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D E C I S I O N
of 23 January 2003

Case Number: T 0070/99 - 3.3.5

Application Number: 93910891.6

Publication Number: 0637998

IPC: B01L 3/00

Language of the proceedings: EN

Title of invention:
Fluid handling in microfabricated analytical devices

Patentee:
TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA

Opponent:
Affymetrix, Inc.

Headword:
Analytical devices/UNIVERSITY OF PENNSYLVANIA

Relevant legal provisions:
EPC Art. 54, 56, 100(b), 100(c), 123(2)(3)

Keyword:
"Admissibility of amendments: yes - generic definition of a subgroup of embodiments by means of a term not literally disclosed but clearly and unambiguously derivable from application as filed"
"Novelty: yes - suitability of prior art device to perform the function to be performed by claimed device not convincingly established"
"Inventive step: yes - after amendment"

Decisions cited:
T 0161/82, T 0383/88

Catchword:
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Case Number: T 0070/99 - 3.3.5

D E C I S I O N
of the Technical Board of Appeal 3.3.5
of 23 January 2003

Appellant: (Opponent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 5 October 1998 rejecting the opposition filed against European patent No. 0 637 998 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairman: R. K. Spangenberg
Members: B. P. Czech
J. H. Van Moer

Summary of Facts and Submissions

I. The appeal is from the decision of the opposition division rejecting the opposition against European patent 0 637 998.

The independent claims 1, 21 and 22 of the patent as granted read as follows:

"1. A device for analyzing a fluid, cell-containing sample, the device comprising:

a solid substrate microfabricated to define:

a sample inlet port; and

a mesoscale flow system defined by chambers and flow passages having cross-sectional dimensions of about 0.1-500 µm wherein chambers in the substrate can also have larger dimensions of a few millimetres, the mesoscale flow system comprising:

a sample flow channel extending from said inlet port; and

a cell handling region for treating cells disposed in fluid communication with said flow channel, said cell handling region comprising a cell lysing structure;

means for inducing flow of cells in said sample through said mesoscale flow channel and said cell handling region to force cells in said sample into contact with said cell lysing structure, thereby to lyse cells in said sample; and means downstream of said cell lysis structure for detecting an analyte in said lysed cell sample."

"21. A device for analyzing a fluid, cell-containing sample, the device comprising:

a solid substrate microfabricated to define:

a sample inlet port; and

a mesoscale flow system defined by chambers and

flow passages having cross-sectional dimensions of about 0.1-500 µm wherein chambers in the substrate can also have larger dimensions of a few millimetres, the mesoscale flow system comprising:

a sample flow channel extending from said inlet port; and

a cell handling region for treating cells disposed in fluid communication with said flow channel, said cell handling region comprising a cell lysing agent;

means for inducing flow of cells in a sample through said mesoscale flow channel and said cell handling region to force cells in said sample into contact with said cell lysing agent, thereby to lyse cells in said sample; and

means downstream of said cell handling region for detecting an analyte in said lysed cell sample."

"22. A method of separating a target subpopulation of cells in a cell-containing liquid sample comprising the steps of:

(A) providing a mesoscale sample flow passage having cross-sectional dimensions of about 0.1 to 500 μm, and comprising a solid wall having immobilized thereon a binding protein specific for a cell membrane-bound protein characteristic of said target population;

(B) passing a cell-containing liquid sample through said passage under conditions to permit capture of members of the cell target subpopulation by reversible cell surface protein-immobilized protein binding, while permitting other cells to pass therethrough; and

(C) changing the conditions in said flow passage to release said target subpopulation of cells."

II. Twelve prior art documents had been cited during the opposition proceedings, including the following:

D1: WO-A-91/13338

D2: Textbook of Medical Physiology, Arthur C. Guyton, 4th edition, 1971, Chapter 8, pages 98 and 105

D3: WO-A-90/04645

D4: WO-A-91/15750

D5: Washizu M. et al., "Handling of biological cells using fluid integrated circuit" IEEE pages 1735 to 1740 (1988)

D6: Kricka L.J. et al, "Liquid Transport in Micron and Submicron Channels", SPIE 1167, 1989, pages 159 to 168.

In addition, the opponent cited

D9: a declaration of Mr Thomas Brendan Ryder.

The opposition division *inter alia* came to the conclusions

- that no added subject-matter was introduced by the terms "lysing structure";

- that the patent in suit met the requirements of Article 83 EPC, and

- that the claimed subject-matter was novel and inventive in view of D1, D3 and D6.

