Soft X-ray Spectromicroscopy
on Hydrated Colloidal and
Environmental Science Samples

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Ulrich Neuhäusler
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Referent: Prof. Dr. G. Schmahl

Korreferent: Prof. Dr. W. Schröter

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Chapter 1

Introduction and Outline

For investigations in science and technology, a large variety of microscopy techniques is available, e.g. electron microscopy, visible light microscopy, atomic force microscopy. These methods often can be subdivided into several modes of operation: Visible light microscopy for instance can be done in bright field, dark field, phase contrast and many other more sophisticated ways.

While images tell about the general spatial distribution, microanalysis goes beyond this to provide more detailed information (e.g. chemical or orientational properties) about a sample. Table 1.1 compares different microanalysis techniques and the particle or radiation beam used for probing the sample as well as the kind of signal being used for data acquisition.

The physical effects used for obtaining information determine their applicability. Every technique has specific advantages and different capabilities.

Soft X-ray microscopes offer especially favorable contrast mechanisms for studying colloidal systems. Unlike in electron microscopy, samples can easily be examined in a hydrated state at atmospheric pressure without any pretreatment like chemically fixing or staining and with a currently approximately five to tenfold higher spatial resolution than achievable in visible light microscopy. The term 'colloidal' covers systems with particle sizes ranging between 1 nm and 1 μm (see figure 1.1), so many systems
Table 1.1: Comparison of different microanalysis techniques (based on [26]).

<table>
<thead>
<tr>
<th>Incident beam</th>
<th>Detected signal</th>
<th>Technique</th>
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<tr>
<td>Electron</td>
<td>Electron</td>
<td>Transmission Electron Microscopy (TEM)</td>
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<td>Electron</td>
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<td>Electron energy loss spectroscopy (EELS)</td>
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<td>Electron</td>
<td>Electron</td>
<td>Auger-electron spectroscopy</td>
</tr>
<tr>
<td>Electron</td>
<td>Photon</td>
<td>X-ray emission spectroscopy</td>
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<td>Photon</td>
<td>Photon</td>
<td>X-ray microscopy (TXM, STXM)</td>
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<td>Photon</td>
<td>Photon</td>
<td>X-ray diffraction</td>
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<td>Photon</td>
<td>Photon</td>
<td>X-ray absorption spectroscopy (XAS)</td>
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<tr>
<td>Photon</td>
<td>Electron</td>
<td>X-ray fluorescence spectroscopy</td>
</tr>
<tr>
<td>Photon</td>
<td>Electron</td>
<td>Ultraviolet photoelectron spectroscopy</td>
</tr>
<tr>
<td>Proton</td>
<td>Photon</td>
<td>Proton Induced X-ray emission (PIXE)</td>
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which are not accessible by light microscopy techniques can be studied in an X-ray microscope.

The energy range of the so called “water window” [145] between the oxygen and carbon K absorption edges (543 eV/2.3 nm and 284 eV/4.4 nm) is of particular interest for studies on hydrated samples, for within this energy range, water is highly transparent for X rays compared to other substances. The “water window” contrast arises from a difference in absorptivity for water and carbonaceous (or other dense inorganic) material (as it can be seen from the linear absorption coefficients shown for water and decane in figure 1.2).

In addition, X-ray absorption edges (in the “water window” energy range e.g. carbon, oxygen, nitrogen, calcium, potassium) can be used to visualize and map compounds containing these elements versus other
compounds. In a small energy range near X-ray absorption edges, the transmission properties change rather drastically for the edge element, while the transmission remains almost constant for all other elements; this can be seen in Figure 1.2, which shows absorption spectra of decane and water. The carbon-K-absorption edge shows clearly for decane at 284 eV and the oxygen-K-absorption edge for water at 543 eV. What is not indicated in Figure 1.2 is the presence of X-ray absorption near-edge structure, or XANES resonances. These resonances are the result of electronic transitions from core to outlying states such as unoccupied molecular orbitals. These resonance-like transitions are very sensitive to different kinds of chemical bonds and can also be exploited in X-ray spectromicroscopy experiments (see e.g. figure 5.6).

The pure existence of these contrast mechanisms as described above alone does not guarantee that experiments actually can be carried out. Figure 1.3 shows the number of photons required to detect either 50 nm of protein or 50 nm of a kaolinite clay mineral in a 10 μm ice or water matrix.
CHAPTER 1. INTRODUCTION AND OUTLINE

Figure 1.2: Linear absorption coefficients for water and decane for the soft X-ray energy range, calculated by using the atomic scattering factors as tabulated in [30]. Oxygen/carbon-K absorption edges clearly show for water/decane. This calculation assumes atoms that are independent of each other and have no molecular bonds. Therefore, near edge absorption resonances that are caused by transitions to molecular orbitals are ignored.

In the “water window” energy range, approximately $10^5$ photons are required for detecting these structures with a reasonable image quality (i.e. with a signal-to-noise ratio of 5). Electron storage rings are X-ray sources that can provide the high brightness needed for high resolution X-ray microscopy. Employing Fresnel zone plates (described in more detail in section 2.1) as X-ray optics actually allow to resolve structures as small as 30 nm.

An overview on the current status of microscopy with soft X rays can be found in review papers by Schmahl et al. [105,106], Kirz et al. [56] and, with more focus on spectromicroscopy, by Ade [4].

The outline of this thesis is as follows:

In section 2, the experimental side of X-ray microscopy, the physics of the hardware and the preparation of fully hydrated samples in a wet specimen chamber, designed and built for the experiments presented is described. The Göttingen transmission X-ray microscope (TXM) at
Figure 1.3: Calculations (from [46]) of the number of detected photons required per resolution element to see either 50 nm of protein in 10 µm ice (top) or 50 nm of a kaolinite clay mineral in 10 µm water (bottom) with a signal-to-noise ratio (SNR) of 5. In both cases a minimum exposure for viewing hydrated material is obtained at a photon energy of about 500 eV, within the “water window”. Good intrinsic phase contrast can also be obtained at photon energies of about 1–10 keV as well [99].
BESSY and the Stony Brook scanning transmission X-ray microscope (STXM) at the NSLS are mentioned as two types of X-ray microscopes operating in the “water window” energy range. Their different potential is put into perspective and a motivation for the experiments is given.

Section 3 deals with the physics of the sample studied in an X-ray microscope and its interaction with X rays. An introduction into X-ray optical interactions is given and model calculations on how to best detect elements near X-ray absorption edges are presented. The basics of near edge absorption spectroscopy and the use of absorption resonances for quantitative elemental mapping are described.

The following chapters show various applications of spectromicroscopy studies on colloidal systems. Besides demonstrating the capability of STXM to examine fully hydrated samples at room temperature, information about these systems was obtained that is not accessible by any other means:

In chapter 4, the interactions between clay mineral suspensions/dispersions and organics are studied. Kaolinite-polyacrylamide aggregates serve as a model system for the flocculation of clay minerals in soils by organics. This is of interest for agronomy, where polyacrylamide is used in irrigation plants as a flocculation agent in order to prevent erosion. Furthermore, polyethyleneglycol-montmorillonite aggregates were studied as systems of current research in colloid chemistry.

Chapter 5 shows two examples of emulsions, investigated in situ, milk and oil-water-emulsions, stabilized with solid colloids. Experiments have been performed both near the calcium-\(L\) and carbon-\(K\) absorption edge and the stabilization mechanisms in these systems could be visualized directly.

Applications already shown in previous sections have an environmental impact, e.g. substituting classical surfactant-based emulsifiers by solid colloids in emulsions or studies on preventing erosion using polyacrylamide. Two examples of studies directly related to environmental issues are presented in chapter 6: Studies on mapping carbonaceous material in contaminated estuarine sediment from NY harbor as well as XANES-
spectroscopy on humic substances were carried out.

The last chapter demonstrates the limitations of the spectromicroscopy technique with soft X rays as they were experienced in the experiments, concludes and gives a perspective on potential future studies.
Chapter 2

X-ray Microscopy

Over the last years, an increasing number of different X-ray microscopes has become available or is about to become available at synchrotron radiation sources all over the world. Most recently, bright synchrotron sources for harder X rays in the energy range of several keV have been established, like e.g., the European Synchrotron Radiation Facility (ESRF) or the Advanced Photon Source (APS) allowing X-ray microscopy experiments beyond the “water window” energy range in phase contrast or X-ray fluorescence detection mode.

In the work presented here, we focus on X-ray microscopy experiments using soft X rays in amplitude contrast, taking advantage of the “water window” contrast for hydrated samples as well as the element specific contrast near X-ray absorption edges. Therefore, two X-ray microscopes operating in this energy range, the Göttingen transmission X-ray microscope (TXM) at electron storage ring BESSY and the Stony Brook scanning transmission X-ray microscope (STXM) at the NSLS are described. To start with, an introduction to Fresnel zone plates as high resolution X-ray optics (used in the TXM as well as the STXM) is given. Both microscopes were the first prototypes in their class and are now operating for almost two decades with evolution and improvements ever since. For this reason, a discussion of their different potential can not be more than a snapshot of the current status. However, putting this into perspective intends to give a motivation for the experiments that were done.
2.1 Fresnel zone plates as high resolution X-ray optics

High resolution optics for soft X rays are not as easy to find and to fabricate as for other kinds of electromagnetic radiation as for instance visible light. Since the refraction index for all materials is close to 1, refractive lenses would need to have a large surface curvature in order to refract significantly. On the other hand, these lenses would need to be thin because of the strong absorption soft X rays suffer when passing through matter. Example calculations [102] show that the numerical aperture (i.e. in an approximation for small angles the ratio of the radius of the lens to its focal length) would be very small and therefore, the spatial resolution achievable would be rather bad (in the range of microns).

Reflective optics, operated under small incident angles to their surface normal, have a very small reflectivity ($\approx 10^{-4} - 10^{-6}$). In principle, this can be improved by fabricating multi-layer mirrors, where the amplitude

![Image](image_url)

Figure 2.1: Geometry of a Fresnel zone plate.
of reflections at the interface of many layers adds up by constructive interference.
Since real part of the refractive index is a little smaller than 1, total reflection occurs for grazing incidence of the X-ray beam [145]. However, it is quite challenging to find techniques to fabricate these mirrors (both for normal and grazing incidence) in the quality necessary for good optical performance for high resolution X-ray microscopy.

Employing Fresnel zone plates turned out to be an increasingly successful optical concept for high resolution X-ray microscopy, ever since they were first used for this purpose in 1976 at DESY [86]. Fresnel zone plates are circular diffraction gratings with radially decreasing line spacing [120]. A zone plate has focusing properties, if the optical ways of rays through neighboring zones differ in multiples $m$ of the wavelength of the incident wave, so radiation coming from these two zones can interfere constructively in the focus point of the $m^{th}$ diffraction order. For so called "amplitude zone plates," the radiation hitting the grating structure material is blocked out while for so called "phase zone plates," the radiation suffers a phase shift when going through the material of (in an ideal case) $\pi$ and makes additional contributions to the intensity in focus compared to amplitude zone plates. Zone plates that are 'optically thin' (definition see e.g. [64,66]) have as amplitude zone plates a theoretical first order diffraction efficiency of $10.1 \% \ (1/\pi^2)$. Phase zone plates diffract in an ideal case $40.5 \% \ (4/\pi^2)$ of the incident radiation in their first order focus [54]. Note, that these numbers do not take into account the absorption caused by the thin foils usually needed as support for the zone plates.

For the exact derivation of the zone plate equations, considerations of imaging a point on the optical axis by a zone plate must be made (see e.g. [101,103]). Here, the assumption of a parallel incident wavefront is made (see figure 2.2), which provides equations that are sufficiently accurate for estimates made for the work shown in this thesis.
Figure 2.2: Rays through part of a zone plate, illustrating the derivation of the zone plate equations. Two corresponding rays of two transparent zones through the points \(a_n\) and \(a_{n-2}\) or \(r_n\) and \(r_{n-2}\) respectively have an optical way difference to the focus of \(\lambda\). This schematic shows the case of an amplitude zone plate, i.e. rays hitting the zone plate structures are blocked out.

\[
f^2 + r_n^2 = \left(f + \frac{n\lambda}{2}\right)^2 = f^2 + n f \lambda + \left(\frac{n\lambda}{2}\right)^2
\]

\[
\Rightarrow r_n^2 = n f \lambda + \left(\frac{n\lambda}{2}\right)^2
\]

For small numbers of zones, the condition \((n \cdot \lambda) \ll f\) is met, so the square term can be neglected:

\[
r_n^2 \approx n f \lambda \quad \Rightarrow \quad f = \frac{r_n^2}{n\lambda}
\]

The position of the zones \(r_n\) is now known, an expression for \(dr_n\) follows from looking at the area of a pair of the \(n^{th}\) and \((n - 1)^{th}\) zone:

\[
A_p = 2 (\pi r_n^2 - \pi r_{n-1}^2) = 2 \pi (n f \lambda - (n - 1) f \lambda) = 2 \pi f \lambda
\]

If the mark-to-space-ratio is 1:1, all zones have the same area:

\[
A_z = \pi f \lambda = 2 \pi r_n \, dr_n \quad \Rightarrow \quad dr_n = \frac{f \lambda}{2 r_n}
\]

Combining equations 2.3 and 2.5 finally results in:
The equations were derived for the first diffraction order but are also valid for higher orders when substituting in the equations $\lambda$ by $m\lambda$.

According to the Rayleigh criterion, the smallest distance $\delta$ of two points that can be resolved using a zone plate with small numerical aperture is:

$$\delta = 1.22 \frac{\lambda f}{D}$$ (2.7)

Substituting $D = 2r_n$, using an expression for $f$ from equation 2.3 and expressing $r_n$ and $n$ by $dr_n$ (equation 2.6) yields for the resolution:

$$\delta = 1.22 \frac{dr_n}{m}$$ (2.8)

Using the first diffraction order ($m=1$), object structures can be made visible that are approximately the same size as the width of the outermost zone of the zone plate used. Equation 2.8 shows that higher resolution can be achieved by using higher diffraction orders $m$ for imaging.

Developing and improving the technology for fabricating Fresnel zone plates has become an own field of research in groups operating X-ray microscopes with zone plate optics. Currently, outermost zone widths as small as 25 nm can be fabricated with good efficiency [92].

The zone plates used with the Göttingen TXM at BESSY are fabricated in house; this is true both for high resolution micro zone plates [93, 138] as well as for condenser zone plates [40, 41]). The Göttingen condenser zone plate KZP7 is also used in TXMs at the Institute for Storage Ring Facilities (ISA) in Aarhus, Denmark and at the Advanced Light Source (ALS), Berkeley, CA, U.S.A.

For acquisition of the data presented in this thesis, the zone plates used were fabricated by S. Spector [121, 122] and C. Jacobeen (SUNY Stony Brook) in collaboration with D. Tennant (Lucent Technologies Bell Laboratories, Holmdel, NJ, U.S.A.).
2.2 The Göttingen transmission X-ray microscope (TXM) at BESSY

The transmission X-ray microscope built and operated by the institute for X-ray physics (Universität Göttingen) is located at a bending magnet beamline at the 800 MeV electron storage ring BESSY in Berlin.

The beamline itself contains no optical elements except a chromium filter (a 50 nm chromium layer on a 150 nm silicon or 300 nm polyimide foil) that is supposed to suppress higher energy light by employing the high absorptivity for energies beyond the chromium-$L_{III}$-absorption edge at 574 eV. More recently, a double mirror system to suppress higher energy photons by making use of the reflectivity dependence from the photon energy has been installed.

A large condenser zone plate, matched in its diameter of 9 mm to collect the synchrotron radiation coming down the bending magnet beamline, serves two purposes. On the one hand, it collects all the X-rays

![Diagram](image)

Figure 2.3: Schematic of the optical setup of the Göttingen transmission X-ray microscope at electron storage ring BESSY.
illuminating its area and focuses them to a spot of several microns in diameter, thus increasing the photon flux density orders of magnitude. On the other hand, the condenser zone plate acts as a linear monochromator. Because zone plate optics have a very strong chromatic aberration \((f \propto 1/\lambda)\), imaging is only possible if monochromatic radiation is used. Assuming a rectangular monochromaticity profile, it can be shown that the degree of monochromaticity has to be in the same range as the number of zones of the zone plate employed for high resolution imaging [129]. For a real monochromaticity spectrum with Gaussian flanks (as shown in [75] and described below), the modulation transfer function (MTF) worsens considerably, as recently shown by D. Weiss [137].

This implies that not only for spectromicroscopy applications, but also for high resolution imaging, good energy resolution is necessary. By placing a pinhole between sample and condenser zone plate, a limited bandwidth of the radiation can be cut out from the broad spectrum of radiation coming from the bending magnet. A simple geometric consideration [83] yields the monochromaticity for a zone plate monochromator as \(\lambda/\Delta\lambda = D/2d\) (\(D\): condenser zone plate diameter, \(d\): pinhole diameter). However, this geometric model assumes a point X-ray source.

Raytracing calculations for the realistic case of an extended BESSY source show that depending on the pinhole diameter, the monochromaticity \(\lambda/\Delta\lambda\) ranges between 195 for a 20 \(\mu m\) pinhole up to 420 for a 10 \(\mu m\) pinhole [75]. Using smaller pinholes increases the monochromaticity only as long as they are not smaller than the demagnified image of the synchrotron source. For photon energies in the “water window” of 400/543 eV (near the nitrogen/oxygen-\(K\)-absorption edge), the source is demagnified 95/75 times to a size of 7.5/9.5 \(\mu m\) (full-width half maximum, Gaussian shape). Therefore, it only makes sense to use pinholes down to 10 \(\mu m\) diameter. It can also be shown that larger pinholes can be used and do not worsen the monochromaticity, as long as only object regions are taken into account that would also be illuminated by a smaller pinhole; the monochromaticity is a function of distance from the optical axis in the object plane.
In a TXM with condenser zone plate monochromator, the monochromaticity would be as high as 900 if source size effects would not have to be taken into account, i.e. if the X-ray source could be regarded as a point source. In this case, the aberrations of the condenser zone plate KZP7 ($\approx 1 – 2 \mu$m) would be the limiting factor.

In the real setup as it can be seen in figure 2.3, two pinholes are used and the sample is mounted in a small, 200 $\mu$m wide air gap in-between these two pinholes. Downstream of the sample, in beam direction, a high resolution micro zone plate with an outermost zone width currently between 25 and 40 nm images the sample onto a backside thinned, cooled CCD camera [142].

