Corrections

PHARMACOLOGY

The authors note that an additional affiliation should be listed for Emanuela Galliera. This author’s affiliations should appear as “Department of Biomedical, Surgical and Dental Sciences, University of Milan, I-20133 Milan, Italy; and Istituto di Ricerca e Cura a Carattere Scientifico (IRCCS) Galeazzi Orthopaedic Institute, I-20161 Milan, Italy.” The corrected author and affiliation lines appear below. The online version has been corrected.


aDepartment of Discovery, Dompé SpA Research Center, 67100 L’Aquila, Italy; bDepartment of Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, 14049-900, Ribeirao Preto, Brazil; cDipartimento di Farmacia, Viale Area delle Scienze 27/A, Università degli Studi di Parma, 43121 Parma, Italy; dALTA Ricerca e Sviluppo in Biotecnologie S.r.l., 67100 L’Aquila, Italy; eDepartment of Biomedical, Surgical and Dental Sciences, University of Milan, I-20133 Milan, Italy; fIstituto di Ricerca e Cura a Carattere Scientifico (IRCCS) Galeazzi Orthopaedic Institute, I-20161 Milan, Italy; gDepartment of Medical Biotecnologies and Translational Medicine, University of Milan, I-20129 Milan, Italy; hHumanitas Clinical and Research Center, Rozzano, 20089 Milan, Italy; and iImunofarmacologia, Departamento de Bioquímica e Immunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, Brazil

www.pnas.org/cgi/doi/10.1073/pnas.1423575112

NEUROSCIENCE

The authors note that the author name Benoit Lebonté should instead appear as Benoit Labonté. The corrected author line appears below. The online version has been corrected.

Georgia E. Hodes, Madeline L. Pfau, Marylene Leboeuf, Sam A. Golden, Daniel J. Christoffel, Dana Bregman, Nicole Rebusi, Mitra Heshmati, Hossein Aleyasin, Brandon L. Warren, Benoit Labonté, Sarah Horn, Kyle A. Lapidus, Viktoria Stelzhammer, Erik H. F. Wong, Sabine Bahn, Vaishnav Krishnan, Carlos A. Bolaños-Guzman, James W. Murrough, Miriam Merad, and Scott J. Russo

www.pnas.org/cgi/doi/10.1073/pnas.1423579112

APPLIED PHYSICAL SCIENCES

The authors note that on page 19269, right column, fifth full paragraph, line 4, “200 ms” should instead appear as “200 μs.”

www.pnas.org/cgi/doi/10.1073/pnas.1423579112
Motionless phase stepping in X-ray phase contrast imaging with a compact source

Houxun Miao, Lei Chen, Eric E. Bennett, Nick M. Adamo, Andrew A. Gomella, Alexa M. DeLuca, Ajay Patel, Nicole Y. Morgan, and Han Wen

*Imaging Physics Laboratory, Biochemistry and Biophysics Center, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892; †Center for Nanoscale Science and Technology, National Institute of Standards and Technology, Gaithersburg, MD 20899; and ‡Microfabrication and Microfluidics Unit, Biomedical Engineering and Physical Science Shared Resource, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD 20892

Edited by Robert M. Stroud, University of California, San Francisco, CA, and approved October 21, 2013 (received for review June 10, 2013)

X-ray phase contrast imaging offers a way to visualize the internal structures of an object without the need to deposit significant radiation, and thereby alleviate the main concern in X-ray diagnostic imaging procedures today. Grating-based differential phase contrast imaging techniques are compatible with compact X-ray sources, which is a key requirement for the majority of clinical X-ray modalities. However, these methods are substantially limited by the need for mechanical phase stepping. We describe an electromagnetic phase-stepping method that eliminates mechanical motion, thus removing the constraints in speed, accuracy, and flexibility. The method is broadly applicable to both projection and tomosynthesis imaging modes. The transition from mechanical to electromagnetic scanning should greatly facilitate the translation of X-ray phase contrast techniques into mainstream applications.

Significance

From diagnostic exams to security screening, a major concern in X-ray imaging is the potential damage from absorbed radiation energy. Phase contrast techniques are being developed to alleviate the concern by detecting the slight refractive bending of X-rays in an object, instead of relying on the attenuation of the beam. A front runner in the development is technologies that require mechanical scanning of a grating in the X-ray beam to attain high-resolution images. This paper reports a motionless, electromagnetic scanning method in place of mechanical scanning. It lifts the constraints on speed and flexibility and reduces the complexity and cost of the technologies, all of which help bring them closer to everyday applications.


