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Three-Dimensional (3D) High-Speed Imaging and Fabrication System Based on Ultrafast Optical Pulse Manipulation

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ABSTRACT

Laser scanning systems for two-photon microscopy and fabrication have been proven to be excellent in depth-resolving capability for years. However, their applications have been limited to laboratory use due to their intrinsic slow nature. The recently introduced temporal focusing concept enables wide-field optical sectioning and thus has potential in both high-speed 3D imaging and 3D mass-production fields. In this paper, we use the ultrafast optical pulse manipulation to generate two-photon excitation depth-resolved wide-field illumination (TPEDRWFI). The design parameters for the illumination were chosen based on numerical simulation of the temporal focusing. The imaging system was implemented, and the optical sectioning performance was compared with experimental result.

Keywords: Two-photon excitation, temporal focusing, 3D lithography, high-speed 3D imaging

1. INTRODUCTION

The two-photon excitation fluorescence microscopy [1, 2], as well as two-photon excitation microfabrication [3-5], have been widely used since it can generate finer 3D images or features than conventional wide-field microscopy and two dimensional (2D) lithography and achieve submicron optical or fabrication resolution. Laser scanners are commonly used to achieve this intrinsic optical sectioning capability based on spatially focusing laser light at the focal point of a high numerical aperture objective. They have broad range of applications in the fields of 3D tissue imaging [6, 7], 3D optical storage [8], tissue scaffold [9], photonic crystal structure [10], and microfluidic devices [11]. However, spatially focused laser spot should be scanned laterally on the sample, resulting in taking long time to complete imaging and fabrication. This limits two-photon excitation system to laboratory use such as microstructure prototyping and ex-vivo or in-vitro imaging despite the very attractive submicron optical lateral resolution and optical sectioning capabilities. Therefore, high-speed two-photon excitation technology has been needed to enable mass production and large volume imaging to be commercially available. Several techniques have been proposed to make imaging and fabrication faster than single focus laser scanning. Line scanning [12] gives lower optical resolution than spot scanning in spite of the easy implementation. The use of multiple foci [13] was proposed, but it causes registration problem between patterns written by different foci. Illumination by interference patterns on the specimen [14] is also quite useful, but its applications are limited since it generates periodic illumination patterns both laterally and axially.

The recently introduced temporal focusing [15, 16] disperses optical pulse into monochromatic waves at different angles on a grating surface and recombines them at focal plane. It is very useful in two-photon excitation depth-resolved wide-field illumination (TPEDRWFI), since the original optical pulses are restored only at focal plane, and several applications in the nonlinear microscopy were proposed [17-20]. However, depth discrimination capability for TPEDRWFI system has not been fully evaluated both theoretically and empirically despite being one of the most important parameters in TPEDRWFI design. Moreover, temporal focusing has not yet been applied to 3D lithographic microfabrication, even though it was originally developed for imaging.

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In this proceeding, we proposed 3D high-speed imaging and fabrication system based on TPEDRWFI. Manipulating ultrafast optical pulse spatially generates temporal focusing, which enables imaging and lithographic process to be depth-resolved with wide-field illumination. We also derive a mathematical model, based on diffraction theory, to predict the axial optical resolution for the two-photon excitation depth-resolved wide-field illumination, and review the design parameters based on numerical simulations to improve axial optical resolution. We implemented the proposed system, and compared experimental result with theoretical calculation in terms of axial optical resolution.

2. TWO-PHOTON DEPTH-RESOLVED WIDE-FIELD ILLUMINATION THEORY

2.1 Principle of temporal focusing

In general, focusing is the process in which the light is concentrated tightly in the space and generates very high power at the focus. With monochromatic wave, there is a unique type of focusing called spatial focusing. With the advent of pulsed laser, another type of focusing has been recently introduced, i.e. temporal focusing. Optical pulse generated by the pulsed laser has bandwidth, which is the set of numerous monochromatic waves. Light does not need to be tied in the space. The only requirement is that all monochromatic waves arrive sharply on the focal plane at the same time. Otherwise, optical pulse is temporally broadened and it no longer generates high instantaneous power. To realize temporal focusing, the optical pulse should be dispursed angularly at the conjugate plane of focal plane. Any angular dispersive element such as diffraction grating, prism, acousto-optic modulator (AOM), and even spatial light modulator (SLM) can be used to manipulate light spatially. In this paper, diffraction grating was selected. This is because it gives high angular dispersion, which ensures tight temporal focusing. It is also cost-effective since optomechanical components are not required in this design.

