Internal distribution code:

(A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 8 February 2012

Case Number: T 1400/08 - 3.4.02

Application Number: 00117522.3

Publication Number: 1180679

IPC: G01N21/64

Language of the proceedings: EN

Title of invention:
A method for sensing fluorescent samples utilizing moment analysis

Applicant:
Evotec OAI AG

Headword:

Relevant legal provisions:
EPC Art. 84, 83

Keyword:
Clarity (yes)
Sufficiency (yes)

Decisions cited:

Catchword:

Case Number: T1400/08 - 3.4.02

D E C I S I O N
of the Technical Board of Appeal 3.4.02
of 8 February 2012

Appellant: (Applicant)
Evotec OAI AG
Schnackenburgallee 114
22525 Hamburg (ALLEMAGNE)

Representative:
von Kreisler Selting Werner
Deichmannhaus am Dom
Bahnhofsvorplatz 1
50667 Köln (ALLEMAGNE)

Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 3 March 2008 refusing European patent application No. 00117522.3 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: A. Klein
Members: M. Rayner
B. Müller

Summary of Facts and Submissions

I. The applicant has appealed against the decision of the examining division refusing European patent application number 00 117 522.3 concerning the sensing of fluorescent samples using moment analysis. The application was amended during prosecution before the first instance by the filing of fresh claims 1 to 22 with the letter of 25 May 2004. At the same time amended pages 1, 1a and 4 of the description were filed. Documents including the following have been referred to in the proceedings before the first instance:

Dl H. Qian et al.: "Distribution of molecular aggregation by analysis of fluctuation moments", Proceedings of The National Academy of Sciences of USA, vol. 87, July 1990, pages 5479-5483, National Academy Of Science. Washington, US, ISSN 0027-8424,

D2: EP-A-O 601 714 (Hamamatsu Photonics K.K.) 15 June 1994.

II. In its letter dated 25 May 2004, amongst other things, the applicant has submitted the following.

Document Dl relates to the study of molecular aggregation by the analysis of fluctuation moments of the fluorescence intensity. Simple model systems, namely mono-disperse suspensions of single species of fluorescent beads as well as two component mixtures of fluorescent beads, are investigated (abstract and first paragraph). In all cases, document Dl measures the first three moments for the complete sample to derive information about the sample from these three quantities only. In the analysis of mixtures, this implies that document Dl is limited to the case of a simple model system, where the full analytical dependence of the moments from the properties of the individual constituents is known. In addition, very high data quality, i.e. long measurement time, is imperative to allow the derivation of multiple sample parameters from only three measured moment values. In fact, it is pointed out that signal acquisition times up to seven minutes were used, even under favourable experimental conditions of highly fluorescent particles with a thirty-fold difference in relative brightness. Even longer measurement times are recommended for typical, less favourable samples. The approach of document Dl is completely unusable in high throughput screening, the application targeted by the present invention, where measurement times must be limited to a few seconds at most. The approach taught by the independent claims of the present invention differs from that of document Dl by a linear superposition of two sets of moments, relating to the cells or soluble or solid supports and the medium, respectively, used to model the behaviour of the composite sample. This is explicitly mentioned in step (c) of claim 1 and the corresponding feature (ii) of claim 16. Consequently, claims 1 and 16 are novel over Dl. This superposition is further not rendered obvious by document Dl in combination with document D2, which does not even disclose the general method of moment analysis. In all cases, the method of document D2 strives directly to classify individual local areas observed based on the locally obtained data. In many practically relevant cases, this simple direct analysis of fluorescence signal will fail because overlaying statistical fluctuations obscure the differences in intensity levels one strives to observe. Document D2 acknowledges this limitation by requiring a sufficiently low background fluorescence signal to ensure reliable discrimination of intensity levels and low concentrations of fluorescence labels.

(a) Average

The power of vector quantities, $\mathbf{n}^i$, is explained in the description (page 2, first formula), as well as in claim 1, as

$$\text{average}(\mathbf{n^i}) = \text{average}(n_1^{i1} n_1^{i2} \ldots).$$

III. The decision under appeal invoked lack of clarity of the claims and insufficiency, the reasons concerned can be summarised as follows.