Requirements of spatial coherence do not have to be met in the hardware setup of the TXM, since the entire image is taken at once and every resolution element of the sample, illuminated incoherently by the condenser zone plate, can be regarded to be a coherent source, imaged by the micro zone plate.

The major research projects currently done with the TXM at BESSY are cryo X-ray microscopy on biological samples [112, 114] also in tomography mode [139, 140], studies on magnetic domains using magnetic circular dichroism [32] and X-ray microscopy on colloid [133] and soil science specimens [63, 89]. These studies are done both in amplitude and phase contrast [107].

2.3 The Stony Brook scanning transmission X-ray microscope (STXM) at the NSLS

Unlike the TXM at BESSY, the X-IA STXM requires a more sophisticated beamline with several optical elements, ensuring high temporal coherence (monochromaticity), which is crucial for spectromicroscopy. In a scanning transmission X-ray microscope, spatial coherence is needed in order to get the highest possible spatial resolution, limited only by diffraction. Figure 2.4 shows a schematic overview of the setup (beamline and
2.1 The X-1A undulator beamline

The X1 undulator (35 periods of SmCo$_5$ hybrid magnets with a period length $\lambda_0$ of 8 cm) at the National Synchrotron Light Source is a tunable bright source of soft X rays for photon energies between 200 and 800 eV. A calculated spectrum for an undulator gap of 39.5 mm (typical for experiments near the carbon-$K$-edge) is shown in figure 2.5. In this energy range, the X1 undulator source emits radiation with a phase space volume $\Phi$ ($\Phi = d \cdot \Theta$, where $d$: source diameter and $\Theta$: emittance angle) of around 100 $\lambda$. Since accepting a phase space larger than 1 $\lambda$ increases only the total flux, but not the coherent flux (see below), nothing is lost in sharing 65% of the undulator beam with another beamline (X-1B) by deflecting part of the beam towards the two branchlines (called “inboard” and “outboard”) of X-1A using a plane mirror. The STXM used for the experiments operates currently at the so-called “outboard branch” of the two branchlines at X-1A.

The toroidal mirror (made of gold-coated silicon, M1O in figure 2.6)
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Figure 2.5: X-1 undulator spectrum for a 39.5 mm gap setting at 2.584 GeV electron energy in the storage ring [47]. This undulator setting is typical for experiments near the carbon-K-\(\alpha\)-absorption edge and allows XANES spectroscopy across the edge in an energy range between 275 and 300 eV without having to change the undulator gap.

![Graph showing X-1 undulator spectrum with energy range and gap setting details.](image)

Figure 2.6: Illustration (from [144]) on the optical elements of the X-1A outboard branch and their focusing properties, viewed from the top (top image) and from the side (bottom image). U: undulator, M10: toroidal mirror, GO: monochromator grating, ENSO: entrance slit, EXSO: exit slit, ZP: zone plate.

![Diagram showing the optical elements of the X-1A outboard branch.](image)
Figure 2.7: Energy resolving power (monochromaticity) available at the outboard branch of the X-1A beamline for different entrance slit sizes, as indicated in the plot (after [144]). For the experiments shown in this thesis, entrance slit sizes of typically 70 to 120 µm have been used.

with a horizontal radius of 71.3 m and a vertical radius of 0.504 m deflects and focusses onto the monochromator entrance slit (ENSO) in the horizontal and onto the exit slits (EXSO) in the vertical plane (as illustrated by the focussing diagram in figure 2.6).

The monochromator consists of a spherical grating (GO) with 900 lines per mm and a radius of 46 m, dispersing linearly in λ and refocussing in the horizontal. The monochromaticity is determined by the entrance slit size (ENSO). Monochromaticities over the “water window” wavelength range for different slit sizes are shown in figure 2.7. Good energy resolution is important for resolving fine structure resonances near absorption edges as discussed in section 2.4.1.

The exit slit (in real life: two slits, one oriented horizontally and one
vertically) determine the spatial coherence (as discussed in more detail below). The degree of coherence is not affected by the control of the energy resolution by changing the width of the entrance slit, because the image of the entrance slit on the exit slit is larger than the exit slit size, so the spatial coherence condition is met for all entrance slit sizes. This makes it possible to trade off energy resolution versus flux (for some experiments flux is more important than energy resolution) at no cost of spatial coherence [143,144].

2.3.2 The Stony Brook scanning transmission X-ray microscope

[Experimental setup] After having passed all beamline optics (as described in the last section), the beam reaches the actual microscope setup [45,48]. The X rays exit the beamline vacuum (10^-9 Torr) through a 100 nm thin, 250 x 250 μm sized silicon nitride window and illuminate the zone plate.

The order sorting aperture (OSA), located between zone plate and sample ensures that unwanted diffraction orders (except for the first order used for imaging) are blocked out. Figure 2.12 illustrates the geometry. In the experiments, the OSA was chosen to be 70 μm in diameter, matched to the 75–80 μm center stop of the 160 μm diameter zone plate used. The OSA must be precisely aligned to the center stop of the zone plate, so that no 0th order light can reach the sample. The zone plate–OSA distance has to be chosen in a way that the first order 'cone' of X rays is not obstructed by the OSA.

The sample can be moved in x- and y-direction (perpendicular to the beam) using either stepper motors (step sizes typically between 1 and 10 μm) for coarse, large image fields (up to several mm), or piezoelectric actuators (with a capacitance feedback system) for high resolution scans of smaller fields (10 to 50 μm). The dwell time per image pixel ranges between 1 msec for quick overview scans and 20 msec for images with good photon statistics. Zone plate and order sorting aperture (OSA) can
In order to detect a certain spatial frequency \( f \) in a sample structure, the image must be sampled with twice the frequency according to the sampling theorem (see e.g. [62]) to detect this frequency \( f \). In piezo scans in STXM, this would require to scan with a step size of half the expected spatial resolution. In the experiments, it was mostly chosen to scan with step sizes between 50 and 100 nm, which is similar to the spatial resolution. Oversampling with finer steps (up to twice the spatial resolution available) make the images look blurred and look subjectively worse than pictures with larger step size. Operating the microscope in the 100 to 200 nm spatial resolution range, and not at the extreme limit of its spatial resolution yields images with good contrast due to good amplitude transfer as described by the modulation transfer function. Furthermore, very often, large structures (aggregates) want to be imaged at once and the limit of the number of pixels in an image and time constraints make
it necessary to choose larger step sizes.

The zone plate and the sample are in air at atmospheric pressure, therefore allowing quick sample change and easy access to the sample, and, more important, experiments on hydrated samples. The distance between exit window and counter might be as large as 1 cm. Since the 1/e-absorption length for air ranges between 1.6 and 3.6 mm for radiation in the wavelength range between 4.4 and 3.1 nm, a constant Helium flow in the detector and sample region ensures higher transmission, because the 1/e-absorption length for He is around 20–25 mm in this wavelength range. However, this He/air mixture has a very large gradient in transmission, if the concentration of He varies only slightly (e.g. by turbulences). The STXM must be covered carefully with a plastic foil to provide constant, undisturbed conditions. The radiation transmitted through the sample is detected by a single-wire or improved multi-wire proportional counter [31], counting with low intrinsic noise and linearly up to 1 MHz count rate. Approaches are currently made towards using solid-state detectors.

The beamline and the microscope are under control of a VAX 4000/90 workstation, controlling the motor and energy scans and the measured data accordingly through a CAMAC electronic interface. Beamline slit motors and other optical elements are command-line controlled while data acquisition of the sample (image scans and energy scans) is performed by a more user-friendly graphical interface.

The major work done with the STXM at X-1A includes XANES-spectroscopy on polymers [5, 6], on biological samples as sperm [148] or amino acids [14], and mapping different calcium compounds in bone [18]. Extraterrestrial organic materials as e.g. interplanetary dust particles or carbon in meteorites are studied [33, 34] as well as organic geochemistry and fuel chemistry questions are addressed using STXM [16, 21].

Temporal coherence (energy resolving power) is important in spectroscopy and will be discussed a little more in the next subsection. Spatial coherence is needed to achieve diffraction-limited resolution and
will be discussed in the following:

**Spatial coherence considerations** A source of light with a certain spatial extension $\sigma$ is spatially coherent for an area of observation $d$ in a distance $r$ to the source (see figure 2.9 for an illustration), if the phase condition amongst all light emitting regions can be regarded constant for the area $d$ over the whole source size $\sigma$. For this consideration, a circular source as well as a circular area of observation are assumed. This condition is true for point sources or for extended sources that can be regarded as point sources for large distances. Furthermore, large incoherent sources are coherent, if the region of observation is chosen to be small enough. From the earth for instance, a circular area of 19 $\mu$m in diameter sees the sun as a coherent source, i.e. for this small area it is not possible to tell the difference between a point source and the spatially extended sun [102]. Therefore, spatial coherence is only a matter of source size, distance of the source to the observer and size of the area of observation. Furthermore, the spatially coherent illuminated region scales linearly with $\lambda$, making the coherently illuminated region much smaller for X rays than for example for visible light when comparing the same geometry of light source size and distance to the observer.

Figure 2.9 illustrates the geometry for spatial coherence considerations: Two source points $S_a$ and $S_b$ of a light source are radiating at a wavelength $\lambda_0$ with statistically distributed phases. The contribution to the electrical field vector of these two source points, observed in two

![Figure 2.9: Schematic of the geometry for spatial coherence considerations.](image-url)
points P1 and P2 in a distance r from the source, can be written as:

\[ E_1 = E_{1a} + E_{1b} \quad (I_1 = E_1 \cdot E_1^*) \]  \hspace{1cm} (2.9)

\[ E_2 = E_{2a} + E_{2b} \quad (I_2 = E_2 \cdot E_2^*) \]  \hspace{1cm} (2.10)

The complex property ’degree of coherence’ is then defined as:

\[ \gamma_{12} = \frac{\langle E_1 \cdot E_2^* \rangle}{\sqrt{I_1 \cdot I_2}} \]  \hspace{1cm} (2.11)

Generalizing the problem to an area source (not just two rays) results in:

\[ |\gamma_{12}| = \frac{2J_1(v)}{v} \quad \text{where} \quad v = \frac{2\pi}{\lambda_0} \alpha d \]  \hspace{1cm} (2.12)

where \( J_1(v) \) is a Bessel function.

If one were to determine the degree of coherence \( |\gamma_{12}| \) of a light source, it would be done by setting up an interference experiment. \( |\gamma_{12}| \) then can be measured experimentally as the contrast between the maxima and minima in an interference pattern generated by a light source with the degree of coherence of \( |\gamma_{12}| \).

There are the following borderline cases for \( |\gamma_{12}| \):

- \( |\gamma_{12}| = 1 \) for \( v = 0 \): completely spatially coherent, i.e. \( \alpha \) and/or \( d \) converge to 0
- \( |\gamma_{12}| = 0 \) (incoherent) if \( v = 3.83 \) (first zero of \( |\gamma_{12}| \)), i.e. \( d = \frac{0.61 \cdot \lambda_0}{\alpha} \)
- \( v = 1 \) is a reasonable estimate for good coherence with

\[ 0.88 \leq |\gamma_{12}| \leq 1 \Rightarrow d = \frac{0.16 \cdot \lambda_0}{\alpha} \]  \hspace{1cm} (2.13)

For spatial coherence considerations at the X-1A beamline, the exit slit size can be regarded to be the effective source size. Equation 2.13 yields that the exit slit size needed to illuminate a 160 \( \mu \)m diameter zone plate placed 1.5 meter downstream of the exit slit coherently would be as small as 13 \( \mu \)m for a wavelength of 4.3 nm. Regardless, if the mechanical properties allow this setting of the slit, this slit setting is too
small regarding the X-ray flux for photon energies near the carbon edge, where exit slits of around 50 to 70 \( \mu \text{m} \) have been used. Therefore, the best case spatial resolution for a 45 nm outermost zone width zone plate (as used in the experiments), namely 55 nm, can only be the lowest limit that can not be achieved for larger slit sizes.

Another approach to understand the effect of large exit slit sizes worsening the spatial resolution is looking at the focus formed by the zone plate as an diffraction limited Airy pattern (point spread function of the zone plate), convolved with the image of the source as imaged by the zone plate. Only for a completely coherent point source (\( \delta \)-function), the convolved airy pattern is still a diffraction-limited Airy pattern when convolved, i.e. multiplied with 1 in Fourier space.

2.4 Spectromicroscopy with soft X rays

2.4.1 Characteristics of STXM versus TXM

Differential imaging at an X-ray absorption edge or, one step further, spectromicroscopy can not be done as easily in a TXM as in a STXM. The TXM was designed and built with a focus on quick (exposure times in the range of seconds instead of minutes as in a STXM), high resolution imaging of hydrated samples at a fixed wavelength. Most of the reasons against this can be overcome in a new design of a TXM for the new storage ring BESSY II and the main points are discussed briefly in the following:

- As already mentioned in section 2.2, the monochromaticity that can be obtained from the TXM zone plate monochromator is not as high as from the STXM grating monochromator. This doesn’t matter so much for imaging across an absorption edge but limits the capabilities of doing spectromicroscopy as illustrated in an example in figure 2.10. The monochromator concept for the TXM at BESSY II will allow to significantly increase the monochromaticity to a \( E/\Delta E \) be-
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Figure 2.10: Polystyrene (PS) C-XANES spectra, taken by measuring the total electron yield (TEY) with a channeltron at a non-spatially resolving EXAFS beamline (HE-PGM3-monochromator, $E/\Delta E \approx 3000$) at BESSY [135]. The data (courtesy of W. Unger) of this reference spectrum (solid line) was used to demonstrate the effects of low monochromaticity for detecting XANES spectra: Calculated spectra that would be measured by illuminating the sample with X rays of monochromaticities as indicated and available in the TXM at BESSY I, namely $E/\Delta E$ of 420/265/195 for 10/15/20 $\mu$m monochromator pinhole diameter, are shown. The convolved spectra show that resonances can still be detected; however, the resonance peak has decreased amplitude and is widened. For a monochromaticity of 100 (just as a low limit example), the shape of the spectrum has completely disappeared. Deconvoluting the spectra with the known monochromator function can help to enhance the flattened peaks of the measured spectrum again and restore them close to their original height. However, high frequencies in the spectrum that were cut off by convolution can not be restored by deconvolution.
between 1000 and 3000, thus improving the performance of the TXM for spectromicroscopy [84,85].

- Changing the wavelength in the TXM can not be done as quickly as in a STXM, but this can be taken into account in the hardware design concept of a new TXM.

- Differential imaging across an absorption edge for mapping the edge element qualitatively or quantitatively requires two images taken at either side of the edge. These two images have to be divided through each other by means of digital imaging processing. This can be done easily in a STXM, because the step size for both images can be set to the identical value. As long as the piezos move reproducibly, the sample doesn’t move and there are no random distortions of the piezos, there is a direct correspondence between certain sample structures and their location when taking two images at different photon energies.

In a TXM, the magnification $V$ varies with the photon energy (in good approximation $V \propto \lambda$), so the raw data can not be processed right away. To correct this, stretching the post-edge image (with smaller magnification) to the same size as the pre-edge image can be achieved by image processing. Another option to correct this varying magnification already in the hardware is changing the CCD camera-micro zone plate distance indirect proportional to the wavelength so as to keep the magnification constant for all photon energies.

- Higher order light (multiples of the actually chosen photon energy) can coincide with the first order focus. In the TXM, the condenser zone plate shows at nitrogen edge photon energies a second order focus (because the mark-to-space ratio of the holographically fabricated zone plate structures is not exactly 1) for twice the energy as well as a third diffraction order for three times the energy. The effect of higher diffraction orders causes problems both in TXM and STXM beamlines and can be overcome by installing a double mirror system that makes use of the energy dependency of the reflectivity.
In the setup of the TXM at BESSY I, the photon flux near the carbon-\(K\)-edge was almost 3 orders of magnitude lower than at the low energy side of the oxygen-\(K\)-edge, so data acquisition was not possible at the low energy end of the “water window”. This problem should have become obsolete at the new electron storage ring BESSY II with its two orders of magnitude increase in flux and the possibility of setting the undulator gap to optimized performance for a certain photon energy.

Against these difficulties, the use of a TXM for spectromicroscopy remains intriguing. While the “stack” imaging mode in STXM (taking a set of images at closely spaced photon energies, discussed in more detail in the next section) is time consuming, a TXM delivers a “stack” dataset quickly by default.

In addition, a new scanning transmission X-ray microscope is currently being designed by the Institute for X-ray physics in Göttingen and will be set up at BESSY II [141]. This will allow to study the same sample with high spatial resolution in a TXM and, if applicable, switch to spectromicroscopy experiments in the STXM within minutes.

Figure 2.11 shows an example of nitrogen mapping in a polymer film with the sample in air, done with the TXM at BESSY I. When taking data at photon energies around the nitrogen edge with the sample in air, the 80 \% \(\text{N}_2\) in air strongly influence the conditions for data acquisition. The ratio of the detected X-ray flux for pre- and post-edge energies without sample grows exponentially with increasing air gap size (assuming an equal incident flux independent of the photon energy). For this reason, nitrogen edge measurements can be carried out in a TXM with a 150–200 \(\mu\text{m}\) air gap, but currently not with the X-1A STXM due to its almost 1 cm air-helium mix path between the exit window of the X rays and the detector. This will change for the new STXM at X-1A, where the entire microscope is mounted inside a chamber that can be pumped down and refilled with Helium [30, 31].
Figure 2.11: Images of a thin section of a varnish film (right half of each image), embedded in araldite resin (left half of each image), taken with the Göttingen TXM at BESSY I at photon energies near the nitrogen-K-absorption edge [75]. A contrast reversal between the nitrogen containing varnish film and araldite (without nitrogen) can be observed for images of different photon energies across the absorption edge. From the original 20 μm image field, only the central part (close to the optical axis) was considered, where no radiation from higher diffraction orders falsifies the data.

Since the work presented in this thesis (carbon and calcium edge spectromicroscopy) can currently not be carried out with the X-ray microscopes running at BESSY I, it was done abroad supported by a fellowship for PhD research studies (Doktorandenstipendium HSP III) from German Academic Exchange Service (DAAD) under a proposal entitled 'Rasterröntgenmikroskopie an wässrigen Proben unter Nutzung des elementspezifischen Kontrastes an Absorptionskanten'.

