Application Note #000385

**KeyWords**
XPS, Biology, Biochemistry, Maple Leaf, Measurements, Surface Analysis

EnviroESCA™

# XPS surface analysis of a leaf with EnviroESCA

**This application note presents the application of EnviroESCA to the field of biology and surface spectroscopy of biological samples. X-ray Photoelectron Spectroscopy (XPS) measurements on different sites of a leaf will be discussed. It will be shown that XPS is able to detect residues of calcium chloride, being used as a fertilizer, on the leafs surface.**

## Motivation

Trees take up nutrients with their roots directly from the soil. Which means all chemicals and substances that get into the soil will be taken up by the tree and are being distributed also into its leaves.
This study shall explain how XPS as a surface analytical method can be applied to a leaf as an example for biological materials.

**Fig. 1 A leaf in the autumn sun. The primary vein and the secondary veins are clearly visible.**

## Method

EnviroESCA utilizes X-ray Photoelectron Spectroscopy (XPS) as its main analytical technique.

Hereby an electron beam is generated inside the X-ray source and focused onto an X-ray anode made of Aluminum. The deceleration of the electrons on the anode leads to the production of X-rays. This X-ray beam is monochromated and focused onto the sample.

X-ray photons impinging the sample excite electrons in the material which are subsequently emitted with specific kinetic energy determined by their binding energy and the photon energy of the X-rays. Thereby only electrons from atoms up to a depth of approx. 10nm are able to leave the surface. These electrons propagate through the lens system of the Electron Analyzer into the hemisphere which acts as a spherical capacitor forcing the electrons onto circular paths with radii depending on their kinetic energy. The electron paths end at an electron sensitive detector where the electrons are amplified and measured as an intensity in counts / second. Sweeping the voltage of the spherical capacitor while measuring the number of electrons per second on the detector results in a photoelectron spectrum. From these spectra a quantitative analysis of the atomic composition of the sample surface can be done.

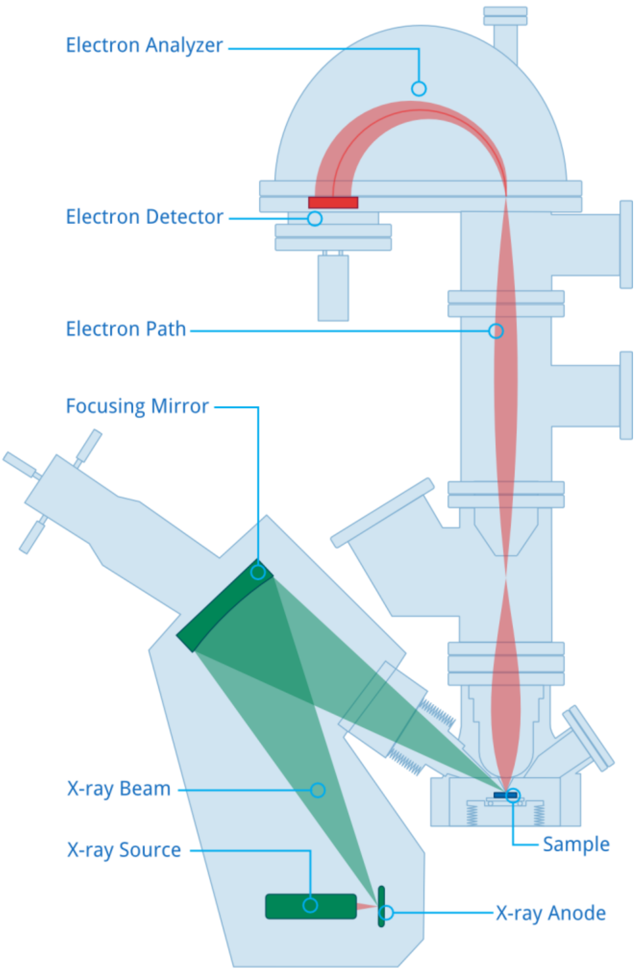


**Fig. 2 XPS with EnviroESCA**

## Experimental Section

The leaf that is used as the specimen in this analysis was found in a park underneath a maple tree growing in a very short distance to a group of apple trees and a crop field.