Conflict of interest statement: H.W. and H.M. are inventors of a pending patent application of the electromagnetic phase stepping method by the National Institutes of Health. This article is a PNAS Direct Submission.

Freedly available online through the PNAS open access option.

1To whom correspondence should be addressed. E-mail: han.wen@nih.gov.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1311053110/-/DCSupplemental.
its effectiveness in imaging studies of rodents and other samples in a bench top system.

Results

A generic grating-based phase contrast imaging system consists of an X-ray tube, a Talbot–Lau interferometer, and an X-ray camera as schematically illustrated in Fig. 1A. The interferometer has two amplitude gratings \(G_0\) and \(G_1\) and one phase grating \(G_2\). In our system, the grating period is 4.8 \(\mu\)m. Grating \(G_0\) splits the X-ray cone beam into a number of thin line sources whose lateral coherent lengths are greater than the grating period at the plane of \(G_1\). Each line source creates an intensity fringe pattern, i.e., fractional Talbot image \((10, 29)\) of \(G_1\), on the plane of \(G_2\). Because the fringe period is usually smaller than the detector resolution, \(G_2\) is used to produce a broader moiré pattern. When the distance between \(G_0\) and \(G_1\) is the same as that between \(G_1\) and \(G_2\), the fringe pattern from each individual line source adds up constructively on the plane of \(G_2\).

If \(G_0\) and \(G_1\) are parallel and \(G_2\) is rotated around the optical axis with respect to \(G_1\) by a small angle \(\theta\), the differential phase information is encoded into the moiré fringes on the detector plane:

\[
I \approx a_0 + a_1 \cos \left( \frac{2\pi d}{\lambda} (x\theta + \frac{\lambda d \partial \phi_b}{\pi \partial y}) + \phi_0 \right),
\]

where \(x\) and \(y\) are coordinates in the detector plane, \(a_0\) is the unmodulated baseline, \(a_1\) is the fringe amplitude, \(p\) is the grating period, \(d\) is the distance between \(G_1\) and \(G_2\), \(\lambda\) is the X-ray wavelength, and \(\phi_0\) is the background instrumental phase, which depends on the positions of the gratings. The desired information is the derivative of the X-ray phase shift caused by the sample, expressed as \(\partial \phi_b / \partial y\) in the detector plane.

The phase-stepping method calculates the differential phase image from several images with different background phases \(\phi_0\). To date, this has required physically moving one of the gratings in the \(y\) direction over multiple steps that cover a grating period, while taking an image at each step. In this process, the moiré pattern in the images visibly moves across the static projection of the object, giving rise to the intuitive term of “fringe scanning” as a synonym of phase stepping.

Recognizing that the essential requirement of fringe scanning is a relative movement between the moiré fringes and the projection image of the object, electromagnetic phase stepping achieves the condition by electromagnetically shifting the focal spot of the X-ray tube in a transverse direction across the fringe pattern. e.g., with an externally applied magnetic field that deflects the electron beam in the X-ray tube (Fig. 1A). Shifting the focal spot causes an opposite movement of the projection of the object on the detector plane, while the fringes can be made to remain stationary or move by a different amount. In our setup of the Talbot–Lau interferometer, the moiré fringes are produced by a slight rotation of the third (analyzer) grating. In this case, the fringes remain stationary despite the shifting focal spot. In the inverted embodiment where the moiré fringes are produced by rotating the first (source) grating, the movement of the fringes will exceed that of the projection image. In all cases, the images can then be digitally shifted back to realign the projections of the object. The result is that the fringes move over a stationary projection image, effectively synthesizing the phase-stepping process (Fig. 1B).

It is worth noting that shifting the focal spot of the cone beam also causes a slight change of the projection angle on the object. This change is negligible for objects that occupy a small fraction of the distance between the source and the camera. When the object thickness is a significant fraction of the source–camera distance, the reconstruction algorithm takes on the characteristics of stereoscopic imaging or tomosynthesis. A more detailed discussion is provided in SI Text.

