CHAPTER 1

Instrumentation

At the beginning of a doctoral study, it is fairly impossible for a student to define the exact subject of his/her work in the next four years, let alone to predict its outcome. Therefore, it would be wrong to give the impression that the presented results were obtained by following a strictly defined scenario or 'mission statement'. This thesis took shape after many discussions with advisors and colleagues, and the followed path had many twists and turns. However, the key issue throughout this study was 'quantitative Thin-Window Electron Probe X-ray Microanalysis (TW-EPMA) of single particles through Monte Carlo simulations'. Readers familiar with the history of the Micro and Trace Analysis Centre (MiTAC) might know this is not the first thesis written on single particle analysis by EPMA, and therefore, it is imperative to discuss the innovative aspects of this work. The next paragraphs summarize recent developments in instrumental technology, elaborating on the relevance of this study.

Table of Contents

1. Recent technological developments in EPMA

Electron microscopy always had to cope with a lot of disadvantages, not only for particle analysis. Since the development of the first electron microscopes (either called electron probe microanalyzers or electron microscopes), researchers have been improving the technology within their instruments. Most of the useful developments were commercialised and put into use for different applications. During the last decades, particle analysis has taken benefit of the technological improvements in the different components of the electron microscope. However, for practical and financial reasons, new technology has not always been accepted or applied very fast in the whole of the particle analysis community (like in many other fields). In environmental particle analysis, for instance, one could say that most of the research goes into the study of the environmental effects of the particles themselves, and that less time and money is spent on testing and applying the latest techniques. Environmental institutes are, therefore, expected to spend money on new technology when the costs are acceptable, or when the need for better, rapidly available data is high. A contrary example is the semiconductor industry, which has always supported the developers of electron microscope because of the mutual, stimulating benefits. New semi-conductor materials could be analysed with electron microscopes, which in return could be improved by developed semi-conductor technology, offering better analytical quality for analysing new materials in the future. MiTAC's strength lies in the fact that it succeeded in following the latest trends in analytical chemistry, and that it has invested in those techniques that were innovative, worth the effort and valuable to a broad range of applications.

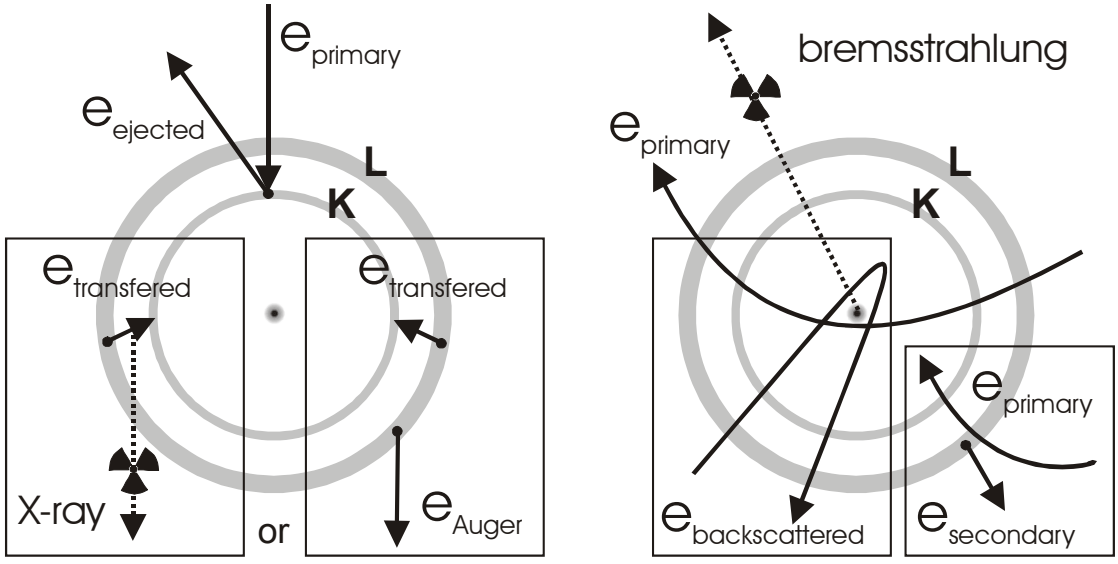

Figure 1: Interaction of primary electrons with atoms (nucleus and electron shells)

In order to discuss the technology in electron microscopy, one should of course point out the principles behind the method. An electron beam is generated by an electron gun and focused on a sample, after which the scattering of the primary electrons in the specimen results in different interaction phenomena (see Figure 1). Elastic scattering affects the trajectories of the electrons inside the specimen without altering their kinetic energy. Inelastic scattering results in the transfer of energy from beam electrons to the atoms in the specimen, causing the excitation of electrons (secondary or Auger), X-ray photons (bremsstrahlung or characteristic radiation), phonons (lattice oscillations) and plasmons (free-electron waves).

Different systems were built to detect the signals caused by these interactions. Elastic scattering is responsible for electron backscattering that forms an important signal for compositional imaging. Auger electrons, phonons or plasmons are not used as signals in scanning electron microscopes, but secondary electrons are used for topographic imaging, and characteristic X-rays offer detailed information on the composition of the sample (in spectra or mappings). This paragraph tries to provide an overview of the recent, major evolutions in electron microscopy technology: from the electron gun to the X-ray and electron detectors. Trends in sample treatment and software development will also be discussed.

1.1 Electron guns

One of the basic parts of an electron microscope is the electron gun. A short discussion about the evolution in the electron gun technology is appropriate in the context of X-ray analysis, since electron beam optics not only play an important role in image formation, but also in X-ray analysis. For example, the size of the electron beam determines the resolution of the electron images, as well as the size of the interaction volume in which the X-rays for microanalysis are generated. A detailed discussion on the principles of electron guns and electron beam optics is beyond the scope of this thesis, but can be found in literature on the basics of electron microscopy.[1] Table 1 gives a more technical overview and comparison of the available technology.

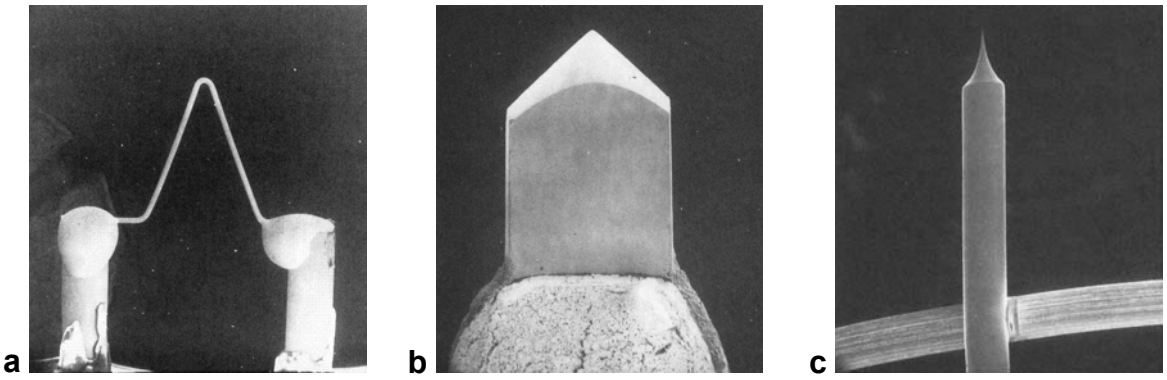

Figure 2: Images of different electron guns
a) tungsten filament, b) lanthanumhexaboride emitter, c) tungsten field emission gun [1]

A lot of electron microscopes “in the field” are still equipped with conventional electron guns based on thermionic emission, requiring a tungsten filament or a lanthanum hexaboride emitter, but much recent research is done on field-emission sources. Although field-emission guns (FEG) were also already developed several decades ago, they are still in the process of gaining more and more acceptance in the particle analysis community. This type of electron gun uses a very small source (e.g. a tungsten hairpin) and requires only simple optics to obtain much narrower and brighter beams (nanometre sizes) than with the thermionic guns (micrometer sizes). This advantage is very useful for high-resolution imaging at low voltages, since thermionic guns show insufficient beam currents and degraded probe sizes under low-voltage conditions, whereas FEGs provide nanometer probes with nanoampere beam currents. The main practical disadvantage, however, lies in the vulnerability of the hairpin emitter to residual gas traces in the vacuum of the specimen chamber.

