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3,567,328

SPECIMEN TRANSPORT MOUNT FOR SPECTROPHOTOMETER

Filed June 2, 1966

2 Sheets-Sheet 1

FIG. 1.

FIG. 2.

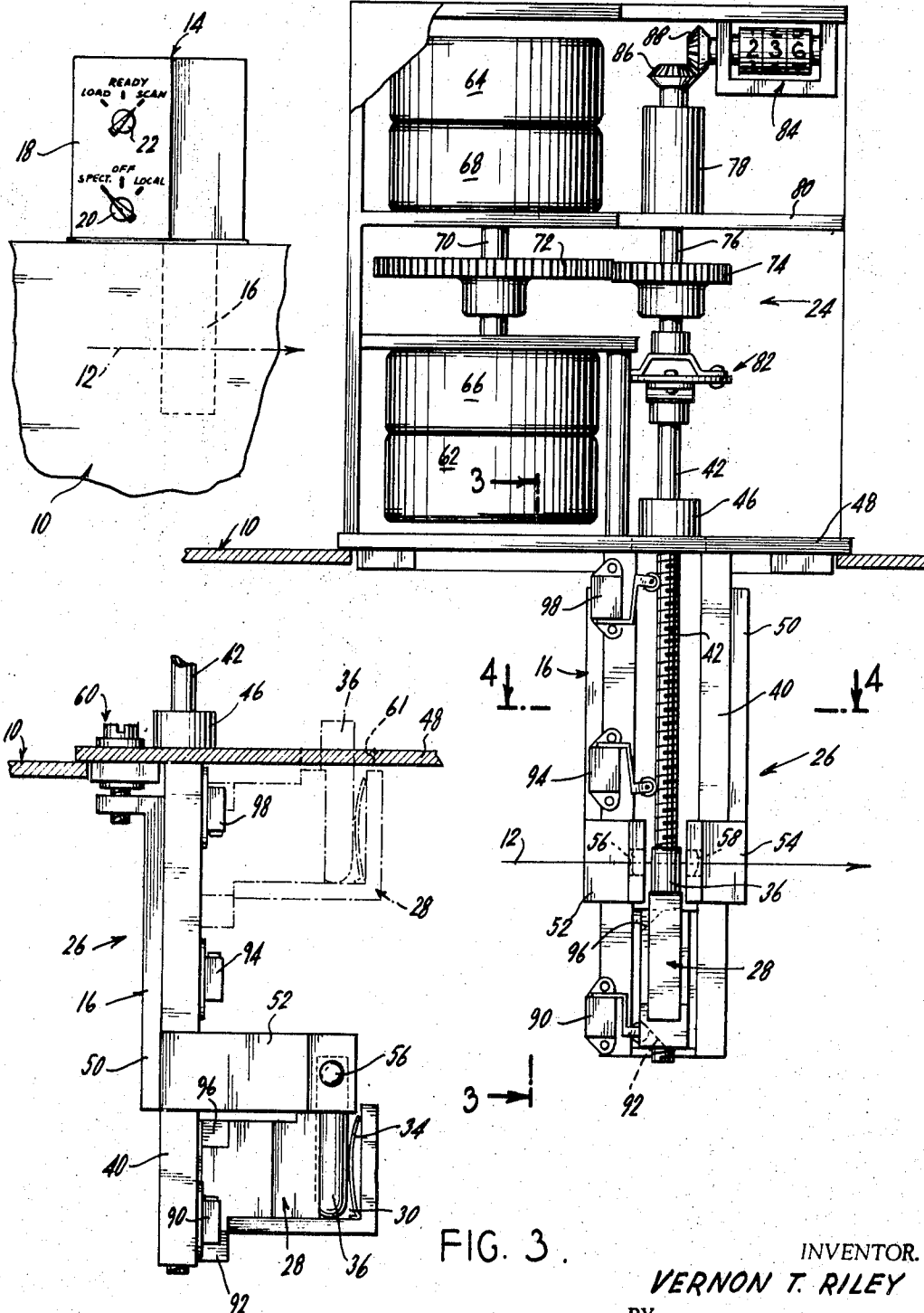


FIG. 3.

