(19) NL Octrooicentrum (11) **2002055**

(12)C **OCTROOI**

(21) Aanvraagnummer: **2002055**

(22) Aanvraag ingediend: **03.10.2008**

(51) Int.Cl.:
B01F 11/00 (2006.01) ***B01F 15/00*** (2006.01)
G01N 21/25 (2006.01)

(43) Aanvraag gepubliceerd:
-

(47) Octrooi verleend:
06.04.2010

(45) Octrooischrift uitgegeven:
14.04.2010

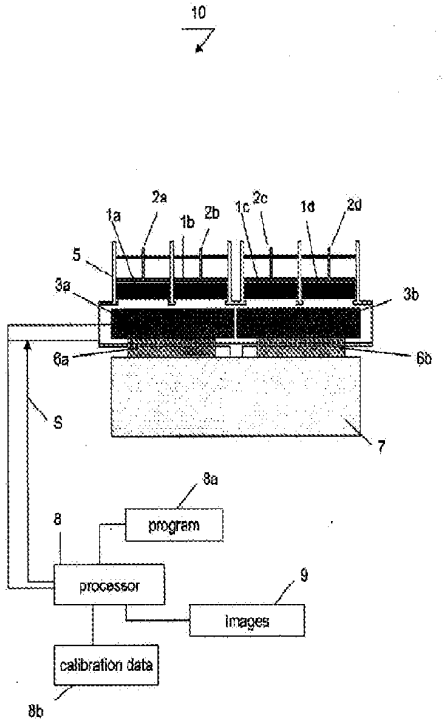
(73) Octrooihouder(s):
Enzyscreen B.V. te Amsterdam.

(72) Uitvinder(s):
Wouter Adriaan Duetz te Heemstede.

(74) Gemachtigde:
Drs. M.J. Hatzmann c.s. te Den Haag.

(54) An apparatus and a method for investigation of microtiter plates subjected to orbital shaking.

(57) The invention relates to an apparatus, a method and a computer program for investigation of microtiter plates subjected to orbital shaking. The apparatus 10 comprises a housing 7, microtiter plates 1a, 1b, 1c, 1d comprising wells, which may be arranged under respective clamp elements 2a, 2b, 2c, 2d arranged to hold the microtiter plate down to the frame 5. The apparatus 10 further comprises a processor 8 conceived to interrupt the motor of the frame 5 for a suitable interrupt period and to trigger the optical devices 3a, 3b for generating two-dimensional images of respective bottoms of the wells present in the microtiter plates 1a, 1b, 1c, 1d. The optical scanning devices 3a, 3b are preferably mounted on respective frames 6a, 6b for support. The frames 6a, 6b may be attached to the housing 7. The processor 8 may be operable using a computer program 8a. The processor may be arranged to store the acquired images in a suitable memory unit 9. Alternatively or additionally, the processor 8 may be arranged to carry-out on-line or off-line analysis of the scan data using calibration data 8b and to adapt shaking mode using a signal S.



NL C 2002055

P86185NL00

Title: An apparatus and a method for investigation of microtiter plates subjected to orbital shaking

FIELD

The invention relates to an apparatus and a method for investigation of the progression of microbial and cell growth in microtiter plates subjected to orbital shaking. The invention further relates to a computer program for enabling investigation of microtiter plates.

BACKGROUND OF THE INVENTION

When working with relatively large numbers of strains or mutant libraries, use of the standard microtiter plate format (with 24 or 96 wells of 0.2 to 11 ml) is attractive because of the availability of compatible robots, multipipettes, readers, and other equipment. Use of microtiter plates for the cultivation of microorganisms has developed rapidly in the last decade, to such an extent that they now are a mature alternative to traditional cultivation vessels such as Erlenmeyer shake flasks, see, for example Duetz, W.A. "Microtiter plates as mini-bioreactors: miniaturization of fermentation methods", TRENDS in Microbiology 2007, 15 (10): 470 – 475. Especially, development of a "sandwich cover" clamped on top of the microtiter plate has been of large importance as it allows the vigorous orbital shaking (at centrifugal forces up to 2.5) in the absence of spilling of the culture fluid or occurrence of cross-contamination between wells. This vigorous orbital shaking is often necessary for sufficient mixing and oxygen supply into the microbial cultures. The small culture volumes in microtiter plates (generally in the range of 0.1-4 ml) allow a large cost saving in terms of amount of culture medium required as well as the number of orbital shakers required for their simultaneous cultivation. However, the disadvantage of small culture volumes is that sample volumes taken from such cultures (e.g. for the determination of the density of the culture) must be extremely small as well, often too small for

reliable measurements. Therefore, there is an increasing need for non-invasive techniques to determine the cell densities is such cultures.