(i) Lack of clarity of the claims - Article 84 EPC

(a) Average

With respect to the formula

$$\text{average}(\mathbf{n^i}) = \text{average}(n_1^{i1} n_1^{i2} \ldots),$$

the fact that the average value of a quantity expressed by a first expression is equal to the average value of a quantity expressed by a second expression does not necessarily imply that said first and second expressions are identical.

(b) Histogram

The subject matter of independent claims 1 and 16 is rendered unclear by the definition used for the moments of the measured photon count numbers,

$$\hat{M}_{\mathbf{i}} = \sum_{\mathbf{n}} \mathbf{n}^{\mathbf{i}} \hat{P}(\mathbf{n}) = average(\mathbf{n}^{\mathbf{i}}) = average(n_1^{i_1} n_2^{i_2} \ldots),$$

wherein the moment of order "**i**" (M_i) is defined as the sum of a factor "n" multiplied by a histogram $\hat{P}(\mathbf{n})$ because it is not clear how a histogram can be multiplied by a factor and then added to other multiplied histograms.

(c) Symbol "**n**"

Moreover, the same symbol "**n**"is used both for the incrementing factor of the sum ∑ and for the number of photon counts per counting time interval. No definition is given for the factor "**n**"or "n_1^{i1}" and "n_1^{i2}".

(d) Vector

Furthermore, there is no mention throughout the whole application of the terms "vector" or "vectorial". Even if bold typeface is currently used for denoting vectors, the mere use of bold typeface does not unambiguously designate a vector, especially as even in claims 1 and 16, bold typeface is used for denoting the parameters $\mathbf{a}_{cell}$and $\mathbf{a}_{medium}$, which are not vectors.

(e) Vector power

Furthermore, the power of vectorial quantities "$\mathbf{n}^{\mathbf{i}}$"is not a generally known concept and thus cannot be considered to provide an indication that "**n**" and "**i**" are vectors, with n_1 and n_2 being components of the vector "**n**" and i_2 and i components of the vector "**i**". In the claims, "**n**" is explicitly defined as a number of photon counts (and a number is not a vector), while "i" is explicitly defined as an order of the moment of the measured photon count numbers (and an order is not a vector). It is therefore not clear what the components of a number "**n**" and the components of an order "**i**" represent.

(ii) Insufficient disclosure - Article 83 EPC

(b) Histogram

The application does not explain how histograms can be multiplied by certain factors and then added.

(c) Symbol "**n**"

Furthermore, claim 3, defining a way of calculating $M_i^{(medium)}$ does not enable the skilled person to carry out the invention because it does not provide any information pertaining to the definition of the "moments of the measured photon count numbers".

(d) Vector

The description does not provide any information capable of clarifying the independent claims and sufficiently disclosing the invention because a large portion of the description merely repeats the wording of the claims, and the example given at the end of the description does not refer to any of the symbols or relationships undefined by the claims. Page 3, last paragraph to page 4, first paragraph of the description describes a two dimensional moment M_{ij} of two photon count numbers n_1 and n_2, which could provide a basis for defining corresponding vectors n (with components n_1 and n_2) and i (with components i and j). While the significance of n_1 and n_2 is indicated (numbers of photon counts determined by means of two photon detectors monitoring emission of different wavelength and/or polarization), the application contains no indication as to the significance of components i and j.

IV. The appellant requested that the decision under appeal be set aside and a patent granted on the basis of the claims as filed on 25 May 2004. Oral proceedings were requested on an auxiliary basis.

