(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 February 2002 (14.02.2002)

PCT

(10) International Publication Number
WO 02/12876 A2

(51) International Patent Classification[7]: G01N 27/447

(21) International Application Number: PCT/GB01/03275

(22) International Filing Date: 20 July 2001 (20.07.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0019500.8 8 August 2000 (08.08.2000) GB

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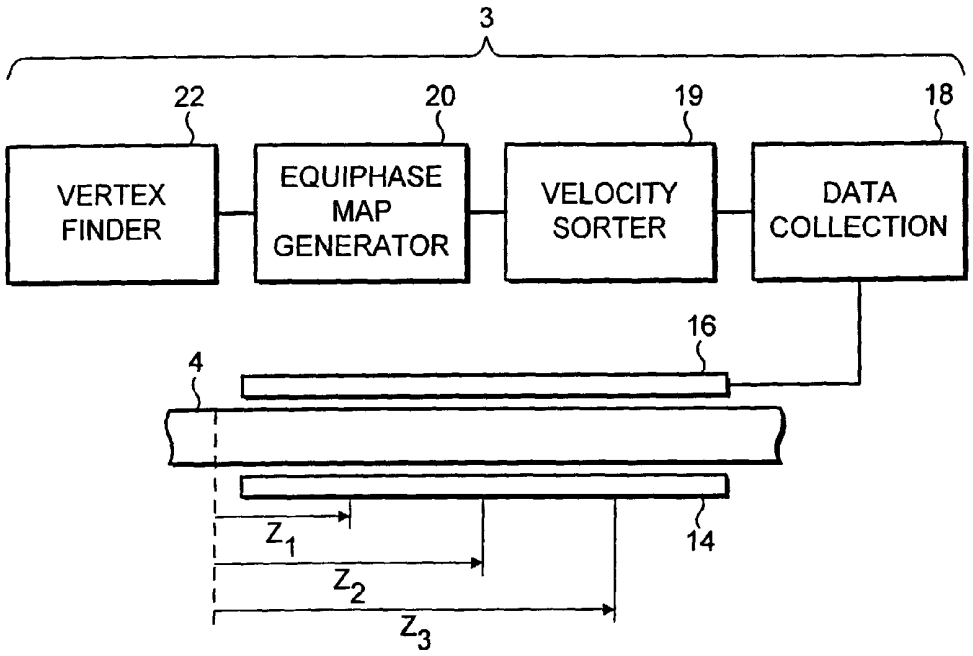
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: SYSTEM AND METHOD



(57) Abstract: An analysis system for and method of enabling determination of the velocities of migrating objects, the system comprising an equiphase space-time map generator for generating an equiphase space-time map of equiphase points from each of a plurality of data sets representative of the signals detected at a plurality of spaced positions, with the velocities of the objects being determinable by fitting the sets of equiphase points in the space-time map corresponding to the respective objects.

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SYSTEM AND METHOD

The present invention relates to an analysis system for and method of determining the velocities of migrating objects, such as sample components travelling through a channel, in particular, but not exclusively, as measured electrophoretically.

Electrophoretic separation techniques are separation techniques in which the components of a sample plug are separated in a separation column by the differences in the migration rates of those sample components on the application of an electric field therealong, where absorption, fluoroescence, electrochemistry, conductivity, radioactivity and mass spectrometry can be all used to detect the electrophoretic separation.

In order to determine the velocities of migrating objects as represented by a set of electropherograms, signal peaks from a plurality of signals as generated by a plurality of spaced detecting elements have to be correlated.

The present inventor has identified that, as the signal peaks and the detecting elements have a finite width, the correlation of the signal peaks is not one to one, and that this non-equiphase correlation leads to a loss of resolution and hence decreased accuracy in determining the object velocities. By way of example, for an object having a linear velocity function, there is a continuous set of slopes in space-time co-ordinates that are incompatible to the set of signal peaks. It would only be if the signal peaks and the detecting elements had infinitesimal width that the correlation of the signal peaks would result in a true straight line in space-time representation.

It is thus an aim of the present invention to provide an improved analysis system for and method of determining the velocities of migrating objects, such as sample components travelling through a channel, and in particular as measured electrophoretically.

Accordingly, the present invention provides an analysis system for enabling determination of the velocities of migrating objects, comprising an equiphase space-time map generator for

generating an equiphase space-time map of equiphase points from each of a plurality of data sets representative of the signals detected at a plurality of spaced positions, with the velocities of the objects being determinable by fitting the sets of equiphase points in the space-time map corresponding to the respective objects.

