

the other lots either showed only slight signs of softening (that in solution 2), or not at all (control in solution 1).

Table 1 shows the carbohydrate content of the date fruit samples before and after experiment calculated in terms of gm. glucose per 100 gm. initial fresh weight.

Table 1

Pretreatment	Reducing sugars	Sucrose	Poly-saccharides	Total
Initial	3.58	22.27	1.06	26.91
6 hr. dis. water + 60 hr. air	6.46	18.84	1.06	26.36
6 hr. 2 per cent NaCl + 60 hr. air	6.80	17.10	1.04	24.94
6 hr. 5 per cent NaCl + 60 hr. air	19.75	3.07	0.95	23.77
6 hr. 10 per cent NaCl + 60 hr. air	19.90	0.93	0.72	21.55

Table 1 reveals that presoaking in distilled water apparently induced decrease in sucrose with a more or less corresponding increase in the reducing sugars. Presoaking in sodium chloride solutions accelerated the disappearance of sucrose and accumulation of hexoses, particularly when 5 and 10 per cent sodium chloride solutions were used. Indeed, the latter not only induced the rapid disappearance of sucrose but also a slight decrease in the polysaccharide content of the dates.

Softening therefore seems to be associated with accumulation of reducing sugars at the expense of sucrose and possibly also some polysaccharides.

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¹ *Nature*, 175, 469 (1955).

A New Method for Determination of Thickness of Microtome Sections

A METHOD for determining the thickness of microtome sections with the same accuracy as the rest of the analytical procedure has long been required in quantitative cytochemistry. Hitherto this has not been possible, and it has generally been necessary to estimate thickness from the microtome setting, a rather unsatisfactory method.

In 1936 Schmaltz described¹ a method for measuring the profile structure of a surface. This method, the so-called light-cut or light-profile technique, has been further developed by Naumann², Menzel³ and Tolansky⁴. Besides the normal microscopical picture of the object, it also gives information about the depth of profile. A microscope for incident illumination is used in which the objective simultaneously acts as a condenser, and in which the illumination is adjusted according to the Kohler principle. An opaque wire or a phase edge is placed in the plane of the field iris diaphragm, the shadow of which is projected on to the surface under examination. By arranging off-central illumination, differences of level in the surface will give proportional displacements of the shadow line, thus giving the profile of the surface.

In an attempt to apply light-profile microscopy for thickness determinations of histological sections

I have encountered certain difficulties. High accuracy is obtained only when the objective has a large aperture. However, the depth of focus is inversely proportional to the aperture, and since the two levels between which the measurement is made have to be relatively sharp in focus, the working-range is thus considerably reduced. In fact, if the highest possible accuracy is required, the maximum thickness to be measured in opaque objects is less than 2μ . For cytochemical work, the thickness of the sections generally exceeds 5μ , and it is therefore impossible to determine the thickness with satisfactory accuracy.

In the new method, the principle of the light-cut and light-profile method is employed; but only as an aid to focusing. The thickness proper (equivalent to the movement of the objective) is obtained by a mechanical measuring instrument, a 'mikrokator' (manufactured by C. E. Johansson, Eskilstuna, Sweden), attached to the microscope.

If a profile-microscope is focused on an even surface, the shadow is seen as a sharp line, slightly curved towards the periphery of the field of view. Defocusing in either direction leads to a parallel displacement of the line, which at the same time becomes unsharp and weaker in contrast. This parallel movement is symmetrical around the middle position as regards movement, sharpness and contrast. If a line in the ocular is superimposed upon the line of symmetry, this parallel displacement allows very accurate and reproducible focusing on two different levels in the object. These levels may, for example, be represented by the upper surface of a microtome section and the upper surface of the object slide, that is, the lower surface of the section. The difference between the two levels of focusing is, as mentioned above, measured by a 'mikrokator'. The measuring point of the 'mikrokator' rests upon a horizontal beam attached to that part of the microscope which is moved by the micrometer screw. A 'mikrokator' measuring a vertical movement of the object down to 0.1μ with an accuracy of ± 1 per cent is well suited for this purpose.

Just as in profile microscopy, the accuracy depends upon the structure of the object, the width, sharpness and contrast of the shadow and the aperture and magnification of the objective. In the present method, however, the accuracy is independent of the absolute thickness of the object, which may be as great as 100μ . The method is well suited for routine work, as one measurement involves only two focusings and two readings on the 'mikrokator', which then give the thickness directly in μ .

As a check upon the absolute accuracy of the method, ten measurements were made upon a layer of aluminium evaporated on glass. The average was: $3.71 \pm 0.04\mu$; stand. dev. = $\pm 0.12\mu$. This compares well with the thickness measured by multiple interferometry: 3.72μ .

Sections of biological material embedded in paraffin, celloidin and plastic may be measured with approximately the same accuracy.

A more detailed report will be published later.

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¹ Schmaltz, G., "Technische Oberflächerkund" (Berlin, 1936).

² Naumann, H., *Bl. Unters. u. Forsch.-Inst.*, 16, 25 (1942).

³ Menzel, E., *Die Naturwiss.*, 14, 332 (1951).

⁴ Tolansky, S., *Nature*, 169, 445 (1952); *Lab. Pract.*, 291 (1953).