DIFFERENTIAL INTERFERENCE CONTRAST FOR X-RAY
MICROSCOPY: FABRICATION AND CHARACTERIZATION
OF TWIN ZONE PLATE OPTICS

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A novel X-ray technique for converting the phase information of weakly absorbing specimen into strong
image contrast similar to Nomarski differential interference contrast (DIC) is presented. DIC for
X-rays is accomplished by the fabrication of a novel X-ray optic (TZP) consisting of two zone plates
(ZPs) on both sides of the same substrate, laterally shifted by about one outermost zone width. The feasibility of DIC for X-rays was proven at the ID 21 X-ray microscopy beamline at the ESRF using
a full-field imaging microscope and a scanning transmission X-ray microscope, which were operated
at 4 keV photon energy. In both microscopes, we observe a tremendous contrast enhancement of up
to a factor of 25. Though first experiments were carried out at 4 keV photon energy, this X-ray DIC
 technique can be adapted to any photon energy where ZPs with appropriate parameters and imaging
performance can be designed and manufactured.

1. Introduction

X-rays were discovered serendipitously and X-ray
imaging has been a subject of intense development
ever since due to their great penetration power. Away from absorption edges, phase shift becomes increasingly dominant with increasing photon energy $E$: absorption contrast scales approximately with $E^{-1}$, whereas phase contrast scales with $E^{-3}$. Thus, different contrast techniques using the real, phase-
shifting part of the refractive index have been suggested, accentuated by the development of third generation synchrotron sources, which produce a high
quantity of coherent X-rays by virtue of their high brilliance and extend X-ray imaging to the multi-keV
and hard X-ray regime. Schmahl, Rudolph et al.\textsuperscript{1} developed Zernike phase contrast X-ray microscopy for
the water window region and demonstrated contrast enhancement of weakly absorbing specimen at the
cost of appearance diffraction halos as known from visible light microscopy. Differential phase contrast
using a configured detector was recently proven by Morrison et al.\textsuperscript{2} X-ray interferometry combined with
high spatial resolution X-ray imaging is proposed, for example by Polack, Joyeux et al.\textsuperscript{3} using a Young
type double slit as wave front divider, but is not
routinely available to drive applications. The equivalent problem for visible light microscopy was solved by Nomarski et al., who introduced differential interference contrast (DIC), which provides good image contrast for phase objects without diffraction halos or the necessity of highly coherent illumination. DIC is based on the idea of creating two images in the object plane using a specially designed objective lens. Both images are laterally separated within the diffraction-limited spatial resolution of the objective. In this case, the two images can interfere without restriction to the coherence properties of the illumination and enhance image contrast in the vicinity of optical path changes.

In this approach, we use the diffraction properties of zone plates in order to divide and recombine wave fronts for X-ray interferometry and DIC with X-rays. This paper is divided into four parts. In the first part, we will describe the non-differential case of X-ray interferometry with zone plates. In the second part, we will analyze the conditions for X-ray DIC using zone plates. The third part describes the fabrication of suited optics. In the fourth part, we present the most recent results of X-ray microscopy in DIC mode.

2. Nondifferential Case of X-Ray Interferometry

The generation of visible light interference patterns by a two-ZP setup dates back to a few decades ago.\(^4\)\(^-\)\(^6\) Figure 1 illustrates an optical arrangement for X-ray interferometry that has been exploited at the ID21 X-ray microscopy beamline of the ESRF.

Two zone plates are positioned in a distance that their back-focal spots are located in the same back-focal plane. The first ZP splits the incident wave in a zero order plane wave and higher order spherical waves. The second ZP interacts with the waves diffracted by the first ZP and creates additional sets of waves and can interfere with waves from the first ZP. In the following, \((n, m)\) denotes the interference of \(n\)th order of the first ZP with \(m\)th order of the second ZP. Assuming that higher diffraction orders can be blocked by suited apertures, only \((0, 0)\), \((1, 0)\), \((0, 1)\), \((1, 1)\) propagate in space. \((0, 0)\) is the transmitted light through the two ZPs. Taking into account that only 1. order creates a real X-ray images and that zero order contributing pairs have higher diffraction efficiency, \((1, 0)\) or \((0, 1)\) has to be used.

