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# Design and potential of instrumented ultramicrotomy

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Ultramicrotomes are generally used for preparation of very thin sections for transmission electron microscopy. Recently it has been shown that when the sample holder of the ultramicrotome is instrumented with a force transducer, it is possible to measure the very small sectioning force during sectioning, and calculate the energy dissipated. In the present work, the instrumentation is further improved. The new sample holder, which uses two piezo-electric force transducers can measure two force components simultaneously. It is not only robust and stiff, but it also shows high sensitivity and reproducibility. It is possible to detect sectioning forces lower than 0.1 mN. The method is demonstrated on two amorphous polymers, poly(methyl methacrylate) and epoxy. Fracture energies in the same order of magnitude as theoretical predictions from chemical bond fracture only are recorded. It is therefore suggested that the method of instrumented ultramicrotomy is a useful tool when information on covalent bond density is needed. Potential future applications are identified including research on nano-scale fracture, characterization of molecular anisotropy and developments of the ultramicrotome. © 1997 Elsevier Science Ltd.

**(Keywords: ultramicrotome; sectioning forces; fracture)**

## INTRODUCTION

An ultramicrotome is used for sample preparation for transmission electron microscopy (TEM). By use of a very sharp knife (diamond or glass), extremely thin sections or 'chips' of, for example, polymers or biological materials are prepared. The chip thickness is typically between 30 nm and 150 nm, and in order to get good sectioning results the sample needs to be in the solid, or preferably in the glassy state.

Since microtome sectioning creates two new surfaces one may look at the sectioning as a crack propagation process. Ericson and Lindberg[1] showed that if the ultramicrotome is instrumented in order to measure sectioning forces it is possible to calculate the sectioning energy during the crack propagation. Since the thickness of the sections are very small, this might give us some insight in very local fracture energy dissipation mechanisms.

Microtomes, which are used for section thicknesses from 0.5 to 10 $\mu$m, have been instrumented by several workers. Vincent *et al.*[2–5] used a rotary microtome fitted with a modified knife holder which was a load cell. They measured cutting forces, and when the thickness of the sections were decreased continuously from 1 mm to 1 $\mu$m the cutting energy also decreased for a number of biological materials. Atkins and Vincent[2] suggested that the sectioning energy extrapolated to zero thickness is equal to the critical fracture energy, $G_C$. No measurements on polymers have been published, but an interesting study on meat was published by Dobraszczyk *et al.*[6]. Wool and Rockhill[7] studied the molecular degradation during microtome sectioning by viscometry. Saubermann *et al.*[8] presented an alternative method to evaluate the sectioning forces on a microtome. However, as seen in the work of Ericson and Lindberg[1] for ultramicrotomes, it is impossible to extrapolate sectioning energies from experiments at the micrometre scale down to zero thickness without significant error. The reason is the dramatic nonlinearity in sectioning energy vs chip thickness in the thickness region below 200 nm. Therefore, in order to measure energy dissipation at a true microscopic scale, one needs to instrument an ultramicrotome.

Several attempts to instrument an ultramicrotome have also been presented in the literature[9–13], all with the purpose to determine sectioning forces. It is found that the techniques for instrumentation vary as much as the reasons for instrumentation. Hodson and Marshall[10] studied the thawing of frozen material during sectioning. They calculated the forces acting on the substrate by measuring the velocity of the specimen arm by a method of electromagnetic induction. When they knew the mass and the retardation of the arm it was possible to calculate the energy dissipated into the material during sectioning. Wikefeldt[11] placed two piezoelectric elements in the drive arm of the ultramicrotome. It was then possible to measure both forces acting perpendicular and parallel to the sectioning direction. The reason for his instrumentation was to record instability vibrations, so called 'chatter',

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developed during sectioning. In his thesis he also sectioned poly(methyl methacrylate) (PMMA) and aluminium, and found very small sectioning forces for small chip thicknesses. Helander[12] measured forces during ultramicrotome sectioning at varying sectioning conditions for epoxy. He instrumented the knife holder with two piezoelectric force transducers and reported forces corresponding to sectioning energies around 20–50 J m$^{-2}$, depending on the chip thickness.

