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Development of ultrafast laser-based x-ray *in-vivo* phase-contrast micro-CT beamline for biomedical applications at Advanced Laser Light Source (ALLS)

Russell Kincaid¹, Andrzej Krol^{1,2}, Sylvain Fourmaux³, Jean-Claude Kieffer³, Cristina Serbanescu³, Marina Servol³, Levon Vogelsang¹, Steve Wilkins⁴, Andrew Stevenson⁴, Yakov Nesterets⁴, Edward Lipson¹, Hongwei Ye¹, and Andrew Pogany⁴

¹ Department of Physics, Syracuse University, Syracuse, NY, 13244 USA

² Department of Radiology, SUNY Upstate Medical University, Syracuse, NY, 13210 USA

³ INRS-EMT, Université du Québec, Varennes, Québec, Canada, J3X 1S2

⁴ CSIRO Materials Science & Engineering Clayton South, VIC, Australia, 3169

Abstract

We are developing and exploring the imaging performance of, an *in vivo*, in-line holography, x-ray phase-contrast, micro-CT system with an ultrafast laser-based x-ray (ULX) source. By testing and refining our system, and by performing computer simulations, we plan to improve system performance in terms of contrast resolution and multi-energy imaging to a level beyond what can be obtained using a conventional microfocal x-ray tube. Initial CT projection sets at single energy (Mo K_{α} and K_{β} lines) were acquired in the Fresnel regime and reconstructed for phantoms and a euthanized mouse. We also performed computer simulations of phase-contrast micro-CT scans for low-contrast, soft-tissue, tumor imaging. We determined that, in order to perform a phase-contrast, complete micro-CT scan using ULX, the following conditions must be met: (i) the x-ray source needs to be stable during the scan; (ii) the laser focal spot size needs to be less than 10 μm for source-to-object distance greater than 30 cm; (iii) the laser light intensity on the target needs to be in the range of 5×10^{17} to 5×10^{19} W/cm²; (iv) the ablation protection system needs to allow uninterrupted scans; (v) the laser light focusing on the target needs to remain accurate during the entire scan; (vi) a fresh surface of the target must be exposed to consecutive laser shots during the entire scan; (vii) the effective detector element size must be less than 12 μm . Based on the results obtained in this research project, we anticipate that the new 10 Hz, 200 TW laser with 50 W average power that is being commissioned at ALLS will allow us practical implementation of *in vivo* x-ray phase-contrast micro-CT.

Keywords

in vivo micro-CT; x-ray phase-contrast imaging; in-line holography; tumor imaging; ultrafast laser-based x-ray source

1. INTRODUCTION

Phase contrast x-ray imaging has come forth as a very promising alternative in the field of biomedical imaging [1–3]. In-line holography x-ray phase-contrast imaging is particularly appealing because it does not require x-ray diffractive optical components that are needed for the alternatives (x-ray interferometry and diffraction enhanced imaging) [1–3]. Therefore, it can be performed with significantly lower x-ray fluence and the system alignment is much simpler than that for alternative methods. This is especially important when this approach is to be extended for use in micro-CT [1–3]. The primary requirement for in-line holography, phase-contrast micro-CT, in the Fresnel diffraction regime, for a given source-to-object distance is an x-ray source with sufficiently small focal spot size and an x-ray detector with sufficient spatial resolution. For application in *in-vivo* micro-CT, the x-ray source needs to provide sufficiently high and stable x-ray flux that would allow uninterrupted acquisition of a complete CT projection set and blank images within 60 minutes (typical *in-vivo* scan duration is around 30 minutes). An ultrafast-laser, plasma-based, x-ray source (ULX) is a promising candidate for a compact x-ray source for phase-contrast imaging. In addition to a small focal spot size, ULX generates narrow x-ray spectra that consist mainly of characteristic lines. Such spectra can be easily tailored (by using laser-beam targets with appropriate chemical composition) to the specific imaging task. Phase-contrast micro-CT allows high-resolution measurements of the spatial distribution of the real (x-ray phase-shift) and the imaginary (x-ray absorption) components of the x-ray refractive index in a living animal. This is in contrast to conventional micro-CT, with a microfocal x-ray tube as an x-ray source, which can only map the 3D distribution of the x-ray absorption coefficient. The ULX source, with its unique spectral qualities and small focal spot size, may allow detection of subtle differences in electron density that is not possible with current clinical x-ray tube based micro-CT scanners. We expect that opening the new channel of information provided by x-ray phase-shift 3D mapping, in addition to a conventional absorption map measurement, will significantly increase soft-tissue low-contrast resolution of micro-CT, thus allowing improved imaging of tumors in small animal models. A ULX source has been proposed and investigated for use in micro-CT and its suitability for in-line, x-ray, phase-contrast imaging has also been reported [8–13]. However, to implement this technology for *in-vivo* use, significant obstacles must be overcome. We have been constructing and exploring imaging performance of an in-line phase-contrast ULX cone-beam micro-CT system for imaging living small laboratory animals. We present here some important details and results of progress we have made toward this goal.

