Optimization of pupil design for point-scanning and line-scanning confocal microscopy

Yogesh G. Patel,1,* Milind Rajadhyaksha,2 and Charles A. DiMarzio1,3

1Electrical & Computer Engineering Department, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts 02115, USA
2Dermatology Services, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 160 East 53rd Street, New York, New York 10010, USA
3Mechanical & Industrial Engineering Department, Northeastern University, 360 Huntington Avenue, Boston, Massachusetts 02115, USA
*ypatel@ece.neu.edu

Abstract: Both point-scanning and line-scanning confocal microscopes provide resolution and optical sectioning to observe nuclear and cellular detail in human tissues, and are being translated for clinical applications. While traditional point-scanning is truly confocal and offers the best possible optical sectioning and resolution, line-scanning is partially confocal but may offer a relatively simpler and lower-cost alternative for more widespread dissemination into clinical settings. The loss of sectioning and loss of contrast due to scattering in tissue is more rapid and more severe with a line-scan than with a point-scan. However, the sectioning and contrast may be recovered with the use of a divided-pupil. Thus, as part of our efforts to translate confocal microscopy for detection of skin cancer, and to determine the best possible approach for clinical applications, we are now developing a quantitative understanding of imaging performance for a set of scanning and pupil conditions. We report a Fourier-analysis-based computational model of confocal microscopy for six configurations. The six configurations are point-scanning and line-scanning, with full-pupil, half-pupil and divided-pupils. The performance, in terms of on-axis irradiance (signal), resolution and sectioning capabilities, is quantified and compared among these six configurations.

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References and links

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Both point-scanning and line-scanning confocal microscopes have proven successful for imaging of human tissues, providing resolution and optical sectioning to observe nuclear and cellular detail. Both technologies are being translated for diverse clinical applications [1–3]. While traditional point-scanning is truly confocal and offers the best possible optical sectioning and resolution, line-scanning is partially confocal but may offer a relatively simpler and lower-cost alternative for more widespread dissemination into clinical settings.

A line-scan is confocal in only one dimension (orthogonal to the line) but not in the other dimension (that is parallel). Consequently, the diffraction-limited optical sectioning is ~20% weaker than that with a point scan [4,5]. However, with a reasonably high numerical aperture, the sectioning is sufficient for imaging nuclear and cellular detail in human tissues such as, for example, the epidermis in skin and epithelium in ovaries [6,7]. Of more serious consequence, is the loss of sectioning with increased imaging depth. The loss of sectioning and loss of contrast due to scattering is more rapid and more severe than with a point-scan.

Interestingly, the optical sectioning and contrast with line-scanning confocal microscopy may be recovered with the use of a divided-pupil, as was experimentally discovered in human skin [8–10]. The divided-pupil configuration was originally pioneered by Koester [11] and is similar to that of the theta microscope which was later developed by Stelzer and Webb [12,13]. More recently, Si et al., Gong et al. and Sheppard et al. reported a theoretical analysis of the divided-pupil configuration with point-scanning, showing improvement in optical sectioning, compared to the full-pupil configuration, under certain detector conditions [14–16]. Liu et al., too, have reported analytical and experimental imaging results, showing stronger sectioning and enhanced contrast in deep tissue with their dual-axes point-scanning design, compared to a conventional single axis [17,18]. The dual axes design mimics the divided-pupil and theta microscope configurations, in which the transmitter and receiver paths are separate and intersect only in the object plane (optical section). The full-pupil is, of course, the standard configuration, in which the transmitter and receiver paths are coaxial.

The results to date indicate that the divided-pupil approach may offer improved imaging performance in scattering tissues, compared to the full-pupil, with either point-scanning or line-scanning. Moreover, as part of ongoing efforts to translate confocal microscopy for detection of skin cancer, we are exploring a simpler and lower-cost line-scanning configuration [7–9] that may offer a practical alternative to currently available point-scanning technology. Thus, to determine the best possible approach for clinical applications, we are...
now developing a quantitative understanding of imaging performance for a set of scanning and pupil conditions.