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FIG. 4.

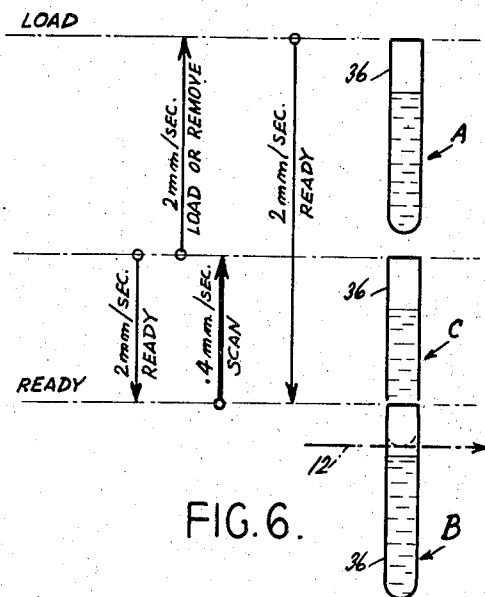
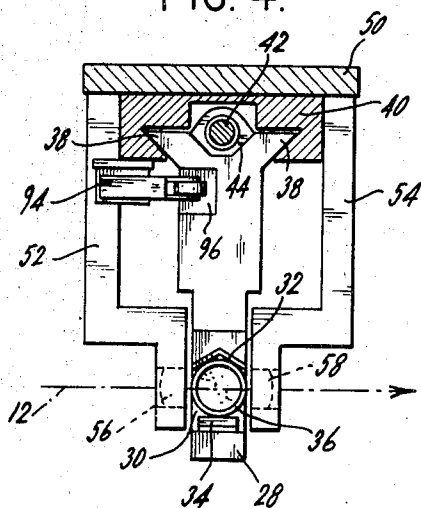


FIG. 6.

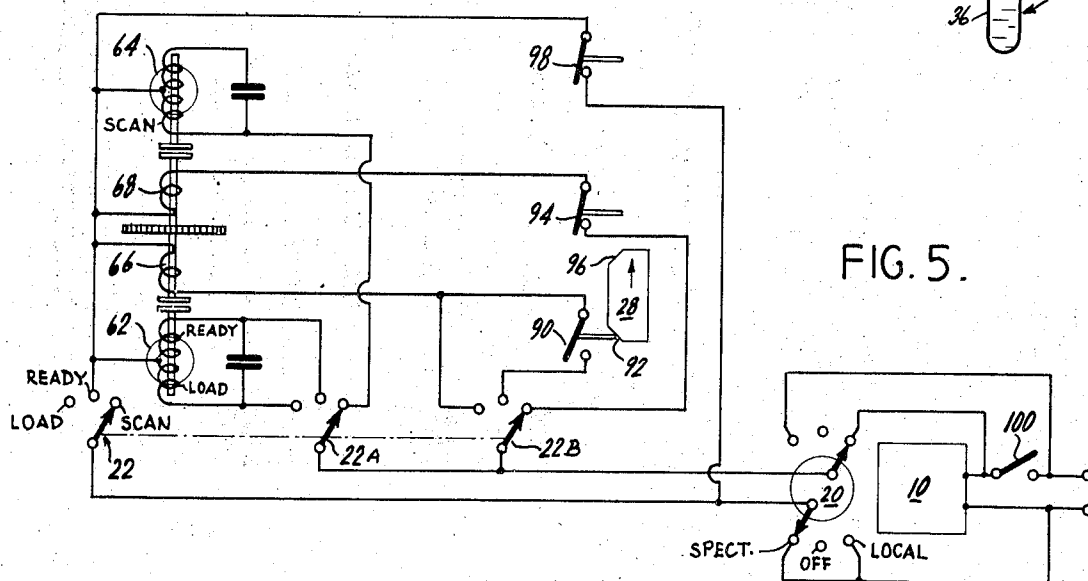


FIG. 5.