The vacuum should be at least better than 10^{-8} Pa in order to obtain reasonably stable emission, since monolayers or thicker coatings of foreign gas molecules on the tip's surface reduce the electron emission. So, the quality of the vacuum determines the stability of the electron gun and the electron beam. Some methods (e.g. 'flashing') can be used to restore stable emission, but repeated use of these methods tends to blunter the sharp tip of the hairpin, which should, therefore, be replaced after many months of operation. Short-term beam instabilities causing streaks in scanned images may be reduced using feedback circuits for compensation.

Table 1: Comparison of electron gun operational properties [1]

	Brightness (A/cm²sr)	Lifetime (h)	Source size (µm)	Energy spread (eV)	Beam noise (%)	Required vacuum (Pa)
W-filament	10^5	40-100	15-100	1-3	1	10^{-3}
LaB_6-emitter	10^6	200-1000	5-50	1-2	1	10^{-5}
W-FEG	10^8	> 1000	< 30.10^{-3}	0.3-1.0	2-5	10^{-7}-10^{-8}

The quality of the images made with field-emission electron guns is very high, and, therefore, more and more authors mention the application of this type of source for making images of single particles in their publications.[2,3,4,5,6] The very narrow probe size, could also be very advantageous for the X-ray analysis of small particles, since the electron interaction volume is also considerably smaller. Many authors, however, use field-emission gun scanning electron microscopy (FEG-SEM) mostly for imaging or for qualitative analysis only. One reason might be that the long-term or short-term instabilities of field emission are expected to hamper quantitative X-ray analysis. Other authors, however, have already used field-emission guns for quantitative particle analysis[6] or to compare the quality of different X-ray detectors for particle analysis.[7,8] FEG-SEM is sometimes referred to as Low-Voltage Scanning Electron Microscopy (LV-SEM), since it can be used for X-ray analysis at low voltages (still offering small enough probe sizes and high enough beam currents); however, this technique is not used very often. One reason is that the analysts should be able to select beam-accelerating voltages that are adequate enough to excite the characteristic peaks of the elements of interest, which is not evident. First of all, the efficiency of characteristic X-ray generation, and evidently also the analytical range, are strongly dependent on the overvoltage U, which is inevitably low for low-voltage analysis. Secondly, the very unpredictable effect of charging becomes also very important at lower voltages, since its influence on the overvoltage is very critical for X-ray generation.[9] A second reason is that low-voltage X-ray analysis also requires the capability to perform light-element detection, which was and is not always straightforward in all applications (as will be further discussed below).

The fact that earlier simulation models for quantitative analysis also did not take into account the different physical behaviour of electrons at low voltages, could have been a third reason for many researchers not to use FEG-SEM or LV-SEM for quantitative analysis. However, many changes in the available software are being made, and, for example, the CASINO Monte Carlo program,[10] which was adapted for quantitative particle analysis in the presented research, was originally developed for this reason, implementing improved functions for better simulating the electron interactions at low voltages. The interest in low-voltage analysis is growing, so further studies using FEGs for quantitative analysis are to be expected in the future.

1.2 X-ray detectors

Although **wavelength-dispersive spectrometers** (WDS) are able to record spectra with very high resolution, their applications for particle analysis are rather limited. Due to its low quantum and geometry efficiency, a higher beam current has to be set in order to cause enough electron interactions which result in a suitable amount of detected X-ray signals. Since WDS also requires long measuring times, the exposure of small or volatile particles could cause damage or even total evaporation.[11] The long measuring times also limit the applicability of WDS, because particle studies mostly involve the analysis of huge amounts of particles, which would require too much time. These disadvantages limit WDS to the analysis of more stable particles, e.g. metal oxides or silicates. [2,3,4] The development and application of new crystals to detect X-rays with longer wavelength have cleared the way for the analysis of light elements, but since most of the scientific research and applications are focussed on energy-dispersive detection (due to the aforementioned disadvantages), this evolution will not be discussed here.
So far, the most interesting technological evolutions can probably be found in the development of **energy-dispersive (semiconductor) spectrometers (EDS)**. These improved detectors which can now also detect the X-rays from light elements (with atomic number $Z < 11$), have proven their usefulness for particle analysis.

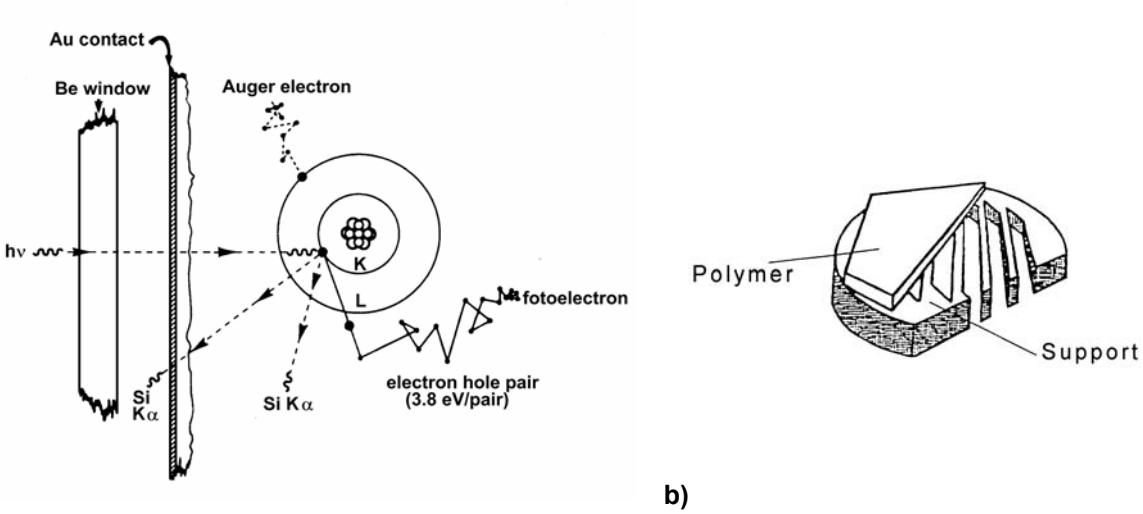

Figure 3: Schematic structure of an energy-dispersive X-ray detector system a) principle with a conventional beryllium window, b) a thin window [12]

The replacement of the beryllium detector window in the earlier models by a much thinner window has almost become the new standard. The first real attempts to use a different detector window set-up for particle analysis were done either by just removing the beryllium window (windowless mode) or by replacing it with the first versions of thin windows, consisting of a thin foil. The latest thin windows mostly consist of a silicon grid supporting a thin polymer foil, coated with a metal film for opacity.
This improved kind of energy-dispersive Si(Li) detectors have been fully commercialised and some recent examples of thin-window electron probe microanalysis (TW-EPMA) can be found in literature. [6,9,13,14,15,16,17,18.] The advantages of semi-conductor EDS over WDS are its better geometry and quantum efficiency, but it always had to cope with many spectral problems (which will be discussed in detail in another paragraph below) due its worse energy resolution. The fact that many of these problems occur in the low-energy part of the spectra, might seem a drawback to use this technique for quantitative analysis in some applications.