Ericson and Lindberg[9] instrumented the sample holder of an ultramicrotome. We showed that an ultramicrotome could be used for measurement of very small sectioning forces in order to calculate the energy dissipation during crack growth in amorphous polymers. The energy dissipated for small chip thicknesses was almost in the same order of magnitude as theoretical predictions based on chemical bond fracture only. This suggests that the method is applicable for nano-scale fracture studies. We used a single piezoelectric force transducer mounted on the sample holder and were able to measure forces in the sectioning direction. However, to increase the sample-to-sample reproducibility and learn more about the chip forming process, we need a new stiffer sample holder, able to measure forces in two directions simultaneously.

The objective of this work is to further develop the instrumented ultramicrotome in order to obtain improved sample-to-sample reproducibility, and higher stiffness of the sample holder, and to measure two force components simultaneously. A new sample holder is designed, tested and evaluated. The method of instrumented ultramicrotomy is demonstrated by tests on two amorphous polymers, PMMA and an epoxy, diglycidyl ether of bisphenol A with curing agent diethylamine trianine (DGEBA/DETA). Potential applications of instrumented ultramicrotomy are suggested.

## EXPERIMENTAL

### *Instrumentation of the sample holder*

It was earlier suggested[1] that two force components are needed in order to study the mechanics of ultramicrotomy. A special instrumented sample holder was therefore designed and built. Two piezoelectric load cells (Model PCB® M209A12 from PCB® Piezoelectronics, USA) with battery-powered signal conditioners (Model 480E09 from PCB®) were mounted between a sample clamping unit and the connection for the ultramicrotome specimen arm. (See the schematic figure and photo in *Figure 1*.) The analogue signals from the signal conditioner were sampled by a computer for further analysis. The two load cells can only measure compressive forces, and were therefore precompressed to approximately 100 N (each) by two pre-stress rods made of a unidirectional glass fibre/epoxy composite.

Calibration of the sample holder was performed by simulating loads as weights in thin strings, fastened where the sample normally is clamped. Loads from 6 mN to 215 mN were used in different loading angles (simulating different sectioning angles). The two signals from the loads cells were then 'calibrated' within the computer so that the two theoretical force components were continuously calculated and sampled. After calibration, the two pre-stress nuts were secured by an epoxy adhesive. The two analogous signals from the signal conditioners were then continuously processed in the computer to give the calibrated response.

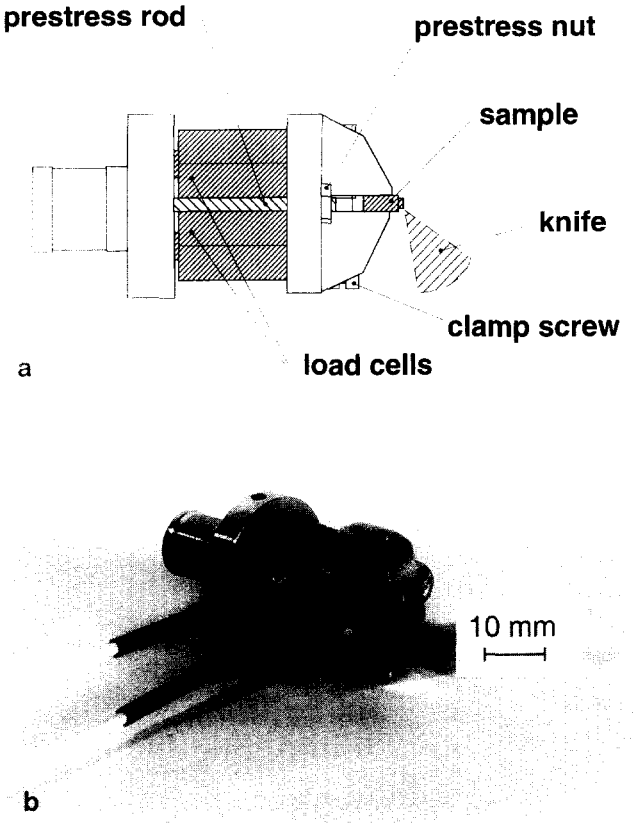


**Figure 1** The instrumented sample holder for ultramicrotome sectioning. (a) Schematic figure. (b) Photo

### *Materials*

For demonstration of the method, two polymers, PMMA and DGEBA/DETA, were sectioned with the new instrumented sample holder. PMMA is a thermoplastic polymer whereas DGEBA/DETA is a thermoset (crosslinked). Both materials are amorphous and in the glassy state at room temperature. For more details about the materials, see ref. 1.