Simplified diagram of 3D lithographic microfabrication system is shown in figure 1. It includes diffraction grating, tube lens and the objective. First, ultrafast optical pulse is transmitted through the grating. Since ultrafast optical pulse consists of multiple wavelengths in the bandwidth, it is dispersed at different angles, depending on wavelength components of the ultrafast optical pulse. As seen in the diagram, its red component is dispersed more than the blue one. Monochromatic waves propagate along different optical paths through lenses. At the focal plane, all the monochromatic waves are combined, which results in restoring them to the ultrafast optical pulse at the focal plane. Out of focal plane, the optical pulse becomes broader than the original one due to the phase mismatch between monochromatic waves, and broad pulse width causes the two-photon excitation intensity to decrease. Therefore, optical sectioning capability can be achieved by controlling optical pulse width. This is different from optical sectioning by the spatial focusing since axial optical resolution is limited by the diffraction regardless of the optical pulse width. In next section, axial optical resolution which determines magnitude of optical sectioning is discussed theoretically.

2.2 Mathematical model for two-photon excitation depth-resolved wide-field illumination

In order to optimize the design of the 3D lithographic microfabrication system, it is important to thoroughly understand the image formation theory underlying this approach. We derive an optical model of light distribution near the focal

Fig 1. Simplified diagram of two-photon excitation depth resolved wide-field illumination model based on spatial light manipulation. It consists of diffraction grating, tube lens and objective. For the illustration purpose, three different color beams are shown.
plane based on diffraction theory [21-24] to evaluate axial optical resolution with axial intensity distribution. This optical model allows us to accurately predict the axial optical resolution that can be achieved. Furthermore, it makes us examine the effects of different design parameter choices in optimizing depth-resolved capability.

Figure 1 shows how each wave propagates through an optical system. To simplify this model, the following assumptions are applied: 1) the optical model is diffraction-limited. 2) all the lenses are perfectly chromatic-aberration-corrected. 3) dispersion occurs only at the diffraction grating. 4) the input beam profile is Gaussian with a width of \( S \) (1/e beam radius), 5) the spectral distribution of the input beam is also Gaussian with a bandwidth of \( K \) in terms of wavenumber, and 6) the ultrafast optical pulse is chirp-free. Then ultrafast optical pulse can be defined as below:

\[
U(x, y, z, t) = \int_{-\infty}^{\infty} U(\tilde{r}, k) e^{i(k \cdot \tilde{r} - \omega t)} d\omega = c \int_{-\infty}^{\infty} U(x, y) e^{i\omega} W(k) e^{-i\omega t} dk
\]

where \( U(x, y) = A_0 \exp\left(-\frac{x^2 + y^2}{S^2}\right) \) and \( W(k) = B_0 \exp\left(\frac{\Delta k^2}{k_0^2}\right) \)

\( A_0 \) and \( B_0 \) are the amplitudes, and \( \Delta k = k - k_0 \) is the difference between \( k = \frac{2\pi}{\lambda} = \frac{\omega}{c} \), the wavenumber for the given wavelength and \( k_0 \), the wavenumber for the center wavelength. Moreover, \( \lambda \) is wavelength, \( \omega \) is optical angular frequency, and \( c \) is the speed of light. Since diffraction theory is valid for monochromatic wave, divide and conquer (D&C) method is applied: optical pulse separates into monochromatic wave components, the electromagnetic wave fields are calculated with diffraction theory, and they merge into the optical pulse in time domain at the focal plane. For each \( k \), the transverse field for each at grating surface [25] can be written as:

\[
U_i(x_i, y_i, k) = U(x, \cos \alpha, y) \cdot \exp\{i \cdot \Delta k \sin \alpha \cdot x\}
\]

\((x_i, y_i)\) is the lateral coordinate at the grating plane. The grating effectively introduces a phase chirp along one direction. \( \alpha = \sin^{-1}\left(\frac{2\pi G}{k_0}\right) \) is the incident angle to the grating with groove frequency \( G \) such that the center wavelength of the input beam propagates along the optical axis. Since the grating and the microscope focal plane is conjugated by a 4-f imaging system, the field can be readily propagated along the optical path. Ignoring the field aperture of the microscope, the field at back aperture of the objective is

\[
U_2(x_b, y_b, k) = -\frac{ik}{2\pi f_i} \int_{-\infty}^{\infty} U_i(x_i, y_i, k) \exp\left\{-i\frac{x_i x_b + y_i y_b}{f_i}\right\} dx_i dy_i
\]