Dirt and sand was blown of the leafs surface with nitrogen before it was mounted to the EnviroESCA sample plate (see figure 3). Carbon tape was used to fix the leaf to the plate and to prevent it from moving during the initial pump down and the final venting of the SampleEnvironment.


**Fig. 3 Leaf fixed on the sample plate**

Three sites on the leaf where selected. The first measurement position was a site on the still green surface areas of the leaf. For the second position a yellow area was chosen and the last measurement position is situated on a brown and partially dissolved area of the leaf. See figure 4 for details.

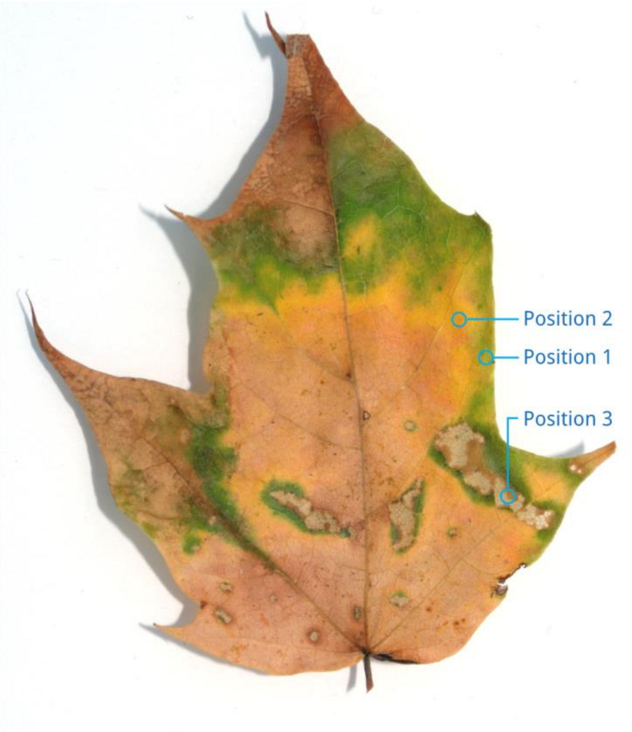


**Fig. 4 Three different measurement positions were chosen**

The leaf as an example for organic material in general is difficult to be analyzed in classical XPS systems that require ultra-high or high vacuum conditions for operation. The main difficulties are the high outgassing rate of the water filled cell structures and the electrical insulation of the material which would immediately lead to a charge up of the surface under X-ray illumination.

With EnviroESCA investigations of such samples are not only possible but also easy to perform. EnviroESCA can work in pressures up to several dozens of mbar, also in water atmosphere, which makes dehydration of the specimens unnecessary. An intrinsic charge compensation method which we call Environmental Charge Compensation makes additional electron or ion sources for charge compensation as in classical XPS systems unnecessary. The gas atmosphere that is surrounding the sample delivers all the free charges, when illuminated with the soft X-rays, that is needed to compensate for surface charging (see figure 3 for an illustration).

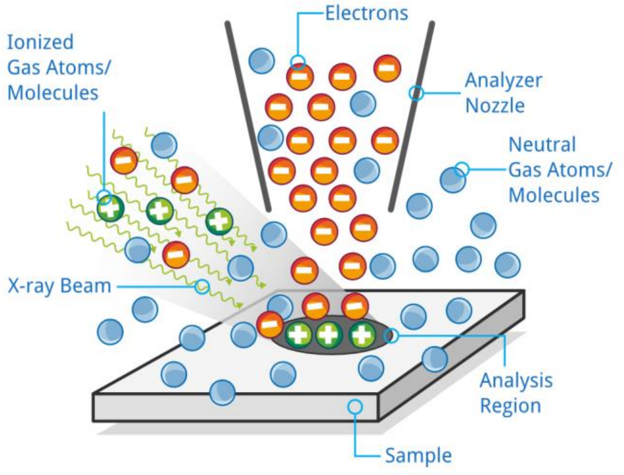


**Fig. 5 Environmental Charge Compensation**

Because the leafs surface would charge up in vacuum conditions a working pressure of 1 mbar was chosen.

