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A Device for the Rapid Measurement of Molecular Model Co-ordinates for X-ray Crystallography

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A device for the rapid and accurate measurement of model molecular co-ordinates, to be used in conjunction with a Richards optical comparator, is described. The device may be operated in either a manual or automatic mode. The manual mode allows an operator to find the co-ordinates of a desired atom by optical superposition of the transmitted image of a small marker light upon the reflected image of the atom to be measured. The automatic mode allows the operator to position the marker light automatically by entering preselected co-ordinates from an electronic console. This mode of operation facilitates the rapid construction and comparison of structures the atomic co-ordinates of which are already known. The device utilizes pulsed stepping motors to position the marker light and incorporates modularized solid-state circuitry throughout. Several applications of the device are described.

1. Introduction

The recent proliferation of X-ray determinations of protein structure has dictated the requirements for rapid and accurate model-building and measuring techniques. Most of the methods currently in use have mechanical limitations which render them inaccurate, or are at least sufficiently tedious so that the prospect of repeated measurement and adjustment of atomic positions required during refinement procedures is contemplated with apprehension. The device described in this communication was constructed to improve the speed and precision with which co-ordinates are measured, and to facilitate construction and comparison of structures the atomic co-ordinates of which are already known.

2. General Operating Description

The automatic co-ordinate hunting engine is used in conjunction with a Richards optical comparator (Richards, 1968). Measurement of co-ordinates is based upon the same principle of optical superposition by which a molecular model is initially constructed from Fourier electron-density maps. A similar instrument utilizing manual positioning and having analogue read-out has been successfully used for model co-ordinate measurement in the laboratory of F. M. Richards and H. W. Wyckoff at Yale University for some years. The digital device described here is essentially an automated version of that device, having additional model-building capabilities. Figure 1 shows a schematic diagram of the co-ordinate engine mounted on a horizontal Richards optical comparator. In order

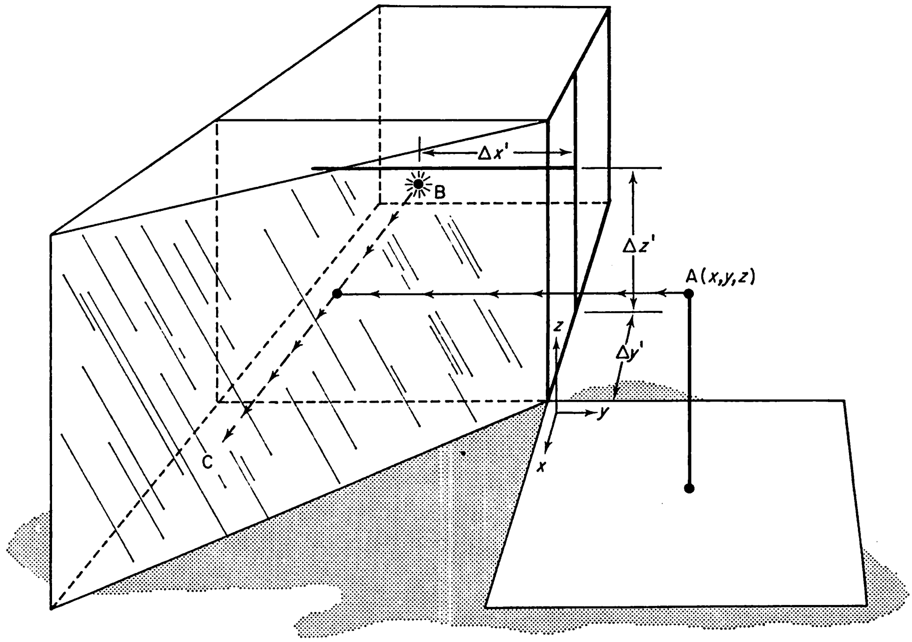

FIG. 1. Schematic drawing of the automatic co-ordinate hunting engine mounted on a horizontal Richards optical comparator. The moving axes of the machine define a left-handed (x', y', z') Cartesian co-ordinate system which is co-incident with the mirror image of the right-handed co-ordinate system in the model (x, y, z) space. Co-ordinate measurement is made by mechanically superpositioning the transmitted image of the marker light B upon the reflected image of the atom at A.

to measure the co-ordinates of an atom A in the model, a small spherical light source is mechanically positioned at B so that its transmitted image superimposes with the reflected image of the atom at A when viewed perpendicular to the $x'z'$ plane at C. Model co-ordinates are measured in a right-handed Cartesian co-ordinate system (x, y, z). Since the reflected image of the model is inverted by the mirror, the machine axes in the Fourier reference frame (x', y', z') define a left-handed co-ordinate system.