In-line holography, x-ray, phase-contrast images, acquired using a ULX source, have been shown to exhibit strong Fresnel diffraction fringes attributable to abrupt changes in the second derivative of the real part of the x-ray refractive index that might occur at interfaces between normal tissue and lesions. It is these fringes that are required to extract the phase-shift component from image data. Our modeling of this experimental configuration has been shown to be reasonably accurate[13]. While we have been unable as yet to image live-animal tumor models, we have performed computer simulations of phase contrast CT imaging of small spherical tumors in soft tissue, and have found that the Fresnel-diffraction fringes at the interfaces between tumors and normal tissue were prominent[18]. We

performed tomographic reconstructions of these simulated CT data; the reconstructed 3D images demonstrated significant improvement in visibility of the tumor boundaries, as compared to tomographic images obtained using CT in absorption regime [18]. Our goal is to obtain experimentally soft tissue tumor images of comparable quality using an in-line holography, phase-contrast, *in-vivo*, micro-CT system beamline with ULX source.

2. MATERIALS AND METHODS

2.1 The Laser Plasma-Based X-ray Source at ALLS

Experiments have been conducted with a femtosecond Ti:Sapphire laser at the Advanced Laser Light Source (ALLS) laser, at the Institut National de la Recherche Scientifique (INRS), University of Québec, Varennes, Canada. The 100 Hz laser provides 110 mJ pulses at the primary wavelength, $\lambda_p = 800$ nm. With a KDP crystal, a high-contrast second harmonic frequency doubling stage generates, a beam of 70 fs pulses with 15 mJ energy (1.5 W average power) and $\lambda_d = 400$ nm. This beam is focused, using a 5 cm diameter, 15 cm focal-length, off-axis, parabolic mirror (Fig. 1) onto a rotating solid target (Fig. 2). The target material is interchangeable for precise matching of the K_α emission line to the imaging task (e.g. animal thickness or imaging probe density or elemental composition). The estimated spatial intensity distribution of the laser light focal spot was Gaussian with $\text{FWHM} = 8 \pm 1 \mu\text{m}$ (Fig. 3). The MTF obtained for the x-ray source is shown in Fig. 4. The intensity of the beam at the focal spot was estimated to be $8 \times 10^{17} \text{ W/cm}^2$. The estimated conversion efficiency, from laser photons to K_α photons was 1.4×10^{-5} . The total flux was estimated to be $8.5 \times 10^8 K_\alpha \text{ photons/(sr}\cdot\text{s)}$. The ns and ps prepulses were 10^{-5} at $\lambda_p = 800$ nm resulting in a contrast ratio better than 10^9 for both ns and ps prepulses.

To limit the Poisson noise to 3%, with source to detector distance equal to 70 cm and detector pixel pitch equal to $24 \mu\text{m}$, at least 1,111 photons per detector element are required per image. Therefore, assuming constant x-ray flux, it would take 18.5 minutes per projection image, and a scan of 360 images would be completed in 6,660 minutes (111 hours) without taking into account time needed for the target change.

