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Three-dimensional image reconstruction of helical structures

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[Plates 40 and 41]

For structures with helical symmetry one electron micrographic image of the structure can provide sufficient information to reconstruct a three-dimensional image provided that, to the resolution to which one is working, only one helical family contributes to each layer plane of the Fourier transform of the structure (DeRosier & Klug 1968; DeRosier & Moore 1970). Mathematically this condition is

$$F(R, \psi, l/c) = G_{n,l}(R) \exp\{\mathrm{i}n(\psi + \tfrac{1}{2}\pi)\} \quad \text{for} \quad R < l/(\text{resolution}). \tag{1}$$

Since the micrographic image represents a projection of the structure, the Fourier transform of the image will be some central section $\psi = \psi_0$ of the transform of the structure:

$$F_{\text{image}} = F_{\text{structure}}(R, \psi_0, l/c),$$

The values of $G_{n,l}(R)$ can thus be obtained from the transform of the image using equation (1) and a three-dimensional image can be calculated using the inverse Fourier–Bessel transformation:

$$\rho(r, \phi, z) = \sum_l \int G_{n,l}(R) J_n(2\pi Rr)\, 2\pi R\, \mathrm{d}R \exp\{+(\mathrm{i}n\phi)\} \exp\{-2\pi \mathrm{i}lz/c\}.$$

In the foregoing treatment it is assumed that the particle imaged has perfect helical symmetry, that the image corresponds to a direction of view which is perpendicular to the helical axis, that the phase origin of the Fourier transform of the image lies on the projected helical axis and that the helical selection rule, $l = tn + um$ (Klug, Crick & Wyckoff 1958), for the structure is known. If such is the case, equation (1) predicts that in the transform of the image, Fourier coefficients at points equidistant from the meridian (the line $R = 0$) and lying on the same layer line should have equal amplitudes and that their phases should differ by $n\pi$.

In practice, the Fourier coefficients of the image do not always have these two properties. Differences between amplitudes at a pair of such points is often the result of inequality in the staining of the near and far sides of the particle. The effect of the inequality on the reconstructed image, however, is generally small. Discrepancies in the phases at a pair of such point can be the result of an accidental tilt of the helical axis out of the plane at right angles to the direction of view. The change in the phases is a function of n, l, R and the amount of tilt. The transform of an image usually contains sufficient information to detect and correct for such an accidental tilt (DeRosier & Moore 1970). It is also possible to purposefully tilt a particle by a known amount in order to verify the selection rule, determine the hand of the structure and assess possible distortions.

The method has been applied to tubes (helical arrays) of catalase, a tetrameric enzyme thought to have 222 point group symmetry (DeRosier, Moore, Klug, Kiselev & Vainshtein, unpublished results). Six different tubes were analysed with the following results. The axes of the tubes were found to be tilted out of the plane normal to the direction of view by 0° to $3\frac{1}{2}$°

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depending on the orientation of the particle. (This tilt was due to a mechanical fault in the microscope—see Kiselev, DeRosier & Atabekov 1969.) The selection rule was found to be $l = 8n + 51m$. Since the direction of the tilt was not known, the hand of the particle could not be determined. The phases and radial position of points were corrected for the tilt as given in DeRosier & Moore (1970). In the reconstructed image each molecule of catalase was found to have four peaks of density which have approximate 222 symmetry (see figure 1, plate 40).

Application of the method to substructures of muscle, namely *F* actin, thin filament (actin plus troponin and tropomyosin), and a complex of thin filament and subfragment 1 of myosin has allowed one, by difference methods, to identify the actin and subfragment 1 in the complex (Moore, Huxley & De Rosier 1970). Figure 2, plate 41, shows three views of the complex of thin filament with subfragment 1.