Preferably, the system further comprises a velocity sorter for determining the nominal velocities associated with the signal peaks in the signals and grouping those signal peaks into sets according to nominal velocity.

Preferably, the system further comprises a vertex finder for identifying at least one vertex from the space-time map, with a single vertex being identified for each group of objects having a common constraint and the velocities of the objects being determinable from the sets of equiphase points in the space-time map as fitted by the respective vertex.

Preferably, the space-time map generator is configured to transform each data set into a set of local slopes and determine the local minima as the minimum absolute local derivatives.

Preferably, the space-time map generator is configured to utilise a corrected time component in generating the space-time map according to a function of the electric current variation.

More preferably, the correction is according to the function $t_c = \int I_0/I(t')dt'$ in the range of 0 to t, where t is the measured time, t_c is the corrected time, I is the measured current and I_0 is the reference current.

In one embodiment the objects are non-labelled objects.

In another embodiment the objects are labelled objects.

Preferably, the objects are migrated through a channel.

More preferably, the channel comprises a separation channel through which the objects are electrophoretically driven.

In one embodiment the objects comprise components from one sample.

In another embodiment the objects comprise components from a plurality of separate samples.

Preferably, the objects comprise molecular components.

Preferably, the objects comprise polymeric components.

More preferably, the components comprise DNA bands.

The present invention also extends to an electrophoresis apparatus including the above-described system.

The present invention also provides a method of enabling determination of the velocities of migrating objects, comprising the steps of: determining equiphase points from the signal peaks of each of the signals detected at a plurality of spaced positions; and generating an equiphase space-time map of the equiphase points, with the velocities of the objects being determinable by fitting the sets of equiphase points in the space-time map corresponding to the respective objects.

Preferably, the method further comprises the steps of determining the nominal velocities associated with the signal peaks in the signals and grouping those signal peaks into sets according to nominal velocity.

Preferably, the method further comprises the step of identifying at least one vertex from the space-time map, with a single vertex being identified for each group of objects having a

common constraint and the velocities of the objects being determinable from the sets of equiphase points in the space-time map as fitted by the respective vertex.

Preferably, the equiphase points are determined by transforming each data set into a set of local slopes and determining the local minima as the minimum absolute local derivatives.

Preferably, a time component corrected according to a function of the electric current variation is utilised in generating the space-time map.

More preferably, the correction is according to the function $t_c = \int I_0/I(t')dt'$ in the range of 0 to t, where t is the measured time, t_c is the corrected time, I is the measured current and I_0 is the reference current.

In one embodiment the objects are non-labelled objects.

In another embodiment the objects are labelled objects.

Preferably, the objects are migrated through a channel.

More preferably, the channel comprises a separation channel through which the objects are electrophoretically driven.

In one embodiment the objects comprise components from one sample.

In another embodiment the objects comprise components from a plurality of separate samples.

Preferably, the objects comprise molecular components.

Preferably, the objects comprise polymeric components.

More preferably, the components comprise DNA bands.

A preferred embodiment of the present invention will now be described hereinbelow by way of example only with reference to the accompanying drawings, in which:

Figure 1 illustrates the detector chip of an electrophoresis apparatus in accordance with a preferred embodiment of the present invention;

Figure 2 illustrates the analysis system of the apparatus of Figure 1;

Figure 3 illustrates a three-dimensional representation of the intensity-time signals of one component of a sample plug as detected at positions z_1, z_2, z_3 spaced along the separation channel of the apparatus of Figure 1;

Figure 4 illustrates the intensity-time signals of three components of a sample plug as detected at positions z_1, z_2, z_3 spaced along the separation channel of the apparatus of Figure 1;

Figure 5 illustrates a space-time map as generated from the intensity-time signals of Figure 4;

Figure 6 illustrates the velocity spectrum as determined from the vertexed space-time map of Figure 5; and

Figure 7 illustrates a space-time map as generated from the intensity-time signals from four separately-injected DNA sample plugs comprising DNA bands having different base pair terminations.

Figures 1 and 2 illustrate an electrophoresis apparatus in accordance with a preferred embodiment of the present invention.

The electrophoresis apparatus includes a detector chip 2 as microfabricated in a substrate chip, and an analysis system 3 for analysing the detection signals generated by the detector chip 2.