Plate I is a photograph of the mechanical positioning unit mounted on the side of the comparator showing the driving components for the marker light.

In the manual mode, the operator positions the marker-light source by means of a portable "joy stick" controlling digital pulse motors which drive the light in the Fourier reference frame. An electronic counting circuit records the number of pulses required to superposition the images of the atom and the marker light and continuously displays co-ordinates relative to a preselected origin. The position of the light is indicated in 0·1 Å increments on the 2 cm/1 Å scale commonly used for the construction of molecular models. The origin is set by "loading" the pulse counting circuits with some predetermined values by means of thumb lever switches. Travel on the axes is restricted by limit switches which shut off the drive motors when contacted. The limit switches may be used to define mechanically the origin of the Cartesian system by driving the motors to their minimum limits and then loading the counter values to $x, y, z = 00{\cdot}0$ by means of the thumb lever switches.

It is possible to position the light automatically at any desired location relative to the origin by switching to the automatic mode, setting the desired co-ordinates on the thumb lever switches, and hitting a "seek" button which activates comparator and pulse generating circuits, which automatically generate the proper number of pulses in the required direction to drive the marker light to the selected position. The largest volume in which co-ordinates may be measured is a cube 94 cm or 47 Å on a side at the 2 cm/1 Å scale.

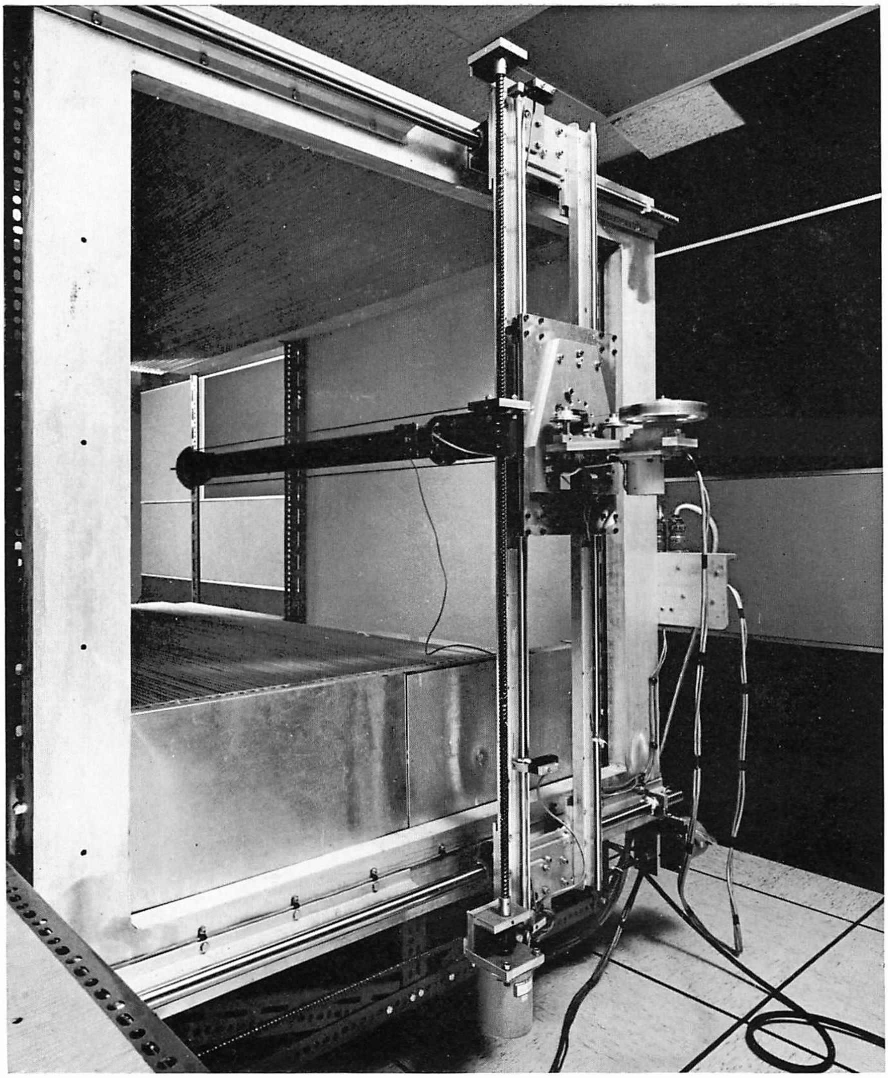

PLATE I. The mechanical unit of the co-ordinate engine consists of a rigid base upon which are mounted the y' axis drive motor and two 1 in. diameter ball-bearing slides which support the vertically mounted z' carriage. The y' axis motor drives a ball-bearing screw meshed to a recirculating ball-nut fixed to the lower end of the z' carriage. The z' carriage mounts the z' drive motor and screw, and two 0·5 in. diameter ball-bearing slides supporting the x' axis beam. The marker light is fixed to a small sled riding on 4 wires strung along the length of the x' beam. The marker light is translated along x' by a cable and pulley arrangement driven by the x' axis digital pulse motor. The small marker light is manufactured from a 1/8 in. diameter piece of plastic fiber light guide fixed to a lens-type 1·5 V penlight bulb.

[*facing p. 698*

3. Applications and Results

(a) *Co-ordinate measurement*

The use of the co-ordinate hunting engine allows an operator to measure molecular model co-ordinates continuously from one end of the chain to the other at a rate of approximately ten amino-acid residues (about 90 atoms) per hour. Use of the automatic mode allows rapid checking in the event of an error in transcription of an atom's position or sequence designation being made.

