High Spatial Resolution Cone Beam X-ray Imaging System Based on a Scanning Electron Microscope

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Abstract

A high-spatial-resolution cone-beam X-ray imaging system has been realized based on the micro spot X-ray source generated by a high-quality electron beam from a Scanning Electron Microscope (SEM). The diameter of the X-ray source was estimated to be 1.0 m based on Geant4 simulation when the primary electron beam size is 10 nm, which is mainly determined by the electron injection range inside the copper target, and the X-ray source was found to follow double Gaussian distribution. The clear X-ray images of a 400-mesh copper mesh have been acquired, and the best spatial resolution of the system was tested to be 7.1 m under magnification factor of 68.8. The X-ray imaging on distinct head regions of a Carebara diversa ant have been realized, the organs like the ant's jaw and eyes were clearly shown, and the imaging range can be adjusted easily. These results demonstrate that this imaging system has high magnifications and precise spatial resolution, and is able to achieve clear and practical X-ray imaging for very small biological specimens.

Full Text

Preamble

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Abstract

A high-spatial-resolution cone-beam X-ray imaging system has been realized based on the micro-spot X-ray source generated by a high-quality electron beam from a Scanning Electron Microscope (SEM). The diameter of the X-ray source was estimated to be 1.0 m based on Geant4 simulation when the primary electron beam size is 10 nm, which is mainly determined by the electron injection range inside the copper target, and the X-ray source was found to follow a double Gaussian distribution. Clear X-ray images of a 400-mesh copper mesh have been acquired, and the best spatial resolution of the system was tested to be 7.1 m under a magnification factor of 68.8. X-ray imaging of distinct head regions of a *Carebara diversa* ant has been realized, with organs such as the ant's jaw and eyes clearly shown, and the imaging range can be adjusted easily. These results demonstrate that this imaging system has high magnification and precise spatial resolution, and is able to achieve clear and practical X-ray imaging for very small biological specimens.

Keywords: cone beam X-ray imaging, SEM, micro spot X-ray source, spatial resolution, magnification.

Introduction

X-ray radiography is a noninvasive imaging technique for visualization of internal structures of various samples. The technique utilizes the intensity attenuation of X-ray beams after passing through the imaging sample. In the medical field, diverse X-ray imaging modalities are applied to general radiography, computed tomography (CT), fluoroscopy, and angiography [1-3]. Chest CT imaging is a standard method used to assess pulmonary damage during the COVID-19 pandemic. High-precision X-ray images spurred extensive research into AI-driven diagnostic methods [4-6]. In addition, X-ray imaging has found critical applications in security screening, agricultural quality control, and industrial defect detection [7, 8]. Cone-beam X-ray imaging, which utilizes a cone-beam X-ray source to acquire 2D images directly, has many advantages and is able to realize 3D reconstruction easily when compared to traditional sector-beam X-ray imaging.

Improving the spatial resolution of X-ray imaging systems has always been a hot topic in the area. The spatial resolution of conventional micro-CT systems is about 0.5 mm, which is not able to clearly resolve some microstructures (e.g., the human stapes posterior crus is 0.19 mm) [9, 10]. To better visualize these small structures, high-quality X-ray images are required that offer high spatial resolution, high contrast, and low noise. All subsystems of the X-ray imaging system must be optimized to achieve high spatial resolution. The spot size of the X-ray source must be as small as possible, while much higher magnification values are preferred, and low-noise detectors are important for increasing contrast.

High-spatial-resolution X-ray imaging methods primarily include phase-contrast imaging based on synchrotron radiation sources and absorption-contrast imaging using microfocus X-ray tubes [11-12]. Recent advancements in X-ray phase-contrast imaging, which employ X-ray microscope plates to exploit diffraction-enhanced imaging, have achieved spatial resolutions below 10 nm [13–16]. However, this method imposes stringent requirements on large-scale scientific devices like synchrotron radiation light sources to generate extremely bright monochromatic X-ray beams, which is very expensive. Compared with X-ray phase-contrast imaging, absorption-contrast imaging features simpler system configuration and lower imaging costs, making it particularly suitable for rapid acquisition of microscale-resolution images. A research team led by Wang Qi at the China Academy of Engineering Physics (CAEP) pioneered the integration of an 8- m microfocus X-ray tube with a microlens array coupling system, successfully performing X-ray imaging on laser inertial confinement fusion targets. Their work attained a spatial resolution of 0.5 m [17]. However, the system also faces challenges including reduced photon flux and shortened exposure time when pursuing higher resolution.

