Desktop X-ray microscopy and microtomography

A. SASOV* & D. VAN DYCK
Visielab, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerpen, Belgium

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Summary
Recent developments in X-ray microtomography have made it possible to miniaturize a CT scanner into a versatile and cost-effective desktop system that fits into any laboratory environment. The possibilities of the technique are demonstrated for a range of applications. It is also shown how an existing scanning electron microscope with an X-ray detector can, with a specially developed attachment, be transformed into an X-ray microscope and microtomograph.

1. Introduction
During the last decades, medical CT scanners have amply proven the power and versatility of X-ray computer tomography. However, the resolution of these instruments is of the order of 1 mm so they can hardly be considered to be microscopes (Cazaux et al., 1994).

Steady progress in the reduction of the focus size of X-ray sources has made it possible to reduce the resolving power by two orders of magnitude (Cazaux et al., 1993, 1994; Anderson et al., 1994; Elliot et al., 1994). It has also proven possible to use the electron beam of a scanning electron microscope (SEM) to generate X-rays which can be used for tomographic reconstruction in the micrometre range (Sasov, 1987).

However, recently with the arrival of a new generation of commercially available compact microfocus X-ray tubes, slow scan CCD cameras and powerful microcomputers it has become possible to construct desktop X-ray microtomographs with a resolution of the order of micrometres, that can fit in any laboratory environment.

Although the principle of the technique is not new, the potential for new applications is great. Indeed, the instrument enables one to look inside the 3D microstructure of an object, under ambient conditions, without any need for specimen preparation, staining, etc. The technique can be used for biological as well as materials objects. If one disposes of an SEM, equipped with an X-ray detector for microanalysis, it is also possible to convert the SEM into an X-ray microscope–microtomograph so as to give a third dimension of information. This is done by using the SEM beam to generate the X-rays in a thin metal foil mounted on the specimen holder and by collecting the X-rays with the detector. Tomographic scanning is carried out by a mechanical rotation of the specimen holder under computer control. This solution is very inexpensive but somewhat limited by the recording time.

2. Principles of X-ray microscopy
X-rays are generated by an X-ray tube with a very small focus and an energy in the order of 10–100 keV. The conical X-ray beam traverses the object and is recorded by an X-ray-sensitive camera where it produces an enlarged radiograph of the object (Fig. 1). The magnification is simply determined by the ratio of the distances from the tube to the detector and to the object. The resolution is limited by the focus of the tube and is optimally of the order of 10 µm.

The interpretation of the image can be done in terms of attenuation of the X-rays in the object.

The attenuation of a material can be characterized by the attenuation length, which is the distance at which the X-ray intensity is reduced to 37% of its original value. Attenuation lengths can differ by a factor of 100 between the lightest and the heaviest materials. The attenuation length is roughly inversely proportional to the mass density of the material and increases with increasing X-ray energy.

For example, the attenuation lengths in graphite and lead are about 30 mm and 0.03 mm, respectively, at 20 keV and about 60 mm and 0.4 mm, respectively, at 60 keV. Hence, to study thick and heavy (high density, high atomic number) materials, one inevitably needs higher energies.

By contrast, lighter materials require a lower energy. In practice, the energy of the X-ray tube is tuned so as to yield optimal contrast. This is the case when the energy is such that the attenuation length is of the same order as the dimensions of the object. A problem can occur when one has an object that consists of a mixture of light and heavy materials.
In materials, one can only reconstruct the 3D structure without artefacts if, in each of the shadow projections, the light material is detected even if it is hidden by the ‘shadow’ of a heavy part of the object. In that case it is clear that the dynamic range of the camera (i.e., the ratio of the highest and lowest signal that can be detected) should be sufficient to detect these little variations in contrast.

If, for instance, the object is imaged by a parallel beam of X-rays with constant intensity, the contrast in the recorded image, in case of attenuation, is then

\[ I(x, y) = \exp[-\int \mu(x, y, z)dz], \]

where \((x, y)\) are the coordinates in the plane of the image, \(z\) is taken along the beam direction and \(\mu(x, y, z)\) is the linear attenuation coefficient at position \((x, y, z)\) in the object. The reciprocal of \(\mu(x, y, z)\) is the attenuation length (mean free path) at the same part in the object.

In order to interpret the image directly in terms of the attenuation coefficient (which is related to the mass density) one has to take the logarithm of (Cazaux et al., 1994), i.e.

\[ -\ln \left( \frac{I(x, y)}{I_0} \right) = \int \mu(x, y, z)dz, \]

\[ \Rightarrow \rho(x, y, z), \]

where

\[ a(x, y, z) = 1/(\lambda(x, y, z)). \]

It is thus clear that \(\rho(x, y, z)\) is the linear superposition of the local attenuation coefficient, integrated along the path of the X-ray. This quantity is called the raysum and will be the basis for the tomographic reconstruction (considered later).

### 3. X-ray microtomography

In X-ray microtomography, the object is rotated so as to obtain radiographic projections from different viewing angles. Each of the projections is recorded and fed into a computer. From these projections the 3D structure of the object can be calculated using a reconstruction algorithm. For this purpose different algorithms exist. In our system we have chosen the filtered back-projection algorithm since it is computationally simple and the calculation can be done during the recording to reduce the overall measurement time.
The desktop X-ray microscope–microtomograph developed in this work contains a microfocus tube, precision specimen manipulator, X-ray TV camera and Pentium computer processor for instrumental control and tomographical reconstruction. The X-ray microfocus tube (10 μm focal spot size) is a compact sealed tube that operates at 7 W power with energies up to 70 kV. The non-cooled X-ray CCD camera (Pulnix) is based on a sensor with on-chip integration possibilities and thermal noise compensation. The X-ray image is converted into light by a high-resolution phosphor layer coated onto a plastic film. With a typical integration time of 200–500 ms per frame this camera produces near ‘real-time’ images on the computer screen. The image formats for shadow projections and reconstructed cross-sections consist of 256 x 256, 512 x 512 or 1024 x 1024 pixels. The total acquisition time ranges from 60 s at low resolution [(256)² pixels, 100 projections] to 2 min at high resolution [(512)² pixels, 100 viewing angles]. The reconstruction time (on a Pentium 200 computer processor) ranges from 5 s per cross-section at

