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Datasheet for the decision of 21 January 2013

Case Number: T 2268/09 - 3.4.02

Application Number: 98926490.8

Publication Number: 988523

IPC: G01N21/64

Language of the proceedings: EN

Title of invention:
METHOD AND APPARATUS FOR DETECTING MICROPARTICLES IN FLUID SAMPLES

Applicant:
EMD Millipore Corporation

Headword:

Relevant legal provisions:
EPC Art. 56

Keyword:
Inventive step - after amendment

Decisions cited:

Catchword:

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Case Number: T 2268/09 - 3.4.02

D E C I S I O N
of the Technical Board of Appeal 3.4.02
of 21 January 2013

Appellant: (Applicant)	EMD Millipore Corporation 290 Concord Road Billerica, MA 01821 (US)
Representative:	Duncan, Garreth Andrew D Young & Co LLP 120 Holborn London EC1N 2DY (GB)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted 9 July 2009 refusing European patent application No. 98926490.8 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: A. Klein
Members: F. Maaswinkel
B. Müller

Summary of Facts and Submissions

I. The appellant lodged an appeal against the decision of the examining division, refusing European patent application 98926490.8. This patent application relates to a device and a method for detecting microparticles in a fluid.

The decision under appeal had been issued as a decision according to the state of the file on request of the applicant. For the reasons of the decision reference was made to the communications of 25 July 2007, 17 March 2009 and 30 June 2009, the latter referring to two telephone conversations held on 10 and 30 June, respectively. According to the consultation by telephone on 30 June 2009 the independent claims of the Main and First Auxiliary Request then on file did not involve an inventive step in view of the teachings of the following documents:

D1: US-A-5 351 118
D8: EP-A-0 068 404
D9: US-A-4 838 688
D10: US-A-5 430 541
D11: WO-A-95 324 24.

II. With the Notice of Appeal the appellant filed a new set of claims to be considered as its single Main Request. In support of this Request arguments were provided on the issues relating to Articles 123(2) EPC, 54 EPC and 56 EPC in a subsequent letter containing the Grounds of Appeal.

III. In a communication under Rule 100(2) EPC the board raised objections under Article 123(2) EPC and 84 EPC 1973 and remarked that an amended set of documents in

which these were overcome could possibly also meet the further provisions of the Convention.

IV. The appellant filed a substitute set of claims 1 to 26 replacing the claims on file and amended description pages.

V. The documents comprising this request include:

Claims: 1 to 26, as received with the letter of 10 December 2012;
Description: pages 1, 2, 4, 5, 9 to 15 as published under the PCT;
pages 3, 3a, 6 to 8 and 16, filed with the letter of 3 December 2012;
Drawings: sheets 1/14, 3/14 - 14/14 as published under the PCT;
sheet 2/14 filed with telefax on 14 December 1999 (with Form 1200).

VI. The wording of independent claim 1 reads as follows:

"1. A single capillary flow cytometer for detecting a microparticle in a sample fluid, the microparticle being tagged with a fluorescent substance, the fluorescent substance emitting fluorescent light when exposed to electromagnetic radiation, the device comprising:

a single capillary tube (202) providing a passageway for the sample fluid;

a fluid delivery system (200) coupled to the single capillary tube, the fluid delivery system capable of causing the sample fluid to flow through the single capillary tube, where in use the sample fluid fills the passageway as it flows therethrough;

a source of electromagnetic radiation (302) for projecting a beam (304) of electromagnetic radiation through the single capillary tube to expose the fluorescent substance to electromagnetic radiation;

a detection device (312, 318) configured to detect fluorescent light emitted from the fluorescent substance when the microparticle is exposed to the beam of electromagnetic radiation; and

a photodiode (316, 320) configured to detect Mie scattered light from the microparticle".