In this paper, we report a Fourier-analysis-based computational model of confocal microscopy for six configurations. The six configurations are point-scanning and line-scanning, with three pupil conditions which are full-pupil, half-pupil and divided-pupil. The full-pupil configuration is with transmitter and receiver through the same pupil. The half-pupil configuration is with transmitter and receiver through the same half-pupil. Although not a practical configuration, it provides useful insight into the degradation of performance with half of the circular aperture. The half-pupil configuration is important in understanding the improved out-of-plane rejection, however, not the best configuration for optimal resolution. Finally, we consider a divided-pupil configuration, with half-pupils separated by an aperture divider, one-half for transmission and the other for receiver detection. Comparative analysis of the half-pupil and divided-pupil configurations allows us to discriminate between the effects of reducing the numerical aperture and separating the two paths. The performance, in terms of on-axis irradiance (signal), resolution and sectioning capabilities, is quantified and compared among these six configurations. We present results of integrated intensity in the coherent-transmission path and incoherent-receive path, which can be used to understand background speckle from scattered light.

2. Theory

Light propagation in the transmitter and receiver paths can be characterized using Fourier methods [19]. Specifically, the field in the pupil plane of the objective lens is proportional to the Fourier transform of the field in the field plane of the objective lens. For example, a uniform plane wave in a circular aperture in the pupil plane is diffracted so that it converges to form an Airy pattern as the point-spread-function (PSF) in the field plane. We use the term “field plane” for the object or image plane.

Coherent light propagation is described by the Fresnel-Kirchoff integral:

\[
E_{\text{Field}}(x_f, y_f; z_f) = \frac{jk}{2\pi} \int_{\text{Aperture}} E_{\text{Pupil}}(x_p, y_p; 0) \frac{e^{jk}}{r} dx_p dy_p
\]

where the distance, \( r \), is between \((x_f, y_f, z_f)\), the field coordinates, and \((x_p, y_p, 0)\), the pupil coordinates. By taking the paraxial approximation of Eq. (1) and expanding the radial distance, \( r \), we obtain the field as a Fourier transform:

\[
E_{\text{Field}}(x_f, y_f; z_f) = \frac{jk}{2\pi z_f} \int_{-\infty}^{\infty} E_{\text{Pupil}}(x_p, y_p; 0) e^{\frac{j2\pi}{\lambda (z_f + z_p)}} e^{-\frac{(x_p x_f + y_p y_f)}{z_f}} dx_p dy_p
\]

where the Fresnel radius, \( r_{\text{f}} \), is given by,

\[
r_{\text{f}} = \sqrt{2\lambda z_f}.
\]

We have assumed \( z_f \) is large enough to neglect a curvature term in \( x_f \) and \( y_f \). In practice, this condition can be satisfied by the use of a Fraunhofer lens (see, e.g [20]). Equation (2) defines the relationship between the pupil function and the field function in terms of a Fourier transform (FT) pair. In order to calculate the field at a location, \( z \), other than the field plane, a defocus parameter, \( Q \), can be applied to the FT pair, where,

\[
\frac{1}{Q} = \frac{1}{z_f} - \frac{1}{z}, \quad r_{\text{f}} = \sqrt{2\lambda Q}.
\]
Equation (2) can be written with the defocus parameter:

\[
E_{\text{Full}}(x_f, y_f, z_f) = \frac{jk e^{ikz_f}}{2\pi z_f} e^{j2\pi \frac{(x_f^2 + y_f^2)}{2z_f}} \int \int E_{\text{pol}}(x_p, y_p, 0) e^{-j2\pi \frac{(x_f x_p + y_f y_p)}{z_f}} d\chi_p d\gamma_p. \tag{5}
\]

The Fourier analysis allows for treating Eq. (3) and Eq. (5) in terms of spatial frequencies, where the spatial frequencies are defined as:

\[
f_x = \frac{x_p}{\lambda z_o}, \quad f_y = \frac{y_p}{\lambda z_o}. \tag{6}
\]

We can describe an optical system by saying that the image is the convolution of the object and the point spread function (PSF). Equivalently, we can say that the FT of the object is multiplied by the transfer function to produce the FT of the image, where the transfer function is the FT of the PSF. For coherent imaging, we consider the object and image to be described by electric fields, and we call the PSF the coherent PSF. For incoherent imaging, the object and image are characterized by irradiance, and we use the terms incoherent PSF, and incoherent transfer function or optical transfer function. Thus optical systems can be analyzed by using only Fourier transforms, inverse Fourier transform, and multiplication.

