Laser divided-aperture differential confocal sensing technology with improved axial resolution

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Abstract: In this study, we found that the axial response curve of divided-aperture confocal microscopy has a shift while the point detector has a transverse offset from the optical axis. Based on this, a novel laser divided-aperture differential confocal sensing technology (LDDCST) with absolute zero and high axial resolution, as well as an LDDCST-based sensor, is proposed. LDDCST sets two micro-regions as virtual pinholes that are symmetrical to the optical axis along the \( x_d \) direction on the focal plane of the divided-aperture confocal system to achieve the spot-division detection and to simplify the detection system, uses differential subtraction of two intensity responses simultaneously detected from the two micro-regions to achieve high axial resolution absolute measurement and low noise, and considers both resolution and measurement range by adjusting virtual pinholes in software. Theoretical analyses and packaged LDDCST sensor experiments indicate that LDDCST has high axial resolution as well as strong anti-interference and sectioning detection capability.

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References and links

1. Introduction

Confocal microscopy (CM) is widely used in microelectronics, materials, industrial precision measurements, and biomedicine for its unique optical sectioning capability and high resolution [1–4]. Koester et al. proposed a CM-based confocal slit divided-aperture microscope for applications in ophthalmology and otology. The microscope used separate portions of the objective aperture for illumination as well as imaging rays that achieve a high degree of optical sectioning [5, 6]. Dwyer et al. developed a confocal theta line-scanning microscope with divided aperture for applications in human skin and oral mucosa imaging in vivo. The obtained lateral resolution was 1.7 μm, and the optical section thickness was 9.2 μm [7, 8]. Sheppard, Si, and Gong et al. proposed a confocal microscope with D-shaped apertures in a comparatively systemic theoretical study. The study included the effects of divider parameters and pinhole size, as well as the three-dimensional coherent transfer function [9–12].

The measurement principles of the aforementioned approaches are generally classified into two types. For sample measurement, the first principle uses the sharp slope of the CM response curve for depth measurement, whereas the second principle uses the peak of the CM response curve for focus tracing. However, these approaches have a number of disadvantages. The absolute displacement measurement is difficult to achieve because of having no absolute zero when the sharp slope of the CM response curve is used. Furthermore, the measurement accuracy is susceptible to the nonlinearity of the sharp slope, disturbances in light source intensity, and to the reflection and dispersion characteristics of the measured sample. The tracing accuracy of CM cannot be further improved during the application of focus tracing because the worst sensitivity arises at the peak of the CM response curve.

In a previous paper we presented a bipolar absolute super-resolution differential confocal microscopy (SDCM) to improve the aforementioned disadvantages [13]. SDCM divided the confocal detection light path into two parts and then placed the two detectors before and after focal plane for differential detection. Thus, bipolar absolute zero tracing measurement can be achieved. However, SDCM had a complicated structure and a stringent adjustment requirement in the offset position of its point detections because of its two optical detection systems.

This paper proposes a new laser divided-aperture differential confocal sensing technology (LDDCST) based on the properties of the axial response curve of a divided-aperture confocal microscopy, which shifts with the point detector as a result of a slight transverse offset from the optical axis. LDDCST uses two micro-regions as virtual pinholes that are set symmetrically in the focus along the x direction on the CCD imaging plane to achieve Airy spot-division detection. LDDCST also uses the differential subtraction of two intensity responses simultaneously detected from the two micro-regions to achieve low noise as well as for high axial resolution absolute measurement. Compared with the aforementioned technologies, LDDCST has an absolute zero and can be used for bipolar absolute measurement. LDDCST can mitigate the effects of the disturbances in light intensity and of the reflection and dispersion characteristics of the measured sample. LDDCST can freely adjust the position and size of pinhole through software settings. The CCD and pinhole adjustment significantly simplify the detection system and eliminate the error attributed to the dissymmetrical placement of pinholes and property difference of the detectors.