This study focuses on optimization approaches for absorption-contrast imaging at high spatial resolution. In this study, the high-precision electron beam of a scanning electron microscope (SEM) was selected to generate X-ray beams with a very small spot size. The electron beam size of the SEM can reach as low as 1 nm, which can effectively reduce the focal size of X-rays. The SEM used in this work is KYKY-EM6200, and the electron beam is approximately 10 nm. The SEM is also equipped with a vacuum chamber and adjustable 5D sample stage, which is modified to create a precise cone-beam X-ray imaging system. The imaging is performed inside the vacuum chamber to ensure that X-rays are not affected by air scattering. A low-noise photon-counting MiniPIX detector is used to acquire the X-ray image in the vacuum chamber [18]. The spot size of the X-ray source is studied by Geant4 simulation, and the performance of the system was evaluated using copper meshes for Transmission Electron Microscope (TEM) and *Carebara diversa* ants.

2.1 Influence of Micro Spot Source on X-ray Imaging Resolution

The principle of cone-beam X-ray transmission imaging is that when cone-beam X-rays pass through an object, X-ray photons might be absorbed or scattered, and the intensity attenuation is connected with the density and thickness of the object. By detecting the transmitted 2D X-ray intensity, the structure of the object can be deduced.

Decreasing the focal size of the X-ray source is a feasible approach to achieve higher spatial resolution X-ray imaging. The principle is shown in Fig. 1.

When the position of the object and the detector are fixed, a larger focal size increases the blurred area of the projected image, resulting in degraded imaging performance.

Figure 1. Blur area of "U" caused by limited focal spot size "S" for cone-beam X-ray imaging.

If the focal size of the X-ray source is S, the distance from the X-ray source to the sample being measured is SSD (Source-to-Sample Distance), the distance from the source to the projection is SDD (Source-to-Detector Distance), the size of the object being measured is D_1, and the projection size is D_2. The formula for the size of the blurry area (U) is shown in Eq. (1). If S is much smaller than SSD, then the magnification factor ($M = D_2/D_1$) of the system can be simplified as SDD/SSD [12]. From Eq. (1), it can be seen that the blurry size U is proportional to the focal size S and positively correlated with the magnification factor M. Therefore, one important way to improve spatial resolution is to reduce the size of the X-ray source and increase the magnification factor of the imaging system. Since the electron beam size of the SEM is very small, it is possible to generate an X-ray beam with a very small focal size by using this electron beam to irradiate a target. In this work, copper material is used as the target, and the Cu characteristic X-rays (8.045 keV and 8.907 keV) are produced for the X-ray source.

2.2 Structure of X-ray Imaging System

Figure 2. Device schematic diagram.

Fig. 2 shows the schematic of various components of the X-ray imaging system, which was constructed based on a KYKY-EM6200 SEM modified to include a specially shaped copper block, a sample holder, and a photon-counting detector. The nanoscale electron beam (diameter: ~10 nm) of the SEM is injected perpendicularly onto the 45° inclined surface of the copper target to generate X-ray beams. The energy and current of the electron beam are set to 30 keV and 170 A to maximize X-ray intensity. The sample holder is placed on the SEM stage, whose position can be adjusted using the 5D SEM positioning system. A MiniPIX detector (Advacam, Czech) is placed on the window flange of the SEM, which has 256 × 256 pixels with a pixel size of 55 m, and the energy threshold is set to 5 keV to suppress noise. A laser level is used to align the system, ensuring that the center of the copper ramp, the sample, and the detector are on the same horizontal plane.

The SDD is fixed at 113 mm in this work, and the magnification value can be adjusted by decreasing SSD. However, when SSD becomes small enough to be comparable with the sample's size, the magnification will be very large, which renders the system very sensitive to mechanical and electrical vibration, and the thicker sample itself would introduce a highly blurred area and degrade the imaging performance heavily. This issue will be discussed in detail in section 3.3.

2.3 Monte Carlo Simulation of the Micro Spot X-ray Source

The focal spot size of the X-ray source is important for the imaging system. However, it is not easy to measure, and it is acquired by Geant4 simulation in this work. Geant4 is a Monte Carlo application software package developed by the European Organization for Nuclear Research based on C++ object-oriented technology, which is widely used to simulate the physical processes of particle transportation in matter [19-22].

