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(54) METHOD TO EXTRACT AND USE TIME-RESOLVED ION CLOUDS FROM A SPECTROMETRIC DATA SET

(57) A method is proposed for extracting single-particle and/or single-cell events from a spectrometric data set comprising intensity values. A respective intensity value is proportional to the number of ions collected by a detector in a given time interval. The method comprises: grouping (22) the intensity values into sets of groups of consecutive intensity values, a respective set being characterised by a distinct group size of intensity values; determining (23) a threshold value separating a background from candidate event intensity values; calculating (24) a dissolved intensity as a mean of the intensity values forming a background signal; carrying out (25) a first candidate event identification by obtaining candidate peak groups based on a feature characterising a respective group; carrying (26) out a second candidate event identification by obtaining ion cloud groups based on the threshold value; carrying out (27) a true event identification by comparing the first and second candidate event identifications to obtain one or more ion cloud group events for a respective group size; and summing (28) the intensity values of a respective ion cloud group event and subtracting the dissolved intensity as scaled from the summed intensity values of the respective ion cloud group event to obtain a total intensity value per particle or cell.

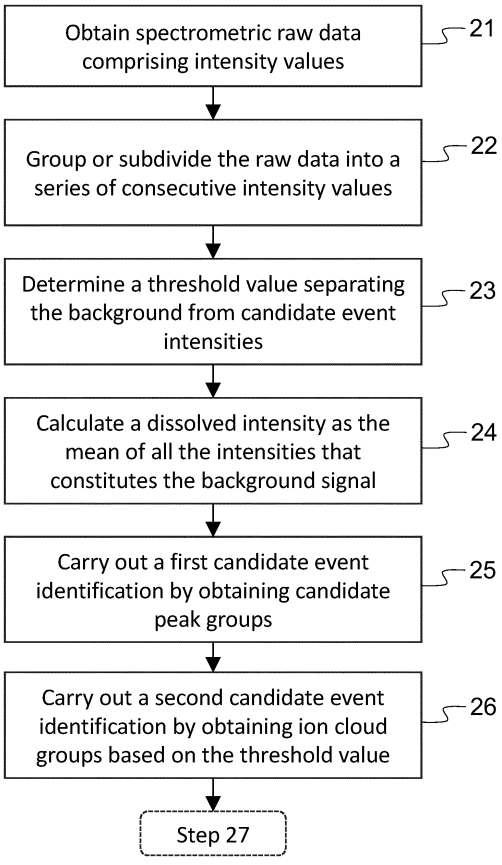

Fig. 21a

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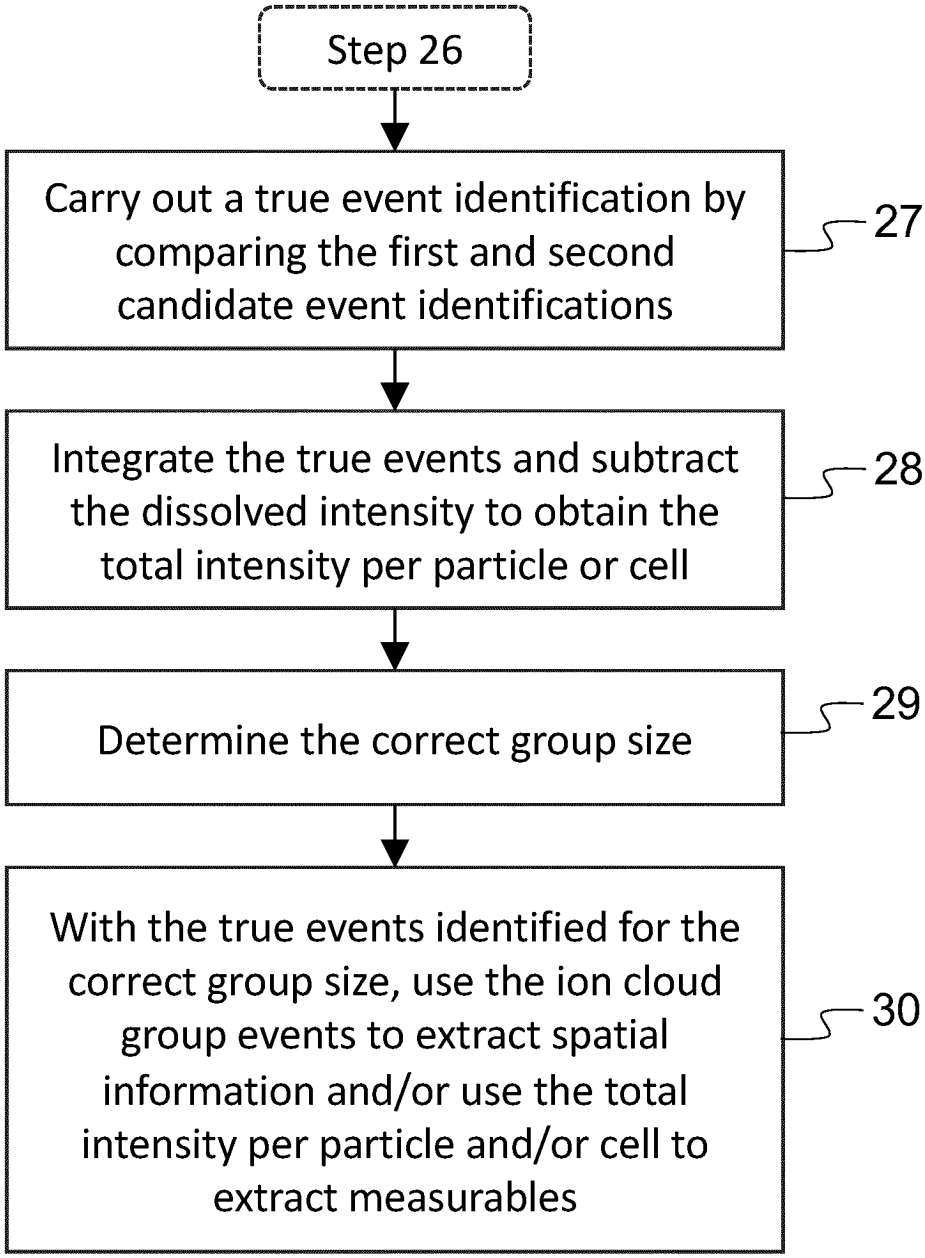
Step 26
Carry out a true event identification by comparing the first and second candidate event identifications
27
Integrate the true events and subtract the dissolved intensity to obtain the total intensity per particle or cell
28
Determine the correct group size
29
With the true events identified for the correct group size, use the ion cloud group events to extract spatial information and/or use the total intensity per particle and/or cell to extract measurables
30

Fig. 21b

Description

TECHNICAL FIELD

[0001] The present invention relates to a method to extract and use time-resolved ion clouds from a spectrometric data set, for instance in the context of a fast scanning inductively coupled plasma mass spectrometry.

BACKGROUND OF THE INVENTION

[0002] Inductively coupled plasma mass spectrometry (ICP-MS) is routinely used to perform (trace) elemental analysis of samples, for example metals in a matrix by determining the concentration of ionic solutions. The ICP-MS system is configured to use a plasma to atomise and ionise samples to be analysed by a mass analyser. The ICP-MS system may include, for example, a peristaltic pump and a nebuliser for sample introduction and aerosol production, which are directed towards a plasma source for atomisation and ionisation. A plasma torch is often configured as a flow through torch with one or more nested concentric tubes. A plasma-forming gas, such as argon, flows through, the outer tube of the torch and is ionised to form a plasma with a sufficient energy source (typically a radio frequency generated by a coil). The aerosols of the sample flow through the torch and are fed to the generated plasma. Introduction of the samples to the high-energy plasma with temperatures greater than 5000 K typically atomises and ionises the introduced samples, which generally carry a positive charge.

[0003] The ions generated by the plasma, containing the information of the elemental make-up of the sample, are extracted and focused into an ion beam which is guided to a mass analyser. The mass analyser may use a time-varied electric field, such as a quadrupole or a series of quadrupoles, or a combination of magnetic fields and electric fields, to spectrally resolve the ions based on their mass/charge (m/z) ratio. Alternatively, a time-of-flight (TOF) tube may be used to accelerate the ions and spectrally resolve them based on their flight times. Resolved ions are then counted by means of an ion detector, such as an electron multiplier, with the counts proportional to the absolute number of elements present in the sample, which gives us the concentration.

[0004] Beyond routine tasks, ICP-MS has found in the past decade two application fields of profound impact: the first in the field of nanotechnology and the second in the (bio)medical field. First, nanotechnology is making a profound impact on a variety of industries, such as (bio)medical, energy engineering, consumer goods, etc. Active (nano)materials, such as microparticles, nanoparticles, nanoclusters, quantum dots, in such technologies are often spatially confined, and are becoming increasingly structurally complex and their characterisation expensive and time-consuming. Second, in the (bio)medical field, i.e., in immunophenotyping, the demand for the classification of rare cell types is becoming more and more urgent as pathologies become more diversified and become better at evading treatment. Faster and more sensitive immunophenotyping approaches are needed.

[0005] ICP-MS can be utilised to quantify elements in particles and cells in a sample by a technique known as a single-particle (SP-)ICP-MS or single-cell (SC-)ICP-MS/mass cytometry. These techniques obtain simultaneously the number of particles or cells, the mass of the quantified elements present per particle or per cell, and the composition distributions of the elements present in individual particles and cells offering the opportunity to identify subsets in populations, i.e., perform immunophenotyping. This is achieved through the fast acquisition of ion intensities of a nebulised, atomised and ionised sample containing particles and/or cells in the microsecond time scale. To accurately quantify the particles and/or cells, their ion intensities must be distinguished from a background signal, i.e., dissolved ionic species. Upon introducing the sample to the ICP-MS, ion clouds are generated by the atomisation and ionisation of particles and/or cells that are spatially correlated and therefore, temporally correlated. These ion plumes or 'particle/cell events' are substantially higher than the background and can be distinguished from the background by deploying an appropriate algorithm to the ICP-MS raw data, to determine the ion intensity as a function of time, obtained by an ion detector of the ICP-MS system.

[0006] Bandura et al. described the possibility to determine single-cell events by means of a TOF-based ICP-MS method in a publication entitled "Mass Cytometry: Technique for Real Time Single Cell Multitarget Immunoassay Based on Inductively Coupled Plasma Time-of-Flight Mass Spectrometry. Anal. Chem. 2009, 81 (16), 6813-6822. Pace et al. described the possibility to count and size particles with a quadrupole mass analyser in a publication entitled "Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry", Anal. Chem. 2011, 83 (24), 9361-9369. Later, Borovinskaya et al. described the possibility to extract short transient signals of particles from a time-of-flight tube mass analyser-coupled ICP, which offered the opportunity to look at a multielement signal within the same pulse, as described in a publication entitled "A Prototype of a New Inductively Coupled Plasma Time-of-Flight Mass Spectrometer Providing Temporally Resolved, Multi-Element Detection of Short Signals Generated by Single Particles and Droplets", J. Anal. At. Spectrom 2013, 28 (2), 226-233. Recently, Koolen et al. described that beyond the particle number concentrations and particle size, particle compositions and structural information of the particles can be accessed as well of either quadrupole or time-of-flight generated particle events as described in a publication entitled "High-Throughput Sizing, Counting, and Elemental Analysis of Anisotropic Multimetallic

Nanoparticles with Single-Particle Inductively Coupled Plasma Mass Spectrometry", ACS Nano 2022. Prior art exists around automated methods to extract particle or single-cell events from the raw signal as disclosed in US2014299763A1, WO2015122920A1, and US11075066B2.

[0007] The prior art comes with two major disadvantages: first, identified single-particle or single-cell events are always integrated into a total intensity discarding valuable information. Second, identification of single-cell or single-particle events are determined based on a static process with a pre-defined group size for intensity values reducing the quality of the event identification.

[0008] First, in order to determine the total mass of an analyte present per particle or cell, the total intensity of the transient signal is integrated (and background subtracted). Although this is an effective method if the total mass is the parameter of interest, it reduces the information that can be extracted from the ion cloud. Beyond the total amount of analyte present, the ion cloud contains spatial information, i.e., a fingerprint of the original 3-dimensional spatial arrangement of the analyte in the particle and or cell as illustrated in Figure 1. This information can be used to extract for instance particle shape and or cell morphology from the ion clouds.

[0009] Referring to Figure 1, ion clouds (or single-particle events) are identified as extracted from ICP-MS raw data generated on cubic and spherical Au nanoparticles of equal mass. A) 920 ion clouds of Au are extracted from the raw data set that are representative of a spherical particle. B) 1123 ion clouds of Au are extracted from the raw data set that are representative of a cubic particle. A clear distinction in the distribution of the intensity (referred to as a "signal" in Figure 1) can be observed for either shape even though the total intensity differs only by ± 5 intensity counts. It can be observed by the length of the intensity distribution that cubic particles generate longer transient signals on average than spherical particles.

[0010] Second, in the prior art, predefined criteria are set by which a signal or series of signals is identified as a peak and thus a particle or cell event. This does not take into account the variability associated with the measurement including variable particle size (with orders of magnitude differences in total mass), the relative height of the ionic background in relation to the particle size, and the number concentration. This can result in false positives in case of high background as illustrated in Figure 2, underestimation of the actual total particle intensity in case of large particles, false negatives in case of small particles, and an overall incorrect number concentration. As especially in (nano)particle manufacturing low ionic backgrounds cannot always be guaranteed, better particle event detection criteria are needed.

[0011] Figure 2 shows an intensity histogram generated from ICP-MS raw data generated for spherical Au nanoparticles with a diameter of 80 nm. A) Prior-art event extraction algorithm finds erroneous events that actually are part of the background, i.e., < 20 counts (generate false positives). B) The event extraction algorithm according to the present invention reduces the false positive rate by a factor of > 100 as becomes clear later.

SUMMARY OF THE INVENTION

[0012] It is an object of the present invention to overcome at least some of the shortcomings identified above relating to extracting single-particle and/or single-cell events from a spectrometric data set, for instance in the context of single-particle ICP-MS or single-cell ICP-MS/mass cytometry.

[0013] According to a first aspect of the invention, there is provided a method of extracting one or more single-particle and/or single-cell events from a spectrometric data set as recited in claim 1.

[0014] The present invention thus proposes a method to extract the full transient signal of an ion cloud, which can be used to extract spatial information or used to learn to identify the shape with artificial intelligence (AI). Furthermore, the present invention may be used as an automated single-particle or single-cell event identification algorithm of ICP-MS raw data that adapts the criteria by which it refutes or accepts an event as a particle or cell event to the experimental conditions using AI.

[0015] According to a second aspect of the invention, there is provided a non-transitory computer program product comprising instructions for implementing the steps of the method according to the first aspect of the present invention when loaded and run on computing means of a data processing device.

[0016] According to a third aspect of the invention, there is provided an apparatus configured to carry out the method according to the first aspect as recited in claim 15.

[0017] Other aspects of the invention are recited in the dependent claims attached hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Other features and advantages of the invention will become apparent from the following description of a non-limiting example embodiment, with reference to the appended drawings, in which:

- Figure 1 shows intensity histograms for two example scenarios: A) 920 ion clouds of Au extracted from the raw data set that are representative of a spherical particle; and B) 1123 ion clouds of Au extracted from the raw data set that are

representative of a cubic particle;

- Figure 2 shows intensity histograms generated from ICP-MS raw data generated for spherical Au nanoparticles with a diameter of 80 nm for two scenarios: A) conventional or static grouping; and B) dynamic grouping according to the present invention;
- Figures 3a and 3b show a flow chart illustrating the proposed method of extracting single-particle or single-cell events;
- Figure 4 illustrates how the raw data set is loaded or received and grouped by z consecutive intensities;
- Figure 5 illustrates the calculation of mean values for individual groups;
- Figure 6 illustrates the calculation of a first threshold value and how it is used to create a new data set of dissolved intensities (background);
- Figure 7 illustrates the thresholding process during which an intensity threshold value Th_R is found that defines what portion of the signal is background signal;
- Figure 8 illustrates the extraction of the background intensity and calculation of the dissolved intensity, which is proportional to the concentration of ions in the solution;
- Figure 9 illustrates the peak recognition algorithm;
- Figure 10 illustrates the calculation of the maximum value I_i within a peak group P_{jz};
- Figure 11 illustrates how a Boolean series is created to identify intensity values I_i that are greater than the threshold value Th_R;
- Figure 12 illustrates how another Boolean series C_i is created providing an identifier for those intensities that may contribute to a candidate ion cloud group or event candidate;
- Figure 13 illustrates how each consecutive value C_i is summed yielding Q_i;
- Figure 14 illustrates the creation of data sets E_{lz} of ion cloud intensities;
- Figure 15 illustrates the integration of the ion clouds to obtain the total intensity per particle or cell event;
- Figure 16 illustrates how mass distribution data sets are generated;
- Figure 17 illustrates how volume distributions are generated;
- Figure 18 illustrates how size distributions are generated;
- Figure 19 illustrates how composition distributions are generated for the measured analytes;
- Figure 20 illustrates how aspect ratio distributions are generated from master data;
- Figures 21a and 21b show a flow chart summarising the flow chart of Figures 3a and 3b;
- Figure 22 shows the outcome of the event extraction algorithm for different group sizes ranging from 1 to 15 and as depicted by their corresponding (total) intensity histograms, and where the group sizes greater than 7 result in a correct event extraction process;
- Figure 23 shows the data workflow once the master data creation is completed, and the ion clouds of the events have been obtained;
- Figure 24 shows examples of size distributions of Au nanoparticles of octahedral, cubic and spherical shape;

- Figure 25 illustrates aspect ratio determination of NaYF4 rod-shaped particles;
- Figure 26 shows ion clouds extracted from mass cytometry data for a sample of antibody metal-tagged stained peripheral blood mononuclear cells;
- Figure 27 shows an atomic composition distribution of a CuAg particle; and
- Figure 28 shows a machine learning prediction of the particle shape based on the ion cloud data (extracted true events), the integrated intensity (total intensity) histogram, the mass and size distributions.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

[0019] In the present disclosure, an automated system and a computer-implemented method are described that can extract single-particle or single-cell events from a spectrometric data set generated for instance on a time-of-flight, single- or multiple-quadrupole, or sector-field mass analyser.

[0020] As utilised herein, "and/or" means any one or more of the items in the list joined by "and/or". As an example, "x and/or y" means any element of the three-element set {(x), (y), (x, y)}. In other words, "x and/or y" means "one or both of x and y." As another example, "x, y, and/or z" means any element of the seven-element set {(x), (y), (z), (x, y), (x, z), (y, z), (x, y, z)}. In other words, "x, y and/or z" means "one or more of x, y, and z." Furthermore, the term "comprise" is used herein as an open-ended term. This means that the object encompasses all the elements listed, but may also include additional, unnamed elements. Thus, the word "comprise" is interpreted by the broader meaning "include", "contain" or "comprehend". Identical or corresponding functional and structural elements which appear in the different drawings are assigned the same reference numerals. It is to be noted that the use of words "first", "second" and "third", etc. may not imply any kind of particular order or hierarchy unless this is explicitly or implicitly made clear in the context.

[0021] Definitions of some terms used in the present description are first given in the following.

[0022] Time-resolved ion cloud: a series of consecutive intensity values measured at short integration times that allows the whole ion cloud to be extracted, i.e., an event including the spatial information of that cloud.

[0023] Spatial information: the information that exists within an ion cloud, the distribution of ions in space that contains the key or fingerprint of the original systematic atomic arrangement in space of the particle or cell, for example a cubic shape.

[0024] Spatially confined: a (nano)material that is defined by its specific volume.

[0025] Background signal: intensities of a spectrometric data set that constitute the background.

[0026] Background: ions in a solution or random noise generating an intensity value.

[0027] Element: an element of the periodic table, such as copper (Cu).

[0028] Analyte: a species under investigation in the sample that may or may not be present, i.e., an ion of a specific mass/charge ratio of a potential element or ion present in the sample.

[0029] Ion: the ionic state of an element, e.g., Cu^{2+} in which 2 electrons have been emitted from the Cu atom.

[0030] Particle: any entity made up of a number of elements within the size range of 10 micrometres (μm) to 1 nanometre (nm).

[0031] Cell: a species of biological origin whose elemental make-up is investigated with an ICP-MS, for instance.

[0032] Transient signal: a series of intensity values above the background that constitutes an event, i.e., an ion cloud generated from a particle or cell.

[0033] Event: an ion cloud generated by ionisation of a spatially confined material, for instance a particle or cell upon its introduction to the plasma and detected using a mass analyser.

[0034] Dwell: time interval in which ions are collected for a given intensity count.

[0035] Threshold: an intensity value, which may or may not be an integer, determining a limit such that intensities equal to, or smaller than the threshold constitute the background signal, and intensities greater than the threshold constitute potential events.

[0036] In the present description, spectrometric raw data (intensity values as a function of time) is processed using a series of algorithms that accurately identify single-particle and single-cell events and extracts the distributions of intensities associated with each event and returns that as a new data set. This extracted true event data set containing the ion clouds of single particles or single cells ionised with an ICP, for instance, can then be used for various algorithmic applications, such as shape detection, mass quantification, composition determination, classification, etc.