In our imaging device, the gratings are rigidly mounted. A solenoid coil is attached to the front surface of the X-ray source (Fig. 1C) and is driven by a 25-V power supply to produce the magnetic field with full digital control and a response time of 200 ms. The resulting focal spot shift causes an opposite movement of the object projection on the camera over a stationary fringe pattern (Movie S1). A magnetic field of 2.4 mT was sufficient to shift the projected image over one period of the moiré fringes (300 \(\mu\)m). The details of the experimental setup are described in Methods.

We created an adaptive image-processing algorithm to extract the differential phase contrast, scatter (dark-field) (20, 30), and...
conventional attenuation images from the EPS data without prior knowledge of the movement of the object projection or of the moiré fringes (SI Text). The algorithm first aligns the projections of the object, generating an image stack in which the object is stationary while the moiré fringe pattern moves over it. The aligned images are then processed as fringe-scanned images by the second part of the algorithm, which measures the phase increments in the phase-stepping process with a Fourier transform method (2, 19, 20, 31). The algorithm can cope with arbitrary and spatially varying phase increments without assuming a priori knowledge, and thus is robust against potential instabilities in the alignment of various components in the imaging system.

Using the bench top system with electromagnetic phase stepping, we first imaged a reference sample containing borosilicate spheres of 5-mm diameter. The full series of images is included in Movie S1. Fig. 2 shows the processed DPC, absolute phase shift (via direct integration of the DPC), and linear intensity attenuation images. The stripe artifacts in the phase shift image result from the lack of low-spatial frequency information in the DPC signal. The linear attenuation at the sphere centers is 0.61 ± 0.02, corresponding to an effective mean X-ray energy of 35.5 ± 0.5 keV. The total phase shift at the sphere centers is estimated to be (3.2 ± 0.2) × 10^{-7} rad, from which the real part of the refractive index decrement of the borosilicate material is estimated to be δ = (3.6 ± 0.3) × 10^{-7}, slightly below the tabulated reference value of 3.7 × 10^{-7} for 35.5-keV photon energy (http://physics.nist.gov/PhysRefData/FFast/html/form.html).

As an example of a biological specimen, Fig. 3 shows the DPC and linear attenuation images of a cricket obtained by electromagnetic phase stepping. The DPC image reveals more detailed structures throughout the head, the body, and the legs of the cricket, owing to its sensitive nature to small changes in the density of the constituents.

Biomedical research routinely involves the imaging of rodents, and the photon energy of the bench top system was sufficient to penetrate the body of adult mice. As examples, under a protocol approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute, we imaged the head region of a euthanized mouse in air and the torso region of another euthanized mouse fixed in 10% (vol/vol) buffered formalin.

Fig. 4 A–D are the processed images of the head region, including the DPC, phase contrast-enhanced, linear intensity attenuation, and scatter (dark-field) linear extinction images. The DPC signal degrades into random phase noise in the areas of the metallic ear tag due to the strong attenuation of the fringes. Phase retrieval by direct integration of the DPC image can lead to substantial errors for reasons. The first relates to the inherent lack of low-frequency information in the DPC data, and the second factor is the random phase values in areas where the interference fringes are strongly suppressed, either due to attenuation (as around the metallic ear tag) or scattering. The first can be addressed by the method of Roessler et al. (32), which merges the low-spatial frequency content of the intensity attenuation with the high-spatial frequency content of the DPC data according to the scaling between the real and imaginary parts of the refractive index of the material (here, soft tissue). The second problem is circumvented by substituting the derivative of the intensity attenuation into the DPC image in areas where the DPC information is missing, again using an appropriate scaling factor. These calculations are described in some detail in Methods. The end result is a phase contrast-enhanced image (Fig. 4B). As indicated by the white arrows, numerous details emerge in the phase contrast-enhanced image that cannot be clearly seen in the intensity attenuation image.

Fig. 5 is a compilation of reconstructed images of the torso region of the mouse. The DPC image highlights weakly absorbing phase objects such as air bubbles (Fig. 5A). The phase contrast-enhanced image (Fig. 5B) contains both the phase and attenuation information. The lungs are most visible in the dark-field (scatter) image (Fig. 5C), owing to their porous microstructures. The high-density bones and the metallic ear tag are clearly visible in the intensity attenuation image (Fig. 5D).