To determine the actual size of the X-ray focal spot, a simplified simulation system is established using Geant4. This system models a perpendicularly incident electron beam on a cubic copper target. Fig. 3 [Figure 3: see original paper] shows the ejected position of the excited X-rays inside the copper cube. The parameters of the electron beam are set according to the experimental setup, which has 30 keV energy and 10 nm beam width.

Fig. 3(a) shows the radial distribution perpendicular to the electron beam. It is found that the X-rays are deviated from the electron beam center and spread to a much wider range. This is because electrons will be scattered heavily inside the target, and about 95% of the X-rays are generated within [-0.82 m, 0.82 m] of the electron beam center. Fig. 3(b) shows the axial distribution along the electron beam direction. The peak exists at 0.2 m, and about 95% of the X-rays are generated within [0, 1.05 m] along the electron beam, which is consistent with the range of about 1.2 m for 30 keV electrons in copper. This indicates that the generated X-ray source size is approximately 1.0 m when the electron beam size is 10 nm, where a significant enlargement in the focal size of X-rays is found when compared to the electron beam size, which is mainly caused by electron penetration and scattering inside the target.

Figure 3. The simulated eject position of the X-rays that are excited by primary electron beam in copper target. (a) Radial distribution perpendicular to the electron beam. (b) Axial distribution along the electron beam. The red lines show the range where 95% X-rays are generated.

To better simulate the experimental setup, a simplified system is constructed using Geant4 to directly characterize the spatial profile and intensity distribution of the X-ray source of the imaging system, which is shown in Fig. 4 Figure 4: see original paper. The target is simplified to a copper block with dimensions of $10 \times 10 \times 5$ mm^3, and a "detector array" consisting of 100×100 units with a pixel length of 200 nm is used to collect X-rays generated by the electron beam, which can demonstrate the shape and intensity of the X-ray source that can be used for imaging. Similar to the experimental setup, the electron beam is injected to the 45° inclined surface of the copper, the diameter of the electron beam is set to 10 nm, and the energy is set to 30 keV. The distance between the electron beam center and the detector array is about 3.6 mm to avoid interference with the copper target and collect as many X-rays as possible. The number of X-ray photons that can pass through the detector array are collected during simulation, and the X-ray intensity by simulating 1$×10^{10}$ electrons is shown in Fig. 4(b). The X-ray source exhibits a circular shape because the X-rays are emitted uniformly from the copper, and it clearly demonstrates the intensity distribution of the X-ray source that can be used for imaging. The X-ray collection efficiency is approximately 0.04% relative to the incident electron count, which means the X-ray flux used for imaging is about 3$×10^{5}$ s^{-1} in this work. To better quantify the structure of the X-ray circular spot, the 1D projection of X and Z directions are respectively shown in Fig. 4(c) and (d). Both are found to follow Gaussian profiles and are in excellent agreement with the expected normal distribution pattern.

The FWHMs are fitted to be 4.02 m in the X direction and 4.03 m in the Z direction. The FWHM is much wider than the X-ray focal size of 1.0 m because the simulated "detector array" is 3.6 mm away from the electron beam center.

Figure 4. (a) The Geant4 setup to simulate the X-ray intensity that can be used for imaging. (b) The collected X-ray intensity from the detector array. (c) X-direction projection of the X-ray intensity. (d) Z-direction projection of the X-ray intensity.

Results and Discussion

3.1 X-ray Image of 400 Mesh TEM Copper Mesh

To test the spatial resolution of this X-ray imaging system, a 400-mesh TEM copper mesh was used. The diameter of the TEM copper mesh is 3.1 mm and the thickness is 30 m. The hole diameter of the copper mesh is 40 m and the minimum grid width is 20 m. The SDD is fixed at 113 mm and the magnifications of the system can be adjusted within 1-120 by changing the SSD.

Fig. 5 [Figure 5: see original paper] shows the TEM images under different magnifications and exposure times. The magnifications of the three columns are 27.5, 68.8, and 118.3, while the exposure times of the two rows are 30 s and 150 s. The circular pores and segmented copper wires are clearly imaged in all cases, and the images are much clearer when magnification is higher. The images become clearer when the exposure time is longer because the signal-to-noise ratio (SNR) can be increased. However, there is an exception for Fig. 5(f), where large distortion and blurred areas are found for the circular pores which have turned into long strips.

After multiple experimental imaging tests, it is found that once the magnification exceeds 118, large distortion appears and becomes much heavier when the exposure time is longer. Specifically, as the exposure time gradually increases, the blur area becomes wider and the distortion becomes heavier. The reason should be connected with vibration of the system, either mechanical vibration of the target or sample, or electrical vibration of the electron beam. The vibrations are always present but only become significant at high magnification and long exposure time.