The detector chip 2 includes a separation channel 4, in this embodiment a meandering, gel-filled channel, through which the components of one or more sample plugs are in use driven by an applied electrophoretic voltage. The separation channel 4 has a length sufficient to allow separation of the components of the sample plugs. Preferably, the separation channel 4 has a width of from 25 to 100 μm and a length of from 20 to 300 mm. The separation channel 4 includes a plurality, in this embodiment first to fourth, spaced sample-injection ports 6, 8, 10, 12 through which sample plugs including a plurality of components, in this embodiment DNA bands having the respective base pair terminations A, T, G and C, are separately injected into the separation channel 4.

The detector chip 2 further includes a light source 14, in this embodiment a UV light source, disposed along a length of one side of the separation channel 4, and a detector 16 disposed along the length of the other side of the separation channel 4 to detect light transmitted through the separation channel 4, with the presence of the migrating components being detected by the change in the detected light intensity as caused by absorbtion of the incident light. By detecting the sample components in this manner, the sample components need not necessarily be labelled. In this embodiment the detector 16 comprises a pixel detector array (PDA) which includes a plurality of pixels providing detecting elements for detecting the transmitted light at a plurality of positions z_1, z_2, z_3 spaced along the length of the separation channel 4 and outputting a plurality of signals S_1, S_2, S_3. For ease of description, the detector 16 is illustrated as including three detecting elements at three positions z_1, z_2, z_3. It will, however, be understood that in practice the detector 16 comprises a plurality of detecting elements at a plurality of positions z_1, z_2, z_3, ... , z_n, which each output a signal S_1, S_2, S_3, ... , S_n. In an alternative embodiment the detector 16 could be provided by a plurality of separate detectors each providing a detecting element. In another alternative embodiment labelled sample components could be used, such as sample components including fluorescent or radioactive labels, which labels would be detected by the detector 16.

The analysis system 3 comprises a data collector 18 for receiving the signals S_1, S_2, S_3 generated by the detector 16 and storing those signals S_1, S_2, S_3 as data sets, a velocity sorter 19 for determining the nominal velocities v_1, v_2, v_3 of the sample components associated with each of the signal peaks SP_1, SP_2, SP_3 of each of the signals S_1, S_2, S_3 and grouping those signal peaks SP_1, SP_2, SP_3 into sets according to nominal velocity, an equiphase space-time map generator 20 for generating an equiphase space-time map of equiphase points from the signal peaks SP_1, SP_2, SP_3 of the signals S_1, S_2, S_3, and a vertex finder 22 for identifying the vertices of the equiphase points of the grouped sets of signal peaks SP_1, SP_2, SP_3. In this embodiment the velocity sorter 19 is provided so as to be operable prior to the equiphase space-time map generator 20. In alternative embodiments the velocity sorter 19 could be provided so as to be operable after the space-time map generator 20 or the vertex finder 22.

Figure 3 is included for the purposes of illustration only and illustrates the signals S_1, S_2, S_3 as including only a single peak SP_1 from a single component of a single sample plug. In reality, however, the signals S_1, S_2, S_3 each include a plurality of signal peaks SP_{1-n}, SP_{1-n}, SP_{1-n}. Figure 4 illustrates the signals S_1, S_2, S_3 as including three signal peaks SP_1, SP_2, SP_3 from three components of a single sample plug.

The velocity sorter 19 is configured to determine the nominal velocities v_1, v_2, v_3 of the sample components associated with each of the signal peaks SP_1, SP_2, SP_3 in each of the signals S_1, S_2, S_3 and then group those signal peaks SP_1, SP_2, SP_3 into sets according to nominal velocity. The nominal velocities v_1, v_2, v_3 can be calculated as the positions z_1, z_2, z_3 of the detector elements are fixed and the elapsed time t is extractable from the signals S_1, S_2, S_3, where the nominal velocities can be expressed as $v_{1-n} = z_{1-n}/t$. By grouping the signal peaks SP_1, SP_2, SP_3 into sets according to nominal velocity, and hence sample component, subsequent analysis is facilitated as the data points associated with each sample component can be fitted without requiring the use of complex data extraction techniques. Velocity sorting is encompassed by our earlier WO-96/35946, the content of which is incorporated herein by reference.