The main presently used non-invasive technique to determine cell densities is based on capability of cells to scatter (change the direction of) visible light, increasingly so at higher cell densities. When an individual microbial cell in a suspended culture is hit by light in the visual area, it will change the direction of this light in a largely random way. This process is commonly referred to as "light scattering" and is caused by a combination of reflection on the cell surface and refraction when the light is not reflected on the surface but travels through the cell and its cytoplasm. This principle of light scattering by cell suspensions has been used for the quantification of cell densities for many decades, mainly by measuring the percentage of transmission of light (often in the area between 500 and 650 nm) through a culture. This method is usually referred to as "optical density measurements" and has also been applied as well for microbial cultures in microtiter plates and similar arrays of wells by various suppliers of per se known laboratory equipment.

In addition to light transmission determinations, measuring the amount of light that is reflected by the culture (typically under angles of between 135 or 180 degrees) for quantifying cell mass may be applied, as described by Mullaney and Dean "The small angle light scattering of biological cells", Biophysical Journal 10: 764-772, 1970, and Kohanovsky "The dependence of the diffuse reflection coefficient of blood on the concentration of red cells", J. Colloid and Interface Science 208: 575-577, 1998, and is also known from EP1730494. In an apparatus known from EP 1730494 a single sensor for each microtiter plate well is used.

The common factor of all the above applications of light scattering for quantifying microbial cell densities is that the equipment used functions with individual light sources (a single beam) for each well in the microtiter plate, and individual sensors for each individual well.

Disadvantages of this approach are i) the relatively large economical costs with increasing number of wells, and ii) no information is obtained on the morphology of the cultures, especially with regard to the homogeneity of the culture and the possible presence of aggregates or clumps of cells.

SUMMARY OF THE INVENTION

It is an object of the invention to provide an apparatus for investigating the progression of eukaryotic cell or microbial growth in orbitally shaken microtiter plates with increased efficiency and, optionally, with increased accuracy.

To this end, in accordance with the invention an apparatus for investigating orbitally shaken microtiter plates comprises:

- a shaker arranged to orbitally shake a plurality of wells present in the microtiter plates;
- an optical scanning device conceived to generate one or more images of the bottom of said plurality of wells and
- a control unit arranged to interrupt the shaker and to cause the optical scanning device to generate said one or more images during a period of said interrupt.

The invention is based on the insight that in order to overcome limitations of apparatus known from the art, it is possible to apply a suitable scanning device, for example a CCD-flatbed scanner device for generating two-dimensional images of the wells present in the microtiter plates positioned in

the shaker. Preferably, a CCD array used in the optical scanning device is one-dimensional. It will be appreciated that suitable commercially available flatbed-scanners may be adapted in terms of steering software, scanning area, light bundling, housing etc. for enabling mounting thereof on an orbital shaker. Preferably, the optical scanning device is arranged to generate visible light, more preferably, the optical scanning device may be arranged to generate light in a range of 400 nm – 650 nm, or even higher, for example, in the infra-red range. It will be appreciated that any other optical devices which may be adapted to enable generation of suitable two-dimensional images of well bottoms may be used. Preferably, the optical scanning device does not undergo shaking. The present invention, thus, concerns an apparatus that allows the orbital shaking of microbial or cell cultures in microtiter plates at a high shaking amplitude and frequency, and enables generation of a high resolution image of the bottom of a suitable plurality of wells of one or more microtiter plates, using an optical scanning device mounted under the microtiter plates (see Figure 1), during a short time interval during which the orbital movement is interrupted.

Preferably, the orbital shaker is combined with a rack system for mounting microtiter plates for shaking in a minimal distance above a statically attached frame of the optical scanning device.

It will further be appreciated that suitable plurality of microtiter plates may be arranged in the shaker, for example, black and white 24-, and 96-round low well microtiter plates with transparent bottoms, as known per se in the art. Preferably, a period of interrupt is set to about 10 - 40 seconds for allowing scanning of the microtiter plates using the optical scanning device, after which the shaker resumes orbitally shaking the microtiter plates. It will be appreciated that duration of the interrupt is dependent on a time necessary for a medium present in the wells to substantially come to rest and a time

necessary for the optical scanning device to generate the two-dimensional images of the wells bottoms.