III. With its statement of the grounds of appeal, the appellant (opponent) filed two further documents:

D14: Szuchet S. et al., "A Simple Cell Disrupter Designed for Small Cells with Relatively large Nuclei", Analytical Biochemistry, 128, 1983, pages 453 to 458; and

D15: Schnaitmann C., "Cell fractionation" Manual of methods for general bacteriology (American Society of Microbiology), 1981, pages 53 to 55 and 61

The appellant referred to its position as already set out in the notice of opposition, and, more particularly,

- maintained its objection under Article 100(c) EPC against the expression "cell lysing structure" comprised in claim 1 as granted;

- maintained that the devices according to independent claims 1 and 21 as granted lacked novelty over the disclosures of D1 and D3, respectively; and

- considered the subject-matter of granted claims 1 and 21 to be obvious in view of the known bench scale techniques and the disclosures of documents D3, D4, D5, D14 and D15.

IV. In its reply, the respondent (patent proprietor) rejected the appellant's objections. In particular, it

- considered that the expression "lysis structure" comprised in claim 1 as granted was supported by the contents of the application as filed;

- submitted that D1 and D3 did not disclose all the features of claims 1 and 3 as granted, respectively; and

- argued that the claimed subject-matter was not obvious in view of the prior art cited.

V. The parties were summoned to oral proceedings.

VI. With a telefax dated 13 January 2003 and referring to the comments made by the board in the annex to the summons, the respondent filed eight sets of amended claims to replace the ones on file, including a set labelled "Main Claim Request" and a set labelled "First Auxiliary Claim Request". The respondent also indicated passages of the application as filed which it considered to support the amendments.

The independent claims 1, 21 and 22 according to the main request are identical with claims 1, 21 and 22 as granted, only the back-references in some of some dependent claims were amended.

VII. Oral proceedings took place on 23 January 2003.

During these oral proceedings, the respondent presented four auxiliary requests consisting of amended claims sets to replace the ones previously on file.

Independent method claim 21 of the first auxiliary request is identical with claim 22 as granted.

Independent device claims 1 and 20 of the second auxiliary request are identical with claim 1 and 21, respectively, as granted, except for the replacement of the term "analyte" by the expression "intracellular molecular component of a cell".

VIII. The parties' oral and written submissions, as far as they are relevant for the present decision, can be summarised as follows.

Concerning claims 22 and 21 according to the main and first auxiliary requests, respectively:

Upon being questioned by the board, the representative of the respondent did not withdraw or modify its earlier acknowledgement of the known bench scale methods as referred to in the contested decision, reasons 5.

The appellant argued that starting from bench-scale methods acknowledged as belonging to the prior art, the skilled person was generally incited by eg D6 to "miniaturise" these methods in view of the obvious advantages obtainable. The feasibility of capturing and fixing cells within microfabricated substrates was demonstrated by D3. On the other hand, the claimed method could also be considered as an obvious modification of the meso-scale cell-sorting methods known from D4 and D5.

The respondent considered D4 and D5 to represent the closest prior art. It argued that D4 and D5, which mentioned other meso-scale cell-sorters, had tackled the miniaturisation problem in a different manner requiring a sensing step/means and could not lead to the claimed invention. D6 was a rather theoretical document relating to the flow of liquids in meso-scale channels and was too general to suggest the claimed miniaturised method. D3 related to the fixation of cells within a substrate by proteins acting in a different, non-selective and glue-like manner, which technique was too different from the one relied upon according to the invention to render the latter obvious.

Concerning the claims according to the second auxiliary request:

The appellant submitted that the expression "lysing structure" comprised in claim 1 was intermediate in scope between the specific mechanical structures disclosed in the application as filed and the broader expression "cell lysing means", which also covered means based on the use of eg pressure shocks, ultrasound, heat or chemical agents. It argued that the effect of the amendment was to create a new group of means, ie the generic sub-group of mechanical structures. Referring to decision T 383/88 of 1 December 1992 (not published), and arguing that such a group was not unambiguously disclosed in the application as filed, it concluded that the expression "cell lysing structure" represented an inadmissible intermediate generalisation of the specific arrangements disclosed.

The respondent submitted that although the term "structure" was not explicitly used in the application as filed, its incorporation into claim 1, together with the other expressions "flow of cells", "to force cells ... into contact with" and "thereby to lyse cells" did not present the skilled person with any new information. Support for this amendment was also to be found on pages 5, 6 and 15 of the description as filed. The intermediate generalisation was thus based on the disclosure of the application as filed.