The average bond lengths and mean deviations for the N—Cα, Cα—C′ (carbonyl) and C′—N interatomic distances obtained from a single measurement of the co-ordinates of a model of cytochrome c_2 (*Rhodospirillum rubrum*, 112 residues) were found to be 1·47 (0·08), 1·58 (0·06) and 1·34 (0·07) Å, respectively (Salemme, manuscript in preparation). These values are to be compared with the comparable values for the bond lengths fixed by the 2 cm/Å Kendrew skeletal models which are N—Cα = 1·44, Cα—C′ = 1·53 and C′—N = 1·32 Å. The observed systematically high mean value for the measured bond lengths is an artifact of the rounding of the interatomic distances to the nearest 0·1 Å by the program used in the calculation of bond lengths from the measured co-ordinates, which were also measured to 0·1 Å precision. The largest deviation found between the calculated mean and fixed values was 0·05 Å for the Cα—C′ bond length, corresponding to 1 mm on the 2 cm/Å scale. This distance is somewhat smaller than the diameter of wire used for the construction of the skeletal models, and about one-third the diameter of the marker light. Remeasurement of atoms having a calculated bond length more than 0·1 Å from the expected value can be rapidly accomplished by utilizing the automatic mode to pre-position the marker light at the initially determined co-ordinates. In this way it is possible rapidly to obtain a precise set of co-ordinates for a protein structural model.

Computer drawings produced from machine measured co-ordinates show symmetrical and flat rings. Consequently, the machine has been useful in preparing computer drawings of model substrate and inhibitor complexes of the enzyme subtilisin (Robertus, personal communication).

(b) *Model building*

When the co-ordinate engine is operated in the automatic mode, it is possible to position the marker light automatically at any predetermined co-ordinates entered from the digital thumb lever switches. This feature has been used to build rapidly analogue substrates of α-chymotrypsin into the active site of the enzyme subtilisin.

In order to compare the detailed tertiary conformation of mitochondrial cytochrome *c* (Dickerson, personal communication) and the photosynthetic cytochrome c_2 of *R. rubrum*, the co-ordinates of cytochrome *c* were transformed and rotated into the proper orientation in the engine co-ordinate system used to measure cytochrome c_2. Small sections of the chain were built using calculated interatomic dihedral angles. These sections were subsequently positioned relative to one another in the Richards box using the automatic mode of the hunting engine. Model building by this technique is quite fast because of the rapidity and ease with which the reference marker light can be positioned.

Interested parties may obtain detailed mechanical and electronic circuit diagrams by writing to the authors.

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