## Results

In the following we are presenting unmodified raw data taken with EnviroESCA. The data was not smoothened or shifted on the energy scale unless otherwise mentioned.

### 1. Position 1 (Green surface area)

Figure 6 shows a photo taken with one of the three digital microscopes at the analysis compartment. It shows the green surface area of the leaf directly underneath the analyzer nozzle. A red pilot laser shot through the lens system of the analyzer helps to navigate with the sample stage and to select the analysis area.

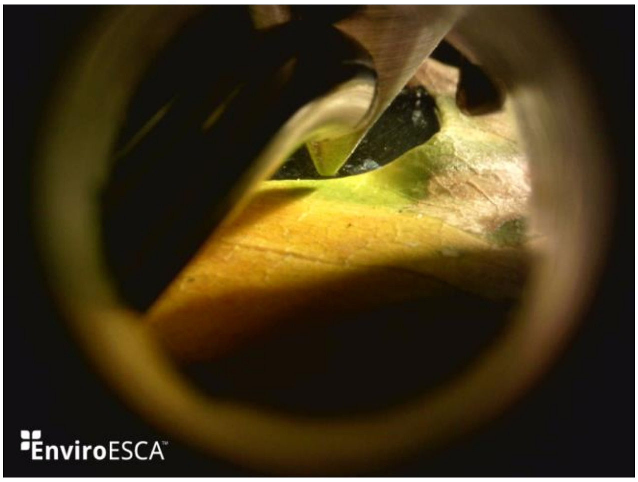


**Fig. 6 The first analysis position underneath the analyzer nozzle**

The first XPS spectrum that was measured right after pump down to 1 mbar is displayed in figure 7. It was measured with a step width of 1 eV in 2 minutes and 29 seconds. Beside the Oxygen KLL Auger the 1s peaks of Oxygen, Nitrogen and Carbon are clearly visible.

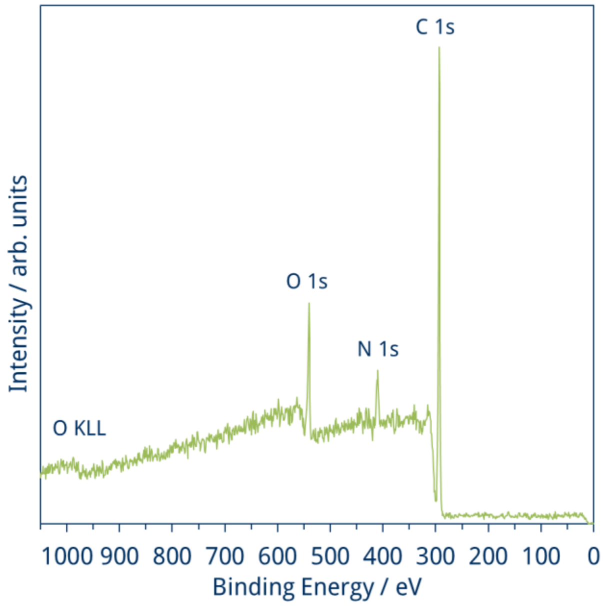


**Fig. 7 Survey XPS spectrum measured on the green surface area (Pos. 1)**

A closer look at the energy region between 150 and 380 eV can be seen in figure 8. In this region only the Carbon 1s is visible. The spectrum was measured in 5 scans with a step width of 0.1 eV in a total measurement time of 19 minutes and 11 seconds.