A new ultrafast laser is being commissioned at ALLS with properties: 20 fs pulses, 5 J per pulse, 10 Hz repetition rate, 50 W average power, and 200 TW peak power. The laser has extremely high fundamental frequency pulse contrast ($>10^9$) that would allow work at fundamental frequency. We plan to transfer our experiment to this laser.

Figure 5 shows the x-ray spectrum we measured for the 100 Hz ULX source with only a $100 \mu\text{m}$ Be filter. The K_α and K_β lines are clearly visible. We estimate that approximately 30% of emitted photons were in the characteristic lines. Because the hot-electron temperature is only 13 keV, the continuous Bremsstrahlung radiation intensity is very low at energies above 40 keV but, unlike the x-ray tube spectrum, there is no well-defined energy cut-off point.

The major problem with the 100 Hz ULX system used so far has been x-ray intensity instability. We kept experiencing a rapid decrease in x-ray power, with characteristic decay half-time ~ 30 s (Fig. 6). Consequently, each time we lost x-ray power, we had to shut down the laser source and we could resume x-ray production only after 5 to 10 minutes delay; this

prevented continuous image acquisition. This problem has been very recently corrected, and presently the 100 Hz ULX system is stable within 20% (Fig. 7).

2.2 The Micro-CT Beamline at ALLS

The micro-CT beamline consists of laser-beam solid target on a rotational and $X'Y'$ translational stage (where X' and Y' denote direction perpendicular and parallel to the focal direction of the parabola), a goniometer (with better than 0.03° absolute accuracy), and a cooled CCD detector (24 μm pitch, Princeton Instruments model PI-SCX:4300 on a XYZ stage). The target (ULX source) is housed in a 61 cm diameter and 51 cm tall cylindrical vacuum chamber. To increase x-ray flux on the sample, we positioned the goniometer in a fixed position inside a recessed port (Fig. 8) with 32.4 cm source-to-isocenter distance (R_I), increasing the x-ray flux by 57%. The isocenter-to-detector distance (R_2) was varied in the 9.1 to 34.0 cm range.

A Gd_2OS_2 screen coupled via a 1:1 optical taper to the CCD camera was used. The estimated detector point-spread function was Gaussian with FWHM = 50 μm .

The Fresnel number is defined as $N_F = a^2/\lambda x$, where a is the characteristic size of the sample, and x is the object to detector distance (here, R_2). For 17–20 KeV x-rays, $\lambda \sim 0.62\text{--}0.73 \text{ \AA}$, and $R_I \sim 5\text{--}50 \text{ cm}$. We assume that $a \sim 10\text{--}20 \mu\text{m}$; therefore N_F is in the 2.74–129 range (i.e. between the *Fresnel* and the *near Fresnel regimes*).

2.3 Alignment of the Micro-CT Beamline

Commercial micro-CT scanners are carefully designed, manufactured, and calibrated, to insure that the source and detector are positioned precisely and consistently. This allows the user to know any detector offsets and to pass this information to the reconstruction software, and thus help minimize reconstruction artifacts. With our prototype ULX micro-CT system, there has not yet been sufficient beam time to allow a full geometric alignment of our system using x-ray images. Before scanning, we made an attempt to align the goniometer axis of rotation vertically, and the detector face vertically and normal to the horizontal isoray. Because we couldn't confirm the accuracy of this alignment before we began scanning, we glued small alignment spheres to the outside of the object, both above and below the isocenter (and the region of interest). These 300 μm diameter carbide and 500 μm diameter stainless-steel alignment spheres functioned as “point” attenuation objects, in order to embed axis-of-rotation data in the projection images on the fly. We used the locations of the spheres in the projection images to estimate the position, in the images, of the projection of the axis of rotation. Detector misalignment was then determined in the pre-reconstruction image-processing step. This method was inspired by somewhat related strategies used by others [20–21]. To determine the detector vertical and horizontal offsets, and angular misalignment, we carefully examined each projection image and, using a macro in ImageJ¹, recorded the positions on the projections of the small alignment spheres. Even though the projected path of a sphere center in the images is not an ellipse, the data can be easily fit to an ellipse, and from that fit one can get five key parameters: the vertical-axis size, the

¹Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/ij/>, 1997–2008.

horizontal-axis size, the tilt angle of the ellipse, and the horizontal and vertical coordinates of the center. Least-squares fits of the coordinates to ellipses were performed. A method similar to that proposed by Noo [21] was used to determine the detector vertical offset, horizontal offset, and angular misalignment.