\( f_i \) is the focal length of the tube lens, and \((x_b, y_b)\) is the lateral coordinate of the back aperture plane. When the waves propagate into the objective, the back aperture size in the objective should be considered, and it is incorporated as:

\[
U_3(x_b, y_b, k) = U_2(x_b, y_b, k) \cdot \text{circ}\left(\frac{\sqrt{x_b^2 + y_b^2}}{D/2}\right)
\]

where \( \text{circ}(r) = \begin{cases} 1 & \text{for } |r| < 1 \\ 1/2 & \text{for } |r| = 1 \\ 0 & \text{Otherwise} \end{cases} \)

\( D \) is the diameter of the back aperture in the objective. The field near the focal plane of the objective can be calculated as:
\[ U_i(x, y, z, k) = -i k \frac{\exp \left\{ ik (2f_1 + z) \right\}}{2 \pi f_1} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} U_i(x', y, z, k) \exp \left\{ -i \pi \frac{x'^2 + y^2 + z^2}{f_2} \right\} \exp \left\{ -ik \frac{x x' + y y' + z z'}{f_2} \right\} \, dx' \, dy \]  

(5)

\((x_i, y, z_f)\) is the coordinate placed on the focus of the objective. Since the tube lens and the objective are chromatic aberration-corrected, the effective optical path lengths (phase terms) are same for the different wavenumber \(k\) at the focal plane.

\[ \exp \left\{ ik \left( f_1 + f_2 \right) \right\} = \text{const} \]  

(6)

To reconstruct the optical pulse, all the monochromatic waves delivered to focal plane of the objective in vector form since they propagate along different path. Therefore, the temporal evolution of the field around focal plane can be shown as:

\[ \mathcal{U}_i(x, y, z_f, t) = \int_{-\infty}^{\infty} U_i(x, y, z_f, k) \left( \tilde{x}_j \sin \beta'(k) + \tilde{z}_j \cos \beta'(k) \right) W(k) \exp \left\{ -i(kct - \phi(x, y, k)) \right\} \, dk \]  

(7)

where \(\phi(x, y, k) = k \left( \frac{z_f}{\cos \beta'(k)} \left[ 1 - \sin \alpha_\beta \cdot M \sin \beta'(k) \right] - \sin \alpha_\beta \cdot M x_f \right)\)

\(\beta'(k)\) is the incident angle for each \(k\) with respect to optical axis after objective, \(\phi(x, y, k)\) is the pulse front delay which comes from diffraction grating, and \(M = \frac{f_1}{f_2}\) is magnification with the lenses. Time-averaged intensity close to the focal plane can be expressed as:

\[ I(x, y, z_f) = f_p \int_{0}^{\sqrt{N}} \left| \mathcal{U}_i(x, y, z_f, t) \right|^2 \, dt \]  

(8)

\(f_p\) is repetition rate of the ultrafast pulsed laser. Since multiphoton excitation is a nonlinear process, multiphoton excitation efficiency is proportional to \(N\)th power of the intensity with \(N\)-photon excitation process.

\[ I^N(x, y, z_f) = f_p \int_{0}^{\sqrt{N}} \left| \mathcal{U}_i(x, y, z_f, t) \right|^{2N} \, dt \]  

(9)

In case of two-photon process in the system, \(N = 2\). With axial intensity profiles obtained in eq. (9), axial optical resolution can be calculated.

### 3. TWO-PHOTON DEPTH-RESOLVED WIDE-FIELD ILLUMINATION EVALUATION

#### 3.1 Simulation for optical axial resolution evaluation

Figure 2 represents simulation results of the axial optical resolution for two-photon depth-resolved wide-field illumination system with following different design parameters (also shown in red in figure 1.): field of view radius \((r=D/2)\), focal length of the tube lens \((f_1)\), the bandwidth of ultrafast optical pulse (FWMH), groove frequency of diffraction grating \((G=1/d)\), and the numerical aperture (NA) of the objective (NA). In this paper, axial optical resolution is defined as full-width-at-half-maximum (FWHM) of axial intensity profile.
Fig 2. The simulation results of axial optical resolution for the given parameters in the depth-resolved wide-filed illumination system: (a) axial optical resolution along \( r \) depending on \( G \), (b) axial optical resolution along \( f_1 \) depending on \( G \), and (c) axial optical resolution along FWHM\( \lambda \) depending on \( G \).
In the simulation, we found interesting behaviors with different parameters. As shown in the figures 2(a), field of view (FOV) size is independent of G with large enough FOV. Axial optical resolution seems to be improved as FOV radius size becomes smaller than 10µm, but smaller FOV approaches to spatial focusing spot size, and it is not wide-field illumination any more. Thus it makes sense that small FOV which approaches to the focal spot provides diffraction-limited axial optical resolution. In the figures 2(b), axial optical resolution is inversely proportional to \( f_1 \), and it is still valid for different Gs and FWHM\(_\lambda\)s. In addition, axial optical resolution is also inversely proportional to FWHM\(_\lambda\), as shown in figures 2(c). It is noted that the optical resolution has asymptotical value (about 750 nm) with large G or FWHM\(_\lambda\), which is close to diffraction limit of spatial focusing spot with given NA of the objective. This is because some of wavelength-resolved light (far-red or far-blue in the ultrafast optical pulse) is clipped due to size of back aperture of the objective determined by NA, even though the optical pulse with large FWHM\(_\lambda\) is very narrow.