### *Sectioning procedure*

The ultramicrotome used was a LKB 2088 Ultrotome® V. The knife was a diamond knife (from Juniper Ultra Micro, Sweden). The radius of the knife edge is, according to the manufacturer, between 5 nm and 7 nm. Specimens were first cut from sheets and then trimmed in the ultramicrotome in order to get an appropriate sectioning block, a so called 'mesa', of about 1 mm$^2$. The chip thickness, $h$, was determined from the interference colour created in the sections on the water surface in the water trough according to *Table 1*. The sectioning speed during measurements was 1 mm s$^{-1}$ and $h$ was varied between 60 and 250 nm at four discrete levels. For each $h$, sectioning forces for at least ten sections were recorded. The total work to section per created surface area, $W_s$, was determined as:

$$W_s = \frac{F_s}{b} \quad (1)$$

where $F_s$ is the sectioning force component in the sectioning direction (see *Figure 2*), and $b$ is the chip width.

**Table 1** Interpretation of interface colours of polymer chips on water surface calculated after Patzelt[14]

| Interference colour | Chip thickness, $h$ (nm) PMMA ($n = 1.5$)[a] | Epoxy ($n = 1.6$)[a] |
|---|---|---|
| Silvergrey | 50–70 | 50–75 |
| Silver | 70–100 | 75–107 |
| Gold | 100–130 | 107–140 |
| Violet | 130–180 | 140–190 |
| Blue | 180–240 | 190–255 |

[a] $n$ is the refractive index

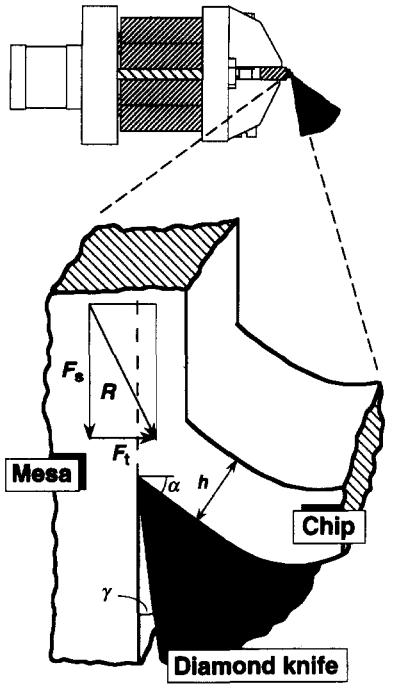


**Figure 2** Schematic figure of the ultramicrotome sectioning and the two force components acting upon the sample. $F_s$ is the sectioning force, $F_t$ the transverse force and $R$ the resulting force

## RESULTS AND DISCUSSION

### *Performance of instrumented sample holder*

The new instrumented sample holder was not only stiff and very sensitive, but also linear in response to loads in a wider range than needed for ultramicrotome sectioning.

Although the load cells were pre-stressed, the large difference in stiffness between the load cells and the pre-stress rods (approximately $350\,N\,\mu m^{-1}$ and $5\,N\,\mu m^{-1}$ respectively) made it possible to fully use the very high sensitivity of the load cells. Since the load cells are positioned symmetrically in the sample holder, it is possible to simultaneously detect both the sectional force, $F_s$, and the transverse force $F_t$ (see *Figure 2*). $F_t$ is proportional to the sum of the forces in the two load cells whereas $F_s$ is proportional to the difference. The smallest possible load to detect for each load cell is 0.22 mN. Due to the built-in lever mechanism, the smallest possible detectable $F_s$ and $F_t$ are as low as 0.05 mN and 0.4 mN respectively. Since the smallest sectioning force per section width for amorphous polymers was found to be about $10\,mN\,mm^{-1}$ [1,9], the sensitivity of the new sample holder is more than sufficient for the desired purposes. Since the maximum allowable load is ±200 N, the sample holder is believed to be very robust. After calibration the sample holder showed very good agreement with simulated loads. Results from the calibration are shown in *Figure 3*, where two different resulting forces, $R$ (13.5 mN and 215 mN) were applied on the specimen holder. When the loading angle, $\phi$, is changed, the system give both $F_s$ and $F_t$ simultaneously. The solid lines are the theoretical values, and the data points are experimental values. The correspondence between simulated loads and recorded values is very good for all loads, $R$ (up to 200 mN) and loading angles, $\phi$, tested.