2.4.2 Spectromicroscopy modes in STXM

Besides imaging at a fixed wavelength, spectromicroscopy can be done in STXM in two different ways:

**Conventional spectroscopy mode** The sample is positioned so that the X-ray focus spot hits the region of interest in the sample. While the sample stays in place, absorption spectrum data are now taken by changing the photon energy and re-focussing the zone plate accordingly and detecting the signal in transmission [147]. A spectrum usually has 512 data points and covers at an absorption edge an energy range of around 30 eV as so to include pre-edge data as well as XANES resonances.
and the edge step. The dwell time per energy step is typically 500 msec, so a spectrum can be acquired by these means in a few minutes.

A normalization spectrum $I_0$ without sample (or just with two silicon nitride windows which are also used as sample support in the wet cell) has to be taken beforehand in order to take into account the undulator spectrum, non-constant efficiencies of the beamline and zone plate optics and, near the carbon edge, absorption by carbon contaminations in the beamline. It should be made sure, that the conditions (e.g. detector-sample distance, helium flow, NSLS ring current, dwell time) for taking the normalization spectrum $I_0$ are the same as for the sample spectrum $I$. Dwell times and NSLS ring current (if significantly differing) can be quantified and taken into account when analyzing the data. If the measured normalization spectrum $I_{0m}$ is a factor of $k$ different from what it actually should be ($I_0$), it adds a constant offset to the absorption spectrum $(-\ln(I/I_{0m}) = -\ln(I/k \cdot I_0) = -\ln(I/I_0) - \ln(1/k))$. Avoiding this effect is particularly important when doing quantitative analysis (see section 3.3.2).

Examples for spectra obtained by the conventional spectroscopy mode can be found in figures 3.12, 4.5, 5.6 and 6.7.

The size of the object area from which a spectrum is obtained is in its extension determined by two factors:

- Imperfections of the motor movement for refocussing the zone plate (and OSA) along the beam axis $z$, i.e. random wiggling movements of zone plate and OSA in $x$ and $y$ make the focus move over the sample. This limits the spatial resolution for acquiring such spectra to about 200 nm. This imperfection can be overcome in principle by using a better hardware setup ($z$ motor for refocussing). If the movement is not random, but reproducible, it could be eliminated by allowing for corrections by moving the sample with the focus in $x$ and $y$.

- If the axis of the $z$ movement of the zone plate/OSA is not parallel to the X-ray beam, the focus moves evenly in one direction over
the sample due to the re-focussing while taking a spectrum. This might be as much as several μm over the energy range of a spectrum (30 eV). The current software allows to correct for movements in x by moving the sample with the focus in x. To do so, the degree of movement in x has to be quantified beforehand by taking two images with the same xy-scan range at different photon energies with known focal distances Δz. The movement Δx of a given sample structure from one image to the other and the known Δz allow to correct the tilt. Unfortunately, the focus movement in y can not yet be corrected in the software, but has to be corrected by adjustment screws, i.e. the entire STXM needs to be aligned to the beam before starting the experiment.

Another possibility for measuring spectra avoiding the problems described in the last two points above is the “stack” data acquisition mode as implemented by C. Jacobsen [49]. An automated routine takes a data set of images (approx. 100) at closely spaced photon energies (usually ΔE ≈ 0.1–0.2 eV). This set of images can be aligned in a computer afterwards using an autocorrelation routine and the wigging and even movement in the x- and y-directions of the focus can be eliminated. The region from which a spectrum is obtained is in this case limited by the microscope resolution. Therefore, spectral information even from very small sample regions (e.g. interplanetary dust particles) can be measured that would not be accessible with the “conventional” spectroscopy technique. Examples of data measured using the “stack” mode are shown in figures 6.4 and 6.8.

In the “stack” mode, it is taken care of the normalization (I₀) by considering regions in the image without sample. For wet cell experiments, these regions have to be in practice at least sample regions that do not contain the absorption edge element. For compounds that do not contain the edge element, the X-ray absorption can be assumed to be constant over a small energy interval. However, the values measured for I₀ are smaller than actually true, because a water absorption contribution is measured over the thickness of the sample where no absorption should
be measured to be correct. In the absorption spectrum (calculated as $-\ln(I/I_0)$), the absorptivity caused by the sample is then calculated to be smaller than in reality. If one is only interested in qualitatively measuring positions of resonances, this influence is not of great importance. But in order to get correct numbers for quantitative analysis, this effect has to be eliminated. More details on how this was handled for the quantitative analysis of calcium in hydrated samples can be found in section 3.3.2.

A disadvantage of the “stack” mode is that it takes a lot of time. Even if “stack” images are chosen to be not as big pixelwise as ordinary STXM images, acquiring a “stack” dataset with the STXM at Brookhaven takes between 2 and 6 hours. But since the procedure is automated, offline time that might not be utilized otherwise can be used for taking data. The advantage of a “stack” data set is that it contains spectra of all image points. Some sample regions might not attract the attention of the user during the experiment, but might turn out to be interesting when analyzing the data set.

2.5 Wet specimen chamber for STXM

As it has already been pointed out earlier, it is especially favorable when running soft X-ray microscopes in amplitude contrast to choose the “water window” energy range for good intrinsic contrast between water and organic (or other dense inorganic) matter.

To exploit this capability, wet specimen chambers of different design have already been used in transmission X-ray microscopes (TXM) [68, 71, 87] and with the X-1A scanning transmission X-ray microscope [36, 95].

In the STXM, one former type of ‘wet’ sample chamber has used rapidly interchangable windows for easy exchange of different cell culture samples and periodic flow of fresh culture medium [95]. In this setup, two silicon nitride windows are mounted at a distance of 1 mm. Microorganisms are grown on one of the windows and in the initial state,
the wet cell is filled with water or culture medium. Of course, the 1 mm water layer is too thick to be penetrated by soft X rays, so the water is drained right before the experiment, assuming that the water held on the biological cells by surface tension will keep them sufficiently hydrated. This chamber can not be used for the presented studies because its ~ 1 mm internal air gap does not provide a fully hydrated, sealed environment.

Other wet cells have used glued-on windows in a configuration that is good for interchange of sub-micrometer samples, but not for the type of specimen studied here. In addition, the sample windows used require more effort in window fabrication than for the wet cell described here [36].

Compared to the TXM used up to the end of November 1999 at BESSY I, the STXM is better coupled to the optics of high resolution
monochromators, and thus better suited to spectromicroscopy applications. However, in STXMs today it is the specimen (not the optics) which is scanned, and this places restrictions on wet chamber dimensions and mass.

For work at the carbon-$K$-absorption edge one requires a chamber able to handle the few hundred $\mu$m working distance from the sample to the order sorting aperture that is typical for high resolution zone plates in STXM. Some wet cell designs (e.g. for the Göttingen TXM [87]) use polymer films to support the sample. For spectroscopy near the carbon edge, it is favorable to use silicon nitride or silicon windows since they do not contain carbon (unlike polymer films).

The wet cell designed [79] was used for the experiments shown in this thesis, as well as by other users for experiments with the STXM at the Advanced Light Source (ALS) in Berkeley [74].

The restrictions and design requirements on the features of the wet cell will be described a little more in detail in the following:
Sample support

The liquid/aqueous sample is sandwiched between two 100 nm thin silicon nitride windows. The Si$_3$N$_4$-windows are thin, uniform, flexible and have a reasonably high transmission for soft X rays as it can be seen in the comparison with other thin foils that can also be used for sample preparation in X-ray microscopy (figure 2.14). While polyimide is not a good choice for spectromicroscopy near the carbon edge, the process of fabricating silicon windows [69] is much more difficult than making silicon nitride windows [10, 91]. However, silicon is from its mechanical properties less brittle than silicon nitride which might be advantageous for some applications.

Aluminium oxide windows (made from commercially available aluminium
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Figure 2.15: Visible light microscope image of an aqueous kaolinite clay mineral suspension (0.2–2 µm fraction, provided by D. Schulz, Agronomy Department, Purdue University), prepared in the wet cell. The square region with a thin sample layer measures approximately 2 x 2 mm.

Capillary forces slightly pull the silicon nitride windows together and 'clamp' the particles, so they can be studied in STXM with the wet cell in a vertical position. The appearance of color fringes caused by visible light interference effects suggest that the layer thickness is in the order of a few times of the wavelength of visible light, i.e. approx. 1 to 2 µm. This is supported by comparing numbers of count rates in STXM with and without wet cell and also by the fact that the clay mineral particles with a maximum diameter of 2 µm can be seen as small 'bumps' in the silicon nitride window surface.

foil) are prone to show small granules of aluminium on them. This is less important for other applications of Al₂O₃ windows (e.g. as vacuum windows), but needs certainly to be avoided when using these windows as a sample support because it might be difficult to tell if structures in the image stem from the sample or from the sample support windows.

The silicon nitride windows used here are 3 x 3 mm in size and sit in the center of a 9 x 9 mm silicon wafer, 200 µm thick and double-side
polished. Reducing the frame size to 8 x 8 mm while increasing the window size to 4 x 4 mm is a good option because some samples can be studied more easily with a larger window area. In addition, a larger number of windows can be obtained out of one 3 inch silicon wafer (which is the material used to make the windows from) by reducing the frame size at the same time.

**Sample layer thickness** The thickness of the aqueous sample layer between the two silicon nitride windows has to be in the range of a few micrometers in order to be able to detect photons of the energy range of the “water window” between 284 and 543 eV in transmission. While the
Figure 2.17: A demonstration of imaging hydrated samples with the wet cell in STXM: Images of bacteria (as part of a natural clay mineral dispersion, sample provided by S. Ziesmer, Institut für Anorganische Chemie, Universität Kiel) in water, taken near the carbon-K-absorption edge. For pre-edge energies, the bacteria absorb as weakly as water and become ‘invisible’ (see right image). There is no more contrast between water and the microorganisms, because the photon energy is outside the “water window” range. It has been double-checked that imaging another time at 290 eV makes the bacteria re-appear. The images have 300 x 300 pixels with a pixel size of 100 nm.

$1/e$ attenuation length for water is 10 µm at 530 eV, it decreases down to 2 µm at 280 eV. Due to the small numerical apertures of the zone plates currently used, the depth of focus is as big as a few microns, so the entire sample is within this range. Figure 2.14 shows that the transmission for a 100 nm thin silicon nitride window is approximately 50 %. Two windows and a water layer of approximately 1–2 µm (50 % transmission) yield an overall transmission of a hydrated sample in the wet cell of 10–15 %. The transmitted radiation through the wet cell sample, measured as a count rate by the proportional counter, ranges (depending on slit sizes and beamline alignment) between 100 and 500 kHz near the carbon edge.

**Space restrictions** The sample must be placed a few hundred microns away from a pinhole (order sorting aperture); this works by using a thin shim metal as a support for one silicon nitride window. Due to these strict working distance requirements, a zone plate with 160 µm in
diameter and 45 nm outermost zone width has been used with a order sorting aperture of 70 \( \mu \text{m} \) in size, placed 1100 \( \mu \text{m} \) from the zone plate. The focal length of this zone plate is 1625 \( \mu \text{m} \) at 280 eV (4.43 nm). The distance between the sample and the flat upstream side of the wet cell is around 250 - 275 \( \mu \text{m} \) (wafer thickness, shim and glue). This leaves 250 \( \mu \text{m} \) space between the OSA and the wet cell. The space restrictions are illustrated in figure 2.16.

**Long time stability** The wet cell is sealed by an O-ring to prevent the sample from drying out; it can be kept hydrated and stable for up to 8 hours. This allows to run the “stack” imaging mode for spectro-microscopy (as described in section 2.4.2) also on hydrated samples as is shown in figure 6.4. Exposure times may vary depending on incident photon flux and image size, but a typical STXM image with good photon statistics (dwell time between 5 and 20 msec) and in the order of 300 x 300 pixels takes between 10 and 30 minutes.

**Weight** The chamber has to be low in mass because it is scanned in the microscope. Therefore, the material chosen for the wet cell is aluminium. Stainless steel has better characteristics regarding its stiffness, but is almost 3 times heavier. The cell must be thin in the beam direction to minimize absorption in the atmospheric pressure air or helium path leading to the detector. For this reason, the cell is as thin as possible so the O-ring can be properly accommodated and the cell parts can be screwed together.

Improvements beyond this first wet cell design could be to try to avoid the shim metal in the upstream part of the cell that has to be re-glued after several preparations, and have an upstream part machined from one piece. However, machining aluminium over an area of more than one square centimeter to a thickness of 0.001" (25 \( \mu \text{m} \)) is very challenging (if possible at all). Technical details on the wet cell can be found in the AutoCAD drawings in appendix B.
Chapter 3

Interactions of soft X rays with matter

3.1 X-ray optical interactions

The interaction between X rays and matter in the soft X-ray range is well described by the high frequency limit of classical anomalous dispersion theory. This theory (see e.g., [38, 50]) gives a form of the refractive index of

\[ n = 1 - \delta - i\beta = 1 - K(f_1 + i f_2) \]  \hspace{1cm} (3.1)

In the refractive index \( n \), the macroscopical optical properties of matter can be brought into connection with atomic properties (oscillator strengths, \( f = f_1 + i f_2 \)) as follows:

\[ \delta = \frac{r_e^2 \lambda^2}{2\pi} N f_1 = \frac{r_e^2 \lambda^2}{2\pi} \frac{N_A \rho}{M} \cdot f_1 \]  \hspace{1cm} (3.2)

\[ \beta = \frac{r_e^2 \lambda^2}{2\pi} N f_2 = \frac{r_e^2 \lambda^2}{2\pi} \frac{N_A \rho}{M} \cdot f_2 \]  \hspace{1cm} (3.3)

where \( r_e = 2.818 \cdot 10^{-15} \text{m} \): classical radius of the electron, \( N \): number density of atoms, \( \rho \): density, \( M \): molar mass, \( N_A \): Avogadro constant.

Here, \( \delta \) resp. \( f_1 \) is the part representing the phase shift and \( \beta \) resp. \( f_2 \) describes the absorption with a proportional factor \( K \) between these...
properties (see equation 3.1) as follows:

\[ K = \frac{1}{2\pi} r_e \lambda^2 N \]  

(3.4)

The atomic scattering factors \( f \) are tabulated in [39] and are also available from [http://www-cxro.lbl.gov].

The treatment of soft X-ray optical interactions as described here is incomplete in that it only considers the attenuation of X-rays by photoelectric absorption, which is the major absorption process for soft X-rays, with an atomic cross section

\[ \sigma_a = 2\lambda r_e f_2 \]  

(3.5)
Figure 3.2: Photon cross section $\sigma$ in barn (1 barn = $10^{-28}$ m$^2$) in carbon as a function of energy, showing the contributions of photoelectric absorption $\sigma_a$, elastic (coherent) scattering $\sigma_e$, and incoherent or Compton scattering $\sigma_i$. These cross sections explain why soft X-ray microscopes are relatively immune to image blurring due to multiple elastic scattering or inelastic scattering. Data from [39, 42], graphic from [46].

and ignores attenuation by elastic scattering with cross section

$$\sigma_e = \frac{8}{3} \pi r_e^2 (f_1^2 + f_2^2)$$  \hspace{1cm} (3.6)

However, this is well justified in the soft X-ray region because $\sigma_e \ll \sigma_a$ (due to the fact that $r_e \ll \lambda$), and furthermore the cross section for inelastic or Compton scattering $\sigma_i$ is significantly lower than $\sigma_e$ or $\sigma_a$ as is shown in Fig. 3.2. Therefore, when compared to electron microscopy one can expect soft X-ray micrographs of thick specimens to be free of any “haze” that could otherwise be caused by multiple elastic scattering, or
improper focusing of inelastically scattered radiation which is improperly imaged using chromatic lenses (zone plates). Nevertheless, X rays suffer a non-negligible phase advance when passing through matter, which makes it possible to operate X-ray microscopes in phase contrast mode [104,107].

When X rays of an incident intensity $I_0$ pass through matter, they suffer an attenuation to an intensity $I$ as described by the Lambert-Beer law:

$$I = I_0 e^{-\mu t}$$

(3.7)

where $\mu = \frac{4 \pi \beta}{\lambda}$ is the linear absorption coefficient (in units of $1/\text{length}$, for soft X rays mostly $1/\mu$) and $t$ is the thickness of the sample. If the sample thickness is not known, the equation can be written using an expression 'mass per area' $m = \rho t$

$$I = I_0 e^{-\frac{\mu}{\rho} m}$$

(3.8)

where $\frac{\mu}{\rho}$ is the linear absorption coefficient $\mu$ divided by the density in units of $[\text{cm}^{-1}]$. Note that the element’s density $\rho$ is needed to write the dependencies in a way suited for further evaluation, but the density does not need to be known explicitly.

This allows one to characterize the sample in terms of a mass density per area $m$ in units of [$\text{g cm}^{-2}$], thus eliminating the sample thickness. $\frac{\mu}{\rho}$ for an element can be calculated from the Henke-Data [39] as follows:

$$\frac{\mu}{\rho} = 2 \cdot \frac{N_a}{M} \cdot r_e \cdot \lambda \cdot f_2(\lambda) = 3.394 \cdot 10^{11} \text{cm} \cdot \frac{f_2(\lambda)}{\text{Mol}} \cdot \frac{\lambda}{\text{cm}}$$

(3.9)

where $N_a$: Avogadro-constant ($6.022 \cdot 10^{23} \text{ mol}^{-1}$) and $M$: molar mass [$\text{g mol}^{-1}$].

$\frac{\mu}{\rho}$ for a compound is the sum of $\frac{\mu}{\rho}$ for all elements in the compound, weighted by the fraction of their contribution to the molecular weight.


3.2 Requirements for detecting elements at X-ray absorption edges

Away from an element’s absorption edge, its linear absorption coefficient varies as \( \mu \propto \lambda^3 \) so that a 1% change in wavelength produces only a 3% change in absorption. On the other hand, at absorption edges the linear absorption coefficient \( \mu \) can change by a factor of ten or more with an energy change of less than 1%, as is illustrated in Fig. 1.2 for decane near the carbon absorption edge (284 eV). This allows one to quantitatively map the concentration of an element by taking two images on either side of its X-ray absorption edge, provided the element is present at a homogeneously distributed bulk concentration of about 1% or more. The bulk concentrations of an element might be much less than 1%, if the element is distributed inhomogeneously in the sample with high detectable local concentrations.