[0037] The proposed process is next explained in more detail with reference to the figures, and in particular to the flow chart of Figures 3a and 3b, and Figures 4 to 20. In step 1, a sample containing particles/cells is in this example introduced to an inductively coupled plasma mass spectrometer (ICP-MS), which in step 2 generates and processes a spectrometric raw data set containing a time-series t_i of ion intensities I_i of length i = 1 to N. I_i thus represents one intensity value in the raw data set. The time interval between two consecutive time instants t_j is in this example constant throughout one experiment.

However, non-constant time intervals throughout one experiment are also possible. In step 3, and as shown in Figure 5, the raw data set is grouped (G_{jz} with j = 1 to [N/z]) by consecutive signals in the time domain with a group size of z with z = 1 to N, with z = N returning the original raw data set in a single group. For instance, if z =2, then the respective group consists of two consecutive intensity values, if z = 3, then the respective group consists of three consecutive intensity values, etc. In other words, in this step the intensity values are grouped into sets (representing the horizontal dimension in Figure 5) of groups (representing the vertical dimension in Figure 5) of consecutive intensity values, where a respective set is characterised by a distinct group size of intensity values, I_i thus represents an intensity value proportional to the total number of ions collected by the detector, which in this example is an electron multiplier, in a given time interval.

[0038] In step 4 and as shown in Figure 5, the mean A_{jz} is calculated for any or some of the groups G_{jz} from z = 1 to N. If z = 1, then the mean $A_{j=i}$ equals the respective intensity value, as in that case each group consists of only one intensity value. In this example, the mean is calculated for all of the groups.

[0039] In step 2.1.1 and as is illustrated in Figures 6 and 7, we define a first or initial threshold Th_1 which is used to determine if a signal should be considered as part of the background or as a particle/cell event. The threshold Th_1 is in this case defined as the mean + 3 times the standard deviation of the raw data set (I_i). In other words, Th_1 = mean(I_i) + 3*sigma(I_i). However, other ways to define the threshold are equally possible. The mean value is thus calculated for the intensity values of column 2 of Figure 6. Having defined the initial threshold, a new data set D_k of length i = 1 to N is created in step 2.1.1 of dissolved (background) intensities I_i such that D_{ik} equals 1 if I_i is smaller than, or equal to the threshold Th_1, or else D_{jk} equals zero. Index k in Figure 6 refers to a counter value of the threshold value as will become clear in the following.

[0040] Having determined the initial threshold Th_1 and intensities D_{ik} below or equal to that threshold Th_1, as shown in Figure 6, the algorithm determines the threshold Th_{k+1} with k = 1 to R by determining the mean for the new data sets D_{ik} with k = 1 to R and i = 1 to N of dissolved intensities that match the criteria that the intensity I_i is smaller or equal to the threshold Th_{k+1}, else D_{ik+1} is zero. This process is repeated until the last determined threshold Th_{k+1} equals the immediately preceding threshold Th_k. The final threshold is than the threshold at convergence, which is the smallest definable threshold. If the final threshold is zero, the last threshold greater than 0 is used instead. At this stage an iterative thresholding process is thus carried out, during which the intensity value is found that defines what portion of the signal belongs to the background signal. However, a non-iterative thresholding process could be used instead. It is to be noted that other thresholding processes may be used instead as known in the prior art. For instance, the thresholding process may be based on a threshold obtained as mean + n*sigma, or the raw signal may be fitted to an exponential according to the teachings of US11075066B2, or the compound Poisson distribution fit may be used according to the teachings of Hendriks et al. "Performance of Sp-ICP-TOFMS with Signal Distributions Fitted to a Compound Poisson Model", J. Anal. At. Spectrom, 2019, 34 (9), 1900-1909.

[0041] In step 2.1.2 and as shown in Figure 8, the intensities D_{ik} (for the last instance of D_{ik}) for which intensities $I_i \leq Th_k$ are set to I_i, otherwise they are set to 0. In step 2.1.3 and as is shown in Figure 8, by using the final dissolved data set intensities D_{ik} with k = R, the dissolved intensity data set. In other words, the background intensity of the dissolved ions is extracted. Intensity values equal to, or smaller than Th_R, which is the sought after threshold value, are considered as being background intensity values, and intensity values greater than Th_R are considered as particle or cell intensities. In other words, for k = R, non-zero intensity values reaching from D_1 to D_N form the dissolved data set, i.e., the background. The "Dissolved", which is proportional to the concentration of ions in the solution, is obtained as the mean of D_{ik} where k = R.

[0042] In step 5 and as illustrated in Figure 9, candidate peak groups are identified. More specifically, based on the determined threshold Th_R, a group G_{jz} is a candidate peak group if $A_{jz} > A_{j-1z}$, and $A_{jz} > A_{j+1z}$, and $A_{jz} > Th_R$. In other words, a group will be recognised as a candidate peak group if the mean value of that group is greater than the mean value of the immediately previous and/or immediately following group and that the mean value of that group is greater than the threshold Th_R. This means that for z = N, it returns exactly P_1 peaks, which in this example is one peak. Thus, at this stage, peak group candidates are identified.

[0043] After recognising the peaks or candidate peak groups, in step 6 and as illustrated in Figure 10, the algorithm determines the maximum value within a candidate peak group P_{jz} with $j = 1 \text{ to } \lceil N/z \rceil$ with z = 1 to N and returns a new data set X_{jz} with j = 1 to $\lceil N/z \rceil$ and z = 1 to N that in the case of z = N contains exactly 1 times the value 1 given that a true maximum can be defined.

[0044] With the candidate peak groups defined, in step 2.2.1 and as illustrated in Figure 11, a Boolean series H_i is created that will have the value of 1 in the case the intensity $I_i > Th_R$ and else 0 is returned. H_i can be interpreted as an intensity value I_i that does not belong to the background. This step is used to identify a series of intensities that are not background and can contribute to an event. In other words, here candidate intensities are identified that will be used to identify candidate events. As is shown in Figure 11, a Boolean with the value 1 is created for each intensity value $I_i > Th_R$. This provides an identifier for those intensities that may contribute to a candidate ion cloud group or candidate event.

[0045] With the Boolean series H_i created, in step 2.2.2 and as illustrated in Figure 12, another Boolean C_i can be created in which the shifted H_{i+1} is compared to H_i with the criterium that it should not be equal to H_i and that $I_i > Th_R$. In such a case, $C_i = 1$, else 0. This is used to find the start codon of the series of intensity values that are considered an event candidate or ion cloud group. In other words, this step ensures that intensity values smaller than Th_R directly following a series of H_i values are included in the candidate event selection.

[0046] In step 2.2.3 and as illustrated in Figure 13, the cumulative sum $Q_i = \Sigma C_i$ is then used to identify those series of consecutive values I_i that are considered candidate particle events. Each series of values, which is equal to another series of values is part of the same candidate event or ion cloud group. This step is used to ensure that the entire event candidate is considered and not merely a portion of it.

[0047] In step 7 and as illustrated in Figure 14, using the maximum value of the peaks and the cumulative sum, for different group sizes, each event is identified as $E_{lz} = I_{lz}$ *if* $\exists\, l_1, l_2 \in \mathbb{Z}^+$ with $l_1 \leq l_2$ such that b' I E $[l_1, l_2]$, $Q_l = Q_{l_1}$ and $\Sigma_l X_{lz} =$ 1 for each z = 1 to N groups, where $\mathbb{Z}^+$ denotes a positive integer numbers set. This step in essence compares the candidates found through steps 2 to 6 on the one hand, and the candidates found through steps 2.2.1 to 2.2.3 on the other hand and accepts the associated candidate ion cloud groups obtained through steps 2.2.1 to 2.2.3 as events if they overlap with the candidate peak groups obtained through steps 2 to 6, i.e., the ion cloud groups contain a maximum of the candidate peak groups. The entire ion cloud group is in that case considered an event E_{lz}. These events E_{lz}, referred to as master data, are then used for further processing, for instance for ion cloud visualisation, shape/morphology extraction, total mass determination, etc.

[0048] With the master data generated, in step 8 and as illustrated in Figure 15, the total intensity S_{lz} of the identified events E_{lz} can be determined by summing over each element in S_{lz} and subtracting the background intensity per dwell for each z = 1 to N groups. The subtraction of the background is considered based on the event length, and therefore the number of intensity values present in the event E_{lz}. In step 9, by iterating over z = 1 to N, the most optimal z can be found, which yields the lowest false positive/negative rate ($z = \alpha$). The obtained $z = \alpha$ can then be fed back to E_{lz} (steps 7 and 8) to extract the correct or preferred events E_l which can be used for processing the spatial information, i.e. morphology in step 10.

[0049] Using the sum of the intensity values with $z = \alpha$ determined, in step 9.1 and as illustrated in Figure 16, mass distributions are calculated using a provided or measured instrument sensitivity of the analyte. In Figure 3b, in connection with steps 9.1 to 9.5, the word "total" refers to the integrated intensity of the consecutive intensity values defined and extracted per particle or cell. Using the mass distributions, in step 9.2 and as illustrated in Figure 17, volume distributions are calculated using a provided or measured density of the analyte. Using the volume distributions, in step 9.3 and as illustrated in Figure 18, size distributions are calculated using a provided or measured shape associated with the analyte. Given that a multitude of analytes are analysed in the same sample, in step 9.4 and as illustrated in Figure 19, composition distributions are generated. In other words, provided that a multitude of elements dj has been measured, and a mol mass MW_d of the element is provided or measured, an atomic composition C_{ld} distribution can be produced per event as shown in Figure 19.

[0050] With the master data generated, in step 9.5 and as illustrated in Figure 20, aspect ratios (RT_{lm}) of the data set can be generated by finding and paring events of matching total intensity (with the intensity count ± 5, or by using any other allowable total intensity difference between the events of the pair) and dividing the largest maximum value of the pair by the smallest maximum intensity of the pair for each corresponding extracted event (E_l) found. The count over each element I in S_l (which is a value) is the total number of events extracted, which can be related to the particle or cell number concentration in step 9.6 using a provided or measured transport efficiency and sample flow rate.

[0051] The above-described method, which is fully or predominantly a computer-implemented method, is next summarised with reference to the flow chart of Figures 21a and 21b. In step 21 and corresponding to steps 1 and 2, ICP-MS raw data, or more broadly spectrometric data, consisting of a series of intensity values is generated or received. In step 22 and corresponding to steps 3 and 4, the raw data is grouped or subdivided into a series or sets of (predefined) consecutive intensity values considering all possible grouping possibilities available in the data set. Each set of intensity values is distinguished from other sets of intensity value by their group size. The group size is thus unique and fixed within a given set, possibly apart from the last group in the set due to the fact that the total number of intensity values when divided by the group size may result in a non-integer value. In step 23 and corresponding to steps 2.1.1 and 2.1.2, a threshold value separating the background from candidate event intensities is determined. This thresholding step may be carried in parallel with step 22, i.e., substantially at the same time as step 22. In step 24 and corresponding to step 2.1.3, a dissolved intensity is calculated as the mean of all the intensities that constitutes the background signal. Step 24 may also be carried out in parallel with step 22, i.e., substantially at the same time as step 22. In step 25 and corresponding to step 5, a first candidate event identification is carried out by obtaining one or more candidate peak groups, which in this case form a first set of candidate events. More specifically, here different groups are compared to another based on a feature characterising a respective group of intensity values, and the groups that contain a series of consecutive intensity values that may

constitute an event are identified. The feature is in this example a mean value, but other features characterising a given group could instead be used. In other words, in this case, one or more first candidate events are formed by intensity values of a given group if their mean intensity value is above the threshold defined in step 23, and if the mean value is greater than the mean intensity value of an immediately preceding group and/or of an immediately following group.

[0052] In step 26 and corresponding to steps 2.2.1 to 2.2.3, a second candidate event identification is carried out by obtaining ion cloud groups based on the threshold value defined in step 23. In other words, to ensure that a complete set of intensity values that construes an event is identified and not only a portion of it, intensity values that are equal to, or below the threshold value but are adjacent to a series of values that are above it are identified and form one or more second candidate events. In step 27 and corresponding to steps 6 and 7, a true event identification is carried out for different sets of intensity values, i.e., for different group sizes, by comparing the first and second candidate event identifications to obtain one or more ion cloud group events. More specifically, the true event identification is carried out by comparing the first and second candidate events such that their overlap forms the true events for a given group size. It is to be noted that quite different ion cloud group events are typically obtained for different group sizes, which may for instance be comprised between 1 and 15. Thus, the true event identification may carried out for all of the group sizes leading to mutually different ion cloud group events.

[0053] In step 28 and corresponding to step 8, the intensity values of a respective ion cloud group event are summed, and the dissolved intensity as scaled based on the number of intensity values in the respective ion group event is subtracted from the summed intensity values of the respective ion group event to obtain a total intensity value per particle or cell. In step 29 and corresponding to step 9, the correct group size z is determined. In this example, the most optimal group size is identified iteratively by means of an AI algorithm trained with a labelled database of experiments with known group size values.

[0054] With the true events identified for the correct group size, in step 30 and corresponding to steps 9.1 and 10, the ion cloud group events are used to extract spatial information and/or the total intensity per particle or cell is used to extract measurables. In other words, with the true events identified for the correct group size, the ion cloud group events can be used to extract spatial information, and/or the integrated intensity per particle or cell can be used to extract measurables such as mass, volume, size, composition and aspect ratio distributions and number concentration.

[0055] According to prior art solutions, a typical approach is to fix z = 5 to group the consecutive intensity values and commence with the peak recognition step. However, as Figures 2 and 22 show, choosing a group size of z = 5 is often not feasible. Figure 22 shows the outcome of the event extraction algorithm with z = 1 to 15 as depicted by their corresponding (total) intensity histograms. The X-axis represents the intensity values, while the Y-axis represents the intensity value count. Only z > 7 results in a correct event extraction process. Especially when particles are small, or the background signal is high, a static group size results in erroneous peak recognition and eventual event extraction. Therefore, the present invention optionally uses a machine learning-based approach to determine the optimal group size per experiment by means of a training set generated on calibrant samples of known sizes. Table 1 below shows the outcome of the predictions for a subset of a series of unseen calibrated data sets of Au, Cu, and Ag, cubic, spherical and octahedral nanoparticles of sizes ranging from 30 nm to 120 nm. Out of 30 experiments, only 19 had the optimal group size z = 5 meaning that conventional algorithms would erroneously group 37.7% of this specific data set resulting in high false positives, false negatives and over/underestimation of the size or particle count.

Table 1. Predicted versus true optimal group sizes z = 3 to 15.

EXPERIMENT ID	GROUP SIZE z =	PREDICTED VALUE	TRUE VALUE
Experiment 1	3	0	0
Experiment 1	4	1	1
Experiment 1	5	0	0
Experiment 1	6	0	0
Experiment 1	7	0	0
Experiment 1	8	0	0
Experiment 1	9	0	0
Experiment 1	10	0	0
Experiment 1	11	0	0
Experiment 1	12	0	0
Experiment 1	13	0	0
Experiment 1	14	0	0
Experiment 1	15	0	0
Experiment 2	3	0	0
Experiment 2	4	0	0

(continued)

EXPERIMENT ID	GROUP SIZE z =	PREDICTED VALUE	TRUE VALUE
Experiment 2	5	0	0
Experiment 2	6	0	0
Experiment 2	7	0	0
Experiment 2	8	1	1
Experiment 2	9	0	0
Experiment 2	10	0	0
Experiment 2	11	0	0
Experiment 2	12	0	0
Experiment 2	13	0	0
Experiment 2	14	0	0
Experiment 2	15	0	0
Experiment 3	3	0	0
Experiment 3	4	0	0
Experiment 3	5	1	1
Experiment 3	6	0	0
Experiment 3	7	0	0
Experiment 3	8	0	0
Experiment 3	9	0	0
Experiment 3	10	0	0
Experiment 3	11	0	0
Experiment 3	12	0	0
Experiment 3	13	0	0
Experiment 3	14	0	0
Experiment 3	15	0	0

[0056] Figures 23 to 28 show some examples illustrating the above-described method and its applications. Figure 23 shows the data workflow once the master data creation is completed, and the ion clouds of the events have been obtained. Figure 24 shows examples of size distributions of Au nanoparticles of octahedral, cubic and spherical shape. Figure 25 illustrates aspect ratio determination of $NaYF_4$ rod-shaped particles. Maxima of events of equal total intensity but with longest duration (lowest maximum) and shortest duration (highest maximum) are used for the short and long axis of the rods, respectively. In this example, the extracted aspect ratio equals 4 (transmission electron microscopy (TEM)), and the determined aspect ratio equals 4. Figure 26 shows ion clouds extracted from mass cytometry data for a sample of antibody metal-tagged stained peripheral blood mononuclear cells. Figure 27 shows an atomic composition distribution of an Ag particle and a Cu particle. Figure 28 shows a machine learning prediction of the particle shape (CUB stands for cube, SPH stands for sphere, THD stands for tetrahedron, and OCT stands for octahedron) based on the ion cloud data (extracted true events), the integrated intensity (total intensity) histogram, the mass distribution and the size distribution.

[0057] An interesting use case of the present invention is next explained. Using a provided expected particle or cell shape, size and/or coefficient of variance (the standard deviation of the size distribution over the mean size), it is possible to construct a virtual expected particle or cell size distribution. By comparing the actual measured particle or cell size distribution to the virtual expected size distribution, it is possible to determine to what degree a particle or cell production process was successful. This allows to tune the parameters of the production process until the measured size distribution matches the expected virtual size distribution. Using this process, the algorithm could be deployed as a quality control tool that monitors the state of the production process. If this is done in-line, one could perform a quality control in-line always guaranteeing that the outcome of the production process would pass quality control. This is possible for all the measurables, i.e., aspect ratio distribution, composition distribution, volume distribution, etc.

[0058] Different advantages and applications of the present invention are summarised below.

- The algorithm can distinguish single-particle and single-cell events from background by means of a background subtraction and single-particle and single-cell event identification process.
- The algorithm can distinguish particle and cell events up to 1000 particles/cells per second.
- The algorithm makes it possible to extract the transient signal of an event of an analyte in a sample.

- The algorithm makes it possible to extract the total intensity of an event of an analyte in a sample.
- The algorithm makes it possible to extract the total mass of an event of an analyte in a sample.
- The algorithm makes it possible to extract the analyte particle sizes for known particle shapes and densities.
- The algorithm makes it possible to extract the analyte particle densities of known particle volumes.
- The algorithm makes it possible to extract composition distributions in the case of a plurality of analytes present in the same particle and/or cell.
- The algorithm makes it possible to perform immunophenotyping on cells that contain a plurality of elements, based on metal tag composition.
- The algorithm makes it possible to determine particle and cell number concentrations using a provided or measured transport efficiency value and sample flow rate.
- The algorithm makes it possible to extract spatial distributions of analytes present in particles and cells.
- The present algorithm makes it possible to extract the shape information for particles and cells.
- The algorithm makes it possible to extract the aspect ratio information for particles and cells.
- The algorithm makes it possible to classify particles and cells based on their shape information.
- The algorithm makes it possible to perform immunophenotyping on cells that contain a plurality of elements, based on metal tag ion cloud distributions, i.e., cell morphology.

[0059] The method steps described above may be carried out by suitable circuits or circuitry when the process is implemented in hardware or using hardware for individual steps. However, the method or at least some of the method steps may also or instead be implemented in software. Thus, at least some of the method steps can be considered as computer-implemented steps. The terms "circuits" and "circuitry" refer to physical electronic components or modules (e.g., hardware), and any software and/or firmware ("code") that may configure the hardware, be executed by the hardware, and or otherwise be associated with the hardware. The circuits may thus be operable (i.e., configured) to carry out or they comprise means for carrying out the required method steps as described above. Different computations may or may not be cloud-computation operations depending on the implementation.

[0060] While the invention has been illustrated and described in detail in the drawings and foregoing description, such illustration and description are to be considered illustrative or exemplary and not restrictive, the invention being not limited to the disclosed embodiment. Other embodiments and variants are understood and can be achieved by those skilled in the art when carrying out the claimed invention, based on a study of the drawings, the disclosure and the appended claims. Further variants may be obtained by combining the teachings of any of the examples explained above.

[0061] In the claims, the word "comprising" does not exclude other elements or steps, and the indefinite article "a" or "an" does not exclude a plurality. The mere fact that different features are recited in mutually different dependent claims does not indicate that a combination of these features cannot be advantageously used. Any reference signs in the claims should not be construed as limiting the scope of the invention.

Claims

1. A method for extracting one or more single-particle and/or single-cell events from a spectrometric data set comprising intensity values, a respective intensity value being proportional to the number of ions collected by a detector in a given time interval, the method comprising:

    - grouping (22) the intensity values into sets of groups of consecutive intensity values, a respective set being **characterised by** a distinct group size of intensity values;
    - determining (23) a threshold value separating a background from candidate event intensity values;
    - calculating (24) a dissolved intensity as a mean of the intensity values forming a background signal characterising the background;

- carrying out (25) a first candidate event identification by obtaining candidate peak groups based on a feature characterising a respective group of intensity values;
- carrying (26) out a second candidate event identification by obtaining ion cloud groups based on the threshold value;
- carrying out (27) a true event identification by comparing the first and second candidate event identifications to obtain one or more ion cloud group events for a respective group size, a respective ion cloud group event being formed by overlapping intensity values from the peak candidate groups and ion cloud groups; and
- summing (28) the intensity values of a respective ion cloud group event and subtracting the dissolved intensity as scaled based on the number of intensity values in the respective ion cloud group event from the summed intensity values of the respective ion cloud group event to obtain a total intensity value per particle or cell.