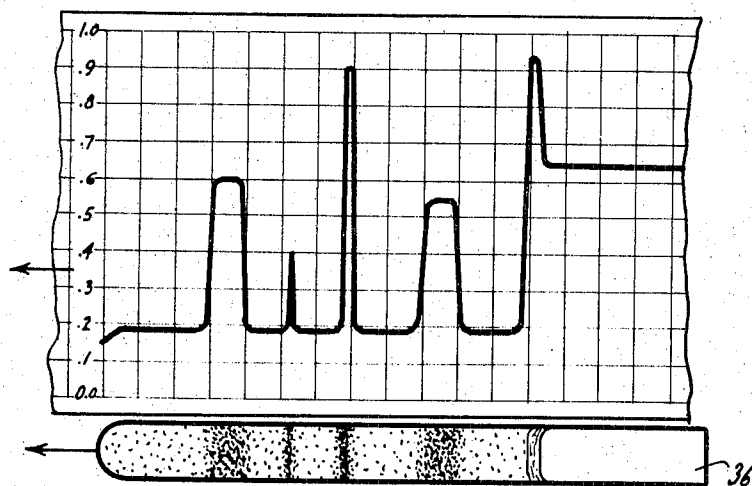


FIG. 7.

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SPECIMEN TRANSPORT MOUNT FOR SPECTROPHOTOMETER

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U.S. Cl. 356—244

6 Claims

ABSTRACT OF THE DISCLOSURE

Spectrophotometric analyses of specimens which have been processed by "density gradient centrifugation" can be performed by the apparatus disclosed herein without having to withdraw fractions of the material from the specimen tubes for separate analysis. The apparatus constitutes an attachment for a conventional spectrophotometer and transports the specimens in a specimen tube through the spectrophotometer's beam of light in a controlled scanning movement parallel to an established density gradient in the specimen.

This invention relates to a method and apparatus for the measurement of the optical density of a specimen which has been subjected to a centrifugal field in an ultracentrifuge and especially to such a specimen when it has been processed in an ultracentrifuge by the method known as "density gradient centrifugation." This method produces bands of specimen material along the length of a specimen tube. The specimen may be placed in a preformed density gradient material, such as a sucrose varying in density from the bottom to the top of a centrifuge specimen tube. Upon being subjected to the action of an ultracentrifuge, the specimen to be examined then seeks the level of density which matches its own molecular density. If it consists of a plurality of molecular species, each having a different density, it is divided into a series of bands located at the corresponding density region in the host liquid. The details of one method of this general type is described in a review by Howard Schachman in *Biochemistry*, vol. 2, p. 887, Sept. 20, 1963.

It has been prior practice to withdraw fractions of the material from the tube, either at various locations of interest or at one end layer by layer, slowly into a series of sample cuvettes and examine same photometrically in a spectrophotometer or other photometric device, or in other ways. Correlating the samples examined with the appearance of optical absorption at a particular wavelength, it was possible to establish the position or the centrifugal radius of the various zones into which the specimen had been decomposed by the centrifugation. It has been particularly advantageous to examine such specimens by spectrophotometry since the component substances can in this way be measured both qualitatively and quantitatively in the spectrophotometer. The optical absorption properties of substances, as is well known, are related to their molecular composition.

Although such examination by density gradient centrifugation has proven to be a useful research tool, I have found marked advantages for a new system, using new equipment, in which the centrifuged specimen, in the host liquid, is investigated in the specimen tube, without any need for it to be disturbed and placed into a series of sample cuvettes and wherein a continuous curve of optical density at a given wavelength may be taken along the length of the specimen tube. By making continuous measurements in situ, many advantages are produced, some of which are as follows: the material in each band cannot be contaminated because it is not disturbed, the specimen can be re-examined often at various wavelengths and the

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curves compared, the investigator cannot make the mistake of missing a possibly important band by selecting examining points above and below it, the materials can be examined in undiluted form, one may easily take measurements of a given sample at various times to produce a full record of migration with size to obtain information as to molecular size, and one can easily obtain information as to an infinite number of fractions over the full range of densities and with several wavelengths of lights. By using the method and apparatus of the present invention, I have found things which if they could have been found at all by prior techniques, would have taken days rather than hours.

