality at the edge of scan range shown in Fig. 9(f) appeared noticeably dimmer and lower contrast than the others. Despite a lighter background showed on the restored images, all of restored images within the scan range appeared sharp and all the spatial details up to the Nyquist frequency (Group 9 Element 1) can be visually discerned. It implies that under the condition that the system is slightly aberrated and exists small kernel mismatch in the deconvolution processing, the full range of the achievable EDOF is still comparable to the scan range.
To quantify the image quality, we analyzed the image of the selected elements on the resolution target corresponding to spatial frequencies of 64, 102, 144, 203, 256, 287, 323, 362, and 406 lp/mm by fitting the central raw profile of each element with a sinusoidal wave to find the maximum and minimum of the oscillation, $I_{\text{max}}$ and $I_{\text{min}}$, respectively. The calculated fringe contrast value of the analyzed element is given as $(I_{\text{max}} - I_{\text{min}})/(I_{\text{max}} + I_{\text{min}})$. The contrast values of the elements in both directions were analyzed and averaged with respect to the selected frequencies. Figure 10(a) presented the analyzed contrast values of the standard in-focused image shown in Fig. 9(a), and the restored images at the midpoint and the edge of the scan range in Figs. 9(b) and 9(f), respectively. The contrast curves of the restored images (in Blue and Magenta) are essentially higher than that of the standard in-focused image (in Black) except at low frequencies in the restored image at the edge of the scan range. With this criterion, the quality of the restored image within the achievable EDOF is considered to be comparable to the standard in-focused image.

However, as the restored images with the presence of artifacts in the background were visually less appealing to the standard image in Fig. 9(a), we furthered calculated the contrast-to-noise ratio (CNR), which accounts for the scales of background noise, for evaluating the restored image quality in the following context. The equation of CNR is given as

$$\text{CNR} = \frac{I_{\text{max}} - I_{\text{min}}}{\sigma},$$

where $I_{\text{max}}$ and $I_{\text{min}}$ are the maximum and minimum of the oscillation, respectively, which were obtained by the same way for determining fringe contrast; $\sigma$ is the standard deviation of the background intensity, which was obtained by taking the standard deviation of the image values within a blank area. Figure 10(b) showed the CNR values of the standard in-focused image in Fig. 9(a), and the restored images at the axial positions corresponding to 0, 1/4, 1/2, 3/4, and 1 of the half-scan range in Figs. 9(b) through 9(f), respectively. The CNR curves of the restored images for the axial positions between the midpoint and 3/4 of the half-scan range (in Blue, Cyan, Green, and Yellow) are essentially lower than that of the standard image (in Black) at low frequencies but higher at high frequencies. Though the CNR curves for the axial positions from 1/4, 1/2, and 3/4 of the half-scan range (Cyan, Green, and Yellow) were not
bouncing around the CNR curve for the midpoint (Blue) as shown in the sub-figures in Fig. 8 and have maximal values alternatively across low spatial frequency to high frequency, the areas below these curves were similar. As expected, the CNR curve for the edge of the scan range (in Magenta) dropped to about halves of the others at corresponding frequencies.

Fig. 10. (a) Analyzed contrast values of the sampled spatial frequencies for the standard in-focused target image shown in Fig. 9(a) and the restored images of the SSFS system shown in Fig. 9(b) and 9(f); (b) Analyzed CNR values of the sampled spatial frequencies for the standard in-focused target image shown in Fig. 9(a) and the restored images of the SSFS system shown in Fig. 9(b) through 9(f).

To demonstrate the axial properties of the restored SSFS image, Fig. 11 plotted the summations of CNR values through sampled frequencies in relation to axial positions for the SSFS system with the scan range of 120 μm and 160 μm. For a given scan range, the summations of CNR curve was relatively flat across the central region until the transition point at an approximate distance of 15 μm from the edge of the scan range and dropped rapidly after that. Regardless of the scan range, the CNR drops to about the half of its maximum when the target is placed at the edge of the scan range (60 μm and 80 μm, respectively, for the two examples). The CNR values at the central region between the transition points show much less variant than the peak values of the PSFacc shown in Fig. 8(b). It implies practically the SSFS system has the capability of tolerating small aberration along with kernel mismatch and provides consistent restored image quality across the axial positions within the central region between the transition points. Like Fig. 8(b), the relative distance between one of the transition point and the corresponding edge of the scan range is approximate to the practically aberrated DOF instead of the diffraction-limited DOF. Therefore the suggested extension of the scan range beyond the desired EDOF in order to improve the restored image quality described in Eq. (9) is now two times of the practical DOF as demonstrated. Figure 11 also demonstrated the effect of the restored image quality as the SSFS system operated with a larger scan range. The CNR values within the central flat region of the SSFS images with 160 μm scan range are smaller than those of the SSFS images with 120 μm scan range. It confirms the predicted quality degradation on the restored images as the scan range increases due to the limited SNR. As discussed in Sec. 3.3, without compromising on shutter speed or spatial resolution, a detector with a higher bit depth may be used for affording a larger EDOF range.