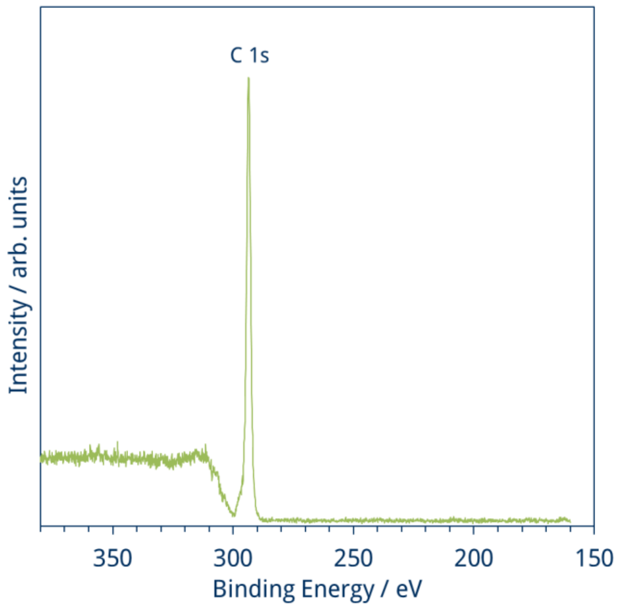


**Fig. 8 The Carbon 1s region between 150 and 380 eV**

## 2. Position 2 (Yellow surface area)

The yellow surface area that was chosen to become the second analysis site can be seen in the photograph of figure 9.

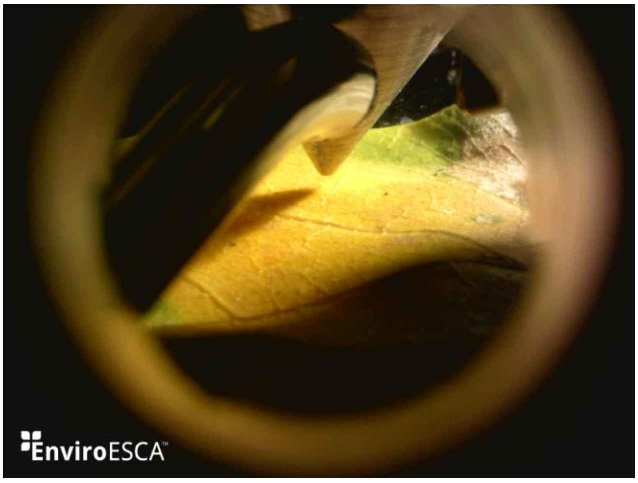


**Fig. 9 The second analysis position**

The XPS survey scan shows a different Carbon to Oxygen ratio than the green region. In addition the Oxygen, Nitrogen and Carbon 1s peaks also additional ones are noticeable (see figure 10 for details)

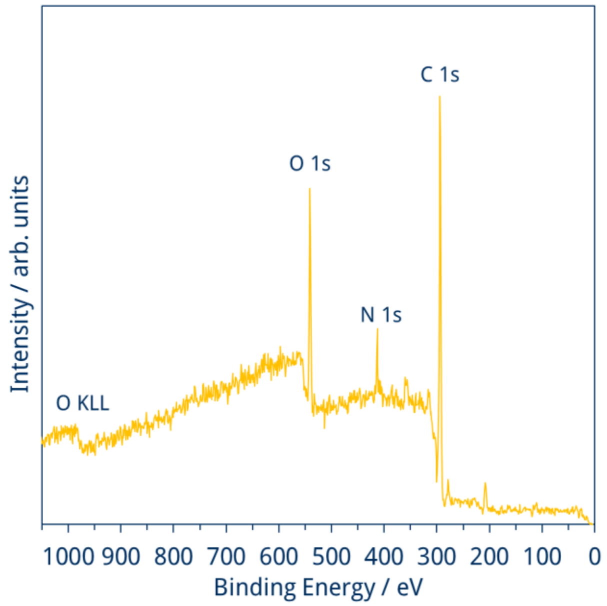


**Fig. 10 XPS survey scan on the yellow surface area**

A detail scan of the Carbon 1s region, presented in figure 11, also reveals the additional peaks. Calcium

(2p) and Chloride (2s and 2p) are visible which could not be found on the green surfaces of the leaf.