2. The method according to claim 1, wherein the method further comprises the step of determining (29) a preferred group size by iterating over different group sizes to obtain the preferred group size, which yields the lowest false positive and/or negative rate in intensity histograms depicting the ion cloud group event.

3. The method according to claim 2, wherein the preferred group size is determined by means of an artificial intelligence algorithm trained with a labelled database of experiments with known group size values.

4. The method according to any one of the preceding claims, wherein the method further comprises the step of using (30) the ion cloud group events to extract spatial information, and/or use the total intensity per particle or cell to extract measurables.

5. The method according to claim 4, wherein the measurables are at least one of the following: a mass distribution, a volume distribution, a size distribution, a composition distribution, an aspect ratio distribution, and a number concentration.

6. The method according to any one of the preceding claims, wherein the feature characterising the respective group of intensity values is a mean value of the intensity values of the respective group.

7. The method according to claim 6, wherein the respective group of intensity values is a candidate peak group if the mean value of the intensity values of the respective group is above the threshold value, and if the mean value of the intensity values of the respective group is greater than a mean intensity value of an immediately preceding group and/or of an immediately following group.

8. The method according to any one of the preceding claims, wherein the dissolved intensity as scaled is obtained by multiplying the dissolved intensity by the number of intensity values in the respective ion group event.

9. The method according to any one of the preceding claims, wherein the ion cloud groups are obtained by including intensity values in a respective ion cloud group that are below the threshold value, but which are adjacent to a series of intensity values that are above the threshold value.

10. The method according to any one of the preceding claims, wherein the spectrometric data is obtained by a scanning inductively coupled plasma mass spectrometer.

11. The method according to any one of the preceding claims, wherein the threshold value is obtained by an iterative thresholding process.

12. The method according to any one of the preceding claims, wherein the threshold value is derived from a standard deviation value of intensity values of a respective set of intensity values, or the threshold value is derived from the intensity values fitted to an exponential, or the threshold value is derived from a compound Poisson distribution fit.

13. The method according to any one of the preceding claims, wherein the method further comprises providing an expected particle or cell shape, size and/or coefficient of variance to construct a virtual expected particle or cell size distribution, and comparing an actual measured particle or cell size distribution obtained from the total intensity per particle or cell to the virtual expected particle or cell size distribution to determine to what degree a particle or cell production process was successful.

14. A non-transitory computer program product comprising instructions for implementing the steps of the method

according to any one of the preceding claims when loaded and run on computing means of a data processing device.

15. An apparatus for extracting one or more single-particle and/or single-cell events from a spectrometric data set comprising intensity values, a respective intensity value being proportional to the number of ions collected by a detector in a given time interval, the apparatus comprising means for:

- grouping the intensity values into sets of groups of consecutive intensity values, a respective set being **characterised by** a distinct group size of intensity values;
- determining a threshold value separating a background from candidate event intensity values;
- calculating a dissolved intensity as a mean of the intensity values forming a background signal characterising the background;
- carrying out a first candidate event identification by obtaining candidate peak groups based on a feature characterising a respective group of intensity values;
- carrying out a second candidate event identification by obtaining ion cloud groups based on the threshold value;
- carrying out a true event identification by comparing the first and second candidate event identifications to obtain one or more ion cloud group events for a respective group size, a respective ion cloud group event being formed by overlapping intensity values from the peak candidate groups and ion cloud groups; and
- summing the intensity values of a respective ion cloud group event and subtracting the dissolved intensity as scaled based on the number of intensity values in the respective ion cloud group event from the summed intensity values of the respective ion cloud group event to obtain a total intensity value per particle or cell.

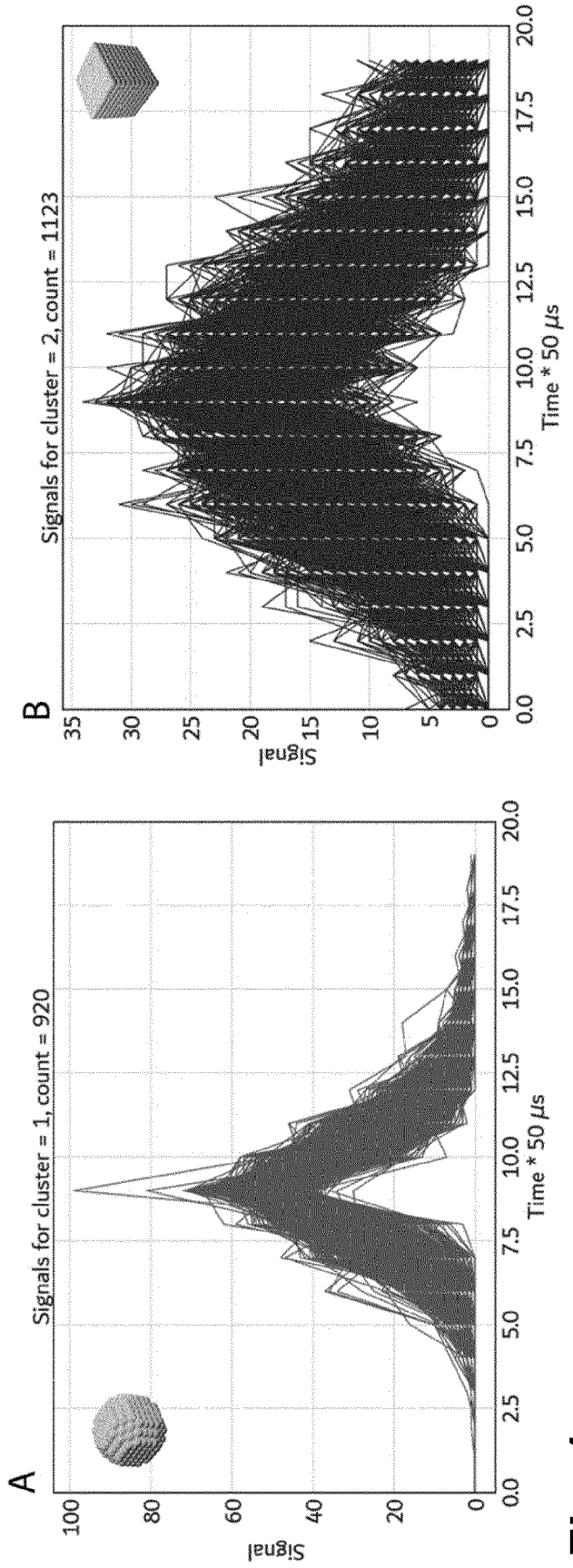
A
Signals for cluster = 1, count = 920
Signal
Time * 50 µs
B
Signals for cluster = 2, count = 1123
Signal
Time * 50 µs

Fig. 1

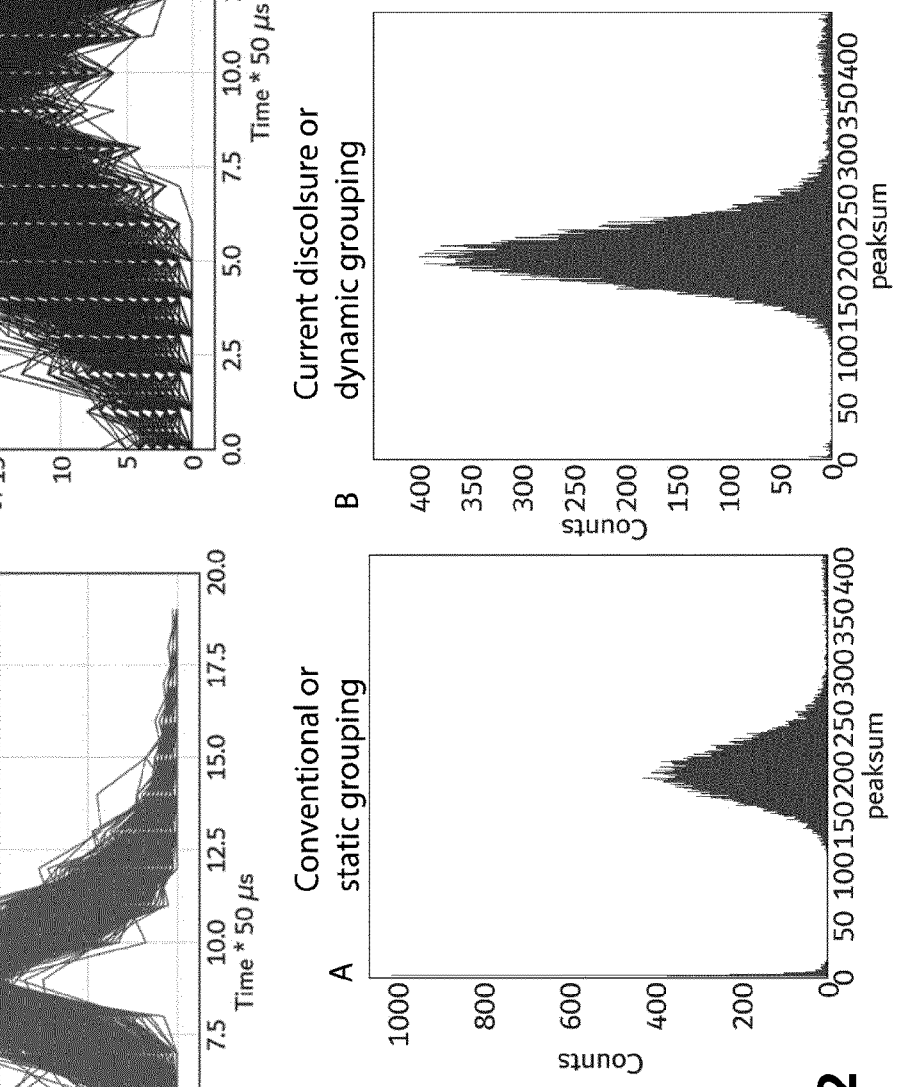
Conventional or static grouping
A
Counts
peaksum
Current disclosure or dynamic grouping
B
Counts
peaksum

Fig. 2

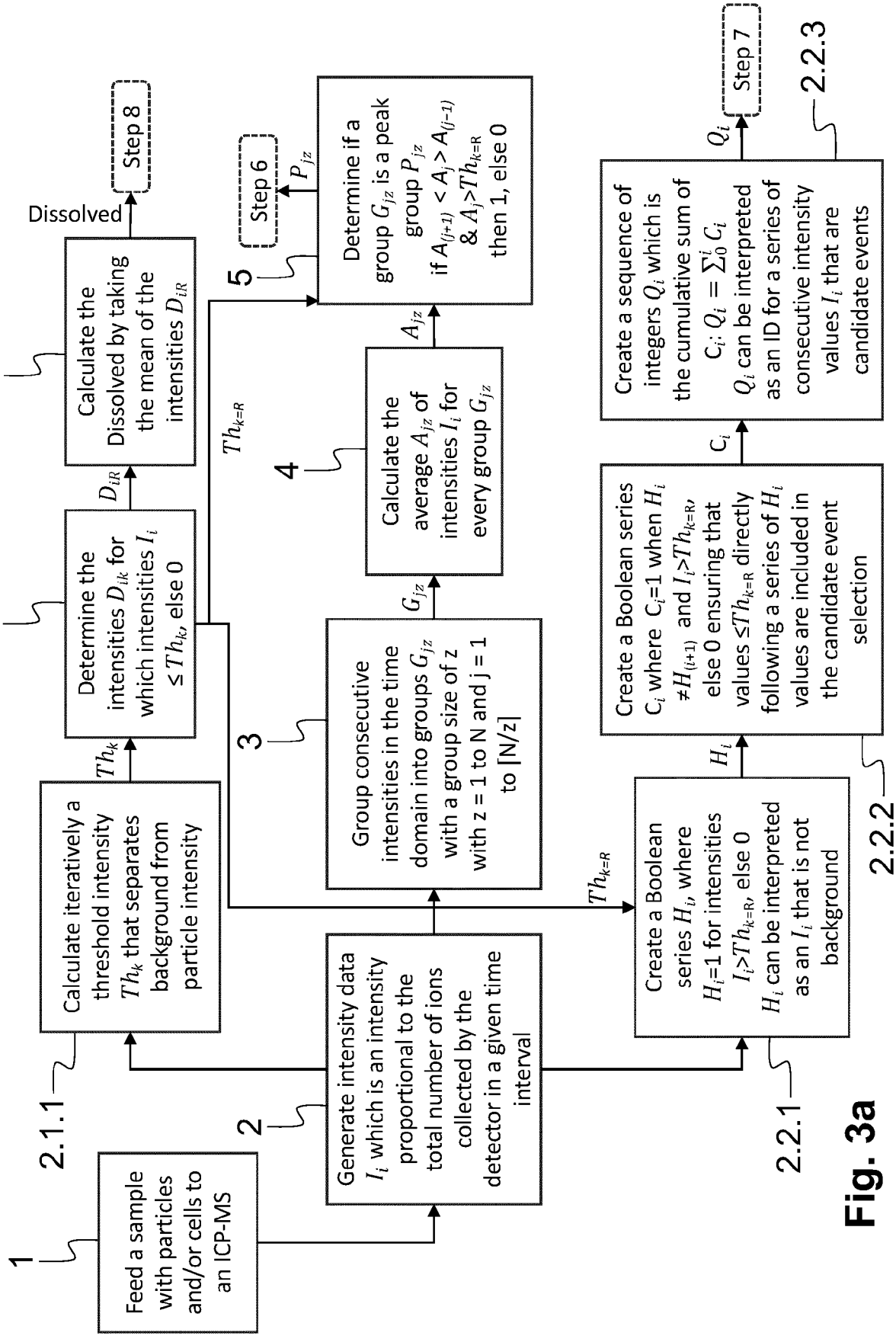
1
Feed a sample with particles and/or cells to an ICP-MS
2
Generate intensity data I_i which is an intensity proportional to the total number of ions collected by the detector in a given time interval
2.1.1
Calculate iteratively a threshold intensity Th_k that separates background from particle intensity
Th_k
2.1.2
Determine the intensities D_{ik} for which intensities $I_i \leq Th_k$, else 0
D_{iR}
2.1.3
Calculate the Dissolved by taking the mean of the intensities D_{iR}
Dissolved
Step 8
$Th_{k=R}$
3
Group consecutive intensities in the time domain into groups G_{jz} with a group size of z with z = 1 to N and j = 1 to ⌈N/z⌉
G_{jz}
4
Calculate the average A_{jz} of intensities I_i for every group G_{jz}
A_{jz}
5
Determine if a group G_{jz} is a peak group P_{jz} if $A_{(j+1)} < A_j > A_{(j-1)}$ & $A_j > Th_{k=R}$ then 1, else 0
P_{jz}
Step 6
$Th_{k=R}$
2.2.1
Create a Boolean series H_i where H_i=1 for intensities $I_i > Th_{k=R}$, else 0
H_i can be interpreted as an I_i that is not background
H_i
2.2.2
Create a Boolean series C_i where C_i=1 when $H_i \neq H_{(i+1)}$ and $I_i > Th_{k=R}$, else 0 ensuring that values $\leq Th_{k=R}$ directly following a series of H_i values are included in the candidate event selection
C_i
2.2.3
Create a sequence of integers Q_i which is the cumulative sum of C_i: $Q_i = \sum_0^i C_i$
Q_i can be interpreted as an ID for a series of consecutive intensity values I_i that are candidate events
Q_i
Step 7

Fig. 3a

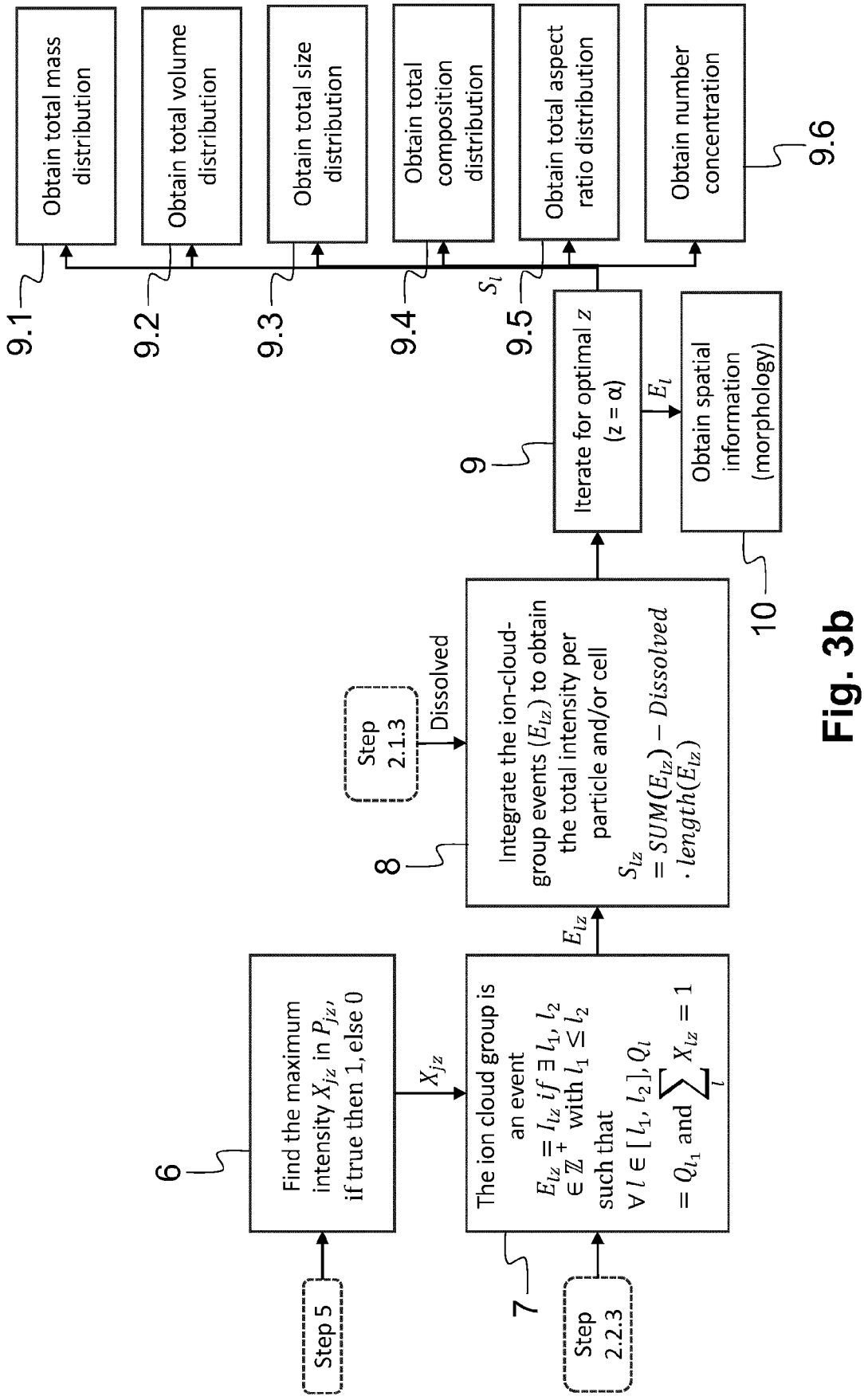
Step 5
Step 2.2.3
Step 2.1.3
Dissolved
6
7
8
9
10
9.1
9.2
9.3
9.4
9.5
9.6
X_{jz}
E_{lz}
E_l
S_l
Find the maximum intensity X_{jz} in P_{jz}, if true then 1, else 0
The ion cloud group is an event $E_{lz} = l_{lz}\ if\ \exists\ l_1, l_2 \in \mathbb{Z}^+$ with $l_1 \leq l_2$ such that $\forall\ l \in [l_1, l_2], Q_l = Q_{l_1}$ and $\sum_l X_{lz} = 1$
Integrate the ion-cloud-group events (E_{lz}) to obtain the total intensity per particle and/or cell $S_{lz} = SUM(E_{lz}) - Dissolved \cdot length(E_{lz})$
Iterate for optimal z (z = α)
Obtain spatial information (morphology)
Obtain total mass distribution
Obtain total volume distribution
Obtain total size distribution
Obtain total composition distribution
Obtain total aspect ratio distribution
Obtain number concentration