Accordingly, it is an object of the present invention to provide an improved method and apparatus for observing a sample which has been treated in an ultracentrifuge. More specifically, it is an object of this invention to provide an attachment for a spectrophotometer which will transport a specimen through the spectrophotometer's beam of light in a controlled scanning movement parallel to an established density gradient in the specimen.

It is further within the contemplation of the present invention to provide an automatic transport mechanism for a liquid specimen for use in conjunction with a spectrophotometer which mechanism moves a specimen across the beam path of a spectrophotometer such that the optical density or light transmission of the specimen may be recorded as a function of the position in the specimen at which the beam of light traverses it.

In accordance with one presently preferred illustrative embodiment of the invention, there is provided a specimen mount for use with a spectrophotometer of the type having a beam of light along a beam path. The specimen mount comprises a frame on which a specimen carriage is mounted by means of appropriate guide means for movement along a controlled vertical path. The specimen carriage has means to secure the specimen in a vertical orientation and is positioned such that the spectrophotometer beam path sweeps along the vertical extent of the specimen as the carriage is moved along its vertical path. Drive means are provided in the device for moving the carriage through a controlled vertical scanning movement to sweep the height of the specimen holder past the beam. The specimen carriage may also be moved at a faster rate to a loading location spaced from the beam path to provide ready access to the carriage for the loading and unloading of specimens. Switching means may be provided for automatically governing the drive means and for denoting the beginning and end of the scanning movement. The improved specimen mount is associated with a recording device to record the signal from the spectrophotometer as a function of movement of the specimen along its scanning path thereby to produce a graph of the optical density of the specimen as it is measured against the relative vertical position of the specimen at which the optical density is recorded.

The above brief description, as well as further objects, features and advantages of the present invention, will be best appreciated by a consideration of the following detailed description of one illustrative embodiment of the invention when taken in conjunction with the accompanying drawings wherein:

FIG. 1 is an elevational view of the attachment in accordance with the present invention shown positioned on a spectrophotometer with a portion of the device shown in phantom. This figure indicates the location of the beam path of the spectrophotometer;

FIG. 2 is an elevational view of the transport device in accordance with the present invention, with most of the covering portions broken away for clarity, and shown mounted in a spectrophotometer and illustrating the posi-

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tion of its various elements with respect to each other and with respect to the spectrophotometer beam path;

FIG. 3 is a partial side sectional view taken at right angles to the view shown in FIG. 2 and taken along the line 3—3 of FIG. 2 and looking in the direction of the arrows;

FIG. 4 is a sectional view taken along the line 4—4 of FIG. 2 and looking downwardly in the direction of the arrows;

FIG. 5 is a schematic diagram of the electrical switching and driving means for the device in accordance with the present invention and illustrating the manner in which electrical interconnection is made to the spectrophotometer;

FIG. 6 is a diagrammatic showing of the relative "Loading" and "Ready" positions of a specimen in the transport device in accordance with the present invention and comprises a diagrammatic showing of the various movements exhibited by a sample positioned in the device; and

FIG. 7 is an illustrative graph of the type produced by a spectrophotometer when using the transport mechanism in accordance with the present invention with a diagrammatic showing of a specimen tube positioned parallel to the horizontal axis of the graph for comparative purposes.