Fig. 3. Bone structure of the inner ear of a cat (see text).
The reconstruction is based on the convoluted back-projection algorithm (see above). The signal to noise ratio is improved by filtering with a Hamming spectral window (parameter 0.54). The maximum diameter of the object is 60 mm. A spatial resolution of up to 10 μm can be obtained. It should be noted that in the case of large objects, the resolution is determined by the number of pixels in the CCD camera and the number of viewing angles rather than by the focus of the X-ray tube. The reconstructed information can be presented as object cross-sections or as pseudo-3D images with possibilities for software ‘rotation’ and ‘slicing’. A special 3D viewer based on liquid crystal shutters can be used for stereoscopic presentation of the internal object structure as a ‘virtual reality’ image on the screen. All software runs using Windows 95. It is interactive and user-friendly and supports image and cross-section printing as half-tone images by any Windows-supported printer and image export in BMP format.

4. Microtomographic attachment for a scanning electron microscope

An existing SEM equipped with an X-ray microanalysis detector can be converted into an X-ray microscope in a rather inexpensive way. For this purpose we developed a microtomographic attachment that consists mainly of three parts: a rotatable specimen holder with a metal target for the generation of the X-rays, an SEM control board for any PC-compatible computer and a software package for X-ray imaging and 3-D microtomographic reconstruction (Fig. 2). The specimen holder contains a polished copper target which is irradiated by the SEM probe to generate a point X-ray source. The target is inclined at 45° with respect to the incident beam. The SEM probe, and hence the X-ray source, can be scanned over the target so as to move the X-ray source along a horizontal line. The X-rays which cross the object are then detected by the standard X-ray detector. The object can be rotated in steps of 2° by a precision rotation mechanism. The rotation of the object allows one to collect the X-ray information from different views, as required for the tomographic reconstruction. The SEM control board can be used with any PC (ISA-bus). It is a half-size card which plugs into the PC. The board contains two digital-to-analog converters (10 bit) for the electron beam scanning control with scan amplifiers, a video preamplifier with adjustable gain/offset/inversion, an 8-bit analog-to-digital converter of the video signal and control circuits for programmable SEM switching to external scans. The software for reconstruction and visualization is the same as for the desktop system. The total acquisition time is dependent on the electron beam current and attenuation in the object. It ranges typically from 10 to 60 min. In case of the reconstruction of one object cross-section, the calculations can be performed within the acquisition time. Shadow images and cross-sections can be displayed as grey-scale or pseudo-colour. According to the requirement of the method used for the microtomographic reconstruction the object should be completely inside the reconstruction.

Fig. 4. Mouse embryo (see text).
Fig. 5. Rough diamond with inclusions (see text).

Fig. 6. Rough diamond with ‘gletses’ (see text).
area, which limits the maximum specimen size to 1–2 mm. The microtomographic attachment was tested on different types of SEMs. 3D reconstruction could be obtained for various inorganic and biological objects with a resolution of 10 \( \mu \text{m} \).

5. Applications

The applications shown below are all made with the desktop microtomograph described in Section 3. The applications are very diverse, covering various biological as well as materials objects.

Biological applications

Figure 3 shows an X-ray projection (a), and different reconstructed cross-sections (b), of the bone structure of the inner ear of a cat. The resolution is sufficient to discriminate...
Fig. 8. Electronic capacitor (see text).

Fig. 9. Polyurethane foam (see text).
details (e.g. the cochlea) with a resolution of the order of 20 \( \mu m \).

Figure 4 shows the X-ray projection (along two viewing directions) (a), and three reconstructed cross-sections (b), of a mouse embryo.

**Diamond**

Figure 5 shows an optical image of a rough diamond (a), the X-ray projection (b), different reconstructed cross-sections from top to bottom (c), the optimized form for the diamond cut (d), and the final cut and polished diamond (e). In the reconstructed cross-sections, different inclusions can be recognized and located with a precision of about 10 \( \mu m \).

Figure 6 shows the optical image of a rough diamond (a), its X-ray projection (b), and three reconstructed cross-sections. In the cross-sections one can observe ‘gletses’. These are 2D growth defects with a thickness of the order of a few micrometres.

**Electronic components**

Figure 7(a) shows the X-ray projection of an integrated circuit with connector wires, encapsulated in plastic. Figure 7(b) shows three reconstructed cross-sections, revealing how pins are connected to the chip by means of wires of about 25 \( \mu m \) thickness.

Figure 8(a) shows the X-ray projection of a capacitor. In the reconstructed cross-sections (Fig. 8b) it can be seen how the aluminium and paper foils are wrapped within the aluminium case.

Figure 9 shows the X-ray projection (a), and several reconstructed cross-sections of polyurethane foam. From the cross-sections quantitative data about the cell structure, porosity, interconnectivity, etc. can be deduced.

**Pencil**

Figure 10 shows the X-ray projection (a), and one reconstructed cross-section (b), of a pencil. The sensitivity of the technique is sufficient to observe the different annual rings of the wooden part of the pencil.

6. **Conclusion**

It is demonstrated that desktop X-ray tomography has become a versatile technique with a large number of possible applications. The technique requires virtually no sample preparation and yields 3D data with a resolution of 10 \( \mu m \).

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**References**