Movies of the mouse head (Movie S2) and torso images (Movie S3) are provided for comparison between phase contrast-enhanced and linear intensity attenuation images.
Discussion
Grating interferometers used with phase stepping enable high-resolution X-ray phase contrast imaging with compact X-ray tubes. However, the stringent requirements for mechanical phase stepping have been a major challenge in bringing phase contrast into common imaging systems. The electromagnetic phase-stepping method and the adaptive processing algorithms presented here effectively replace the precision mechanical scanning system and its associated engineering challenges with a simple solenoid coil attached to the X-ray source, providing substantial advantages in speed, accuracy, and flexibility. The near instantaneous control of the focal spot could also enable real-time compensation for instrumental instabilities, including thermal drift and vibrations. The transition from mechanical to electromagnetic scanning also reduces the cost of parts and maintenance and should improve reliability, all of which may contribute to the translation of phase contrast techniques into mainstream applications. For biomedical imaging, gratings periods of a few hundred nanometers are being developed for greater phase sensitivity (33, 34). Here, electromagnetic phase stepping may become a necessity, as precise mechanical movement at the nanometric level may be difficult to achieve outside the most favorable settings.

Methods
Technical Specifications of the Imaging System. We used a tungsten-target X-ray tube (S8-80-1k; Source Ray) operating at a peak voltage of 55 kV and a current of 1 mA as the source. The focal spot of the tube was ∼50 μm. The Talbot–Lau interferometer consisted of three gratings of 4.8-μm period (Fig. 1A). The design photon energy was 27.5 keV. Gratings G₀ and G₂ were intensity-modulating (amplitude) gratings, G₁ was a π-phase shift grating. The grating lines were oriented horizontally. The amplitude gratings (Micro-works) had gold-filled trenches of 60-μm nominal depth in a polymer substrate (35, 36). They were rotated around the vertical axis by 45° to increase the effective gold height (37). The phase grating had unfilled trenches etched into a silicon substrate using the Bosch process (38), with an etch depth of 27 μm. It was also rotated by 45° to be parallel with the other gratings and resulted in an effective depth of 38 μm, or a phase shift of 1.08π at the design photon energy. The gratings were positioned at equal spacing over a total distance of 76 cm. The third grating (G₃) was slightly rotated around the optical axis to create vertical moiré intensity fringes of ∼300-μm period on the detector plane. With this arrangement, the moiré fringes are largely independent of the position of the X-ray source (see Movie S1 for a demonstration). The silicon substrates of the gratings effectively added 1.77 mm of silicon filtration to the X-ray beam. The X-ray camera (PI-SCX-4096; Princeton Instruments) had a pixel size of 30 μm and a pixel matrix of 2,048 × 2,048.

For electromagnetic phase stepping, a home-made solenoid coil of copper wire (60-mm inner diameter, 200 turns) was attached to the front surface of the X-ray tube housing (Fig. 1B). The coil was driven by a digital power supply which provided up to 2.0 A of current at up to 8 W of power. The corresponding peak magnetic field was calculated to be 3.1 mT at the location of the electron beam inside the X-ray tube. The field strength was verified experimentally with a magnetometer. The electron beam is oriented vertically. The magnetic field shifted the focal spot by up to 380 μm (with 1.5-A current applied) in the horizontal direction, perpendicular to the moiré fringes. The deflections of the focal spot at various levels of input current into the coil were measured experimentally as shown in Fig. S1.

Image Acquisition and Processing in Electromagnetic Phase Stepping. The method of electromagnetic phase stepping obtains three types of information.
1. Smith-Bindman R, et al. (2012) Use of diagnostic imaging studies and associated ra-
diation exposure for patients enrolled in large integrated health care systems, 1996–
2015–2025.
x-ray phase contrast microscopy by coherent high-energy synchrotron radiation.
11. Momose A (2003) Phase-sensitive imaging and phase tomography using X-ray inter-
156–160.
and X-ray phase tomography with Talbot interferometer and white synchrotron ra-
55–57.
26. Fowler CA (1998) Old radar types never die; they just phased array or ... 55 years of
New York).
imaging in X-ray scatter correction and phase contrast imaging using structured il-
Nat Mater 7(10):134–137.
Proc SPIE 8313:831354.
105007.
high aspect ratio SU8 submicron structures. (Translated from English). Microsyst
Microsyst Technol 14(9-11):1311–1315.
108(5):051101.