Integrated light-sheet illumination using metallic slit microlenses

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Abstract: Light sheet microscopy (LSM) - also known as selective plane illumination microscopy (SPIM) - enables high-speed, volumetric imaging by illuminating a two-dimensional cross-section of a specimen. Typically, this light sheet is created by table-top optics, which limits the ability to miniaturize the overall SPIM system. Replacing this tabletop illumination system with miniature, integrated devices would reduce the cost and footprint of SPIM systems. One important element for a miniature SPIM system is a flat, easily manufactured lens that can form a light sheet. Here we investigate planar metallic lenses as the beam shaping element of an integrated SPIM illuminator. Based on finite difference time domain (FDTD) simulations, we find that diffraction from a single slit can create planar illumination with a higher light throughput than zone plate or plasmonic lenses. Metallic slit microlenses also show broadband operation across the entire visible range and are nearly polarization insensitive. Furthermore, compared to meta-lenses based on sub-wavelength-scale diffractive elements, metallic slit lenses have micron-scale features compatible with low-cost photolithographic manufacturing. These features allow us to create inexpensive integrated devices that generate light-sheet illumination comparable to tabletop microscopy systems. Further miniaturization of this type of integrated SPIM illuminators will open new avenues for flat, implantable photonic devices for in vivo biological imaging.

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1. Introduction

Optical microscopy is an increasingly powerful tool for biology thanks to a growing library of fluorescent reporters and optical methods to manipulate cell activity [1–6]. Accordingly, new microscopy systems are being developed to quickly and efficiently deliver and collect light within three-dimensional volumes of tissue [7–9]. To improve 3D imaging speed, several groups have demonstrated a technique known as light-sheet microscopy (LSM) [1,10–12] or selective plane illumination microscopy (SPIM) [13–16] that focuses excitation light not to a single spot but rather a thin 2D plane. The light sheet is then adjusted and aligned to precisely match the focal plane of the objective lens that collects an image. Because only the imaging plane is illuminated, SPIM reduces the photo damage and stress induced on a living sample. Furthermore, SPIM provides good sectioning capabilities by reducing the background signal from out-of-plane fluorescence [1,13].

One can expand the experimental capabilities of optical microscopes by miniaturizing them to create microscope arrays that increase experimental throughput [17–20] or wearable or implanted “miniscopes” that can visualize activity in freely moving animals [19,20]; however, researchers have yet to demonstrate a miniature SPIM microscope. One challenge for miniaturizing SPIM systems is creating planar illumination with a compact, light-weight device. Typically planar illumination is achieved using cylindrical lenses that are several millimeters thick. To miniaturize the illumination system, we explore flat lenses that can be easily manufactured using photolithography.
Recently, several types of flat lenses have been proposed and demonstrated including zone plate lenses [21,22] and Fresnel lenses [23,24], and meta-lenses based on sub-wavelength features. The major drawback of the zone plate is that it absorbs or reflects a significant part of the incident power, resulting in low transmission. This low transmission is particularly problematic for miniature devices that operate on a limited power budget. A Fresnel lens consists of many concentric segments of variable height and typically has higher transmission than a zone plate lens. However, Fresnel lenses are difficult to fabricate at the microscale due to their complex 3D shape. More recently, meta-lenses have shown performance similar to Fresnel lenses based on planar nanofabrication processes [25,26]. While these lenses show great promise for a number of applications, their requirement for subwavelength features that necessitates nanofabrication processes like electron beam lithography can significantly increase the cost of the lenses, making it more difficult to create arrays of low-cost SPIM systems. Metallic nano-slit arrays can also be used as a lens [27–32]. Verslegers et al. [27] have demonstrated a nano-slit lens consisting of optically thick gold film with air slits. The phase shift imposed on light as it passes through each slit is sensitive to length, width, and the materials inside the slit. By properly setting the phase shifts, light can be focused as though it has passed through a lens. Compared to a zone plate lens, the nano-slit lens can transmit more power because surface plasmon polaritons (SPPs) excited on the metallic film can guide some of the incident light through the slits. However, the major limitation is that the polarization direction of the incident light must be perpendicular to the metal/dielectric interface (TM mode) to excite SPP waves and a given nano-slit microlens only works properly for a narrow wavelength range. In addition, the fabrication process is similar to meta-lenses, requiring time-consuming, high precision lithography and etching techniques due to the high aspect ratio of the slits.