Finally, Fig. 12 demonstrated the effects of the extended scan range method suggested in Eq. (9) for an example of a ± 60 μm desired EDOF. The resolution target was placed at 60 μm from the origin, 5 raw SSFS images of the target were captured, corresponding to a scan range of 120 μm, 130 μm, 140 μm, 150 μm, and 160 μm, and their corresponding EDOF images were restored using the same post-processing as those shown in Fig. 9. For each of the restored images, the same spatial frequencies as those used in Figs. 10 and 11 were sampled and their corresponding CNR values were computed using Eq. (11). Figure 12 plotted the CNR values as a function of the spatial frequencies for the restored SSFS images at the axial
positions of 60 μm from the midpoint with the various scan ranges from 120 to 160 μm. As shown in Fig. 12, the lowest CNR curve (in Blue) corresponds to the scan range of 120 μm, where the achievable EDOF is equivalent to the desired EDOF. The highest CNR curve (in Yellow) corresponds to the scan range of 150 μm, which extends the full range of the desired EDOF by 2DOF as suggested in Eq. (9). As the CNR curve corresponding to the scan range of 160 μm (in Magenta) appeared noticeably lower than the blue curve corresponding to 150 μm scan range, it illustrated that a further extension of the scan range does not improve the restored image quality further. When extending the scan range beyond the range suggested in Eq. (9), the effect of low SNR due to the increased scan range starts to dominate the effect of kernel mismatch near the edges of the scan range and degrades the restored image quality within the desired EDOF.

Fig. 11. Summations of CNR values through sampled frequencies in relation to axial positions for the SSFS system with the scan range of 120 μm and 160 μm.

Fig. 12. Analyzed CNR values of the sampled spatial frequencies for the restored SSFS images of the target placed at the axial position of 60 μm from the midpoint with various scan ranges from 120 μm to 160 μm.

7. Conclusion

Through analytical modeling under the condition of being aberration free except defocus we have examined the properties and limitations of a single-shot focus scanning (SSFS) system, and quantified the achievable EDOF range and restored image quality in relation to the axial position of the object being imaged, the operated scan range, and signal-to-noise. By examining the SR, MTF, as well as the NRMSD properties, the system responses of a SSFS system demonstrated high degrees of insensitivity to the axial position of an object within the scan range. The theoretically achievable EDOF was found equivalent to the half of the scan range based on the same criterion of the axial SR as being used in a standard microscope. We further suggested extending the scan range beyond the full width of the desired EDOF range by a small fraction, two times of the standard DOF, can minimize the effect of kernel mismatch. In practice, because both the contrast and SNR of the acquired image in a SSFS system degrade with the increase of scan range, the practically achievable EDOF range is limited by the detector characteristics. Without compromising on shutter speed or spatial resolution, detectors with low quantization errors and high dynamic range can afford larger
EDOF range. These predicted imaging properties were experimentally verified via a 10 × 0.25 NA prototype system under a practical condition that the system is not diffraction-limited and an aberration-free deconvolution kernel was used instead of the experimental kernel in the deconvolution processing. With the experimental results, we have demonstrated the SSFS system has capability of tolerating small aberration along with kernel mismatch. For example, in a focus-scanning microscope with a ±15 μm standard DOF, a 120 μm focus scan range can obtain a ±60 μm EDOF, but a 150 μm scan range affords noticeably better EDOF images for the same EDOF range. A further extension of the scan range degraded the restored image quality due to the lower SNR of the acquired image. Based on the guidelines developed in this paper, our future work will focus on optimizing and implementing an EDOF system for a variety of microscopy applications.

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