To conclude, the new sample holder shows several advantages compared with the sample holder described in refs 1 and 9. Firstly, the earlier sample holder had to be taken apart between a change of samples. The pre-stress in the load cell was then altered and therefore also its response, resulting in poor sample-to-sample reproducibility. The new sample holder is never dismounted and therefore does not suffer from this. Secondly, by use of two load cells, it is possible to detect two force components simultaneously, with much higher sensitivity on a wide load range (from 0.2 mN to much higher than the tested 200 mN). It should therefore also be suited for microtome measurements. Thirdly, the new sample holder is much stiffer. The load cells used here are almost 100 times stiffer than the old sample holder

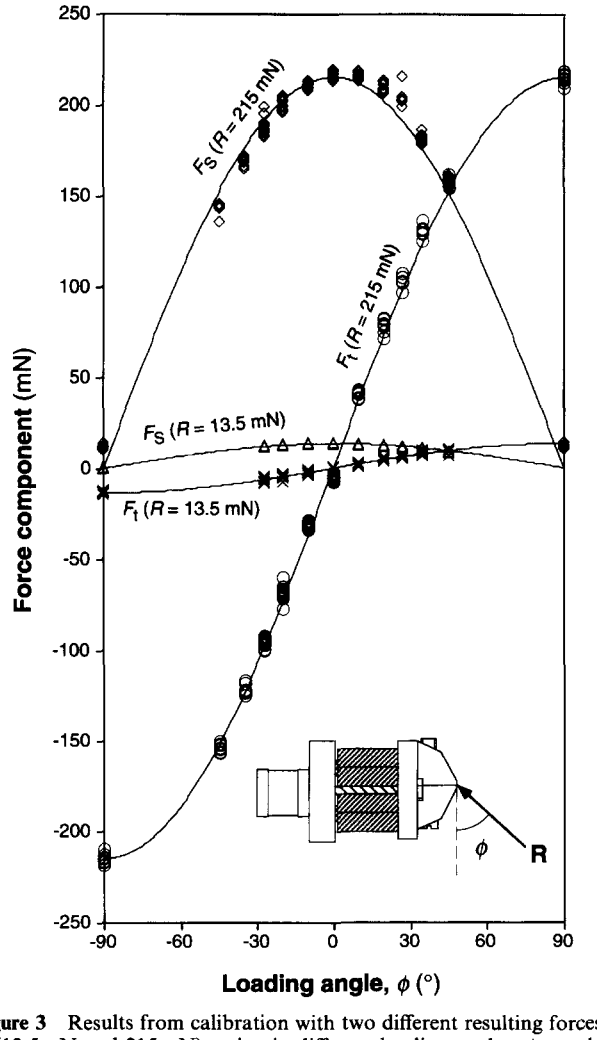


**Figure 3** Results from calibration with two different resulting forces, $R$ (13.5 mN and 215 mN) acting in different loading angles, $\phi$, on the specimen holder. For each loading angle two force components, $F_s$ and $F_t$, are given from the instrumented sample holder. Solid lines are expected values

described in refs 1 and 9, 350 MN m$^{-1}$ compared with 4 MN m$^{-1}$. This will make it less inclined to chatter, and easier to handle.