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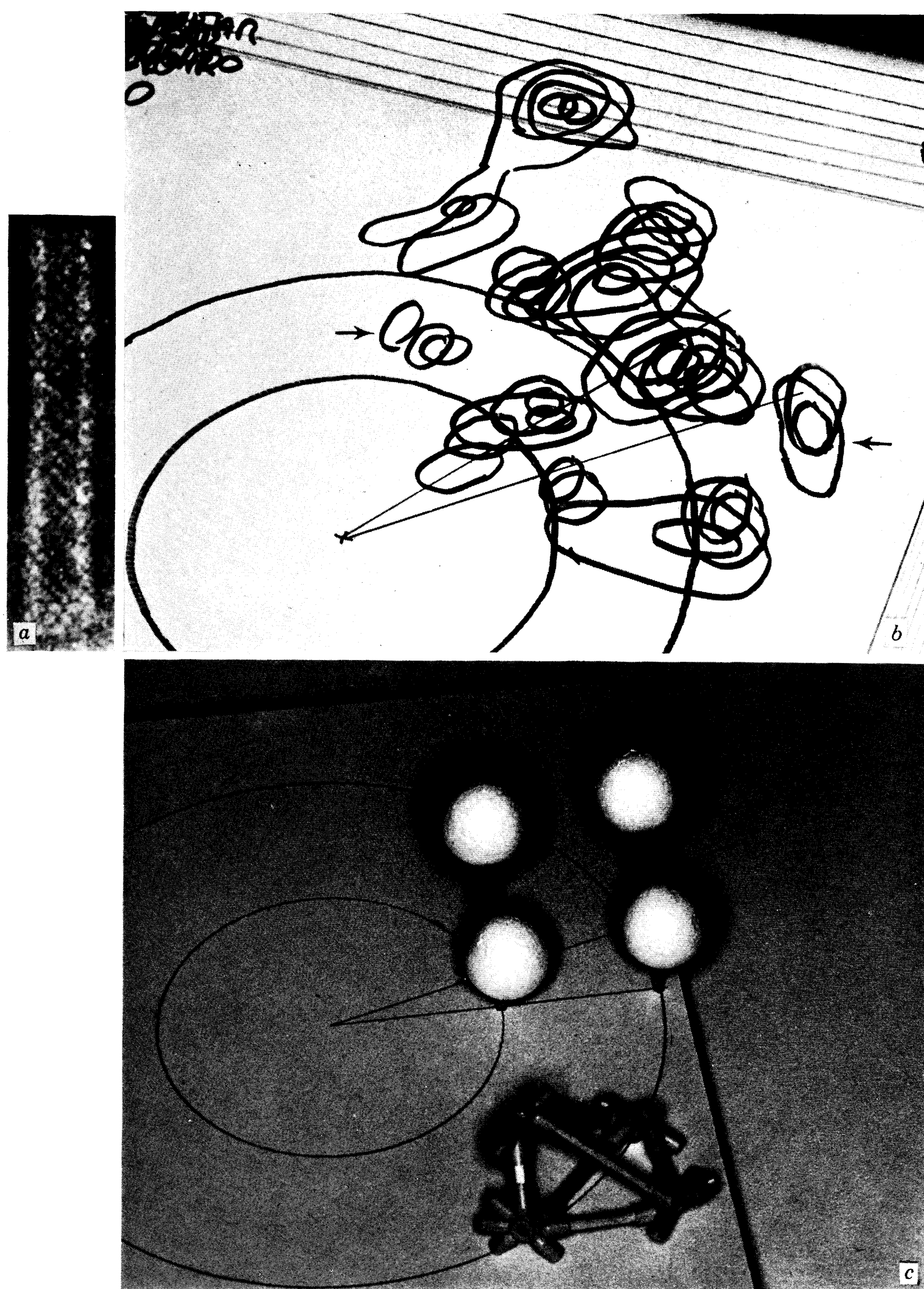

FIGURE 1. (*a*) An electron micrograph image of a tube of catalase (from Kiselev, Shpitzberg & Vainshtein 1967). (*b*) A sector of the three-dimensional image reconstructed using the image shown in 1*a*. The sector contains a complete molecule plus portions of the molecule just above and below it. (*c*) A ball model and a stick model shown at the same size and in the same orientation as the image. Note there are two peaks of density which are not accounted for by the model (see arrows). These presumably represent peaks of noise.