V. In support of its request, the appellant advanced arguments including the following.

(i) Clarity

(b) Histogram

$\hat{P}(\mathbf{n})$ is indeed a "histogram", i.e. a function that assigns a real and scalar value to each of the discrete coordinates **n**. A "bar chart" is a special case of a histogram for one dimension which assigns a real value P(k)to each of a set of integer values k. Correspondingly, $\hat{P}(\mathbf{n})$ denotes a multidimensional histogram with the vector **n** specifying a coordinate in multidimensional space. A summation of $\hat{P}(\mathbf{n})$ over a range of values for **n** would simply produce a real and scalar value. In the summation of

$$\hat{M}_{\mathbf{i}} = \sum_{\mathbf{n}} \mathbf{n}^{\mathbf{i}} \hat{P}(\mathbf{n}) = average(\mathbf{n}^{\mathbf{i}}) = average(n_1^{i_1} n_2^{i_2} \ldots),$$

each sum term is the product of a real number $\hat{P}(\mathbf{n})$ and an integer $\mathbf{n}^{i} = n_1^{i1} n_2^{i2}$. All sum terms, and the resulting moment $\hat{M}_{\mathbf{i}}$ are therefore real and scalar numbers.

(c) Symbol "**n**"

Vector **n** represents the number of photons registered per counting time interval by one or more photon detectors in a plurality of sensed volumes. This fact is further exemplified for the case of two components, n1, n2 in the specification on page 3 last paragraph to page 4 first paragraph of the application as filed and in claim 8. Denoting **n** as the index in the sum in the formula

$$\hat{M}_{\mathbf{i}} = \sum_{\mathbf{n}} \mathbf{n}^{\mathbf{i}} \hat{P}(\mathbf{n}) = average(\mathbf{n}^{\mathbf{i}}) = average(n_1^{i_1} n_2^{i_2} \ldots),$$

means that the sum is calculated over all values of **n** This is in agreement with established mathematical notation. For a vector **n**, this means that the sum is calculated over all combinations of component values n_1, n_2 etc. This use of **n** is therefore not in contradiction with **n** being a vector.

(d) Vector

Bold typeface in the formulas does unambiguously refer to vector quantities. The examining division is mistaken in stating that $\mathbf{a}_{cell}$ and $\boldsymbol{a}_{medium}$are examples for the use of boldface to designate non vector quantities. As stated throughout the application, $\mathbf{a}_{cell}$ refers to parameters of the cells or solid supports, and $\boldsymbol{a}_{medium}$ refers to parameters of the medium, whereby reference is made to the plural "parameters" in referring to either quantity. The vector **i** is used to denote multi-dimensional moments.

(e) Vector power

The equation in claims 1 and 16 defines the meaning of **i** and its components in the general case, and the bridging section on pages 3 and 4, as well as claim 8, illustrates the special case of two dimensions. The components i_1, i_2,... of the vector **i** denote the power of the individual photon count components n1, n2, … in the multi-dimensional moment. For the two dimensional case, the components are designated i and j.

(ii) Sufficiency

The example discloses a determination of the brightness of the membranes of cells expressing the β-2 adrenergic receptor in the presence of FL-CGP12177. On page 9, paragraph 3, it is disclosed that photon counts are recorded for each of several sensed volumes within the sample and that the fluorescence intensity is recorded and the frequency of particular photon counts is plotted. Further, a photon count histogram comprising the fluorescence intensity measured of ligand molecules not bound to cells and the fluorescence of ligands bound to the cells is disclosed. For example the term "photon count histogram" refers directly to the subject matter of claims 1 and 16, in particular to step (b) of claim 1. Throughout the example the terminology used corresponds to that used in the claims so that the person skilled in the art can without doubt relate the disclosure of the example to the subject matter of the claims. Further, in view of the example, a person skilled in the art gets the information that the photon count distribution from a sample containing a suspension of cells comprises two components: the contribution of the free ligand in the liquid medium and the contribution from sensed volumes situated in or on the cells where ligand is bound to its receptor in the cell membrane. The example discloses the separation of the calculated moments into the respective moments deriving from these two contributions, according to step (c) of claim 1 and feature (ii) of claim 16.