Concerning novelty of the device according to claim 1, the appellant, relying on the contents of the declaration D9, argued that the apparatus and conditions disclosed in D1 were suitable for lysing at least some kinds of cells. Some fraction of cells having cross-sectional dimensions larger than the ones of the channels (3) disclosed in D1 would inevitably be lysed

upon passage through the openings of the channels, depending on the cell membrane properties. The extent of cell lysis could be easily varied by varying the operating conditions of the apparatus. More particularly, during the use of the apparatus as disclosed in D1, some "old" red blood cells would inevitably be lysed. Haemoglobin released from the lysed cells would be detected by the camera means (6) which were to be considered as being arranged at least partly downstream of the lysis means. Although intended for different ends, the device of D1 thus showed all the features of granted claim 1. Concerning novelty of the subject-matter of claim 21 over D3, the appellant merely referred to the notice of opposition, without submitting any arguments as to why the appreciation of D3 by the opposition division was to be considered as wrong.

The respondent argued that the channels in the D1 device could not be called cell lysis structures simply because some cells may hypothetically happen to lyse when passing through them. Moreover, the devices disclosed in D1 did not operate at pressures high enough to cause cell lysing, and they did not comprise any detection means downstream of the cell lysing structures. Concerning novelty over D3, it referred to the conclusions arrived at by the opposition division.

Concerning inventive step, the appellant argued that methods and apparatus for the bench scale cell lysis and analysis, as exemplified in eg D14 or D15, were to be considered as the closest prior art. The underlying problem was the miniaturisation of the bench-scale processes, so that microvolumes of sample could be analysed. Miniaturising one part of the bench-scale operation of D14, ie the cell lysing means and the corresponding inlet channel, and putting the two discrete bench scale steps of lysing and detecting

together was obvious in the light of documents D3, D4, D5 and D6 which all related to bench scale cell handling operations miniaturised to a solid substrate.

The respondent pointed out that the cell disrupter of D14 was not associated to detection means and was not, unlike the device according to the claimed invention, adapted for an integrated system, even on the bench scale, let alone in a microfabricated system. None of D3 to D6 related to the lysing of cells and to the subsequent detection of an analyte in the lysed cell sample. Hence, there was no obvious way in which the equipment of D14 could be scaled down to arrive at a microfabricated substrate comprising a mesoscale flow system containing a lysing structure or agent, let alone at an integrated system further including detection means and which could be operated in a flow-through manner.

IX. The appellant requested that the decision under appeal be set aside and that the patent be revoked.

The respondent requested that the decision under appeal be set aside and that the patent be maintained

- on the basis of claims 1 to 24 submitted with letter of 13 January 2003 (main request) or, in the alternative,

- on the basis of claims 1 to 23 filed during the oral proceedings as first auxiliary request (first auxiliary request), or

- on the basis of claims 1 to 20 of the first auxiliary request (second auxiliary request), or

- on the basis of claims 1 to 22 submitted at the oral proceedings as second auxiliary request (third auxiliary request), or

- on the basis of claims 1 to 19 of the second auxiliary request submitted at the oral proceedings (fourth auxiliary request).

Reasons for the Decision

Main request

1. *Claim 22 - Inventive step*

1.1 Closest prior art

Claim 22 is directed to a method for separating a subpopulation of cells from a cell-containing liquid sample. During the oral proceedings before the opposition division the respondent had admitted that, on the bench scale, it was known "to separate sub-population of cells by flowing the cells through columns containing a binding protein specific for a cell membrane-bound protein characteristic of said target subpopulation and to elute then the bonded cells", see the contested decision, reasons 5. Although the file does not contain printed documents illustrating this prior art, the board has no reason to doubt that the said techniques indeed belonged to the prior art. Hence, on the bench scale, the essence and the underlying principle of the concept relied upon for the separation of a cell sub-population according to claim 22 was known before the filing date of the present patent. Consequently, the board shares the view

of the appellant that the known bench-scale technique can be considered to represent the closest prior art for the purpose of assessing inventive step.

1.2 Technical problem

In the board's view, the only feature which is not at least implicitly addressed in the acknowledgement, in the contested decision, of the bench-scale prior art, is the dimensioning of the flow channels through the column. The respondent has not pointed out or argued any further difference between the bench-scale technique and the subject-matter of claim 22. Assuming in the respondent's favour that the flow passages through the columns used on the bench scale have cross-sectional dimensions which are larger than the 500 µm mentioned in present claim 22, the technical problem can be seen in the provision of a further process with all the steps of present claim 22, but being suitable for treating samples of very small volumes. Apart from potential savings in terms of the size of the apparatus to be used for the separation of the cells, the board sees no further, let alone unexpected advantage that could be attributed to the flow channel related feature "cross-sectional dimensions of 0.1 to 500 µm".