Fig. 3b

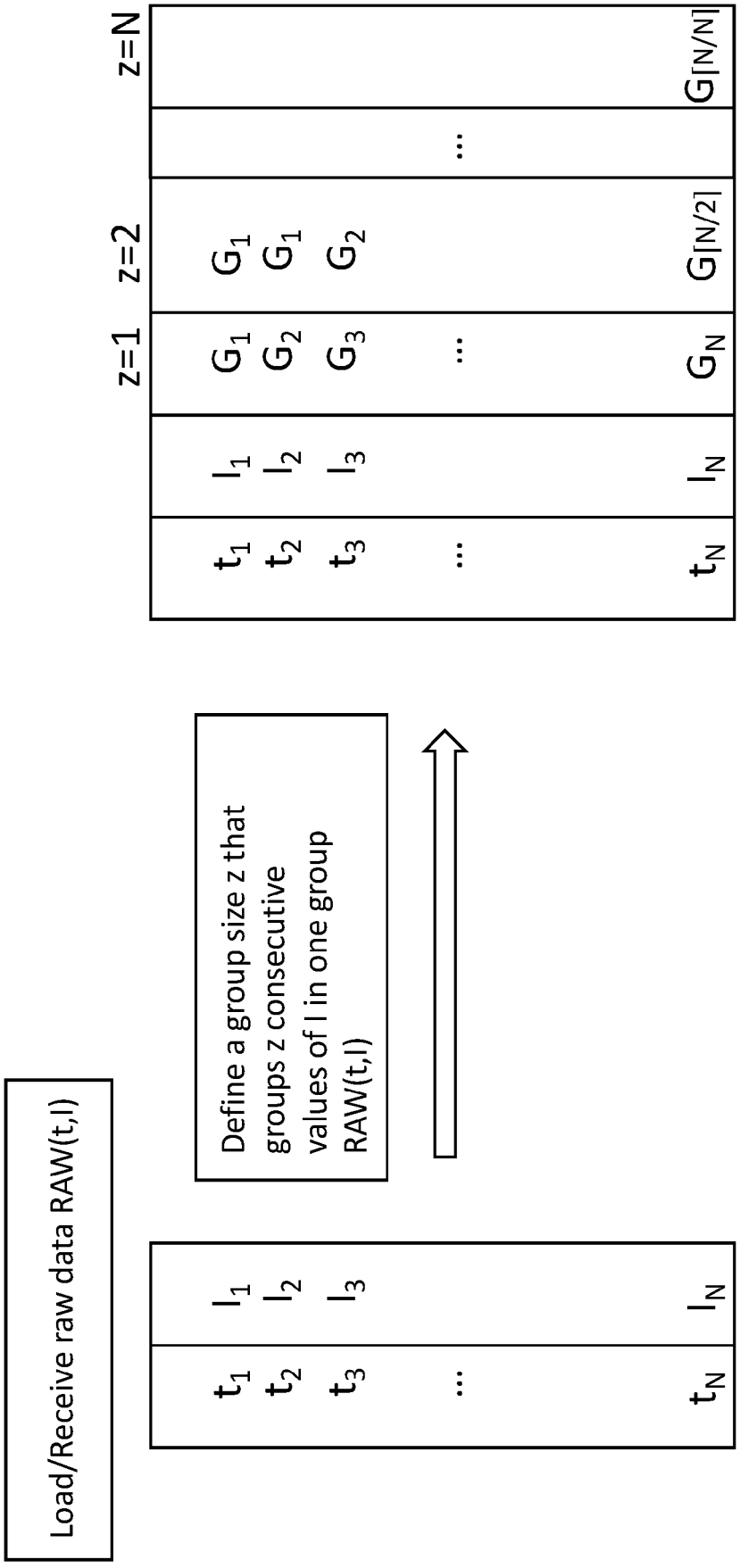
Load/Receive raw data RAW(t,I)
Define a group size z that groups z consecutive values of I in one group RAW(t,I)
z=1
z=2
z=N
t1 t2 t3 ... tN
I1 I2 I3 ... IN
G1 G2 G3 ... GN
G1 G1 G2 ... G[N/2]
G[N/N]

Fig. 4

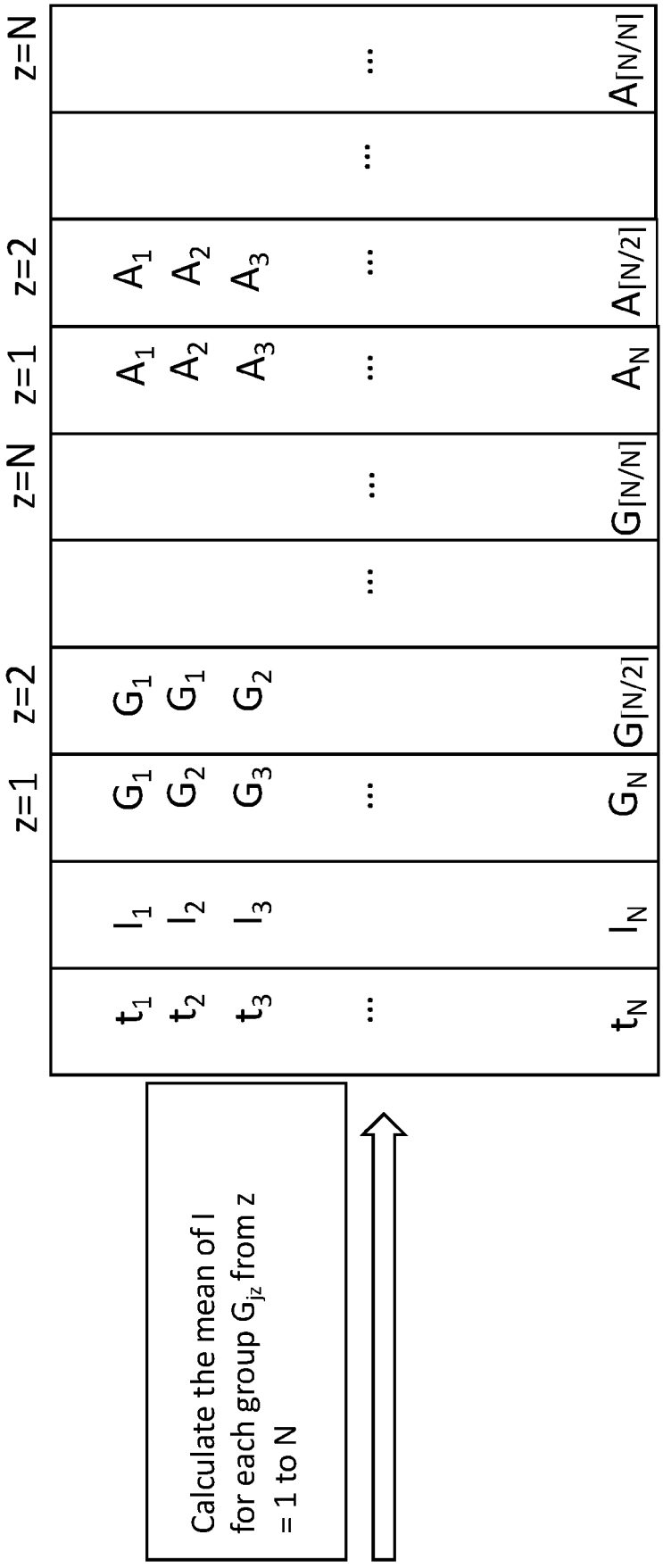
z=1
z=2
z=N
z=1
z=2
z=N
t1 t2 t3 ... tN
I1 I2 I3 ... IN
G1 G2 G3 ... GN
G1 G1 G2 ... G[N/2]
... G[N/N]
A1 A2 A3 ... AN
A1 A2 A3 ... A[N/2]
... A[N/N]
Calculate the mean of I for each group Gjz from z = 1 to N

Fig. 5

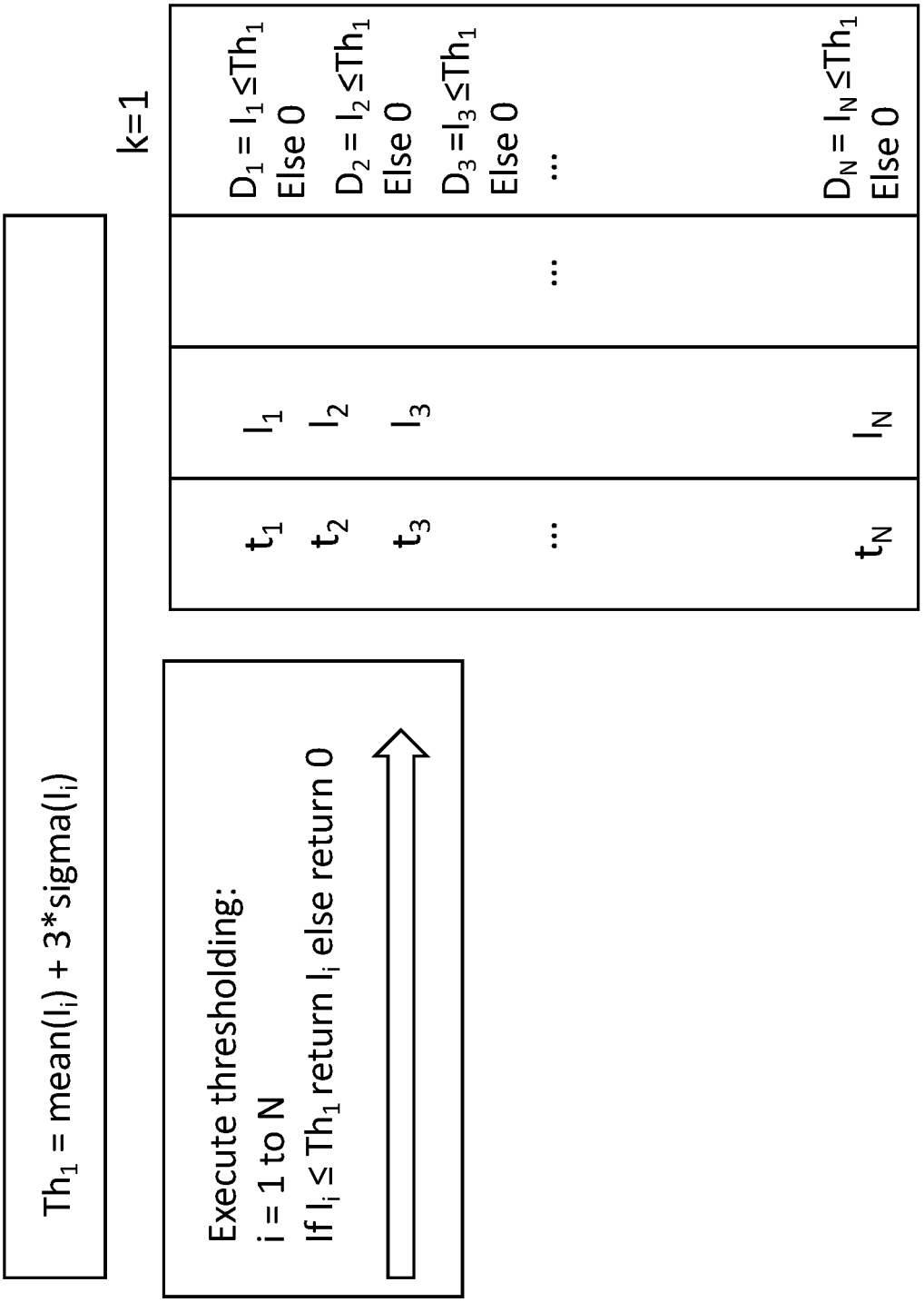
$Th_1 = mean(I_i) + 3*sigma(I_i)$
k=1
Execute thresholding:
i = 1 to N
If $I_i \leq Th_1$ return I_i else return 0
t_1 I_1 $D_1 = I_1 \leq Th_1$ Else 0
t_2 I_2 $D_2 = I_2 \leq Th_1$ Else 0
t_3 I_3 $D_3 = I_3 \leq Th_1$ Else 0
... ...
t_N I_N $D_N = I_N \leq Th_1$ Else 0

Fig. 6

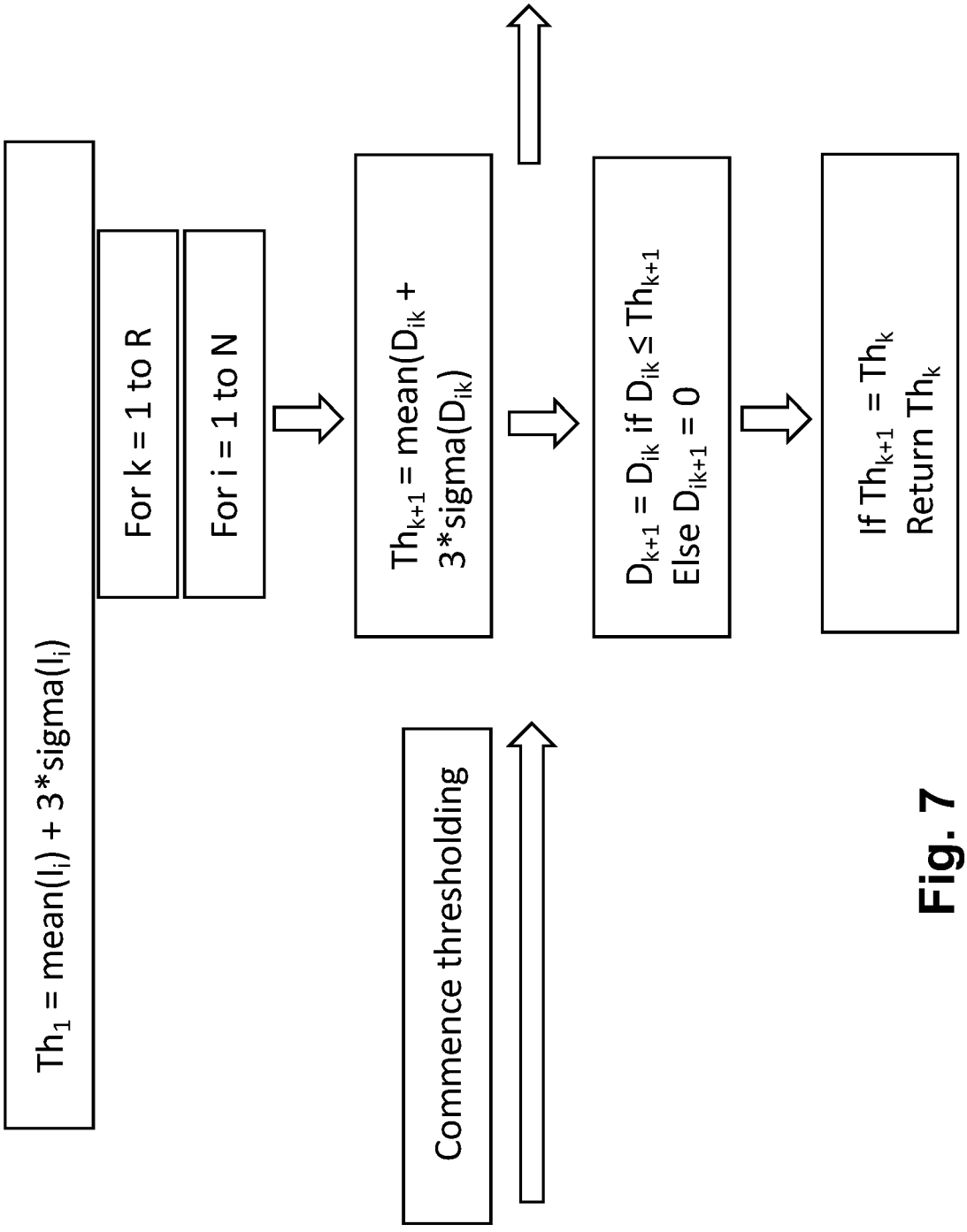
Commence thresholding
$Th_1 = mean(I_i) + 3*sigma(I_i)$
For i = 1 to N
For k = 1 to R
$Th_{k+1} = mean(D_{ik} + 3*sigma(D_{ik}))$
$D_{k+1} = D_{ik}$ if $D_{ik} \leq Th_{k+1}$
Else $D_{ik+1} = 0$
If $Th_{k+1} = Th_k$
Return Th_k

Fig. 7

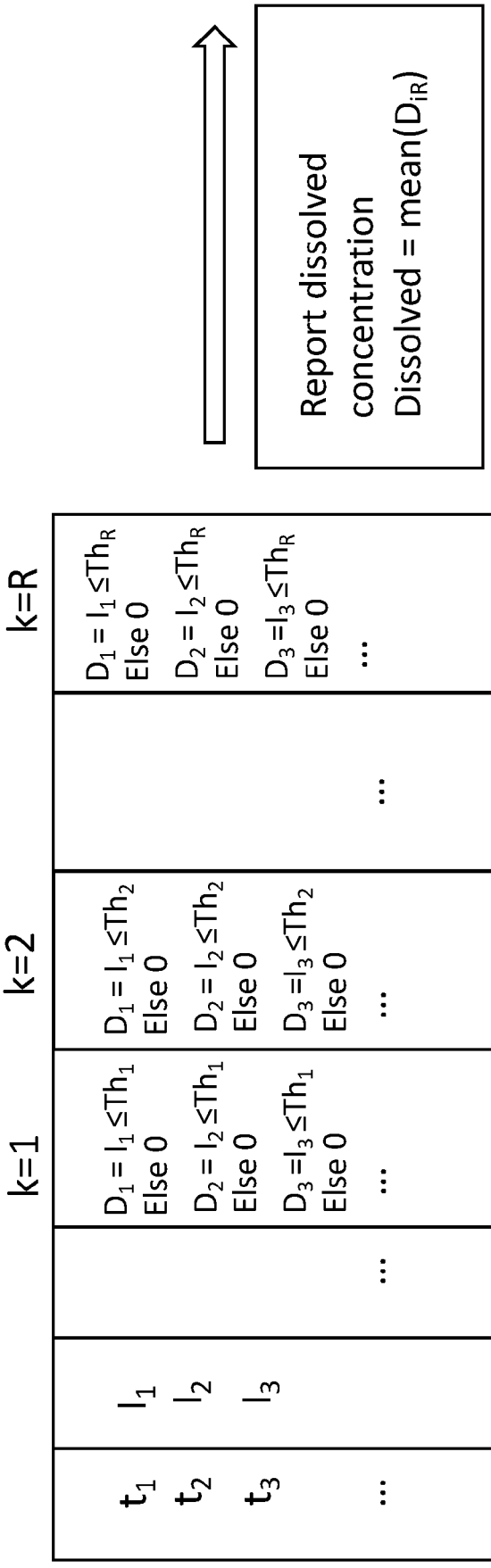
k=1
k=2
k=R
t_1
t_2
t_3
t_N
I_1
I_2
I_3
I_N
$D_1 = I_1 \leq Th_1$ Else 0
$D_2 = I_2 \leq Th_1$ Else 0
$D_3 = I_3 \leq Th_1$ Else 0
$D_N = I_N \leq Th_1$ Else 0
$D_1 = I_1 \leq Th_2$ Else 0
$D_2 = I_2 \leq Th_2$ Else 0
$D_3 = I_3 \leq Th_2$ Else 0
$D_N = I_N \leq Th_2$ Else 0
$D_1 = I_1 \leq Th_R$ Else 0
$D_2 = I_2 \leq Th_R$ Else 0
$D_3 = I_3 \leq Th_R$ Else 0
$D_N = I_N \leq Th_R$ Else 0
Report dissolved concentration
Dissolved = mean(D_{iR})

Fig. 8

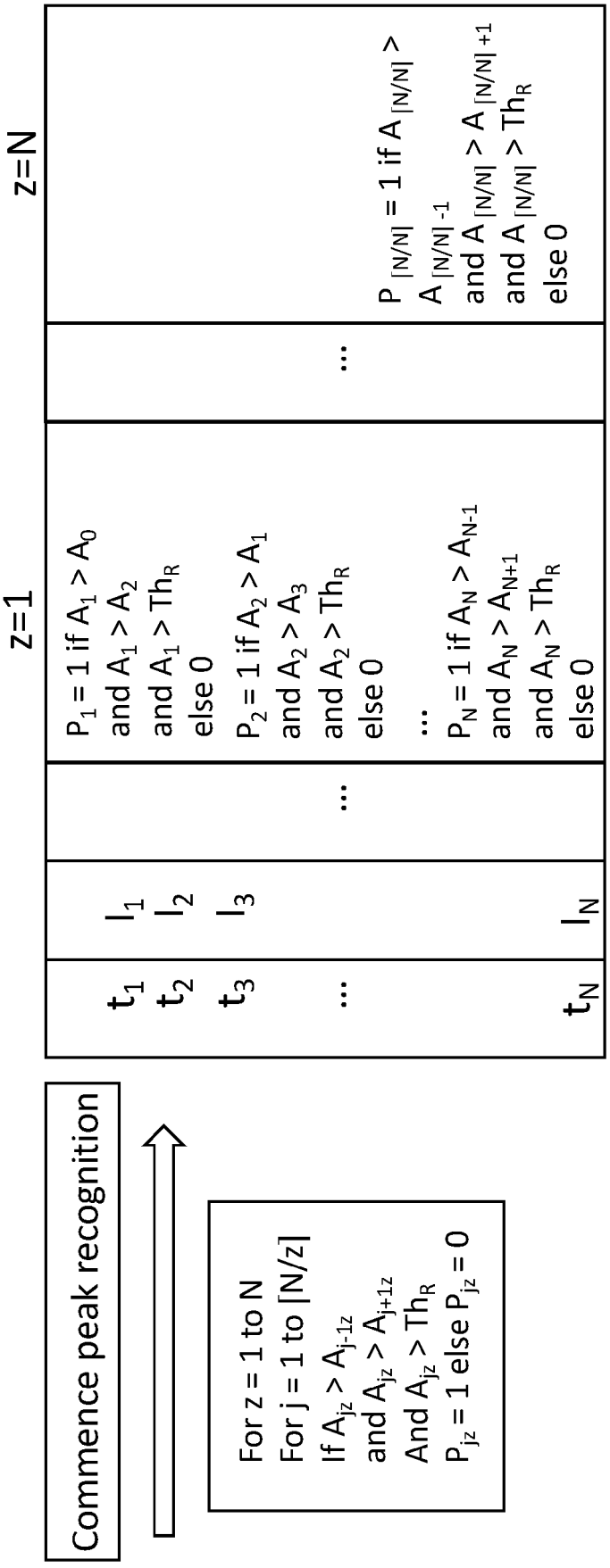
Commence peak recognition
For z = 1 to N
For j = 1 to ⌈N/z⌉
If $A_{jz} > A_{j-1z}$
and $A_{jz} > A_{j+1z}$
And $A_{jz} > Th_R$
$P_{jz} = 1$ else $P_{jz} = 0$
z=1
z=N
t_1 l_1 $P_1 = 1$ if $A_1 > A_0$ and $A_1 > A_2$ and $A_1 > Th_R$ else 0
t_2 l_2 $P_2 = 1$ if $A_2 > A_1$ and $A_2 > A_3$ and $A_2 > Th_R$ else 0
t_3 l_3 ...
... ... $P_N = 1$ if $A_N > A_{N-1}$ and $A_N > A_{N+1}$ and $A_N > Th_R$ else 0
t_N l_N $P_{\lceil N/N \rceil} = 1$ if $A_{\lceil N/N \rceil} > A_{\lceil N/N \rceil -1}$ and $A_{\lceil N/N \rceil} > A_{\lceil N/N \rceil +1}$ and $A_{\lceil N/N \rceil} > Th_R$ else 0