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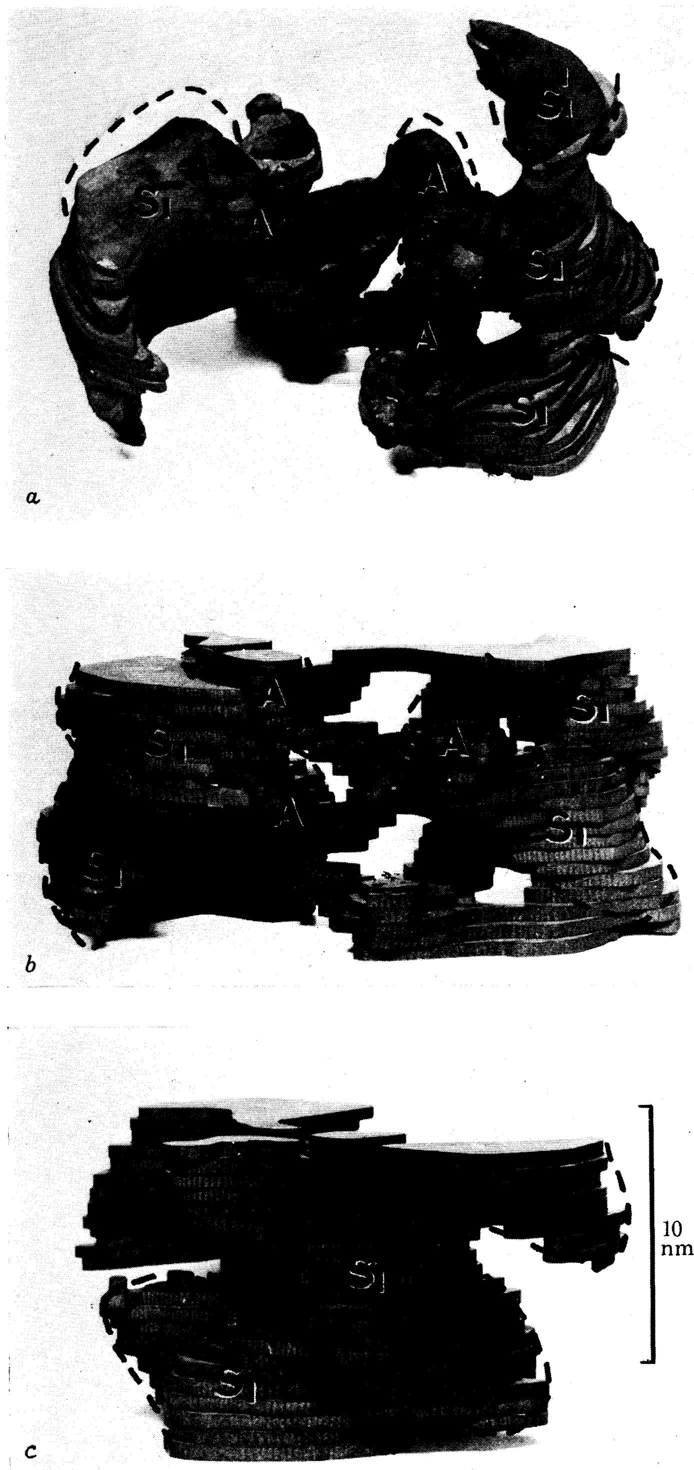

FIGURE 2. Three views of a model of the thin filament complex with subfragment one are shown. The dashed portions labelled A are actin monomers and those marked S_1 are the molecules of subfragment 1.