The equiphase space-time map generator 20 is configured to determine the local minima of the signal peaks SP_1, SP_2, SP_3 in the signals S_1, S_2, S_3 detected at the detection positions z_1, z_2, z_3 and generate an equiphase map M in space-time dimensions from the determined local minima. Figure 5 illustrates the space-time map M generated from the local minima extracted from the signal peaks SP_1, SP_2, SP_3 of the signals S_1, S_2, S_3.

In this embodiment each electropherogram is transformed into a set of local slopes, where a triangular slope sequence defines a signal and the local extreme is the minimum absolute local derivative.

Also, in this embodiment the time component of the detected signals S_1, S_2, S_3 is corrected as a function of the integrated electric current variation. Owing to the variation of various factors in electrophoretic detection, the temperature being one of the most significant, the characteristics of the separation medium, in this embodiment a gel, are altered. Firstly, the resistivity of the gel changes, leading to variations in the potential difference between the electrodes and a given point in the gel and fluctuations in the electric current. Secondly, the sieving properties of the gel change, affecting the mobility of the electrophoresed components. By monitoring the electric current, the time component of the space-time map M can be corrected as set out hereinbelow. Specifically, the time component is curved as a function of the integrated electric current variation.

The velocity of a sample component is:

$$v = dz/dt \qquad (1)$$

For a transformation of the measured time component to a corrected time component $t \rightarrow t_c$, it follows that $dt \rightarrow dt_c$ and $v \rightarrow v_c$. Thus:

$$v_c/v = dt/dt_c \qquad (2)$$

The transformation $v \rightarrow v_c$ can be defined as:

$$v_c/v = I(t)/I_0 \qquad (3)$$

where I is the measured current and I_0 is the reference current which corresponds to the frame where all velocities and time components are projected.

From equations (2) and (3), it follows:

$$dt_c = I_0/I(t)dt \qquad \rightarrow \qquad t_c = \int I_0/I(t')dt' \text{ for } 0 \text{ to } t \qquad (4)$$

The justification for the velocity transformation (3) is that the velocity is approximately proportional to the applied electric field, which in turn is proportional to the electric current in the separation channel 4. This correction factor has been found to work well for small current changes, with the integral of equation (4) providing for an accurate time transformation.

The vertex finder 22 is configured, in this embodiment by the use of rotational matrices, to identify the vertices V of the equiphase points of the grouped sets of signal peaks SP_1, SP_2, SP_3 as determined by the equiphase space-time map generator 20, where the components of each injected sample plug have a common vertex V by virtue of being time and/or spatially separated in the space-time dimension. All of the sample components injected in a single sample plug are uniquely identified by a single vertex V in space-time co-ordinates, thus allowing for the identification of the sample components from each of a plurality of separately-provided sample plugs. Figure 5 illustrates the vertex V as determined from the generated space-time map M. This space-time map includes only a single vertex V as all of the components were provided in a single sample plug.

By using each vertex V as a constraint to extract the velocity spectrum of the sample components, the resolution is approximately proportional to $\sqrt{n}$, where n is the number of components. In this way, the velocity of one component is calculated using the velocities of all of the other components from the same sample plug, and thus, as the number of

components in a sample increases, the resolution of the analysis increases accordingly. Such space parameterisation which results in multiple vertex formation in the form of intensity enhanced regions in space-time co-ordinates is particularly suited to the cases of multiple sample injections and multiple column correlation. The power of this technique has been demonstrated on DNA samples which include large numbers of fragments (>100) having lengths of one base pair difference, thereby providing a sequencing technique having a greatly extended dynamic range.

From the determination of the vertices V in the space-time map M, high resolution of the electrophoresis data is achieved, allowing accurate determination of the velocities of the sample components as illustrated in Figure 6.

Use of the above-described electrophoresis apparatus to sequence DNA samples having the base pair terminations A, T, G and C will now be described hereinbelow.