1.3 Obviousness of the solution

1.3.1 For various known reasons, and in particular cost reasons (see eg the "Benefits" associated with the features "Small Size" and "Microscale" in Table I on page 4 of the patent in suit), miniaturisation was something like a trend in the field of devices for the (bio)chemical analysis of samples and of devices for the manipulation of cells. Even before the priority dates of the contested patent, a skilled person was thus incited to "pack" known methods into smaller devices such that they become suitable for handling

small sample volumes. This is confirmed eg by the passages on page 2, lines 12 to 16 and lines 23 to 33 of the patent. Moreover, a particular interest in the provision of flow systems microfabricated in solid substrates for applications such as the "modification and separation of biological cells" has already been generally formulated before the priority date of the contested patent, see D6, first page, "abstract" and the first paragraph under the heading "2. Introduction", which document specifically refers to a "Washizu biological cell shift register", the latter also being addressed in D5 (see point 1.4.3. below).

1.3.2 Moreover, the feasibility of cell retention on solid walls within microfabricated mesoscale flow systems was already known from D3, even though for other purposes and by means of substances acting more like a glue, see Figure 7 and page 12, lines 4 to 19. The physical and/or chemical techniques required for immobilising cell binding moieties on (flow channel) surfaces were also known as such, see eg the contested patent, page 6, lines 32 to 38.

1.3.3 Documents D4 and D5 both disclose methods for sorting individual cells, and hence for separating the cells of a sample into two subpopulations, by means of a microfabricated device comprising mesoscale channels for the transport of the cells through the device, see D4, the entire document and D5, pages 1735 and 1739. Whereas the presently claimed method is of a batch type (separate retention and elution steps) and relies on the physico-chemical properties of the cell surfaces and their bonding affinities to given cell capturing moieties, D4 and D5 relate to the sorting of individual cells by means of a continuous flow-through method requiring sensing means for characterising individual cells and generating a signal for actuating

mechanical or electrical sorting means. Due to these differences in terms of the separation principle relied upon, and contrarily to the position of the respondent, the board thus takes the view that D4 and D5 are less appropriate starting points for the assessment of inventive step. Nevertheless, D4 and D5 clearly show that the manipulation of cells, and more particularly the separation of subpopulations of biological cells, is feasible in microfabricated devices within mesoscale channels.

1.3.4 The board thus holds that the skilled person would consider a reduction of the cross-sectional dimensions of the flow passages as used in the known bench-scale method involving columns as a generally desirable measure, eg to save on the sample volume and the reagent costs, and was encouraged to tackle the miniaturisation by prior art such as D6. Moreover, as illustrated by eg documents D3, D4, D5 and D6, the feasibility of the miniaturisation of flow-channels, cell handling, cell retention and cell sorting on the mesoscale had already been demonstrated before the priority dates of the contested patent. Hence, the skilled person would consider carrying out the known bench-scale method within flow passages of mesoscale dimensions, the microfabrication of which was known as such, as an obvious solution of the stated technical problem.

1.4 Hence, the subject-matter of claim 22 is not based on an inventive step as required by Article 56 EPC. Consequently, the main request cannot be allowed.

First auxiliary request

2. Claim 21 according to this request is identical in wording with claim 22 according to the main request.

Hence, for the same reasons as given here above concerning claim 22 of the main request, the subject-matter of claim 21 of the first auxiliary request is not based on an inventive step as required by Article 56 EPC. Consequently, the first auxiliary request cannot be allowed either.

Second auxiliary request

3. *Amendments*

3.1 Claim 1

3.1.1 During substantive examination of the patent in suit, claim 1 as originally filed was modified by incorporating several features taken from dependent claims 2 (flow inducing means), 3 (cell lysing), and 7 (detection means), as well as from page 5 of the description (definition of mesoscale flow system) as filed. In present claim 1, the expression "cell lysing means", as contained in claim 3 as originally filed is replaced by the expression "cell lysing structure". According to the appellant this amendment is not sufficiently based on the application documents as originally filed and, therefore, objectionable under Article 100(c) EPC.

In the discussion of this issue, it was common ground -

- that the term "structure" has no literal basis in the application as filed;

- that this term relates to mechanical lysing means only, and does not encompass cell lysing means relying on other principles such as the use of heat, ultra-sound or chemical compounds;

- that the term "structure" is, therefore, narrower in scope than the expression "lysing means" as used in claim 1 of the application as filed;

- that the specific embodiments disclosed in claims 4 to 6, in the paragraph bridging pages 5 and 6 and on page 15, second paragraph, of the application as filed are all "structures" in the sense of granted claim 1; and

- that "lysing structures" fall somewhere between "lysing means" in general and the said specific embodiments, and that the expression "lysing structure" could therefore be considered as "intermediate" generalisation" of the said specific embodiments.