Experimentally, elemental mapping can be done either by measuring differences in the transmission signal at either side of an absorption edge or by measuring increases in a fluorescence signal when tuning the X-ray energy across the edge (for earlier discussions, see e.g., [13, 28, 55]).

We wish to calculate the illumination signal needed to detect a number concentration of \( n_z \) atoms per volume within a matrix of thickness \( t \). We will assume that the matrix has an absorption coefficient \( \mu_m \) which does not change significantly across the absorption edge. The important physical properties for this calculation are the difference in the \( f_2 \) value across the edge as well as the fluorescence yield. Table 3.1 shows these properties for different elements and their edge energies.

3.2.1 Differential edge contrast in transmission

The transmitted signal at the high energy side of the absorption edge will be

\[
N_{\text{high}} = N_0 \exp(-\mu_m t) \exp(-2\tau_c \lambda n_z f_2, Z_{\text{high}} t)
\]  

(3.10)
whereas on the low energy side of the edge the signal will be

\[ N_{\text{low}} = N_0 \exp[-\mu_{mt}] \exp[-2r_e \lambda n_Z f_{2, Z, \text{low}} t] \]  

Therefore the signal difference will be

\[ \Delta N = N_0 \exp[-\mu_{mt}] \left( \exp[-2r_e \lambda n_Z f_{2, Z, \text{low}} t] - \exp[-2r_e \lambda n_Z f_{2, Z, \text{high}} t] \right) \]

\[ \simeq N_0 \exp[-\mu_{mt}] 2r_e \lambda n_Z \Delta f_2 t \]

where we have defined \( \Delta f_2 = f_{2, Z, \text{high}} - f_{2, Z, \text{low}} \) and assumed a low concentration for the edge element (good approximation for 1% concentration [55]) and moderate sample thicknesses in the last step. With this linear approximation \( (\exp(x) \simeq 1 + x) \), the contrast \( C \) for differential imaging simplifies to

\[ C = \frac{N_{\text{low}} - N_{\text{high}}}{N_{\text{low}} + N_{\text{high}}} = r_e \lambda n_Z \Delta f_2 t \]

For differential absorption imaging, this signal difference must be detected against a background of statistical fluctuations \( \sqrt{N_{\text{high}} + N_{\text{low}}} \) which in the low contrast limit is \( \sqrt{2N_0 \exp[-\mu_{mt}]} \). A measurement of differential absorption will therefore have a signal-to-noise ratio \( \text{SNR} \) of

\[ \text{SNR}_{\text{dn}} = \frac{\Delta N}{\sqrt{2N_0 \exp[-\mu_{mt}]}} = \sqrt{\frac{N_0}{2}} \exp[-\mu_{mt}/2] 2r_e \lambda n_Z \Delta f_2 t. \]

To detect a given concentration of atoms \( Z \), we therefore require illumination with

\[ N_{0, \text{dn}} = \frac{\text{SNR}_{\text{dn}}^2}{2 \exp[-\mu_{mt}] (r_e \lambda n_Z \Delta f_2 t)^2} \]

detectable photons per resolution element for differential absorption mapping.

When looking at \( d(\text{SNR}_{\text{dn}})/dt \), the SNR shows a local maximum for a sample thickness of \( 2/\mu_m \). To make the SNR for a fixed number of X-ray photons used as high as possible and the number of photons necessary to obtain a given SNR as low as possible, the ideal specimen thickness would be two times the attenuation length \( a_t \) for the matrix material.
Table 3.1: Values of $f_2$ at absorption edges in the range $100 \text{ eV} \leq E \leq 1300 \text{ eV}$. The approximate energy of the edge is shown, as is $f_2$ on the high energy side of the edge and the increase $\Delta f_2$ from $f_2$ at the low energy side of the edge. Data on $f_2$ are from [39], while data on fluorescence yields is from [39]. Tabulations of X-ray absorption edges of all elements, sorted by energy can be found in [153].

<table>
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<tr>
<th>Element</th>
<th>$Z$</th>
<th>$E$ (eV)</th>
<th>$f_2$</th>
<th>$\Delta f_2$</th>
<th>Yield $Y$</th>
<th>$Z$</th>
<th>$E$ (eV)</th>
<th>$f_2$</th>
<th>$\Delta f_2$</th>
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<td>14.67</td>
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3.2.2 Edge contrast using fluorescence

For fluorescence analysis, we will assume that the extra photons which are absorbed as one crosses an absorption edge will give rise to fluorescence with a yield $Y$ \cite{55} which we will then detect with an efficiency $\eta$. Tabulations of the fluorescence yield $Y$ are shown in table 3.1. The number of detected fluorescent photons $N_t$ is therefore

$$N_t = \eta Y \Delta N = \eta Y N_{0,t} \exp[\mu t] \Delta f 2 \lambda n Z \Delta f t$$  \hspace{1cm} (3.17)

Assuming sufficiently low background signal, and approximating Poisson statistics with Gaussian statistics, the signal-to-noise ratio in detecting fluorescent photons is $\text{SNR}_f = N_t / \sqrt{N_t} = \sqrt{N_t}$, so the number of illuminating photons required is

$$N_{0,t} = \frac{\text{SNR}_f^2}{\eta Y \exp[\mu t] \Delta f 2 \lambda n Z \Delta f t}$$  \hspace{1cm} (3.18)

Considerations of detector solid angle and quantum efficiency give $\eta = 0.01$ as a typical detection efficiency \cite{55}.

The optimum sample thickness for maximum SNR for fluorescence imaging is $1/\mu m$, i.e. the attenuation length $a_t$ of the matrix material. For the detection of fluorescence radiation, the background due to X-ray scattering can be effectively reduced if the detector is at 90° with respect to the beam in the plane of the beam’s polarization. Practically, the sample is for symmetry reasons at an angle of 45° to the incident beam \cite{127}. In this geometry, it must be taken into account that the actual sample thickness $t$ is seen by the beam projected with a larger thickness of $\sqrt{2}t$.

3.2.3 Discussion of the model calculations

Calculations on photon numbers required when mapping elements using X rays in differential absorption or fluorescence mode have already been performed by Kirz \cite{55} in combination with dosage calculations. Here, we want to take an approach discussing the contrast that can be expected
for differential absorption edge imaging and how to optimize the signal-to-noise ratio by matching the sample thickness. Figure 3.3 illustrates that good edge contrast does not necessarily mean good quality data, because of the thickness dependence of the signal-to-noise ratio.

The dependencies shall be demonstrated and discussed with a concrete example. We assume our matrix material to be a kaolinite clay mineral having a composition of Si$_2$Al$_2$O$_5$(OH)$_4$. Table 3.2 shows the 1/\(\mu_m\)-attenuation lengths for this matrix material for the energies of different absorption edges.

Figure 3.4 shows contrast calculations for differential absorption edge imaging in transmission, following equation 3.14.

Contrast can not be defined similarly for fluorescence detection, because
CHAPTER 3. INTERACTIONS OF SOFT X RAYS WITH MATTER

Table 3.2: 1/e-attenuation lengths $\alpha$ of the kaolinite matrix material for the absorption edge energies of different elements in the range between 100 and 1000 eV. In figures 3.4 and 3.5, it will be referred to the attenuation length and the elements listed. In absorption edge contrast imaging, the SNR can be maximized if the sample thickness is optimized to $\alpha_t$ for transmission detection or $\alpha_f$ for fluorescence detection.

<table>
<thead>
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<th>edge energy [eV]</th>
<th>attenuation length $\alpha$ [nm]</th>
<th>element</th>
<th>atomic charge Z</th>
<th>absorption edge</th>
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<td>15</td>
<td>L</td>
</tr>
<tr>
<td>162.6</td>
<td>69</td>
<td>S</td>
<td>16</td>
<td>L</td>
</tr>
<tr>
<td>200.1</td>
<td>104</td>
<td>Cl</td>
<td>17</td>
<td>L</td>
</tr>
<tr>
<td>248.5</td>
<td>162</td>
<td>Ar</td>
<td>18</td>
<td>L</td>
</tr>
<tr>
<td>294.7</td>
<td>234</td>
<td>K</td>
<td>19</td>
<td>L</td>
</tr>
<tr>
<td>346.3</td>
<td>337</td>
<td>Ca</td>
<td>20</td>
<td>L</td>
</tr>
<tr>
<td>398.8</td>
<td>470</td>
<td>Sc</td>
<td>21</td>
<td>L</td>
</tr>
<tr>
<td>453.9</td>
<td>642</td>
<td>Ti</td>
<td>22</td>
<td>L</td>
</tr>
<tr>
<td>512.2</td>
<td>857</td>
<td>V</td>
<td>23</td>
<td>L</td>
</tr>
<tr>
<td>574.2</td>
<td>283</td>
<td>Cr</td>
<td>24</td>
<td>L</td>
</tr>
<tr>
<td>638.8</td>
<td>369</td>
<td>Mn</td>
<td>25</td>
<td>L</td>
</tr>
<tr>
<td>706.9</td>
<td>475</td>
<td>Fe</td>
<td>26</td>
<td>L</td>
</tr>
<tr>
<td>778.2</td>
<td>607</td>
<td>Co</td>
<td>27</td>
<td>L</td>
</tr>
<tr>
<td>852.8</td>
<td>771</td>
<td>Ni</td>
<td>28</td>
<td>L</td>
</tr>
<tr>
<td>932.6</td>
<td>967</td>
<td>Cu</td>
<td>29</td>
<td>L</td>
</tr>
</tbody>
</table>

Photons are absorbed as one crosses an absorption edge, giving rise to fluorescence, but there is no significant pre-edge signal that could be compared to the post-edge signal.

In figures 3.5, 3.6, we compare the minimum number of illuminating X-ray photons needed to detect 1% of an edge element with an atomic charge $Z$ in the kaolinite matrix using either transmitted X rays or fluorescence X rays. For figure 3.5, it is assumed that the matrix thickness
Figure 3.4: Contrast for soft X-ray differential absorption edge imaging (detection in transmission). The example specimen is a kaolinite matrix containing 1% of the edge element with the atomic charge $Z$ at the $K$ and $L$ absorption edge of element $Z$. Different sample thicknesses are shown: The solid line shows calculations for optimized SNR with a sample thickness of $2 \alpha_l$ (attenuation length of the matrix material, as listed in table 3.2). Sample thicknesses of 50 nm (dotted line) and 1000 nm (dashed line) are also shown in comparison. Oxygen ($Z=8$) is not shown, because of its presence in the kaolinite matrix. The elements with atomic charge $Z=11-14$ do not have X-ray absorption edges in the considered energy range between 100 and 1000 eV, thus do not appear in the plot. Thin samples (e.g. 50nm, dotted line) show in general weak contrast, as already demonstrated in figure 3.3.

matches the attenuation length $\alpha_l (1/\mu_m)$ optimum thickness for fluorescence or the $2 \alpha_l (2/\mu_m)$ optimum thickness for differential absorption at the energy of the edge.

For biological samples, the system to be studied determines the thickness of the sample, changes can not be made without disturbing the system. Embedding chemically fixed cells in resin and preparing thin
Figure 3.5: Number of X-ray photons required to detect 1% of an edge element with atomic charge Z in a kaolinite clay mineral matrix. All elements shown have either a K or a L edge in the energy range 100-1000 eV (see also table 3.1). Oxygen (Z = 8) is not shown because of its presence in the matrix. The elements with atomic charge Z=11-14 do not have X-ray absorption edges in the considered energy range between 100 and 1000 eV, thus do not appear in the plot. For each element, it is assumed that the matrix thickness matches the $1/\mu_m$ optimum thickness for fluorescence or the $2/\mu_m$ optimum thickness for differential absorption at the energy of the edge.

sections (down to 50 nm in thickness) is a widely used technique in transmission electron microscopy and provides useful information, however not without artifacts.

Therefore, matching the sample thickness at no cost of information remains only an option for material or colloidal science experiments.

The expression “matrix of optimized thickness for max. SNR” in figure 3.5 should be read in a way that the SNR of 5 is the maximum possible. All photon number calculations made have a signal-to-noise ratio of 5, which is also known as the Rose criterion [97].
Figure 3.6: Number of X-ray photons required to detect 1% of an edge element with atomic charge $Z$ in a kaolinite clay mineral matrix. The sample thickness is assumed to be constant, 1 μm in the top plot and 50 nm in the bottom plot.
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Figure 3.6 shows a comparison for 50 nm and 1 µm sample thickness. However, in these calculations, the SNR of 5 is not the maximum and higher SNRs would be achievable when choosing appropriate sample thicknesses. Therefore, the photon numbers for non-optimized sample thicknesses are always greater or equal to the numbers calculated for optimized thickness.

In all cases, differential absorption edge mapping seems to be preferable over fluorescence mapping. This is on the one hand due to the relatively low fluorescence yields in the low Z region (only absorption edges between 100 and 1000 eV have been considered). Detecting in fluorescence mode is much more promising in the harder X-ray regime of several keV, where the fluorescence yields increase compared to lower energies. On the other hand, fluorescence is preferable over transmission to detect very low concentrations. While $n_z$ (number concentration per volume) is in the fluorescence case linear in the denominator of equation 3.18, it is a square term for the transmission case (equation 3.16). This makes the photon numbers needed increase more drastically for transmission than for fluorescence, when going to very small concentrations.

While the difference in the photon number requirements have been discussed in the last paragraph, the dependencies of the number concentration $n_z$ for the case of equal photon numbers for fluorescence and transmission detection shall be discussed in the following. Equations 3.16 and 3.18 yield the number concentration $n_z$, for which transmission and fluorescence detection require equal numbers of photons. This number concentration $n_z$ is given by:

$$n_z = \frac{\text{SNR}_T^2}{\text{SNR}_F^2} \cdot \frac{\eta Y}{r_e \lambda \Delta f_2 t}$$  \hspace{1cm} (3.19)

Note, that for an equal sample thickness $t$ and equal photon numbers, the SNRs are not necessarily equal for the fluorescence or transmission mode.

Equation 3.19 shows, as expected that the fluorescence mode becomes more competitive to the transmission detection mode also for higher num-
ber concentrations $n_Z$ of the element to be detected if the fluorescence yield $Y$ gets larger and if a higher detection efficiency $\eta$ would be possible. Furthermore, $n_Z$ increases for decreasing $\lambda$. This implies that for harder X rays, fluorescence is not only advantageous because of the higher fluorescence yield, but also because of the higher energy itself. A statement on the dependencies of the sample thickness $t$ can not be made in equation 3.19, because the SNR is a function of $t$, as it has been shown in figure 3.3.

### 3.3 XANES-spectroscopy

#### 3.3.1 Basic principles of near edge absorption fine structure

Chemical bonds between single atoms are formed by outer-shell electrons with energy levels of several eV. Although their energy levels are orders of magnitude lower than inner shell electron levels, they are able to influence the binding energies of electrons in the inner shells.

During the formation of a covalent chemical bond, atom orbitals overlap and their wave functions superimpose and split into a binding molecular orbital MO (for constructive superposition) and a anti-binding molecular orbital (for destructive superposition) as shown in figure 3.7. Since the energy level is lower for the binding case, the binding MO is filled first and the antibinding MO is unoccupied in many cases.

![Diagram of the splitting of two 1s atom orbitals into a binding ($\sigma{1s}$) and antibinding ($\sigma^*{1s}$) molecular orbital, each having different energy levels.](image-url)
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In single atoms, photoelectrons generated by the absorption of an X-ray photon can make transitions to unoccupied atom orbitals of the outer shells, while in molecules, the photoelectrons make transitions to unoccupied (mostly antibinding) molecular orbitals (see figure 3.8). These transitions occur for different energies and thus can be measured by using monochromatic X rays (in real life X rays with a small energy band-
Figure 3.9: Illustration of the scattering of a photoelectron that was emitted by X-ray absorption in the center atom (after [7]).

**Left:** For low kinetic energy of the photoelectron (XANES), it suffers multiple scattering at its neighboring atoms.

**Right:** For higher kinetic energies of the photoelectron (EXAFS), single scattering is the pre-dominant effect. The photoelectrons manage to escape rather far away from the emitting atom.

Similar effects can be found in a scanning electron microscope. Secondary electrons with low energy ($\approx 50$ eV), generated by inelastic scattering, leave the surface of the sample much closer to the atom of their origin than backscattered electrons with higher energy. Therefore, secondary electrons are much better suited for obtaining an electron microscope image with high spatial resolution [96].

If the X-ray energy exceeds the binding energy of the electron, the electron is emitted as a photoelectron, taking up the difference between binding energy and the X-ray photon energy as kinetic energy. The photoelectron suffers increased scattering for increased X-ray energies beyond an absorption edge; that is, for increased kinetic energy of the electron.

Therefore, the fine structure near X-ray absorption edges can be subdivided into two classes that continuously follow on each other:

- **XANES** (X-Ray Absorption Near Edge Structure) or, equivalently, the term **NEXAFS** (Near Edge X-Ray Absorption Fine
**CHAPTER 3. INTERACTIONS OF SOFT X RAYS WITH MATTER**

Figure 3.10: Schematic illustrating the mechanism of the oscillation of the absorption in EXAFS. The photoelectron emitted by the center atom suffers scattering at the neighboring atoms. Different incident X-ray energies ($E_1 > E_2$) cause different de Broglie wavelengths ($\lambda = \frac{h}{mv}$) of the emitted photoelectron ($\lambda_1 < \lambda_2$). Depending on the phase conditions, the incident and backscattered photoelectron wave can interfere either constructively (left side of the figure) or destructively (right side of the figure). The quantum mechanical probability for constructive interference of the de Broglie photoelectron wave is higher than for the destructive interference, therefore resulting in a slightly modulated absorption. The absorption periodically oscillates along the X-ray energy axis (and with that along the energy axis of the kinetic energy of the photoelectron) between constructive and destructive interference.

**Structure** is used: Region of 10 - 40 eV within the edge. The photoelectrons with low energy (for this reason, the original name was 'low energy Kossel structure' [57, 58]) suffer scattering with a high cross section, causing the strong modulations of absorption.

- **EXAFS** (Extended X-Ray Absorption Fine Structure). This region follows on XANES and lies about 40 eV above the absorption edge. The modulation of the absorption is much weaker due to the smaller cross section for scattering. This region was originally
named 'high energy Kronig structure' [23–25].