Here we demonstrate an easily manufactured beam shaping element for a miniature SPIM system by combining an LED with a flat metallic slit lens (Fig. 1). Specifically, we show that a metallic slit microlens bonded to an LED can operate as a tiny, low-cost replacement for the illumination arm of a SPIM system. Compared to zone plate lenses and plasmonic nano-slit lenses we find that optical throughput through a micron-scale metallic slit is much higher while producing similar light sheet illumination patterns. Figures 1(b) and 1(d) show FDTD simulations of light transmitting through a zone plate lens, plasmonic nano-slit lens, and a single metallic slit lens, each designed for an 8 μm focal length. When these fields are normalized to the incident power, we find that diffraction from a single slit leads to higher light throughput than a zone plate lens and plasmonic nano-slit lens while creating comparable planar illumination, which means the metallic slit microlens is more efficient in focusing. It should be noted that because our simulations and experiments use metals to create the slit lens, some of the transmission losses are due to absorption, which could lead to undesired device heating. To reduce absorption losses one could adjust the radiation pattern of the source to better fill the aperture, or exploit more advanced fabrication techniques to produce metasurfaces based on high-aspect-ratio and low absorption loss titanium dioxide [25].
Fig. 1. Integrated SPIM illumination using a metallic slit microlens. (a) Illustration of the light sheet produced by a metallic slit microlens. FDTD simulations of the light field produced by (b) a zone plate lens, (c) a nano-slit plasmonic lens, and (d) a single slit metallic lens. Each lens was designed for an 8 μm focal length. All simulations used the same incident power and the color axis is normalized to the incident power. Scale bar is 2 μm. Photographs of (e) metallic slit microlens and (f) microlens integrated onto a blue LED above a US penny. Scale bar in (e) is 3 mm. (g) Schematic showing the optical setup for the integrated light sheet illumination system.
2. Physical model

Fig. 2. Radiation pattern of the metallic slit microlens is well described by QCW theory. FDTD calculations showing the real part of the magnetic field at a wavelength of 488 nm for (a) a single QCW, as expected for diffraction from a sub-wavelength-scale (0.1 μm wide) metallic slit and (b) interference between QCWs spaced by 0.32 μm. This interfering QCW radiation pattern is expected for a metallic slit microlens with a width of 1 μm. (c) Calculated intensity distribution of light diffracting through a metallic slit with 6 μm slit width based on our analytical model. (d) FDTD simulations of light intensity for the same metallic slit show close agreement to the analytical results. (e) Cross section of the intensity at the focal plane and (f) cross section along the propagation direction, show good agreement between FDTD simulations (red line) and analytical model (black line). The positions of the cross sections are marked as dashed lines in (c) and (d). Scale bar in (c) is 5 μm.

By approximating light diffracting through the metallic slit as interfering quasi-cylindrical waves (QCW) we can create an analytical model to calculate the metallic microlens performance [31–37]. In this two-dimensional approximation we define the slit width as \( d \) and assume that the slit is infinitely long along the y-axis. We then model a silver film with dielectric constant \( \varepsilon_m \) and beside the metal surface consider a semi-infinite dielectric medium with a relative permittivity of \( \varepsilon_d \) [Fig. 1(d)]. Note that although silver films support SPPs, it has been shown that these fields decrease exponentially in the propagation direction away from the metal/dielectric interface [38–40] and thus they can be neglected in the far field.