However, despite these remaining, but improved, spectral limitations, TW-EPMA currently appears to be the best, commercially available option to perform particle analysis over a broad range of elements (from low-*Z* to high-*Z*) in a straightforward, fast way.
Another very recent kind of energy-dispersive detector is the **microcalorimeter** (sometimes called 'bolometer'), which could hardly have been unnoticed by particle analysts.[19,20] Microcalorimeters combine the advantages of both WDS and conventional EDS, since their excellent energy resolution (comparable with WDS) allows straightforward identification of closely spaced X-ray peaks in complicated spectra at fast operation times (comparable with EDS). X-rays are detected by the temperature increase that results from their absorption in a metallic target or absorber, as Figure 4 shows schematically.

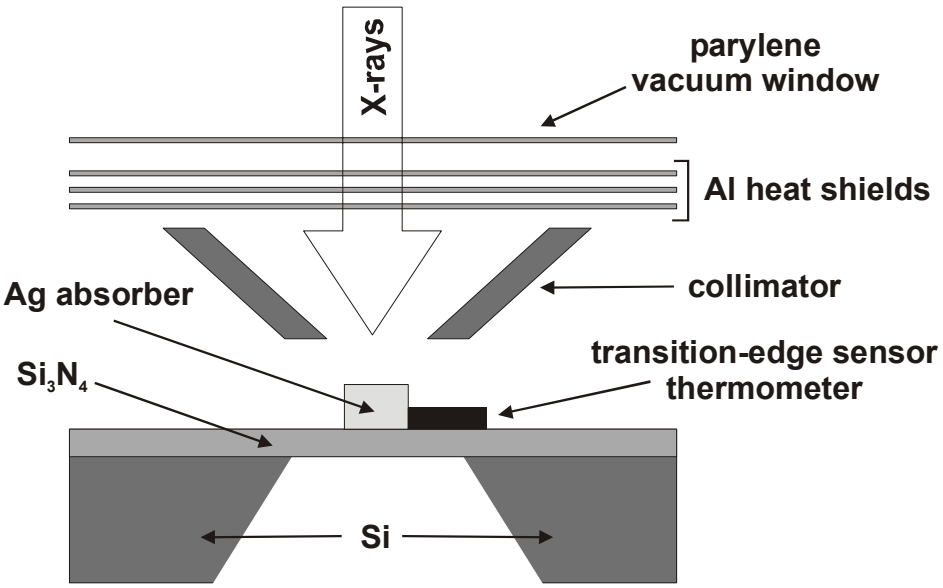

Figure 4: Schematic layout of a microcalorimeter detector [21]

Wollman et al.[22] used a microcalorimeter detector for the analysis of sub-micrometer particles down to 0.1 µm on silicon wafers. Besides proving that microcalorimeter EDS (µcal-EDS) is suitable for this kind of analysis (even in non-optimal cases where the electron beam diameter is larger than the analysed particle), they also performed chemical shift analysis on particles. Changes in electron binding energies with the chemical environment of atoms result in "chemical" shifts of peaks in the acquired spectra, providing information on oxidation states. With the X-ray information on chemical bonding, the analysts were able to differentiate between chemical species present in investigated particles. Chemical shift analysis is also possible with WDS, but this was never routinely performed because of the long scanning times involved. Therefore, when the much faster, high resolution microcalorimeters would be further improved and commercialised, they would undoubtedly provide significant benefits for particle analysis, certainly if they would be combined with FEG-SEM in order to analyse small particles.
In order to perform X-ray analysis with FEG-SEM, Newbury et al.[8] equipped a microcalorimeter detector with a polycapillary X-ray optic to increase the solid angle of the instrument by a factor of 300 (dependent on photon energy), since the standard solid angle of the detector was too small to obtain statistically useful spectra with a nanoampere beam within 100-1000 s. However, the use of polycapillary optics is not straightforward and might complicate the interpretation of the acquired spectra, because of demanding requirements for the alignment of the optic and the X-ray absorption in the capillary.[21]

The developers often publish on the status of the performance of the microcalorimeter,[23] and in the future, efforts to improve the detector resolution, counting rates, low-photon cut-off and the solid angle are to be expected. A similar type of high-resolution energy-dispersive detection can be found in the **superconducting tunnel junction detector** (STJ-EDS), in which X-rays are detected through the tunnelling current produced by their absorption near a junction. Until now, this technique has never really been considered for commercial use, since it has a lot of technical drawbacks, though technological improvements have been achieved and its capabilities are still being investigated. [24,25]
A qualitative comparison of the different detectors is shown in Table 2. In view of the technological capabilities for X-ray analysis, an "ideal" spectrometer should meet the five criteria given in the top row. The *energy resolution* and the *peak-to-background ratio* (P/B) are of course important in view of the spectrum quality. The *dispersive character* is related to the ability of parallel detection of X-rays over a wide energy range. The *count rate capability* is related to the ability to handle sequences of incoming signals. The *detection and geometric efficiencies* are respectively related to the solid angle of collection and the loss of signals due to the instrumental set-up.

Table 2: Comparison of X-ray detectors used for single particle analysis

	Energy resolution and P/B	Dispersive character	Count rate capability	Detection efficiency	Geometric efficiency
WDS	+++	+	+++	+	+
EDS	+	+++	+	+++	+++
µcal-EDS	+++	++	++	+ ©	++ ©

+++ = best, ++ = intermediate, + = worst, © = using a capillary optic; information based on data in references 1 and 21

A technique, which has been reported to use a different X-ray detector geometry, rather than new detector technology, is **Grazing-Exit EPMA** (GE-EPMA). In this technique, which will be discussed later, the X-ray detector (WDS or EDS) is positioned at low take-off or exit angles in order to minimize the effect of the substrate on the acquired spectra, whether by tilting the sample or by moving the detector with a step-motor. Tsuji et al.[26,27,28] have shown that it offers better detection of X-rays coming from very small particles or thin-layers. An additional aspect regarding particle analysis is the specific detection of signals coming from the top layers of a particle only. Coated particles or particles with a core-shell structure could be analysed using GE-EPMA at negative exit-angles, if the particles are positioned at the edge of a tilted specimen substrate. By increasing the exit angle in small steps, the particle structure is then revealed layer by layer.