Further details of XANES (NEXAFS) spectroscopy are discussed in the widely read monograph by Stöhr [126]. An introduction to X-ray spectroscopy is given by Agarwal [7]. EXAFS spectroscopy is explained in its basics by Teo [128]. Applications of EXAFS in chemistry can be found in [12] and [70].

3.3.2 Quantitative elemental mapping using XANES features

Model calculations on the dependencies of mapping elements near X-ray absorption edges have been discussed in section 3.2. In this section, we want to focus on how this quantitative elemental analysis was done experimentally near the calcium-L-absorption edge.

Appendix A sketches how to evaluate the data when imaging across an absorption edge step at two wavelengths ($\lambda_1/\lambda_2$). First, the absorption properties of calcium (molar mass 40.078 g/Mol) need to be calculated at these two wavelengths using the Henke data (equations 3.9 or A.3):

Table 3.3: Absorption coefficients of calcium across the calcium-L-absorption edge.

<table>
<thead>
<tr>
<th>i</th>
<th>Energy [eV]</th>
<th>Wavelength [nm]</th>
<th>$f_2$</th>
<th>$(\mu\mu/\rho)_y$ [m$^2$/g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>356.0</td>
<td>3.4827</td>
<td>14.44</td>
<td>4.2589</td>
</tr>
<tr>
<td>2</td>
<td>346.1</td>
<td>3.5824</td>
<td>1.568</td>
<td>0.4757</td>
</tr>
</tbody>
</table>

Equation A.2 then yields the mass per area of the edge element.

This analysis allows us to quantify the mass per area of calcium in STXM images of aqueous Ca/Al layered double hydroxide (LDH) suspensions and in emulsions using Ca/Al LDH as emulsifying agent (as described in more detail in section 5.3). The spectrum of Ca/Al LDH in Fig. 5.6 (a) shows that the XANES absorption features are much more pronounced than the actual absorption edge step. This suggests the use of absorption features for quantitative mapping because of better data statistics [18]: the absorption cross section is much higher for a resonance
Figure 3.11: Graphic (from [19]) illustrating the limitations of quantitative mapping near absorption edges using a calcium-L-edge XANES resonance with a natural full-width-half-maximum peak width of 0.4 eV. For high calcium concentrations, the ratio of measured to true peak height drops because of thickness effects [90]. For low energy resolving power, the ratio decreases because of the convolution of the spectral feature with the monochromator spectrum (also illustrated in figure 2.10).

(e.g. at 352 eV) than for post-edge energies (e.g. at 355 eV). The strong Ca-L near-edge absorption resonances [22] help enhance the sensitivity of calcium maps. Doing quantitative analysis on calcium using only pre- and post edge energies would yield a noisy calcium map due to the small edge step.

While just imaging across the absorption edge can be done also with a moderate monochromaticity and for high edge element concentrations, the use of resonances for quantitative mapping is limited to low concentrations and requires high energy resolution [19]. The ratio of absorption
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peak height to the edge step as calculated by the Henke data is no longer constant if the monochromaticity of the radiation used for data acquisition is too low and if the calcium concentration is too high as illustrated in figure 3.11. This is due to the fact that in regions where the absorption coefficient $\mu$ varies rapidly, the absorption is no longer described by the simple exponential function of the Lamber-Beer law, but needs to be written as

$$I_1 = \int_0^\infty I_0(E) G(E) \exp(-L(E)\mu c) dE$$  \hspace{1cm} (3.20)

where $I_0(E)$ describes the variation in $I_0$ over the energy width of the monochromator function $G(E)$, $L(E)$ is a normalized Lorentzian describing the shape of the XANES peak and $\mu$ is the value of the peak at its center.

The conditions of high monochromaticity and calcium concentrations in the range of micrograms per square cm have been met in our experiments. To quantify calcium, we took images at a pre-edge energy (346 eV) and a XANES resonance energy (352 eV) as are shown in Figs. 5.4 and 5.8, and used the step-edge normalized calcium spectrum of Fig. 5.6(a) at an energy of 356 eV for calcium quantification.

In practice, the analysis was done as follows: From the absorption spectrum of Ca/Al layered double hydroxide (as shown in figure 5.6 a), we derived the proportional factor between the edge step (356 eV) and the resonance (352 eV). The images taken at 352 eV (the left images in figures 5.4 and 5.8) were divided by this proportional factor as so to normalize the image taken at a resonance to what they would have looked like quantitatively if they were taken at 356 eV. Combining the absorption properties of calcium as listed in table 3.3 with the intensities in the images taken at 346 eV (pre-edge) and the normalized resonance image (taken at 352 eV, but normalized to 356 eV) in equation A.2 allowed to quantitatively link the absorption peak height to a certain amount of calcium.

The problem of the absorption contribution of water for quantitative analysis (as described in the point 'Spectromicroscopy in “stack” mode’
in section 2.4.2) was eliminated by normalizing the calcium map to a calcium amount of zero in regions without solid sample, knowing that there is no, or at least orders of magnitude less calcium in solution, compared to the solid, calcium rich double hydroxide.

### 3.3.3 XANES versus EELS

In electron energy loss spectroscopy (EELS), the kinetic energy distribution of inelastically scattered electrons is measured, providing information about the electronic structure of the specimen. EELS will not be described in detail here, but we want to point out that there is a direct agreement between EELS and XANES spectra, as it can be seen in figure 3.12.

EELS data is available on many compounds and in spectromicroscopy experiments, it can be helpful to take EELS data into consideration. Knowing the precise position of absorption peaks is for example useful for calibrating the beamline monochromator using e.g. CO$_2$, N$_2$, O$_2$ gas (flowing around the sample region). When planning STXM experiments on samples containing several different compounds of the same edge element, the EELS spectra can help to predict if XANES contrast will be possible by comparing the energy position of the absorption peaks.

A large library of EELS spectra already exists and building up a library of XANES spectra is done at the moment (see figure 3.12). Although EELS and XANES are in agreement, it is not redundant to collect XANES spectra, because the EELS spectra can be only measured easily for gases, volatile liquids and thin sections, and the absorption characteristics of many other samples are only accessible over X-ray measurements. In addition, in the raw data of EELS, small signals are measured on a large background of plural inelastic scattered electrons while in XANES spectra, absorption resonances with a large amplitude and no background are measured.
Figure 3.12: Comparison between the XANES and EELS spectra of CO₂ gas near the carbon-K-edge and O₂ near the oxygen-K-edge.

*Top row:* EELS spectra (from http://xray.uu.se/hypertext/corexdb.html)

*Bottom row:* XANES spectra, taken in STXM

*Left column:* CO₂, C edge.  *Right column:* O₂, O edge

The XANES spectra are noisy due to short dwell times, although better statistics can be obtained by integrating over several spectra. The strong noise between 533 and 537 eV in the O₂ XANES spectrum is due to the very low incident photon flux. This is caused by the order sorting mirrors in the beamline, consisting of quartz (SiO₂) that is absorbing strongly at the oxygen edge as well as by the low reflectivity of the mirrors in this energy range. Due to the fact that normalization reference spectra are taken without the sample beforehand, the quartz does not greatly influence the measured spectrum of the O₂ gas.

EELS data of many compounds are available at http://xray.uu.se/hypertext/corexdb.html

Building up a database of XANES spectra is in progress; see http://xray1.physics.sunysb.edu/xas/xas.html.
Chapter 4

X-ray microscopy studies of hydrated clay-organic interactions

4.1 Introduction to clay minerals

4.1.1 Terminology and structure

Clays are loose sedimentary rocks, consisting of mineral particles with a diameter smaller than 20 μm. Amongst these particles, plate-shaped silicate clay minerals with a diameter below 2 μm are predominant.

In their crystalline structure, the silicate clay minerals are composed of [SiO₄] tetrahedrons and [M(O,OH)₆] octohedrons (M: metal, e.g. Al, Mg, Fe). These silicate clay minerals can be divided into two subgroups: the 1:1 sheet silicates (with one tetrahedral and one octahedral sheet) and the 2:1 sheet silicates (with two tetrahedral sheets and one octahedral sheet between them, see figure 4.1).

Depending on the charge of the metal ion in the octahedron, minerals are called 'dioctahedral' (M³⁺, here Al³⁺) or 'trioctahedral' (M²⁺, here Mg²⁺). If the overall charge is neutral, only two thirds of all lattice sites are occupied for a dioctahedral mineral while all sites are occupied for the trioctahedral case.

The layers of the 2:1 sheet silicates may carry negative charge. Since this layer charge is (in two systems studied with X-ray microscopy - see sections 5.3 and 4.2) responsible for their behavior, the effects leading to
Figure 4.1: Schematic (from [51]) of the layer structure of the 2:1 sheet silicates.  
Right Three layers are shown with their negative charge and the positively charged cations in the interlayer to compensate for this negative charge.  
Left Detail of one layer, consisting of two tetrahedral sheets (T) and one octahedral sheet (O).  
The situation is different for the 1:1 sheet silicates (not shown in the figure). The layers do not carry charge, so there are no interlayer cations. Each individual layer builds up from only one tetrahedral and one octahedral sheet.  
this layer charge shall be listed a little more in detail:  

- trivalent metal ions of the octahedral sheet get replaced by bivalent ones (dioctahedral minerals, e.g. montmorillonite: $\text{Al}^{3+} \rightarrow \text{Mg}^{2+}$)

- bivalent metal ions of the octahedral sheet get replaced by ones having single valency (trioctahedral minerals)

- not all octahedral lattice sites (2/3 for the dioctahedral and 3/3 for the trioctahedral case) need to be occupied

- $\text{Si}^{4+}$ ions in the tetrahedral sheet might have been substituted by $\text{Al}^{3+}$

All of the above results in an excess of negative charge. This negative charge for the 2:1 sheet silicates is compensated by positive interlayer cations, usually sodium or calcium ions (shown in figure 4.1). Montmorillonite, a representative of the 2:1 sheet silicates, can then be classified
into sodium- or calcium-montmorillonite, depending on the kind of interlayer cation. Different forms of montmorillonite can be transformed into each other by ion exchange, e.g. the sodium montmorillonite described in section 4.2 has initially been a calcium montmorillonite. In addition, water and other agents like organics can penetrate into the interlayer, which explains the ability of this kind of clay mineral to swell as well as the altering distance between the sheets.

The 1:1 sheet silicates (e.g. kaolinite, see next section) almost do not swell at all and have therefore a well defined layer distance of 0.7 to 0.73 nm. Another important characteristic of the 1:1 sheet silicates is that their layers do not carry any charge and are kept together only by dipole-dipole forces, hydrogen bonds and van-der-Waals forces.

### 4.1.2 Delamination and Coagulation

The colloidal properties of clay mineral dispersions very much depend on the layer charge, the kind of interlayer cations, the temperature and on the kind and the concentration of ions (as well as other substances as e.g. polymers) in the solution.

A reaction taking place e.g. in sodium montmorillonite dispersions is the delamination in water and in solutions with low ion concentrations: The silicate layers that usually come as stacks of up to 10 sheets (so called 'tactoids') completely fall apart into single silicate layers (approximately 1 nm thick) and form a colloidal dispersion. Figure 5.5 shows a STXM image of a sodium montmorillonite dispersion that is delaminated to a high degree, resulting in a very weak contrast of diffuse structures that are too small to be resolved in the X-ray microscope.

Another important effect in 2:1-sheet mineral dispersions is coagulation. The negatively charged clay mineral sheets are surrounded by positively charged ions in solution (as shown in figure 4.2). Due to thermal movement, the cation concentration in solution decreases exponentially with the distance to the clay mineral layer. The gradient of this exponential decrease is a function of temperature, ion charge and concentration.
CHAPTER 4. CLAY-ORGANIC INTERACTIONS

Figure 4.2: Illustration of the dependence of the in-solution-concentration of counterions (cations, \( n^+ \)) and the co-ions (anions, \( n^- \)) in regions close to a negatively charged layer of a colloidal particle. The cation concentration decreases exponentially (see figures at the top) with increasing distance to the clay mineral particle, while the anion concentration in solution increases for increasing distance, due to repulsion by the negatively charged clay mineral surface. Apart from a clay mineral particle, the anion and cation concentration both converge to the equilibrium of cations and anions, the anion/cation concentration in the solution \( (c_a, c_b) \) respectively. The left/right side shows the low/high \( (c_a < c_b) \) ion concentration case: For increasing ion concentrations, the diffuse ion layer surrounding the colloidal particle gets compressed.

For high concentrations and/or bivalent or trivalent cations, the ion concentration in the diffuse cation layer drops so rapidly with the distance from the clay mineral layer that different clay mineral particles can come very close to each other. For small distances between the particles, the repulsive electrostatic forces can be overcome by attractive, short range van-der-Waals forces, thus resulting in a coagulation of the particles. The minimum concentration of a certain cation needed to cause this coagula-
tion for a given temperature is called ‘critical coagulation concentration (c.c.c.)’. The interaction between these clay mineral particles and the energy balance between van-der-Waals attraction, Born repulsion (which is taking into account that the particles can’t overlap) and electrostatic repulsion is described in the so called ‘DIVO theory’ (after Derjaguin, Landau, Verwey and Overbeek, who formulated this theory).

The last two subsections are a brief summary following a textbook on clay minerals edited by Jasmund and Lagaly [51]. The intention of these subsections is to define terms and to give motivation needed in the following for understanding and interpreting the spectromicroscopy experiments. Further reference on a large variety of colloidal systems (including clay minerals) can be found in a recently published book by Lagaly [61]. An introduction to the physical-chemical basics of colloids is given by Brezesinski and Mögel [17].

4.2 Colloid chemistry: TMA-PEG–montmorillonite

In collaboration with Sönke Ziesmer (Institut für Anorganische Chemie, Universität Kiel), an aqueous dispersion of 0.1-% sodium montmorillonite and 10 g/l trimethylammonium-polyethyleneglycol (TMA-PEG) with a molecular weight of 20000 has been analyzed using STXM in its hydrated state near the carbon absorption edge.

The sodium montmorillonite used in the studies has been processed from a crude Bavarian calcium bentonite (M47, Südchemie) by exchanging the calcium ions for sodium ions. Furthermore, iron oxides, carbonates and particles larger than 2 μm have been removed. However, the treatment for getting rid of organics (mainly humics, but also microorganisms can be found in the sample as it can be seen in figure 2.17) was skipped to leave the clay mineral as much as possible close to its natural state.

In the STXM experiments, only differential imaging across the carbon-K-absorption edge has been applied. This method is not sensitive for
the differentiation of several carbonaceous compounds in the sample. However, it is well justified here, because the clay mineral contains only 1-5 % organics. This results in the clay mineral-PEG system in a ratio of carbonaceous PEG versus carbon 'contaminants' (humics etc.) of 100:1, so non-PEG carbon can be neglected [150].

The PEG molecules are modified chemically by substituting the OH end groups by cationic trimethylammonium (TMA) groups, yielding a positively charged trimethylammonium polyethylene glycol (TMA-PEG). These positively charged TMA-PEGs interact with the clay mineral dispersion by attaching to the negatively charged clay mineral surface.

Polymers can be used to stabilize delaminated clay mineral dispersions by preventing coagulation mechanisms caused by ions as described in the previous section. In the so called 'steric stabilization', large polymer molecules attach to the clay mineral surface either by electrostatic or covalent binding, cover the clay mineral particle and, due to the large size of the polymers, generate a buffer so that the minimal distance these particles can have is too large for van-der-Waals forces to have effect, making the so-stabilized clay mineral dispersion insensitive to solutions with high ion concentrations. This stabilization mechanism depends very much on the kind of solvent and the type and molecular weight of the polymer macromolecules. An important application of such systems are drilling fluids in geotechnical applications, e.g., underwater oil drilling fluids that should have the same characteristics under all ionic conditions.

Polymers can also cause flocculation in clay mineral dispersions (as shown in the next section). The flocculation can be due to polymer molecules that can bridge different clay mineral particles. Furthermore, flocculation can be caused by flocculating ions (particularly calcium or aluminum ions), if the clay mineral surface is not covered sufficiently with polymer molecules.

The colloid chemical properties of these systems have been studied for different clay mineral dispersions and different TMA-PEGs. For the particular system studied here, a slight effect of sterical stabilization has been measured [151].
Figure 4.3: STXM images of an aggregate formed by an aqueous dispersion of 0.1-
\% sodium montmorillonite and 10 g/l trimethylammonium-polyethyleneglycol (TMA-
PEG with a molecular weight of 20000), taken near the carbon-K-absorption edge
[77, 82]. The images have $330 \times 330$ pixels with a pixel size of 1.20 nm.
Taking advantage of carbon absorption edge contrast in STXM, the details of this interaction between sodium montmorillonite and TMA-PEG can be revealed. The left bottom image in figure 4.3, taken at weak absorption for carbon, shows light structures (b) in the interface between a huge aggregate of almost 50 µm in diameter and the surrounding water. This is an excess of liquid TMA-PEG with an absorption coefficient at that energy that is even lower than that of water. Since the absorption for the TMA-PEG is so low, the absorption in the homogeneous spherical-shaped aggregate structure (a) must be caused by the montmorillonite. On length scales of our spatial resolution, there are no inhomogeneities visible. This suggests that the dispersed montmorillonite is either sterically stabilized by the TMA-PEG and/or the montmorillonite and PEG built a very homogeneous network-like structure. When tuning the X-ray energy to high carbon absorption, the carbon within the TMA-PEG can be highlighted. We can see the strong absorption of the excess of TMA-PEG in the aggregate-water interface, but in addition, we see the absorption of the TMA-PEG in the aggregate itself. In the aggregate-water interface, we can now see aggregate structures (see region (c) in the top right image in figure 4.3). The negative logarithm of the ratio of the image taken at 290 eV and the image at 280 eV (calculated by means of digital image processing) yields a qualitative carbon map (right bottom image of figure 4.3). In this carbon map, the amount of carbon scales linearly with the grey scale of the image. The pictures show in an unambiguous fashion the high affinity between the montmorillonite clay mineral and the TMA-PEG polymer molecules.

4.3 Agronomy: PAM–kaolinite

Beyond colloid chemistry, clay-organic interactions also play an important role in agronomy.

In field studies over the last years, it turned out that adding polyacrylamide (PAM, a biodegradable polymer, soluble in water) to irrigation water is a successful concept to prevent erosion [11, 119, 146]. A non-
negligible part of the earth’s crop lands (15–17 %) are irrigated and erosion is a serious problem in many cases. Furrow outflow soil losses of 5–50 tons/(ha,year) are common in the U.S. Pacific Northwest [119].