In use, four sample plugs comprising DNA bands having different length and one of the base pair terminations A, T, G and C are separately introduced into the ports 6, 8, 10, 12 of the separation channel 4, and electrophoretically driven therealong. In one mode of use, the sample plugs are introduced simultaneously into the ports 6, 8, 10, 12 which are spatially separated along the separation channel 4. In another mode of use, the sample plugs are introduced sequentially into one of the ports 6, 8, 10, 12 so as to be time spaced. The signals $S_1, S_2, S_3, \ldots, S_n$ detected by the detector 16 as the DNA bands pass the detecting elements at the detecting positions $z_1, z_2, z_3, \ldots, z_n$ are collected by the data collector 18. The velocity sorter 19 then determines the nominal velocities $v_1, v_2, v_3, \ldots, v_n$ of the sample components associated with each of the signal peaks $SP_1, SP_2, SP_3, \ldots, SP_n$ of the signals $S_1, S_2, S_3, \ldots, S_n$ and groups those signal peaks $SP_1, SP_2, SP_3, \ldots, SP_n$ into sets according to nominal velocity. The equiphase space-time map generator 20 then determines the local minima of the signal peaks $SP_1, SP_2, SP_3, \ldots, SP_n$ of the signals $S_1, S_2, S_3, \ldots, S_n$, and generates an equiphase space-time map M. The vertex finder 22 then identifies the vertices V_A, V_T, V_G, V_C of the determined local minima for each of the grouped sets of signal peaks $SP_1, SP_2, SP_3, \ldots, SP_n$. In this embodiment the space-time map M includes four vertices V_A, V_T, V_G, V_C as four

sample plugs were separately injected into the separation channel 4, each being attributable to DNA bands having one of the base pair terminations A, T, G and C. In this way, the DNA sample can be sequenced, with the lengths of the DNA bands being determined from the migration velocities.

Finally, it will be understood that the present invention has been described in its preferred embodiment and can be modified in many different ways without departing from the scope of the invention as defined by the appended claims.

CLAIMS

1. An analysis system for enabling determination of the velocities of migrating objects, comprising an equiphase space-time map generator for generating an equiphase space-time map of equiphase points from each of a plurality of data sets representative of the signals detected at a plurality of spaced positions, with the velocities of the objects being determinable by fitting the sets of equiphase points in the space-time map corresponding to the respective objects.

2. The system of claim 1, further comprising a velocity sorter for determining the nominal velocities associated with the signal peaks in the signals and grouping those signal peaks into sets according to nominal velocity.

3. The system of claim 1 or 2, further comprising a vertex finder for identifying at least one vertex from the space-time map, with a single vertex being identified for each group of objects having a common constraint and the velocities of the objects being determinable from the sets of equiphase points in the space-time map as fitted by the respective vertex.

4. The system of any of claims 1 to 3, wherein the space-time map generator is configured to transform each data set into a set of local slopes and determine the local minima as the minimum absolute local derivatives.

5. The system of any of claims 1 to 4, wherein the space-time map generator is configured to utilise a corrected time component in generating the space-time map according to a function of the electric current variation.

6. The system of claim 5, wherein the correction is according to the function $t_c = \int I_0/I(t')dt'$ in the range of 0 to t, where t is the measured time, t_c is the corrected time, I is the measured current and I_0 is the reference current.

7. The system of any of claims 1 to 6, wherein the objects are non-labelled objects.

8. The system of any of claims 1 to 6, wherein the objects are labelled objects.

9. The system of any of claims 1 to 8, wherein the objects are migrated through a channel.

10. The system of claim 9, wherein the channel comprises a separation channel through which the objects are electrophoretically driven.

11. The system of any of claims 1 to 10, wherein the objects comprise components from one sample.

12. The system of any of claims 1 to 10, wherein the objects comprise components from a plurality of separate samples.

13. The system of any of claims 1 to 12, wherein the objects comprise molecular components.

14. The system of any of claims 1 to 13, wherein the objects comprise polymeric components.

15. The system of claim 14, wherein the components comprise DNA bands.

16. A method of enabling determination of the velocities of migrating objects, comprising the steps of:
determining equiphase points from the signal peaks of each of the signals detected at a plurality of spaced positions; and
generating an equiphase space-time map of the equiphase points, with the velocities of the objects being determinable by fitting the sets of equiphase points in the space-time map corresponding to the respective objects.

17. The method of claim 16, further comprising the steps of determining the nominal velocities associated with the signal peaks in the signals and grouping those signal peaks into sets according to nominal velocity.

18. The method of claim 16 or 17, further comprising the step of identifying at least one vertex from the space-time map, with a single vertex being identified for each group of objects having a common constraint and the velocities of the objects being determinable from the sets of equiphase points in the space-time map as fitted by the respective vertex.

19. The method of any of claims 16 to 18, wherein the equiphase points are determined by transforming each data set into a set of local slopes and determining the local minima as the minimum absolute local derivatives.