*Sectioning forces and work to section*

In *Figure 4* typical sectioning force signals for DGEBA/DETA are presented. Both $F_s$ and $F_t$ are more or less constant over the load pulse. The decrease in both $F_s$ and $F_t$ with time is due to the decay of the signal from piezoelectric load cells. This is a characteristic feature of the load cells, and since it is the variation in load when entering or leaving the sample that is of interest, it is not a problem here. The fluctuations in the forces during sectioning, as seen in *Figure 4*, could be chatter as well as material variations, or a result from discontinuous crack propagation.

The sectioning force per unit width, $F_s/b$, which is equal to the work to section ($W_s$), and transverse force, $F_t/b$, as a function of section thickness $h$ is presented in *Figure 5* for PMMA and DGEBA/DETA. As observed previously[1], $W_s$ decreases down to less than 30 J m$^{-2}$ for the two materials. Keeping in mind that the macroscopically determined fracture energy, $G_{ic}$, for PMMA and DGEBA/DETA is 500 J m$^{-2}$ [15] and 130 J m$^{-2}$ [16], respectively, it is remarkable that the $W_s$ values for the two polymers do not differ more. The theoretically determined minimum fracture energy, the so-called intrinsic fracture energy, $G_0$, is about 1 J m$^{-2}$ for PMMA[17]. $G_0$ is the energy required to cut the chemical bonds at the fracture plane, and is believed not to differ significantly for DGEBA/DETA. Since $W_s$ for very thin sections and $G_0$ are in the same order of magnitude, we believe that the total energy dissipated during sectioning is dominated by chain scission. Other possible energy dissipation mechanisms include chain stretching prior to scission and chain pull-out after bond rupture[18,19]. However $F_t/b$ differs significantly between PMMA and DGEBA/DETA. We believe that differences in other energy dissipated mechanisms, such as friction, may cause this. In order to estimate this effect of other energy dissipation mechanisms, one needs to model the chip formation process and the frictional forces. This is done elsewhere by Ericson and Lindberg[20] for five different amorphous polymers.

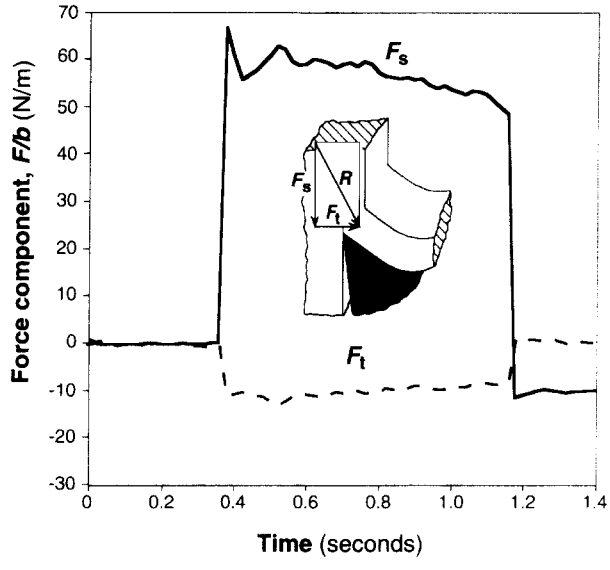


**Figure 4** Typical output of $F_s$ and $F_t$ during sectioning of DGEBA/DETA with $h \approx 220$ nm. Sampling frequency is 50 Hz and nominal sectioning speed is about 1 mm s$^{-1}$

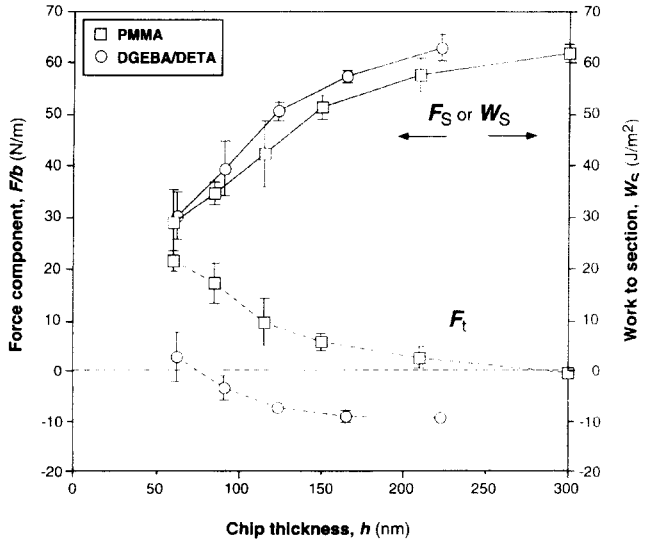


**Figure 5** Work to section, $W_s$, and sectioning force components, $F_s/b$ and $F_t/b$ for PMMA and DGEBA/DETA. The sectioning speed is 1 mm s$^{-1}$