2.4 Tomographic Acquisition Scans of the Phantoms and the Mouse

Tomographic projection sets were acquired for a cylindrical phantom composed of small spheres of different sizes and compositions, embedded in epoxy resin. The small spheres in the phantom were made of 2.4, 4.8, and 5.1 mm diameter glass, 3.2 mm diameter polystyrene, 2.4 mm diameter Nylon, and 1.6 mm diameter acrylic, respectively. Various imaging geometries were investigated including magnification as near to unity as possible, and magnification greater than 2.0. Projection images were acquired every 10° over 360°.

Projection images were also acquired using a small euthanized mouse (Fig. 9) of mass 5 g. Because of problems with ULX stability, this scan resulted in an incomplete CT projection set. Only a limited set of consecutive projection images was acquired with 1° increment over 26° total arc.

2.5 Processing the Data

Plugins in ImageJ were used to translate and rotate the image data to compensate for misalignment revealed by our “on the fly” alignment method. Dark-current image subtraction and flat-field normalization were also performed using an ImageJ plugin.

2.6 Tomographic Reconstructions

Tomographic reconstruction was done using commercial implementation of 3D CT reconstruction of the Feldkamp [25] filtered back-projection algorithm (COBRA by Exxim²). Since the images of the small mouse were acquired over only 26° total arc, a high quality reconstruction was not possible. A limited scan angle tomosynthesis reconstruction provided encouraging results.

2.7 Comparison with Commercial micro-CT

A tomographic projection set was acquired for the same cylindrical phantom, composed of small glass and plastic spheres embedded in epoxy resin, using a commercial micro-CT scanner (microCAT II, Siemens; located at SUNY Upstate Medical University, Syracuse, NY). This micro-CT scanner is equipped with a 40 W microfocal x-ray tube source, with W anode and Al filters. The peak energy is adjustable within the 35–80 kVp range. The maximum anode current is 500 μ A. The focal spot size is specified to be less than 50 μ m. A CCD camera (DALSA, air-cooled, 12 μ m pitch) was used, with a Gd₂OS₂ screen coupled via a 2.2:1 optical taper (26 μ m effective pitch). Projection images were acquired every 1° over 360°. We used a source-to-isocenter distance, $R_1 = 34.8$ cm, and an isocenter-to-detector distance, $R_2 = 7.9$ cm ($M = 1.23$). A second sparse projection set was extracted from every tenth view (every 10° over 360°) of this scan, to be used for appropriate comparison to the limited projection set acquired with the ULX system.

²<http://www.exxim-cc.com/products.htm>

3. RESULTS

The 100 Hz ULX source at ALLS proved to be inadequate for phase-contrast micro-CT scanning for two main reasons: (i) it exhibited temporal instability in a 30 s time scale, and (ii) the average power was too low.

We compared results of tomographic reconstructions of a phantom containing spheres composed of glass, polystyrene, and Nylon. These reconstructions were performed for data acquired using the 100 Hz ULX (Fig. 10a) and a commercial micro-CT scanner (Fig. 10b). Because of an insufficient number of views, streak artifacts are very prominent in both images, and the images are very noisy. We observe a satisfactory geometrical agreement (i.e. sphere size and location) between images obtained using both methods. Additionally, we compared these images with those obtained via tomographic reconstructions of projection data acquired every degree, over 360° total arc by means of a commercial micro-CT scanner (Fig. 10c). We established that a complete data set allowed low-noise high-resolution reconstruction with all three spheres visible. The tomographic data acquired with an ultrafast-laser-based x-ray source produced results in reasonable agreement with tomographic data acquired with a commercial scanner. We have detected phase-contrast fringes in the images attributable to abrupt interfaces in the object.