If the origin of the two spherical waves created by one of the pairs is in-plane-displaced (perpendicular to the optical axis), the fringe pattern consists of almost straight lines.

Figure 2 shows images of a 50-μm-thick Kapton foil edge acquired with a full-field imaging microscope operated at 4 kEV photon energy in absorption (a) and non-differential interference contrast mode (b, c, d) with different in-plane displacements of the two ZPs. The phase jump or optical path change is made visible by the curvature of the interference pattern. Of special interest is (d), where one fringe covers the entire image field. The strong contrast enhancement compared to (a) gives hint for DIC imaging.\(^7\)
It has to be emphasized that nondifferential interferometry and interference contrast imaging requires highly spatial and temporal coherent illumination.

3. Differential Interference Contrast with Zone Plates

3.1. General considerations

In difference to the nondifferential interference contrast described above, DIC makes use of a separation of the two ZPs, which is far below the diffraction-limited resolution. Thus, only one image is practically visible in the image field. DIC does not convert absolute phase values into contrast, as for example the Zernike technique, but the differentiation of the phase with respect to the direction of the two differentially displaced images. Therefore, DIC leads to contrast enhancement of optical path differences like changes in thickness or refractive index.

3.2. Coherence considerations

DIC imaging is in general less demanding in terms of coherence than other interferometric methods. The reason is that light within an Airy pattern is intrinsically coherent per definition. Differential displacement within the diffraction-limited spatial resolution means that the two Airy disks generated by the two ZPs overlap. In terms of temporal coherence, it can be derived that the monochromaticity necessary for zone plate imaging changes from $\lambda/\Delta \lambda \geq N$ for a single ZP to $\lambda/\Delta \lambda \geq N + 1$ for a twin ZP, where $N$ denotes the zone number. This difference is negligible for typical zone numbers of a few hundred. In practice, no additional precautions have to be considered compared to a single ZP in terms of spatial and temporal coherence.

3.3. Optical setup for X-ray imaging in differential interference contrast

3.3.1. Full-field imaging geometry

The X-ray beam is monochromatized by a Si crystal monochromator. A condenser ZP focuses the X-ray beam onto the sample. The twin ZP as objective lens downstream of the sample generates two differentially displaced images, which are recorded by a CCD camera.

3.3.2. Scanning transmission microscope geometry

In the scanning X-ray microscope, the twin ZP forms two differentially displaced microprobes and...
Fig. 5. Scheme of the zone plate doublet. Two zone plates, identical in geometry, are created on both sides of the same substrate. The thickness of the substrate is far below the depth of focus of the zone plates. An incident plane wave is split into a set of waves behind the first zone plate, ZP1, as described in Sec. 1. The second zone plate, ZP2, uses the 0. Diffraction order of ZP1 to form the second spot in the back-focal plane. Only relevant diffraction order pairs (+1, 0) and (0, +1) are displayed. All other diffraction orders, including the transmitted light (0, 0), can be blocked by apertures. The lateral displacement $a$ leads to two spots in the common focal plane that can be treated as origins O1 and O2 of two almost spherical waves with small shear. The interference of these waves is observed in the detector plane (cf. Ref. 10).

The specimen is raster-scanned across this probe. The interference of the two microspots appears as fringes in the far-field detector plane. With a large detector, the signal from the two microspots is integrated to a bright-field image. When a small aperture is placed in front of the detector and aligned onto the slope of one interference fringe, the setup is highly sensitive to optical path changes introduced by the specimen, which are observed as shifts of the interference fringes.

4. Fabrication and Characterization of the Twin ZP

Operation of an X-ray microscopy in DIC mode requires a modification of the optics setup demonstrated in Fig. 1 as follows:

(i) The two ZPs have the same focal length and thus the same outermost zone width $\Delta r$.
(ii) The ZPs are separated along the optical axis within the depth of focus $\text{DOF} = \pm 2\Delta r^2/\lambda$.
(iii) The ZPs are in-plane-displaced within the diffraction-limited spatial resolution $1.22 \Delta r$ (Rayleigh criterion).