The wording of independent claim 16 reads as follows:

"A method for detecting microparticles tagged with a fluorescent substance, comprising:

providing a single capillary tube having a passageway for the microparticles in a sample fluid;

transporting the sample fluid to a selected location in the single capillary tube, wherein the sample fluid fills the passageway when it flows therethrough;

irradiating the fluorescent substance tagged to the microparticles passing through the selected location,

measuring fluorescent light emitted from the fluorescent substance at the selected location; and

measuring Mie scattered light scattered from the microparticles using a photodiode".

Claims 2 to 15 and 17 to 26 are dependent claims.

VII. The appellant's arguments may be summarised as follows:

Claim 1 on file differs from claim 1 addressed in the decision under appeal by specifying that the device is a single capillary flow cytometer and in that the

sample tube is a single capillary tube. Basis for this amendment can be found at page 3, line 14 and page 11, line 1 of the PCT application as published. In addition, the limitation of a syringe pump introduced into claim 1 of the refused Main Request has been deleted. Corresponding amendments have been made to dependent claim 11 and method claim 16. Furthermore, it will be noted that none of the features objected to by the examining division in the Communication of 30 June 2009 are now present in the claims. Claims 1 and 16 have also been amended in that the second detection device is restricted to a photodiode which is configured to detect Mie scattering. Basis for this amendment is at page 7, lines 16 to 20. Finally the word "elongate" has been deleted in claim 16. Accordingly, it is submitted that the claims fulfil the requirements of Articles 84 EPC 1973 and 123(2) EPC.

In the summons to oral proceedings before the examining division document D1 had been considered to anticipate the subject-matter of former claim 1. Present claim 1 is directed to a single capillary flow cytometer which employs a single capillary tube to feed a sample fluid, to provide a flow passage or detection region, and to discharge the sample fluid passing through the detection region. The skilled person would readily understand the term "single" to mean "consisting of one piece, one part", "not divided", "unbroken" and the like. Similarly, he would understand the term "capillary tube" to mean a "tube" having a fine bore. In contrast, document D1 teaches a flow cytometer using a cuvette consisting of two body members, each of which has a planar facial surface, to provide a flow passage for a sample fluid containing particles to be analyzed. One body member has an elongated groove. An elongate flow passage is formed by pressing the two body members

against their planar facial surfaces using mechanical means. This cuvette would not be considered by the skilled person to comprise a "single capillary tube". The subject-matter of the claims is therefore novel over D1.

With respect to inventive step document D1 is considered to represent the closest prior art. The present independent claims differ from D1, at least, in that the claimed device and method employ a single capillary tube in place of the two-component cuvette of D1. A problem exists with the cuvette of D1 in that complex mechanical means such as spring loading means are required to compress the two body members or seal the cuvette, see Figure 6 of D1, which shows the complexity of the mechanical means used for compressing the two body members 3 and 4. Notwithstanding the complexity of the mechanical means, the cuvette may still cause sample fluid leakage due to improper positioning or aligning of the two body members. Furthermore, in D1 a separate diluent is added to the flow passage via a feeding passage. This complicates direct counting of particles because multiple fluids must be accurately controlled. In the cases where the volume of a diluent is much larger than that of the sample fluid, extreme accuracy in controlling the diluent flow is required, making the measurement problematic. Therefore, the objective problem addressed by the present invention is to modify the device of D1 to simplify the construction of the device, avoid potential problems of sample leaking, and facilitate direct measurement of particle concentration by controlling the constant flow rate or known volume of the sample fluid. This problem is solved by providing a device having a single capillary tube as defined in the independent claims. There is nothing in the prior art