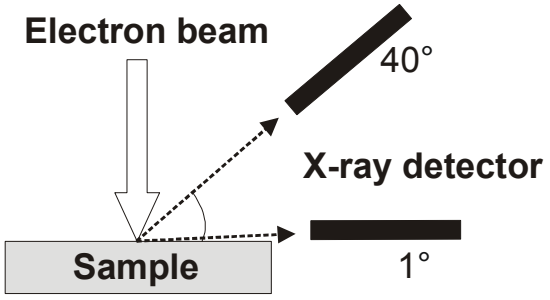

Figure 5: Grazing-Exit EPMA

Another option for the analysis of structured particles is **Dual-Voltage EPMA** (DV-EPMA) in which particles are analysed at two or more different voltages (e.g. 5 and 15 kV) using the change in information depth to study particle layers.[29] This technique will also be further discussed in another chapter.

1.3 Sample treatment

Regarding sample treatment during analysis, developments have been reported on the use of low-vacuum, environmental or **Variable Pressure SEM** (VP-SEM). The difference with SEM can be found in the specimen chamber, which is not under vacuum conditions, but contains a gas mixture in order to perform electron microscopy at higher pressure.[30]
The technology behind VP-SEM depends on a combination of differential pumping and an electron beam transfer system, allowing an electron beam to be formed in vacuum and being transferred to the specimen in the gas medium. The advantage of this technique lies in its possibility to study wet, volatile and even non-conducting samples, since the gas medium preserves the samples from evaporation, and it acts as a charge dissipating means which frees the sample from conductive coatings or chemical treatments. Since these advantages would be able to broaden the range of applications for X-ray analysis in electron microscopy, this technique appears to be very promising. However, most of the applications reported concern visual analysis in morphology studies (using special imaging detectors adapted to the gas medium), since X-ray analysis is still rather difficult. One reason is electron "skirting", a process in which electrons transfer energy to the gas medium. The energy loss, which is difficult to predict or to quantify, affects all the electron interactions with the specimen. The lack of suitable standards is also reported to be a drawback for X-ray analysis in VP-SEM,[31] and, therefore, one can only find examples of particle imaging in literature. However, VP-SEMs now account for 50 % of the market for non-field emission SEMs, so more is to be expected from this technique.[32] A recent development, for example, is the combination with FEG-SEM by Kim and Lee.[33]

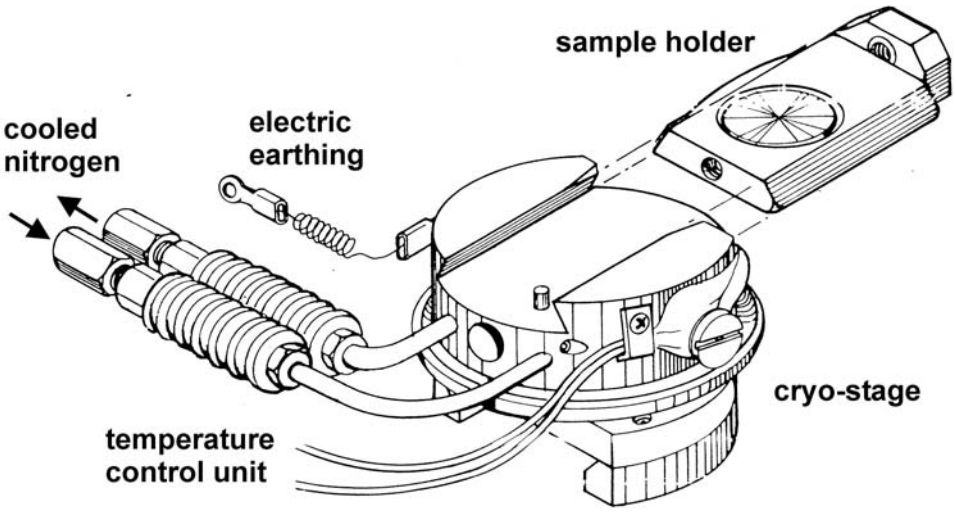

Figure 6: Cryo-cooling stage (Oxford Instruments Inc.)

Another example of enhanced sample treatment can be found in **Cryogenic SEM** (Cryo-SEM) in which the samples are cooled down to low temperatures using special sample stages.[34] A stream of nitrogen gas flows through the stage, after it has been cooled down with liquid nitrogen (–193 °C). This technique offers the possibility to study more volatile species, like ammonium sulphate and nitrate. In environmental analysis, these compounds are very important since their abundance in atmospheric particles is very high.[11,17] The reduction of the beam damage effects allows to analyse small particles (down to 0.3 μm), which will be discussed in another chapter.

1.4 Imaging detectors

Only few reports on new electron imaging devices can be found in literature, maybe because possible technological improvements have been introduced without much notice to the user. One of the most recent developments is probably the **variable pressure secondary electron detectors** that can withstand the environment within VP-SEM, described before (typical pressures of 2-133 Pa). [35]

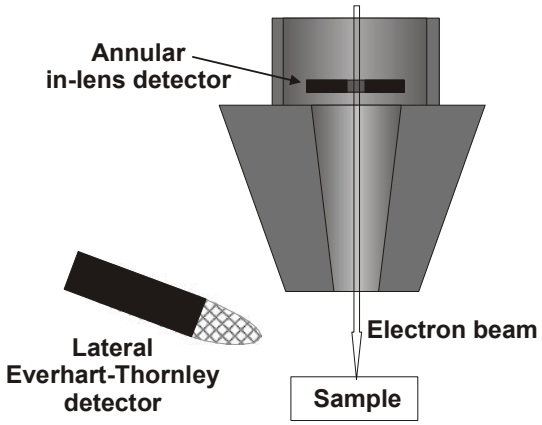

Figure 7: Schematic layout of secondary electron detector configurations

Annular in-lens secondary electron detectors were also quite recently introduced, compared to the conventional lateral Everhart-Thornley detectors. In the in-lens set-up, secondary electrons are accelerated to a high energy by the field of the electrostatic lens and then focussed on the annular detector, which is positioned above the objective lens inside the column (see Figure 7). This provides high-resolution information, an increased signal-to-noise ratio, an improved dynamic range and the prevention of aging effects on the detector material. However, most instruments with in-lens detectors are still equipped with a lateral detector for optimum topographic information.

1.5 Software development

The reader probably needs no reminder that the evolution within computer technology has lead to revolutionary improvements in many scientific fields. The best example is of course the ultra-fast storage and processing of data, which has almost become evident. Nowadays, the technology behind electron microscopes has been integrated in "user-friendly" software systems, which are claimed to be compatible with most of the commonly used operating systems.

Single particle analysis often requires fast and automated analysis of huge numbers of particles, which means that the analyst needs specific software to locate and analyse the particles in a computer-controlled way. Over the past decades, MiTAC developed its own particle analysis programs, mostly based on the backscattered electron signal (BES).[36, 37] Particles are mounted or sampled on a well-chosen type of substrate, in order to obtain a good contrast between the BES from the particles and that from the substrate. During the analysis, the electron beam scans the samples, and when the measured BES is higher than a pre-set threshold value, a particle is considered as detected. When the contours of the located particle are found, the morphology (shape factor) and size (geometric equivalent diameter) are determined, and an X-ray spectrum is recorded after which the scanning of the sample is continued. Although now some of the software environments and program languages seem "old-fashioned", the main advantage of self-made software is that the analysts have a lot of freedom in how they obtain and treat their data (e.g. most of the input and output files are in ASCII format).