The operation of the apparatus according to the invention is based on the following insights. It is found that the most significant fraction of light scattered by particles and cells is refracted and reflected in the forward direction of the incident light beam. That implicates that a major part of light returning from a suspension (scattering in the opposite direction of the incident light beam) must have been refracted by various cells. Assumed that each refraction event alters the initial direction of a light beam less than 30° and all particles are randomly distributed, numerous particles are necessary for a 180° turn of the light beam. Furthermore it can be concluded that the higher the concentration of a suspension, the more evenly will be the dispersion of the fractions of each light-beams in all directions. Absorption or reflection effects at the boundaries of a suspension influence the intensity of light re-emitted from the suspension. Theoretically, this has a major impact on light-scattering measurements in non-infinite suspensions such as microtiter plate wells. Hence, the influence of the suspension filling height, vessel-diameter and color of the vessel-wall and the influence of the area of illumination was investigated in order to establish a better understanding of light-scattering phenomena in cell-suspensions and in a practical point of view to detect potential sources of error for light-scattering measurements in microtiter plates.

Preferably, in the apparatus according to the invention, the shaker frame is arranged to receive microtiter plates with dimensions of 86 x 128 mm^2. This has an advantage that the standard, most frequently used, mictrotiter plates may be utilized, improving usability of the apparatus according to the invention in laboratories.

In a further embodiment of the apparatus according to the invention, it further comprises a processor arranged to determine a growth-related parameter for a culture present in respective wells of said microtiter plates based on the images and pre-determined calibration data.

It is found preferable to enable the apparatus according to the invention with some analytic features, for example with features pertaining to determination of a growth-related parameter. Although the apparatus according to the invention may be arranged for storing the image data for later analysis, it is also possible to arrange the apparatus according to the invention to carry-out an on-line analysis of the growth-related parameter. Preferably for the growth-related parameter respective cell-mass concentrations are selected.

In order to avoid inaccuracies pursuant to characteristics of a microtiter array under consideration, it is preferable to provide a dedicated calibration data for each type of well of the microtiter plates to be analyzed in the apparatus.

For example, a suitable calibration data may be based on a calibration equation, comprising a suitable number of empirically determined variables, for example setting out a relation between image density and a corresponding cell-mass concentration. For example, a suitable calibration equation for the light scattering progressions may have a following analytical form:

$$f(x) = a + b(x + c)^d,$$

wherein

f(x) is light scattering as a function of cell mass in the well,

a, b, c, and d – are parameters, empirically determined for each combination of specific microtiter plate and type of microbial or eukaryotic cells.

For example, the calibration curve f(x) may be different for black 24-low well microtiter plates, white 24-low well microtiter plates, grey 24-low well microtiter plates, black 96-low well microtiter plates, white 96-low well microtiter plates, and so on, see Figure 4.

It has been found that there is a good agreement between the calibration curves f(x), and the light scattering progressions demonstrated that a function of only four parameters is sufficient to establish a mathematical correlation between the image data as measured by the optical scanning device of the apparatus according to the invention and the amount of cell-mass per ml present in the microtiter plates.

Preferably, in the apparatus according to the invention the processor is arranged to select pre-determined calibration data from a plurality of pre-determined calibration data based on a type of the microtiter plates present in the shaker.

The invention further relates to the method for investigating orbitally shaken microtiter plates. Advantageous embodiments of the method according to the invention are recited in claims 11, 12 and 13.

The invention further relates to a computer program product for causing a processor to carry out the steps of a method as set forth in the foregoing.

These and other aspects of the invention will be discussed with reference to drawings. It will be appreciated that drawings are presented for illustrative purposes and may not be used to limit the scope of the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents a schematic view of an embodiment of an apparatus according to the invention.

Figure 2 presents a schematic view of a detail of the apparatus shown in Figure 1.

Figure 3 presents a schematic view of an exemplary embodiment of images provided by the apparatus according to the invention.

Figure 4 presents in a schematic way calibration data that may be used by the apparatus of Figure 1.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1 presents a schematic view of an embodiment of an apparatus according to the invention. The apparatus 10 comprises a housing 7 onto which respective constructive parts of the apparatus may be mounted. In accordance with the invention, suitable microtiter plates 1a, 1b, 1c, 1d comprising wells (not shown) may be arranged under respective clamp elements 2a, 2b, 2c, 2d arranged to hold the microtiter plate down to the frame 5 for preventing displacement of the microtiter plates with respect to the frame 5 during shaking. The orbital shaking is enabled by the frame 5 which is suitably driven by a motor (not shown). The motor may be adaptable with regard to the shaking velocity and shaking amplitude.

