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**Datasheet for the decision  
of 16 March 2012**

**Case Number:** T 1409/09 - 3.4.02

**Application Number:** 00950844.1

**Publication Number:** 1203218

**IPC:** G01N21/64, G01N33/543

**Language of the proceedings:** EN

**Title of invention:**

SYSTEM AND METHOD FOR MONITORING CELLULAR ACTIVITY

**Applicant:**

CALIFORNIA INSTITUTE OF TECHNOLOGY

**Headword:**

**Relevant legal provisions:**

EPC Art. 54, 56

**Keyword:**

**Decisions cited:**

**Catchword:**



Case Number: T1409/09 - 3.4.02

**D E C I S I O N**  
**of the Technical Board of Appeal 3.4.02**  
**of 16 March 2012**

**Appellant:** CALIFORNIA INSTITUTE OF TECHNOLOGY  
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**Decision under appeal:** **Decision of the Examining Division of the  
European Patent Office posted 22 December 2008  
refusing European patent application No.  
00950844.1 pursuant to Article 97(2) EPC.**

**Composition of the Board:**

**Chairman:** A. Klein  
**Members:** F. Maaswinkel  
D. Rogers

## **Summary of Facts and Submissions**

- I. The appellant (applicant) lodged an appeal against the decision of the examining division refusing the European patent application No. 00950844.1.
- II. In its decision, the examining division held that the subject-matter of the independent claims of the requests then on file did not meet the requirement of novelty of Articles 52(1) and 54 EPC in view of the disclosure of document D1 (WO-A-96/22521).
- III. With the statement of grounds of appeal the appellant requested that the decision of the examining division be set aside and that a patent be granted upon the basis of the claims as filed on 17 October 2008 (Main Request) or on the basis of the sets of claims according to the 1st or 2nd Auxiliary Requests filed with the statement of grounds of appeal. In addition the appellant filed an auxiliary request for oral proceedings.
- IV. In a Communication pursuant to Article 15(1) RPBA accompanying the summons to oral proceedings the Board raised objections under Article 84 EPC against the claims of all requests. With respect to the issues of novelty and inventive step the Board expressed its preliminary opinion that it tended to concur with the position of the examining division in the decision under appeal.
- V. In a further letter filed on 19 December 2011 the appellant filed sets of claims according to a new Main Request and Auxiliary Requests 1 - 6.

VI. Oral proceedings took place on 16 March 2012. At the oral proceedings the appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of claims 1 - 22 of the Main Request, or subsidiarily according to the claims of one of the Auxiliary Requests 1 - 6, all such Requests submitted under cover of the letter dated 19 December 2011, or according to the claims of the new Auxiliary Request submitted at the oral proceedings before the Board. The Board gave its decision at the end of the oral proceedings.

VII. The wording of claim 1 of the Main Request reads as follows:

" A method of monitoring cellular activity in a cellular specimen, comprising:  
applying a plurality of different excitable markers to the specimen;  
applying light to the specimen from a laser microscope to excite a region of the specimen and cause fluorescence to be radiated from the region by the markers in that region;  
separating the fluorescence into wavelength bands using a spectral analyzer; and  
detecting the fluorescence through an array of detectors, with each detector receiving one of the wavelength bands and generating a corresponding signal ".

The wording of claim 1 of Auxiliary Request 1 is as that of claim 1 according to the Main Request, only differing in the expression "... separating the radiating fluorescence into wavelength bands using a spectral analyzer; and ~~detecting~~collecting the fluorescence..."

(*underlining here and in the following applied by the Board*).

The wording of claim 1 of Auxiliary Request 2 is as that of claim 1 according to the Main Request, only differing in the expression "~~...separating~~dispersing the fluorescence into wavelength bands using a spectral analyzer...".

The wording of claim 1 of Auxiliary Request 3 reads as follows:

" A method of monitoring cellular activity in a cellular specimen, comprising:  
applying a plurality of different excitable markers to the specimen;  
applying light to the specimen from a laser microscope to excite a region of the specimen and cause fluorescence to be radiated from the region by the markers in that region;  
dispersing the fluorescence into wavelength bands using a spectral analyzer;  
detecting the fluorescence through an array of detectors, with each detector receiving one of the wavelength bands and generating a corresponding signal;  
and  
wherein the spectral analyzer includes a grating or a prism ".

The wording of claim 1 of Auxiliary Request 4 is as that of claim 1 according Auxiliary Request 2, only differing in the expression "...detecting the fluorescence through a linear array of detectors;...".