Fig. 9

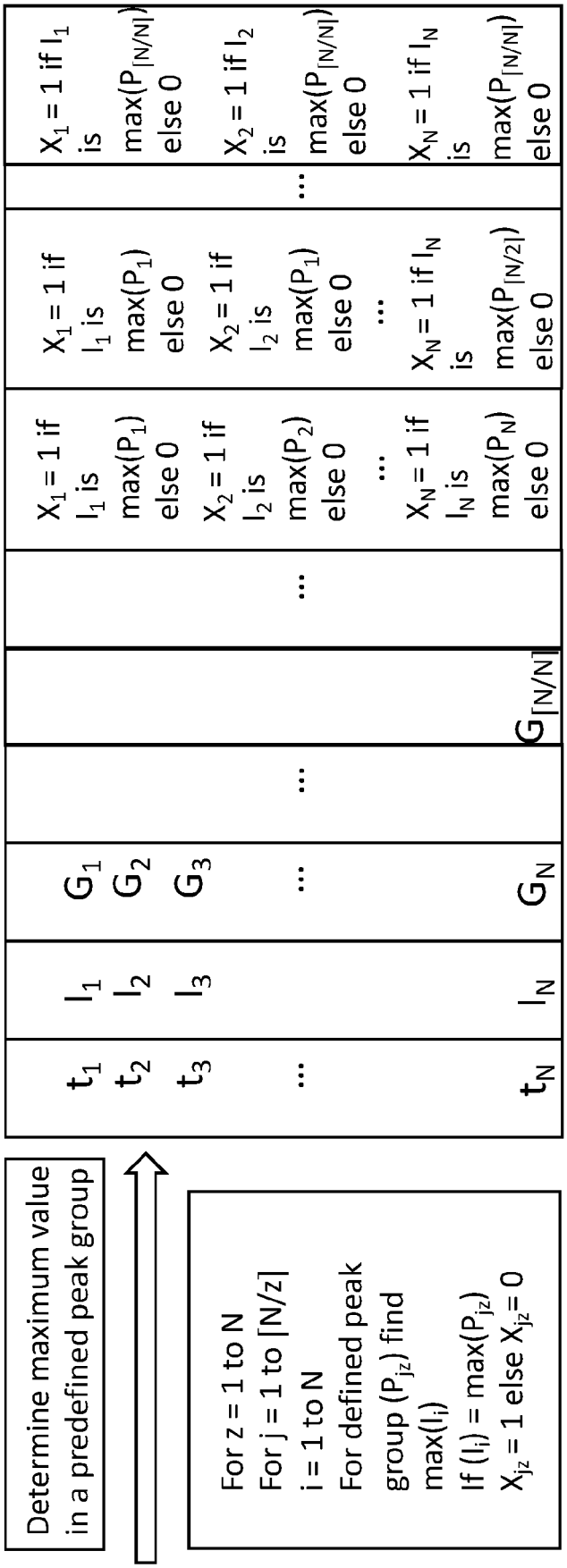
Determine maximum value in a predefined peak group
For z = 1 to N
For j = 1 to ⌈N/z⌉
i = 1 to N
For defined peak group (P_{jz}) find max(I_i)
If (I_i) = max(P_{jz})
X_{jz} = 1 else X_{jz} = 0
z=1
z=N
z=1
z=2
z=N
t_1 t_2 t_3 ... t_N
I_1 I_2 I_3 ... I_N
G_1 G_2 G_3 ... $G_{⌈N/N⌉}$
X_1 = 1 if I_1 is max(P_1) else 0
X_2 = 1 if I_2 is max(P_2) else 0
X_N = 1 if I_N is max(P_N) else 0
X_1 = 1 if I_1 is max(P_1) else 0
X_2 = 1 if I_2 is max(P_1) else 0
X_N = 1 if I_N is max($P_{⌈N/2⌉}$) else 0
X_1 = 1 if I_1 is max($P_{⌈N/N⌉}$) else 0
X_2 = 1 if I_2 is max($P_{⌈N/N⌉}$) else 0
X_N = 1 if I_N is max($P_{⌈N/N⌉}$) else 0

Fig. 10

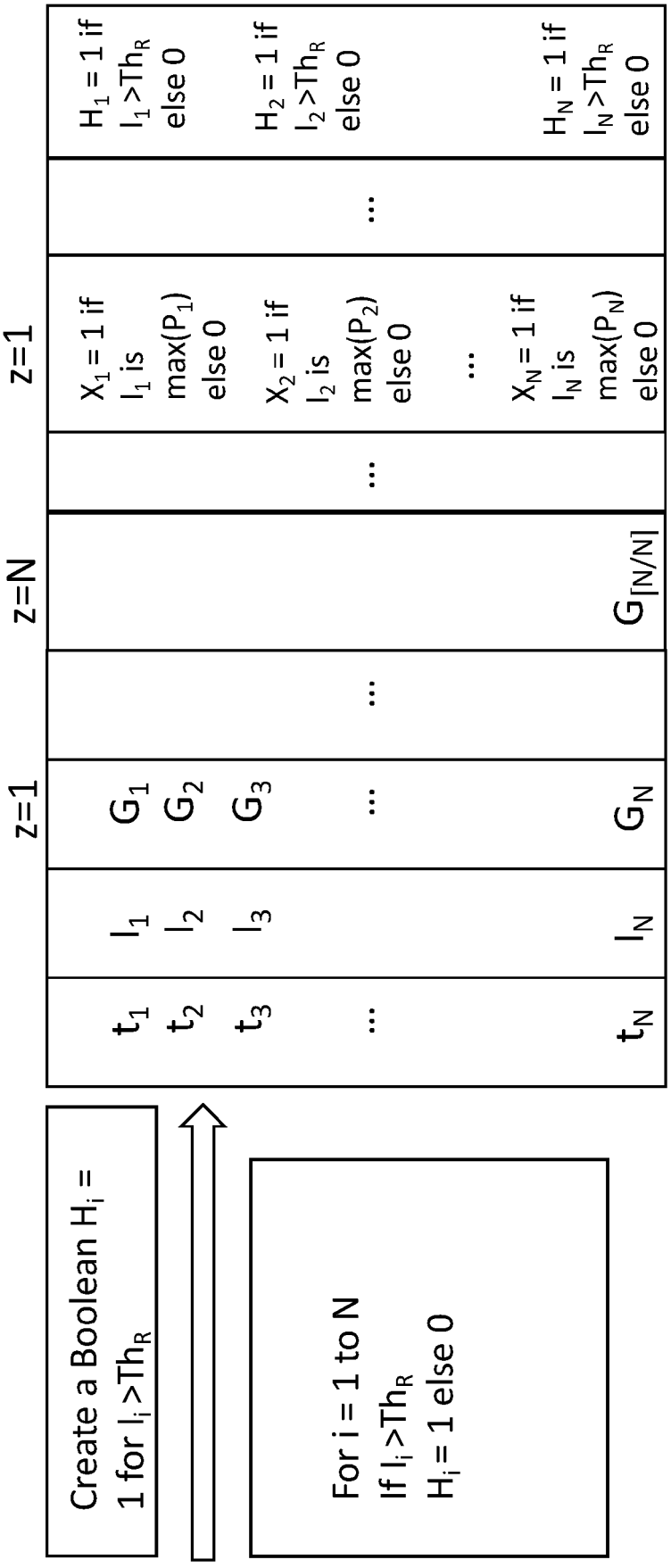
Create a Boolean H_i = 1 for I_i>Th_R
For i = 1 to N
If I_i>Th_R
H_i = 1 else 0
z=1
z=N
z=1
t_1 I_1 G_1
t_2 I_2 G_2
t_3 I_3 G_3
... ...
t_N I_N G_N
$G_{\lceil N/N \rceil}$
X_1 = 1 if I_1 is max(P_1) else 0
X_2 = 1 if I_2 is max(P_2) else 0
... ...
X_N = 1 if I_N is max(P_N) else 0
H_1 = 1 if I_1>Th_R else 0
H_2 = 1 if I_2>Th_R else 0
... ...
H_N = 1 if I_N>Th_R else 0

Fig. 11

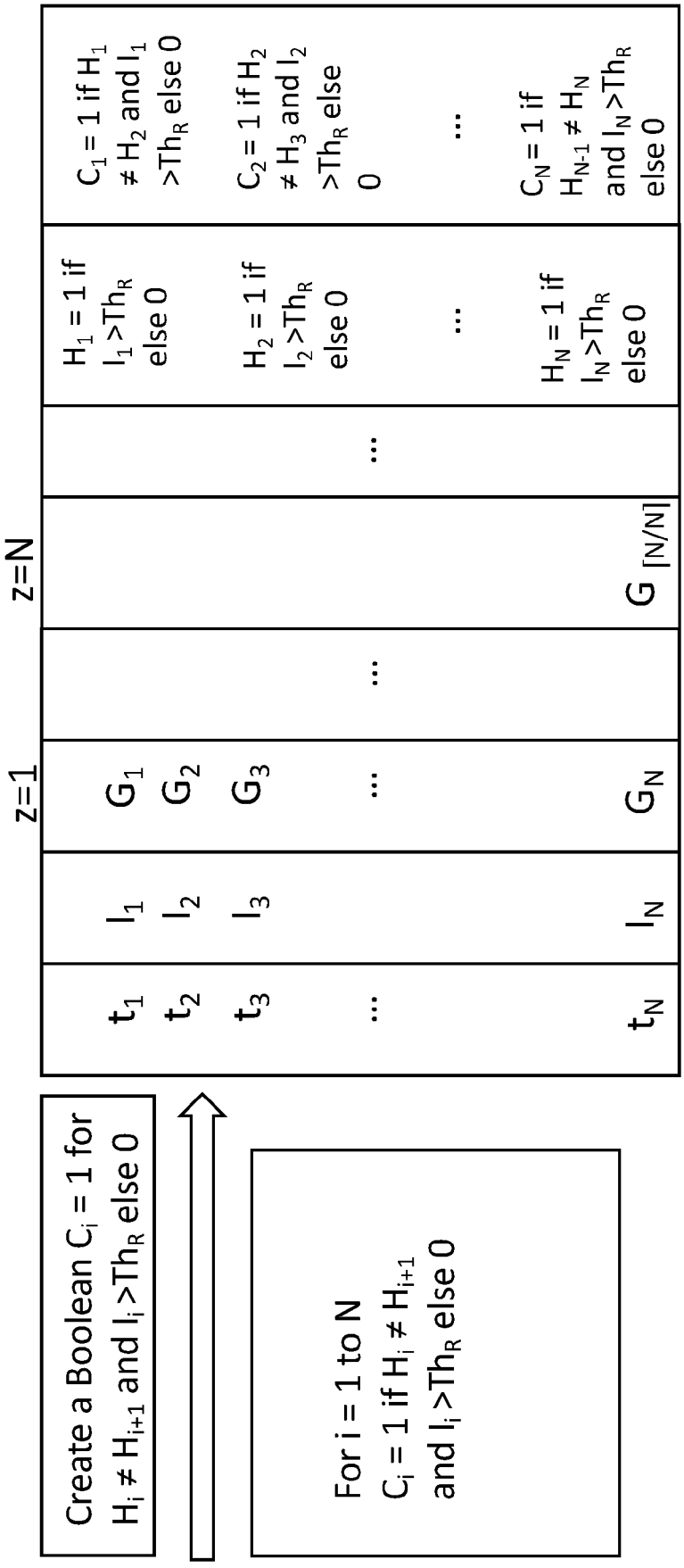
Create a Boolean C_i = 1 for $H_i \neq H_{i+1}$ and $I_i > Th_R$ else 0
For i = 1 to N
C_i = 1 if $H_i \neq H_{i+1}$ and $I_i > Th_R$ else 0
z=1
z=N
t_1 t_2 t_3 ... t_N
I_1 I_2 I_3 I_N
G_1 G_2 G_3 ... G_N
... ... $G_{[N/N]}$
H_1 = 1 if $I_1 > Th_R$ else 0
H_2 = 1 if $I_2 > Th_R$ else 0
... ...
H_N = 1 if $I_N > Th_R$ else 0
C_1 = 1 if $H_1 \neq H_2$ and $I_1 > Th_R$ else 0
C_2 = 1 if $H_2 \neq H_3$ and $I_2 > Th_R$ else 0
... ...
C_N = 1 if $H_{N-1} \neq H_N$ and $I_N > Th_R$ else 0

Fig. 12

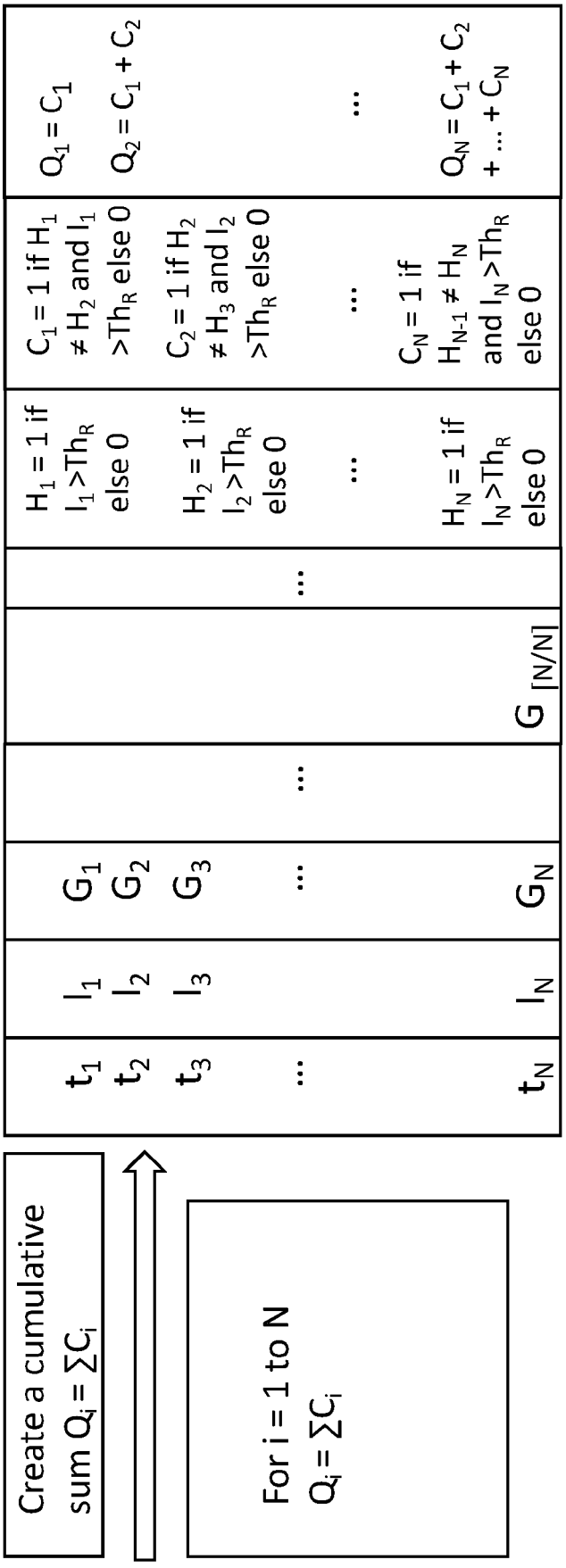
z=1
z=N
t_1 t_2 t_3 ... t_N
I_1 I_2 I_3 ... I_N
G_1 G_2 G_3 ... G_N
$G_{[N/N]}$
$H_1 = 1$ if $I_1 > Th_R$ else 0
$H_2 = 1$ if $I_2 > Th_R$ else 0
$H_N = 1$ if $I_N > Th_R$ else 0
$C_1 = 1$ if $H_1 \neq H_2$ and $I_1 > Th_R$ else 0
$C_2 = 1$ if $H_2 \neq H_3$ and $I_2 > Th_R$ else 0
$C_N = 1$ if $H_{N-1} \neq H_N$ and $I_N > Th_R$ else 0
$Q_1 = C_1$
$Q_2 = C_1 + C_2$
$Q_N = C_1 + C_2 + ... + C_N$
Create a cumulative sum $Q_i = \sum C_i$
For i = 1 to N
$Q_i = \sum C_i$

Fig. 13

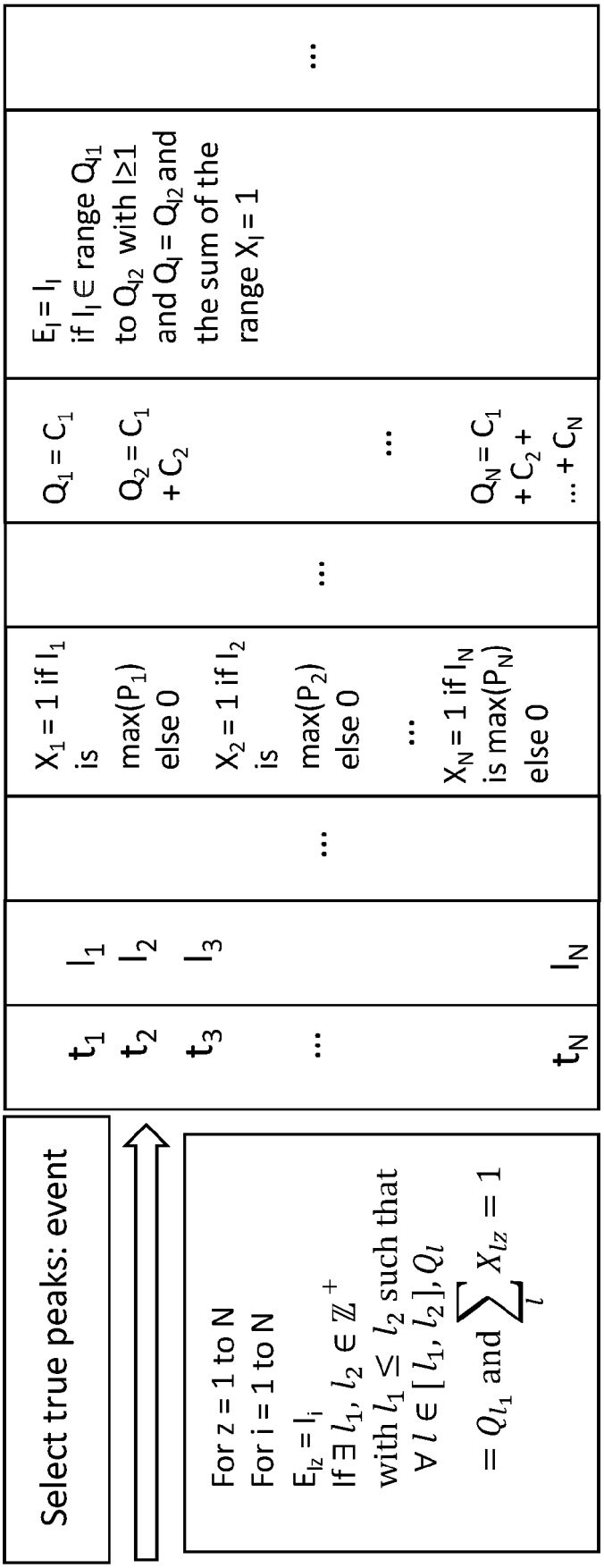
Select true peaks: event
For z = 1 to N
For i = 1 to N
$E_{lz} = l_i$
If $\exists\, l_1, l_2 \in \mathbb{Z}^+$
with $l_1 \leq l_2$ such that
$\forall\, l \in [l_1, l_2], Q_l$
$= Q_{l_1}$ and $\sum_l X_{lz} = 1$
z=1
z=1
t_1 l_1 $X_1 = 1$ if l_1 is max(P_1) else 0 $Q_1 = C_1$ $E_l = l_l$ if $l_l \in$ range Q_{l1} to Q_{l2} with $l \geq 1$ and $Q_l = Q_{l2}$ and the sum of the range $X_l = 1$
t_2 l_2 $X_2 = 1$ if l_2 is max(P_2) else 0 $Q_2 = C_1 + C_2$
t_3 l_3
... ...
t_N l_N $X_N = 1$ if l_N is max(P_N) else 0 $Q_N = C_1 + C_2 + ... + C_N$

