LETTERS TO THE EDITORS

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High-Speed Counting with the Flying Spot Microscope

In the flying spot microscope the specimen is scanned by a single spot and the resulting pictures displayed on a television monitor tube[1]. If the microscope specimen consists of a number of particles, and if the output of the microscope is taken directly to a high-speed counter, each particle will be crossed by the spot more than once, and the counter will register the total number of intercepts, giving a count greater than the number of particles in the field. In order to obtain a count equal to the number of particles in the field, the following development has been made.

A birefringent crystal is mounted between the objective and the specimen, and a 'polarizing cube' below the condenser. An additional photocell is mounted at right-angles to the original photocell. An anti-coincidence circuit is connected between the photocell outputs and the counter. The counter start and stop mechanisms are connected to the microscope frame-blanking amplifier, via differentiating circuits.

Thus the flying spot from the objective is split into two spots by the birefringent crystal, and therefore the specimen is now scanned by two spots instead of one. The spots are adjusted one line-width apart, and are polarized at 90° to one another. The transmitted light from these spots is now collected by the condenser and passed to the photocells via the polarizing cube, which transmits the light from one polarized spot to one photocell and reflects the light from the other polarized spot to the other photocell. Therefore each photocell only receives light from one spot, and never from the other. Consider that the two spots are approaching a microscopic particle, scanning from above, one spot being one line-width above the other. First of all one spot will hit the particle and be obscured. The result is that a signal will be generated by its associated photocell the output of which is, say, positive. This pulse then passes through the anti-coincidence circuit to the counter which then registers one. When this spot returns to the same particle on the next line, it will be one line lower and its companion spot will have taken its former place, one line-width above. The result is that both spots are now obscured by the particle and both photocells generate pulses, which are prevented from operating the counter circuit by the anti-coincidence circuit. Therefore no count is made. This will continue until the time when the bottom spot is off the particle, the top spot being obscured. A signal is now passed by the anti-coincidence circuit to the counter; but as this signal is made negative, it will not operate the counter. Therefore only when the lower spot *alone* is on the particle will the counter operate.

To prevent the counter from repetitive counting of successive frames, it is necessary for it to be started at one end of a frame and switched off at the other end. (This is done by the frame-blanking pulses from the frame-blanking amplifier.) The lagging edge of the frame-blanking pulse is used for operating the start mechanism of the counter, and the leading edge of the succeeding frame pulse to operate the stop mechanisms. It is therefore only necessary to press the reset button of the counter to obtain a complete count of all the particles in the field. Counts of particles at a speed of a million a second have been obtained at accuracies of the order of less than 1 per cent. Multiple-spot operation appears to offer considerable further possibilities in automatic sizing of particles, and this application is now being investigated.

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F. Roberts
J. Z. Young

Department of Anatomy,
University College,
London, W.C.1. April 30.

[1] Roberts, F., and Young, J. Z., *Nature*, **167**, 231 (1951).
[2] Walton, W. H., *Nature*, **169**, 518 (1952).

Resolving Power in Diffraction Microscopy with Special Reference to X-Rays

Rogers's[1] elementary explanation of Gabor's[2] technique for microscopy treats the hologram as a generalized Fresnel zone plate. I have used this idea for deriving a resolution criterion. Two point objects separated by a distance y produce two Fresnel zone patterns at the hologram with centres separated by a distance z, where $z = p_a y/(p_a - q_a)$, if p_a and q_a are the distances from the illuminating point source of wave-length λ_a to the hologram film and from the film to the object, respectively. The subscript a refers to parameters of the 'analysis' while r will refer to the 'reconstruction'.

If the hologram is enlarged photographically by a factor M, the distance between the centres of the reconstruction patterns, when the hologram is used like a positive lens, is $(p_r + q_r)Mz/p_r$, where p_r and q_r are distances from the illuminating point source of wave-length λ_r to the hologram and from the hologram to the reconstruction, respectively. For resolution in the reconstruction plane, we require that the central maximum of the diffraction pattern due to one object coincide with the first minimum of the diffraction pattern due to the other. According to Myers[3], the angular separation, β, between these two is $\beta = \dfrac{1{\cdot}22\ \lambda}{a}$, where a is the effective aperture of the Fresnel zone pattern being used. These considerations lead to

$$y = \frac{1{\cdot}22\ \lambda_a q_a M}{a_r}$$

Thus, the minimum resolvable distance, y, seems to depend upon the wave-length, λ_a, of the analysis, and the aperture, a_r, of the Fresnel zone pattern used in the reconstruction. So far, the expression does not contradict what the usual lens criterion would lead one to believe.

However, two real factors which limit the aperture of the zone pattern are the resolving power, N, of