In Fig. 2(a) we calculate the real part of the magnetic intensity at the wavelength of 488 nm diffracted by a silver slit with width of 0.1 μm using the FDTD method. The resulting radiation pattern represents a single QCW. In Fig. 2(b), we increase slit width to 1 μm and the diffraction pattern can be considered as the interference of three QCWs scattered from the slit edges, which will lead to the focusing of light to a sheet similar to zone plate and nano-slit plasmonic lenses.
To explain the focusing properties of the metallic slit microlens analytically, we can write the single slit diffraction pattern according to Helmholtz equations and QCWs as originally outlined in [41].

We start with the time varying harmonic field described by \( \exp(-iwt) \), and solve the 2-D Helmholtz equation as follows:

\[

[\nabla^2 + k^2]A(x,z) = 0

\]

(1)

Where \( \nabla^2 = \partial^2 / \partial x^2 + \partial^2 / \partial z^2 \), \( k = 2\pi / \lambda \) and \( A(x,z) \) is the amplitude of the wave propagating in x-z space. Assuming the exit of the slit is at \( x = 0 \), the diffracted wave will propagate into the space defined by \( x > 0 \). Based on the angular spectrum method [41,42], we can derive the field \( A(x,z) \) to be:

\[

A(x,z) = \frac{1}{k} \int_{-\infty}^{\infty} \overline{A}(k_x) \exp(i k_x z) \exp[i x(k^2 - k_x^2)^{1/2}] dk_x

\]

(2)

Where \( k_x \) is the wave vector along \( z \) direction. The spectral amplitude function \( \overline{A}(k_x) \) is the Fourier transform of the field at \( x = 0 \):

\[

\overline{A}(k_x) = \frac{k}{2\pi} \int_{-\infty}^{\infty} A(0,z) \exp(-i k_x z) dz

\]

(3)

By substituting (3) into (2), we can get

\[

A(x,z) = \int_{-\infty}^{\infty} E(x,z-z') \overline{A}(0,z') dz'

\]

(4)

Where

\[

E(x,z) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \exp(i k_x z) \exp[i x(k^2 - k_x^2)^{1/2}] dk_x

\]

(5)

We can then rewrite this expression in terms of a Hankel function [43] as:

\[

E(x,z) = \frac{ikx}{2(x^2 + z^2)^{1/2}} H^{(1)}_1[k(x^2 + z^2)^{1/2}]

\]

(6)

Where \( H^{(1)}_1 \) is the Hankel function of first kind and first order, which represents a cylindrical wave of first order [43].

According to Eq. (4) and Eq. (6), the diffraction field \( A(x,z) \) can be expressed as:

\[

A(x,z) = \int_{-\infty}^{\infty} \frac{ikx}{2[\sqrt{x^2 + (z-z')^2}]} H^{(1)}_1\left[k\left(x^2 + (z-z')^2\right)^{1/2}\right] \overline{A}(0,z') dz'

\]

(7)

Equation (7) shows clearly that the diffracted field consists of the summation of cylindrical harmonic waves with origins within slit.

In the case of a normally incident plane wave, \( A(x',0) \) is a \( A_0 \). The diffracted field from an incident plane wave can then be written as:

\[

A(x,z) = A_0 \int_{-d/2}^{d/2} \frac{ikx}{2\sqrt{x^2 + (z-z')^2}} H^{(1)}_1\left[k\left(x^2 + (z-z')^2\right)^{1/2}\right] dz'

\]

(8)

Where \( d \) is the width of the slit. With Eq. (8) we can calculate the intensity distribution of the diffraction field from a single slit.
In Figs. 2(c)-2(f), we compare FDTD simulation of single slit diffraction with our theoretical model in Eq. (8) for a plane wave with wavelength of 488 nm normally incident on a silver slit with a width of 6 μm. The agreement between both FDTD simulations and our theoretical model in Eq. (8) verifies the accuracy of the QCW approximation. What’s more, the intensity distribution shows beam focusing behavior with focal length of 34 μm [Fig. 2(e)] and a light sheet thickness (full width at half maximum) of 2.2 μm at the focal point [Fig. 2(f)]. By tuning the width of the slit we can adjust the effective focal length of the slit as is shown in Fig. 1(d) and the predicted focal length is found to be in consistent with FDTD simulations.