Referring now to the drawings, there is shown in FIG. 1 an elevational view of a portion of a photometer 10, specifically, a spectrophotometer 10, with the location of its light beam diagrammatically indicated by the arrow 12. A specimen mounting and transport device, generally designated by the numeral 14 and constructed in accordance with the present invention, is positioned on the spectrophotometer 10 with its downward extension 16 extending downwardly into the spectrophotometer 10 and interrupting the beam path 12. The device 14 has a front plate 18 on which is positioned a power source switch 20 and a mode of operation switch 22.

Basically, the transport device comprises a driving mechanism, generally designated by the numeral 24, located in the upper portion of the device and a specimen carriage and carriage guide mechanism, generally designated by the numeral 26, located below the driving mechanism 24 and mounted on the downward extension 16. The specimen carriage itself, designated by the numeral 28, is seen in side view in FIG. 3, in front elevational view in FIG. 2 and in top view in FIG. 4 and includes a specimen-containing location 30 having a V-shaped back wall 32 and specimen-retaining spring 34 biased toward the back wall 32. A specimen tube 36, having a closed lower end and adapted to contain the specimen to be analyzed, fits securely between the V-shaped rear wall 32 and the spring 34 and rests on the carriage 28 at the bottom of the specimen-containing space 30. The specimen tube 36 is constructed of material which is of good optical quality so that it does not cause any variation in the beam of light in the spectrophotometer as it is moved vertically through that beam.

At the rear of the specimen carriage 28, there are provided a pair of outwardly or sidewardly extending guide wings 38 which are received in complementary shaped elongated notches or ways formed in a vertical support member 40. The vertical support member 40 comprises a portion of the frame of the transport device 14 and the interaction of the wings 38 on the carriage 28 and the complementary slots in the support member 40 provide a smooth, reproducible vertical path of movement for the carriage 28.

Extending downwardly from the driving mechanism 24 is an accurately machined lead screw 42 which is engaged within a complementary threaded aperture 44 formed vertically in the carriage 28 between the two wing portions 38. As may be best seen in FIG. 4, the support member 40 is cut away to provide room along its length for the portion of the body of the carriage 28 which in-

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cludes the threaded opening 44. It will be readily appreciated that as the lead screw 42 turns in a clockwise direction (as viewed from above) the carriage 28, and a specimen mounted therein, will be moved vertically upwardly whereas counterclockwise rotation will produce downward vertical movement of the carriage 28.

The lead screw 42 providing the drive means for the carriage 28 is mounted by means of a bushing 46 secured on the bottom plate 48 of the drive mechanism 24 and provide both axial and transverse support for the lead screw 42. The threaded aperture 44 of the carriage 28 aids the bushing 46 in securing the lead screw 42 against transverse movement.

As may best be seen in FIGS. 2, 3 and 4, a bracket 50 is provided extending downwardly from the plate 48 and has a pair of forwardly directed arms 52, 54, each of which is bent toward the other and terminates at a point aligned with the beam path 12. The arms 52, 54 carry appropriate lens elements 56, 58 to focus and/or align the beam of light along the beam path 12. As seen in the view of FIG. 4, the lenses 56, 58 are positioned along either side of the location of the specimen tube 36. Of course, the arms 52, 54 and the lenses 56, 58 mounted therein remain stationary during the entire operation of the device 14. An adjustment assembly 60 (see FIG. 3) is provided to adjustably mount the bracket 50 on the bottom plate 48 so that exact alignment of the lenses 56, 58 with the beam path 12 may be easily accomplished.

Prior to a discussion of the details of the driving mechanism 24 and the microswitches which control the driving elements and therefore the movement of the carriage 28, reference will be made to FIG. 6 for a description of the various movements of the carriage 28.