As it is difficult to align two separated ZPs within their depth of focus (a few $\mu$m) and displace them by within sub-100 nm, both ZPs were generated on both sides of the same substrate as shown in Fig. 5.10

4.1. Fabrication of a twin ZP

The ZPs were generated by e-beam lithography and nanostructuring techniques.11–13 ZP patterns were generated using a Leica Cambridge electron beam lithography system EBMMF 10cs/120, at accelerating energy of 50 keV and a beam current of 0.5 nA. Electron beam field size was set at 327.68 $\mu$m. The resist used was PMMA 7% 900 K molecular weight whose thickness, per each side of the membrane, was 0.6 $\mu$m for Gold FZP. The sample was developed in 1:3 methyl-isobutyl-ketone:isopropyl-alcohol (MIBK:IPA) for 1 min at 20°C.

The final metallic features were obtained by electroplating FZP for each side, on a silicon nitride membrane 1.5 $\mu$m thick and 0.4 $\mu$m of gold. The detailed steps of the process are the following, as shown in Fig. 6: (a) alignment markers definition in both sides (front and back side) of a silicon nitride membrane; (b) exposure of the first ZP aligned to the front markers; (c) back side resist coating of the membrane and exposure of the second ZP aligned to the back markers with a desired misalignment; (d) electroplating of Au and final result: one ZP in the front side and the other in the back side of the membrane.

Fig. 6. Different steps of the generation process of the twin ZPs.
It is important to note that we developed a custom alignment procedure to minimize the misalignment errors during the multilevel fabrication process. An analysis at the scanning electron microscope (SEM) and the subsequent optical testing of the FZP indicated that the misalignment between the two ZPs on the opposite sides can be controlled under 50 nm. Figure 8 shows a SEM micrograph of a fabricated ZP on one side of the membrane.

4.2. Characterization of the twin ZPs

The diffraction efficiency, i.e. the fraction of incident light that is diffracted into the order used for imaging, was measured with the scanning X-ray microscope at the ID21 beamline of the ESRF. The first order was selected by introducing an aperture in the back-focal plane. The flux through this aperture is normalized with the flux incident on the twin ZP. A first order diffraction efficiency of 9.6% was measured at 4 keV photon energy. Taking into account the absorption of the ZPs, this is close to the theoretically expected value.

The spatial resolution was determined with the full-field imaging microscope by imaging a Siemens star test pattern in W with smallest linewidths of 100 nm. Periods of 320 nm could be resolved, which is close to the theoretical value when the Rayleigh criterion is applied.

5. Experimental Results

With these twin ZPs and the above-described setups, images of different test objects and biological samples were acquired.

Figure 9 shows X-ray images of 2-μm-thick PMMA structures with a transmission of 98.8% at first order diffraction efficiency of 9.6% was measured at 4 keV photon energy. Taking into account the absorption of the ZPs, this is close to the theoretically expected value.

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4 keV photon energy. The images were acquired with the full-field imaging microscope on the ID21 beam-line of the ESRF operated at 4 keV photon energy. The left images were recorded in absorption contrast, the right images in X-ray DIC mode. In both cases, we observe an increase in image contrast of up to a factor of 25.

Figure 10 shows an X-ray image of similar test structures in 2-μm-thick PMMA acquired with a scanning X-ray microscope. Also with type of microscope, we observe a tremendous contrast enhancement.14

6. Conclusion
We successfully introduced a DIC technique to X-ray microscopy, which can be used for full-field imaging as well as for scanning X-ray microscopy. Spatial resolutions of less than 200 nm have already been achieved. The experiments were performed at 4 keV photon energy, but the technique can be extended to any photon energy, where ZPs are available with suited imaging performance. In addition, there are plans for new optical ZPD designs in order to simplify the manufacturing process, accomplish better lateral displacement control, and avoid the anisotropic appearance (shadowing effect) of the X-DIC.

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References