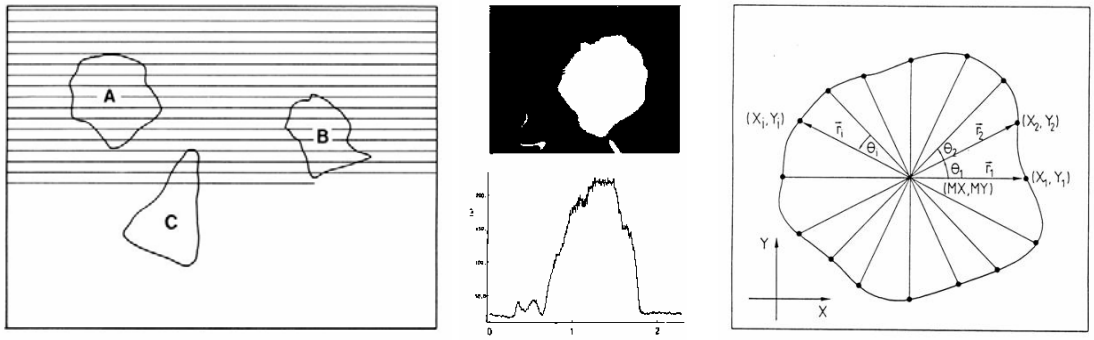

Figure 8: Principle behind the strategy to find single particles using the BES (scanning the sample; BE image and line profile of a particle; particle morphology scan)

Nowadays, manufacturers sell so-called "feature analysis programs", which are mostly based on the same principles as the MiTAC software. The analyst is offered a lot of possibilities which seem quite helpful, like automatic identification and classification of certain features, automatic X-ray peak identification and integration, extended image collection in combination with graphic tools, easy and uniform reporting, etc. However, it is our experience that: 1) the user has "to believe" the results of automatic calculations and interpretations, since there is often no possibility to check how they were obtained or to correct possible mistakes; 2) the user is bound to the file formats offered in the developed programs, which are sometimes not easy to decipher and often incompatible with the systems sold by other companies. Although the offered information technology is indeed most helpful, one could get the feeling that producers are transforming electron microscopes into "black boxes". Manufacturers are now even developing so-called "digital" microscopes: no more buttons to click, because the computer does most of the work itself (the analyst only needs to put the samples inside the instrument). This appears to be a dangerous evolution, since scientists will have more and more difficulties to check what is really happening during their analyses and data processing, and because in the end, only (well-paid) technicians will have the know-how to discover and to correct possible mistakes in software and technology.

2. Competitive and complementary methods for (single) particle analysis

In the past years, many authors have made reviews about the available techniques for the analysis of particulate matter, especially for single particle analysis.[38,39,40,41] Therefore, in order to avoid endless repetition of conclusions and data that were published before, the reader is referred to these papers and to all references therein. The aim of this paragraph is to provide an overview of the recent trends in individual particle analysis, rather than an extensive summary of analytical methods.

2.1 'Chemical' versus 'physical' properties

Governmental authorities need fast techniques for environmental monitoring, preferably on-line, at low cost and comparable with methods in other countries. So far, most of the methods that have been standardized by national and international laws for the monitoring and analysis of particulate matter (certainly for atmospheric aerosols) provide information on physical properties. Some of these methods are optical (e.g. particle counters [42]), but some methods determine mass concentrations gravimetrically, either on-line (e.g. tapered element oscillating microbalances) or afterwards with ultra-sensitive balances under controlled conditions.[43] However, the need for chemical information is becoming increasingly important, especially in studies of the epidemiological effects of dust particles. For many years, scientists have studied the effect of particulate matter on human health using gravimetric and optical information, but methods for chemical analysis are now required to really understand which pollutants are harmful and interact with the human body.[44]

2.2 'Micro' versus 'bulk' analysis

Particles could be analysed by bulk techniques, which means that qualitative or quantitative information is obtained for the whole of the sample and not for each collected particle separately. Some of these techniques allow direct and non-destructive analysis, like X-ray fluorescence (XRF) or particle-induced X-ray emission (PIXE), but other methods require the extraction of the sampled matter into a liquid, like chromatography and mass spectrometry.

Besides applying these bulk techniques, much effort was also put into the development of microanalytical methods. Single particle analysis proved to have high potential in e.g. aerosol studies, because it allows characterizing both the composition and the morphology of each individual particle, which is often important to understand the chemistry behind atmospheric processes. Among the variety of microanalytical techniques, electron probe X-ray microanalysis (EPMA) is by far the most commonly used; in its automated mode it can analyse large numbers of individual particles with very high analytical efficiency in a very short time. Using an electron beam for the generation of characteristic X-rays and backscattered electrons, EPMA is capable of simultaneously detecting the chemical composition and the morphology of microscopic volumes, and the combination with cluster analysis and/or multivariate techniques makes it a powerful tool for single particle analysis.

However, thorough environmental research requires a multi-analytical approach to guarantee profound insights, which means that many different complementary bulk or micro techniques have to be combined.

2.3 'Elemental' versus 'molecular' information: the road to hyphenation

Conventional energy-dispersive X-ray (EDX) detectors were not suitable for light element analysis, because their beryllium window absorbed low-energy X-rays (see the previous paragraph). Quantification methods for particle analysis used to be limited, because of the lack of spectral information on light elements like carbon, nitrogen and oxygen, for which concentration values could only be approximated from estimated stoechiometry. Therefore, for several decades, competing and complementary methods have been developed for single particle analysis.[45,46,47,48,49] For example, the better detection limits of mass spectrometry, and the additional information on light elements, always were advantages over conventional EPMA. In laser microprobe mass spectrometry (LMMS) or secondary ion mass spectrometry (SIMS), individual particles are first vaporized and ionised by interaction with a high-power laser pulse or a primary ion beam respectively, after which the formed ions are detected by an appropriate analyser. The obtained mass spectra are then used for inorganic speciation and organic fingerprinting, which makes it interesting to use such techniques on e.g. atmospheric aerosols.
It will be shown in this thesis that, using TW-EPMA, we are now able to obtain elemental concentrations, based on the X-ray intensities, the size and the estimated density of the analysed particle. Starting from the calculated quantitative information, more detailed data on the possible speciation of simple inorganic species can be obtained. TW-EPMA is a useful method to study environmental particles, since it could offer additional or new information on the various chemical reactions to (trans)form them. However, TW-EPMA will always have the disadvantage of not being able to provide "real" molecular speciation, since it can only detect elements. Therefore, manufacturers are now investigating the possibilities of combining it with different techniques. In the past, most of the (sporadically) combined or 'hyphenated' techniques in electron microscopy were also based on the irradiation of the sample with electrons, since this only required installing an extra detector (e.g. for scanning Auger microscopy). Recently, Jeol inc. and Renishaw pcl. have adapted their EPMA/SEM and micro-Raman technology for combining them in one instrument (SEM-Raman).[50] Therefore, TW-EPMA can now be combined with micro-Raman analysis, which is another single particle method, based on photon irradiation and providing information on the presence of molecular groups.[51] There should be no doubt about the high potential of SEM-Raman, and more is to be heard of its applications. This example shows that hyphenation could be the answer to the need for molecular speciation in SEM/EPMA.