3.2 Image Signal-to-Noise Ratio Analysis

SNR is a key factor affecting image quality. In this work, the detector's energy threshold is set to 5 keV to minimize detector noise. The predominant noise then originates from scattering of electrons and X-rays within the imaging system. The electron beam and X-ray photons might be scattered several times in the environment before entering the detector, creating noise in the images.

The contrast-to-noise ratio (CNR) and coefficient of variation (COV) are representative factors that can quantitatively measure the noise level of an image and are widely applied in denoising algorithms in the computer vision field [1], which are used as key indicators to quantitatively evaluate the performance of the X-ray imaging system in this work. CNR is defined in Eq. (2), which represents both the contrast and noise of two distinct areas within an image [23, 24]. The COV is expressed by comparing the noise with signal values in a given region of interest (ROI), and the definition is shown in Eq. (3) [25, 26]. The image quality is better for higher CNR and lower COV values [27, 28].

In Eqs. (2) and (3), _R and σ_R are the mean value and standard deviation for the established target region of interest (ROI_R), while _B and σ_B are the mean value and standard deviation for the established background region of interest (ROI_B).

Fig. 6 [Figure 6: see original paper] presents the images used for quantitative analysis. The first row displays the three original raw images at magnifications of 27.5, 68.8, and 118.3 (which are enlarged sub-images of those shown in Fig. 5), while the second row demonstrates the denoised images of the first row after median filtering. Similar to Fig. 5, much more detailed information can be deduced at higher magnification. The images become much clearer after median filtering, which indicates that image quality can be further improved. The ROIs (ROI_R and ROI_B) used for calculating CNR and COV are shown as red boxes and blue boxes in Fig. 6. A small homogeneous area along the Y-direction and X-direction of the copper mesh was selected as ROI_{R1} and ROI_{R2}, while the pore center area was chosen as ROI_B for comparison.

Figure 6. The images used for quantitative analysis, which are enlarged sub-images of Figure 5 at three magnification values. The second row shows the filtered images of the first row after median filtering.

The calculated CNR and COV values are shown in Fig. 7 [Figure 7: see original paper]. As the magnification factor increases, the original images exhibit higher CNR and lower COV, indicating improved image quality. When the magnification increases from 27.5 to 118.3, the average CNR for ROI_{R1} and ROI_{R2} increases from 0.15 to 0.35, while the average COV values decrease from 2.6 to 1.12. When compared to the original images, the images after median filtering demonstrate significant enhancement in SNR, particularly at lower magnification levels. Under the magnification of 27.5, the average CNR value increased from 0.15 to 3.54 after median filtering, which is 23.60 times higher than that of the original image, while the COV value has been reduced to 25% of its original value.

The improvement is more significant at small magnification and becomes less effective at high magnifications. This is because the image performance at small magnification is much worse for the original images, and median filtering is highly effective. This phenomenon can be attributed to the reduced source-to-sample distance (SSD) at higher magnification, where more X-rays are absorbed by the copper mesh when SSD is smaller, thereby reducing the number of scattered photons from the irrelevant environment reaching the detector's sensitive area. In conclusion, it indicates that the median filtering method has a significant effect on noise reduction. Median filtering makes it easier to measure stable image resolution in the images.

Figure 7. The contrast-to-noise ratio (CNR) and coefficient of variation (COV) values of the TEM mesh image shown in Fig. 6 at three magnification conditions.

3.3 Imaging Resolution Analysis

The spatial resolution is calculated by the Knife-Edge Method using the edge of the mesh grid. The basic principle is that a slight line as the "ideal" curve in Fig. 8 [Figure 8: see original paper] can be found for a perfect imaging system, while a blurred area can be found for a system with limited spatial resolution. The spatial resolution can be deduced from the blurred area of the edge.

Figure 8. Principle of spatial resolution calculation.

Fig. 9 [Figure 9: see original paper] shows the spatial resolution calculation algorithm. Fig. 9(a) shows the region of interest (ROI) within the imaging field, which encompasses 2 grid edges of nearby pores. Fig. 9(b) is the 1D intensity curve along the Y-axis of the ROI, and the two grid edges are clearly shown. The deviation of the 1D curve is calculated and shown in Fig. 9(c). Two peaks shown in Fig. 9(c) are connected with the blurred area of the two grid edges, which are fitted with Gaussian functions, and the fitted Full Width at Half Maximum (FWHM) can be used to represent the spatial resolution. The fitted Gaussian curves at various magnification conditions and exposure times are shown in Fig. 9(d) for comparison, and the X-axis has been changed to length units. In Fig. 9(d), the peaks are much more significant for longer exposure time, which is consistent with the results of Fig. 5 that longer exposure time helps reduce statistical error.