In accordance with the invention, the apparatus 10 further comprises a processor 8 conceived to interrupt the motor of the frame 5 for a suitable interrupt period, for example for 10 – 40 seconds, and to trigger the optical devices 3a, 3b for generating two-dimensional images of respective bottoms of the wells present in the microtiter plates 1a, 1b, 1c, 1d. Although in this embodiment two optical scanning devices are illustrated, it will be appreciated that any number of the optical scanning devices may be applicable, including a sole optical scanning device. Preferably, the optical scanning device comprises a one-dimensional array of detector elements (not shown). Preferably, for the optical scanning device a flatbed scanner is used. The optical scanning devices

3a, 3b are preferably mounted on respective frames 6a, 6b for support. The frames 6a, 6b may be attached to the housing 7.

After respective scans are generated, the frame 5 resumes orbital shaking of the microtiter plates 1a, 1b, 1c, 1d. It will be appreciated that in accordance with the invention, the orbital shaking of the microtiter plates comprising wells provided with a suitable biologic material is interrupted during period of cell growth for providing intermediate data on cell-mass concentrations and/or morphology. For example, shaking amplitude may be set to 50mm and a shaking velocity may be set to 300rpm to reach oxygen transfer rates in the order of 30-40 mmol O2/L/h. At such conditions the aerobic bacterial strain *Pseudomonas putida CA-3* may reach cell densities up to 9 g (dry weight) L-1 during growth on a glucose mineral medium.

Preferably, the processor 8 may be operable using a computer program 8a which comprises suitable instructions for interrupting the shaker frame 5, for enabling the optical scanning devices 3a, 3b to generate two-dimensional images of the bottom of the wells present in the microtiter plates 1a, 1b, 1c, 1d and for resuming orbital shaking by the frame 5.

The processor may be arranged to store the acquired images in a suitable memory unit 9. Alternatively or additionally, the processor 8 may be arranged to carry-out on-line or off-line analysis of the scan data using calibration data 8b. The calibration data are provided per microtiter plate type and relate image density of two-dimensional images generated by the optical devices 3a, 3b with a growth parameter of a cell culture present in the microtiter plates. Preferably, for the growth parameter a cell-mass concentration is used.

Preferably, the apparatus 10 according to the invention is arranged to suitably modify operational parameters of the motor of the orbital shaker, causing the frame 5 to correspondingly alter the shaking mode to be applied to the microtiter plates. For example, the processor 8 may be provided with reference data corresponding to a desirable growth curve (not shown). When, based on the latest acquired two-dimensional images, it is found that the growth curve of cultures, currently present in the wells of the microtiter plates, deviates from the reference data, the shaking mode can be modified. For this purpose, the processor 8 may provide a signal S to the motor of the shaker to adapt the shaking velocity and/or the shaking amplitude. After suitable adaptations are fulfilled, orbital shaking may be resumed.

Figure 2 presents a schematic view of a detail of the apparatus shown in Figure 1. The microtiter plates 2 may comprise a cover 24, preferably a rubber cover, for limiting evaporation of a medium from culture 22 present in the wells. The microtiter plates 2 comprise walls 25 for mutually separating individual wells of the microtiter plate. Preferably, a bottom of the microtiter plate 26 is transparent for reducing loss of light generated by a light source 28 of the optical scanning device as well as loss of the reflected light and scattered light. The apparatus 20 may comprise a protective transparent plate 27 for protecting scanning elements of the optical scanning device. Preferably, the optical scanning device is mounted on a frame 29, which supports the light source 28 and a CCD element 29a, which may be a one-dimensional array. Preferably, the frame 29 is adjusted for allowing a distance D between the light source and the bottom of the wells of the microtiter plates to be as small as about 10 mm. During scanning, the optical elements 29 may be translated along a direction L and the source 28 is arranged to illuminate respective wells so that in incidence angle θ of about 30 degrees is enabled. This angle of about 30 degrees prevents direct reflections from the bottom of the microtiter plates

to reach he CCD, which would take place at some spots in case a two dimensional CCD (as present in photo cameras) were applied.

Figure 3 presents a schematic view of an exemplary embodiment of images provided by the apparatus according to the invention. Images 30 depict subsequent two-dimensional images of respective bottoms of 6 wells from a 96-well microtiter plate having wells with white walls and a transparent bottom. The images are generated at times 0, 3 hours, 9 hours, 13 hours and 16 hours. The 6 wells have been inoculated with various bacterial strains at time t = 0 into Nutrient Broth medium, and were shaken at 300 rpm, 50 mm amplitude at 30 degrees Celsius. The shaking was interrupted for 30 seconds each hour, allowing the two-dimensional images to be automatically acquired in accordance with the invention. It is clearly visible that the cell density in well A1 is gradually increasing in time, see items 31, 32, wherein the cell culture is homogeneous at all times. In contrast, the microbial strain in well B2 is growing in a non-homogeneous way, see items 34, 35; wherein image portion 35 clearly visualizes aggregates of cells. The apparatus and the method according to the invention have, next to accurate and dynamic monitoring of cell growth, an additional advantage, as information on aggregates is provided. Such information is not obtainable by the apparatus of the prior art due to technical limitations therein, notably use a single beam/sensor system.