VI. Independent claims 1 and 16 submitted by the appellant are worded as follows.

"1. A method for sensing fluorescent samples of cells, or soluble or solid supports in a medium, said method comprising the steps of:
(a) monitoring intensity of fluorescence emitted from said fluorescent samples by measuring with the help of one or more photon detectors numbers of photon counts **n** per counting time interval in a plurality of sensed volumes,
(b) calculating a series of moments $\hat{M}_{\mathbf{i}}$ of the measured photon count numbers, defined as

$$\hat{M}_{\mathbf{i}} = \sum_{\mathbf{n}} \mathbf{n}^{\mathbf{i}} \hat{P}(\mathbf{n}) = average(\mathbf{n}^{\mathbf{i}}) = average(n_1^{i_1} n_2^{i_2} \ldots),$$

where $\hat{P}(\mathbf{n})$ denotes a normalized histogram of photon count numbers, and
(c) estimating characteristic parameters of the cells, or soluble or solid supports $\mathbf{a}_{cell}$, and of the medium $\mathbf{a}_{medium}$ from said series of moments $\hat{M}_{\mathbf{i}}$ using a set of equations corresponding to different orders of the moment **i**, of a general form

$$\hat{M}_{\mathbf{i}} = P_{cell} M_{\mathbf{i}}^{(cell)}(\mathbf{a}_{cell}) + (1 - P_{cell}) M_{\mathbf{i}}^{(medium)}(\mathbf{a}_{medium}),$$

where
P_{cell} denotes the probability that the sensed volume is situated in or on the cell, or soluble or solid support,
$M_{\mathbf{i}}^{(cell)}(\mathbf{a}_{cell})$ denotes the expected **i**-th moment of the photon count number provided the sensed volume is situated in or on the cell, or soluble or solid support, as a function of the parameters $\mathbf{a}_{cell}$, and
$M_{\mathbf{i}}^{(medium)}(\mathbf{a}_{medium})$ denotes the expected **i**-th moment of the photon count number provided the sensed volume is

situated in the medium, as a function of the parameters $\mathbf{a}_{medium}$.

16. An apparatus for working the method according to claims 1 to 15 comprising:

- a stage for supporting a sample in an examination site, the sample comprising cells, or soluble or solid supports in a medium;
- a light source positioned to deliver light to the sample in the examination site;
- a detector positioned to receive fluorescence emitted from the sample in the examination site and constructed so that it determines numbers of photon counts **n** per counting time interval in a plurality of sensed volumes and delivers the numbers **n** to an evaluation device;
- an evaluation device coupled to the detector for receiving and evaluating the numbers **n**; characterized in that
- the evaluation device is adapted to

(i) calculate a series of moments $\hat{M}_{\mathbf{i}}$ of the measured photon count numbers, defined as

$$\hat{M}_{\mathbf{i}} = \sum_{\mathbf{n}} \mathbf{n}^{\mathbf{i}} \hat{P}(\mathbf{n}) = average(\mathbf{n}^{\mathbf{i}}) = average(n_1^{i_1} n_2^{i_2} \ldots),$$

where $\hat{P}(\mathbf{n})$ denotes a normalized histogram of photon count numbers, and

(ii) estimate characteristic parameters of the cells, or soluble or solid supports $\mathbf{a}_{cell}$, and of the medium $\mathbf{a}_{medium}$ from said series of moments $\hat{M}_{\mathbf{i}}$ using a set of equations corresponding to different orders of the moment **i**, of a general form

$$\hat{M}_{\mathbf{i}} = P_{cell} M_{\mathbf{i}}^{(cell)}(\mathbf{a}_{cell}) + (1 - P_{cell}) M_{\mathbf{i}}^{(medium)}(\mathbf{a}_{medium}),$$

where

P_{cell} denotes the probability that the sensed volume is situated in or on the cell, or soluble or solid support,

$M_{\mathbf{i}}^{(cell)}(\mathbf{a}_{cell})$ denotes the expected **i**-th moment of the photon count number provided the sensed volume is situated in or on the cell, or soluble or solid support, as a function of the parameters $\mathbf{a}_{\text{cell}}$, and

$M_{\mathbf{i}}^{(medium)}(\mathbf{a}_{medium})$ denotes the expected **i**-th moment of the photon count number provided the sensed volume is situated in the medium, as a function of the parameters $\mathbf{a}_{\text{medium}}$."

The board observes that claims 20 and 21 directed to an assay both include the feature "amounts of fluorescence...estimated according to any of claims 1 to 15."