Figure 1(a) shows the normalized cross-sectional intensity distributions of the beam after passing through the metallic planar microlens where the beam propagates along the x axis. The diffraction field can be considered as a light sheet illumination. The minimum width of the beam in z direction represents the minimum light sheet thickness which would be used for imaging. The field of view increases with the length of the slit in y direction.

3. Focusing properties

![Fig. 3](image.png)

Fig. 3. Focal length and depth of focus can be tuned by changing the slit width. (a) The radiation pattern of the metallic slit microlens with 6 μm slit width as calculated by an FDTD simulation. Scale bar is 10 μm. (b) Intensity distribution at the center of the metallic slit as a function of distance from the slit (x), which represents the white dash line in (a). The FWHM of the axial intensity distribution is defined as the depth of focus. (c) Calculated focal length (blue squares) and numerical aperture (red circles) versus slit width. (d) Light sheet thickness (blue squares) and depth of focus (pink circles) versus slit width.

To analyze the light sheet thickness and depth of focus/field of view created by microlenses of different slit widths, we calculate the focal length, light sheet thickness and depth of focus using FDTD simulations (Fig. 3). We quantify the light sheet thickness as the beam waist diameter, which is the full width at half maximum (FWHM) of the focal spot. The focal length is the distance between the incident plane and the focal plane. The depth of focus is defined as the FWHM of the axial intensity distribution shown in Fig. 3(b) [1]. As expected, the focal length, light sheet thickness, and depth of focus are found to increase with the slit width of the microlens. However, the numerical aperture drops with the increase of the slit width, which can be derived from the ratio of half the illumination width over the focal length. Choosing a narrow slit that acts as a high NA lens maximizes the optical sectioning ability by minimizing the sheet thickness, but significantly reduces the uniformity of illuminated area, which can limit the optical field of view [Fig. 3(c)]. Choosing a low NA lens by increasing the slit width extends the focal depth of the sheet, but increases the sheet thickness resulting in reduced optical sectioning capabilities [Fig. 3(d)]. Thus by tuning the effective NA of the metallic slit lens, we can easily adjust the lens properties for a specific application.

4. Broadband focusing and polarization independence

Metallic slit microlenses are significantly less sensitive to polarization and wavelength compared to the nano-slit grating arrays. For example, an example metallic slit microlens can focus single wavelengths of light across the entire visible spectrum [Figs. 4(a)-4(d)] and the shift of the focal length was much smaller than the depth of focus over all wavelengths [Fig. 4(f)], which suggests that the metallic slits can be used as a broadband microlens with a
common focusing region throughout the entire visible range. This broadband operation implies that the focusing properties of the lens will be maintained for white light illumination.

Fig. 4. The metallic slit microlens can focus over the visible spectrum. (a-d) The calculated focal distribution for a 5 μm wide metallic slit microlens operating at wavelengths of 400, 500, 600, and 700 nm. Dashed lines denote the focal plane. Scale bar is 5 μm. (e) Over the visible spectrum the change of the focal length is smaller than the depth of the focus. Green shaded region shows the depth of focus range for each wavelength throughout the visible spectrum. (f) FDTD simulations along the center axis at different wavelengths show the shift if focal length is smaller than the depth of field.