20. The method of any of claims 16 to 19, wherein a time component corrected according to a function of the electric current variation is utilised in generating the space-time map.

21. The method of claim 20, wherein the correction is according to the function $t_c = \int I_0/I(t')dt'$ in the range of 0 to t, where t is the measured time, t_c is the corrected time, I is the measured current and I_0 is the reference current.

22. The method of any of claims 16 to 21, wherein the objects are non-labelled objects.

23. The method of any of claims 16 to 21, wherein the objects are labelled objects.

24. The method of any of claims 16 to 23, wherein the objects are migrated through a channel.

25. The method of claim 24, wherein the channel comprises a separation channel through which the objects are electrophoretically driven.

26. The method of any of claims 16 to 25, wherein the objects comprise components from one sample.

27. The method of any of claims 16 to 25, wherein the objects comprise components from a plurality of separate samples.

28. The method of any of claims 16 to 27, wherein the objects comprise molecular components.

29. The method of any of claims 16 to 28, wherein the objects comprise polymeric components.

30. The method of claim 29, wherein the components comprise DNA bands.

31. An electrophoresis apparatus including the system of any of claims 1 to 15.

32. An analysis system for enabling determination of the velocities of migrating objects substantially as hereinbefore described with reference to the accompanying drawings.

33. A method of enabling determination of the velocities of migrating objects substantially as hereinbefore described with reference to the accompanying drawings.

