examined. I am grateful to the authorities of the British Museum (Mineralogical Department) for the loan of the crystals, and to the British Council for a scholarship.

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[1] Verma, A. R., *Nature*, **167**, 939 (1951); **168**, 430 (1951); **168**, 783 (19 1); *Phil. Mag.*, **42**, 1005 (1951).
[2] Burton, W. K., Cabrera, N., and Frank, F. C., *Phil. Trans. Roy. Soc.*, **243**, 299 (1951).
[3] Bragg, W. H., and Bragg, W. L., "X-Rays and Crystal Structure" (4th edit., 1924).
[4] Tolansky, S., "Multiple-beam Interferometry of Surfaces and Films" (Oxf. Univ. Press).

Interference Microscopy and Mass Determination

WE are interested to find that Dr. R. Barer, in his letter dated October 20, 1951[1], has evolved the method of mass determination in cells by interferometry. We have already described what is apparently this method at a meeting of the Society for Cell Biology in September 1951[2]; but, as our conclusions may not be generally known, we would like to state them briefly here.

Cell interferometry provides a new method of measuring growth, and we have been trying the method with the view of obtaining, in living cells, measurements of total substance (other than water) so as to correlate these with measurements[3,4] of total quantity of ultra-violet absorbing substance. In contrast to ultra-violet microscopy, cell interferometry will not injure living cells, and hence a quantitative record may be obtained of the change of substance in one cell during a long period of time, and during intracellular changes.

The usual formula, for solutions, connecting quantity of dissolved substance with optical path-length applies also for concentrated gels and dry substances, and may be expressed in the form

$$M = \frac{\varphi}{\chi},$$

where M is the mass of substance per unit area, φ is the optical retardation produced by the substance. χ for solutions is 100 α, where α is the specific refractive increment. χ for a dry substance is $(\mu_1 - \mu_2)/\rho$, where μ_1 is the refractive index of the substance, μ_2 is the refractive index of the immersion medium, ρ is the density of the substance.

As Barer has pointed out, values of α for solutions of proteins and deoxyribose nucleic acid are $\sim 0{\cdot}0019$ ($\chi = 0{\cdot}19$). The figures in the accompanying table show, however, that for other cell constituents χ does not vary much, and hence one may expect cell interferometry to give approximate dry weights of cells and cell constituents, *independent of composition*. In fact, the very non-specificity of light refraction makes cell interferometry a useful complement to specific cytochemical methods. Large proportions of lipoid in protein, even fats themselves, and carbohydrates will seldom introduce an error which is likely to be greater than that involved in the optical measurements. Ovalbumin and tobacco mosaic virus contain, respectively, small and large proportions of aromatic amino-acids, but the value of χ is not significantly different. Salts sometimes give markedly different values of χ, but this will seldom introduce error as the concentrations are low, and the effect is likely to be cancelled out by similar concentrations in the isotonic medium in which the cells are immersed.

Compound	Physical state	Density	Refractive index	χ
β-Lipoprotein[5] (75% lipid)	dil. sol.			0·17
Fats[6]	Nat.	0·93 av.	1·46 av.	0·14 av.
Sucrose[6]	2% sol.	1·006	1·3359	0·141
Sucrose[6]	Cryst.	1·588	1·558	0·141
Starch[6]	Solid	1·50	1·53	0·133
Ovalbumin[7]	1·61% sol.			0·187
Ovalbumin[7]	6·45% sol.			0·188
Tobacco mosaic virus[8]	dil. sol.			0·17
Tobacco mosaic virus	dry solid	1·335 av.[9]	1·534[10]	0·151
Gelatin[6]	dry solid	1·27	1·525 av.	0·151
Sodium chloride[6]	5·25% sol.	1 035	$1{\cdot}34_3$	0·163
Sodium chloride[6]	Cryst.	2·165	1·544	$0{\cdot}097_5$
Potassium chloride[7]	10% sol.	1·07	1·3449	0·115
Potassium chloride[6]	Cryst.	1·984	1·490	0·079
Calcium chloride[6]	17% sol.	1·143	1·374	0·21
Calcium chloride[7]	Cryst.	2·512	1·52	0·075
Yeast nucleic acid[11]	Approx. 2% sol.			0·168

The second important aspect of the method is that it gives dry weights *independent of concentration*. Even in cells where much of the nucleus and cytoplasm contains as little as 10 per cent or less dry weight, the nucleoli may contain $\sim$ 70 per cent dry weight. If the substances involved obeyed the Lorenz–Lorentz relation[5], χ would increase somewhat as the concentration increased. However, such approximate data for dry proteins as exist indicate that χ decreases instead of increasing with concentration.

Inhomogeneous distribution of substance, and resulting light scatter, diffraction, etc., will introduce errors in cell interferometry in much the same way as in microspectrography, and an analysis of these errors is in progress. However, from what has been said above, a general rule of some importance to microscopists emerges. In cells, differences of composition (with dry weight per unit area remaining constant), such as difference in proportion of nucleic acid and protein, will not produce large percentage differences of optical path-length. Hence, interference microscope and phase-contrast pictures of cells are generally pictures of dry-weight variation.

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[1] Barer, R., *Nature*, **169**, 366 (1952).
[2] Davies, H. G., and Wilkins, M. H. F., Cytochemistry Commission of the Society for Cell Biology: "Physical Aspects of Cytochemical Methods" (Stockholm), September 1951 (in the press).
[3] Walker, P. M. B., and Davies, H. G., Farad. Soc. Discuss. No. 9 (1950).
[4] Walker, P. M. B., and Yates, H. B. (in the press).
[5] Armstrong, jun., S. H., Budka, M. T. E., Morrison, K. C., and Hasson, M., *J. Amer. Chem. Soc.*, **69**, 1747 (1947).
[6] "Handbook of Chemistry and Physics" (Rubber Company, Ohio).
[7] Perlman, G. E., and Longsworth, L. G., *J. Amer. Chem. Soc.*, **70**, 2719 (1948).
[8] Oster, G. (private communication).
[9] Bawden, F. C., and Pirie, N. W., *Proc. Roy. Soc.*, B, **123**, 274 (1937).
[10] Bernal, J., and Fankuchen, I., *J. Gen. Physiol.*, **25**, 111 (1941).
[11] Davies, H. G., and Wilkins, M. H. F. (unpublished).