2. LDDCST principle

Figure 1 is a schematic optical diagram of the LDDCST, where the pupil plane of objective L is divided into illumination pupil S1 and collection pupil S2. The collimated parallel light is focused on the measured sample by objective L after passing through pupil S1. The measurement light reflected from the sample is focused on focal plane P by collection lens Lc after passing through pupil S2. The Airy spot on plane P is magnified on the CCD imaging...
plane by lens $L_M$ with a magnification of $\beta$. Figure 1 shows that $(x,y,z)$ is the coordinate of objective $L$ in the image space, $(\eta,\xi)$ is the coordinate of the pupil of objective $L$, $(x_d,y_d,z_d)$ is the coordinate of the CCD imaging plane.

The scalar paraxial theory can be applied to the theoretical deduction when N.A. < 0.7. The point spread functions of the illumination and collection systems are $h_i(v_x,v_y)$ and $h_c(v_x,v_y)$, respectively [14].

\[
h_i(v_x,v_y,u) = \int_{S1} P(v_x,v_y) \exp \left[ \frac{iu}{2} \left( v_x^2 + v_y^2 \right) \right] \exp \left[ i \left( v_x v_y + v_x v_y \right) \right] dv_x dv_y,
\]

\[
h_c(v_x,v_y,u,v_M) = \int_{S1} P(v_x,v_y) \exp \left[ \frac{iu}{2} \left( v_x^2 + v_y^2 \right) \right] \exp \left[ i \left( v_x + v_y \right) v_x + v_y \right] dv_x dv_y,
\]

where $v_x = 2\pi \sin \alpha / \lambda$, $v_y = 2\pi \sin \alpha / \lambda$, and $u = 8\pi \sin^2(\alpha/2) / \lambda$ are the normalized optical coordinates of objective $L$ in the image space. $v_M = 2\pi \sin \alpha / \lambda$ is the normalized optical coordinate of the transverse offset, $M$ is the transverse offset of the detector on plane $P$ from the optical axis, $\alpha$ is the semi-aperture angle of objective $L$ in the object space, $\alpha_d$ is the semi-aperture angle of collection lens $L_c$, $P(v_x,v_y)$ is the pupil function of objective $L$, and $\eta$ and $\xi$ are the normalized radii of objective $L$.

Figure 1 shows that the three identical micro-regions are set as A, O, and B with radius $r$ on the CCD image plane. Micro-region O is set at the focus, and two micro-regions A and B are set symmetrically at the focus along the $x_d$ direction on the CCD imaging plane. The three responses $I_A(u,-v_M)$, $I_O(u,0)$, and $I_B(u,v_M)$ those correspond to micro-regions A, O, and B are then obtained by CCD.

Axial intensity response $I_i(u,v_M)$ that is obtained from point detector $i$ with offset $v_M$ is

\[
I_i(u,v_M) = \left| h_i(0,0,u) \cdot h_c(0,0,u,v_M) \right|^2.
\]

Let the wavelength of the light source $\lambda = 632.8$ nm, the numerical aperture of objective $L$ N.A. = 0.42, and the normalized radii of pupils $S_1$ and $S_2$ be $r_d = 0.5$. Figure 2 shows the normalized axial intensity response curves $I_A(u,-4)$, $I_A(u,0)$, and $I_B(u,4)$, which $I_O(u,0)$ is the normalized axial intensity response of micro-region O with an offset $v_M = 0$, $I_A(u,-4)$ and $I_B(u,4)$ are the respective normalized axial intensity responses of micro-regions A and B with an offset $v_M = 4$. 

Fig. 1. LDDCST principle.
Fig. 2. Theoretical axial response curves with $v_M = -4$, 0, and 4.

Figure 2 shows that the shapes of curves $I_A(u,-4)$ and $I_B(u,4)$ are almost the same as that of $I_D(u,0)$. A shift is only observed in the $u$ direction of $I_D(u,0)$ when the point detector has slight transverse offset $v_M$. That is, the offset of the point detector in the $x_d$ direction results in a shift of the divided-aperture confocal microscope axial intensity response. LDDCST is based on this feature.