An access port 61 is provided in the plate 48, in alignment with the specimen-receiving cavity 30 of the carriage 28, and when the carriage 28 is in its uppermost position (the LOAD position shown in phantom in FIG. 3), the operator of the spectrophotometer may easily place the specimen tube 36 into its appropriate spot on the carriage. In a diagram of FIG. 6, the upper edge of the specimen tube has been arbitrarily selected as the reference point, it being understood that this is merely a diagrammatic illustration and any given point could have been chosen as a starting reference. In position A, the LOAD position, the specimen tube is shown at the point it is loaded into the carriage. By operation of the driving mechanism 24, through a switch 22, the carriage 28 is moved downwardly from its loading position to its lowermost or READY position, labeled B in the diagram of FIG. 6. At this position, the beam path 12 is approximately aligned with the meniscus of the specimen such that as the tube 36 is raised upwardly slowly through the scanning movement of the carriage 28, the beam will pass through the specimen along the entire vertical extent of the specimen. Scanning movement, which is much slower than that of the movement from the LOAD to the READY position, is initiated by placing the mode of operation switch 22 into its SCAN position. At the completion of the scanning movement, the carriage 28 is located at an intermediate position along its path of vertical movement at position C in FIG. 6). At this point, the operator may set the mode switch 22 to its READY position to move the carriage 28 back to the READY position in order to prepare it for another scanning movement; alternatively, the operator may move the mode switch 22 to its LOAD position which will bring the carriage 28 up into the LOAD position as shown in dotted lines in FIG. 3 such that the specimen may be removed.

A pair of motors 62, 64 and a complementary pair of electromagnetic clutches 66, 68 are housed within the upper chamber of the device 14 and are connected to a common shaft 70 on which is mounted a main driving gear 72. The main driving gear 72 on the shaft 70 is connected to a driven gear 74 which is rigidly secured to an aux-

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iliary shaft 76 which is axially aligned with the lead screw 42 and is mounted for rotation on a bearing 78 positioned on an intermediate shelf 80 in the driving mechanism 24. A universal flexible joint 82 interconnects the secondary shaft 76 with the portion of the lead screw 42 which extends above the bearing 46. Accordingly, it will be appreciated that rotation of either one of the motors 62, 64, when their respective clutches 66, 68 are engaged, will cause rotation of the secondary shaft 76 and hence the lead screw 42 and thereby will produce movement of the carriage 28. The intermediate shaft 76 at its upper end drives a counter 84 by the interaction of the bevel gears 86, 88. The counter 84 provides convenient visual indication of the location of the carriage 28 along its vertical path.

A set of three microswitches is mounted along the support member 40 of the downward extension 16 and are positioned to be engaged by the carriage at the LOAD position, at the READY position and at the position at the top of the scanning movement. Specifically, a ready microswitch 90 is mounted at the bottom of the supporting member 40 and engages a first inclined surface 92 (see FIG. 2) of the carriage 28 when the carriage has moved to its lowest position. The microswitch 90 is normally closed and thus a circuit is opened when the carriage arrives at the READY position thereby to de-energize the driving mechanism 24. A second microswitch 94 is mounted intermediate the length of the supporting member 40 and is positioned to engage a second inclined surface 96 on the carriage 28 when the carriage 28 has completed its scanning movement. This second microswitch 94 is also of the normally closed variety and is effective to open a circuit at the end of the scanning movement to de-energize the driving means. A third or LOAD position microswitch 98 is located at the upper end of the supporting member 40 and engages the second inclined surface 96 of the carriage member 28 when the carriage member has been transported up to the LOAD position. This microswitch is also of the normally closed type and opens the circuit to de-energize the driving mechanism 24 when the carriage 28 has reached the LOAD position.