The board also accepts that in view of the language of claim 1 as amended, the cell lysing means to be used are in fact restricted to those mechanical structures which require the cells to be flowed in contact with and past them in order to be lysed by this (mechanic) interaction, see the expressions "flow of cells", "into contact with said lysing structure", "thereby to lyse cells", and "downstream of said cell lysing structure". On the other hand, the board holds that in view of language used in the applications as filed in connection with the description of this type of cell lysing mechanical structures, and in particular in view of the terms "eg" and "may" as used on page 6, lines 1 and 6, the disclosure of the application as filed is **not** restricted to the specific embodiments described in the passages quoted here above, which are considered as examples forming a non-exhaustive list. Other embodiments of lysing means working according to the same lysing principle, ie requiring the flow of the cells to be lysed along and past a mechanical type

means, ie something that can be labelled a "structure", are in no way excluded. In view of the above, the board is convinced that the skilled person is clearly and unambiguously being presented with the information that, within the broader group of all kinds of lysing means, means comprising a mechanical means, ie a mechanical structure, against and past which the cells are to be flowed to be lysed, are a preferred sub-group of lysing means. The mechanical means shown in the examples given are sufficient in number and detail to illustrate the lysing principle and to further support and justify the use of the more generic term "structure", ie the intermediate generalisation addressed by the appellant. Therefore, this finding is also in compliance with decision T 383/88 cited by the appellant, see reasons 2.2.2, first paragraph thereof.

Summarising, the board comes to the conclusion that the replacement, in claim 1, of the expression "lysing means" by the narrower expression "lysing structure", together with the incorporation of the further features specifying the operating principle of these particular lysing means, finds sufficient support in the application as filed, and is not objectionable under Article 123(2) EPC.

3.1.2 After some discussion at the oral proceedings concerning the original disclosure of the combination of features comprised in claim 1, the latter was further amended by the incorporation of features taken from claim 3 as granted. In comparison with the wording of claim 3 as granted, the term "the presence of" has been suppressed in the expression now reading "detecting an intracellular molecular component of a cell". The appellant has pointed out this fact without, however, indicating why this omission could justify its corresponding objection under Article 100(c) EPC. In the board's view, the amendment further restricts the

claim, ie with respect to the detection means encompassed, and it is sufficiently based on claim 3 as granted (claim 7 of the application as filed). Since the detection of a molecular component requires its presence, the omission of the expression "the presence of" does not imply any change in meaning. The board is thus satisfied that this amendment is also not objectionable under Article 123(2) or (3) EPC.

3.2 Claim 20

The board is satisfied, and it was not in dispute, that claim 20 as amended during the oral proceedings is based on claims 1, 2, 3 and 7, and on the second paragraph of page 15 of the application as filed (claims 1 and 3 and page 5, lines 24 to 32 of the contested patent). Concerning the omitted expression "the presence of", the same considerations apply as in the case of claim 1. Hence, the amendment meets the requirements of Article 123(2) and (3) EPC.

3.3 Dependent claims

The wording of the remaining dependent claims 2 to 19 is the same as to the one of the corresponding claims 2 and 4 to 20 of the granted patent, except for their re-numbering and for the adaptation of all and the restriction of some of the back-references. The respondent has indicated description passages and claims of the application as filed forming a basis for these claims and for the amendments carried out during the appeal proceedings. It was not disputed, and the board sees no reason for questioning, that the amendments carried out in the dependent claims are in compliance with requirements of Articles 123(2) and (3) EPC.

4. *Sufficiency of disclosure*

In its earlier notice of opposition, the appellant had raised an objection under Article 100(b) EPC. In its grounds of appeal it maintained its positions set out in the notice of opposition. However, during the entire appeal proceedings the appellant did not specify any reasons for which it considered the decision of the opposition division to be wrong in this respect. The board is not aware of any reason that would justify the overturning of the decision of the opposition division, and therefore shares the latter's view that the disclosure of the patent is sufficiently clear and complete for it to be carried out by a person skilled in the art.