The wording of claim 1 of Auxiliary Request 5 is as that of claim 1 according Auxiliary Request 2, with the

additional feature at the end of the claim "(...and generating a corresponding signal) ; and processing the signals by linear unmixing".

The wording of claim 1 of Auxiliary Request 6 is as that of claim 1 according to the Main Request, only differing in the expression "... applying light to the specimen from a multi-photon laser microscope...".

The wording of claim 1 according to the New Auxiliary Request submitted at the oral proceedings before the Board is as follows:

" A method of monitoring cellular activity in a cellular specimen, comprising:  
applying a plurality of different excitable markers to the specimen;  
applying light to the specimen from a laser microscope to excite a region of the specimen and cause fluorescence to be radiated from the region by the markers in that region;  
dispersing the fluorescence into wavelength bands using a spectral analyzer; and  
detecting the fluorescence through an array of detectors as measured spectrum, with each detector receiving one of the wavelength bands and generating a corresponding signal, retrieving characteristic spectra for the different fluorescent markers (104), and decomposing the measured spectrum into the different component marker spectra (106) ".

All Requests include independent apparatus claims and further dependent claims, which, however, are not relevant for the purpose of the present Decision.

VIII. The arguments of the appellant may be summarised as follows.

The examining division refused the Main Request for not meeting the requirements of Article 54 EPC in view of document D1. This opinion was based on the disclosure on page 14, lines 23 et seq. of this document which discloses a system with two or more detectors, wherein the detectors use appropriate spectral filters tuned for the different wavelength bands corresponding to the different fluorescent dyes, and the dyes are used for specific staining of the particles to be analyzed.

However, according to claim 1 the fluorescence of the different markers is separated in wavelength bands using a spectral analyzer, which enables to obtain knowledge of the respective spectra of the markers and not just one signal per marker as in the arrangement in document D1. Therefore the claimed method enables to differentiate between different markers (for instance, dyes), even if there is a partial spectral overlap between the spectra of the dyes. This is further supported by the description on page 2, l. 31 - 37, and page 4, l. 22 - 28. The spectral analyzer may be a tunable filter, which enables to sweep through the spectrum by tuning. This is not the same as the filter of the arrangement in D1, which is tuned to a specific fixed wavelength and does not allow to sweep through the spectrum. The subject-matter of claim 1 according to the Main Request is therefore novel.

The method according to this claim also involves an inventive step for the following reason. In view of the above-indicated difference between D1 and the subject-matter of the present application, the objective problem to be solved is to provide a method that

efficiently analyses fluorescent probes present at a specimen in a test sample. However, the section in D1 on page 14, lines 23 et seq.. relates to a different object, namely to discriminate non-specific photon-counts: D1 teaches that the detector can detect one or more photons of the fluorescence emission coming from one particle. The detector receives background fluorescence caused by free molecules in the sample, from scattering and from thermal noise. In particular, document D1 uses correlation analysis of the single photon counts, wherein an auto-correlation analysis is based on registration of the time intervals between the photon counts from each detector (receiving the respective wavelength bands of the fluorescence), whereas a cross-correlation analysis is used in the case of two or more independent photon detectors on the basis of various correlation parameters, which have to be adjusted for optimal discrimination of non-specific photon counts. Hence, document D1 cannot prompt the skilled person to amend its disclosure in such a way as to arrive at the subject-matter of the present application.

## **Reasons for the Decision**

1. The appeal is admissible.
2. *Main Request- Novelty*
  - 2.1 The wording of claim 1 of the Main Request is essentially identical to that of claim 1 filed with the letter of 17 October 2008. This claim had been addressed in the Decision under appeal under "Auxiliary



- Request 2". In point 7 of the Decision the examining division considered that the subject-matter of this claim lacked novelty over the disclosure in document D1.
- 2.2 In point 6 of its decision the examining division considered that document D1 concerns a flow cytometer for analysing and classifying cells stained with one or several fluorescent dyes. That device and method concerned the detection of rare events or small particles in biological fluids wherein the term "particle" referred to any biological particle including mammalian cells, blood cells, bacterial cells, cell organelles and viruses (referring to p.2, l. 34 - p.3, l. 5 of D1). For the particular embodiment relevant to the subject-matter of claim 1 the Decision made reference to the passage on page 14, l. 24 - 32 of this document (*hereinafter referred to as the embodiment or arrangement on page 14*).
- 2.3 The appellant has argued that the method defined in claim 1 employs a spectral analyzer to separate the fluorescence into wavelength bands. Thus spectral information from the markers (dyes) is obtained which allows to distinguish between the different dyes. For instance, according to claims 4 and 5 tunable filters may be employed which allow sweeping across the spectrum. According to the appellant, in contrast, the apparatus disclosed in document D1 in the paragraph on page 14 referred to in the Decision only contains detectors with tuned filters, which can only detect a single pre-set wavelength and therefore do not anticipate a spectral analyzer as defined in claim 1.
- 2.4 The Board does not concur with this position of the appellant. According to the passage in question in