3. Fourier optics model

The Fourier-analysis computational model was developed for two scanning modes, point-scanning and line-scanning. In each mode, we evaluated the performance for three pupil configurations: full-pupil, half-pupil and divided-pupil. In the full-pupil, the transmitter and receiver path are through the entire pupil. For the half-pupil configuration, the transmitter and receiver path are through the same half of the pupil. The half-pupil is a useful intermediate step between the full-pupil and divided-pupil configuration. It provides the same pupil geometry as the divided-pupil, and therefore the same resolution, and beam profiles. It is not a practical approach to microscopy, but provides insight into resolution and sectioning. The divided pupil enhances contrast, because the transmitter and receiver paths are through opposite halves of the pupil, leading to rejection of light scattered by objects far from the field plane at \( z_f \).

![Fig. 1. Fourier-analysis computational model flowchart.](image)

A complete Fourier-analysis computational model flowchart, in three components, is shown in Fig. 1: (I) the transmission irradiance at the sample, (II) the receiver function at the...
detector, and (III) the aperture configuration at the pupil plane. Each row in Fig. 1 will be discussed in the following sections.

3.1. Coherent-transmitter path

For the transmitter’s figure of merit, we compute the on-axis irradiance along the coherent-transmitter path of a confocal point-scanning or line-scanning microscope in order to optimize the profile of a Gaussian beam in a full-pupil or divided-pupil.

We define the pupil diameter as $D$ and the Gaussian beam diameter at $1/e^2$ is $hD$. The variable parameter, the fill-factor, $h$, is the ratio of the $1/e^2$ diameter of the Gaussian beam to the diameter ($D$) of the full-pupil. From Fig. 1, on the transmitter side, we start with a coherent source, $E_{Pupil}(x_p, y_p, 0)$, at the pupil plane (Ia) and compute the field $E_{Field}(x_f, y_f, z_f)$ in the field plane (Ib) according to Eq. (5). The irradiance (Ic), is $|E_{Field}(x_f, y_f, z_f)|^2$, the square of the magnitude of the field (Ib).

3.2. Incoherent-receiver path

For the receiver side, we use incoherent calculations because the detected signal is proportional to $|E|^2$. Incoherent analysis for a single unresolved scatterer is acceptable because we can use coherent or incoherent. For a collection of scatterers, which may be out-of-focus, to determine clutter, incoherent is the right approach. Therefore, we need the incoherent transfer function and the incoherent-point spread function. The image in incoherent receiver is calculated by convolving the object irradiance with the incoherent-point spread function (PSF).

We therefore need a description of the receiver in the field plane. In the absence of diffraction, the receiver would be described as a geometrical optics image of the pinhole, or specifically a function that is non-zero inside a finite radius. A complete description of the receiver function is given as the convolution of this pinhole function with the incoherent-PSF of the optical system. Equivalently, in the pupil plane, we multiply the Fourier transform of the pinhole function by the incoherent transfer function.

The pinhole function is shown in Fig. 1(IIa), along with its Fourier transform (IIb). Recalling that (1) the incoherent optical transfer function is the Fourier transform of the incoherent PSF, (2) the incoherent PSF is the square of the coherent PSF, and (3) the coherent PSF is the inverse Fourier transform of the coherent transfer function, we calculate the optical transfer function as shown in line III of Fig. 1.

The coherent transfer function, given by the aperture for (IIIa) is inverse Fourier transformed to the produce the coherent PSF (IIIb). Next, we compute the magnitude squared of the coherent PSF to obtain the incoherent PSF and then Fourier transform it to obtain the optical transfer function (IIIId). We multiply this optical transfer function by the Fourier transform of the pinhole (IIb) and inverse Fourier transform to obtain the receiver function (IV). Now we have the transmitter irradiance in (Ic) and the receiver function in (IV). Multiplying these two, we obtain the sensitivity of the microscope (V) to a point target of scattering cross-section. We can then (1) examine the maximum value of this function to determine signal strength, (2) measure its variation with $x$ (or $y$) to determine transverse resolution, or (3) integrate it over all $x$ and $y$ to determine the response to a thin target. Finally, we can vary $z_o = z_f + z$, and determine how the thin target signal degrades with defocusing, $z$, in order to evaluate sectioning ability.