Small amounts of 10 ppm (a tablespoon of PAM for every 750 gallons, i.e. 2840 liters of irrigation water) show the desired effect of reducing erosion. There are two mechanisms making this work:

- PAM is a settling agent and flocculates finely dispersed clay particles carried in the water flow and makes them settle down. These fine particles are not transported away any more and can no longer clog pores of the soil where the water is applied. This characteristic of PAM is already used in water treatment plants so as to clean up the water by precipitating small particles (similar to the use of iron chloride for flocculating humics in sewage water [109], see also section 6.3.3).

- PAM stabilizes the topmost few millimeters of the soil it is applied on by aggregating the soil colloids. This topmost layer is most critical for erosion processes and it is sufficient to treat this layer; the PAM does not need to be mixed into the soil [11]. Therefore, the treatment is very efficient and only small amounts of around 1 kg/ha need to be applied.

In 1995 (the first year of commercialization), PAM was used on 20000 ha of furrow irrigated land, halting an estimated 900 000 tons of soil by reducing the sediment in runoff and promoting the ability of soil for water-uptake.

In collaboration with D. Schulze (Agronomy Department, Purdue University) and D. Stott (Agricultural Research Service, U.S. Department of Agriculture), we visualized the flocculation of a KG a-1 kaolinite clay suspension model system at various concentrations of polyacrylamide that are in good agreement with the concentrations needed for field applications. X-ray microscopy provides a very direct way of visualizing the effects of the flocculant. Figure 4.4 shows STXM images of fully hydrated
aggregates consisting of 0.2–2 \( \mu \)m kaolinite clay mineral particles at different concentrations of polyacrylamide (PAM), suspended in an aqueous solution of 0.01 M CaCl\(_2\). The structures become more and more dense for increasing PAM concentration.

Figure 4.5 shows an X-ray absorption spectrum of hydrated polyacrylamide as well as an image of a polyacrylamide solution. The polyacrylamide studied is a macromolecule with a molecular weight of 18 million g/mol. In the image, sample structures of spherical shape with a diameter between 100 and 300 nm can be seen. Assuming the radius of an atom to be 0.1 nm, the diameter of a solid sphere with the volume of 18 million atoms would be around 50 nm. Since the packing of the 18 million atoms is neither solid, nor hexagonally close-packed, but more determined by the band- or curled network-like structure of a polymer, it is very likely that the spheres seen in the STXM images are individual macromolecules or aggregates of very few macromolecules.

An attempt was made to visualize the carbonaceous PAM versus the inorganic kaolinite clay mineral particles in the clay mineral-PAM aggregate by carbon absorption edge contrast; however, this attempt was
CHAPTER 4. CLAY-ORGANIC INTERACTIONS

Figure 4.5: Left top: Absorption spectrum (taken in defocussed mode so as to minimize radiation damage effects; see section 7.1.1) of hydrated polyacrylamide (PAM) molecules with a molecular weight of 18 million g/mol. The spectrum does show a well defined edge step, but does not show any prominent sharp absorption resonances. Left bottom: Valence bond structure of acrylamide, the monomer of polyacrylamide. Right: STXM image of polyacrylamide (PAM) macromolecules, dissolved in water. The image has 300 x 300 pixels with a pixel size of 50 nm.

not successful. The clay mineral concentration in the sample (0.01 g/ml) is more than 3 orders of magnitude higher than the highest PAM concentration (0.005 mg/ml), making it hard or impossible to detect PAM within the dense clay mineral aggregate. Furthermore, PAM turned out to be very sensitive to radiation damage (as described in section 7.1.1), so the small amounts of PAM in the aggregate might have been destroyed by radiation during the experiment.
Chapter 5

Spectromicroscopy on emulsions

5.1 Introduction and motivation

Emulsions are systems of non-mixable liquids dispersed in each other. Since they are non-mixable, they are not stable over time unless they are stabilized. The main destabilization mechanisms of emulsions are shown in figure 5.1. Flocculation and creaming (or sedimentation in case of higher density of the dispersed phase) are reversible processes when a small amount of energy is supplied (for example by shaking), coalescence and breaking destroy the emulsion and only the supply of a large amount of energy is able to restore the initial state.

Here, we are focusing on oil-in-water-emulsions (o/w-emulsions), where the major constituent is water and oil is dispersed in the aqueous phase. In our everyday life we encounter o/w-emulsions in many different forms, both in nature (e.g. milk) as well as in industrial products like cosmetics, pharmaceuticals, and foods.

In order to be stable, oil-water emulsions require some kind of emulsifying agent which prevents destabilization mechanisms as shown in figure 5.1. While the liquid fats in milk are stabilized naturally by proteins and protein-like compounds (see section 5.2), industrial emulsions usually require high amounts of additives called surface active agents. These surfactants can be hazardous to humans and the environment and it is desirable to substitute them with harmless substances. One approach is to use solid colloids as emulsifying agents and X-ray microscopy studies
on emulsions stabilized in this way are shown in section 5.3. This short introduction is intended as a motivation for the X-ray microscopy experiments. A more detailed discussion on the colloid chemistry of emulsion and their stabilization can be found in [61].

5.2 Milk as a natural emulsion

In milk, the liquid fat droplets dispersed in the aqueous phase are stabilized by proteins and protein-like substances that are located in the interface between fat and water. Using X-ray microscopy, milk was imaged across the carbon-\(K\)-absorption edge in its natural hydrated state as shown in figure 5.2. For an energy of 290 eV, where carbonaceous material absorbs strongly, the liquid fat droplets dispersed in water appear black (left image of figure 5.2). For pre-edge energies (280 eV), the contrast between fat and water reverses compared to higher energies and
the oil droplets come out transparent and can be unambiguously identified as organic (right image of figure 5.2). What can be also seen in the right image of figure 5.2 is a small black rim in the interface between oil and water, possibly caused by absorption of the proteins. Although the proteins are way to small to be resolved individually in the STXM, they might contribute to the overall absorption of the resolution element they are in.

What can be seen as a side effect are small black horizontal stripes in the left image of figure 5.2. This is an effect of artifact structures of very small droplets moving in the hydrated environment and will be discussed in more detail in section 7.1.2.
5.3 Oil-water emulsions stabilized by solid colloids

It has been known for a long time that solid colloidal particles such as clay minerals can act as stabilizers in emulsions [94]. However, the surface of the clay mineral usually has to be modified by organic cations in order to obtain a stable emulsion. The oil-water emulsions examined here have been stabilized only by a clay mineral (sodium montmorillonite, Wyoming) and calcium/aluminium layered double hydroxide (LDH) without any additional surface active agents [1, 3, 15, 37]. The idea behind was that pronounced stabilization will be attained when two types of particles with different charges are used: clay minerals with negatively charged layers and double hydroxides with positively charged layers. Due to the interactions between clay mineral and layered double hydroxides, heterocoagulates form. A model for the heterocoagulation process is shown in figure 5.3. These heterocoagulates surround and cage the oil droplets in the emulsion and prevent them from coalescing and forming a separate oil/water phase. This new technique of stabilizing emulsions is of interest for a large variety of applications in cosmetics and pharmaceutical products, because it is desirable to avoid surfactants from an environmental and toxicological point of view.

5.3.1 Materials

The experiments on solid-stabilized emulsions were carried out as a collaboration with S. Abend (Institut für Anorganische Chemie, Universität Kiel). The process of making these solid stabilized oil-water emulsions and measurements on the parameters that are interesting from the colloid chemistry point of view like rheology and coalescence properties were done by S. Abend and are described in [3]. Here, X-ray microscopy experiments on these emulsions are shown [76]. The emulsions have also been studied with the Göttingen transmission X-ray microscope (TXM) at BESSY [130], but without any spectral information about the compounds. The emulsions analyzed with STXM contained water, 10 %v/v
Figure 5.3: Model of emulsions stabilization by heterocoagulation of oppositely charged particles (from [3]), namely by negatively charged clay mineral (sodium montmorillonite) and positively charged Ca/Al layered double hydroxide (LDH). From image a to d, the concentration of LDH increases from 0 to 100 % of the overall amount of solid emulsifier. For mixtures of clay mineral and LDH, the oil droplets are surrounded by a heterocoagulate network of oppositely charged particles (images b and c), thus impeding coalescence. X-ray microscopic images of this kind of system, which contains the same amount of montmorillonite and LDH are shown in figure 5.7.

For exclusively using LDH as emulsifier, the particles are stabilized by envelopes of hydroxide particles (image d), located right in the interface between oil and water (classical Pickering emulsion). STXM images of this kind of system are shown in figure 5.8.

Using only clay mineral as colloidal emulsifier (image a) yields the least stable emulsions. These systems were not studied in the X-ray microscope. Unlike the case shown in image d (only LDH), the plate like shape of the clay mineral particles does not allow the formation of a Pickering emulsion with colloidal particles right in the interface between oil and water.
paraffin oil and a total amount of 1 %w/w solid emulsifier in different ratios of clay mineral and LDH. For the preparation in the wet cell, the emulsions have been diluted with water to 0.1 %w/w emulsifying agent and 1 %v/v oil. Two emulsions are shown here: one with the same amount of clay and LDH (called X05), the other (called in the following X1) containing only LDH as emulsifying agent.

Besides demonstrating the capability of STXM for measurements on hydrated colloidal systems, our goal was to use X-ray microscopy to study the structural properties of these emulsions by imaging and spectroscopic analysis in order to better understand the stabilization process and its dependencies and compare it to existing models from colloid chemistry.

5.3.2 Imaging the single compounds of the emulsions

To be able to compare the structures of the clay mineral and LDH in the emulsions heterocoagulate to that of the single components, pure aqueous sodium montmorillonite and layered double hydroxide (LDH)

Figure 5.4: STXM images of an aqueous dispersion containing 0.5 %w/w Ca/Al layered double hydroxide, taken at the photon energies indicated in figure 5.6 (a) near the calcium absorption edge. The calcium map (right image) is calculated from the left and center image (strong and weak absorption for calcium); the grey scale bar indicates the amount of calcium in µg/cm². The images have 300 x 300 pixels with a pixel size of 80 nm.
dispersions have been imaged separately (figures 5.4, 5.5).

The center image of figure 5.4 was taken at 346 eV and shows that for weak calcium absorption, the layered double hydroxide is almost as transparent as water, so there is almost no contrast between water and LDH. This is a fortunate situation, because when imaging the clay mineral/LDH aggregate at an energy of 346 eV, it can be assumed that only the clay mineral structures are visible in the aggregate (see figure 5.7 a).

The contrast for the pure clay mineral dispersion shown in figure 5.5 is very weak due to the fact that the sodium montmorillonite is delaminated to a high degree. As a result, the clay mineral does not appear in the form of aggregates, but as small tactoids. The black spherical particles in the image appear to be quartz particles that are a natural component of this montmorillonite dispersion. Sodium montmorillonite dispersions have also already been studied in a hydrated state in the Göttingen TXM at BESSY [88].

Figure 5.5: STXM image of an 1% w/w aqueous sodium montmorillonite dispersion, taken at a photon energy of 290 eV (250 x 250 pixels, 70 nm pixel size).
5.3.3 XANES spectroscopy on paraffin oil and Ca/Al double hydroxide

In order to find photon energies suited for absorption edge contrast imaging, the absorptivity of oil/LDH was characterized first by taking absorption spectra near the carbon and calcium edges (figure 5.6). Photon energies chosen for imaging are marked with vertical dashed lines.

The absorption peak for paraffin oil at around 287.5 eV is rather wide due to the overlap of many resonances caused by transitions from many different bonding states.

For the spectra of the LDH near calcium edge, the absorption peaks corresponding to the $L_{II}/L_{III}$ shell electron transitions are shifted by 3 eV to higher energies compared to tabulated values [39] due to the ionic state of calcium in the sample.

5.3.4 Differential imaging near absorption edges

In a single image of the emulsion, taken at one photon energy, it is not possible to unambiguously identify oil droplets versus water and clay or quartz particles versus LDH concentrations. Therefore, differential imaging at different photon energies has been applied both near carbon and calcium X-ray absorption edges.

Taking advantage of absorption edge contrast for emulsion X05, oil could be distinguished from water near the carbon-$K$-edge and the Ca/Al layered double hydroxide from clay (contains no calcium) near the calcium-$L$-edge by taking images at photon energies with characteristic absorption properties for carbon and calcium respectively (figure 5.7).

A contrast reversal between water and oil can be observed when comparing images (c) and (d) in figure 5.7. This is in agreement with the absorption coefficients as calculated and shown in figure 1.2, though decane groups are only a part of paraffin oil.

For image (a) in figure 5.7, the calcium-rich LDH is transparent and only the clay mineral aggregates are visible in the aqueous phase. They
(a) X-ray absorption spectrum of Ca/Al layered double hydroxide suspended in water near the calcium-L-absorption edge. The spectrum is normalized in order to quantitatively determine the height of the absorption edge step as indicated by the horizontal dashed lines. (b) X-ray absorption spectrum of paraffin oil (bulk sample) near the carbon-K-absorption edge. For both plots, the photon energies used for taking the images shown in figure 5.7 and 5.8 are marked with vertical dashed lines.
Figure 5.7: STXM images of emulsion X05, which contains equal amounts of clay and layered double hydroxide, near the calcium and the carbon X-ray absorption edges. The photon energies of the X-rays used are indicated in Figure 5.6. a) Calcium weakly absorbing, the clay mineral visible. b) Calcium strongly absorbing, LDH visible. c) Carbon weakly absorbing, the paraffin oil droplet is transparent. d) Carbon strongly absorbing, the oil droplet appears black. The images were taken over a time period of 2 hours in the sequence a,b,d,c. During the experiment, the oil droplet remains at a fixed position, where heterocoagulate is caging the oil droplet (lower part of the image), but can disperse in regions without stabilizing envelope (upper part). The images have 300 by 300 pixels with a pixel size of 70 nm. The data acquisition time was 10 (20) min for each image taken near the calcium (carbon) edge.
CHAPTER 5. SPECTROMICROSCOPY ON EMULSIONS

Figure 5.8: STXM images of Pickering emulsion X1 (which contains only LDH as a solid compound), taken near the calcium-L-edge. From left to right: Strong/weak absorption for calcium, quantitative calcium map (the grey scale bar indicates the amount of calcium in µg/cm²). The image has 96 x 96 pixels with a pixel size of 75 nm.

Look more dense than in the pure montmorillonite dispersion (figure 5.5) because of aggregation caused by calcium and aluminium ions of the LDH and formation of the heterocoagulate. For image (a) and (b) in figure 5.7, carbon is in both cases strongly absorbing, because the calcium-L-edge is at higher energies than the carbon-K-edge. Therefore, the oil droplet appears also black in these two images.

Figure 5.8 shows a classical Pickering emulsion (X1) that is stabilized only by one solid compound, namely LDH. At a photon energy where calcium is strongly absorbing, it is not possible to decide if this is only a LDH aggregate or a stabilized oil droplet. The image taken for high calcium transmission reveals the oil droplet inside the LDH envelope. Calcium has been mapped quantitatively in the Pickering emulsion X1 (as described in section 3.3.2), indicated by the grey scale bar in figure 5.8.

For emulsion X1, the colloidal particles are located right in the interface of oil and water (figure 5.8) as predicted for Pickering emulsions by theory, while the heterocoagulate structures extend rather far away from the oil/water interface for the emulsion X05 (figure 5.7).
Chapter 6

Environmental applications

6.1 Introduction

Environmental aspects have already been playing a role in connection with experiments described in previous sections:

In section 4.3, studies on the interaction between hydrated polyacrylamide macromolecules and kaolinite clay mineral has been described. This interaction is already interesting by itself when studying clay-organic interactions, but the actual background is an environmental one: understanding the aggregation processes when adding PAM to irrigation water in order to prevent erosion.

In section 5.3, the environmental impact of substituting surface active agents as classical emulsifiers by colloids has been mentioned in a context of presenting STXM experiments on solid-stabilized oil-water emulsions.

In the next sections, more applications of soft X-ray spectromicroscopy that are directly connected to environmental questions are to be shown.

One characteristic that distinguishes these studies from the other ones is that the samples are no longer well defined laboratory systems, but are of natural origin and very heterogeneous. Therefore, interpreting the data is more difficult and one might need supplementary information provided by other techniques.
6.2 Decontamination of dredged estuarine sediment from NY/NJ harbor

The bay areas surrounding New York/New Jersey Harbor are naturally shallow, thus catching river-transported sediments and solids from surface point and non-point sources. Approximately 4 million cubic meter of sediment have to be dredged from navigational channels and berthing areas each year to ensure safe ship navigation. A large fraction of that is contaminated with PCBs (polychlorinated biphenyls), PAHs (polyaromatic hydrocarbons), dioxines and furanes [123]. After the regulations for off-shore ocean disposal of dredged estuarine sediment have become more stringent in 1992, only 25% of this material still pass the test criteria for unrestricted disposal. There are several concepts, what to do with the contaminated sediment. These include [124]:

- Solidification/stabilization by addition of cement, lime/fly ash, and/or proprietary chemicals to create solid aggregates where the contaminants are immobilized.

![Figure 6.1: New York harbor scene, viewed from the south](image)
Table 6.1: Sediment Mineralogy of Newtown Creek (from [53]).

<table>
<thead>
<tr>
<th>Mineral Species</th>
<th>Chemical Formula</th>
<th>Weight Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartz</td>
<td>SiO$_2$</td>
<td>66 to 75</td>
</tr>
<tr>
<td>Muscovite (Mica)</td>
<td>K$_2$O · 2MgO · Al$_2$O$_3$ · 8SiO$_2$ · 2H$_2$O</td>
<td>11 to 15</td>
</tr>
<tr>
<td>Amorphous Phase</td>
<td>Organics</td>
<td>3 to 13</td>
</tr>
<tr>
<td>Kyanite</td>
<td>Al$_4$O$_7$ · SiO$_2$</td>
<td>6 to 7</td>
</tr>
<tr>
<td>Hydrated Aluminium Silicate</td>
<td>19Al$_2$O$_3$ · 1.73SiO$_2$ · 9H$_2$O</td>
<td>5 to 6</td>
</tr>
<tr>
<td>Cronstedtite</td>
<td>4FeO · 2Fe$_2$O$_3$ · 3SiO$_2$ · 2H$_2$O</td>
<td>4 to 6</td>
</tr>
</tbody>
</table>

- Soil washing using proprietary surfactants, chelating agents and high pressure collisions to remove both organic and inorganic (e.g. heavy metals) contaminants.
- Thermal desorption: Removing surface contaminants by heating the sediment up to 550° C. No complete removal of carbon contaminants.
- High temperature thermal destruction: Complete destruction of carbon compounds and fusing the inorganic material into a stable matrix (temperatures for this process range from 750 to 3000° C). Beneficial re-use of this material as cement or glass.
- Creation of an artificial manufactured soil by adding compost, manure, and other materials to the de-contaminated or weakly contaminated sediment and using this 'soil' in suited regions (e.g. to cover Superfund sites, mining sites).

