Integration of a cryo ultramicrotome and a specially designed cryo AFM to study soft polymers and biological systems

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At present, the study of the ultrastructure and properties of soft and hydrated materials (plastics, engineering resins, polymers, which exhibit liquid crystallinity, nanoliquids etc.) is a rapidly developing field. These types of materials have advantages over metals and ceramics, because of their low processing costs, low weight and useful characteristics, such as transparency, tensile strength, elongation and impact strength which form unique combinations. Almost all biological polymers may be assigned to material class as well. The commercial importance to understand the relationships between the manufacturing processes, the structure produced, and the resulting physiological properties of the biomaterials for medical and pharmaceutical applications cannot be overestimated [1].

In a (bio-) polymeric blend the bulk morphology can be considered the main factor that determines its properties. In order to obtain information about the internal ultrastructure of the investigated material one has to proceed with two main steps. The first step is the preparation of an ultrathin section by ultramicrotomy at temperatures lower than the glass transition temperature (Tg). For most soft polymers Tg is significantly below 0°C. The second step is a microscopy measurement of the sample. There are two main microscopic techniques that allow obtaining structural information of an object in the nanometer range: 1) transmission electron microscopy (TEM) including scanning TEM (STEM), and 2) scanning probe microscopy (SPM) including atomic force microscopy (AFM) [2]. TEM is nowadays the most widely spread technique used for the investigation of polymer blends and composites, although the low contrast of biological and polymer samples, the necessity to use a two-dimensional projection of the sample volume, and the issue of electron beam damage strictly limit the material range and abilities of the technique. Alternatively, the AFM has a nondestructive character, and since it is primarily a surface characterization technique, the radiation damage of the sample surface is absolutely excluded [3]. The possibility to obtain information about the location, architecture, and mechanical properties of macromolecules or polymer chains in a nanometer range directly from the surface of the section or block face makes this technique extraordinary useful for the investigation of local changes within the sample that take place during dynamic processes as well as the whole ultrastructure in general.

Recently a new device has been developed, which is based on the integration of a specially designed scanning probe microscope and a Leica UC6NT ultramicrotome and is working under ambient conditions at room temperature. This integration enables the direct monitoring of a block face surface immediately following each sectioning cycle of the ultramicrotome. Consequently, this device can be applied for serial section tomography of a wide range of hard biological and polymer materials [4]. However this device cannot be used

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for many soft polymers and biological hydrated systems because a section preparation of those materials requires cryo conditions.

In this work we demonstrate the development of a new technique using a cryo AFM directly mounted in the cryogenic chamber of the ultramicrotome. This combination of instruments will allow one to scan a sample immediately after cryosectioning, so that structural changes can be avoided as the whole structure will be stabilized by cold. A direct observation of the block face surface of the sample by cryo AFM will provide information about the native structure of the bulk polymer which was not chemically or mechanically modified during sample preparation or observation. At the same time, if the cryo section has a good quality, it can be collected and used for cryo TEM analyses, so that TEM and AFM information can be obtained from the same particular specimen area.

The presented cryo AFM uses chemically etched metal (W, PtIr) tips attached to quartz tuning forks as AFM probes (Fig. 1A). This approach was proven to deliver decent results in ambient and cryo conditions on polymer materials (Fig. 1B) and allowing us to overcome a number of complications concerned with use of conventional cantilever probes with an optical detection scheme for low temperature measurements.

In addition, the construction of such a device provides the possibility to investigate samples in a wide range of temperatures (from –190ºC to 100ºC), where the heating of the sample can be performed by the microtome control.

The general goal of the presented device is the coherent operation of cryo AFM and cryo ultramicrotomy for the possibility of a 3D reconstruction of serial measurements and compatible cryo TEM analysis.

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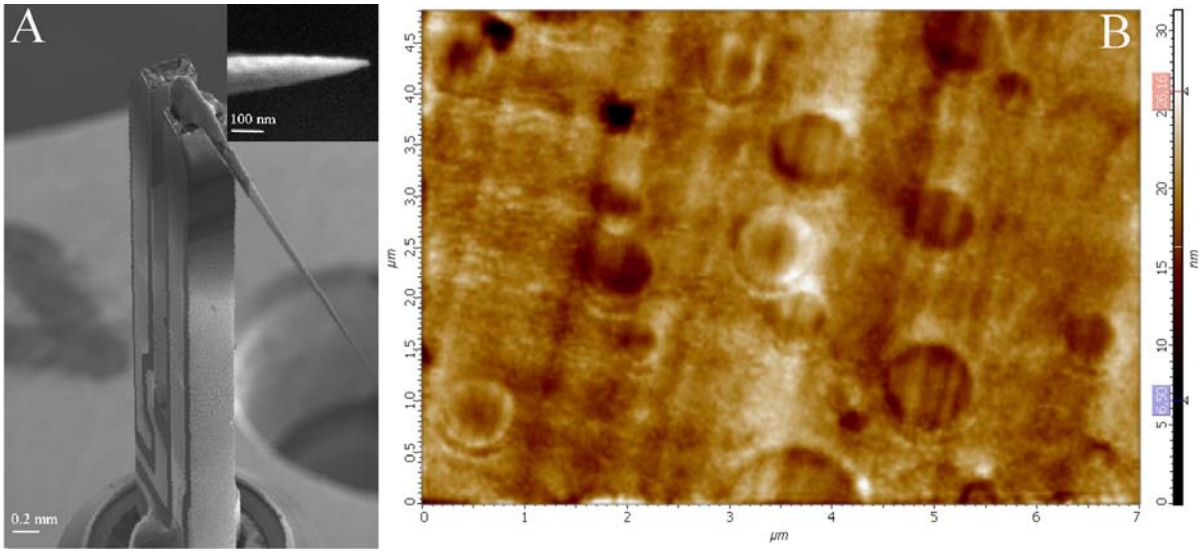

Figure 1. SEM image of the tuning fork with an attached tungsten needle (A). The inset represents the needle edge at high magnification. Figure (B) shows an AFM topographical image of the block face surface of ABS/PA6 copolymer. The image was acquired at -190°C.

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