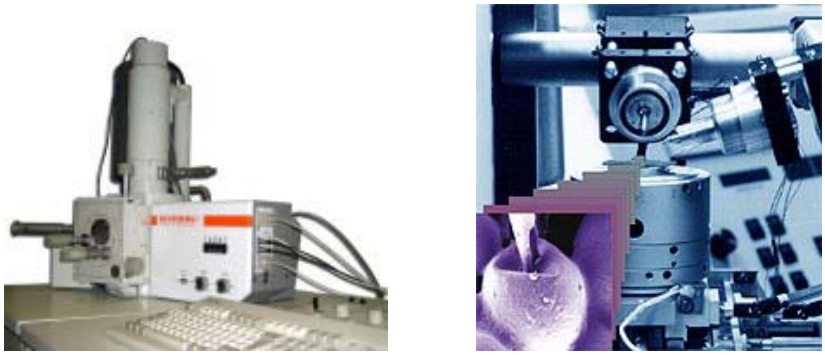

Figure 9: SEM-Raman instrument (left) and nanomanipulators (right) [52,53]

Another more complicated method for analysing particles with a combination of different techniques, is the use of nanomanipulators to transport particles from one sample holder to another using glass needles. Kaegi and Holzer [54] used this method for the analysis of the same atmospheric particles by VP-SEM and TEM, but it might be possible to use the method for more types of analysis.

2.4 'Off-line' versus 'in-situ' analysis

Like EPMA, many of the methods mentioned above, were originally developed for off-line use, i.e. the particles were first collected on filters or foils and analysed some time afterwards. Many efforts were also put into the development of in-situ analysis of aerosols.[55,56,57,58] One of the most recent techniques is aerosol time-of-flight mass spectrometry (ATOFMS), which was developed for measurements "in the field" using lasers for real-time aerodynamic particle sizing and adsorption/ionisation time-of-flight mass spectrometry.[59] This fast technique is not dependent upon particle collection using a filter or impactor preceding the actual chemical characterization, so it should be able to minimise sample detoriation. Therefore, ATOFMS is a powerful technique, able to directly reveal the complex nature of airborne particles and with capabilities to elucidate chemical processes in the atmosphere. However, due to its poor reproducibility, the technique can now only provide qualitative determination on chemical species in individual particles.
Although TW-EPMA is an off-line method, it is, compared to other techniques, easier to automate, less expensive, and, therefore, well distributed among research institutes. TW-EPMA is also less destructive compared to the mass spectrometric methods, since the impact of the electron beam is smaller. Cooling down the samples to –193°C using a cold stage with a pre-cooled flow nitrogen gas could further reduce the damage caused by the energy impact of the electron beam. The particles can be exposed much longer to the beam and they can be analysed several times. Although techniques like ATOFMS are expected to become more important in the future, they are still in their infancy, while TW-EPMA still offers a solid solution to many analytical problems.

Figure 10: Mobile aerosol mass spectrometer (ATOFMS) [60]

2.5 From micrometer to nanometer

In many single particle applications, a trend has been set to find ways to analyse very small particles. A well-known technique for nanometer or submicrometer particles is transmission electron microscopy (TEM), especially in the study of atmospheric aerosols or powder materials.[61,62,63,64] Although the procedures for the preparation and analysis of single particles are not always easy in TEM, it has never been out of the picture, since SEM/EPMA could only analyse particles down to 0.2 μm. However, some recent developments could enable the analysis of particles with even smaller diameters. Firstly, the electron beam from field-emission guns (FEG) is very narrow, and therefore its smaller interaction volume could allow improved analysis of smaller particles (see paragraph 1). Secondly, recent experiments by Laskin and Cowin [6] suggest that the use of TEM grids for SEM/EPMA could also help in extending the size range. The analysis of particles collected on a thin film, reduces the bremstrahlung continuum in the X-ray spectra, and the contribution or interference by the substrate is extremely small. The capability of this technique was also tested, and the results will be discussed in this thesis.

3. Conclusions

As stated in its introduction, the purpose of this chapter was to discuss the innovative aspects of the presented work in regard of the instrumentation for single particle analysis. The recent technological developments in EPMA, as well as in other analytical methods were summarized, which leads us to the conclusions below.

3.1 New insights using better technology

Considering the developments discussed in *paragraph 1*, one could conclude that at this moment, many different types of recently developed techniques have shown to be very promising within EPMA methodology. However, among these techniques, TW-EPMA with a thermionic electron source and the possibility for cryo-cooling offers the most mature, stable and easily available technology for particle analysis over a broad range of sample types.
The Micro and Trace Analysis Centre (MiTAC) bought its first electron microscope in the seventies, and although this Jeol JXA-733 is quite old, it is still in use upon this moment, and it plays a key role in many scientific projects. A second microscope, a Jeol JSM-6300, was bought some time later and the conclusions in many Ph.D. dissertations were based on the results obtained with this instrument. Table 3 gives a short, technical comparison of both electron microscopes. Some aspects will be discussed in more detail when these properties are important in view of quantitative analysis.

Table 3: Properties of MiTAC's electron microscopes

	Jeol JXA-733	Jeol JSM-6300
Electron beam		
Electron gun	Tungsten filament	Tungsten filament
Accelerating voltage	1-50 kV	0.2 - 30 kV
Beam current	10^{-12} - 10^{-5} A	10^{-12} - 10^{-5} A
Detection		
Electrons	Everhart-Thornley detector for secondary electrons (lateral resolution 7 nm); annular backscattered electron detector	Everhart-Thornley detector for secondary electrons (lateral resolution 3.5 nm); annular backscattered electron detector
X-rays (EDX)	Oxford Instruments thin-window detector (take-off angle 30°) with window conditioner; Tracor conventional detector (take-off angle 40°)	Princeton Gamma Tech (PGT) thin-window detector (take-off angle 30°)
Software		
Operating system	MS DOS	Windows/Unix
EM/EDX software	733 (MiTAC)	Imix (PGT)
Sample chamber		
Vacuum conditions	Typically 10^{-5} mbar or lower	Typically 10^{-5} mbar or lower
Pumps	Oil rotation and diffusion	Oil rotation and diffusion
Specimen tilting	-5° – 60° (max.)	-5° – 90° (max.)
Cryo-cooling	Oxford cryo-cooling system	Not installed

Over the past decades, MiTAC was able to develop software for fully automated single particle analysis based on backscattered-electron signals, which made it one of the leading research centres on the study of environmental particles in the world. At first, both its instruments were equipped with conventional energy-dispersive detectors, but quite recently these were replaced by thin-window versions, which lead to the research presented in this thesis. An initial literature study showed that only a few (preliminary) articles had appeared in literature on the use of TW-EPMA for single particle analysis. Therefore, the initiative was taken to investigate the capabilities of TW-EPMA for at least semi-quantitative analysis of individual (sub)micrometer-sized particles. The next chapters describe the steps that were taken to achieve this goal.

Figure 11: MiTAC's electron microscopes (JSM-6300 left, JXA-733 right)

3.2 A multi-analytical approach

Among the different techniques available for the analysis of particulate matter, of which a selection was discussed in *paragraph 2*, EPMA is without any doubt still one of the key methods in the field. One reason is probably that it is commercially available at a reasonable price, and since it can be applied to a large variety of samples (not only single particles), it is possible to get a fairly easy return-of-investment. The mature technology of TW-EPMA, combined with the achieved expertise, now offers the possibility to obtain new insights in environmental chemistry. However, since MiTAC also has access to a variety of other (micro)analytical methods, our knowledge could even be much further extended. Some examples are given in the applications discussed at the end of this thesis.