Figure 4 presents in a schematic way calibration data that may be used by the apparatus of Figure 1. In particular, calibration curves for relating the cell density in Pseudomonas putida cell-suspensions to the green-light scattering-intensities, as measured by the optical scanning device in the centre of the wells of polystyrene 24-well microtiter plates. The calibration data, presented as calibration curves, may have measurement data on intensity of scattered light, varied between I_0 and I_{max} for with call masses in the range of M_0 and M_{max}. It will be appreciated that suitable selection of the range M_0 -

M_{max} lies within skill of the artisan. It will be further appreciated that the calibration data may not be limited to measurement data, as respective curves may be fitted with a suitable analytic equation for extrapolating the calibration data beyond the measurement results. Curve A represents data for white walls of the wells, curve B represents data for grey walls of the wells, curve C represents data for black walls of the wells. It is found that microtiter plates with white walls are especially suitable to measure low densities at a high accuracy. Microtiter plates with black-walled wells are suitable for relatively high cell densities, with a relatively poor response at low cell densities. Microtiter plates with grey walls give a good accuracy at low cell densities, but also result in an well measurable increase in the green-light scattering-intensities at high cell densities.

It will be appreciated that while specific embodiments of the invention have been described above, that the invention may be practiced otherwise than as described. In addition, isolated features discussed with reference to different figures may be combined. In addition, although in the specification the invention is explained with reference to a 96-well microtiter plate, other microtiter plates may also be analyzed. The invention is not limited to the flatbed scanner featuring an embodiment of an optical scanning device as is set forth in the foregoing.

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CONCLUSIES

1. Inrichting voor het onderzoeken van eukaryote en microbiële celcultures die orbitaal geschud zijn in microtiter platen, omvattende:

- een schudder ingericht om een meervoudig aantal van in de microtiter platen aangebrachte putten orbitaal te schudden;

met het kenmerk dat

de inrichting verder omvat een optische scaninrichting ingericht om één of meer tweedimensionale afbeeldingen van het genoemde meervoudig aantal van putten te genereren en een besturingseenheid ingericht om de schudder te onderbreken en om de scaninrichting de genoemde één of meer afbeeldingen te doen genereren tijdens de genoemde onderbreking.

2. Inrichting volgens conclusie 1, waarin de optische scaninrichting een *flatbed* scannende eenheid omvat.

3. Inrichting volgens één der voorgaande conclusies, verder omvattende een processor ingericht om een groeigerelateerde parameter te bepalen voor een cultuur aanwezig in de genoemde putten op basis van de afbeeldingen en een vooraf bepaalde ijkgegevens.

4. Inrichting volgens conclusie 3, waarin de processor is ingericht om de vooraf bepaalde ijkgegevens te selecteren vanuit een meervoudig aantal van ijkgegevens op basis van een type van een microtiter plaat aanwezig in de schudder.

5. Inrichting volgens conclusie 4, waarin de processor is ingericht om een type van de microtiter plaat te bepalen op basis van de afbeeldingen gegenereerd door de optische scaninrichting.

6. Inrichting volgens één der voorgaande conclusies 3 – 5, waarin de processor is nader ingericht om een schudmodus aan te passen op basis van een waarde van de bepaalde groeigerelateerde parameter.

7. Inrichting volgens één de voorgaande conclusies, omvattende een meervouding aantal van microtiter platen voorzien van putten.

8. Inrichting volgens één der voorgaande conclusies, waarin de optische scaninrichting is ingericht om zichtbaar licht te genereren.

9. Inrichting volgens één der voorgaande conclusies 1 – 7, waarin de optische scaninrichting is ingericht om licht in het bereik van 400 nm – 650 nm te genereren.

10. Inrichting volgens conclusie 7, waarin de putten zijn voorzien van transparante bodems en witte, grijze of zwarte wanden.

11. Werkwijze voor het onderzoeken van orbitaal geschudde putten aanwezig in microtiter platen, omvattende de stappen van:

- het orbitaal schudden van de microtiter platen, die putten omvatten welke zijn voorzien van een cultuur om daarin te worden gegroeid;
- het onderbreken van het schudden van de microtiter platen;
- het genereren van een meervoudig aantaal van tweedimensionale afbeeldingen van de putten;
- het hervatten van het schudden.

12. Werkwijze volgens conclusie 11, verder omvattende de stap van:

- het analyseren van de genoemde afbeeldingen voor het bepalen van een groeigerelateerde parameter.