4. Results

We will define all transverse distances in terms related to the pupil diameter, $D$. We have already mentioned the beam diameter, $d = hD$. In the divided-pupil configuration we can vary the width of the divider, $w = a_wD$, and the center position of the light source, $(x_c, y_c)$, given as $x_c = a_xD$ and $y_c = a_yD$. In the full-pupil configuration, symmetry dictates that the center position of the Gaussian beam light source, $(x_c, y_c)$, is optimally set at $(0,0)$ for maximum irradiance at the sample, as shown in the pupil plane of Figs. 2a, 2b, 2c.
4.1. Coherent-transmitter path

Numerous parameters are available for optimizing the coherent-transmitter path, such as image irradiance and peak irradiance, resolution, contrast and signal to noise ratio. The choice, of course, will usually be an optimum compromise among these parameters that will depend on the desired application and. For our work, optimization of the coherent-transmitter path is achieved by maximizing the image field irradiance and peak irradiance. To maximize image field irradiance, the goal is to optimize fill-factor, $h$, and $xy$-position ($\alpha_x, \alpha_y$), for a given $a_w$. If loss of power is eliminated, optimal $h \rightarrow 1.00$, however, a compromise is made by using $h$ slightly greater than the $h_{optimal}$ to account for power.

4.1.1. Full-pupil point-scanning system

The transmitter pupil irradiance (W/mm$^2$) and field irradiance (W/μm$^2$) for varying fill factors, $h$, are shown in Fig. 2. The Gaussian beam is optimally centered at ($\alpha_x = \alpha_y = 0$) for maximum irradiance at the sample as shown in Fig. 2. We show three specific cases, with an under-filled pupil (Fig. 2a), a moderately filled pupil (Fig. 2b), and an over-filled pupil (Fig. 2c). The corresponding transmitter irradiance maps are shown in Figs. 2d, 2e, 2f, respectively.

![Fig. 2. Transmission pupil and field irradiance for a full-pupil point-scanning system.](image)

With the Fourier-analysis computational model, the fill factor, $h$, is varied $0 \leq h \leq 5$, and the on-axis image irradiance is plotted in Fig. 3a. For small values of $h$, $(h < 1)$, as in Figs. 2a and 2d, we can integrate $E_{Pupil}(x_p,y_p,0)$ to infinity because it approaches zero before reaching the edge of the aperture and a Gaussian-beam approximation is valid. To confirm the correctness of the Fourier analysis we verify good agreement between the numerically computed on-axis image irradiance and this Gaussian approximation. The irradiance increases with increasing $h$ because the image diameter of the Gaussian beam becomes smaller in inverse proportion to $h^2$. For large values of $h$, the Gaussian beam is approximately constant over the aperture and the image field is an Airy function. Again, image irradiance of the Airy function agrees with the Fourier analysis. The irradiance decreases as $h$ increases because the large Gaussian beam overfills the pupil of the objective by increasing amounts. The diffraction pattern does not change shape or size but the image irradiance decreases because the total power through the aperture decreases.

Using the numerical computation, the optimum fill factor, $h$, is near the intersection of the computed Gaussian beam and uniform source. More exactly, the optimum is at $h = 0.89$ and the fields shown in Figs. 2b and 2e are optimal.
4.1.2. Full-pupil line-scanning system

We analyzed the full-pupil line-scanning configuration using the same approach. The optimal position, \((x_c, y_c)\), for the line-source is again \((0,0)\) by symmetry, with the computed optimum fill factor, \(h = 1.02\), shown by the peak (o) in Fig. 3b. The pupil is slightly overfilled to produce the highest on-axis irradiance in the image plane. This result is not surprising. As the Gaussian beam diameter at the pupil increases for the point-scanner, the area of the beam in the field plane decrease according to \(1/h^2\). In line-scanning, it decreases according to \(1/h\). Therefore, optimization occurs at (o) for larger \(h\).