5. *Novelty*

5.1 Independent claim 1 - Document D1

5.1.1 Document D1 relates to a device for measuring the rheological properties of biological cells, eg the locations, velocities and deformations of red blood cells as occurring during their passage through capillaries. The apparatus disclosed undisputedly comprises a silicon substrate micromachined to comprise a mesoscale flow-system with channels (3) having a length of typically 100 µm and a cross-section of eg 5 µm x 5 µm and reservoirs (5) having a depth of 15 µm, as well as flow regulating means (9), eg a piezoelectric pump, for applying a pressure differential across the channels, ie for inducing the flow of a liquid cell sample through inlet and outlet ports, the reservoirs and the channels. The pressure differentials applied across the channels are said to be typically in the physiological range of 2 to 20 mm Hg. Moreover, the device disclosed comprises a microscope and a video camera (6) for monitoring the

position, shape and/or speed of the cells moving within the channels. In the case of red blood cells, the contrast of the images is improved by the use of illumination in the far visible violet range, which is absorbed by the haemoglobin in the cells. See in particular claims 1, 4, 6 and 7, Figures 2, 3 and 4, page 1, lines 2 to 4, page 3, line 29 to page 4, line 11, page 4, line 29 to page 5, line 4 and page 6, line 14 to page 11, line 10.

5.1.2 It was not disputed that the dimensions of the channels (3) described in D1 fall within the mesoscale range and are regions of restricted cross-section as referred to in present claim 2, alternative (c). Moreover, the board accepts that the camera (6) shown in D1 is, in the broadest sense of the wording of claim 1, a means suitable for merely detecting the presence of the intra-cellular molecular component haemoglobin, irrespective of whether lysis of the cells occurs or not. However, lysing of cells or any dedicated means for this purpose are not mentioned in D1. D1 cannot, therefore, be considered to generally disclose devices suitable for lysing cells. Since D1 relates to devices for monitoring the rheology of cells, *inter alia* within the microfabricated channels, the skilled reader would understand that lysing of the cells to be observed upon entrance in, as well as within the channels is to be avoided, see also Figure 4 and page 9, line 29 to page 10, line 2. Therefore, it would not consider channels (3) to be lysing structures. The question that needs to be answered in the examination of novelty is thus whether or not the devices specifically disclosed in D1 show all the constructional features of present claim 1 and could be considered to be suitable for the purpose of lysing certain kinds of cells. Since it is common ground that the devices disclosed in D1 are intended for a different purpose, the subject-matter disclosed in this document could only accidentally fall

within the wording of present claim 1, without there being a common technical problem. Therefore, a particularly careful comparison has to be made between what can fairly be considered to fall within the wording of claim 1 and what is effectively shown in D1, see also decision T 161/82, OJ 1984, 551, reasons 4.

5.1.3 By means of the declaration D9 the appellant alleges that a certain fraction of cells having larger cross-sectional dimensions than the 5 µm by 5 µm opening of the channels (3), such as eg red blood cells or white blood cells, would inherently lyse in the device of D1 under the conditions described therein, due to the high shear forces acting on the cells forced against the sharp channel opening. Moreover, the extent of lysis achieved for a given type of cell could be easily manipulated by varying the system pressure, the dimensions of the channel, the cell suspending liquid medium and could also depend on pre-treatment of the cells, see sections 4 to 6 of D9.

5.1.4 It was not disputed that cells could in principle be lysed by forcing them, under pressure, through regions of cross-sectional dimensions smaller than the dimensions of the cells concerned, see eg the contents of D14 discussed below. However, D1 itself shows that the particular size and shape of the inlet openings of the capillary channels (3), which are not explicitly said to have sharp edges, are not as such sufficient to necessarily have a lysing effect on cells having a greater cross-section. Under the flow conditions referred to in D1, the larger cells, eg red blood cells, deform and pass these constrictions, see Figure 4 and page 9, line 29 to page 10, line 2. This behaviour of red blood cells is also confirmed by D2, see page 98, right-hand column, second paragraph. It is thus clear that particular conditions such as a relatively high pressure would be necessary to lyse

cells by forcing them through the inlet openings of the channels (3). The pressure differences to be applied according to D1 are "typically" in the physiological range, see page 9, lines 25 to 28. Although, as pointed out by the appellant, this particular wording does not strictly limit the pressure differences to the physiological range indicated ("2-20 mm H_2O"), the board does not see any reason, and the appellant has not indicated any, for which this sentence should be regarded as a disclosure of means suitable for generating greater pressure differences, with more profound effects on cell integrity. The board also takes the view that D1 does not disclose devices having other specific channel dimensions, ie cross-sectional dimensions and lengths larger or smaller than the ones mentioned on page 6, lines 25 to 27 and page 7, lines 14 to 25.

5.1.5 In view of the foregoing considerations, the board takes the view that the conclusions drawn by the appellant in declaration D9, cannot be accepted in the absence of supporting evidence conclusively demonstrating that specific types of larger cells, eg white blood cells, would inevitably lyse in significant amounts upon entry into the specific 5x5x100 µm channels of D1 at low pressure differentials of from 2 to 20 mm H_2O, rather than changing their shape and entering the channels without lysing, or merely plugging the inlet opening without lysing.