document D1, the "two or more fluorescence detectors" comprise "appropriate spectral filters, tuned for the different wavelength bands" (emphasis by the Board). This implies that for detecting the signals of each of the "two or more" fluorescent dyes, every filter is tuned to the appropriate wavelength of the respective dye, i.e. the filters must be tunable for every particular dye. Indeed, in this arrangement of D1, by detecting different parts of the radiated fluorescence spectrum by the respective "two or more" detectors equipped with appropriately tuned filters, a spectral discrimination and separation of the fluorescence spectrum is obtained. Also, an arrangement comprising two or more detectors meets the qualification of an "array" of detectors (see, for instance, page 5, l. 1 of the published patent application: "...a detector array 42 consisting of individual detectors 43"). Therefore, in the Board's view, this embodiment in D1 anticipates the method step in claim 1 of the Main Request "... separating the fluorescence into wavelength bands using a spectral analyzer; and detecting the fluorescence through an array of detectors, with each detector receiving one of the wavelength bands and generating a corresponding signal".

- 2.5 With respect to the appellant's argument that the filters further specified in claims 4 and 5 of this Request may be actively tuned and thereby able to sweep through the wavelength spectrum, it is observed that, neither in the claims, nor in the originally filed patent application, there appears to be a disclosure that the filters are actively tuned for sweeping through the fluorescence spectrum during the monitoring method. Since this method step is not disclosed, it cannot be used to distinguish the claimed method from the prior art.

2.6 For these reasons the Board concurs with the finding in the Decision under appeal that the method of claim 1 of the Main Request is not novel.

2.7 Accordingly, the Main Request is not allowable for lack of novelty.

3. *First Auxiliary Request*

3.1 The wording of claim 1 of this Request differs from that of claim 1 of the Main Request in the feature "separating the radiating fluorescence into wavelength bands" and in "collecting" (instead of: detecting) "... the fluorescence".

3.2 With respect to the term "radiating fluorescence" it appears that the phenomenon "fluorescence" is intrinsically a radiation process (*namely: a process in which an excited species, for instance a molecule of dye, releases a part of the excitation energy in a radiation process*). Therefore each fluorescence necessarily involves a "radiating fluorescence". Hence, the new wording equally applies to the fluorescence in the detection method of document D1.

3.3 The feature "collecting the fluorescence" must be interpreted in the light of the disclosure of the present patent application. In the particular embodiment of Figure 5, addressed on page 6, l. 6 - 22, the system includes a light collector substantially surrounding the specimen and collecting the light emitted by the fluorescent dyes. However, since in this particular embodiment the radiation is not collected by "an array of detectors" as specified in the claim, the term "collecting" does not imply a collection in the

total solid angle. Rather, in the further embodiments of Figures 1, 2, 3 and 4, the light is "collected" by collection optics, in the same way as disclosed in document D1, page 6, l. 14 and 15, which discloses that the system employs a "high numerical objective lens for excitation and detection". Therefore the feature "collecting the respective fluorescence bands by an array of detectors" is known from the embodiment addressed on page 14 of document D1.

3.4 Hence, Auxiliary Request 1 is not allowable for lack of novelty.

4. *Auxiliary Request 2*

4.1 In claim 1 according to this Request the feature in claim 1 of the Main request "...separating the fluorescence into wavelength bands using a spectral analyzer " has been amended to "...dispersing the fluorescence into wavelength bands using a spectral analyzer". The basis for this feature can be found, e.g., in the embodiment of Figure 2, addressed on page 4, l. 22 - 28 of the published patent application; and in original claim 29. Therefore the amendment is not contrary to the requirements of Article 123(2) EPC. Furthermore, since in this Auxiliary Request the claims addressing embodiments in which the fluorescence is separated by tunable filters (*and not by the effect of dispersion*) are deleted, there is also no conflict with Article 84 EPC. Therefore claim 1 of this Request is formally admissible.