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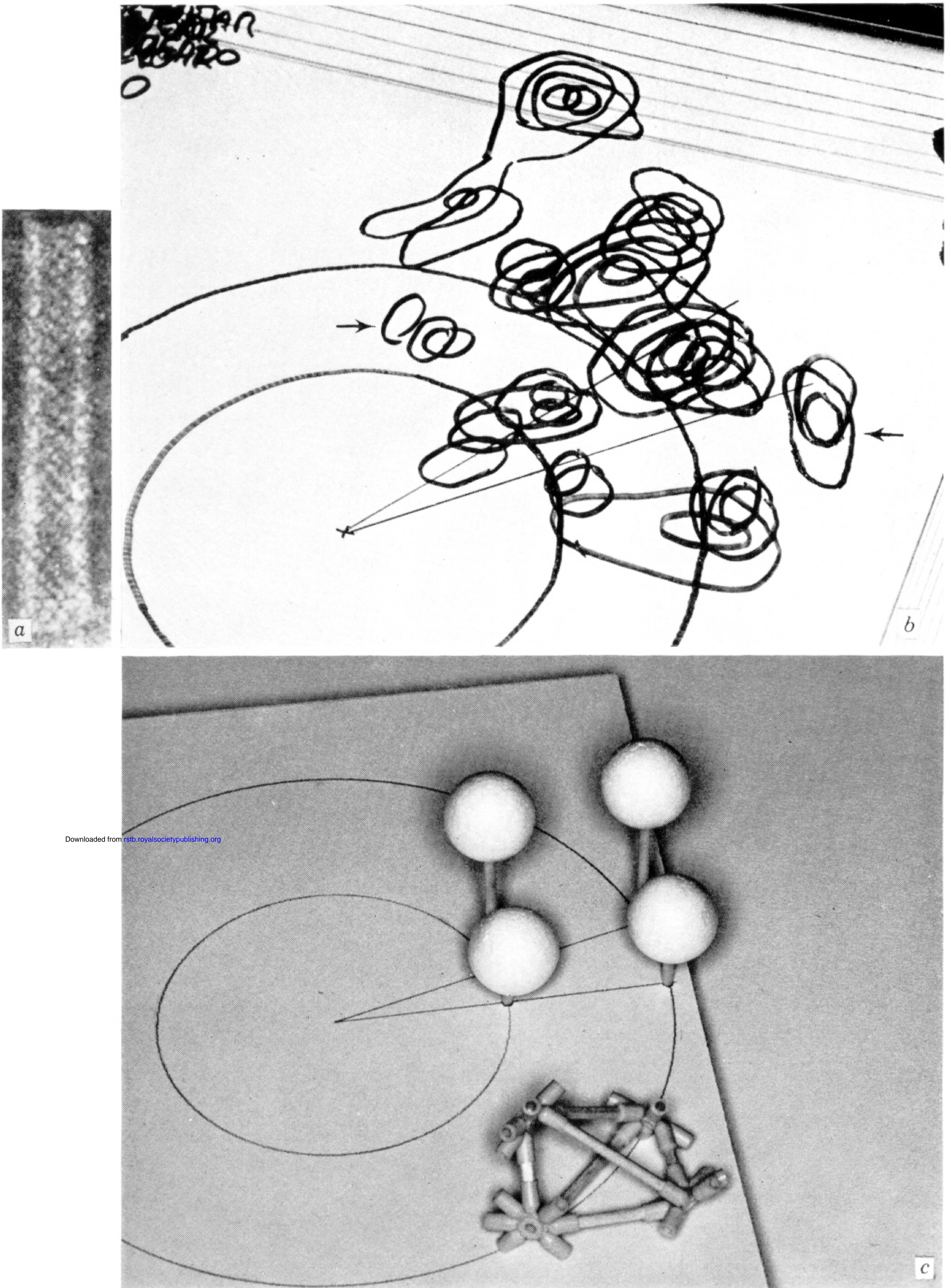

