Hard X-ray scanning transmission microscopy with a 2D pixel array detector

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Abstract. X-ray microscopy is an invaluable tool for the characterization of life and materials science samples on the submicron length scale. Particularly in case of hard X-rays, it combines high penetration power with elemental, chemical, and magnetic specificity. However, contrast decreases drastically at higher photon energies, and imaging modalities beyond mere absorption measurements become increasingly attractive. We discuss the use of a fully pixelated detector for hard X-ray scanning microscopy of a biological specimen in absorption, phase contrast, and dark-field imaging mode.

1. Introduction
In scanning transmission X-ray microscopy (STXM) the photon flux past the specimen is recorded in order to determine the specimen’s transmission. Spatial resolution is due to the limited extent of the tightly focused illuminating X-rays. More advanced detection schemes that offer complementary image information exist [1–8]. However, their everyday use is often hindered by the need of dedicated segmented detectors [1–5] or typically slow and noisy readout of fully pixelated 2D detectors such as charge-coupled devices (CCDs) [6–8].

Yet detector development has seen significant progress in recent years. For instance, 2D arrays of single-photon counters are now available. The PSI-developed PILATUS is such a detector [9]. A PILATUS module has ~ 100,000 pixels, each of which is $172 \times 172 \mu m^2$ in size and is operated in single-photon counting mode. The PILATUS allows frame rates beyond 100 Hz without readout noise. Its quantum efficiency in the energy range of $4–10 \text{keV}$ is > 90%.

2. Experimental
Figure 1 shows a STXM scan of a single unstained red blood cell on a Si membrane. The measurements were taken at the cSAXS beamline at the Swiss Light Source (SLS), Paul Scherrer Institut (PSI), Villigen, Switzerland. The photon energy was 6.2 keV corresponding to the wavelength $\lambda = 2\AA$. We used a Fresnel zone plate with $100 \mu m$ diameter and 100 nm finest zone width as focusing device. Data were collected “on the fly,” i.e., while the sample stage was moving along the fast horizontal axis of the scan. Acquisition time per pixel was 20 ms, followed by 8 ms dedicated for readout. Image pixels are $100 \times 100 \text{nm}^2$. The sample–detector distance was 7.19 m bridged by a flight tube, which was flushed with He gas at atmospheric pressure.
As demonstrated in the transmission image (a) and its histogram (b), there is essentially no discernible absorption contrast. The image has been median-filtered in order to decrease noise distortions. Nevertheless, statistics are not sufficient to reliably “see” the specimen. The signal-to-noise ratio (SNR) of the transmission measurement is $\sim 1 : 8$, which was determined after having located the specimen using phase contrast, discussed below, Fig. 1 (e).

In the absence of a specimen the detector is illuminated by an annulus corresponding to the light diffracted by the zone plate and the central stop. A gradient in the phase shift, $\phi$, caused by a specimen gives rise to a refraction angle $\alpha_x = \frac{\lambda}{2\pi} \frac{\partial \phi}{\partial x}$. Thus, measuring the shift of the illumination on the detector yields differential phase contrast (DPC). Figures 1 (c) and (d) show such measurements in the horizontal ($x$) and vertical ($y$) directions, respectively. No filtering or image enhancement has been performed. We note that virtually the entire image information is due to shifts by less than a single detector pixel. The statistical noise is in the order of 0.5 $\mu$rad in $x$ and 0.7 $\mu$rad in $y$, i.e., even in case of this weakly interacting specimen the SNR in DPC measurements is $\sim 30 : 1$.

Such quantitative DPC measurements can reliably be integrated [10–12]. We use the real part of Eq. 4 of Ref. 11,

$$\phi = i\mathcal{F}^{-1} \left\{ \frac{q_x \mathcal{F} \phi^x + q_y \mathcal{F} \phi^y}{q_x^2 + q_y^2} \right\},$$