4.1.3. Divided-pupil point-scanning system

For the half-pupil and divided-pupil configurations, the coherent-transmitter path is the same, therefore the Fourier-analysis computational model for the coherent-transmitter path was repeated for only the divided-pupil configuration of a point-scanning and line-scanning system. Now we need to optimize \(h\) and \(a_x\) simultaneously. The optimal values are dependent on \(a_w\), but, \(a_y = 0\) still by symmetry. In the present work we consider \(a_w = 0\) to maximize transmitter area. The plot of the on-axis image irradiance for the divided-pupil point-scan is shown in Fig. 3c. The peak (o) for the computed divided-pupil point-scan on-axis image irradiance is at \(h = 0.66\) with \(a_x = 0.35\). For completeness, the Gaussian beam, uniform source, and the full-pupil point-scanning on-axis image irradiance are plotted. As expected, the peak is smaller than for the full-pupil case.
4.1.4. Divided-pupil line-scanning system

The optimum fill factor, \( h \), for the divided-pupil line-scanning system, as determined by the peak (\( \circ \)) in Fig. 3d is \( h = 0.52 \) with \( a_x = 0.35 \). The Gaussian beam, uniform source, and the full-pupil line-scanning on-axis image irradiance are plotted for comparison. Again, the optimal \( h \) is smaller for line-scanning than point-scanning as expected (Table 1).

| h | \( a_x \) | \( \text{Irradiance} \) |
|---|---|---|
| 0.52 | 0.35 | 1.5 W/mm\(^2\) |

4.2. Transverse resolution measurements

Resolution is defined as the ability to discern that two objects are distinct. It depends on signal-to-noise ratio and desired statistics; therefore, it is complicated to define exactly and no standard criterion exists. Whatever the choice of definition for resolution, it can be computed from the point spread function (PSF) in the field plane (V) as seen in Fig. 1. We arbitrarily choose to use the full width at half maximum (FWHM), which is the distance between the points where the irradiance is equal to 50% of the maximum irradiance, to determine the transverse resolution for a point-scan, \( \Delta x_p \), or line-scan, \( \Delta x_l \).

4.2.1. Transverse resolution for point-scanner

The irradiance is plotted as a function of transverse distance, \( x \), for three point-scanning systems in focus (\( z = 0 \mu m \)) in Fig. 4 with a detector pinhole diameter, \( d_{\text{pinhole}} = 2.5* d_{\text{AiryDisc}} \) and NA = 0.90. The transmitter image irradiance, the receiver function in the field plane, and the product are shown. For this pinhole (a typical choice), the transverse resolution is limited only by the transmitter beam diameter. The computed transverse resolution when in focus is \( \Delta x_p \approx 0.22 \mu m \) for the full-pupil (\( h = 0.89 \)), Fig. 4a, and \( \Delta x_p \approx 0.50 \mu m \) for the half-pupil and divided-pupil (\( h = 0.62 \)), Figs. 4b and 4c. From Table 2, we observe that the transverse resolution remains consistent for half-pupil or divided-pupil configurations for all configurations with different values of NA and \( d_{\text{pinhole}} \), given optimal fill factor, \( h \), and x-position, \( a_x \). For a line-scan, the transverse resolution is better in all configurations, irrespective of the pupil configuration, NA, and \( d_{\text{pinhole}} \). The reason

![Image](Fig. 4. Normalized image irradiance for point-scanning system, for \( z = 0 \mu m \) with NA = 0.90, \( d_{\text{pinhole}} = 2.5* d_{\text{AiryDisc}} \): (a) full-pupil point-scan, (b) half-pupil point-scan, and (c) divided-pupil point-scan. Note: See Fig. 6 for the same curves at \( z = -0.75 \mu m \).)

| \( z = 0 \mu m \) | \( d_{\text{pinhole}} = 0.1* d_{\text{AiryDisc}} \) | \( d_{\text{pinhole}} = d_{\text{AiryDisc}} \) |
|---|---|---|
| NA = 0.50 | NA = 0.90 | NA = 0.50 | NA = 0.90 |
| \( \Delta x_p \) | \( \Delta x_l \) | \( \Delta x_p \) | \( \Delta x_l \) |
| Full-Pupil | 0.41 | 0.38 | 0.14 | 0.14 | 0.59 | 0.51 | 0.23 | 0.20 |
| Half-Pupil | 0.72 | 0.70 | 0.28 | 0.27 | 1.00 | 0.98 | 0.38 | 0.38 |
| Divided-Pupil | 0.72 | 0.70 | 0.28 | 0.27 | 1.00 | 0.98 | 0.38 | 0.38 |

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for the improvement in transverse resolution relates to the fill factor of line-scanning \((h = 1.02)\) versus point-scanner \((h = 0.89)\). Because the coherent-transmitter path of the line-scanner results in a larger \(h\) in the pupil, the corresponding PSF produces improved transverse resolution.