6.2.1 Carbon mapping in the contaminated sediment

The contaminated estuarine sediment sample studied in STXM stems from Newtown Creek that is running off the East River and is the boundary between Brooklyn and Queens (somewhere on the right hand side in
Table 6.2: List of the compound groups in Newtown Creek estuarine sediment (from [53]).

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Concentration (µg/g dry basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sulfides</td>
<td>7830</td>
</tr>
<tr>
<td>Total Organic Carbon (TOC)</td>
<td>73200</td>
</tr>
<tr>
<td>Total Polychlorinated Biphenyls (PCB)</td>
<td>5.26</td>
</tr>
<tr>
<td>Total Chlorinated Pesticides</td>
<td>0.462</td>
</tr>
<tr>
<td>Total Polyaromatic Hydrocarbon (PAH)</td>
<td>117</td>
</tr>
<tr>
<td>Bis-2-ethylhexylphthalate</td>
<td>48.6</td>
</tr>
<tr>
<td>Fluoranthenane</td>
<td>10.3</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>6.5</td>
</tr>
<tr>
<td>Others (24)</td>
<td>51.6</td>
</tr>
<tr>
<td>Total Dioxins</td>
<td>0.00645</td>
</tr>
<tr>
<td>Total Furans</td>
<td>0.0165</td>
</tr>
</tbody>
</table>

The natural carbon content (biological material like microorganisms and their degradation products like humics) in the sample is around 2% while the overall carbon content is 10% [52].

The carbon contaminants as described above are present in the sample in the parts-per-million (ppm) range, so although the sediment contains contaminants in concentrations that makes it harmful for humans and animals, the concentration is much too low to be detected in STXM. The major part of the non-biological carbon material is oil and tar. However, there is a connection between the contaminants and oil and tar since they act as carrier for the contaminants. Volatile carbon compounds if they were originally present are no longer present in the sample.

In collaboration with K. Jones and H. Feng (Department for Applied Science, Brookhaven National Laboratory), the contaminated sediment was analyzed in STXM at photon energies near the carbon-K-absorption edge and compared to a sediment that underwent a cleaning procedure,
Our goal was to map the carbonaceous material in the hydrated marine sediment sample by X-ray microscopy near the carbon absorption edge and to visualize the structures, density and distribution of the carbon material in the sample as well as to learn how this material interacts with other, non-carbonaceous material in the sample (as e.g. mineral particles). For instance, one important question for studies on remediation techniques for the contaminated sediment was whether the sediment particles are coated by a layer of carbonaceous contaminants or not. Fur-
thermore, it should be proven that also this kind of sample can be studied in the STXM near the carbon absorption edge.

Figure 6.2 shows two object regions of the contaminated sediment sample suspended in water at either side of the carbon-K-absorption edge. Carbon can be clearly seen in more dense spots (as indicated by the 'a' in the image), most likely tar or biological material, and in more extended liquid phases (b), most likely oil [77, 80]. A carbon coating could not be shown on length scales of the spatial resolution.

The carbon mapping done by just imaging across the carbon absorption edge is a good tool to prove the existence of carbon in the sample, but it is insensitive to the kind of carbon compound. Beyond that, more information on the chemical speciation with high spatial resolution was obtained by taking XANES-spectra.

6.2.2 Comparison of XANES-data of contaminated and treated sediment

The contaminated sediment was treated by a proprietary soil washing technique developed by BioGenesis Enterprises. This washing technique includes several steps of physical and chemical treatment (shown in figure 6.3) and is capable of removing PAHs, dioxins, furans, metals and PCBs with an effectiveness between 80 and 100%. The treated sediment (see step 6 in figure 6.3) was analyzed spectroscopically and compared to the contaminated sediment (figure 6.4). Comparing spectrum 2 (treated sediment) in figure 6.4 with spectrum 3 (contaminated sediment) in the same figure shows that they look alike. The interpretation of this is not unambiguous and allows two possible explanations:

- the contaminant has not been removed completely by the cleaning procedure and is still present in the treated sample (in agreement with bulk measurements on the treated sample)
- a carbonaceous material with a chemically indentical fingerprint (XANES-spectrum), e.g. a detergent, was induced by the cleaning
CHAPTER 6. ENVIRONMENTAL APPLICATIONS

Figure 6.3: Schematic of the contaminated sediment cleaning procedure developed by BioGenesis Enterprises (from [123]).
Figure 6.4: Comparison of contaminated and treated sediment by XANES image sequences. Two images of the “stack” dataset are shown as representatives for the whole data set, a pre-edge (weak carbon absorption) and an edge image (strong carbon absorption) and their respective absorption spectra (right side). The regions the spectra were obtained from are marked with an ‘x’. The upper and the middle row shows treated sediment data while the lower row shows data taken on a contaminated sediment sample.
process

Spectrum 1 and 2 of the treated sediment sample (figure 6.4) point to carbon in a different chemical environment and confirm the heterogeneity of the sample. A final conclusions would require better data statistics (more “stack” datasets than just the few ones shown here) and a combination and comparison with other analytical techniques. However, studying these samples with high spatial resolution and chemical sensitivity in an X-ray microscope might help to better understand the cleaning procedure and its efficiency and to optimize the process parameters.

6.3 XANES-spectroscopy of humics and its applications

Humic substances (also called 'humics') are anionic polyelectrolytes generated by degradation of organic matter in terrestrial and aquatic systems. Since a large fraction of the carbonaceous material in these systems is present in the form of humics, studying this class of compounds is important. A textbook by Stevenson [125] gives a thorough introduction to humus chemistry. German readers might want to have a look at the book written by Ziechmann [149].

6.3.1 Introduction and Motivation

The compound class of humics is very heterogeneous and contains a large variety of different chemical groups. The terminological distinction between 'humic acid', 'fulvic acid' and 'humins' is made by the molecular weight and solubility in water. Fulvic acids have a lower molecular weight and are much more soluble than humic acids. Humins are not soluble in water at all (see also figure 6.9).

In a context of environmental science, the following questions have been addressed:
CHAPTER 6. ENVIRONMENTAL APPLICATIONS

Figure 6.5: Proposed chemical structure of a humic acid (from http://www.ar.wroc.pl/~weber/kwasy2.htm).

- XANES-spectra near the carbon absorption edge have been taken in order to prove that the difference in the chemical characteristics of humin and fulvic acid can be measured with spectromicroscopy (see subsection 6.3.2).

- Transport phenomena of heavy metals in ground water aquifers are bound to humics that can act as a complexing agent for metals. The interaction of humics with colloidal iron particles has been studied in STXM (see subsection 6.3.3).

- In soils, besides other organic material like microorganisms, humic substances are a major constituent. A schematic on the division of carbon compounds in soil can be found in figure 6.9. Carbon in an aqueous soil aggregate has been mapped using STXM (see subsection 6.3.4).

These projects have been initiated by J. Thieme (Institut für Röntgenphysik, Universität Göttingen) within a general user proposal entitled 'Wavelength-sensitive X-ray microscopy studies of colloidal dispersions' at X-1A beamline at the NSLS.
6.3.2 Discussion of the XANES-spectra

The humin studied here originates from the A₃-horizon of the calcic phaeozem near Göttingen. This humin was extracted by sodium pyrophosphate, following a procedure described in [125], in order to induce as few structural changes as possible. The sample was provided by J. Niemeyer (Fachbereich VI-Geowissenschaften, Universität Trier).

The humin was analyzed spectroscopically in a wet and dry state and compared to a dry sample of a synthetic fulvic acid, provided by A. Knöchel and K. Franzas (Institut für Anorganische und Angewandte Chemie, Universität Hamburg). In figure 6.9, humins are defined as insoluble in water. Speaking of 'hydrated humins' means in this context that the humins studied were present in the hydrated sample as colloidal particles and not in a dissolved state.

Figure 6.7 shows the significantly different looking spectra of these samples:

- The resonance peak at 289 eV (characteristic for the C=O double bond), due to the more acidic character of fulvic acid, is more prominent for fulvic acid than for the humin.
- The two resonances at around 300 eV can be assigned to potassium. Due to dilution effects, these resonances are only present in the dry humin sample, not in the hydrated humin sample.
Figure 6.7: STXM XANES-spectra of dry and hydrated humin and dry fulvic acid, covering a photon energy range from the carbon-K to the potassium-L absorption edges [109, 131]. For better comparability, the data has been plotted shifted along the y axis.
Table 6.3: A possible assignment [109,131] of the resonances in the humin and fulvic acid spectra (figure 6.7) to functional chemical groups using reference data from [43]. The potassium resonances are shifted 3.5 eV to higher energies compared to the values as tabulated in the Henke-Data [30], because of the ionic state of the potassium.

<table>
<thead>
<tr>
<th>energy [eV]</th>
<th>type of resonance</th>
</tr>
</thead>
<tbody>
<tr>
<td>285.9</td>
<td>( \pi_1 ) C_6H_6</td>
</tr>
<tr>
<td>287.0</td>
<td>3s CH_3, CH_2</td>
</tr>
<tr>
<td>287.9</td>
<td>3s CH_3, CH_2</td>
</tr>
<tr>
<td>288.4</td>
<td>( \pi^* ) C=O, ( \pi_2^* ) C_6H_6</td>
</tr>
<tr>
<td>292.0</td>
<td>( \sigma^* ) C - C</td>
</tr>
<tr>
<td>293.9</td>
<td>( \sigma_1^* ) C_6H_6</td>
</tr>
<tr>
<td>298.1</td>
<td>K L_{III} (Henke: 294.6 eV)</td>
</tr>
<tr>
<td>300.8</td>
<td>K L_{II} (Henke: 297.3 eV)</td>
</tr>
</tbody>
</table>

Comparing the dry humin sample to the hydrated humin sample, it can be seen that the resonances between 285 and 288 eV differ in height and position. The process of transferring the sample into a hydrated state affects the bonding states of the humin.

Knowing the XANES-spectra of humin might make it possible in future studies to distinguish humin from other organic matter in soils by picking photon energies of distinct resonances for imaging in STXM.

### 6.3.3 Interaction of iron particles with humics

In ground water aquifers, transport mechanisms of heavy metals are often bound to humics, where humics influence and induce aggregation processes.

In anaerobic groundwater aquifers, iron is present in a reduced bivalent state. When it gets in contact with aerobic water, an oxidation takes place and the trivalent iron ions can form insoluble compounds that appear as colloidal particles. These particles can act as nucleation sites for
additional aggregation processes, e.g., with humic substances. A similar process is used in water treatment plants, where iron chloride helps to precipitate organics from sewage. The flocculation of humics by iron chloride has been also studied in STXM [109].

To study these processes, humic substances were added to an aerobic groundwater (containing iron ions in solution), which was oxidized afterwards, thus generating a precipitate of colloidal iron particles. The colloidal structures formed in consequence due to the interaction between iron and humics were then imaged in a dry state using the “stack” spectromicroscopy mode (as described in section 2.4.2). The black dots in
image “2 of 118” in figure 6.8 are presumably iron particles around which the humic substances are aggregated in a fluffy shape.

These experiments were done for iron and humics as well as for iron and microorganisms, where the colloids tend to accumulate at the microorganisms (left half of figure 6.8). The difference in the C-XANES-spectra was shown for humic substances versus cell walls (see spectra in figure 6.8), so it should be possible to distinguish microorganisms against humics in a heterogeneous sample by tuning the photon energy to specific resonances for humics or the microorganisms.

The spectrum for the humics shown in figure 6.8 is in its shape in agreement with the spectrum of dry humic substance as taken with the standard spectra acquisition mode as shown in figure 6.7, although it seems to be slightly shifted along the x axis (energy) due to possible drifts in the beamline monochromator calibration at that time.

### 6.3.4 Mapping carbon in aqueous soil aggregates

Soil colloids consist of inorganic particles such as clays and iron oxides, and organic components as listed and distinguished a little more in detail in figure 6.9.

Chemical and physical reactions are generally enhanced in colloidal systems due to the large surface areas (e.g. in clay minerals up to 800 m²/g and humic substances up to 1000 m²/g) of the colloidal particles. The available surface in soil and the pore structure of the soil determines the ability of soils for water uptake (see also the discussion on prevention of erosion in subsection 4.3). Capillary forces that increase with decreasing pore size have an effect on the water in soils. Very fine pores can hold water within the soil with a pressure of up to 15 bar, making this water unavailable for plants [100]. For this reason, plants almost cannot grow on pure clays with fine pores. Coarse pores in turn cannot hold the water against gravity so the water is seeping into deeper layers. Medium sized pores in soils (0.2–10 µm in diameter) are the main source of water for plants. In a healthy soil, there must be a balanced distribution of
pore sizes. In farming, plowing makes sure that soils stay loosely-packed and are sufficiently ventilated. Furthermore, the bioavailability of nutrients as well as toxicants for plants is determined by the surface properties of soil colloids.

X-ray microscopy is a particularly advantageous technique for studying hydrated samples because the size of soil colloids and their structural association (pores) are in a range accessible by this technique. X-ray microscopy might help to make contributions for a better understanding of the chemical and physical properties of soils, especially when using the possibility of mapping carbon by spectromicroscopy.

X-ray microscopy studies on soil samples have already been carried out with the Göttingen transmission X-ray microscope at BESSY. The dynamical behavior of the colloidal fraction of different aqueous soil samples have been studied, i.e., different electrolytes have been added while ob-

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**Organic compounds of soil**

- Living organisms (edaphon)
- Soil organic matter
  - Unaltered materials (undecayed plant and animal tissues)
  - Transformed products (humus)
- Nonhumic substances (e.g. carbohydrates, lipids, amino acids)
  - Fulvic acids (water soluble under all conditions)
  - Humic acids (soluble in water for pH < 2)
  - Humin (non-soluble in water)

---

Figure 6.9: Organic compounds of soil (after http://www.ar.wroc.pl/~weber/def2.htm).
serving the changes induced by changing the chemical environment [98]. Furthermore, the interaction of colloidal soil particles with humics and cationic detergents were studied [132].

First results of carbon mapping in a hydrated soil sample are shown in figure 6.10. The images clearly demonstrate the capability of STXM to investigate this kind of sample and show the inhomogeneity of the carbon distribution within the aggregate. However, in this data, there is not yet any sensitivity for distinguishing the large variety of different carbonaceous compounds (as listed in figure 6.9).

As already mentioned earlier, a perspective for future studies is to distinguish humics in soil aggregates from other soil organic matter taking advantage of the known absorption XANES features of humics.
Chapter 7

Conclusion and Perspectives

7.1 Limitations of the technique

It has been proven that X-ray spectromicroscopy is a well suited tool to investigate colloidal systems. However, there are some limitations of this technique, some of them are general for X-ray microscopy as

- Radiation damage
- Artifact structures of small particles in hydrated samples

and some are related to the characteristics of colloidal systems:

- size of sample components below resolution limit (e.g. microemulsions)
- size of particles in the sample is too large (preperability, transmission)

7.1.1 Radiation damage

Imaging samples with good photon statistics in high resolution soft X-ray microscopes exposes them to very high radiation doses $D$ in the range of $10^7$ Gy. This due to the fact that soft X rays and matter interact rather strongly with each other. The desirable goal to increase spatial resolution $t$ makes in addition the radiation dose $D$ go up drastically as the dose depends from the resolution as $D \propto 1/t^4$. 

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What effects this radiation exposure will be showing depends very much on the kind of material examined and its molecular chemistry. Some polymers for example might be very sensitive to radiation exposure while others are not. Polypropylene gets destroyed instantly (in less than 1 second) when exposed to the beam provided by the condenser zone plate of the TXM at BESSY. On the other hand, polyimide resists the same kind of X-ray beam for many hours and is therefore used as thin carrying foil for the BESSY TXM wet specimen chamber [113].

In biological samples, these high radiation doses often induce visible changes to the sample. Chemical bonds get broken up and the structures change and get blurred in the image by movement of the molecule fractions. This movement is driven by diffusion, transporting these fractions in timescales of milliseconds within length scales of the resolution of an X-ray microscope [110].

A solution to overcome this problem is found by applying cryo-techniques. The hydrated biological sample is deep frozen rapidly and kept at liquid nitrogen temperature during the experiment, therefore increasing the sample’s radiation tolerance many orders of magnitude. This takes advantage of two effects. A more minor effect is that the number of chemical bonds broken per energy unit of radiation absorbed decreases with temperature. The major effect is that the molecule fractions generated can not be transported away by diffusion since they are fixed within a matrix of amorphous ice. This allows one to expose the cryo fixed sample to many orders of magnitude more radiation before visible changes occur (limitation in this case is the radiolysis of water at $10^{10}$ Gy) [110,111].

Chemical fixatives (as glutaraldehyde) can be used to treat biological cells in order to look at them at room temperature. However, these fixatives increase the radiation tolerance much less than cryo techniques do, and in addition the fixatives are likely to induce artifacts in a cell.

Cryo techniques have been applied to X-ray microscopy with the Göttingen TXM at BESSY [112,114,115] and more recently with the Stony Brook STXM at the NSLS [65].
CHAPTER 7. CONCLUSION AND PERSPECTIVES

Figure 7.1: STXM images of an aggregate of hydrated polyacrylamide (PAM) showing structural changes due to radiation damage. After the first/second image, a spectrum was taken in region a/b. Since these images were meant to be for cross-check purposes only, the image quality is reduced. The images have 80 x 80 pixel with a pixel size of 100 nm.

For almost all the data shown in previous chapters, we do not have any evidence that the samples suffer radiation damage. This might be partially due to the fact that the radiation dose delivered to the specimen is lower in STXM, where the specimen is followed by an X-ray detector, than in the TXM, where a 10–20 % efficient zone plate and the detector follows the specimen.