References

[1] Goldstein J.I., D.E. Newbury, P. Echlin, D.C. Joy, A.D. Romig Jr., C.E. Lyman, C. Fiorgi and E. Lifshin, *Scanning electron microscopy and X-ray microanalysis*, Plenum press, New York (1992)

[2] Ortner H.M., P. Hoffmann, F.J. Stadermann, S. Weinbruch and M. Wentzel, *Chemical characterization of environmental and industrial particulate samples*, Analyst, 123, 833-842 (1998)

[3] Ortner H.M., *Sampling and characterization of individual particles in occupational health studies*, J. Environ. Monit., 1, 273-283 (1999)

[4] Höflich B.L.W., M. Wentzel, H.M. Ortner, S. Weinbruch, A. Skogstad, S. Hetland, Y. Thomasssen and V.P. Chaschin, *Chemical composition of individual aerosol particles from working areas in a nickel refinery*, J. Environ. Monit., 2, 213-217 (2000)

[5] Jones T.P., B.J. Williamson, K.A. Bérubé and R.J. Richards, *Microscopy and chemistry of particles collected on TEOM filters: Swansea, south Wales, 1998-1999*, Atm. Environ., 35, 3573-3583 (2000)

[6] Laskin A. and J.P. Cowin, *Automated single-particle SEM/EDX analysis of submicrometer particles down to 0.1 μm,* Anal. Chem., 73, 1023-1029 (2001)

[7] Wollman D.A., S.W. Nam, G.C. Hilton, K.D. Irwin, N.F. Bergren, D.A. Rudman, J.M. Martinis and D.E. Newbury, *Microcalorimeter energy-dispersive spectrometry using a low voltage scanning electron microscope*, Journal of Microscopy, 19, 37-44 (2000)

[8] Newbury D., D. Wollman, K. Irwin, G. Hilton and J. Martinis, *Lowering the limit of detection in high spatial resolution electron beam microanalysis with the microcalorimeter energy dispersive X-ray spectrometer*, Ultramicroscopy, 28, 73-88 (1999)

[9] Newbury D.E., Measures *for spectral quality in low-voltage X-ray microanalysis,* Scanning, 22, 345-351 (2000)

[10] Hovington P., D. Drouin and R. Gauvin, CASINO: *A New Monte Carlo Code in C Language for Electron Beam Interaction – Part I: Description of the Program*. Scanning, 19,1-14 (1997)

[11] Szalóki I., J. Osán, A. Worobiec, J. de Hoog and R. Van Grieken, *Optimization of experimental conditions of thin-window EPMA for light element analysis of individual environmental particles*, X-Ray Spectrom., 30, 143-155 (2001)

[12] Goldstein J.I., D.E. Newbury, P. Echlin, D.C. Joy, C.E. Fiori and E. Lifshin, *Scanning electron microscopy and X-ray microanalysis*, Plenum press, New York (1981)

[13] Diebold A.C., P. Lindley, J. Viteralli, J. Kingsley, B.Y.H. Liu and K.-S. Woo, *Comparison of the submicron particles analysis capabilities of Auger electron microscopy, time-of-flight secondary ion mass spectrometry, and scanning electron microscopy with energy dispersive X-ray spectroscopy for particles deposited on silicon wafers with 1 μm thick oxide layers*, J. Vac. Sci. Technol. A16, 1825-1831 (1998)

[14] Roth B., and K. Okada, *On the modification of sea-salt particles in the coastal atmosphere*, Atmosph. Environ., 32, 1555-1569 (1998)

[15] Paoletti L., M. Diociaiuti, B. De Berardis, S. Santucci, L. Lozzi and P. Picozzi, *Characterization of aerosol individual particles in a controlled underground area*, Atmosph. Environ., 33, 3603-3611 (1999)

[16] Ro C.-U., J. Osán and R. Van Grieken, *Determination of low-Z elements in individual environmental particles using windowless EPMA*., Anal. Chem., 71, 1521-1528 (1999)

[17] Osán J., I. Szalóki , C.-U. Ro and R. Van Grieken, *Light element analysis of individual microparticles using Thin-Window EPMA*, Mikrochim. Acta, 132, 349-355 (2000)

[18] Szalóki S. Szegedi, K. Varga, M. Braun, J. Osán and R. Van Grieken, *Efficiency calibration of energy dispersive detectors for application in quantitative X- and γ-ray spectrometry*, X-Ray Spectrom. 30, 49-55 (2001)

[19] Wollman D.A., K.D. Irwin, G.C. Hilton, L.L. Dulce, D.E. Newbury and J.M. Martinis, *High-resolution, energy-dispersive microcalorimeter spectrometer for X-ray analysis*, Journal of Microscopy, 188, 196-223 (1997)

[20] Diebold A.C., D.A. Wollman, G.C. Hilton, K.D. Irwin, J.M. Martinis and B.Y.H. Liu, *Applications of microcalorimeter EDS X-ray detectors to particle analysis*, Solid State Phenomena, 65-66, 199-202 (1999)

[21] Kenik E.A., D.C. Joy and D. Redfern, *Microcalorimeter EDS detectors and their application in low-voltage SEM-based microanalysis*, Mikrochim. Acta, 145, 81-86 (2003)

[22] Wollman D.A., G.C. Hilton, K.D. Irwin, L.L. Dulcie, N.F. Bergren, D.E. Newbury, K.-S. Woo, B.Y.H. Liu, A.C. Diebold and J.M. Martinis, *High-resolution microcalorimeter energy-dispersive spectrometer for X-ray microanalysis and particle analysis,* in *Characterization and Metrology for ULSI technology: international conference 1998* (Eds. D.G. Seiter, A.C. Diebold, W.M. Bullis, T.J. Shaffner, R. McDonald and E.J. Walters - international conference), 799-804 (1998)

[23] Wollman D.A., S.W. Nam, D.E. Newbury, G.C. Hilton, K.D. Irwin, N.F. Bergren, S. Deiker, D.A. Rudman and J.M. Martinis, *Superconducting transition-edge microcalorimeter X-ray spectrometer with 2 eV energy resolution at 1.5 keV*, Nucl. Instr. Meth. A444, 145-150 (2000)

[24] Frank M., L.J. Hiller, J.B. le Grand, C.A. Mears, S.E. Labov, M.A. Lindeman, H. Netel and D. Chow, *Energy resolution and high count rate performance of superconducting tunnel junction x-ray spectrometers*, Rev. Sci. Instrum., 69, 25-31 (1998)

[25] Angloher G., P. Henttl, M. Huber, J. Jochum, F. von Feilitzsch and R.L. Mössbauer, *Energy resolution of 12 eV at 5.9 keV from Al-superconducting tunnel junction detectors*, J. Appl. Phys., 89, 1425-1429 (2001)

[26] Tsuji K., K. Wagatsuma, R. Nullens and R. Van Grieken, *Grazing exit electron probe microanalysis for surface and particle analysis*, Anal. Chem., 71, 2497-2501 (1999)

[27] Tsuji K., R. Nullens, K. Wagatsuma and R. Van Grieken, *Elemental X-ray images obtained by grazing-exit electron probe microanalysis (GE-EPMA)*, J. Anal. At. Spectrom., 14, 1711-1713 (1999)

[28] Tsuji K., Y. Murakami, K. Wagatsuma and G. Love, *Surface studies by grazin-exit electron probe microanalysis (GE-EPMA)*, X-Ray Spectrom., 30, 123-126 (2001)