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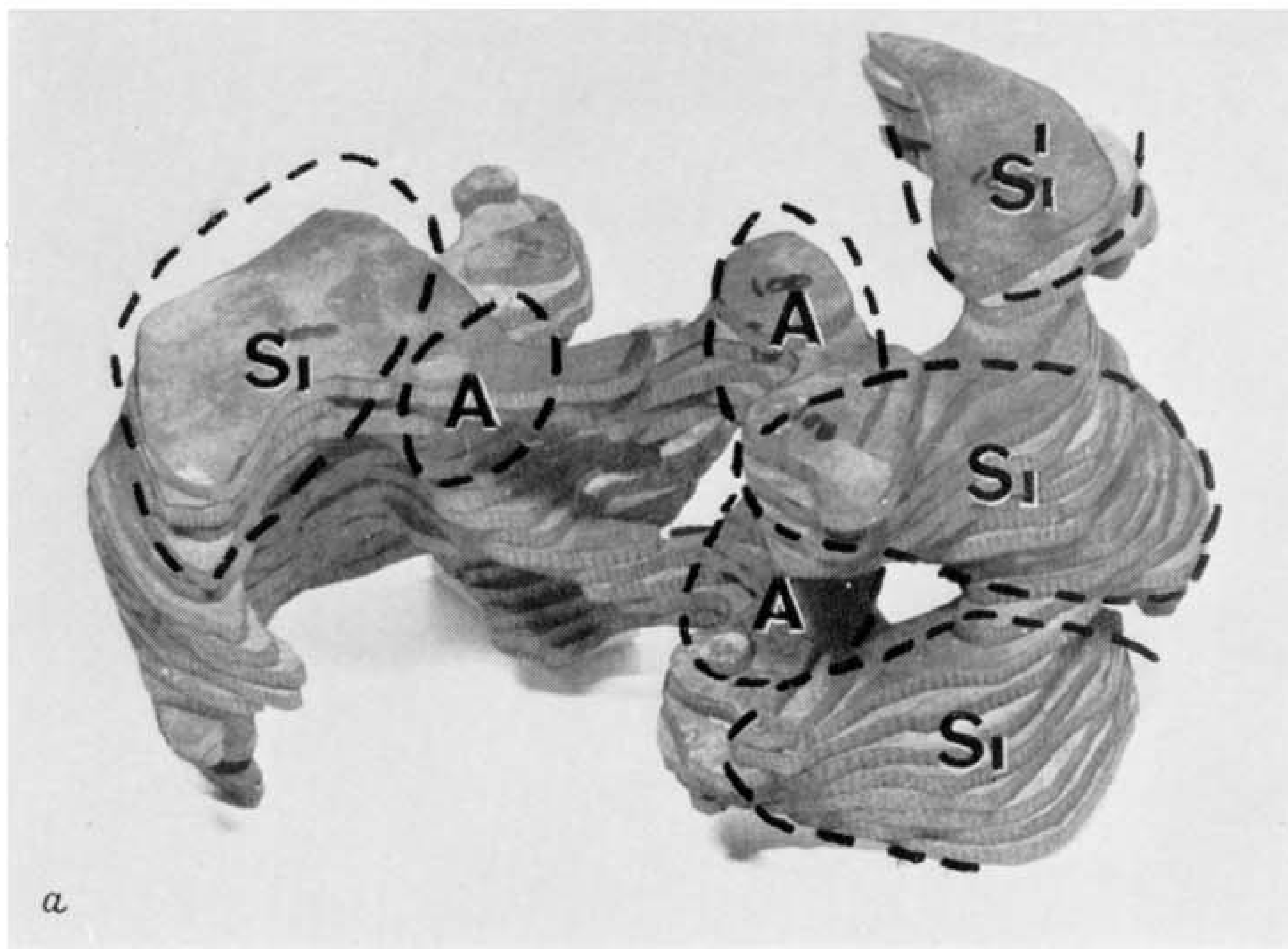