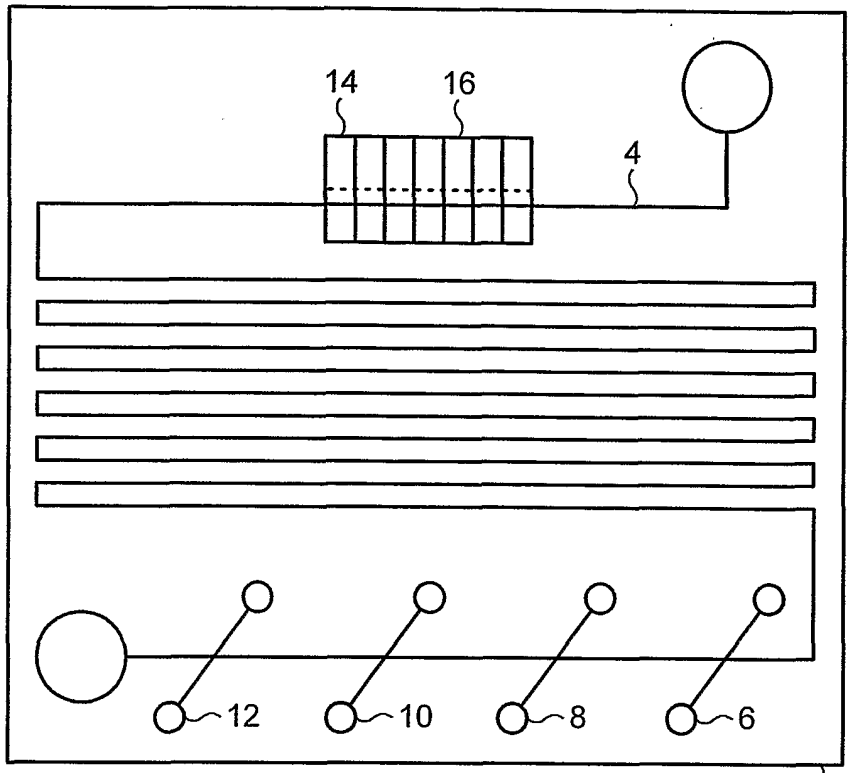
14
16
4
12
10
8
6
2

FIG. 1

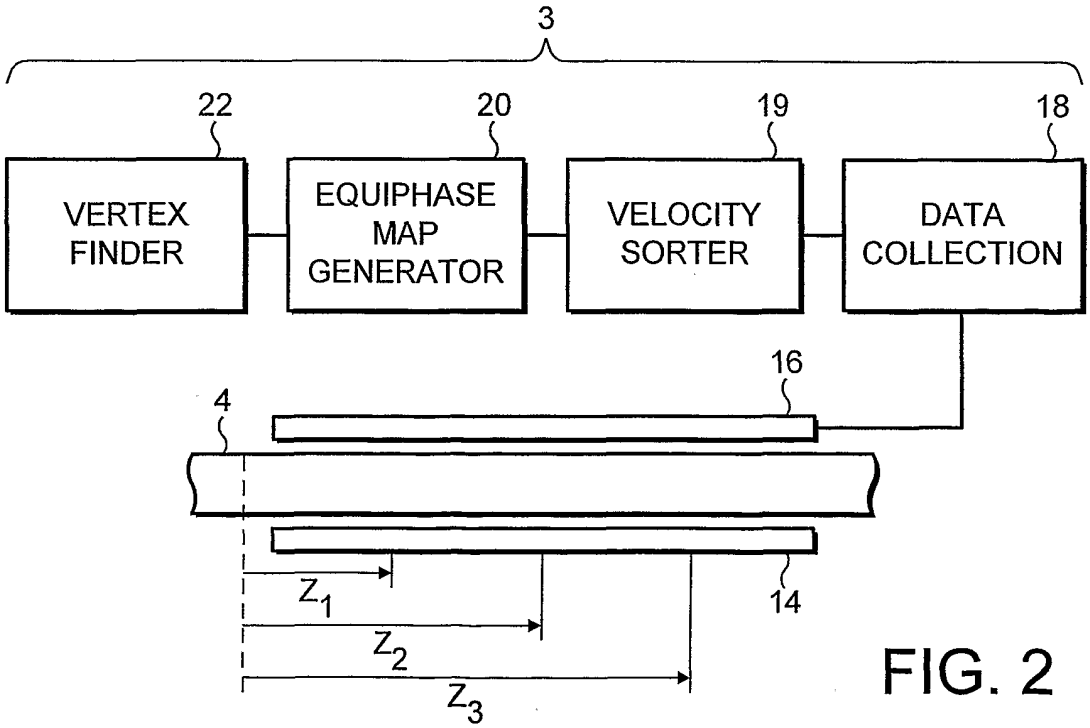
3
22
20
19
18
VERTEX FINDER
EQUIPHASE MAP GENERATOR
VELOCITY SORTER
DATA COLLECTION
16
4
14
Z_1
Z_2
Z_3