13. Werkwijze volgens conclusie 12, verder omvattende een stap van het aanpassen van een schudmodus van de microtiter platen op basis van een bepaalde waarde van de groeigerelateerde parameter.

14. Computerprogrammaproduct voor het laten een processor de stappen van de werkwijze volgens conclusies 11, 12 of 13 uit te voeren.

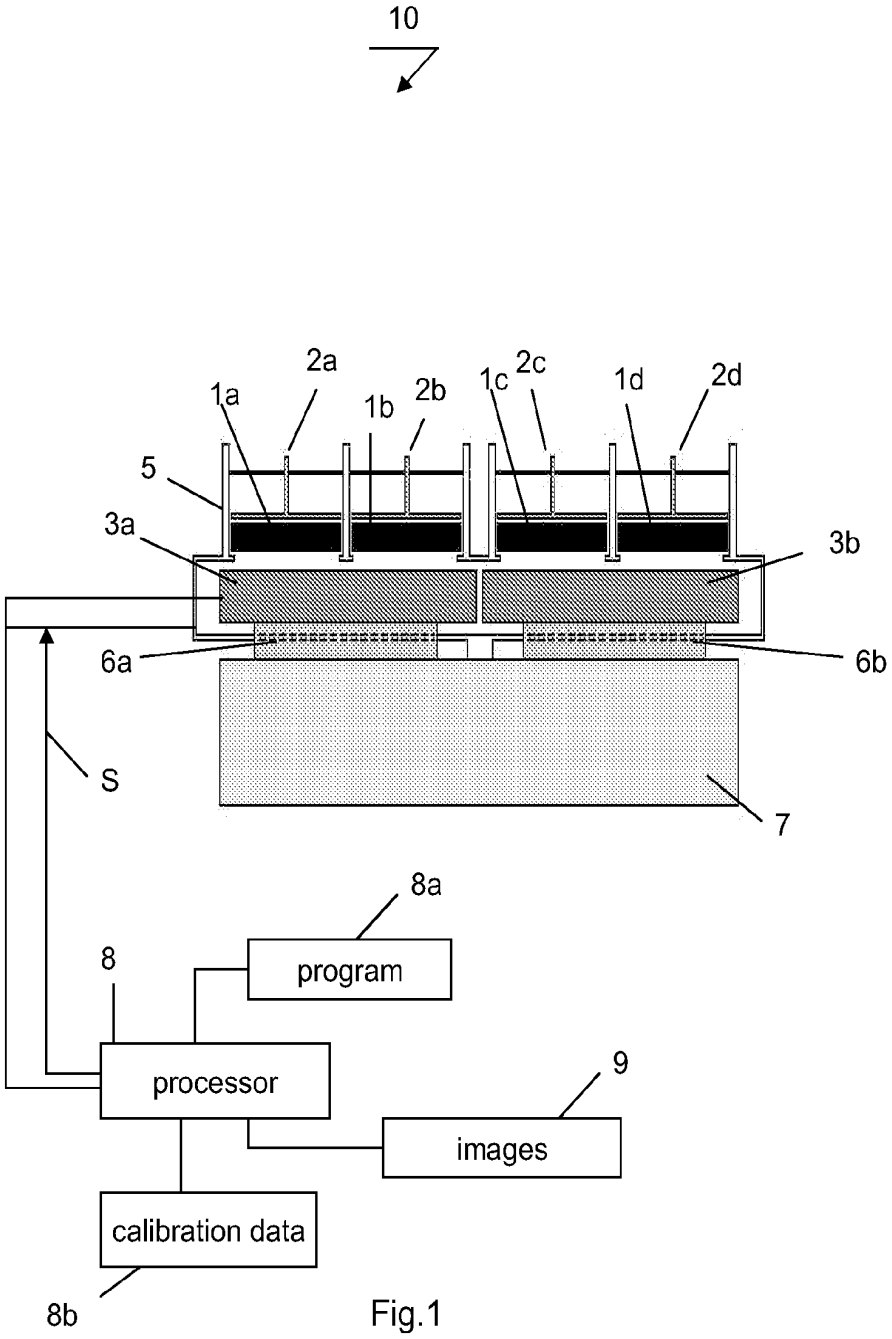

10
2a
1a
1b
2b
1c
2c
1d
2d
5
3a
3b
6a
6b
S
7
8a
8
program
processor
9
images
calibration data
8b