Fig. 14

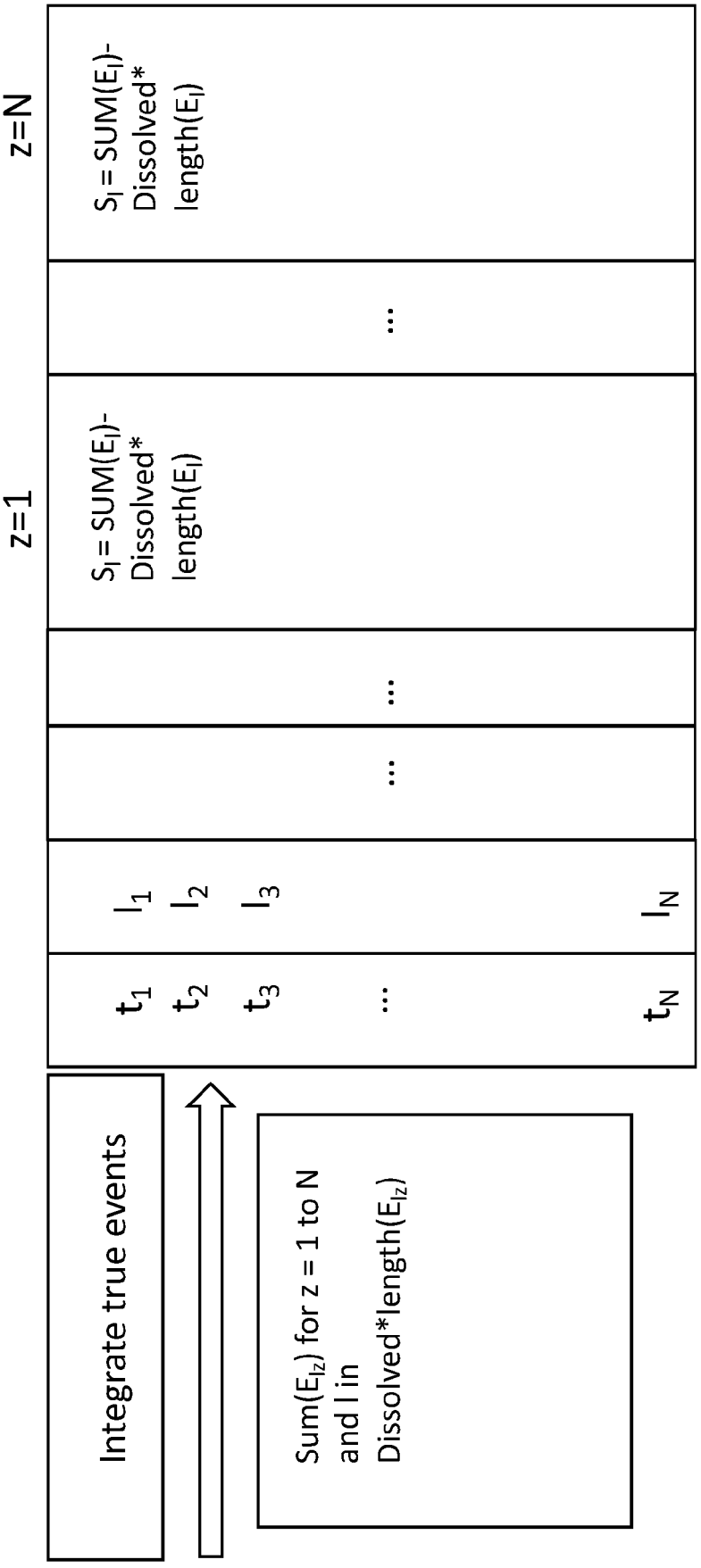
z=1
z=N
S_I = SUM(E_I)-Dissolved* length(E_I)
S_I = SUM(E_I)-Dissolved* length(E_I)
t_1 t_2 t_3 ... t_N
l_1 l_2 l_3 ... l_N
Integrate true events
Sum(E_{Iz}) for z = 1 to N and l in Dissolved*length(E_{Iz})

Fig. 15

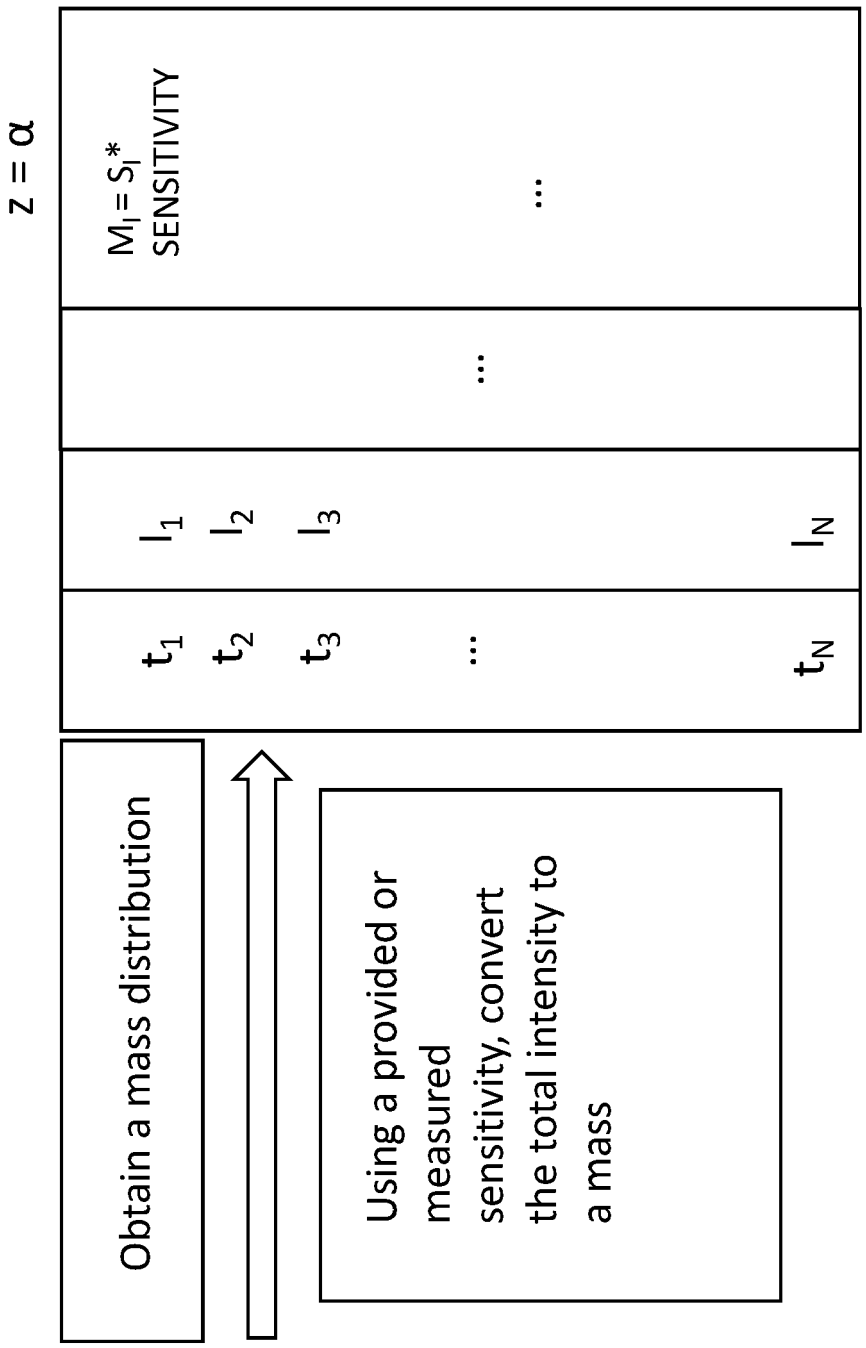
z = α
Obtain a mass distribution
Using a provided or measured sensitivity, convert the total intensity to a mass
t1 t2 t3 ... tN
I1 I2 I3 ... IN
... ...
MI = SI* SENSITIVITY

Fig. 16

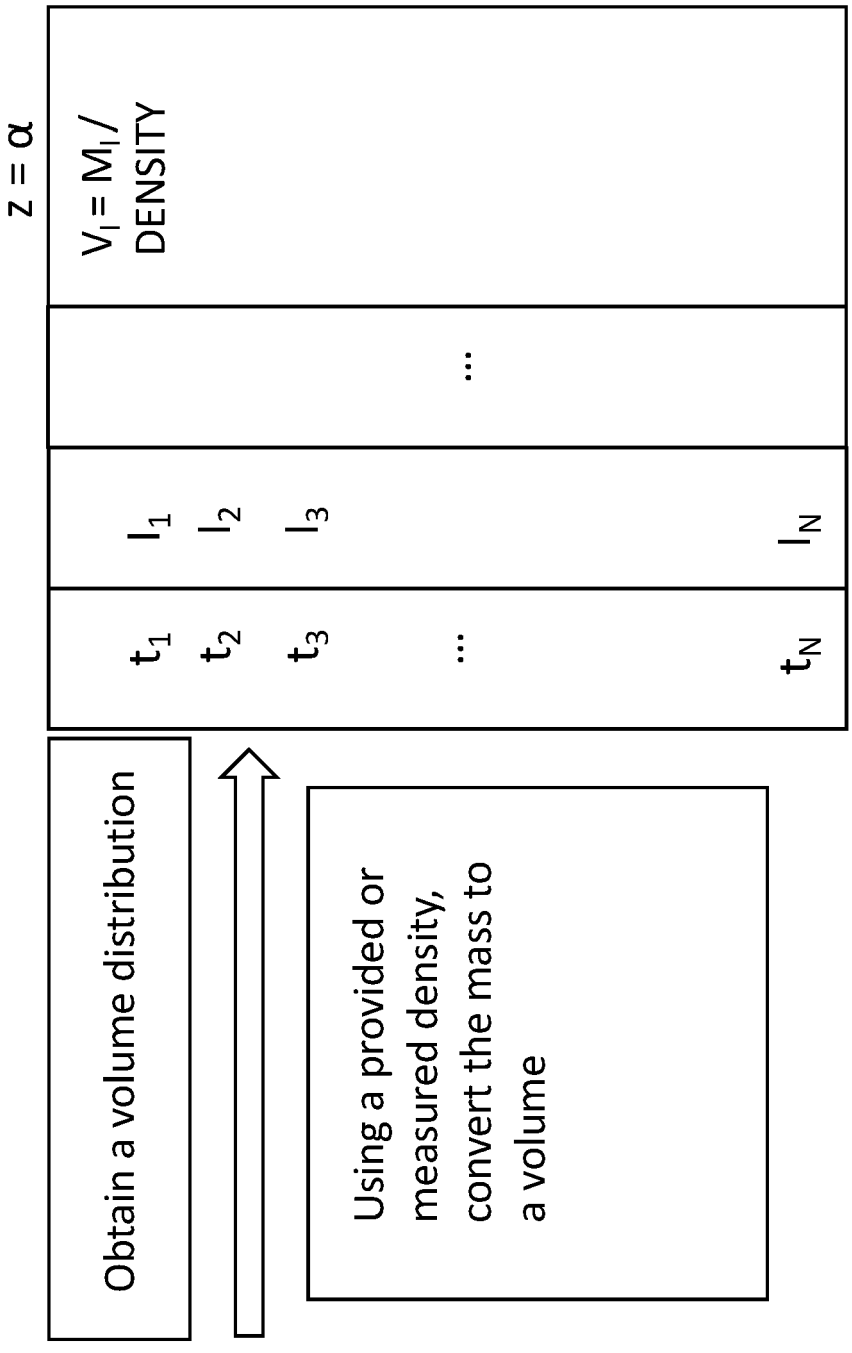
z = α
$V_I = M_I /$ DENSITY
t_1 t_2 t_3 ... t_N
I_1 I_2 I_3 ... I_N
Obtain a volume distribution
Using a provided or measured density, convert the mass to a volume

Fig. 17

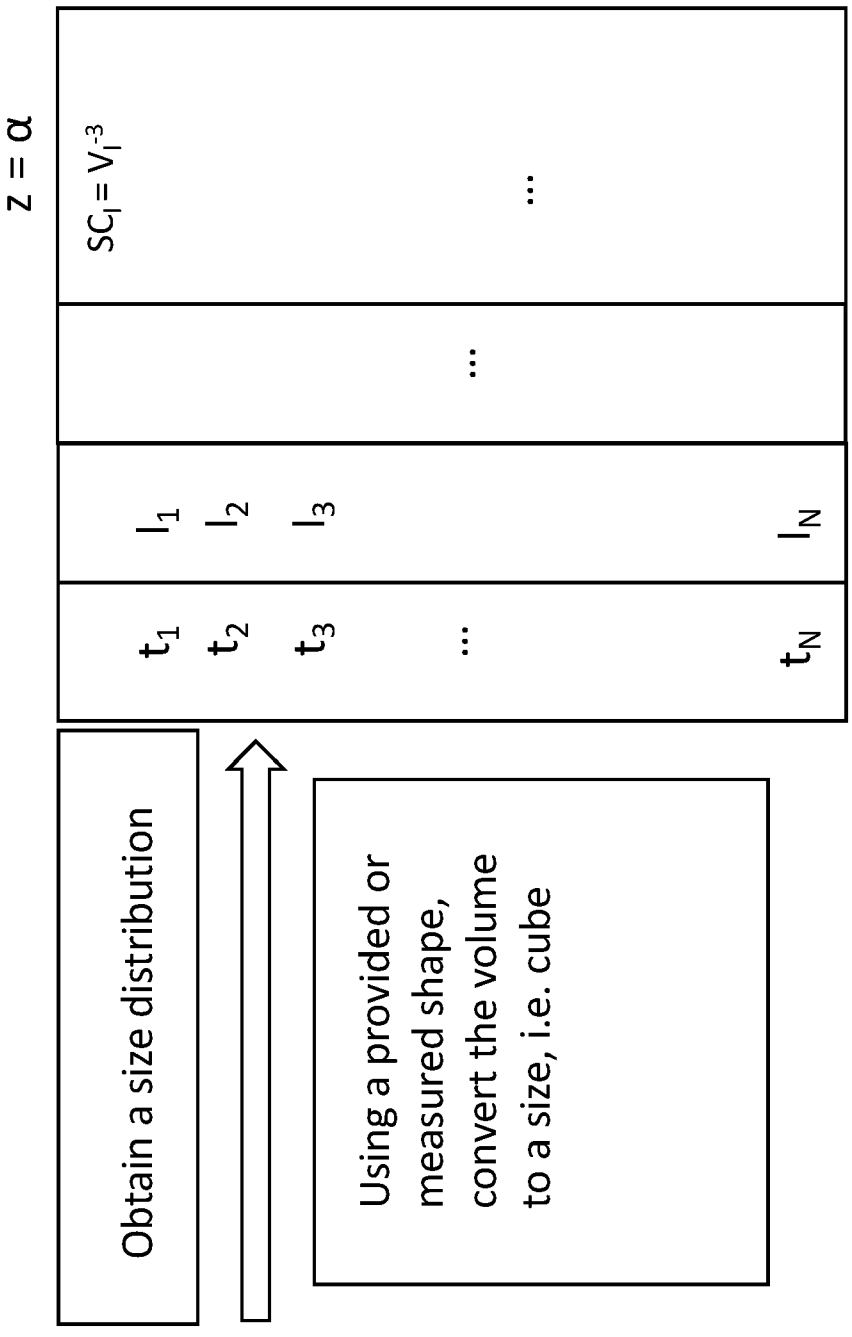
z = α
SCl = Vl-3
...
...
l1
l2
l3
lN
t1
t2
t3
...
tN
Obtain a size distribution
Using a provided or measured shape, convert the volume to a size, i.e. cube

Fig. 18

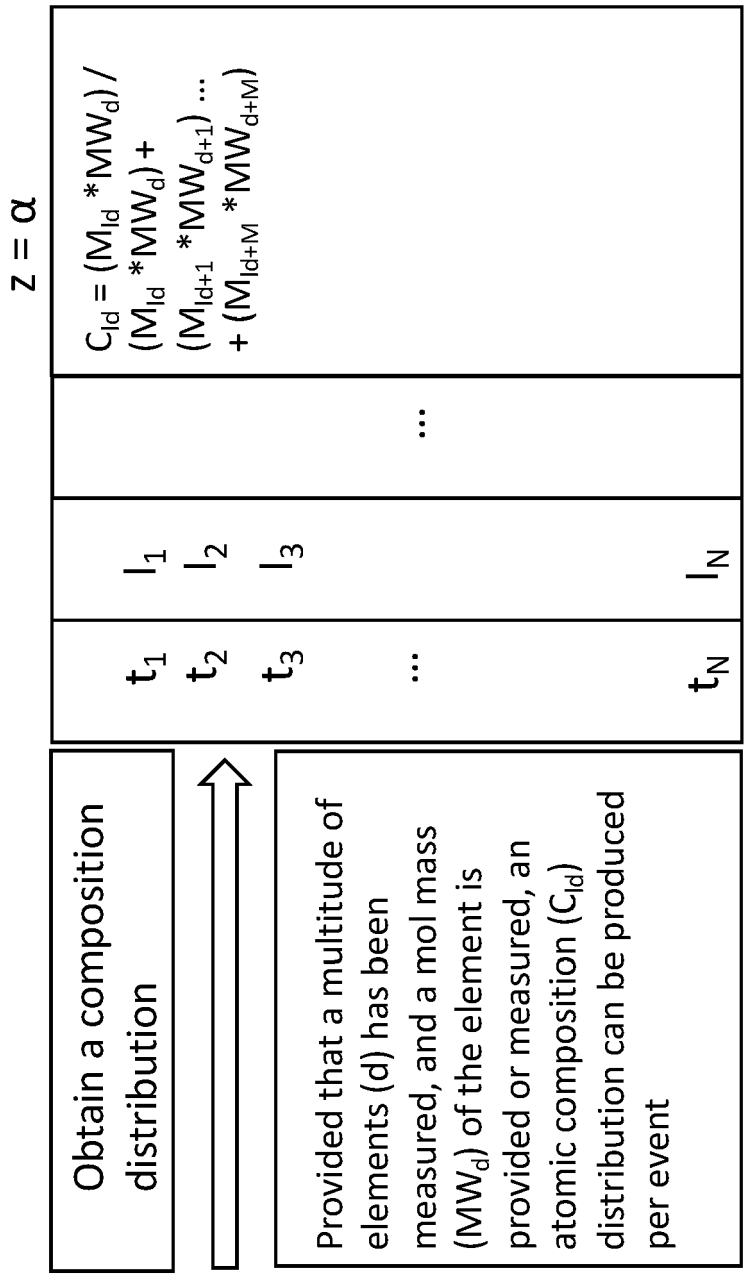
z = α
Obtain a composition distribution
Provided that a multitude of elements (d) has been measured, and a mol mass (MW_d) of the element is provided or measured, an atomic composition (C_{Id}) distribution can be produced per event
t_1 t_2 t_3 ... t_N
I_1 I_2 I_3 ... I_N
$C_{Id} = (M_{Id} * MW_d) / (M_{Id} * MW_d) + (M_{Id+1} * MW_{d+1}) ... + (M_{Id+M} * MW_{d+M})$

Fig. 19

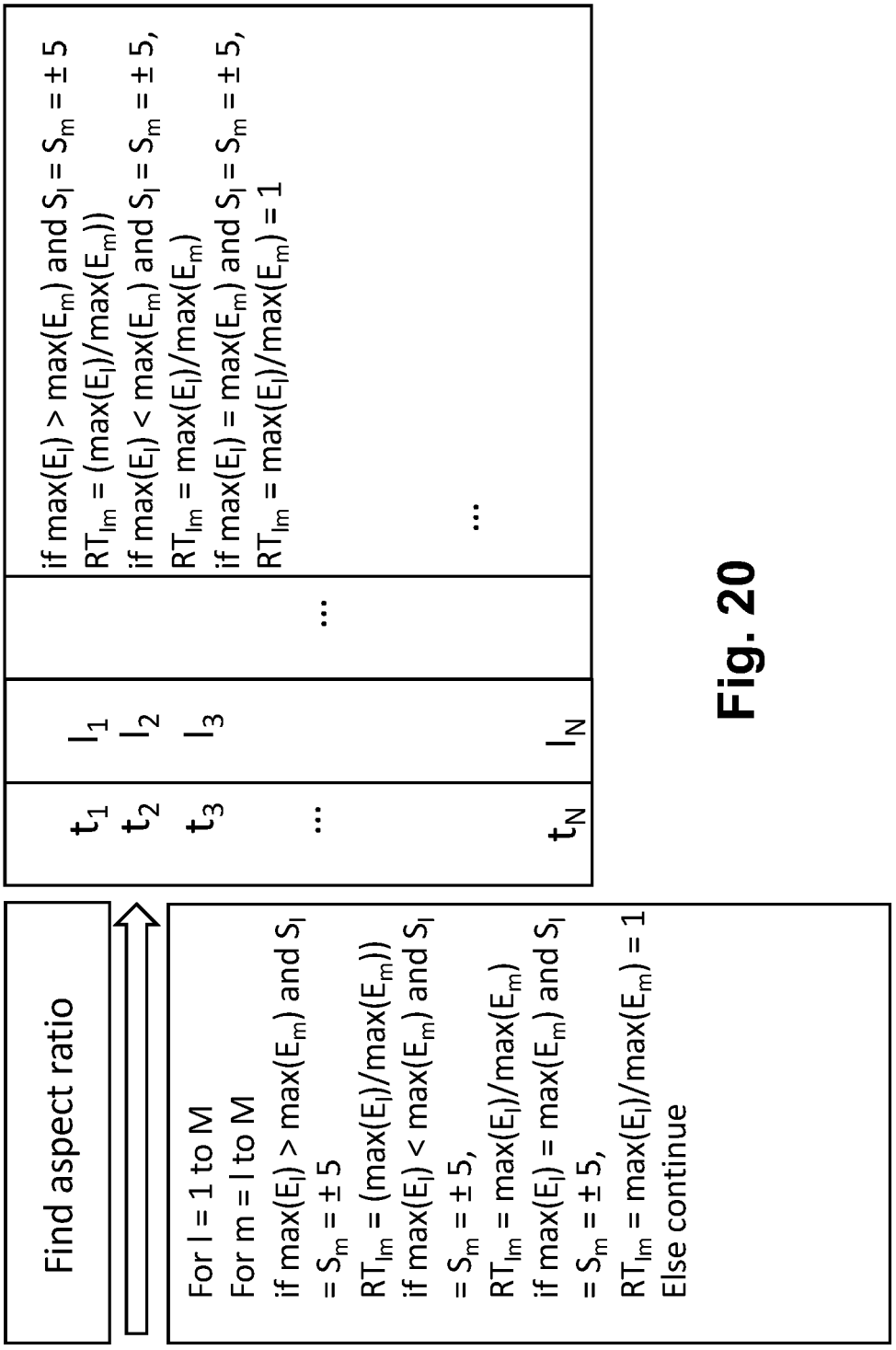
Find aspect ratio
For I = 1 to M
For m = I to M
if max(E_I) > max(E_m) and S_I = S_m = ± 5
RT_Im = (max(E_I)/max(E_m))
if max(E_I) < max(E_m) and S_I = S_m = ± 5,
RT_Im = max(E_I)/max(E_m)
if max(E_I) = max(E_m) and S_I = S_m = ± 5,
RT_Im = max(E_I)/max(E_m) = 1
Else continue
z = α
t1 I1
t2 I2
t3 I3
... ...
tN IN
if max(E_I) > max(E_m) and S_I = S_m = ± 5
RT_Im = (max(E_I)/max(E_m))
if max(E_I) < max(E_m) and S_I = S_m = ± 5,
RT_Im = max(E_I)/max(E_m)
if max(E_I) = max(E_m) and S_I = S_m = ± 5,
RT_Im = max(E_I)/max(E_m) = 1
...

