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# A vacuum-compatible wet-specimen chamber for compact X-ray microscopy

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**Key words.** Compact X-ray microscopy, wet cell, wet-specimen chamber.

**Summary**

Soft X-ray microscopy is a powerful tool for investigations of, for example, polymers or soils in their natural liquid environment. This requires a wet-specimen chamber. Compact X-ray microscopy allows the horizontal mounting of such samples, thereby reducing the influence of gravitational forces. We have developed a wet-specimen chamber for such compact X-ray microscope. The chamber is vacuum compatible, which reduces the exposure time. The vacuum sealing is achieved by a combination of mechanical sealing and sealing by bio-compatible glue. With the wet-specimen chamber the specimens can be kept in an aqueous environment in a vacuum of $10^{-4}$ mbar for several hours. Imaging of lipid droplets in water demonstrates the function of the wet-specimen chamber.

## Introduction

Soft X-ray microscopy in the water-window region ($\lambda = 2.3$–$4.4$ nm; approximately 0.3–0.5 keV) is an attractive technique for high-resolution biological imaging owing to the possibility to study thick unstained objects (Schmahl *et al.*, 1980; Kirz *et al.*, 1995). Such microscopes rely on the natural contrast between water and, for example, carbon-containing material. Current operational X-ray microscopes are based on synchrotron radiation sources which unfortunately limit the accessibility and thus applicability for, e.g., biological research. We have developed the first compact X-ray microscope with a liquid-jet laser-plasma source (Berglund *et al.*, 2000; Johansson *et al.*, 2002). Such a table-top microscope shows promise for use in the small-to-medium-scale application laboratory, thereby increasing the impact of X-ray microscopy.

The properties of X-ray microscopy make it uniquely suited for high-resolution studies, which require the sample to be in an aqueous environment. This is particularly important for, for example, polymer (Hitchcock *et al.*, 2005) and soil science (Neuhäusler *et al.*, 1999), where the structure is determined by, for example, hydration forces, but also in some cases for cell imaging (Vogt *et al.*, 2000). Such applications are presently performed at synchrotron-based microscopes at dedicated beam lines (Warwick *et al.*, 1998). Several wet-specimen chambers allowing the sample to be in its natural aqueous environment have been developed for this purpose (Medenwaldt *et al.*, 1994; Niemann *et al.*, 1994; Meyer-Ilse *et al.*, 1998; Neuhäusler *et al.*, 2000). To support the specimens silicon or silicon nitride windows or polymer films are used. The specimens are clamped in between two of these windows or films to avoid dehydration. However, in synchrotron-based microscopes the sample is mounted vertically, resulting in gravitational forces, which influence the sample before and during exposure.

Compact X-ray microscopes can be built vertically, allowing the sample to be mounted horizontally. This is an intrinsic advantage when imaging samples in a wet environment. For a compact X-ray microscope a polymer-film-based wet-specimen chamber has been developed (Rudolph *et al.*, 1994). Like the wet-specimen chambers for synchrotron-based microscopes it was designed for operation in atmospheric air pressure. However, the available X-ray flux from the compact sources is significantly lower than from the synchrotron sources. To avoid unnecessary losses and thereby achieve acceptable exposure times the number of vacuum windows and the optical path length in air should be minimized. Thus, the sample in our vertical compact microscope (Johansson *et al.*, 2002) is mounted in vacuum, which requires sealing of the wet-specimen chamber against liquid losses. This together with the small working distances of the imaging optics, typically a few hundred micrometers, makes the task to design a wet-specimen chamber demanding.

In this paper we describe the design and function of a vacuum-compatible wet-specimen chamber. The chamber is horizontally mounted and can be used for working distances

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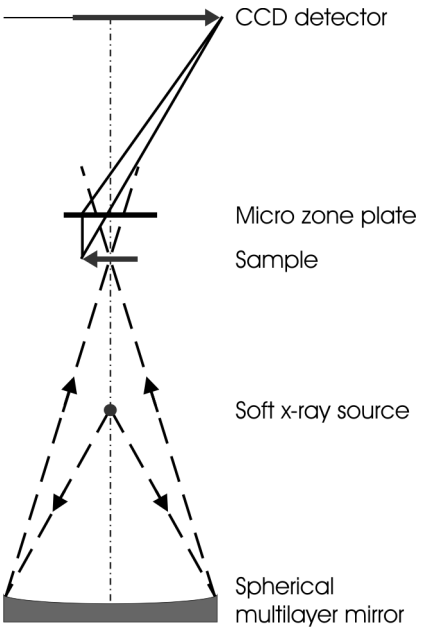


**Fig. 1.** Experimental arrangement of the compact X-ray microscope.

as small as 350 $\mu$m. The first images with the wet-specimen chamber are presented.

### Vacuum-compatible wet-specimen chamber

The compact X-ray microscope used in this experiment is described in Johansson *et al.*, 2002. Figure 1 shows its experimental arrangement. The microscope is based on a 100-Hz, negligible-debris, high-brightness methanol liquid-jet laser-plasma source (U. Vogt *et al.*, 2004). The microscope operates at the strong $\lambda = 3.37$ nm carbon line with a narrow line width ($\lambda/\Delta\lambda \approx 500$) (Wilhein *et al.*, 1997). The source is combined with a spherical normal-incidence Cr/Sc-multilayer condenser (Stollberg *et al.*, 2006). The sample is imaged by a nickel micro zone plate with an outermost zone width of 30 nm onto a back-illuminated $24 \times 24$ $\mu$m$^2$ pixel CCD camera.