[29] Ro C.-U., K.-Y. Oh, J. Osán, J. de Hoog, A. Worobiec and R. Van Grieken, *Heterogeneity assessment in individual CaCO3-CaSO4 particles using ultrathin window electron probe X-ray microanalysis*, Anal. Chem., 73, 4574-4583 (2001)

[30] Danilatos G.D., *Environmental scanning electron microscopy and microanalysis,* Mikrochim. Acta, 114-115, 143-155 (1994)

[31] Griffin B.J., C.E. Nockolds, M.R. Philips and G. Remond, *New needs for imaging and X-ray microanalysis standards: ESEM, CHIME and low voltage microanalysis*, Inst. Phys. Conf., 165, 395 (2000)

[32] Mohan A., N. Khanna, J. Hwu and D.C. Joy, *Secondary electron imaging in the*

variable pressure scanning electron microscope, Scanning, 20, 436-441 (1998)

[33] Kim G.M., and DH-H. Lee, *FEG-SEM investigation of micromechanical deformation processes in ultrafine monospherical SiO_2 particle-filled polymer composites*, J. Appl. Polym. Sc., 82, 785-789 (2001)

[34] Gregory C.L., H.A. Nullens, R.H. Gijbels, P.J. Van Espen, I. Geuens and R. De Keyzer, *Automated particle analysis of populations of silver halide microcrystals by electron probe microanalysis under cryogenic conditions*, Anal. Chem., 70, 2551-2559 (1998)

[35] Thiel B.L., *Image formation in low-vacuum SEM*, Mikrochim. Acta, 145, 243-248 (2003)

[36] H. Nullens, *unpublished software developed at MiTAC*

[37] Raeymaekers B., *Characterization of particles by automated electron probe microanalysis*, Ph.D. thesis, University of Antwerp (1986)

[38] Van Grieken R. and C. Xhoffer, *Microanalysis of individual environmental particles*, J. Anal. Atom. Spect., 7, 81-88 (1992)

[39] Jambers W. and R. Van Grieken, *Present and future applications of beam techniques in environmental microanalysis,* Trends in Anal. Chem., 15, 114-122 (1996)

[40] Jambers W., L. De Bock and R. Van Grieken, *Recent advantages in the analysis of individual environmental particles*, Analyst, 120, 681-691 (1995)

[41] De Bock L., *Microanalysis of indoor and marine aerosols and cloud particles*, Ph.D. thesis, University of Antwerp (1998)

[42] Ankilov A., A. Baklanov, M. Colhoun, K.-H. Enderle, J. Gras, Yu. Julanov, D. Kaller, A. Lindner, A.A. Lushnikov, R. Mavliev, F. McGovern, T.C. O'connor, J. Podzimek, O. Preining, G.P. Reischl, R. Rudolf, G.J. Sem, W.W. Szymanski, A.E. Vrtala, P.E. Wagner, W. Winklmayr and V., Zagaynov, *Particle size dependent response of aerosol counters*, Atmos. Res., 62, 209-337 (2002)

[43] Ayers G.P., M.D. Keywood and J.L. Gras, *TEOM vs. manual gravimetric methods for determination of PM2.5 aerosol mass concentrations*, Atmos. Environ., 33, 3717-3721 (1999)

[44] Dockery D.W., J. Schwartz and J.D. Spengler, *Air pollution and daily mortality: associations with particulates and acid aerosols*, Environ. Res., 59, 362-373 (1992)

[45] Klaus N., *SIMS analysis of aerosols collected during a weather period with low aerosol concentrations*, Sci. Total Environ., 41, 1-12 (1985)

[46] Dierck I., D. Michaud, L. Wouters and R. Van Grieken, *Laser microprobe mass analysis of individual North Sea aerosol particles*, Environ. Sci. Technol., 26, 802-808 (1992)

[47] Murphy D.M. and D.S. Thomson, *Laser ionization mass spectroscopy of single aerosol particles*, Aeros. Sci. Techn., 22, 237-249 (1995)

[48] Tourmann J.L. and R. Kaufmann, *Laser microprobe mass spectrometry (LAMMS) of coal mine dusts: single particle analysis and toxicity correlation*, Intern. J. Environ. Anal. Chem., 52, 215-227 (1993)

[49] Van Ham R., A. Adriaens, P. Prati, A. Zucchiati, L. Van Vaeck and F. Adams, *Secondary ion mass spectrometry as a new analytical tool for measuring atmospheric particles on insulating substrates*, Atmos. Environ., 36, 899-909 (2002)

[50] Nielsen C., *Micro-Raman analyser*, European Microscopy and Analysis, 83, 51 (2003)

[51] Davis E.J., S.D. Rassat and W. Foss, *Measurement of aerosol/gas reaction rates by microparticle Raman spectroscopy*, Aeros. Sci., 23, S492-S432 (1992)

[52] Renishaw, United Kingdom; http://www.renishaw.co.uk

[53] Klocke Nanotechnik, Germany; www.nanomotor.de

[54] Kaegi R. and L. Holzer, *Transfer of a single particle for combined ESEM and TEM analysis*, Atmos. Environ., 37, 4535-4359 (2003)
[55] Kievit O., J.C.M. Marijnissen, P.J.T. Verheijen and B. Scarlett, *On-line measurement of particle size and composition*, J. Aerosol Sci., 23 – Suppl. 1, S301-S302 (1992)
[56] Mansoori B.A., M.V. Johnston and A.S. Wexler, *Matrix-assisted laser desorption/ionisation of size- and composition-selected aerosol particles*, Anal. Chem., 68, 3595-3601 (1996)
[57] Reilly P.T.A., R.A. Gieray, M. Yang, W.B. Whitten and J.M. Ramsey, *Tandem mass spectrometry of individual airborne microparticles*, Anal. Chem., 69, 36-39 (1997)
[58] Liu D.-Y., D. Rutherford, M. Kinsey and K.A. Prather, *Real-time monitoring of pyrotechnically derived aerosol particles in the troposphere*, Anal. Chem., 69, 1808-1814 (1997)
[59] Gard E., M.J. Kleeman, D.S. Gross, L.S. Hughes, J.O. Allen, B.D. Morrical, D.P. Fergenson, T. Diepes, M.E. Gälli, R.J. Johnson, G.R. Cass and K.A. Prather, *Direct observation of heterogeneous chemistry in the atmosphere*, Science, 279, 1184-1187 (1998)
[60] TSI Incorporated, United States of America; www.tsi.com
[61] Taniguchi I., C.K. Lim, D. Song and M. Wakihara, *Particle morphology of spinel $LiMn_2O_4$ powders synthesized using ultrasonic pyrolysis method*, Solid State Ionics, 146, 239-247 (2002)
[62] Kitajima M.-A. and Y. Fukai, *Measurement of size and number concentration of polystyrene particles*, Colloids and Surfaces A, 153, 467-469 (1999)
[63] Aragón Piña A., G. Torres Villaseñor, P. Santiago Jacinto and M. Monroy Fernández, *Scanning and transmission electron microscope of suspended lead-rich particles in the air of San Luis Potosi, Mexico*, Atmos. Environ., 36, 5253-5243 (2002)
[64] Smekens A., *Morphological, microstructural, and chemical characterisation of individual soot aggeragates from different sources by electron microscopical techniques*, Ph.D. thesis, University of Antwerp (2000)