Reasons for the Decision

1. The appeal is admissible.

2. Clarity

2.1 The examining division has not challenged the clarity of claim 1 insofar as it concerns sensing fluorescent samples in a medium by monitoring intensity of fluorescence by measuring photon counts or digitised values per unit time in a plurality of sensed volumes. Also unchallenged is calculating moments as such and taking account of cell and medium moments in estimation of parameters.

2.2 The lacks of clarity seen by the division concern notation and the application of normalised histogram $\hat{P}(\mathbf{n})$ as set out in the sections of the Facts and Submissions above referenced III(i)(a) to (e). The view of the board is as follows.

2.2.1 Average (section III(i)(a))

The examining division gave no specific example of what it understood to be obscure in the use of the equals sign in the reference to average in the expression

$$\hat{M}_{\mathbf{i}} = \sum_{\mathbf{n}} \mathbf{n}^{\mathbf{i}} \hat{P}(\mathbf{n}) = average(\mathbf{n}^{\mathbf{i}}) = average(n_1^{i_1} n_2^{i_2} \ldots),$$

so that the board cannot appreciate how a general remark that equals may not be identical calls into question that all component values of power are treated as set out by the appellant in sections II(a) and V(i)(e) of the Facts and Submissions above. The Board therefore concluded that the case of the appellant is more convincing.

2.2.2 Histogram (section III(i)(b))

It was not clear to the examining division how a histogram can be multiplied by a factor and then added to other multiplied histograms. However the expression given in claims 1 and 16 is clear to the skilled person and corresponds to the explanation given by the appellant in section V(i)(b) of the Facts and Submissions above. Consequently, the board does not identify any lack of clarity of the kind alleged by the examining division.

2.2.3 Symbol **"n"** (section III(i)(c))

Denoting **n** as the index in the sum in the formula means, consistent with normal mathematical usage and as submitted by the appellant, that the sum is calculated over all values of **n**. Consequently, the board does not identify any lack of clarity of the kind relied on by the examining division.

2.2.4 Vector (section III(i)(d))

Boldface in the formulas unambiguously refers to vector quantities which is not called into question by boldface $\mathbf{a}_{cell}$ and $\mathbf{m}_{edium}$ as argued by the examining division, because these parameters, contrary to the opinion of the examining division, do represent vectors. Consequently, the board does not identify any lack of clarity of the kind alleged by the examining division.

2.2.5 Vector power (section III(i)(e))

The skilled person knows from the formula in the independent claims that vector **i** denotes the power of the individual photon count components n1, n2, … in the multi-dimensional moment. In a two dimensional case described in the paragraph bridging pages 3 and 4, the components are designated i and j. In other words, neither **n** nor **i** are just numbers not vectors, numbers are what a specific count of specific order is. Consequently, the board does not identify any lack of clarity of the kind alleged by the examining division.

3. Sufficiency

The board is satisfied that the example given in the description concerning the beta-2 adrenergic receptor in the presence of a fluorescently labelled ligand for this receptor is sufficient teaching for the subject matter given in section 2.1 above. It is true that a precise calculation of the moments and parameter values in, say tabular form, is not given for the example. However, since the method of moments is well known in statistics, especially photoelectron statistics, the skilled person knows how to implement the method without such detailed calculation and parameter values. This view is not really challenged by the position of the examining division because the considerations advanced by the division amount, in essence, to a repetition of the clarity objections to which the board does not agree.

4. Patentability

The board has examined the contents of the file in relation to patentability and neither disagrees with the submissions of the made during the examination proceedings (see section II of the Facts and Submissions) nor considers the available prior art to call patentability into question.

5. Procedure

5.1 In view of the foregoing, the board considers it appropriate to exercise powers within the competence of the first instance and order grant of a patent. In these circumstances, the oral proceedings requested on an auxiliary basis are not necessary.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to grant a patent based on the following documents:

   Description

   Pages 2,3,5 to 10 as originally filed,

Pages 1, 1a, 4 received with the letter of 25 May 2004

Claims

1 to 22 received with the letter of 25 May 2004, and

Drawing Sheets

1/3 to 3/3 as originally filed.

The Registrar: The Chairman:

M. Kiehl A. Klein

Decision electronically authenticated