Reference will now be made to the schematic electrical diagram of FIG. 5 for a description of the electrical circuitry employed in the driving and switching mechanisms of the transport device 14. The power source switch 20 is connected to appropriate power lines either directly (when the switch is set in its "LOCAL" position) or through the spectrophotometer 10 (when it is set in its "SPECT." position). In its "OFF" position, of course, no power is sent to the unit. The "SPECT." position allows the operator to set up the transport device 14 in position to operate as soon as an appropriate switch on the spectrophotometer is actuated. This control is schematically illustrated by the switch 100. The mode of operation switch 22 has two active arms 22A and 22B which control the mode of the motors 62, 64 and clutches 66, 68, respectively. Specifically, in the position of the various switches as shown in FIG. 5, the carriage 28 is in its READY position and the switch 22 has just been turned to the SCAN position to energize the low speed scanning motor 64 and the scanning clutch 68 to drive the carriage 28 through its scanning movement. Specifically, it will be seen that a power circuit exists from the power switch 20 through the motor control branch 22A of the switch 22, through the motor 64 and then, by a return circuit, through the third microswitch 98. The scanning clutch 68 is similarly energized through the clutch control branch 22B of the switch 22, through the second microswitch 94 to the clutch 68 and then, by a similar return circuit, through the third microswitch 98. It will be appreciated that as the carriage 28 moves upwardly, its second inclined surface 96 will engage the second microswitch 94 thereby opening it and thereby de-energizing the scanning clutch 68 to halt the scanning operation. When the car-

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riage 28 has reached this point, the operator may either return the carriage to the READY position to make another SCAN or may bring it upwardly to the LOAD position to change the specimen. In order to repeat the scanning procedure, the operator turns the switch 22 to its "READY" position which, of course, deenergizes both the scanning motor and clutch circuit. It will be obvious that the motor control element of the switch 22A energizes one-half of the high speed motor 62 (its upper half in the schematic of FIG. 5) and the circuit through that motor is completed through the main branch of the switch 22. Similarly, the clutch 66 is energized through the clutch element 22B of the switch 22, through the normally closed first microswitch 90, through the solenoid of the clutch 66 and through the main elements of the switch 22. The motor 62 is built to rotate approximately five times more rapidly than the motor 64 thereby to make the return movement of the carriage 28 substantially faster than its scanning movement. When the carriage 28 has returned to the READY position, its first inclined surface 92 contacts the first microswitch 90 thereby de-energizing the clutch 66 and halting movement of the carriage 28. The operator may then repeat the scanning movement by moving the mode switch 22 to its "SCAN" position as described above.

The operator may bring the specimen carriage 28 to the LOAD position (seen in dotted lines in FIG. 3) at any time by moving the mode switch 22 to its "LOAD" position. The motor portion 22A of the mode switch 22 energizes the high speed motor 62 to rotate in a direction which will move the carriage 28 upwardly and the clutch portion 22B of the mode switch 22 energizes the clutch 66 directly (not through the first microswitch 90) and through the third microswitch 98. The carriage 28 moves upwardly to the top of its path of travel until the inclined surface 96 contacts the microswitch 98 thereby opening this circuit and deenergizing both the clutch 66 and the motor 62, thereby halting movement of the carriage 28. After a new specimen has been placed in the carriage 28, it is returned to its READY position by moving the mode of operation switch 22 to the "READY" setting which, as stated above, energizes the high speed motor 62 in a downward direction as well as the clutch 66, thereby to transport the carriage 28 all the way down to the READY position at which time the microswitch 90 is engaged, halting further downward movement of the carriage 28. The scanning movement may then proceed in the manner described above.

From the foregoing, it will be appreciated that an investigator using an otherwise conventional recording spectrophotometer, but provided with the specimen transport device of the present invention, may obtain a great deal more information than was previously available to him. On the other hand the increased information can be obtained with little or no more difficulty than was encountered in spectrophotographic examinations according to the prior art. Specifically, the investigator may obtain a chart or graph generally similar to that shown in FIG. 7 wherein optical density is plotted on the vertical axis and location along the length of the specimen tube 36 is plotted along the horizontal axis. At each band of high density, the graph will give an appropriate reading. Thus, in addition to simply determining the various optical densities of the portions of the specimen, the investigator will have specific information as to the relative locations and sizes of those bands and thus have significantly more information as to each specimen than could be obtained from prior art investigations.