which would motivate the skilled person to consider modifying the device of D1 to arrive at the present claims, for the reasons outlined below. Dealing first with D1 itself, the teaching of D1 is directed to solving an entirely different problem, namely avoiding clogging or blocking of the flow passage. Such clogging or blocking can occur when the cross sectional dimension of the flow passage is sized to commensurate to the particles to be analyzed in order to provide individual particle presentation. D1 therefore teaches the use of a two-body member cuvette which is adapted to be separated, an additional passage for feeding the sample fluid and for receiving an agitating wire, which is machined in one of the body members, and an additional passage for feeding diluents which is also machined in the cuvette body member. The two-body member cuvette, which is adapted to separate once assembled, is the essence of D1. At col. 24, l. 62 - 65, D1 states that the two-part cuvette can eliminate blocking of the flow passage by opening the cuvette, and this is believed to constitute "an extremely advantageous feature of the measuring system as compared to the prior art measurement systems, which lack this advantageous feature or capability". The feeding passage perpendicular to the flow passage for feeding sample fluid and for receiving an agitating wire is also taught as an essential feature as compared to prior art devices which use "centrally" fed particles suspended in a liquid (see col. 9, l. 34 -40). Indeed, D1's two-body member cuvette design intends to prevent clogging of the flow passage, to provide a base for machining separate perpendicular feeding passages for the sample fluid, diluents, or discharged fluid. To replace the two-body member cuvette of D1 with a single capillary tube as suggested by the examining division would be contrary to the

intent, purpose, or function of D1's device. A single capillary tube would not be adapted to "be separated" to prevent clogging. A single capillary tube would not provide a base for machining separate perpendicular passages for feeding sample fluid, for receiving agitating wire, for feeding diluents, or for discharging fluid. There is therefore no motivation for the skilled person to adapt the device of D1 in this manner. Also the further documents D8 - D10 referred to by the examining division provide no guidance to the skilled person towards the solution proposed by the present invention. This is because D8 teaches a sheath flow cytometer, which is different from the presently claimed single capillary flow cytometer in both construction and operation. Document D9 requires that a light beam be projected into a flow cell along the longitudinal axis of the flow cell. The reason for such arrangement is to prevent scatter light caused by the sample when irradiated by incident light from reaching the detector to improve the sensitivity of the detector. Rather than being blocked, the scatter light is measured in the appellant's device using a detector for counting the number of microparticles passing through a detection region. D9 does not teach and is not suitable for individual particle measurement. Similarly, D10 requires an opaque beam blocker 123 to block scatter light off the sample from reaching the detector - see column 7 lines 1 - 30. This document does not teach and is not suitable for individual particle measurement. Both D9 and D10 teach a flow cell directly connected to an outflow from a chromatography column or apparatus. Neither D9 nor D10 could possibly teach or suggest tagging microparticles with a fluorescent substance as recited in the claims of the present application as amended. Document D11, which had only been cited because it discloses a second detector,

would not be of assistance in solving the above technical problem. For the above reasons, the appellant submits that the claims now meet the requirements of the EPC.

Reasons for the Decision

1. The appeal is admissible.

2. *Amendments*
The board is satisfied that the set of claims finds support in the patent application as originally filed documents.

3. *Patentability - Claim 1*

3.1 *Novelty*

3.1.1 According to the communications referred to in the decision under appeal document D1 was considered to disclose the most relevant prior art because it disclosed nearly all features of claim 1 then on file. This document discloses a flow cytometer and a method suitable for detecting microparticles being tagged with a fluorescent substance, see col. 3, l. 58 - 60. As illustrated in Figure 1, the device comprises a source of electromagnetic radiation (lamp 90) to expose the fluorescent substance to electromagnetic radiation; and a detection device (photodetectors 94 and 96) to detect fluorescent light emitted from the fluorescent substance. Although document D1 refers in general terms that also "light scatter" analyzing methods may be applied (col. 4, l. 43 and 44), the particular device of D1 does not include a photodiode configured to detect Mie scattered light from the microparticle.

3.1.2 As argued by the appellant, the present independent claims relate to a particular flow cytometer comprising a single capillary tube. This type of flowmeter is rather different from the flow meter disclosed in document D1 which comprises a cuvette including a capillary passage defined by two parallel plates 3 and 4 (see figures 2, 6 and 8), one of the plates having a longitudinal groove 1 and defining the capillary passage. These plates are displaceable or movable with respect to each other (see col. 18, l. 26 - 28).