4.2 *Novelty*

In the embodiment disclosed on page 14 of document D1, the different parts of the fluorescence spectrum are separated by means of a detector array with respective

tuned filters in front of the detectors. Hence, in that embodiment the fluorescence spectrum is not dispersed. The subject-matter of this claim is therefore novel over the prior art arrangement in D1.

#### 4.3 *Inventive step*

4.3.1 In the context of the Main Request the appellant had argued that the objective problem addressed by the difference between the claimed method and the embodiment on page 14 of document D1 resided in providing a method for efficiently analyzing the output of fluorescent probes applied to a specimen in a test sample. According to the appellant, the embodiment in document D1 relates to a different object (the discrimination of non-specific photon-counts), therefore the skilled person would not have had a motivation to modify the arrangement of D1 and arrive at the solution defined in claim 1.

4.3.2 The Board concurs with the appellant insofar as a detection of the fluorescent radiation after dispersing the spectrum allows for a more complete analysis of the spectral components. Therefore, starting from the arrangement on page 14 of D1, the objective problem can be seen as improving the detection of the fluorescent radiation. Since the improvement of a monitoring method or apparatus is an aim routinely pursued by the person skilled in the art, in this context being an optical instrumentation physicist or engineer, the formulation of the objective problem as such does not involve an inventive activity.

4.3.3 With respect to the argument that the disclosure in D1 on page 14 relates to "a different object", whence the skilled person would not have been incited to modify

that arrangement in the way as defined in claim 1, the Board wishes to point out that the final "object" or application of this arrangement should not be confused by addressing the issue of the "objective" problem and solution: rather, the relevant question is, whether the skilled person, taking knowledge of the disclosure in document D1, in particular on page 14, would have considered on the basis of his general knowledge to modify that apparatus in the manner as defined in claim 1.

4.3.4 The passage addressing the relevant embodiment on page 14 of D1 starts by explaining that the apparatus should be optimised for better discrimination between the background counts and true counts of the particles. In order to improve the discrimination it is recommended to specifically stain the particles by the use of two or more dyes. In the subsequent sentence the disclosure continues that "...the system can incorporate two or more detectors respectively...". The paragraph ends with the teaching that these detectors may include appropriate spectral filters, tuned for the different wavelength bands corresponding to the different fluorescent dyes.

4.3.5 The Board understands from this paragraph in D1 that if -in order to further improve the discrimination between counts- the skilled person decides to apply staining with two or more dyes, he may run into the problem that the fluorescent spectra of the different dyes may partially overlap. Therefore, firstly, for each dye to be detected at least one detector is necessary. However, even if appropriate spectral filters, tuned for the different wavelength band corresponding to each dye, are used, an unambiguous discrimination between the respective dyes may become difficult. For instance, if a larger number, e.g. ten, different dyes are

applied. In such a case the inclusion of a spectrograph, instead of spectral filters, is well known in the technical field. As an example reference is made to point 4 of the International Preliminary Examination Report ("IPER"), where in addressing original claims 2 and 3 it was explained that "Grating, prism; dispersive spectral analyzer" were well known alternatives to filters in this field. In particular reference was made to document D2 (US-A-5,891,738), col. 15, l. 40 - 55.

4.3.6 This patent document (from the same inventor as document D1) discloses that the spectral features specific for microparticles ... can be registered with several photon detectors with appropriate spectral filters (col. 15, l. 43 and 44). In the subsequent sentence the disclosure continues: "Alternatively, registration of the spectrum ...can be made using e.g. a conventional grating spectrometer ..., which are coupled to an array detector".

4.3.7 Hence, it is concluded that the skilled person will select the type of spectral analysis instrumentation depending on the particular requirements: if only one or two dyes are to be detected, one detector with an appropriate spectral filter per dye may be adequate for unambiguously detecting the signals. However, if "more than two" dyes should be detected, possibly with partially overlapping spectra, the use of a "conventional grating spectrometer" coupled to an "array detector" may be a preferable alternative, as indicated in the passage in document D2 referred to in the IPER.

4.3.8 Therefore the Board considers that the subject-matter of claim 1 of this Request does not involve an inventive step.

5. *Auxiliary Request 3*

5.1 Claim 1 of this Request includes the method step of claim 1 of the 2nd Auxiliary Request "dispersing the fluorescence..." and, in addition, the features that the spectral analyzer includes a grating or a prism.

5.2 As set out in point 4.3.7 supra, to employ a spectral analyzer including a grating spectrometer as an alternative to spectral filters is well known in the art, as e.g. documented in D2, col. 5, l. 43 - 51. Furthermore, it is also well known to use a prism spectrometer as an alternative to a grating spectrometer.