The LDDCST intensity response $I_{LDDCST}(u,v_M)$ is obtained through the differential subtraction of responses $I_A(u,-v_M)$ and $I_B(u,v_M)$, by using Eq. (1) to Eq. (3), and

$$I_{LDDCST}(u,v_M) = I_A(u,-v_M) - I_B(u,v_M).$$

Figure 3 shows the theoretical response curves of LDDCST, which $I_A(u,0)$, $I_A(u,-4)$, and $I_B(u,4)$ are the respective axial intensity responses of micro-regions O, A, and B, and $I_{LDDCST}(u,v_M)$ is the intensity response of LDDCST with $v_M$.

CM axial resolution can be calculated from the slopes of the linear range of response curves. In LDDCST, the axial resolution can also be calculated from the slopes of the linear range of response curves. Gradient $k(u,v_M)$ of $I_{LDDCST}(u,v_M)$ on $u$ is
where \( k(u,v_M) \) is determined in the linear range, while the gradient in the linear measurement range of \( I_{LDDCST}(u,v_M) \) can be expressed in \( k(0,v_M) \) at \( u = 0 \) as follows

\[
\Delta_{\text{axial}} = k(u,v_M) = \frac{\partial I_{LDDCST}(u,v_M)}{\partial u},
\]

(5)

Figure 4 shows the value of \( k(0,v_M) \), where \( \lambda = 632.8 \text{ nm} \) and N.A. = 0.42. The largest value is calculated at \( v_M = 4 \). Thus, LDDCST has the best axial resolution when micro-regions A and B are at offset \( v_M = 4 \).

Let the fluctuation factor attributed to the variations in the light source and the reflectivity of the sample is \( \eta \), and the noise attributed to the environmental variation and the electrical noise is \( \varepsilon \). The intensity responses received from micro-regions A and B are \((\eta I_A(u,-v_M) + \varepsilon)\) and \((\eta I_B(u,v_M) + \varepsilon)\). The property equation of LDDCST can be expressed as

\[
I_x(u,v_M) = \frac{[\eta I_A(u,-v_M) + \varepsilon][\eta I_B(u,v_M) + \varepsilon] - [\eta I_A(u,v_M) + \varepsilon][\eta I_B(u,-v_M) + \varepsilon]}{[\eta I_A(u,v_M) + \varepsilon][\eta I_A(u,-v_M) + \varepsilon] + [\eta I_B(u,v_M) + \varepsilon][\eta I_B(u,-v_M) + \varepsilon] + 2\varepsilon / \eta}.
\]

(7)

LDDCST is often used for high-precision measurement where \( \varepsilon \rightarrow 0 \). Thus, \( 2\varepsilon / \eta \) is negligible relative to the sum of \( I_A(u,-v_M) \) and \( I_B(u,v_M) \). Equation (7) can be simplified as

\[
I_x(u,v_M) = \frac{I_A(u,-v_M) - I_B(u,v_M)}{I_A(u,v_M) + I_B(u,v_M)}.
\]

(8)

Figure 5 shows the LDDCST property curves with N.A. = 0.42 and different offset \( v_M \) that are obtained through Eq. (8). Figure 5 shows that LDDCST sensitivity increases and its measurement range decreases when \( v_M \) increases to 4, whereas LDDCST sensitivity decreases and its measurement range increases when \( v_M \) further increases.
In LDDCST, $v_M$ is optional in the software. Thus, the appropriate LDDCST property curve can be selected through optimal $v_M$ to meet the different requirements of sensitivity and measurement range. In this paper, LDDCST uses the property curve with a higher sensitivity and a smaller measurement range when used for focus tracing measurement, $v_M = 4$ is the optimum value between sensor resolution and measurement range.

Figure 6 shows LDDCST property curve $I_L(u, v_M = 4)$ and CM property curve $I_{CM}(u, 0)$ for N.A. = 0.42, where bevel intervals $ab$ and $cd$ with same length of $\Delta u$ in $u$ direction are the linear areas used for measurements.