FIG. 2

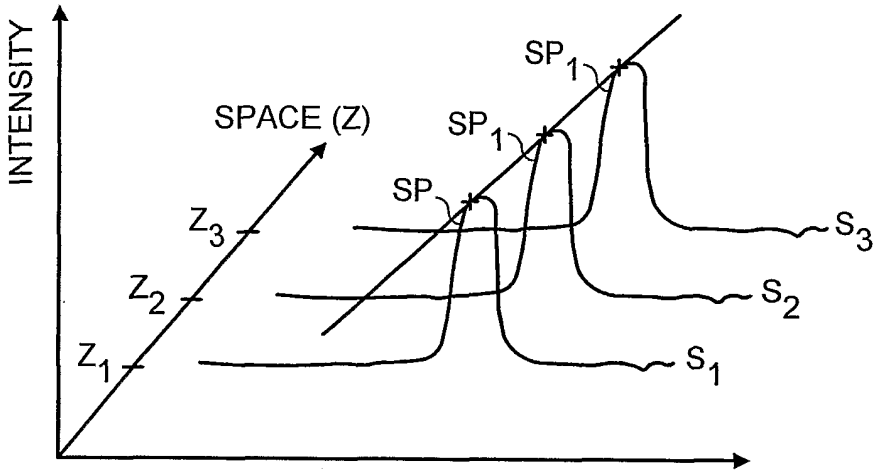

FIG. 3

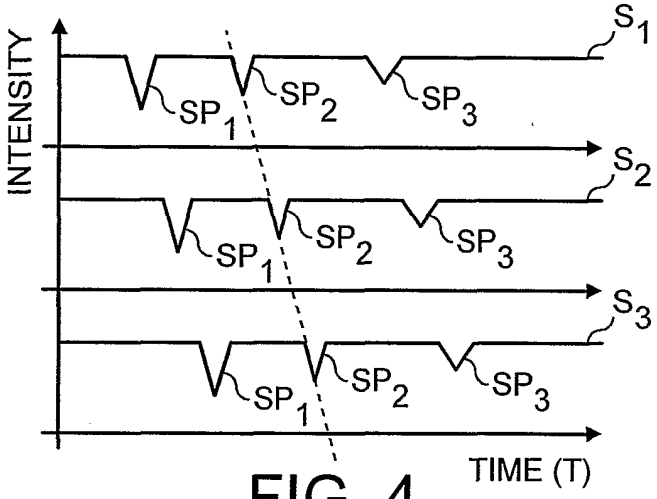

FIG. 4

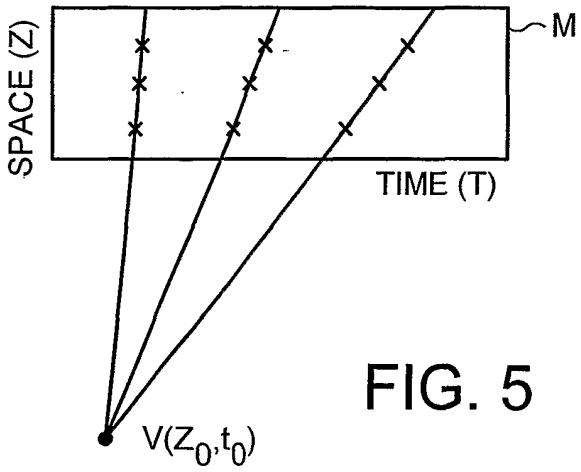

FIG. 5

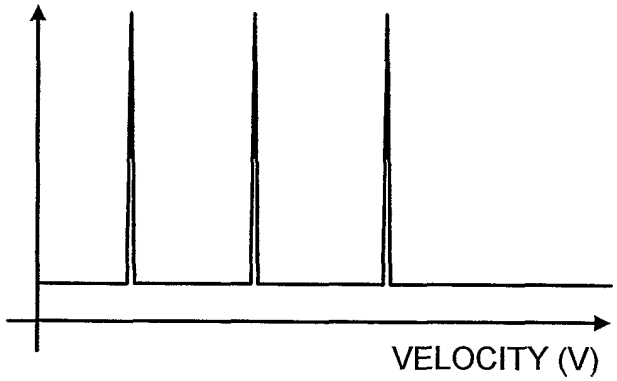

FIG. 6

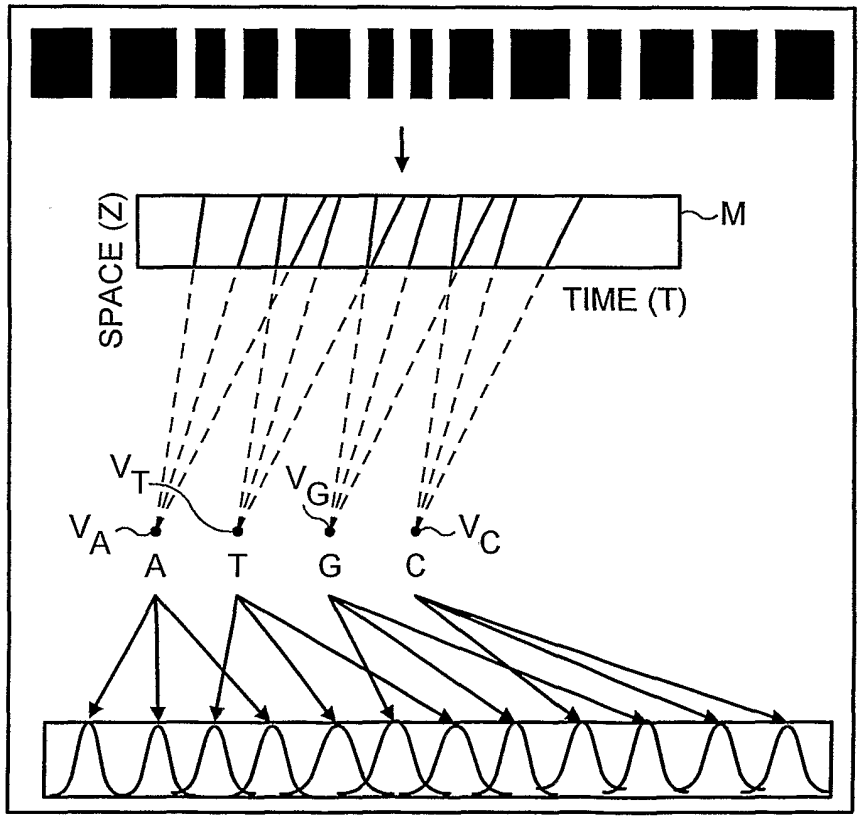

FIG. 7