5.1.6 According to another line of argument of the appellant based on D9, some unknown fraction of "old" blood cells, or of some other unspecified and possibly pre-treated cells could hypothetically be lysed when flowed against and through the inlet opening of the channels under appropriate conditions. The passage of D2 (page 105, the paragraph bridging the two columns) quoted by the appellant in this connection is, however,

silent about the dimensions of the "tight spots" referred to that could lead to the rupturing of fragile red blood cells in the human body. Hence it cannot be gathered from D2, and the appellant has not submitted any evidence showing that "old" red blood cells would indeed be lysed in significant amounts at the inlet of the channels (3) as described in D1 at physiological pressure differences.

5.1.7 Moreover, the board is convinced that a skilled person would not consider an apparatus, or a particular part thereof, wherein, upon operation, an small but unknown proportion of old or otherwise shear-sensitive cells of unidentified properties may hypothetically lyse as a "lysing structure". Rather, the expression "lysing structure" must be understood to stand for an apparatus or a distinct part of an apparatus which is clearly suitable for reliably and reproducibly lysing a significant proportion of a sample of a certain type of cells passing therethrough. In the context of D1, these structures must, moreover, be able to lyse cells at physiological pressures. The appellant has not, however, submitted evidence showing that specific kinds of cells, having cell membranes so weak that they would lyse when flowed against and through the 5x5 µm channel inlet openings (3) at physiological pressures as described in D1, would mainly be lysed at this location, rather than in all the other parts of the device where shear forces occur due to the flow of the suspension medium, such as in the sample reservoirs, pump means, conduits, and upon entrance and within capillaries.

5.1.8 Summarising, the board is not convinced that the declaration D9 and the arguments submitted by the appellant suffice to establish that D1 provides a clear and unambiguous disclosure of an apparatus comprising distinctive means that could be considered to

inevitably act as cell lysing means when operated at physiological pressure differences with at least one specific kind of cell. Hence, the device according to present claim 1 is found to be novel over the disclosure of D1.

5.2 Independent claim 21 - Document D3

Upon filing of the appeal, the appellant had again challenged the novelty of the device according to claim 21, basing its objection on the disclosure of D3. However, upon being questioned by the board during the oral proceedings, it did not present any argument as to why the detailed reasoning of the opposition division (see impugned decision, reasons 4.2) was wrong. The board itself sees no reason to question the assessment, by the opposition division, of the novelty over D3, or the positive result of this assessment. The finding of the opposition division is not affected by the further restricting amendment carried out in this claim during the appeal proceedings. Hence, the board upholds the decision of the opposition division, that the subject-matter of claim 21 is novel over the disclosure of D3. The board also holds that under the present circumstances, it need not give any further detailed reasons for its decision.

5.3 The board is also convinced that none of the other prior art documents relied upon by the appellant discloses devices with all the features of the present independent claims 1 or 20, let alone of the dependent claims 2 to 19. The differences between the claimed devices and the devices disclosed in the most relevant prior art documents will also become apparent from the following discussion of inventive step.

5.4 The board concludes that the subject-matter of claims 1 to 20 is novel over the prior art cited by the appellant.

6. *Inventive step*

6.1 Construction of claims 1 and 21

During the oral proceedings before the board, it was common ground that the flow inducing means and the detection means need not necessarily be microfabricated and/or integrated with the lysis on a same solid substrate. However, as argued by the respondent, the different parts of the claimed device need to be adapted to be put together in fluid communication in a manner permitting the flow-through operation of the device, thereby constituting an "integrated" system. As far as the integration of the detection means is concerned, this interpretation is clearly supported by the use of the term "downstream" in claim 1.

6.2 Closest prior art

6.2.1 As accepted by the appellant, documents D1 and D3 do not have the explicit objective of providing a device for carrying out the methods associated with the devices of present claims 1 and 20. Hence, these documents which concern the examination of the behaviour of living cells (see also D3, page 3, lines 2 to 23) cannot represent the closest prior art in the assessment of inventive step.

6.2.2 The board concurs with the appellant that the known bench-scale devices for lysing cells of a sample and subsequently detecting intra-cellular molecular analytes in the lysed sample can be considered as the

closest prior art for the purpose of assessing inventive step. Such bench-scale devices and techniques are, for instance, disclosed in D14 and D15.