In Figs. 3, 5 and 6, we can observe that

1) LDDCST has an absolute zero, which has the maximum sensitivity and corresponds to the LDDCST focus. Thus, LDDCST is suitable for focus tracing measurement;

2) LDDCST has a slope in the bevel interval $cd$ double as that in the bevel interval $ab$ in comparison with CM under the same conditions. Thus, the axial resolution of LDDCST is improved;
3) The linearity of LDDCST in the bevel interval $cd$ is better than that of CM in the bevel interval $ab$. The linear measurement range of LDDCST is extended;

4) LDDCST uses the differential detection and anti-noise process to suppress effectively the common-mode noise that results from environmental differences, disturbance in the intensity of the light source, and from electrical drift of the detector, etc.;

5) LDDCST considers both resolution and measurement range. Different work modes can meet the requirements of resolution and measurement range by adjusting $v_M$ in the software.

3. LDDCST sensor

Figure 7 shows the LDDCST sensor that we developed. Figure 7(a) shows the schematic diagram of the LDDCST sensor, whereas Fig. 7(b) shows the photo of packaged sensor. The laser source is a semiconductor laser CPS180 produced by Thorlabs Inc. The diameter of the laser beam is $\phi 5$ mm. Microscope objective $L$ is SPAHL-50 with long work distance produced by SIGMA KOKI. The N.A. of SPAHL-50 is 0.42. The diameter of the illumination and collection pupils is $\phi 2.5$ mm. Collection lens $L_C$ is a convex lens with a focal length of 150 mm. Lens $L_M$ is a standard microscopic objective with magnification of $\beta = 10 \times$. The CCD used as detector is a WATEC 902H2 Ultimate with effective pixels of 752(H) $\times$ 582(V), and the pixel size is 8.6 $\mu$m (H) $\times$ 8.3 $\mu$m (V). The mirrors are used to reduce the LDDCST sensor size through folding light path.

Micro-regions A and B with a diameter of 6 pixels and offset $C$ are set on the CCD imaging plane, where $C = \beta \nu_M/2\pi \sin \alpha = \nu_M C_0$ is the lateral offset of the point detector, based on the calculation with actual parameters, and $C_0$=7.5 pixels. Thus, the best offset is $C=30$ pixels when $\nu_M = 4$. By using $u = 8\pi \sin^2(\alpha/2)/\lambda$ and $C = \nu_M C_0$, Eq. (8) can be described as

$$I_L(z,C) = \frac{I_A(z,-C) - I_B(z,C)}{I_A(z,-C) + I_B(z,C)} = \frac{I_A(z,-\nu_M C_0) - I_B(z,\nu_M C_0)}{I_A(z,-\nu_M C_0) + I_B(z,\nu_M C_0)}.$$  

(9)
4. Experiments and analyses

4.1 Axial properties

An experimental setup is established to verify the LDDCST principle and to calibrate the axial properties of the LDDCST sensor. In the experiments, the movement of the sample is achieved by using Picomotor Actuators produced by NEWFOCUS to drive the flexible hinge. The XL80 laser interferometer produced by RENISHAW is used to measure the movement displacement. The grey summations of pixels within two micro-regions A and B are respectively calculated when the measured sample moves along the $z$ direction. The axial intensity responses $I_A(z,C)$ and $I_B(z,C)$ are then obtained. Next, the LDDCST sensor response curve $I_{LDDCST}(z,C)$ and property curve $I_L(z,C)$ are respectively obtained by using Eq. (4) and Eq. (9).
Figure 8 shows the LDDCST sensor experimental response curves $I_{LDDCST}(z,C)$ and property curves $I_2(z,C)$ for $C = 10, 20, 30, \text{ or } 40$ pixels. Figure 8(a) shows that $I_{LDDCST}(z,C)$ has the best axial resolution of $\Delta_{axial} = 5.5 \text{ nm}$ when $v_M = 4 \text{ (i.e., } C=30 \text{ pixels).}$ It agrees with the theoretical analyses in Fig. 4 and Fig. 5. Figure 8(b) shows that the measurement range of $I_2(z,C)$ decreases as $C$ increases to 30 pixels, whereas increases as $C$ further increases. So, the improvement of the resolution increases as $C$ increases to 30 pixels, whereas decreases as $C$ further increases. The results follow the theoretical analyses shown in Fig. 5.