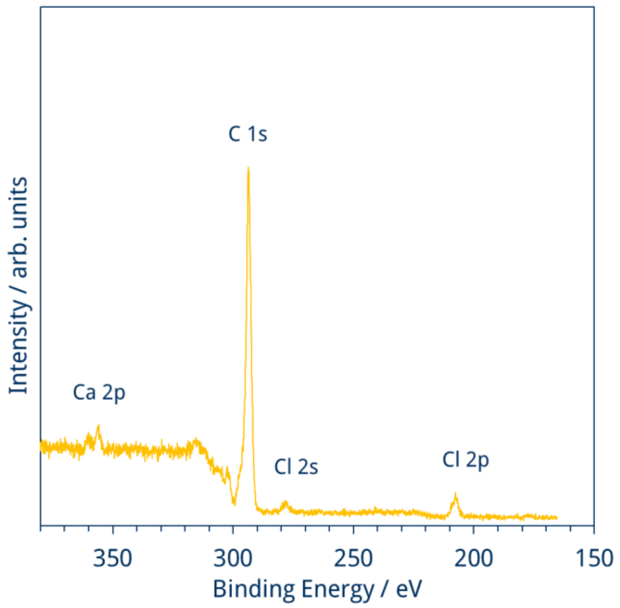


Fig. 11 The Carbon 1s region of the yellow surface area

## 3. Position 3 (Brown surface area)

As the third analysis position a brown and partially dissolved surface area was chosen. The exact position is shown in the camera image displayed in figure 12.

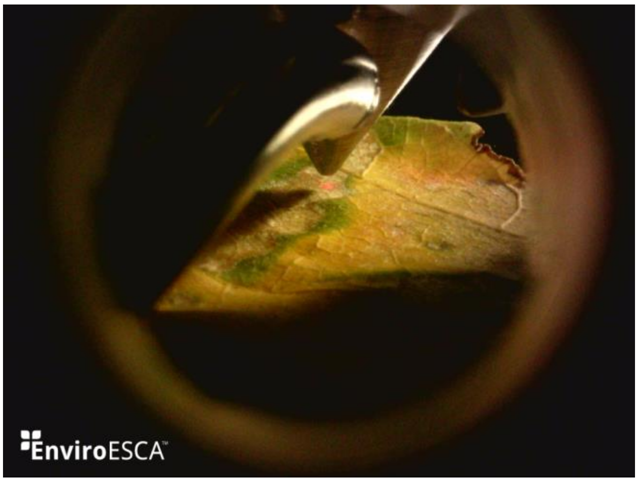


Fig. 12 The third analyis position (please note the red dot oft he pilot laser)

At this position more Oxygen and less Carbon compared to the spectra on the green and yellow surfaces is visible. Also the additional peaks are even more pronounced than in the spectra of the yellow region.

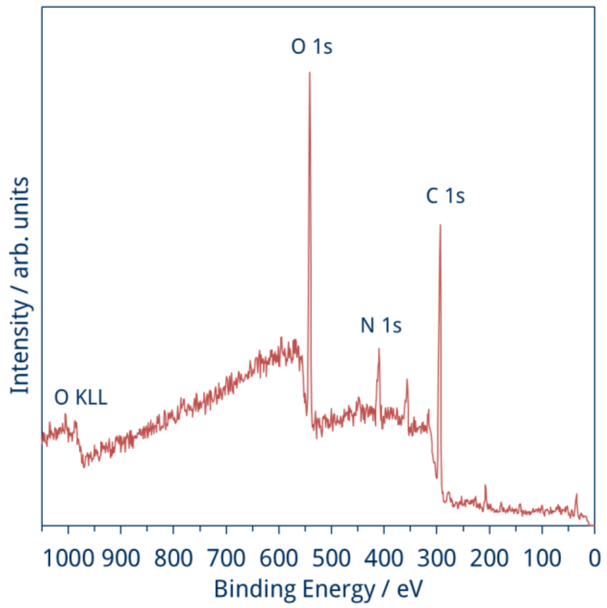


Fig. 13 Survey spectrum recorded at the brown surface of the leaf

The spectrum recorded with better statistics around the Carbon 1s (see figure 14) shows more intense Calcium and Chlorine peaks.