The X-axis units of Fig. 9(b) and (c) are in pixels, which need to be converted to length units in Fig. 9(d). This is done by using the TEM pore diameter in these images. The pore diameter (in number of pixels) can be measured from the image, the pixel width of the detector is known to be 55 m/pixel (L_d), then the imaged diameter of these pores can be calculated. Since the actual diameter of the TEM pore is 40 m, the magnification (M) is calculated as the ratio of the imaged diameter to the actual diameter. The calibration factor is then derived using L_d and M. Subsequently, the number of pixels (N) can be converted to length units (L) based on this calibration factor, as shown in Eq. (4). The calculated FWHM at various magnifications and exposure times are summarized in Table 1 .

Figure 9. (a) The ROI used for spatial resolution calculation for magnification of 27.5. (b) 1D intensity along Y-axis of the ROI. (c) Deviation of the 1D intensity curve, which are fitted with Gaussian function. (d) Fitted curves under various magnification and exposure time, and X-axis has been shown in length units.

Table 1. Actual resolution of X-ray imaging system under various magnifications and exposure time.

Magnification	Resolution (m) for Exposure time of 30 s	Resolution (m) for Exposure time of 150 s
27.5	12.3	10.8
68.8	8.5	7.1
118.3	9.2	11.4

In Table 1, the spatial resolutions are within the range of 7–12 m, and the spatial resolutions are sensitive to magnifications and exposure time. As the magnification increases, the spatial resolution initially improves but becomes worse at very high magnification due to increased sensitivity to vibration. The best resolution is found under magnification of 68.8, which can be further improved with longer exposure time of 150 s. The spatial resolution for the X direction is slightly better than that of the Y direction, because the Y direction is more sensitive to vibration. The apparent contradiction observed at 118.3 magnification with 150 s exposure time arises because the X-ray intensity distribution along the Y-axis deviates from the expected Gaussian profile at this setting. Taken together, the best spatial resolution is found to be 7.1 m under magnification factor of 68.8 and exposure time of 150 s.

3.4 X-ray Imaging of Small Animals

The cone-beam X-ray imaging has been found to have micro-scale spatial resolution, which indicates it is capable of imaging small animals. Images of an ant's head were taken by this system, and the results are shown in Fig. 10 [Figure 10: see original paper]. The *Carebara diversa* ant was chosen for tests because it has a sturdy head structure and higher imaging contrast. Its widest upper jaw is about 2.5 mm long, and the head is about 4.2 mm long. The field of view is very sharp under high magnification, and the sensitive imaging area can be adjusted to acquire the full ant head. A total of 8 images of the ant's head (along the orange line in Fig. 10(a)) are shown in Figs. 10(b)-(i). The exposure time of 120 s was chosen to obtain images with acceptable quality. The jaw and eyes of the *Carebara diversa* ant can be clearly seen in Figs. 10(b) and (i), while the structures in the brain are found in Figs. 10(d)-(h). These demonstrate that this X-ray imaging system can achieve clear imaging of small animals.

Figure 10. (a) The imaging area in head of the *Carebara diversa* ant. (b)-(i) The original images of the ant, with exposure time of 120 s.

Conclusion

This study investigates a cone-beam X-ray imaging system employing a high-precision electron beam from a KYKY-EM6200 SEM. The focal spot of the X-ray source was carefully studied using Geant4, revealing a spot diameter of approximately 1.0 m, which is mainly connected with the electron injection range inside the copper target. The system was evaluated through X-ray imaging experiments on a 400-mesh TEM copper mesh under three magnification factors (27.5, 68.8, and 118.3) and various exposure times (30–150 s). Median filtering algorithm was used to enhance signal-to-noise ratio (SNR). Quantitative analysis demonstrated the best spatial resolution of the system is 7.1 m under magnification factor of 68.8. Practical validation was achieved through micrometer-scale cone-beam X-ray imaging of a *Carebara diversa* ant's head region, highlighting the system's utility in biological studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgment

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Note: Figure translations are in progress. See original paper for figures.