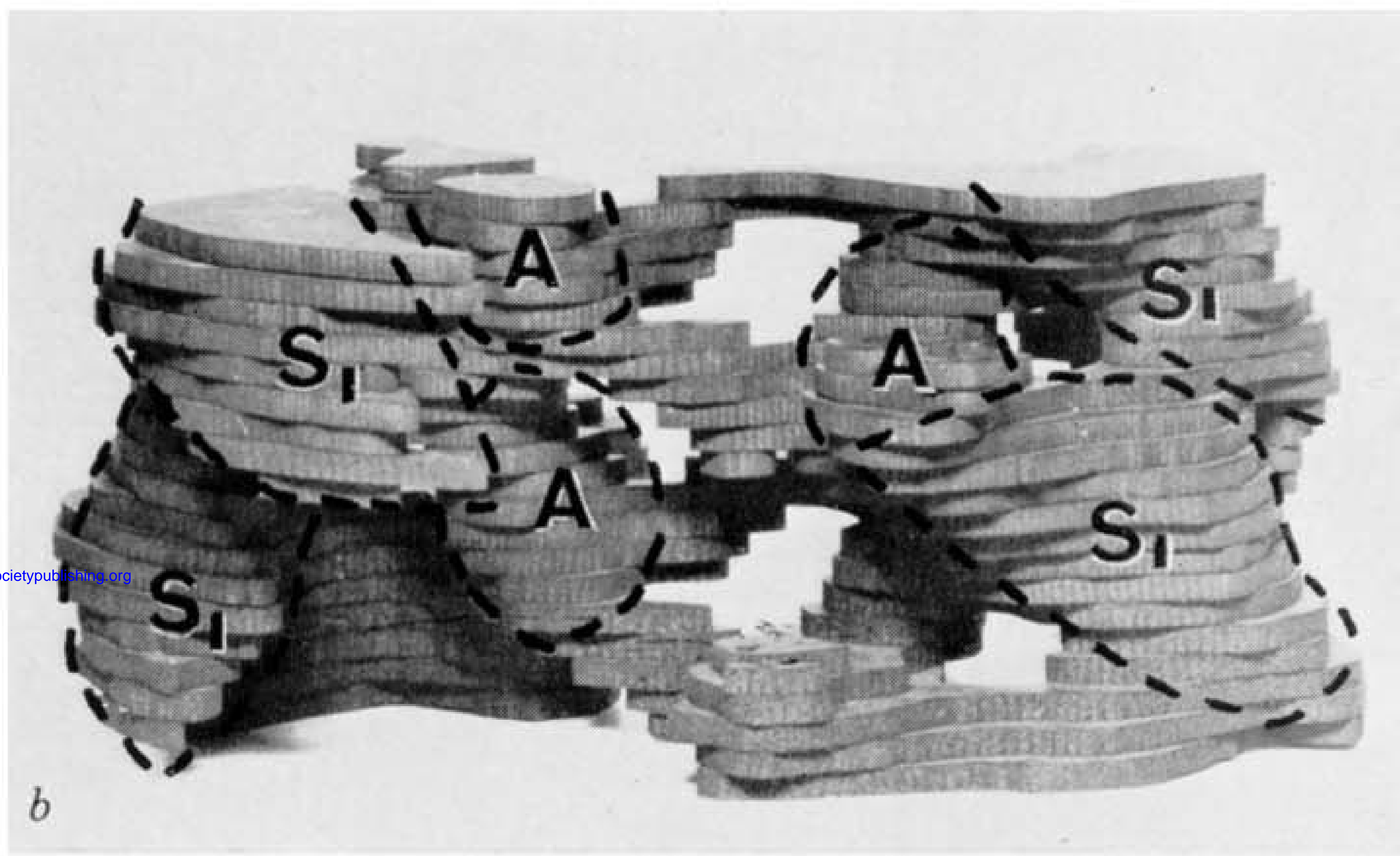

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