One reason why the metallic slit microlens performance is relatively insensitive to polarization and wavelength is the fact that it does not rely on SPPs, which are typically highly dispersive and polarization dependent [39]. To compare the influence of polarization sensitivity of the metallic slit microlens and a nano-slit plasmonic lens, we have simulated the focusing field of TM and TE incident field after passing through a 17 slits nano-slit plasmonic lens and a metallic microlens with 4 μm slit width. As shown in Figs. 5(a) and 5(c), both nano-slit plasmonic lens and metallic slit microlens have similar focusing properties for TM polarized illumination light. However, the nano-slit plasmonic lens fails to focus the TE polarized incident light while the metallic slit lens maintains the focusing properties as shown in Figs. 5(b) and 5(d), respectively. Therefore, the focusing property of the nano-slit plasmonic lens is sensitive to the incident polarization, which only works for TM polarization. While for the metallic slit microlens, both TM and TE incident light show nearly identical radiation patterns, which implies that the focusing ability of the metallic slit microlens is polarization insensitive.

Fig. 5. Metallic slit microlens performance is robust to changes in polarization. (a, b) Intensity distributions produced by a nano-slit plasmonic lens when illuminated by TM and TE polarized light, respectively. (c, d) Intensity distributions produced by 4 μm wide metallic slit microlens when illuminated by TM and TE polarized light, respectively. All the intensities are normalized to the incident intensity. Scale bar in (a) is 2 μm.
5. Light sheet illumination in agarose samples

To confirm that metallic slit diffraction can indeed form sheet illumination we fabricated and tested single slit microlenses with slit widths of 10 μm, 12 μm and 14 μm and compared our experimentally measured diffraction patterns to the simulation results. Based on Eq. (8), we calculated the diffraction intensity distribution for each slit, which is shown in Figs. 6(b)-6(d). We also collected the experimental measurements of the field diffracted by three different slits [Figs. 6(e)-6(g)] using the experimental setup depicted in Fig. 6(a). We found excellent agreement between the experimental data and our calculations based on the QCW model. We also found good agreement between theory and experiment when comparing the intensity distribution in the focal cross section along the x and z direction [Figs. 6(h)-6(k)].

To compare the focal length of our fabricated lenses to theory we plotted the intensity distribution averaged over the three columns of pixels in the center of the beam as a function of x, where the resolution of each pixel is 0.75 by 0.75 μm. The intensity distribution along the propagation direction for our theoretical model [Fig. 6(h)] closely matches the distribution we measured from the single slit diffraction [Fig. 6(i)].

![Fig. 6. Experimental measurements of metallic microlens performance match theory. (a) Experimental setup for testing the microlens and measuring the diffraction pattern. Agar sample and the microscope with detector 2 are on separate x-y-z translation stages. Calculated radiation patterns normalized to unit power for slit widths of (b) 10 μm, (c) 12 μm and (d) 14 μm (incident wavelength 488 nm). Experimentally measured radiation patterns for slit widths of (e) 10 μm, (f) 12 μm, and (g) 14 μm. (h) Calculated and (i) measured intensity distributions, as measured along the propagation direction in the focal cross section along the x direction. (j) Calculated and (k) measured intensity distributions measured along the z direction at the focal point show the minimum thickness of the light sheet. Intensities are normalized to the peak values. Scale bar is 100 μm.]

6. Integrated SPIM illumination for fluorescence imaging

6.1 Integrated photonic device for light sheet illumination

To create a fully integrated SPIM illumination module, we integrated the microlens onto a flat blue LED by using UV curable epoxy (NOA 85, Norland). Before curing, we need to align the microlens to the LED center by using a three axis micromanipulator. Figure 1(f) shows the microlens and the integrated photonic device. Without a microlens, the LED illumination diffuses over large volume of the sample as shown in Fig. 7(b). After integrating the metallic slit microlens, we can see in Fig. 7(c) clearly the light sheet illumination into the fluorescein dye solution, with a minimum light sheet thickness around 10 μm and the depth of focus...
around 400 μm, which indicates such an integrated photonic device can be effectively used for light sheet illumination [43].