4.3. Optical sectioning measurements

We begin our analysis of sectioning by examining how the signal, integrated over \(x\) and \(y\) varies as the planar diffuse object is moved out of focus. We demonstrate this approach for a line-scanner, using the optimum fill factor, \(h\), for a full-pupil line-scan, \(h = 1.02\), with NA = 0.90, and \(d_{\text{pinhole}} = 2.5*d_{\text{AiryDisc}}\). Figure 5a shows results in the field plane for the full-pupil. The upper left panel shows the transmitter, which is a vertical line. The upper right shows the receiver function, dominated by pinhole diameter, and the bottom left is the product. The lower right shows a slice through \(y = 0\). The width is controlled by the transmitter because the pinhole is large.

![Transmitter Irradiance and Receiver Irradiance](image)

(a) Full-Pupil Configuration \((z = 0\mu m)\)  
(b) Full-Pupil Configuration \((z = 1\mu m)\)  
(c) Half-Pupil Configuration \((z = 0\mu m)\)  
(d) Divided-Pupil Configuration \((z = 0\mu m)\)

Fig. 5. Transverse resolution for line-scan, with \(h = 1.02\), NA = 0.90, and \(d_{\text{pinhole}} = 2.5*d_{\text{AiryDisc}}\) for (a) full-pupil configuration \((z = 0\mu m)\), (b) full-pupil configuration \((z = 1\mu m)\), (c) half-pupil configuration \((z = 1\mu m)\), (d) divided-pupil configuration \((z = 1\mu m)\).

The most important characteristic of a confocal microscope is sectioning. Sectioning rejects out-of-focus scatter and thereby increases contrast. Contrast is limited by all the light from out of focus. Therefore, optical sectioning can be characterized by integrating the signal over the area for different defocusing. The transmitter and receiver functions become progressively wider when the target is out-of-focus. Figure 5b shows that the broadening of the transmitter and receiver functions leads to a reduced signal. The integral under the product...
is less than it is in Fig. 5a. Thus, out-of-focus scattering contributes less to detected power than does in-focus scatter.

Figure 5c shows the same for half-pupil. Transverse resolution and axial sectioning are both worse because only ~1/2 of the aperture is used. However, the divided-pupil recovers the sectioning performance. The transmitter and receiver are both centered on the axis in focus, but are displaced in opposite directions with increasing distance as the object is moved out of focus. Therefore, the product is lower than for the half-pupil and its integral is lower as well. The effect is similar for the point-scan illustrated in Fig. 6. Quantitatively, the integrals are plotted for all configurations in Fig. 7. The axial resolution (defined by the FWHM) for a full-pupil line-scanning configuration ($\Delta z_{sl} \approx 1.70\mu m$) is better than for a half-pupil configuration ($\Delta z_{sl} \approx 2.0\mu m$), as expected. The loss in axial performance with the half-pupil is recovered in the divided-pupil line-scanning configuration ($\Delta z_{sl} \approx 1.30\mu m$) providing better sectioning as more out-of-focus light is suppressed by the confocal slit or pinhole. More importantly, Fig. 7 shows that the optical sectioning improves with the divided-pupil. In fact it is even better than for the full-pupil by a factor of six at $z = -1\mu m$. For the line source, even the axial resolution is improved with the divided-pupil configuration.