and note that its imaginary part can be used to signify inconsistencies between the measured DPC signals $\phi^x$ and $\phi^y$. $q_x$, $q_y$ denote the momentum transfer, and $\mathcal{F}$ and $\mathcal{F}^{-1}$ are the forward and inverse Fourier transform operators. Zero padding the DPC signals and applying Eq. 1 yields the absolute phase shown in Fig. 1 (e), where the gray scale is in radians. Thus we can gain complete knowledge of the specimen’s complex-valued optical X-ray transmission function, a fact which allows essentially arbitrary imaging modes or “omni-microscopy” [13].
Finally, since the detector we used for these studies is fully pixelated it allows virtually arbitrary detector responses. Figure 1 (f) shows a dark-field image, where the ratio of light scattered beyond the radius of the illuminated annulus to total detected intensity is plotted. A constant background has been subtracted. This imaging mode probes small-length-scale density fluctuations, which in case of the red blood cell apparently do not reveal additional structural information. It has been remarked earlier [3, 14] that, despite the low count rates used for this measurement, it often yields excellent SNR. Here, SNR $\sim 20 : 1$.

3. Conclusions
The advent of a new generation of pixel detectors has made possible imaging measurements in unprecedented flexibility and efficiency. Here, we used such a detector for scanning X-ray transmission microscopy. Using imaging modes, such as phase contrast and dark-field imaging, we have demonstrated the viability of such hard X-ray measurements even in case of weakly absorbing biological specimens. The excellent SNRs of these modes enable the microscopist to drastically reduce the dose imparted to the specimen.

More advanced imaging modalities are possible. Instead of merely integrating the scattered light, as we have done here for our dark-field measurement, one can quantitatively analyze the small-angle X-ray scattering pattern for each point of the raster scan. Such studies, including the correlation of scattering behaviour and tissue types and properties are ongoing and will be published elsewhere. One can also use the area detector to “oversample” a coherent diffraction pattern at each point. This allows ptychographic image reconstruction methods, which improve the resolution towards the diffraction-limited case [15–17].

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References
[1] Dekkers N and de Lang H 1977 Philips Technical Review 37 1–9
[2] Hawkes P W 1978 J. Opt.-Nouv. Rev. Opt. 9 235–241
[3] Morrison G R and Browne M T 1992 Rev. Sci. Instrum. 63 611–614
[4] Feser M, Jacobsen C, Rehak P and De Geronimo G 2003 J. Phys. IV 104 529–534
[5] Feser M, Hornberger B, Jacobsen C, De Geronimo G, Rehak P, Hoff P and Struder L 2006 Nucl. Instrum. Methods Phys. Res. Sect. A-Acel. Spectrom. Dect. Assoc. Equip. 565 841–854
[6] Morrison G and Niemann B 1998 X-Ray Microscopy and Spectromicroscopy ed Thiema J, Schmahl G, Rudolph D and Umbach F (Berlin: Springer Verlag)
[7] Morrison G, Eaton W J, Barrett R and Charalambous P 2003 J. Phys. IV 104 547–550
[8] Kaulich B et al. 2006 Proc. 8th Int. Conf. X-ray Microscopy vol 7 pp 22–25
[9] Broennimann C et al. 2006 J. Synchrotron Radiat. 13 120–130
[10] Arnison M R, Larkin K G, Sheppard C J R, Smith N I and Cogswell C J 2004 J. Microsc.-Oxf. 214 7–12
[11] Kottler C, David C, Pfeiffer F and Bunk O 2007 Opt. Express 15 1175–1181
[12] de Jonge M D, Hornberger B, Holzner C, Legnini D, Paterson D, McNulty I, Jacobsen C and Vogt S 2008 Phys. Rev. Lett. 100 163902
[13] Paganin D, Gureyev T E, Mayo S C, Stevenson A W, Nesterets Y I and Wilkins S W 2004 J. Microsc.-Oxf. 214 315–327
[14] Chapman H N, Jacobsen C and Williams S 1996 Ultramicroscopy 62 191–213
[15] Rodenburg J M and Faulkner H M L 2004 Appl. Phys. Lett. 85 4795–4797
[16] Rodenburg J M, Hurst A C, Cullis A G, Dobson B R, Pfeiffer F, Bunk O, David C, Jefimovs K and Johnson I 2007 Phys. Rev. Lett. 98 034801
[17] Thibault P, Dierolf M, Menzel A, Bunk O, David C and Pfeiffer F 2008 Science 321 379–382