4. CONCLUSIONS

In order to perform a phase-contrast complete micro-CT scan using ULX, the following conditions must be met: (i) the x-ray source must be stable during the scan; (ii) focal spot size must be smaller than 10 μm for a source-to-object distance larger than 30 cm; (iii) the laser light intensity on the target must be in the range of 5×10^{17} to $5 \times 10^{19} \text{ W/cm}^2$; (iv) the ablation protection system must allow an uninterrupted scan; (v) laser light focusing on the target must remain accurate during the entire scan; (vi) a fresh surface of the target must be exposed to consecutive laser shots during the entire scan; (vii) effective detector element size must be smaller than 12 μm .

Based on the results obtained in this research project, we anticipate that the new 10 Hz, 200 TW laser with 50 W average power that is being commissioned at ALLS will allow us practical implementation of *in vivo* x-ray phase-contrast micro-CT.

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Fig. 1.

Interior of target chamber, showing rotating target holder, parabolic mirror (5 cm diameter), ring with film in center (for protection of the parabolic mirror from ablation debris from hot plasma, 15 cm diameter), and output port.

Fig. 2.
Solid Mo target (5 cm diameter) is rotated and translated precisely during the CT scan.
Craters due to laser beam impact are clearly visible.

Fig. 3.

Image of the focal spot of 100 Hz ultrafast laser beam at $\lambda_d = 400$ nm on the target, obtained at magnification $M = 45$ (~8 micrometers FWHM, color calibration to Gaussian profile).

Fig. 4.

Modulation Transfer Function (MTF) estimated using line-pair phantom images. The upper (blue) data points correspond to MTF in the horizontal direction (smaller dimension of the elliptical focal spot). The lower (red) data points correspond to MTF in the vertical direction (larger dimension of the elliptical focal spot).

Fig. 5.

X-ray spectrum acquired using Amptek spectrometer at fundamental frequency at 110 mJ, and second harmonics at 14.6 mJ. Hot-electron temperature is 13 keV. K_{α} and K_{β} lines are clearly seen on spectrum. A Be filter (100 μm) was used.

Fig. 6.
X-ray beam intensity vs. time [seconds] for 100 Hz ULX before correction for compression-grating thermal loading

Fig. 7.
X-ray beam intensity vs. time [seconds] for 100 Hz ULX after correction

Fig. 8. Recessed port installed in x-ray vacuum chamber (30 cm diameter). Goniometer will be mounted on the flat plate, adjacent to output window.

Fig. 9.

Projection image of small mouse, acquired at ALLS laboratory with 100 Hz ultrafast laser-based x-ray source with Mo target and Be filter. Source-to-object distance, $R1 = 32.4$ cm; source-to-detector distance $R2 = 9.1$ cm; $M = 1.28$. A cooled CCD camera ($24\text{ }\mu\text{m}$ pitch) was used, with a Gd_2OS_2 screen coupled via 1:1 optical taper.

Fig. 10.

Reconstructed slice of 11.6 mm diameter cylindrical PMMA experimental phantom with various spheres embedded in epoxy, composed of glass (attenuates most), polystyrene (attenuates least), and nylon (with darker less dense core): (a) image obtained at ALLS laboratory with 100Hz ultrafast laser-based x-ray source with Mo target and Be (100 μm) filter; reconstruction from 36 projections, acquired every 10°. (b) Reconstruction from 36 projections, acquired every 10°, and (c) Reconstruction from 361 projections, acquired every 1°; images obtained at SUNY Upstate Medical University with Siemens microCAT II scanner, with microfocal x-ray tube, W target, and Al (0.25 μm thick) filter. (d) Legend G: 2.4 mm diameter glass sphere; P: 3.2 mm diameter polystyrene sphere; N: 2.4 mm inch diameter nylon sphere; E: epoxy.