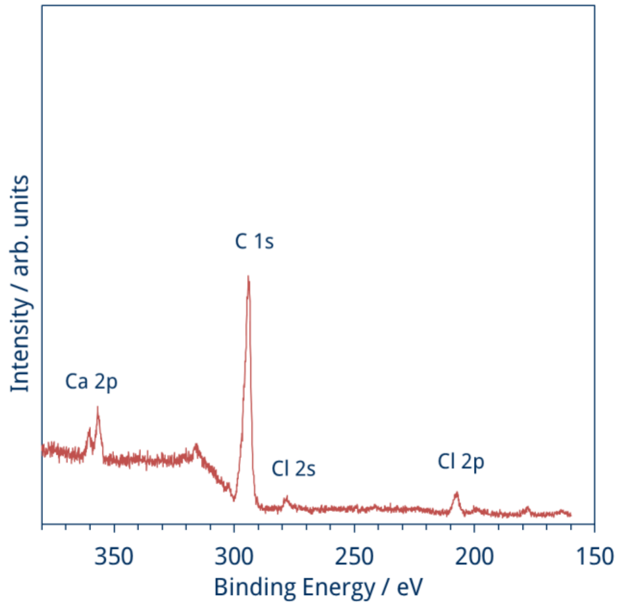


Fig. 14 The Carbon 1s region of the brown surface area

## Discussion

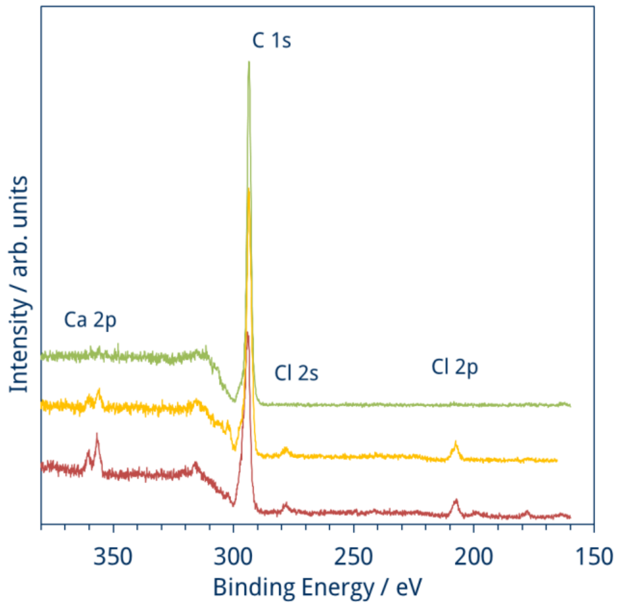


**Fig. 15 All three Carbon 1s regions plotted for comparison.**

A comparison of all three C 1s detail spectra clearly shows that the intensity of the Calcium and the Chlorine peaks increase when changing the measurement position from the green to the yellow and finally to the brown regions on the leaf.

Calcium chloride is being used as a fertilizer for fruit trees and in crop production. Both could be found in close distance to the maple tree where this leaf was found.

The signal intensity of Calcium and Chlorine increases when changing the measurement position from the green surface with intact cell structures to the yellow surface and to the brown with less intact or dissolved cell membranes. An interpretation of the results could be that the water solvable fertilizer was taken up by the roots of the trees and channeled into the leaves and cells and therefore cannot be found on the surface of the green regions.
Whereas on the yellow regions with less intact cells the quantity of calcium chloride on the surface is higher because less cells can be seen as intact.
Finally on the brown and dissolved areas the fertilizer stays as a residue even if the cell membranes are gone and is traceable with XPS.