5.3 Accordingly it is found that the subject-matter of claim 1 of this Request does not involve an inventive step for essentially the same reasons as discussed for claim 1 of the higher Request.

6. *Fourth Auxiliary Request*

6.1 Claim 1 according to this Request also includes the feature of claim 1 of the 2nd Auxiliary Request "dispersing the fluorescence..." and, in addition, the feature that the array of detectors is a linear array.

6.2 With respect to the first feature, reference is made to points 4 and 5 supra. The feature that the detector array is a linear array appears an obvious choice, in particular if the spectrum is to be analysed with a grating spectrometer and an array detector, as



disclosed in document D2, col. 15, l. 46 - 51, since a linear array is the preferred choice if only the detection of the dispersed spectrum is of interest, and if no spatial resolution is needed (in which case a two-dimensional detector array might be preferred).

6.3 Therefore the subject-matter of claim 1 of this Request does not involve an inventive step.

7. *Fifth Auxiliary Request*

7.1 The wording of claim 1 of this Request is based on that of claim 1 of the 2nd Auxiliary Request with the additional feature at the end of the claim "...and processing the signals by linear unmixing".

7.2 The Board notes that a claim including the feature relating to "linear unmixing" had not been present in the sets of claims addressed in the Decision under appeal. According to the appellant in its letter of 19 December 2011, support for the feature that the signals are processed (by a processor) by linear unmixing is in original claim 28 and page 6, l. 23 of the published patent application.

7.3 In fact, the only evaluation of a claim including this feature in the earlier proceedings is found in point 4 of the IPER. Against original claim 28 it had been objected: "Linear unmixing of signals. D2 (col. 8, l. 40 - 50) and D5 (col. 8-9) suggest that resolution of a total spectrum into its components representing respective fluorophores is well-known in the art".

7.4 It is noted that during the earlier proceedings the applicant has not argued against the above finding and also has not forwarded any arguments in support of the

patentability of a claim including these features. Since, furthermore, according to the passage on page 6, l. 22 - page 7, l.10, linear unmixing is just one of the known techniques of reducing spectra having spectral overlap (the other being principal component analysis, PCA) and no arguments have been presented that applying linear unmixing would result in an unexpected technical effect, the Board follows the position taken in the IPER.

7.5 For this reason, the subject-matter of claim 1 of this Request does not involve an inventive step.

8. *Sixth Auxiliary Request*

8.1 Claim 1 of this Request is identical to claim 1 of the Main Request, with the additional limitation that the laser microscope is a multi-photon laser microscope.

8.2 In point 6 of the Reasons for the Decision the examining division had already pointed out that the confocal set-up disclosed in document D1 included the possibility of two-photon excitation. Indeed, this is disclosed in greater detail in the Section "Realisation of the invention with two-photon excitation", see page 9, l. 14 of D1. It is also defined in claim 1 of this document.

8.3 It is concluded that claim 1 according to this Request lacks novelty over the disclosure in document D1.

9. *New Auxiliary Request*

9.1 This Request had been submitted during the oral proceedings before the Board. The claim defines in particular the new features "...retrieving characteristic

spectra for the different fluorescent markers (104), and decomposing the measured spectrum into the different component marker spectra (106)". At the oral proceedings the appellant explained that the wording of this feature finds its basis in the last paragraph on page 6 of the published patent application and in Flow Chart shown in Figure 6.

9.2 The Board observes that the new features in this claim have not been the subject of any claim filed in the First Instance proceedings. It is therefore doubtful whether these features have been addressed in the International Search Report.

9.3 In the Communication annexed to the summons to oral proceedings under Article 15(1) RPBA the Board had drawn the attention of the appellant in relation to amendments of its case is directed to Article 13(3) of the Rules of Procedure of the Boards of Appeal, which recites:

"Amendments sought to be made after oral proceedings have been arranged shall not be admitted if they raise issues which the Board or other party or parties cannot reasonably be expected to deal with without adjournment of the oral proceedings."

9.4 Since, in order to investigate the patentability of claim 1 of the New Auxiliary Request including the unsearched features, a remittal of the Case to the First Instance would be necessary, the Board does not admit this Request.

10. In conclusion, none of the Requests can either be allowed or admitted. Therefore the appeal is to be dismissed.

**Order**

**For these reasons it is decided that:**

The appeal is dismissed.

The Registrar:

The Chairman:



M. Kiehl

A. Klein

Decision electronically authenticated