The above brief description of one presently preferred embodiment of the invention is merely illustrative. The scanning mechanism described moves the specimen with respect to the beam of light; it is apparent that the beam could be moved with respect to a stationary specimen in order to use the teachings of the disclosed invention. Furthermore, although the example given above uses a

spectrophotometer as the measuring device, other devices may be used to scan and measure the optical qualities of the specimen without departing from the gist of the invention. Many other structures can be constructed to incorporate the basic teachings of the invention departing in major or minor detail from that shown in the drawings and described above. Accordingly, the following claims should be construed broadly in a manner consistent with the spirit and scope of the invention.

What is claimed is:

1. An improved specimen mount and transport device for use in a spectrophotometer having a beam of light along a beam path comprising

a frame,

a downwardly extending vertical support member attached to the frame for extending downwardly into said spectrophotometer a distance sufficient to interrupt the beam path thereof, said support member having elongated ways formed therein,

a specimen carriage,

means on said specimen carriage for securing a specimen in a vertical orientation at a specimen location in said beam path,

outwardly extending guide wings formed in the specimen carriage to be received in the elongated ways of said vertical support member, the interaction of said wings and complementary ways providing a smooth, reproducible vertical path of movement transverse to said beam path for said specimen carriage,

drive means for moving said carriage upward, through a scanning movement at a scanning speed from one vertical side of the beam path to the other side thereof, for returning the carriage to a ready or lowermost position prior to scanning and for moving the carriage to a load or uppermost position, and

switch means for governing said drive means for effectively de-energizing said drive means at the completion of said scanning movement.

2. A specimen mount and transport device as defined in claim 1 wherein said elongated ways defines a first path of scanning movement from below said beam path to above said beam path and a second path which is a linear extension of said first path for moving said carriage to a load position at which a specimen may be easily loaded therein.

3. A specimen mount and transport device as defined in claim 1 wherein said switch means comprises micro-switches mounted along said vertical support member in a position so as to be actuated by the specimen carriage at the ready position prior to scanning of the specimen, at the load position and at the end of said scanning movement to halt said scanning movement.

4. A specimen mount transport device as defined in claim 1 including indication means operatively connected to said drive means providing an indication of the position of a portion of said specimen whereby, on spectrophotometric measurement of a plurality of portions of said specimen, said position may be identified with said portion.

5. A specimen mount and transport device as defined in claim 1 including a pair of parallel arms extending horizontally from said vertical support member and secured to said vertical support member at a point corresponding to the beam path of the spectrophotometer, each of the arms having an opening therethrough at a point in the path of said beam of light and lenses mounted in said openings of said arms to collimate said beam of light coming from the spectrophotometer.

6. An improved specimen mount transport device for use in a spectrophotometer having a beam of light along a beam path comprising

a frame,

a downwardly extending vertical support member attached to the frame for extending downwardly into said spectrophotometer a distance sufficient to interrupt the beam path thereof, the support member having elongated ways formed therein,

a specimen carriage,

means on said carriage for securing a specimen in a vertical orientation in said beam path,

outwardly extending guide wings formed in the specimen carriage to be received in the elongated ways of said vertical support member, the interaction of the wings and complementary ways providing a smooth, reproducible vertical path of movement transverse to said beam path for said specimen carriage, the beam path sweeping the vertical extent of said specimen,

driving means for moving said carriage upward at a scanning speed from one vertical side of said beam path to the other vertical side thereof, for returning the carriage to a ready or lowermost position prior to scanning and for moving the carriage to a load or uppermost position, said driving means comprising a pair of power means connected through clutches to a common shaft, one power means providing a first and slow speed for scanning movement and the other power means a second and faster speed for movement of said carriage to said ready and load positions, a main driving gear rigidly mounted on said common shaft,

a driven gear in mesh with said driving gear,

an auxiliary shaft to which the driven gear is rigidly secured,

a lead screw in axial alignment with said auxiliary shaft and secured thereto at its upper end, the lower end of said lead screw being operatively connected to said specimen carriage such that, on rotation of said screw, the specimen carriage is moved in a vertical direction, and

switch means for automatically governing said drive means for effectively de-energizing said drive means at the completion of said scanning movement.

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U.S. Cl. X.R.

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