In the STXM studies shown here, there was only one substance, namely polyacrylamide (PAM), that showed changes in its structure and its XANES-spectrum due to exposure to X rays as illustrated in figure 7.1. Despite this sensitivity of PAM to radiation, useful spectra of polyacrylamide could be obtained. Since the sample does not contain any other carbonaceous material besides polyacrylamide and the sample aggregate extends a couple of microns, it was possible to take spectra using an X-ray beam that is defocused to around 2 μm in diameter, thereby decreasing the dose several orders of magnitude. After acquisition of the several XANES-spectra, double-checking the last spectrum showed that the sample was not damaged. In the defocussed mode, radiation damage starts to take place much later, after having repeatedly taken spectra.
7.1.2 Artifact structures of small particles in hydrated samples

In Figure 7.2 (as well as in Figure 5.2), black horizontal lines can be seen that are exactly oriented in the direction of the scanning movement in the microscope. These structures cannot be real, because they cannot be reproduced. Effects like this one can occur if the sample slightly touches and scratches along either the proportional counter or the order sorting aperture during scanning. But this would affect also other, larger image structures that are imaged properly. After excluding this possibility, the effect was determined to be caused by small particles that are not clamped between the two silicon nitride windows and can be floating around in the aqueous environment. The two possible explanations for that are explained in the following.

Brownian molecular motion

Since the sample is scanned linewise horizontally, these black stripes could be very small droplets or particles that happen to move (due to their small size driven by Brownian molecular motion) within the aqueous environment at almost the same speed but opposite direction as the sample holder.

The Brownian molecular motion occurs randomly distributed in all directions and with different velocities, depending on the size of the particles. This allows some of them to stay with the X-ray focus and therefore, a line-shaped absorption occurs, faking a line-shaped particle.

Tests show that this effect occurs between 1 and 20 msec dwell time per pixel, i.e. for velocities of the sample movement between 100 and 5 \( \mu \text{m/s} \) for 100 nm pixel size.

For a given temperature, the velocities of the particles (mass \( m \)) are distributed proportional \( 1/\sqrt{m} \) over a wide range. The square average movement of a particle over a time period \( t \), driven by Brownian molecular motion can be calculated using the Einstein-Smoluchowski equation [27],

\[
\bar{x}^2 = 3 D t = \frac{k T}{2 \pi \eta r} t
\]  

(7.1)
CHAPTER 7. CONCLUSION AND PERSPECTIVES

Figure 7.2: STXM images of an aqueous latex dispersion, consisting of a polystyrene matrix with $-COOH$ and $-SO_3^-$ functional groups. Small particles can move within the hydrated environment, causing artifact structures of small particles (horizontal stripes) in the image. The sample was provided by R. Zimel (Institut für Anorganische Chemie, Universität Kiel). The left/right image has 250 x 250 / 200 x 200 pixels. Both images have a pixel size of 100 nm.

where $D$: diffusion coefficient, $T$: temperature, $k$: Boltzmann constant ($1.380658 \cdot 10^{-23} \text{J/K}$), $\eta$: viscosity of the medium the movement happens in (for water at 293 K: 1.002 mPa·s [60]) and $r$: radius of the particle.

From electron microscope measurements on the dry Latex sample [152], it is known that the particles might be as small as 35 nm in diameter, resulting in a displacement (according to the equation above) of around 6 $\mu$m per second. This is in the same range as the movement of the sample holder in STXM.

**Inertia of small particles within the accelerated wet specimen chamber**

Another approach to explain the effect shown in figure 7.2 is looking at a small particle as wanting to sit still due to its own inertia, while the scanning stage drags the fluid inside the wet cell past the particle [44]. This happens at each new scan line, when the scanning stage slows down
at the end of the finished scan line and then accelerates again in the opposite direction for the next scan line. At the onset of scanning motion, the particle will soon see a flow of fluid going past. Friction forces will accelerate the particle. The question is, in which timescales this happens.

Considerations for the same example parameters as in the last paragraph (assuming a density of the particle of 1 g/cm³, a velocity of the scanning stage of \(v = 10^{-5} \text{ m/sec}\) for 100 nm steps every 10 msec, a radius of the particle of \(r = 17.5 \cdot 10^{-9} \text{ m}\), and a viscosity of \(\eta = 10^{-3} \text{ Pa s}\)) yields for the Reynolds number

\[
N_R = \frac{\rho v r}{\eta} = 1.75 \cdot 10^{-7}
\]  

This Reynolds number is very low so there is no turbulent flow. The drag force on the particle is then given by Stokes’ law:

\[
F = 6\pi rv\eta
\]  

If this horizontal friction force is in the same order of magnitude as the gravitational force on the particle, the effect should not be observed. For the example particle of 35 nm diameter, the friction force according to the Stokes law is \(3.3 \cdot 10^{-15} \text{ N}\), the gravitational force \(G\) is \(2.2 \cdot 10^{-19} \text{ N}\), orders of magnitude smaller than the friction force, so the scenario described should be possible.

The friction force should produce an acceleration of the particle, so we can write

\[
ma = m\frac{dv}{dt} = \frac{4}{3} \pi r^3 \frac{dv}{dt} = 6\pi rv\eta
\]  

This yields a differential equation for the movement of the particle:

\[
\frac{1}{v} dv = \frac{9\eta}{2r^2 \rho} dt
\]  

Thus we’ll have an exponential change in velocity (that is, the particle will soon be dragged along with the fluid) with a characteristic time of
The characteristic time for the acceleration of a 35 nm diameter particle would be $6.8 \cdot 10^{-11}$s. For larger particles, this characteristic time increases as expected, for instance a particle with a diameter of 200 nm would have a characteristic acceleration time of 2 nsec. This is considerably more, but the time scale of accelerating these small particles is much smaller than what we would need to explain the artifact structures in the STXM images. To the best of our knowledge, we think that this effect must be caused by Brownian molecular motion.

In the X-ray microscopy community, there have always been efforts of pushing the achievable spatial resolution to smaller numbers by improving zone plate nanofabrication technology. In the context of in water dispersed systems however, this demand must be considered carefully. Due to the limitations of molecular motion, pushing the imaging of hydrated samples to higher resolution only makes sense, if the small sample structures of the sample are either cryo-fixed (and prevented from moving around) or, at room temperature, hydrated and embedded in a large, non-moving matrix as for example an aggregate. The issue of spatial resolution will be discussed from other perspectives a little more in the next section.

### 7.1.3 Size of structures in the samples and resolution

The issue of small particles moving around has already been addressed in the previous section: Small particles, floating around freely in an aqueous medium, might possibly be large enough to be resolved in the X-ray microscope, but actually are not, because of their movement.

Besides this effect, domains or particles might be really too small to be resolved: In section 1, the term ‘colloidal’ has been defined as systems with particles or phases ranging in size from 1 μm down to 1 nm, so not all colloidal systems can be studied in an X-ray microscope. Microemulsions
CHAPTER 7. CONCLUSION AND PERSPECTIVES

Figure 7.3: STXM images of a microemulsion that has phase separated. The domain that can be seen in the image is almost 20 µm in diameter, which is unrealistic for this kind of system. However, effects in the interface between the two phases can be seen when imaging across the carbon absorption edge. The images have 300 x 300 pixels with a pixel size of 80 nm.

For example, where the domains are sub-100 nm down to several nm in size [61], are bridging this limit, so some systems might be studied, others not.

Microemulsions are thermodynamically stable mixtures of water, oil (here used as a general term for hydrocarbons) and surfactants. Their spontaneous formation and transparency makes them distinct to regular emulsions. They contain different carbonaceous compounds which make them in principle predestined to be studied by spectromicroscopy techniques in STXM near the carbon absorption edge. But, as mentioned before, the domains in many microemulsions might be much too small to be studied by X-ray microscopy. Figure 7.3 shows a microemulsion made by S. Abend (Institut für Anorganische Chemie, Universität Kiel) following a procedure as described in [61], page 273: 10 mL toluene, 2 mL water, 0.97 g sodium oleate are mixed and give a cloudy mixture
that clears by forming a microemulsion when titrated with approx. 1.4 mL pentanol. Although the images look interesting and changes in the interface between the two phases can be observed (most likely due to the sodium oleate molecules that act as emulsifier), these pictures are not of interest for colloid chemists, because the microemulsion has not formed correctly or separated to a 'macroemulsion' afterwards [35].

Going to the other extreme on the dimensions scale, very large structures, large aggregates or large single clay mineral particles for instance may be hard or impossible to study. First of all, particles over a diameter of 10 μm can not be easily prepared in the wet cell and break the silicon nitride windows quickly. But even if such samples can be prepared in the wet specimen chamber, it would not make any sense to study them with soft X rays. Unlike a biological cell, that might be up to 10 μm thick and can be imaged in the “water window” because of its large water content (up to 80 %), the transmission is almost zero for dense mineral materials of 10 or more μm in diameter.

Using higher energy X rays (in the range of several keV) makes this kind of samples accessible for studies, because the absorption coefficient decreases drastically and samples with a thickness of up to the mm-range can be studied. But for such studies, the sample preparation must be completely different.

### 7.2 Summary and Outlook

Fully hydrated colloidal systems, prepared in a wet cell based on silicon nitride windows [79] have been studied employing soft X-ray spectromicroscopy near the carbon-K and calcium-L X-ray absorption edges for the first time in the X-1A scanning transmission X-ray microscope at the NSLS.

The interpretation of the data is straightforward for well defined laboratory systems from colloid chemistry (see sections 4 and 5.3). Information about the structure and association of the single components in
hydrated aggregates was revealed that is currently not accessible by any other means and can help to support or disprove theoretical models about the interaction of these components.

There are certainly still a lot more colloid chemistry systems that can be studied with spectromicroscopy near the carbon-\(K\)-absorption edge. Currently, experiments dealing with the heterocoagulation of positively charged polystyrene latex and negatively charged montmorillonite are in progress [2].

Model calculations (see chapter 3) showed the requirements of detecting elements near X-ray absorption edges: With the electron storage rings currently available as high brightness X-ray sources, elements can easily be detected in a concentration down to 1 %. Trace element mapping will require much higher photon rates and is more likely to be successful in fluorescence mode for elements with their absorption edges in the several keV range. This has already been demonstrated by micro-XANES without the use of focusing optics in the micrometer resolution range on soils and rice leaves [116,118].

Due to their heterogeneity, it is much more difficult to draw final conclusions when studying samples from environmental science (see section 6), especially when investigating systems with different unknown carbonaceous compounds near the carbon absorption edge. However, also in this case, at least useful information about size, shape and association of the carbon phases in the sample can be obtained. Using other techniques supplementary should be considered. FTIR (Fourier transform infrared spectroscopy) for instance is chemically much more sensitive than XANES spectroscopy, however with a reduced spatial resolution. Furthermore, X-ray spectromicroscopy in the several keV energy range becomes more and more available for users and allows to access the absorption edges of many more higher \(Z\) elements, thus opening potential new sources of information.

'In situ' measurements on hydrated samples with high spatial resolution in combination with the capability of highlighting elements naturally by their absorptivity are currently not possible by any other technique
and are one of the most intriguing advantages of X-ray microscopy.
Appendix A

Quantitative elemental analysis near X-ray absorption edges

Figure A.1: Schematic illustration (from [29]) of the mass absorption coefficients for the wavelength range around the X-ray absorption edge of a certain element. The edge element's absorption properties are shown in the graph with the solid line, all other elements in the sample contribute to the absorptivity as indicated by the dashed line (y: index for the absorption edge element, r: index for the overall contribution of all other elements, $m_y/m_r$: mass densities [g/cm$^2$], $\mu_{jk}/\rho$: mass absorption coefficients [cm$^2$/g])

Equations for evaluating the quantitative analysis near X-ray absorption edges are derived following a method as described in [29] that does neither require the sample
APPENDIX A. QUANTITATIVE ELEMENTAL ANALYSIS

thickness to be known, nor requires explicit knowledge of the densities, but express the properties per sample area.

The attenuation of X rays passing through the sample are described by the Lambert-Beer-law (indices are explained in figure A.1). Transmitted intensity at the short wavelength ($\lambda_1$) side of the absorption edge:

$$I_{11} = I_{01} e^{-(\frac{\mu}{\rho})_y m_y - (\frac{\mu}{\rho})_y m_e}$$

Transmitted intensity at the long wavelength ($\lambda_2$) side of the absorption edge:

$$I_{12} = I_{02} e^{-(\frac{\mu}{\rho})_y m_y - (\frac{\mu}{\rho})_y m_e}$$

Eliminating $m_e$ from these equations allows to write the unknown $m_y$ as:

$$m_y = \frac{\ln \left( \frac{I_{11}}{I_{11}'} \right) - \left( \frac{\mu_1}{\rho} \right)_y \cdot \ln \left( \frac{I_{12}}{I_{12}'} \right) \cdot \left( \frac{\mu_2}{\rho} \right)_y \cdot \left( \frac{\mu_1}{\rho} \right)_y \cdot \left( \frac{\mu_2}{\rho} \right)_y}{\left( \frac{\mu_1}{\rho} \right)_y - \left( \frac{\mu_2}{\rho} \right)_y \cdot \left( \frac{\lambda_1}{\lambda_2} \right)} \quad (A.1)$$

For wavelengths $\lambda_1$ and $\lambda_2$ from a narrow interval, the expression $(\frac{\mu}{\rho})_y/(\frac{\mu}{\rho})_y$, can be approximated by $(\frac{\mu}{\rho})_y/(Z\lambda_2)^\gamma$, whereas for $f(Z\lambda_2)$, a value of 3 can be assumed, because of $\mu \approx \text{const.} Z^\gamma \lambda^\gamma$.

Taking this into account, $m_y$ can be written as:

$$m_y = \frac{\ln \left( \frac{I_{11}}{I_{11}'} \right) - \left( \frac{\lambda_1}{\lambda_2} \right)^3 \cdot \ln \left( \frac{I_{12}}{I_{12}'} \right) \cdot \left( \frac{\lambda_1}{\lambda_2} \right)}{\left( \frac{\mu_1}{\rho} \right)_y - \left( \frac{\mu_2}{\rho} \right)_y \cdot \left( \frac{\lambda_1}{\lambda_2} \right)} \quad (A.2)$$

The less $\lambda_1$ and $\lambda_2$ differ from each other, the better the approximation $(\frac{\lambda_1}{\lambda_2})^\gamma \approx 1$ is met. Using this approximation, equation A.2 can be even more simplified if in addition the incident intensities $I_{01}$ and $I_{02}$ are equal or are normalized to equal values:

$$m_y = \frac{\ln \left( \frac{I_{12}}{I_{11}} \right)}{\left( \frac{\mu_1}{\rho} \right)_y - \left( \frac{\mu_2}{\rho} \right)_y}$$

However, in our experiments, the photon energies across the edge differ significantly from each other, so equation A.2 has been used for quantitative analysis. The mass absorption coefficient $(\frac{\mu}{\rho})_y$ for the edge element to be mapped can be calculated from:

$$\frac{\mu}{\rho} = 2 \cdot \frac{N_a}{M} \cdot r_e \cdot \lambda_i \cdot f_1(\lambda_i) = 3.394 \cdot 10^{14} \cdot \frac{\text{cm}}{\text{Mol}} \cdot \frac{f_1(\lambda_i)}{M} \cdot \lambda_i \quad (A.3)$$
whereas $N_a$: Avogadro constant ($6,022 \cdot 10^{23} \frac{1}{mol}$), $M$: molar mass, $r_e$: classical radius of the electron ($2,88 \cdot 10^{-15} m$), $f_I$: imaginary part of the atomic scattering factor (tabulated in [30]). All other variables (wavelengths and intensities) in equation A.2 are data, known from the experiment.
Appendix B

Drawings of the wet specimen chamber

The current wet cell design uses two aluminium parts (see figures B.3 and B.4) with an O-ring (inner diameter 3/4", outer diameter 7/8", width 1/16") between them. The two parts are screwed together (figure B.2) with flat head screws (#0-80 thread, 1/8" long). The silicon nitride windows are made of 200 micron thick, both side polished 3" silicon wafers and have a 100 nm thick silicon nitride membrane. As window sizes, 3 x 3 mm windows in a 9 x 9 mm silicon wafer frame as well as 4 x 4 mm in a 8 x 8 mm frame have been used. One of the windows is glued airtight with nailpolish to the downstream part (figure B.4) of the wet cell, the other to the shim part (figure B.1), which is in turn glued to the upstream aluminium part (figure B.3).

Figure B.1: Circular stainless steel shim part, 0.001" (25 microns) thin, with two punched holes in it to be glued with nailpolish on the upstream part of the wet cell. The printout scale of the drawing corresponds approximately to the real size of the shim.
Figure B.2: AutoCAD drawing of the wet cell assembly.
Figure B.3: AutoCAD drawing of the upstream part of the wet cell.
Figure B.4: AutoCAD drawing of the downstream part of the wet cell.
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Lebenslauf

Ulrich Neuhäusler

Kontaktadresse:
Birkenstraße 24
D-86496 Bonstetten
Tel. ++49-8233-321


1976 - 1980 Besuch der Grundschule Adelsried
1987 Stipendium 'Schülerbegabtenförderung in Bayern'
Juni 1989 Abitur

1989 - 1997 Ersatzdienst im Katastrophen schutz, Krankentransport, Rettungsdienst und der Jugendarbeit des Bayerischen Roten Kreuzes, Kreisverband Augsburg-Land

1989 - 1992 Studium an der TU München
1992 - 1994 Studium an der Universität Göttingen
(Leiter Prof. G. Schmahl) der Universität Göttingen
Titel: 'Untersuchungen zum elementspezifischen Kontrast an Absorptionskanten mit dem Röntgen mikroskop'
Mai 1996 Diplom in Physik

11/1996 - 3/1999 Forschungsaufenthalt im Rahmen eines Doktorandenstipendiums
HSP III des Deutschen Akademischen Austauschdienstes (DAAD)
bei der 'X-ray optics group' an der State University of New York at Stony Brook (Betreuer Prof. C. Jacobsen)
Experimente an der National Synchrotron Light Source
(Brookhaven National Laboratory), Upton, NY, U.S.A.

seit 4/1999 Fortführung der Promotion am Institut für
Röntgenphysik der Universität Göttingen
(Leiter Prof. G. Schmahl)
Titel der Dissertation: 'Soft X-Ray Spectromicroscopy on Hydrated Colloidal and Environmental Science Samples'