Table 3 summarizes the calculated axial resolution for each source and for all configurations with different values of NA and $d_{\text{pinhole}}$, given optimal fill factor, $h$, and x-position, $a_x$. For a half-pupil line-scan, with NA = 0.50 and $d_{\text{pinhole}} = 0.1* d_{\text{AiryDisc}}$, $\Delta z_{sl} = 2.50\mu m$, which is greater than for a full-pupil ($\Delta z_{sl} = 1.93\mu m$) or divided-pupil ($\Delta z_{sl} = 1.65\mu m$) configuration. A point-scan for full-pupil and divided-pupil is equal ($\Delta z_{sl} = 1.64\mu m$ or 0.50$\mu m$) for small $d_{\text{pinhole}}$ for all NA (NA = 0.50 or 0.90), respectively. As $d_{\text{pinhole}}$ increases, the optical section measurement of a point-scan improves as NA increases. For a line-scan with a small $d_{\text{pinhole}}$, the optical sectioning is best in the divided-pupil configuration. For large $d_{\text{pinhole}}$ of a line-scanner, the axial resolution is best in the full-pupil configuration, irrespective of the NA.
Table 3. Summary of axial resolution measurements

| Pupil Configuration | \( d_{\text{pinhole}} = 0.1 d_{\text{AiryDisc}} \) | \( d_{\text{pinhole}} = d_{\text{AiryDisc}} \) |
|---------------------|---------------------------------|---------------------------------|
|                     | \( \Delta z_p \) | \( \Delta z_d \) | \( \Delta z_p \) | \( \Delta z_d \) | \( \Delta z_p \) | \( \Delta z_d \) | \( \Delta z_p \) | \( \Delta z_d \) |
| Full-Pupil          | 1.64   | 1.93   | 0.50   | 0.25   | 2.23   | 2.31   | 0.51   | 0.34   |
| Half-Pupil          | 2.22   | 2.50   | 0.51   | 0.36   | 3.49   | 5.37   | 0.56   | 0.79   |
| Divided-Pupil       | 1.64   | 1.65   | 0.50   | 0.24   | 2.74   | 3.20   | 0.50   | 0.46   |

4.4. Numerical aperture and pinhole diameter

As the numerical aperture (NA) of the objective lens increases, the signal increases proportionately, as shown in Fig. 8a. The full-pupil configuration gives the largest signal regardless of source. The line-scan gives the smallest signal regardless of the pupil configuration. As the pinhole diameter, \( d_{\text{pinhole}} \), increases, the irradiance increases, as shown in Fig. 8b. However, for values of \( d_{\text{pinhole}} > 2.5 d_{\text{AiryDisc}} \) the irradiance approaches an asymptote; the pinhole is collecting about the light scattering from the focused transmitter spot.

Fig. 8. Image Irradiance versus (a) numerical aperture at focal plane, (b) pinhole diameter at focal plane.

5. Discussion

We have presented a Fourier-analysis computational model for optimal pupil design of confocal microscopy. Note that the model is purely for pupil configurations and does not explicitly account for object conditions. Thus, the inherent assumption is that the object is optically homogeneous and clear. In actual practice, the choice of pupil design for the best imaging performance will depend on the optical properties of the desired object and application. In our particular application for imaging skin cancer, the effects of scattering and aberration must be considered.

For optimization of the transmitter path for our system using the computational model, the optimum value for fill-factor, \( h \), of the four confocal microscopy configurations: (1) full-pupil point-scanning, (2) full-pupil line-scanning, (3) divided-pupil point-scanning, and (4) divided-pupil line-scanning are 0.89, 1.02, 0.66, and 0.52 respectively.

Our results show the transverse resolution worsens from the full-pupil configuration to the half-pupil and divided-pupil configurations, as expected in confocal microscopy. For optical sectioning, the divided-pupil configuration is ideal, even outperforming the full-pupil. Therefore, there is greater rejection of out-of-focus light in the divided-pupil configuration and higher contrast. The axial resolution degrades from the full-pupil to the half-pupil configuration, as expected. The divided-pupil recovers at least some resolution, and is even better than the full-pupil configuration in some cases. Even if resolution is worse, optical
sectioning is always better with the divided-pupil configuration. The image irradiance at the
detector increases by several orders of magnitude as numerical aperture increases and to a
lesser extent when the detector pinhole, $d_{\text{pinhole}}$, increases. Exact values of resolution depend
on numerical aperture (NA) and pinhole size. The divided-pupil recovers some or all the lost
resolution, depending on NA and pinhole. Because the resolution of confocal microscope is
naturally better than required for imaging subcellular detail in skin, the improved sectioning
of a divided-pupil configuration, and particularly a line-scanner, provides an improvement
that is more important than the small degradation of resolution that arises from using a
fraction of the aperture.

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