*Potential applications for instrumented ultramicrotomy*

Results from instrumented ultramicrotome studies are encouraging. We strongly believe that instrumented ultramicrotomy opens room for several new applications for ultramicrotomes, for material properties characterization as well as for sectioning optimization (sectioning parameters or the equipment). A number of potential applications are presented below.

1. *Nano-scale crack growth studies.* Since the measured work to section, $W_s$, is in the same order of magnitude as theoretical predictions based on chemical bond fracture only, we believe that the method is applicable to research on nano-scale crack growth. In addition to fundamental research on molecular fracture, this might be useful in the development of new toughened materials. However, the crack propagation processes and the chip forming process are at present not very well understood. An increased insight in the different energy dissipation mechanisms and the deformation mechanisms are needed.
2. *Characterization of molecular anisotropy.* Since the energy dissipated is proportional to the density of covalent bonds, the method is also sensitive to molecular anisotropy for macromolecular materials such as polymers. Ericson and Lindberg[20] showed that $W_s$ varied significantly when a highly oriented amorphous polymer was sectioned in different directions. The variation in $W_s$ corresponded well to variations in Young's modulus and dimensional recovery after heat treatment.
3. *Determination of cell wall fraction in cellular solids.* Since $W_s$ is proportional to the sectioned area, it may be possible to quantify the volume fraction of cell walls in cellular materials.
4. *Optimization of ultramicrotome sectioning parameters.* In order to get high quality sections with an ultramicrotome, one has to set several sectioning parameters, such as sectioning speed, rake angle of

the knife etc. We believe that the best quality implies a minimum of sectioning energy. This was also suggested for microtomes by Willis[21] and Atkins and Vincent[2] and Willis and Vincent[5]. Therefore it may be possible to set optimum sectioning parameters from measurements of dissipation energies for different setting for sectioning parameters. Mechanical chatter can also be detected as force variations, as shown by Wikefeldt[11].

5. *Knife sharpness.* As shown by Ericson and Lindberg[20], it is possible to detect when the knife is dull. For a dull knife the $W_s$ does not decrease significantly for small $h$.
6. *Measurement of chip thickness.* Since the work to section strongly depends on $h$ (especially for small $h$, see *Figure 5*) one can read the chip thickness from the level of $W_s$ *if* the relation between $h$ and $W_s$ is known. This may be useful when the material is not transparent, for example aluminium, or when the sections are so thin that they do not give any interference colour at the water surface.
7. *Automation of knife approach.* One critical moment during ultramicrotome sectioning is the approach of the very sharp and sensitive knife edge onto the sample. The knife is easily damaged if the first contact with the sample is not very gentle. Since the sample holder easily detects forces in the mN range, the approach could be motorized, and stopped when a critical force is exceeded.

## CONCLUSIONS

Developments in the design of the instrumented ultramicrotome resulted in significant improvements. By use of two piezoelectric load cells in the sample holder it was possible to measure two force components simultaneously during sectioning. The new instrumented sample holder showed improved sample-to-sample reproducibility and sensitivity. The smallest load that was possible to detect was less than 0.1 mN. The method was successfully demonstrated by sectioning of PMMA and an epoxy (DGEBA/DETA). Very small levels of work to section were measured (less than $30\,J\,cm^{-2}$) for both materials. It is suggested that the method of instrumented ultramicrotomy may be useful whenever information on chemical bond density in a material is of interest. For instance, fracture energies at the molecular level and molecular anisotropy can be studied with the method. Furthermore, developments such as *in situ* chip thickness determination and an automatic knife approach are possible with the new sample holder.

## ACKNOWLEDGEMENTS

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