## Conclusion

The results presented in this application note clearly shows that X-ray photoelectron spectroscopy (XPS) especially in near ambient pressure (NAP-XPS) or as it is often called ambient pressure photoelectron spectroscopy (AP-PES or AP-XPS) can deliver results even on surfaces of organics and biomaterials that are not vacuum compatible.

The application of the well-established technique XPS to this new field of application opens up the door for a wide variety of experiments in biology and biochemistry that could not profit of this powerful technique with its unraveled surface sensitivity and its great element detection capability.

# Related Applications

**Here we present some related application of EnviroESCA you might be also interested in.**

**For more information about other fields of application feel free to visit www.EnviroESCA.com or contact us under info@specs.com.**

## Liquids

Water and aqueous reagents are essential in any biological process or system. But apart from a few special low vapor-pressure cases, liquids have not been accessible to any technique requiring UHV conditions. EnviroESCA opens up this exciting field of applications.

## Gaseous and Liquid Environments

The interaction of gases and liquids with surfaces plays a key role in many different fields ranging from biological and catalytic systems to construction materials. EnviroESCA offers the possibility of investigating surfaces in contact with gases and liquids, such as salt water, acidic rain, wastewater, or gaseous atmospheres with high humidity.

## Astrochemistry and Astrobiology

The interaction of organic molecules with water and ice surfaces in atmospheres that can be found on distant planets is a vital field of research. EnviroESCA can create sample environments that realistically simulate conditions in planetary atmospheres such as on Mars, where the pressure ranges from $10^{-6}$ mbar to 7 mbar.

## Biological Material

With the capability of operating in the near ambient pressure regime EnviroESCA offers an entirely new opportunity to investigate biological materials and processes, making ESCA more versatile than ever before.

## Soils and Minerals

XPS analysis is widely used in soil and mineral research for characterizing surface organic films, mineral decomposition and redox transformations. Until now these studies were limited to UHV compatible samples. EnviroESCA overcomes this constraint and offers new exciting possibilities.

## Archaeology and Archaeometry

The analysis of priceless ancient artifacts with surface science techniques like XPS and NAP-XPS allows delivering results about the surface composition of metallic and non-metallic specimens without damaging or destroying them. EnviroESCA offers the possibility to load large and uneven samples and to perform the analysis in environmental conditions which will preserve the delicate relics.

**Enviro**ESCA™

## Technical Specifications

| **Enviro**ESCA | |
|---|---|
| Electron Spectrometer | ▪ Hemispherical electron analyzer with 150 mm mean radius<br>▪ Differentially pumped lens system<br>▪ Delayline detector with up to 400 channels |
| X-ray Source | ▪ Al $K_\alpha$ micro-focused monochromator<br>▪ Rowland circle diameter of 600 mm<br>▪ Spot sizes of 200 µm-1 mm optimized to analysis area |
| Charge Neutralization | ▪ System immanent charge compensation by X-ray photoionization |
| Ion Source (optional) | ▪ Scannable small spot ion source (200 eV-5 keV) or gas cluster ion source |
| Pumping System | ▪ Turbomolecular pumps<br>▪ Oil-free backing pumps |
| Pressure Range | ▪ Defined by analyzer aperture (up to 100 mbar with an aperture of 300 µm; other aperture sizes on request) |
| Gas Dosing | ▪ Two separated mass flow controlled gas dosers at analysis position |
| Cameras | ▪ 3 digital microscopes for sample navigation and documentation |
| Automation and Software | ▪ Fully automated vacuum and gas dosing system<br>▪ Advanced software package |


| SampleEnvironment (standard, others on request) | |
|---|---|
| Samples Stage | ▪ High precision 3-axis stage |
| Sample Size | ▪ Up to 120 mm in diameter and 40 mm in height<br>▪ 50 mm inner diameter addressable |
| Gas Dosing | ▪ Mass flow controlled process and purge gas |
| Cleaning | ▪ Downstream RF plasma cleaner |
| Camera | ▪ Digital microscope for sample observation and documentation |