(i) D14 discloses a cell disrupter comprising a stainless steel cloth (screen) and its use in the isolation of cell components such as membranes and nuclei. Cells of 10 to 20 µm are disrupted by shearing forces set up as the cells suspended in liquid are forced through the small pores (in the micrometer range) of the metal screen by applying a positive N_2 pressure of 40 psi. A microchemical assay is used for separately measuring the DNA in the lysed sample after centrifugation thereof. See in particular the abstract on page 453, page 453, left-hand column, last full sentence, Figure 1 on page 454, page 454, right-hand column to page 455, left-hand column, the section labelled "Description", and page 454, right-hand column, the section labelled "DNA measurements".

(ii) D15 generally refers to various bench-scale methods and devices for lysing cells. More particularly, it refers to physical methods such as, *inter alia*, pressure shearing, where high shear forces are generated as the cell suspension is forced through a small orifice under high pressure, but also to methods involving chemical agents, such as enzymatic or osmotic lysis. D15 also mentions the examination of the lysed cell sample for eg nucleic acids after centrifugation. See in particular page 53, left-hand column, first and second full paragraph and the section labelled "5.1.1 Pressure Shearing" and page 55, the sections labelled "5.1.5 Muramidase Digestion" and "5.1.6 Osmotic Lysis".

(iii) D14 and D15 do not disclose devices comprising a lysing structure or agent arranged within a mesoscale flow system and arranged upstream of detection means in a flow-through manner.

6.3 Technical problem

The contested patent mentions various "objects of the invention" each consisting in providing analytical systems which fulfill a multitude of requirements, see page 2, lines 42 to 48. Considering the features actually contained in independent claims 1 and 20 and their appropriate interpretation, the technical problem to be solved in view of the closest prior art can in any case be seen in the provision of further analytical systems suitable for rapid, automated and economic analysis of microvolumes of cell containing samples and detection of intra-cellular molecular components therein. The board finds it plausible, and it has not been disputed during the appeal procedure, that the said technical problem is solved by the devices according to claims 1 and 20.

6.4 Non-obviousness of the solution

6.4.1 Documents D14 and D15 do not address the issue of the analysis of particularly small samples or the automation of the separate method steps disclosed.

6.4.2 D6 illustrates the general interest in providing flow systems of very reduced size, including flow inducing means, for use in diverse applications such as the "modification and separation of biological cells", and the "detection and quantitation of macromolecules". Specifically, D6 mentions applications of the type

disclosed in D1 and D5, see Figure 2, sheet 4, fourth paragraph and sheet 6, last paragraph. However, D6 is silent about the detection of intra-cellular molecular component of cells previously lysed.

6.4.3 As already mentioned above, D4 and D5 disclose devices for individually measuring a characteristic cell parameter and sorting/separating the cells in response to the measured signal. Although these documents illustrate the feasibility of cell manipulations within mesoscale flow systems, they do not relate to the analysis of intracellular molecular components at all, let alone of analytes made accessible to detection by previously lysing the cells.

6.4.4 The methods and devices disclosed in D3 concern the examination of effects of cell affecting agents on the metabolism of living cells retained within a microfabricated substrate comprising a mesoscale flow system. Means are provided for flowing a solution of the cell affecting agent through the flow system. The metabolism of the cells is monitored by measuring extra-cellular electric potentials by means of appropriate sensors. See eg page 3, lines 2 to 23. D3 is not, however, concerned with the detection of intracellular molecular components of cells. In Example 20 particularly relied upon by the appellant, the production of acidic metabolites is monitored by measuring the pH outside of the cells, but no particular intracellular molecular component of the cells is detected. Hence, the board holds that the objectives of D3 are so different from the ones of the present patent that the skilled person would not consider the contents of D3 at all when looking for a solution to the stated technical problem.

6.4.5 Even considering that miniaturisation of analytical devices was generally desirable before the priority dates of the contested patent, the particular combination and **integration** of lysing, intra-cellular analyte detecting and flow-inducing means into a **flow-through** device, wherein a sample flow-channel and a cell handling region comprising the lysing structure or agent are microfabricated into a solid substrate, could thus not be derived in an obvious manner from the disclosures of documents D14, D15 and D3 to D6, whether taken alone or in combination, by a skilled person having no knowledge of the present invention.

6.4.6 The board is convinced, and it was not disputed, that the prior art disclosed in the other documents cited by the appellant does not come closer to the invention and that these documents do not contain any more relevant information.

6.5 Hence, the subject-matter of claims 1 and 20, and consequently of dependent claims 2 to 19, according to the second auxiliary request is found to be based on an inventive step.

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 maintain the patent with the following documents:

   - Claims 1 to 20 (second auxiliary request)

   - A description and drawings to be adapted.

The Registrar: The Chairman:

U. Bultmann R. Spangenberg