3.1.3 Therefore the subject-matter of claim 1, and similarly the method defined in claim 16, differs from the cytometer and analyzing method in document D1 in the type of the measuring section of the cytometer, which in the independent claims is a single capillary tube, whereas the device of D1 comprises a cuvette formed by two movable plates with a capillary groove in one of the plates; and by a photodiode for measuring Mie scattered light.

3.1.4 It is concluded that the subject-matter of claims 1 and 16 is novel over the disclosure in document D1.

3.1.5 With respect to the further documents D8 to D11 cited in the communications referred to in the appealed decision, document D8 does not disclose the use of a single capillary tube but relies on providing a particle-free sheath liquid (36, Figure 3) which is produced by "embedding" a particle stream 33 to be investigated in a surrounding particle-free sheath liquid, see also Figure 6.

3.1.6 Document D9 discloses the use of a transparent capillary tube 30 (see col. 3, l. 45 - 51) in which the

light beam is coaxial with the tube axis. The fluorescence is measured with an integrating sphere completely surrounding the midsection of the capillary (col. 1, l. 59 - 63). Because of the integration, individual particles cannot be detected (there is no positional resolution). Also because of this arrangement, the detection of scattered light is not possible.

3.1.7 Document D10 discloses a cytometer comprising a tubular capillary 11 (see col. 5, l. 59 to col. 6, l. 1). The fluid/particle beam is exposed in a region 12 and the fluorescence is collected by a collector 113 (Al-coated hemispherical mirror) and detected by a photomultiplier 114. Because of this construction, in order to prevent that the light reflected by the collector reaches parts of the capillary tube outside of the exposing region 12, the rest of the tube is blocked by an opaque beam blocker 123. This also blocks scattered light from the tube and an independent detection of such light is not possible.

3.1.8 Document D11 relates to a cytometer for detecting blood samples. It discloses the use of a "flow cell 14 in a conventional means" (p. 8, l. 4) and therefore not a single capillary tube. Scattered light (i.e. having the excitation wavelength, here 488nm) is detected by a first photodetector 28; and the fluorescent light can be detected with two detectors 36 (green) and 42 (red).

3.1.9 It is concluded that the subject-matter of the independent claims is novel.

3.2 *Inventive step*

3.2.1 If, as identified by the examining division with respect to former claim 1, document D1 is considered to disclose the closest prior art, the subject-matter of the independent claims differs from this disclosure in the type of the capillary section of the cytometer and by the photodiode for measuring Mie scattered light (see point 3.1.3 supra).

3.2.2 The board concurs with the arguments by the appellant that the very specific two-body cuvette with movable plates in the device of D1 has intentionally been constructed in order to avoid blocking of the flow passage and offering the possibility of cleaning the cuvette with a cleaning brush 51 (Figure 6). Since this arrangement is the gist of this device, the skilled person would have no reason to modify the arrangement of document D1 by replacing the movable plate capillary cuvette by a single capillary tube.

3.2.3 Documents D9 and D10 disclose devices comprising capillary tubes. However, as set out in point 3.1.6 for document D9 and 3.1.7 for document D10, by their particular constructions these devices do not allow the detection of scattered light of individual particles and there would therefore be no reason to include a photodiode configured to detect Mie scattered light from the particle.

3.2.4 Hence, the subject-matter of the independent claims 1 and 16 is not obvious and involves an inventive step.

3.3 *Claims 2 - 15 and 17 to 26.*

3.3.1 These claims are dependent claims and are equally allowable.

4. For the above reasons, the board finds that the appellant's request meets the requirements of the EPC and that a patent can be granted on the basis thereof.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

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

   Claims: 1 to 26, as received with the letter of 10 December 2012;

   Description: pages 1, 2, 4, 5, 9 to 15 as published under the PCT;
   pages 3, 3a, 6 to 8 and 16, filed with the letter of 3 December 2012;

   Drawings: sheets 1/14, 3/14 - 14/14 as published under the PCT;
   sheet 2/14 filed with telefax on 14 December 1999 (with Form 1200).

The Registrar: The Chairman:

M. Kiehl A. Klein

Decision electronically authenticated