Fig. 7. Integrated SPIM illumination for fluorescence imaging. (a) Schematic of setup for integrated SPIM imaging in phantom sample. (b) shows LED illumination in x-z cross section in the fluorescein dye solution without microlens. (c) illustrates the light sheet illumination through the integrated photonic device at the x-z plane. Scale bars in (b) and (c) are 200 μm. The left column in (d) represents maximum intensity projections of 1 μm fluorescent beads in agarose gel based brain phantom cross (i) x-y, (ii) x-z and (iii) y-z planes in (a), respectively. 1 μm fluorescent microsphere in the center of the FOV selected for PSF analysis are indicated by yellow-squared box. The right column in (d) show the zoomed-in images of the cross sections of the selected 1 μm fluorescent bead in x-y, x-z, and y-z plane.

6.2 Imaging in fluorescent brain phantom

Using an agar based fluorescent brain phantom to estimate the scattering properties of the tissue, we found that our light-sheet illuminator enabled imaging up to 400 μm deep into the tissue phantom [16]. We experimentally characterized the fluorescence point spread function (PSF) by doing z-stack imaging of the brain phantom that has the approximate scattering properties of the brain, which is made of a 1% agar solution with a suspension of 1 μm diameter fluorescent and nonfluorescent polystyrene microspheres. As shown in Fig. 7(a), we can collect individual images of fluorescent microspheres at different depths within the brain phantom sample by moving the sample along z direction, which is driven by the picomotor assembled micromanipulator stage. Figure 7(d) shows an x-y-z 3D projection of the brain phantom using a metallic slit microlens with 12 μm slit width and 500 μm slit length, which would have a focal length of 136 μm. The field of view (FOV) is about 400 μm by 400 μm by 400 μm in x-y-z directions, which can be improved by using longer slit. Cross sections through the PSF in the center of the FOV in each dimension are shown in the right column of Fig. 7(d). The resolutions can be characterized by the full width half maxima (FWHM) of the fluorescence intensity cross the center of the microsphere, which are measured to be 4.5 μm, 4.5 μm and 10.0 μm in x’, y’, z’ directions, respectively.
7. Experiments and methods

7.1 Fabrication and measurement

The microlens was fabricated through a lithography, deposition, liftoff process. Before fabrication, the 3 inch fused silica wafers were sonicated in acetone for 20 min and then rinsed by isopropyl alcohol (IPA) for 5 min and deionized (DI) water for 2 min to wash away the solvent residue. Washing was followed by a N2 blow dry process for 2 min, the wafers were placed on a hotplate at 150 °C for 5 min to bake off any water residue. Next, 5 minutes of oxygen plasma cleaning was applied to remove impurities and contaminations from the surfaces. The microlens pattern with height of 2 μm was created on the substrate by using 3D direct laser lithography system (Nanoscribe, Germany) with high resolution negative photosensitive resist (IP-L). Next, 5 nm chromium film and 100 nm silver film were sputtered onto the substrate surface. The liftoff process would be finished by sonicating the samples in acetone for 30 min to remove the photoresist underneath. A dicing saw (Disco DAD-321) was used to divide up the processed wafers into individual microlens pieces, which is shown in Fig. 1(e).

7.2 System setup

To measure the diffraction patterns and focusing properties of the microlenses, we used a Coherent Genesis MX 488-1000 STM laser with a wavelength of 488 nm coupled to a single-mode optical fiber. We then illuminated the sample by collimating the output of the single mode fiber and aligning this collimated beam onto the metallic slit through the backside of the fused silica substrate. The diffracted light then propagated through a suspension of agar in water (1% bacterial agar powder w/v in milli-Q water). The agar hydrogel weakly scattered the diffracted light allowing us to measure the diffraction pattern without significantly attenuating the light. To position the collimated beam onto a lens, we positioned a detector (Imagingsource DFK 72BUC02) above the sample and used a Nikon 10X (NA = 0.30) objective and tube lens to visualize the sample surface [Fig. 6(a)]. To ensure plane wave illumination, we confirmed that the output was collimated and the spot size was much larger than any particular lens. We then aligned the laser spot to the center of the microlens using a three-axis translation stage. To image the diffraction pattern we used a Point Grey (GS3-U3-2S6M-C) detector combined with a Mitutoyo 20X (NA = 0.42) objective and tube lens focused on the side of the agar hydrogel using another three-axis translation stage [Fig. 6(a)].