![Figure 8](image.png)

**Fig. 8.** Axial properties experimental curves of LDDCST sensor (a) experimental curves of $I_{LDDCST}(z,C)$ with different $v_M$ and (b) experimental curves of $I_2(z,C)$ with different $v_M$.

### 4.2 Lateral properties

An experimental setup based on the Knife-Edge Test verifies the LDDCST principle and then calibrated the lateral properties of the LDDCST sensor. In the setup, the movement of the sample is achieved by using a high-precision electric motorized translation stage with a feed of 0.08 $\mu$m to drive the air bearing slider. The XL80 laser interferometer is used for displacement measurement. The focal point moves across the knife-edge when the measured...
sample moves along the x direction. The distance that corresponds to the step change of axial intensity responses \( I_a(x, C) \) and \( I_b(x, C) \) can represent the LDDCST sensor lateral resolution.

Figure 9 shows LDDCST sensor lateral properties curves \( I_a(x, C) \) and \( I_b(x, C) \) for \( C = 30 \) pixels, which have step changes when the knife-edge passing through the focal point. The larger horizontal skip distance of the step between the two identification points is \( 0.84 \mu m \), which can be considered to be the LDDCST lateral resolution. However, the evaluation method is very strict, and the actual LDDCST lateral resolution is better.

![Figure 9. Lateral properties curves of LDDCST sensor.](image)

4.3 Standard step measurement

The properties of LDDCST sensor are verified by measuring the standard step HS-500MG produced by Budget Sensor. Figure 10 shows the measurements of the standard step obtained by using the Dimension 3100 atomic force microscope (AFM). The step height between two identification points is approximately \( 477.29 \text{ nm} \), and the horizontal skip distance of the step between the two identification points is \( 0.9375 \mu m \).

![Figure 10. Measurement of standard step by AFM.](image)
The HS-500MG is measured as the sample by the LDDCST sensor. The movement of the sample is achieved by using a high-precision electric motorized translation stage with a feed of 0.08 μm to drive the air bearing slider. The XL80 laser interferometer is used to measure the sample movement along the x direction.

Figure 11 shows the intensity response $I_{\text{LDDCST}}(x,C)$ measured by the LDDCST sensor for $C = 30$ pixels and $I_{\text{AFM}}$ measured by AFM on the same piece of HS-500MG. The step height obtained by the LDDCST sensor is approximately 489 nm, and is generally in line with the height obtained by AFM. The obtained horizontal skip distance of the step between the two identification points is 1.74 μm, including the slope of the step itself at 0.9735 μm. The LDDCST sensor lateral resolution is approximately 0.77 μm. The further test results of the LDDCST sensor follow aforementioned obtained properties.

![Figure 11. Measurements of standard step scanned by the LDDCST sensor.](image)

5. Conclusions

A new LDDCST is proposed in this paper. This new LDDCST uses two micro-regions as virtual pinholes on the CCD imaging plane to achieve virtual detection, can freely adjust the position and size of the pinhole. LDDCST significantly simplifies the structure and the pinhole adjustments, mitigates the error caused by pinhole adjustment, uses the differential subtraction of two intensity responses to achieve absolute measurement and low noise, has improved axial resolution and an extended measurement range, can consider both resolution and measurement range, and effectively suppresses the effects of the disturbances in light intensity and of the reflection and dispersion characteristics of the sample. An LDDCST-based sensor has been developed. Preliminary experiments indicate that the LDDCST sensor has an axial resolution of approximately 5.5 nm and a lateral resolution of approximately 0.77 μm, which follows the theoretical analyses.

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