Fig. 20

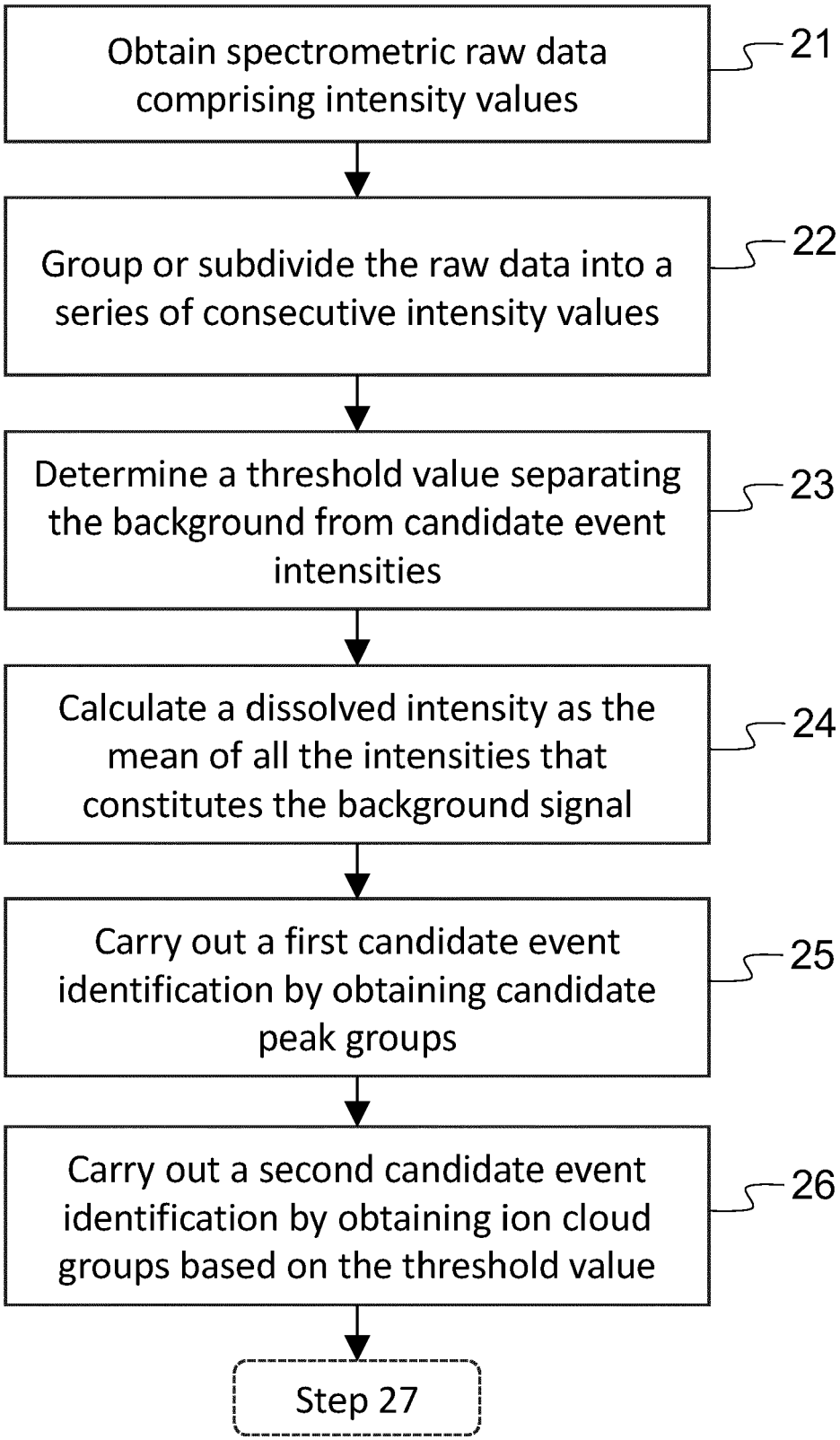
Obtain spectrometric raw data comprising intensity values
21
Group or subdivide the raw data into a series of consecutive intensity values
22
Determine a threshold value separating the background from candidate event intensities
23
Calculate a dissolved intensity as the mean of all the intensities that constitutes the background signal
24
Carry out a first candidate event identification by obtaining candidate peak groups
25
Carry out a second candidate event identification by obtaining ion cloud groups based on the threshold value
26
Step 27

Fig. 21a

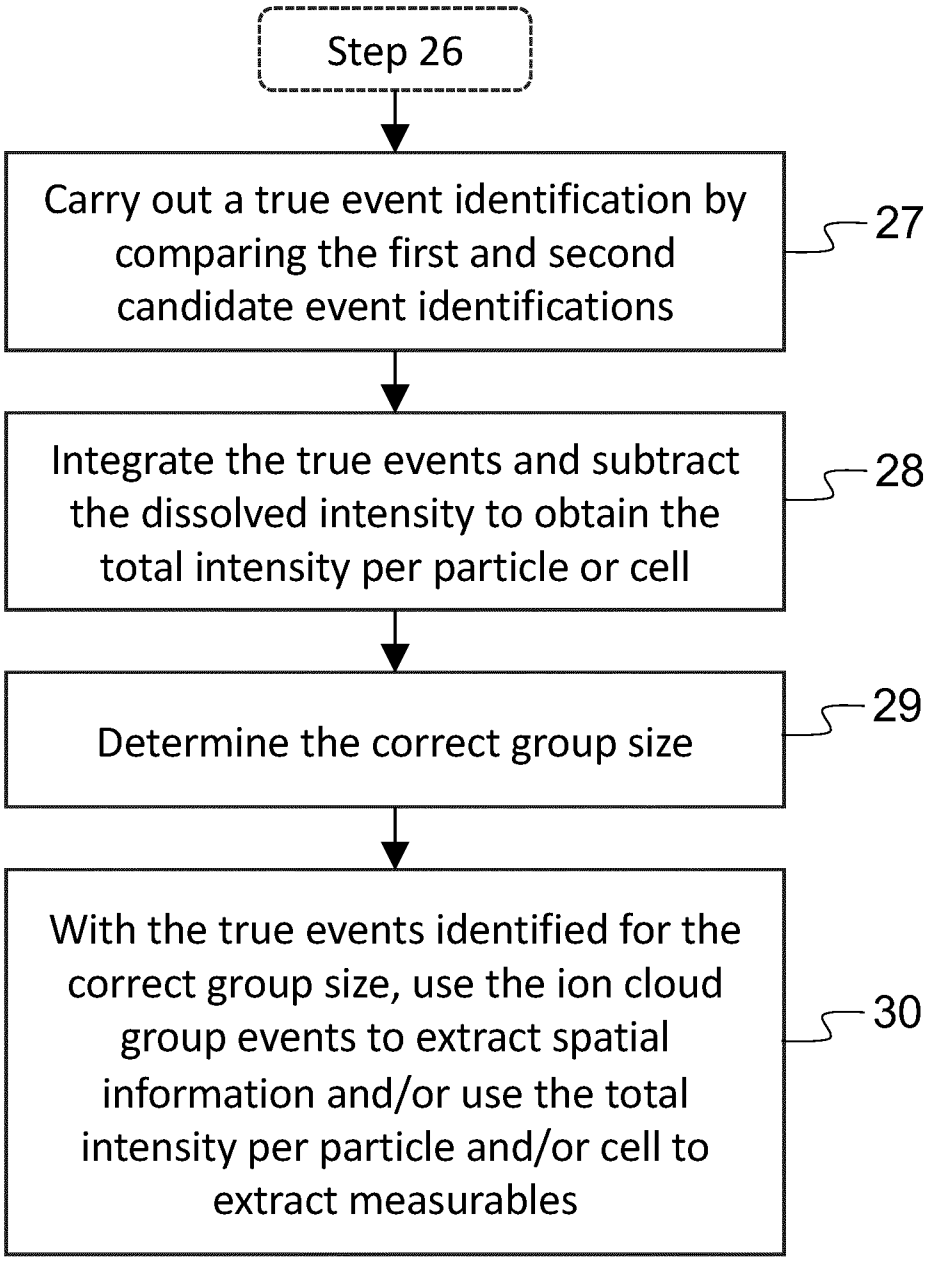
Step 26
Carry out a true event identification by comparing the first and second candidate event identifications
27
Integrate the true events and subtract the dissolved intensity to obtain the total intensity per particle or cell
28
Determine the correct group size
29
With the true events identified for the correct group size, use the ion cloud group events to extract spatial information and/or use the total intensity per particle and/or cell to extract measurables
30

Fig. 21b

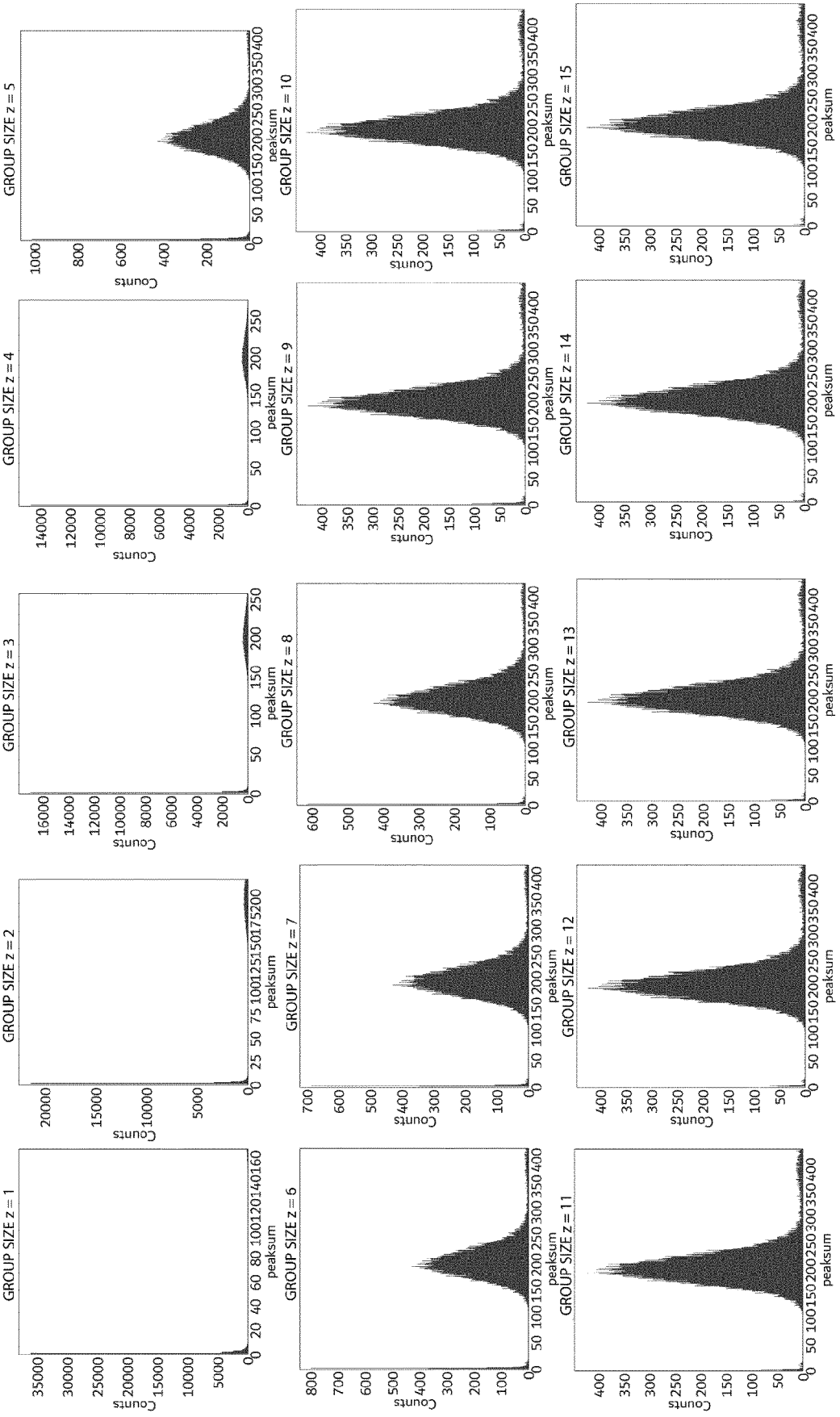
GROUP SIZE z = 1
GROUP SIZE z = 2
GROUP SIZE z = 3
GROUP SIZE z = 4
GROUP SIZE z = 5
GROUP SIZE z = 6
GROUP SIZE z = 7
GROUP SIZE z = 8
GROUP SIZE z = 9
GROUP SIZE z = 10
GROUP SIZE z = 11
GROUP SIZE z = 12
GROUP SIZE z = 13
GROUP SIZE z = 14
GROUP SIZE z = 15
Counts
peaksum

Fig. 22

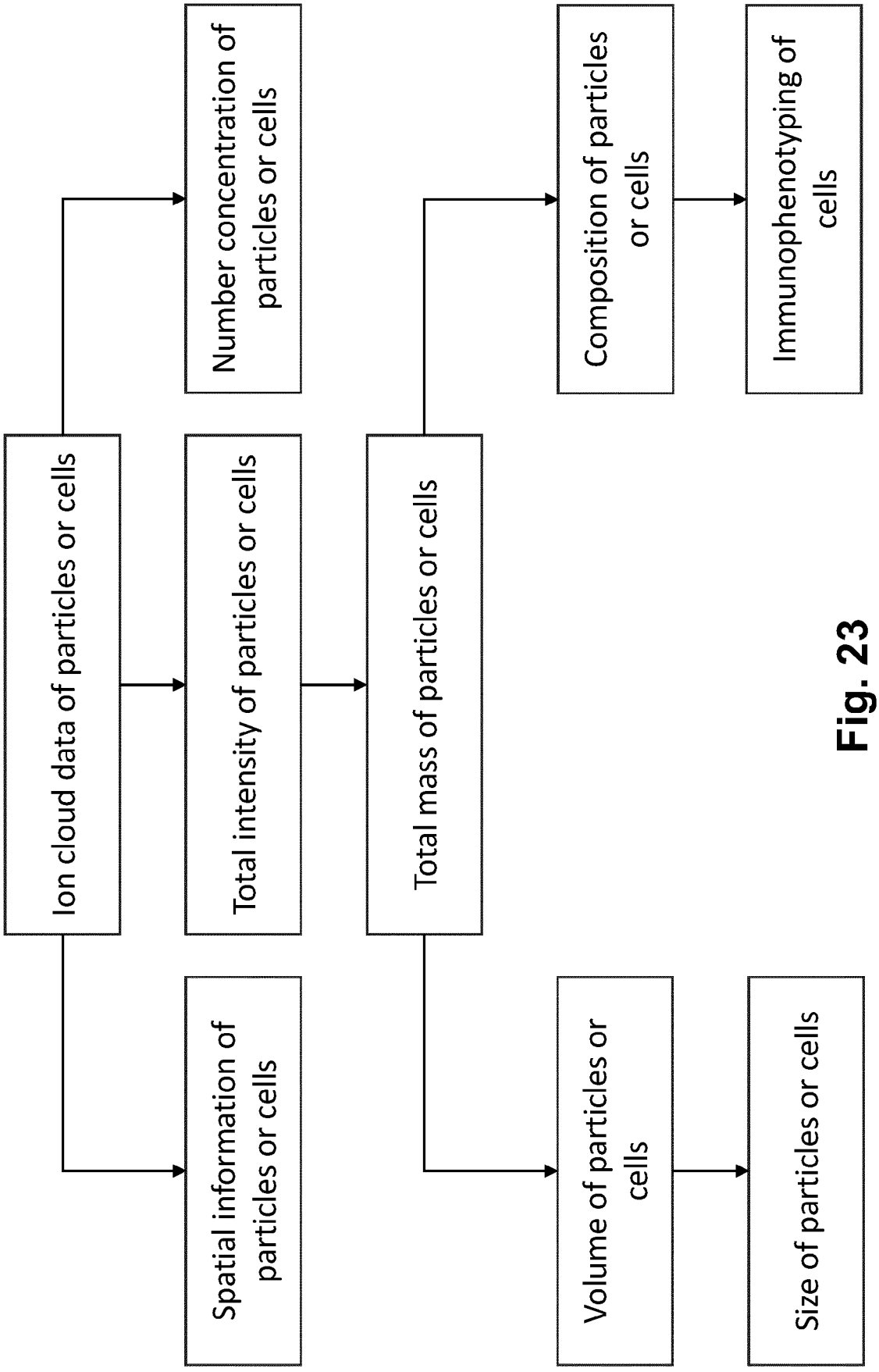
Ion cloud data of particles or cells
Number concentration of particles or cells
Total intensity of particles or cells
Spatial information of particles or cells
Total mass of particles or cells
Composition of particles or cells
Immunophenotyping of cells
Volume of particles or cells
Size of particles or cells