The wet-specimen chamber for the compact X-ray microscope is shown in Fig. 2. The specimen is placed between two silicon nitride windows with a thickness of 100 nm. The upper window is $200 \times 200$ $\mu$m$^2$ centred on a $10 \times 10$ mm$^2$ silicon wafer; the lower one is $100 \times 100$ $\mu$m$^2$ on a $5 \times 5$ mm$^2$ silicon wafer. Together these windows have a transmission of 40% at $\lambda = 3.374$ nm. The liquid layer between the silicon nitride windows should not be thicker than a few micrometers to minimize losses (3 $\mu$m of water corresponds to 45% transmission at $\lambda = 3.374$ nm). The upper wafer is glued onto a metal shim, providing support and sealing against liquid losses in vacuum. The metal shim is only 75 $\mu$m thick, since the object distance between sample and micro zone plate is a few hundred micrometers and the silicon-wafer thickness is 200 $\mu$m. The distance between the

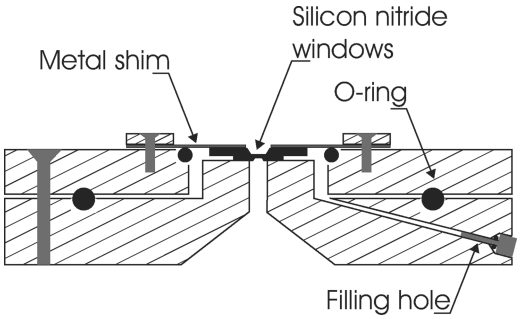


**Fig. 2.** Principle arrangement of the wet-specimen chamber. In order to show the main features the dimensions are not in scale. Only one of the two filling holes is shown.

sample and the objective micro zone plate can be as small as ~300 $\mu$m. The lower membrane is glued onto the lower part of the wet-specimen chamber. To avoid liquid losses in the vacuum environment the sealing between the two parts of the chamber is done by O-rings. To avoid the expansion of air in the wet-specimen chamber in vacuum the air is replaced by non-compressible liquid. The liquid is filled in the chamber through one filling hole, releasing the air through a second one right beside the first one. These holes are then sealed with O-rings. With the wet-specimen chamber it is possible to keep the specimen hydrated for hours in the microscope vacuum of $10^{-4}$ mbar. The typical exposure times for experiments with the wet-specimen chamber are less than 300 s.

### Results and discussion

To ensure that the thickness of the wet-cell liquid layer is small, the first experiments were performed with only water in the wet-specimen chamber. In order to obtain a thin layer of water the silicon wafers were cleaned by a weak oxygen reactive ion etch, making them hydrophilic. With this treatment a layer thickness below 2 $\mu$m could be produced. Comparative absorption measurements in the X-ray microscope were used to determine the thickness.

Figure 3 illustrates the applicability of the wet-specimen chamber for investigation of polymers and lipids in aqueous environment. Here a 10% solution of fish oil in water (Omegaven, *Fresenius Kabi*) is imaged. Figure 3 shows two samples with different droplet sizes. The low contrast in the images is caused by smaller droplets between the large droplets in the solution. The smallest droplets can not be resolved due to Brownian motion during the exposure time (300 s) needed for a sufficient signal-to-noise ratio. The effective resolution in the experiments with the wet-specimen chamber is thus limited by diffusion to about 1 $\mu$m.

In summary, we have demonstrated a wet cell that can be operated in the vacuum sample chamber of a vertical compact X-ray microscope. The major advantage of this arrangement is the reduction in exposure time for present compact microscopes and the horizontal sample mounting,

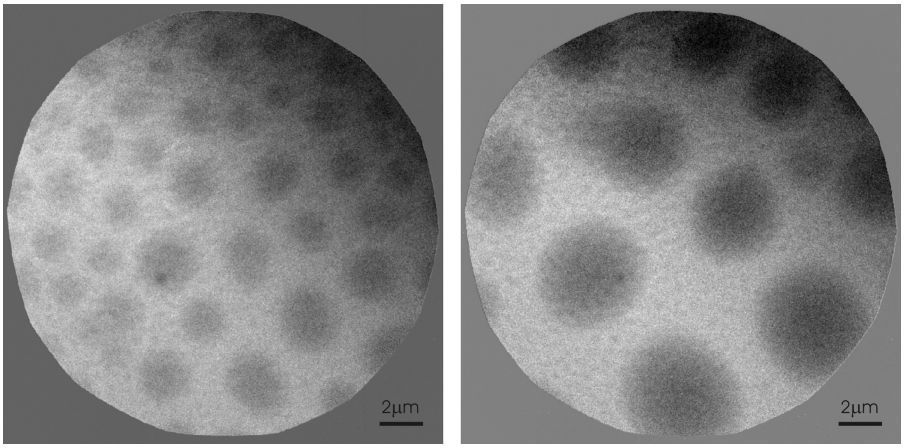


**Fig. 3.** Images of a 10% solution of oil droplets of different sizes in water taken by the compact X-ray microscope with a 1000× magnification at $\lambda = 3.374$ nm with an exposure time of 300 s.

which reduces the influence of gravity on the sample. The wet-specimen chamber has applications in studies of, for example, polymers, lipid systems, environmental science and possibly studies of cells under different environmental conditions.

The present long exposure time is both inconvenient and disturbing since Brownian motion reduces the effective resolution. We are currently planning for a laser upgrade that will reduce the exposure times by a factor of 10, thereby reducing the diffusion. Such shorter exposure time will also reduce the effect of accumulated radiation damage to the sample.

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