7.3 Brain phantom preparation

The brain phantom samples were created by suspending blue/green fluorescent (TransFluoSpheres Carboxylate-Modified Microspheres, 1 μm diameter, blue-green fluorescent (488/560), Polysciences Inc., FluorSpheres Sulfate Microspheres, yellow-green fluorescent (505/515), 3.6 × 1010 beads/ml) and nonfluorescent polystyrene microspheres (1 μm diameter, 4.55 × 1010 beads/ml, Polysciences Inc.) in agar/milli-Q water solution (1% bacterial agar powder wt/v in 18.2 MΩ·cm water). 50 mg 1% wt/v bacterial agarose powder was weighted and added into the 4.4 ml milli-Q water in 10 ml tubes. The milli-Q water was heated to boiling to activate the agar by putting into a microwave for 1 min. Then the agar/milli-Q solution was vortexed to ensure that the agar was evenly distributed. After the solution cooling down at room temperature for 15 min, we added 10 μl fluorescent beads and 0.6 ml nonfluorescent scattering beads into the solution since the beads could not be heated above 97 °C without deformation. The sample was vortexed again to ensure that the beads were distributed uniformly into the sample suspension before transferred into the cuvettes by syringes. Then the samples in the cuvettes were kept in refrigerator for 30 min to coagulate the sample. For the brain phantoms, the concentration of the nonfluorescent scattering microspheres were 5.46 × 109 beads per ml, which yielded the reduced scattering
coefficient of around 1 mm$^{-1}$ at 488 nm. These values were chosen to simulate the range of reported scattering coefficients in mouse brain tissue [5].

7.4 Fluorescein solution preparation

First we added 5 ml milli-Q water into the test tube and heated the tube for 30 seconds in a microwave. Then 50 mg agrose powder was added into the milli-Q and heated for 60 seconds in the microwave until the solution was transparent and uniform. This was followed by dissolving 1 mg fluorescein sodium salt (Sigma Aldrich) into the solution, which was then vortexed until evenly mixed.

7.5 Measurement synchronization

We used a Picomotor piezo linear actuator (Picomotor Actuator 8742, Newport Cor.) to precisely control and move samples at the constant speed of 1 step/sec by connecting to a three-axis micromanipulator. The fluorescence images were taken between picomotor steps. Each step was 750 nm and the step variation was 30 nm.

8. Conclusions

In conclusion, a single metallic slit can serve as a simple and inexpensive focusing element for miniature integrated light-sheet illumination modules. The focusing properties of these lenses can be explained by plane wave diffraction through an opaque slit as described by the 2-D Helmholtz equation and interference of QCWs induced by the slit edges, which shows excellent agreement with the FDTD calculations. Furthermore, we can tune the focusing properties by adjusting the slit geometry, and the predicted lens performance match our experimental measurements. These microlenses perform well across entire visible range and the focal fields have high tolerance to the polarization state. These properties allow us to create integrated SPIM illumination modules by combining metallic slit microlenses with LEDs. In particular, we have shown that the compact and inexpensive SPIM illumination enabled by integrated module enables optical sectioning and 3D fluorescent imaging in brain tissue phantoms. The features of high optical throughput, broadband focusing capability, polarization insensitivity, miniaturized size, and low-cost fabrication suggest that the metallic slit microlenses can enable low-cost miniature SPIM systems for biological imaging.

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