To summarize, FOV size is irrelevant of optical resolution as long as the system maintains wide-field illumination. Angular dispersion (dispersion angle per wavelength), which can be controlled by \( f_1 \) and G, is the most important design parameter to optimize axial optical resolution, but is limited by diffraction limit associated with NA. Therefore, angular dispersion determined by G and FWHM\(_\lambda\) is one of the key factors to control the axial optical resolution, which is also diffraction limited.

### 3.2 Comparison between simulation and experiment result for axial optical resolution

![Schematic diagram for two-photon excitation depth-resolved wide-filed illumination system.](image)

Fig 3. Schematic diagram for two-photon excitation depth-resolved wide-field illumination system. The ultrafast optical pulse (780nm, \( \sim \) 100 fs) was delivered from tunable Ti:Sapphire pulsed laser. To control illumination field of view, beam expander is used. Reflective diffraction grating disperses the optical pulse in the space, and it is restored after the high NA objective at the focal plane. Fluorescence signal is collected backward, and it is detected with intensified CCD (iCCD) at the image plane.

To validate the simulation results, axial optical resolution should be measured after TPEDRWFI is set up. As a light source, the ultrafast optical pulse (the center wavelength of 780 nm, the pulse width of about 100 fs, and repetition rate of about 80 MHz) was delivered from the tunable Ti:Sapphire pulsed laser (Tsunami, Spectra-Physics, Mountain View, CA) pumped by the diode-pumped solid-state (DPSS) laser (Millennia Xs, Spectra-Physics, Mountain View, CA). The beam diameter can be controlled with the beam expander, resulting in scaling the field-of-view on the specimen. At the surface of the reflective diffraction grating with the groove frequency of 600 g/mm (53004BK02-35IR, Richardson Co., Ltd.).
Grating Lab, Rochester, NY), ultrafast optical pulse is dispersed spatially, and it is restored after the objective (Fluar, 403/1.30 Oil, Zeiss MicroImaging, Thornwood, NY) in the inverted microscope (Axiovert S100TV, Zeiss MicroImaging, Thornwood, NY) through NIR achromatic doublet with the focal length of 250mm (AC254-250-B, Throlabs, Newton, NJ) used as a tube lens to reduce chromatic aberration. The fluorescence on the specimen is collected backward through the objective. At the beam splitter (700dcxr, Chroma Technology, Rockingham, VT), the fluorescence is reflected, and it is imaged at the intensified CCD (PI-MAX, Princeton Instrument, Trenton, NJ). Specimen is placed on the stage controlled laterally in the submicron range, and the objective is also controlled axially by piezoelectric actuator to move the focal plane at the specimen.

To measure optical resolution, 0.1µm diameter yellow-green (505/515) fluorescent polystyrene microspheres (F-8803, Invitrogen, Carlsbad, CA) were used, each of which can be considered as a point light source. Figure 4 shows the axial optical resolution in the simulation with design parameters from the developed system and the axial optical resolution in the experiment. Measured resolution (figure 4(b)) is very close to simulated resolution (figure 4(a)). It confirms that the simulation results are well matched with measurement and shows the potential to achieve diffraction-limit optical resolution practically with different design parameters.

**Fig 4.** Axial resolution with 0.1µm fluorescent microsphere: (a) axial optical resolution from the numerical simulation (b) axial optical resolution from the measurement (red dots), which is fitted to Lorentzian function (blue line).

### 4. CONCLUSION

In summary, TPEDRWFI can be applied to both imaging and fabrication systems. First, mathematical model for TPEDRWFI was derived based on optics theory. With this model, numerical simulation was performed to figure out the dominant parameters for determining axial optical resolution and to optimize the proposed system design. Depth-resolved wide-field microscopy was incorporated with image sensor, and simulation results were well matched with axial optical resolution measurements. In the future, it will lead to using high-speed imaging with high sensitivity in the live cell such as image correlation spectroscopy (ICS) applications, and 3D lithographic microfabrication in the photonics and biomedical applications such as 3D optical circuit with photonic crystal structures and 3D tissue scaffold for artificial organ regeneration.

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