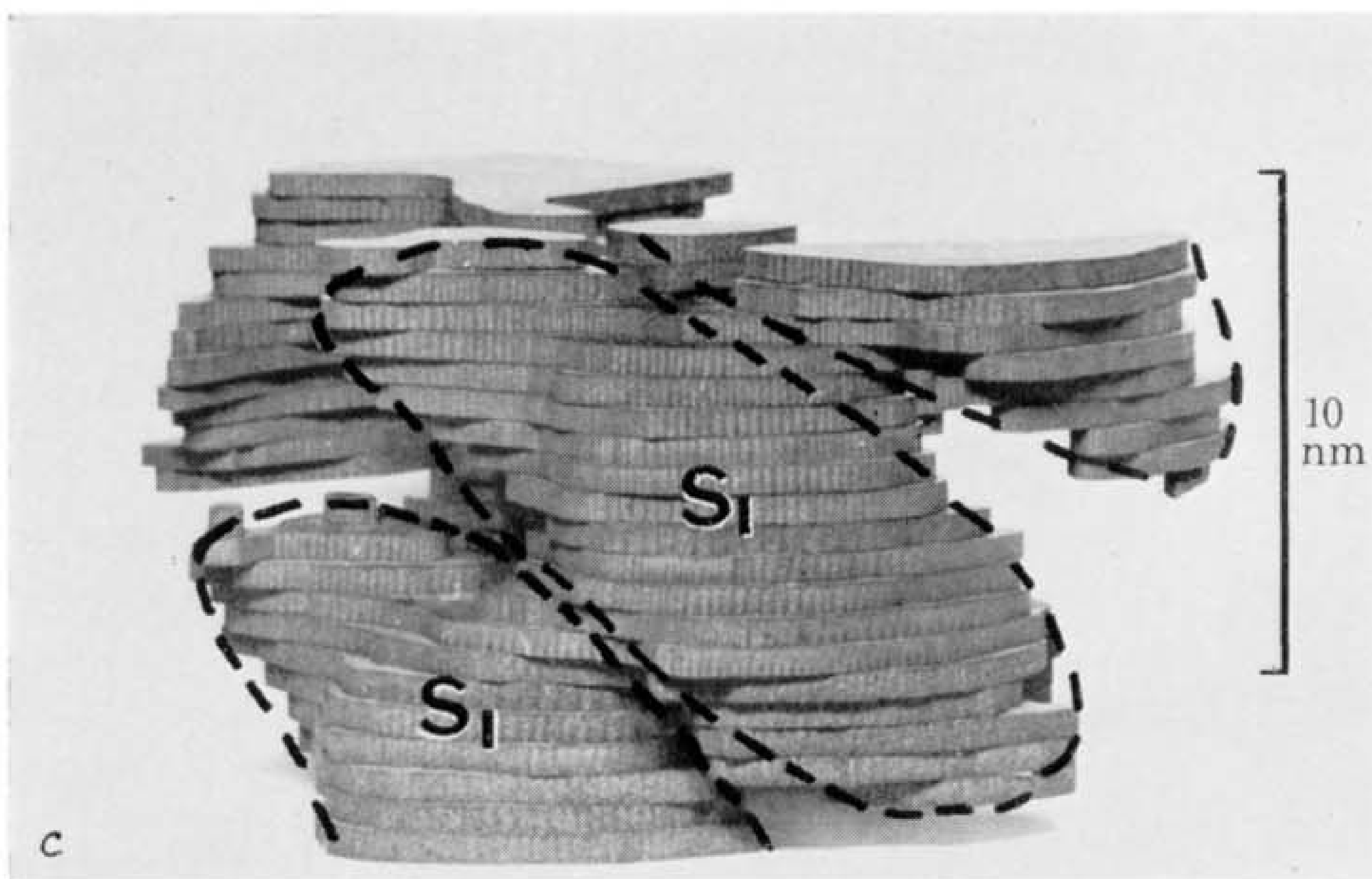

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