Fig. 23

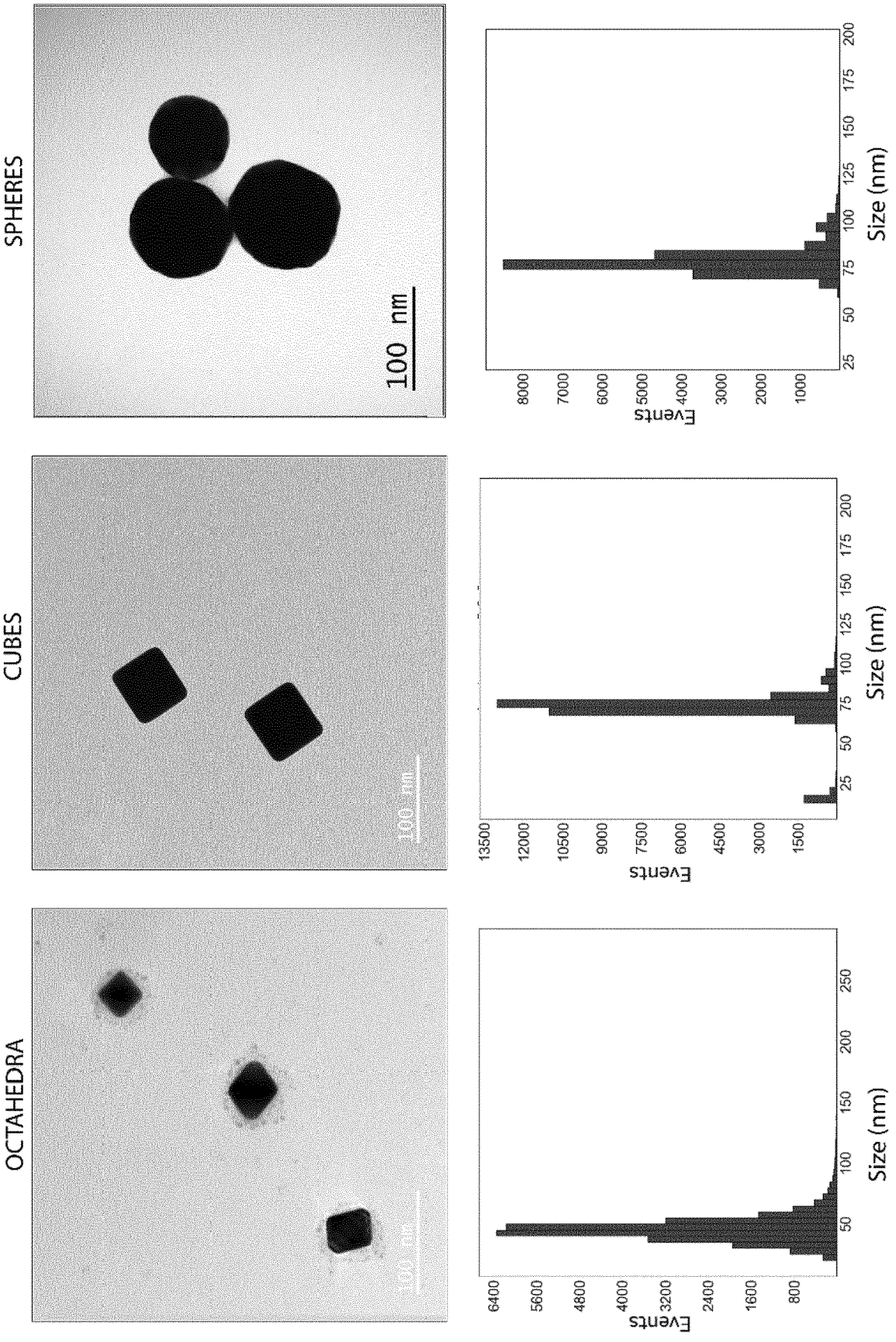
SPHERES
CUBES
OCTAHEDRA
100 nm
100 nm
100 nm
Events
Size (nm)
8000
7000
6000
5000
4000
3000
2000
1000
25
50
75
100
125
150
175
200
13500
12000
10500
9000
7500
6000
4500
3000
1500
6400
5600
4800
4000
3200
2400
1600
800
250

Fig. 24

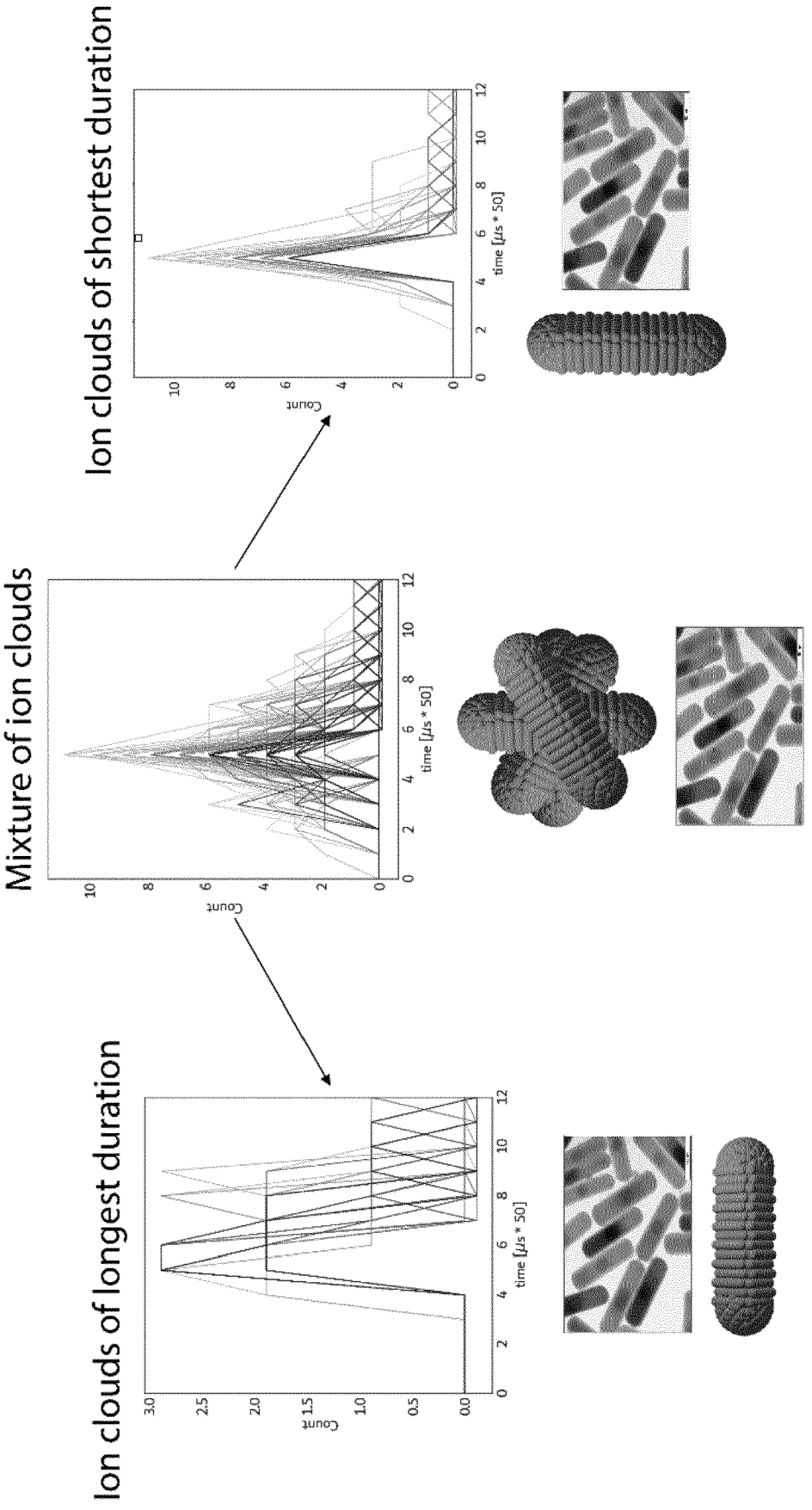
Ion clouds of shortest duration
Mixture of ion clouds
Ion clouds of longest duration
Count
time [µs * 50]

Fig. 25

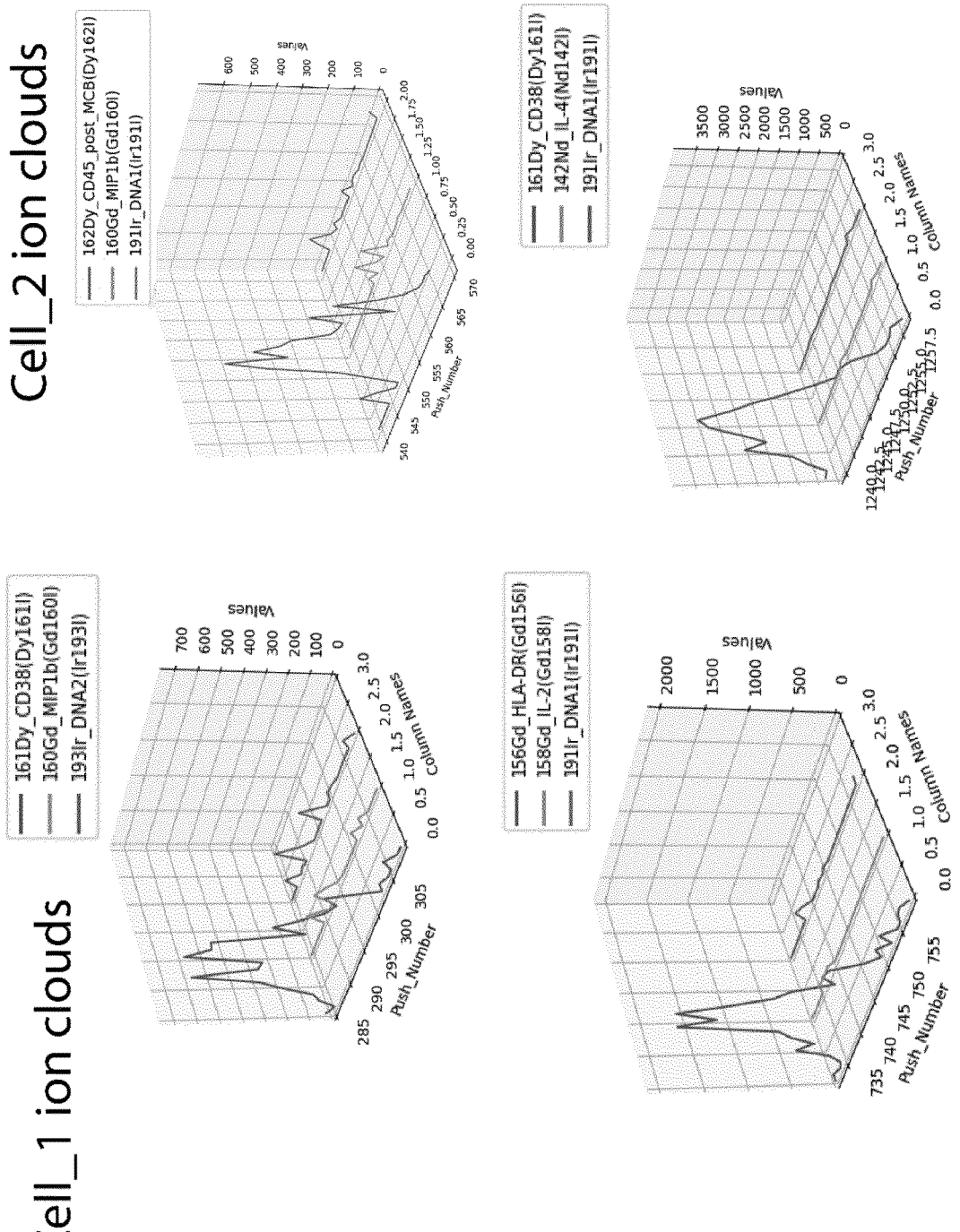
Cell_2 ion clouds
162Dy_CD45_post_MCB(Dy162l)
160Gd_MIP1b(Gd160l)
191Ir_DNA1(Ir191l)
Values
Push_Number
Cell_1 ion clouds
161Dy_CD38(Dy161l)
160Gd_MIP1b(Gd160l)
193Ir_DNA2(Ir193l)
Values
Push_Number
Column Names
Cell_4 ion clouds
161Dy_CD38(Dy161l)
142Nd_IL-4(Nd142l)
191Ir_DNA1(Ir191l)
Values
Push_Number
Column Names
Cell_3 ion clouds
156Gd_HLA-DR(Gd156l)
158Gd_IL-2(Gd158l)
191Ir_DNA1(Ir191l)
Values
Push_Number
Column Names

Fig. 26

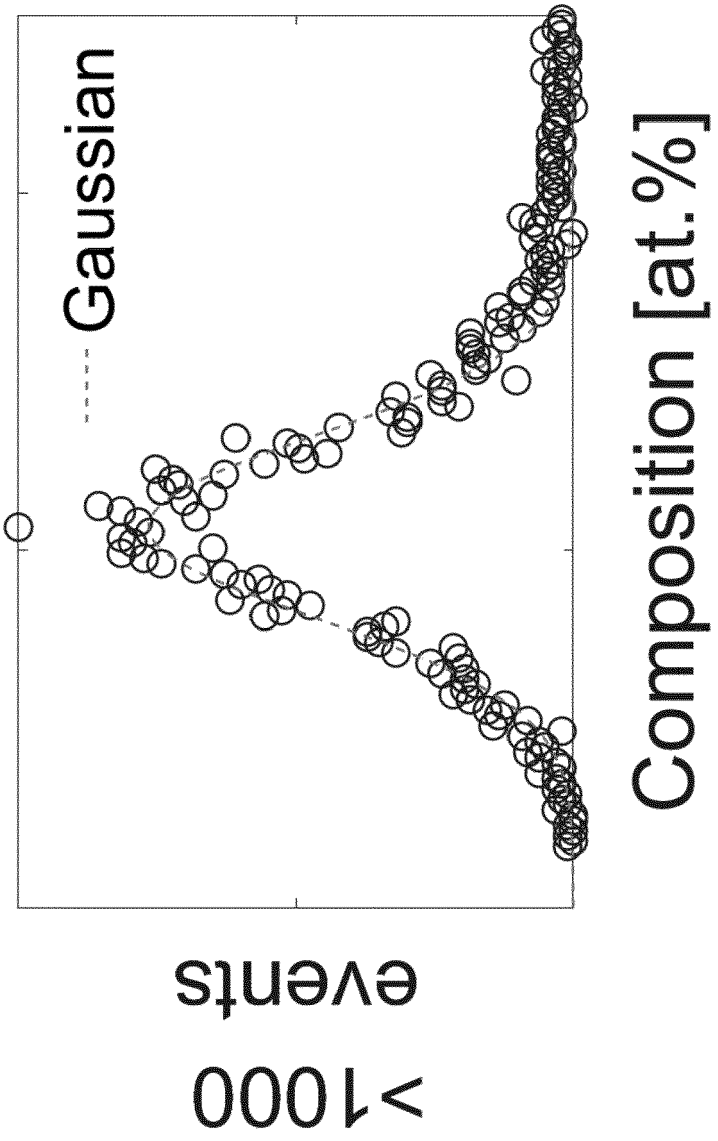

Fig. 27

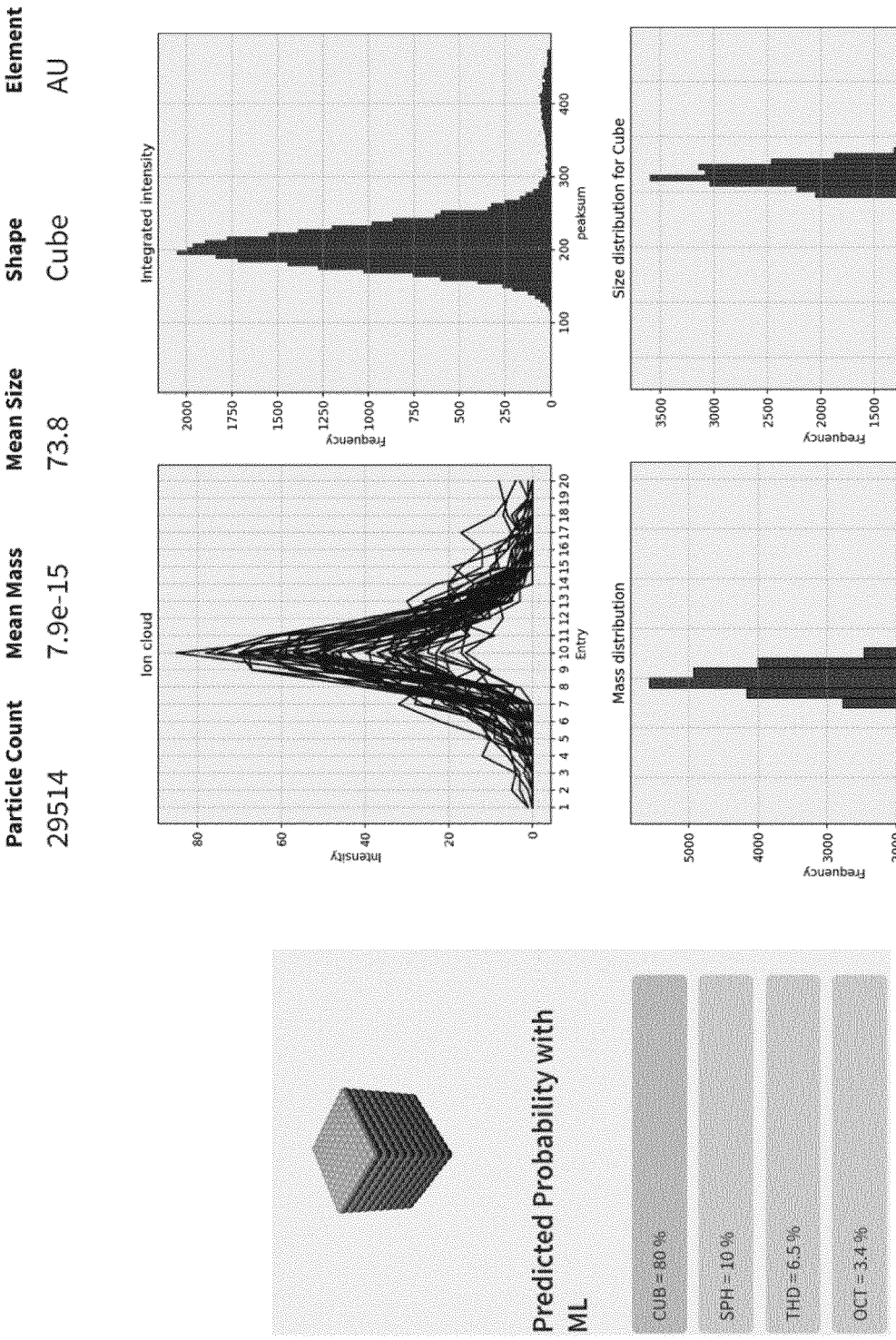
Particle Count
29514
Mean Mass
7.9e-15
Mean Size
73.8
Shape
Cube
Element
AU
Integrated intensity
Frequency
peaksum
Size distribution for Cube
Frequency
Size [nm]
Ion cloud
Intensity
Entry
Mass distribution
Frequency
Mass [gram]
1e−14
Predicted Probability with ML
CUB = 80 %
SPH = 10 %
THD = 6.5 %
OCT = 3.4 %

Fig. 28

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EUROPEAN SEARCH REPORT

Application Number
EP 23 21 8927

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
A,D	US 2015/235833 A1 (BAZARGAN SAMAD [CA] ET AL) 20 August 2015 (2015-08-20) * paragraphs [0012] - [0022] * * paragraphs [0033] - [0037], [0056] - [0078]; figures 1-5 *	1-15	INV. H01J49/00
A	WO 2023/095020 A1 (ECOLE POLYTECHNIQUE FED LAUSANNE EPFL [CH]) 1 June 2023 (2023-06-01) * the whole document *	1-15	
A	Steve Wilbur ET AL: "Characterization of nanoparticles in aqueous samples by ICP-MS White paper Authors", Argilent Technologies, 31 July 2017 (2017-07-31), pages 1-10, XP055652992, Retrieved from the Internet: URL:https://www.agilent.com/cs/library/whitepaper/public/ICP-MS_5991-5516EN-nanoparticles.pdf [retrieved on 2019-12-16] * the whole document *	1-15	TECHNICAL FIELDS SEARCHED (IPC) H01J
A	MONTAÑO MANUEL D ET AL: "Single Particle ICP-MS: Advances toward routine analysis of nanomaterials", ANALYTICAL AND BIOANALYTICAL CHEMISTRY, SPRINGER BERLIN HEIDELBERG, BERLIN/HEIDELBERG, vol. 408, no. 19, 23 June 2016 (2016-06-23), pages 5053-5074, XP035992049, ISSN: 1618-2642, DOI: 10.1007/S00216-016-9676-8 [retrieved on 2016-06-23] * the whole document *	1-15	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	10 June 2024	Loiseleur, Pierre

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

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The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

10-06-2024

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2015235833 A1	20-08-2015	US 2015235833 A1	20-08-2015
		US 2017358438 A1	14-12-2017
WO 2023095020 A1	01-06-2023	NONE	

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 2014299763 A1 **[0006]**
- WO 2015122920 A1 **[0006]**
- US 11075066 B2 **[0006] [0040]**

Non-patent literature cited in the description

- Mass Cytometry: Technique for Real Time Single Cell Multitarget Immunoassay Based on Inductively Coupled Plasma Time-of-Flight Mass Spectrometry. *Anal. Chem.*, 2009, vol. 81 (16), 6813-6822 **[0006]**
- Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry. *Anal. Chem.*, 2011, vol. 83 (24), 9361-9369 **[0006]**
- A Prototype of a New Inductively Coupled Plasma Time-of-Flight Mass Spectrometer Providing Temporally Resolved, Multi-Element Detection of Short Signals Generated by Single Particles and Droplets. *J. Anal. At. Spectrom*, 2013, vol. 28 (2), 226-233 **[0006]**
- High-Throughput Sizing, Counting, and Elemental Analysis of Anisotropic Multimetallic Nanoparticles with Single-Particle Inductively Coupled Plasma Mass Spectrometry. *ACS Nano*, 2022 **[0006]**
- **HENDRIKS et al.** Performance of Sp-ICP-TOFMS with Signal Distributions Fitted to a Compound Poisson Model. *J. Anal. At. Spectrom*, 2019, vol. 34 (9), 1900-1909 **[0040]**