Fig.1

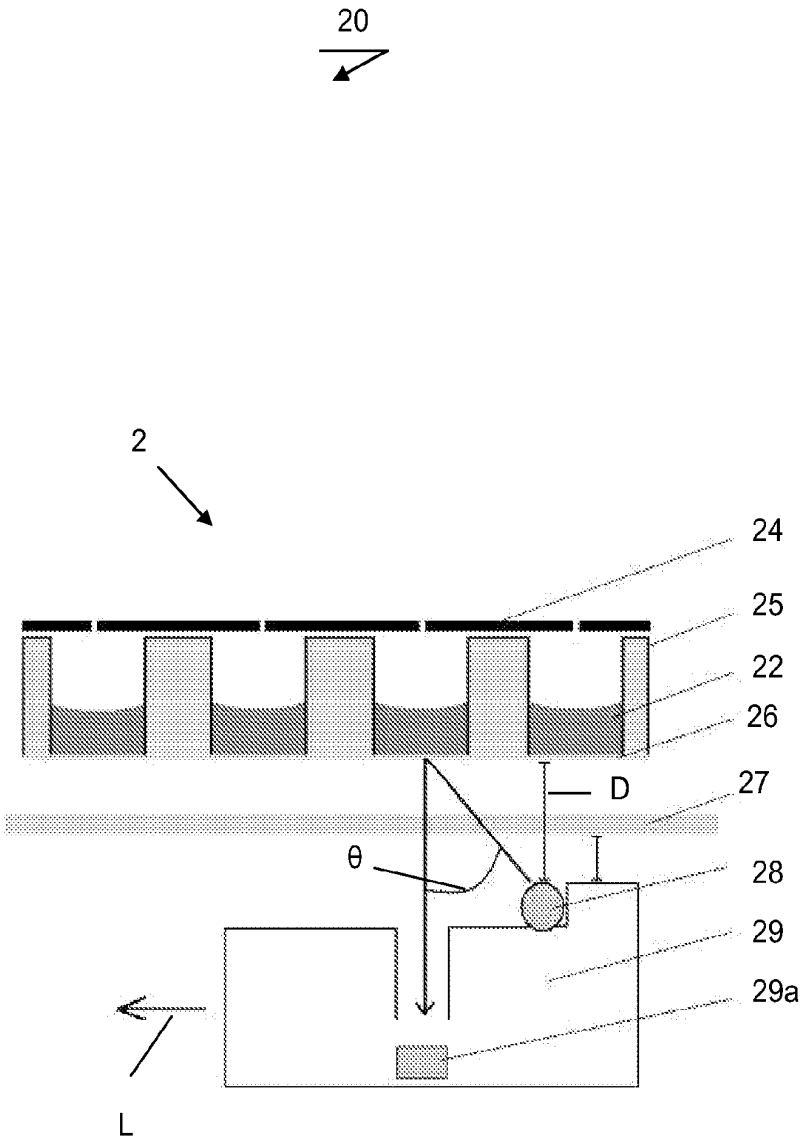


Fig.2

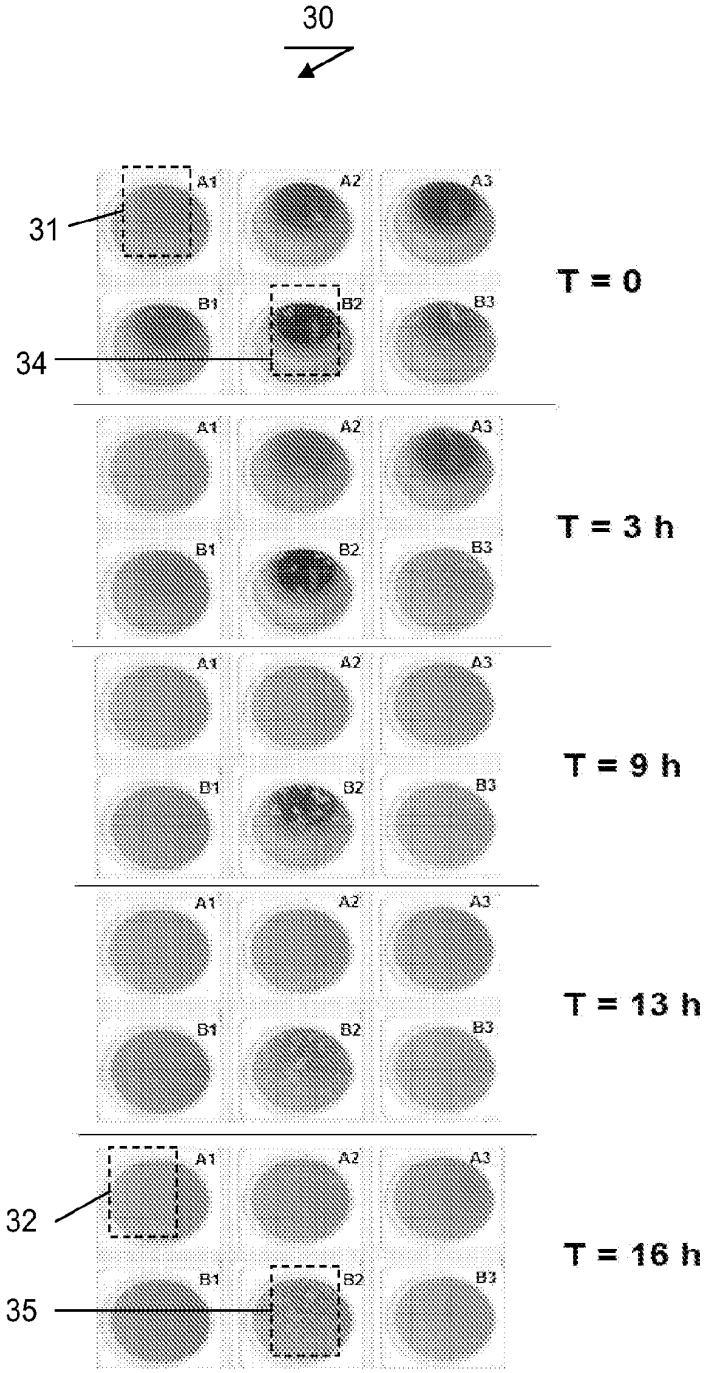


Fig.3

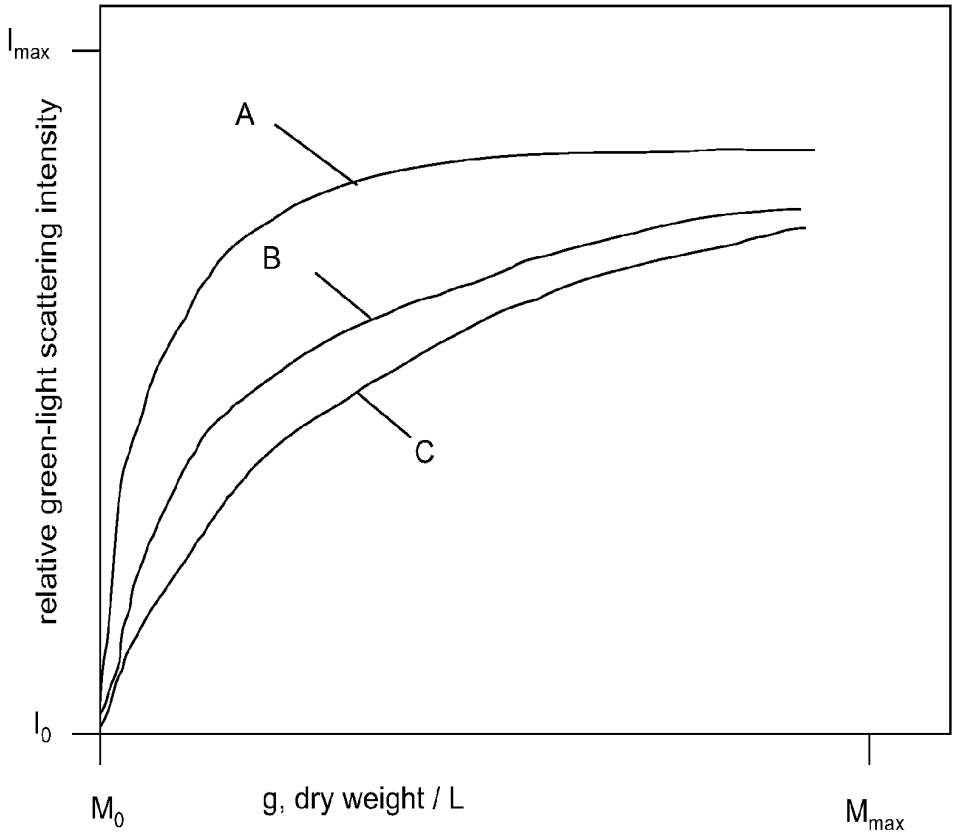
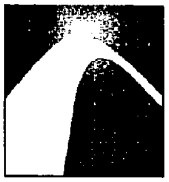

I_{max}
relative green-light scattering intensity
A
B
C
I_0
M_0
g, dry weight / L
M_{max}

Fig. 4



OCTROOICENTRUM NEDERLAND

OCTROOIAANVRAAG NR.:
NO 136362
NL 2002055

ONDERZOEKSRAPPORT

BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK

RELEVANTE LITERATUUR

Categorie[1]	Literatuur met, voor zover nodig, aanduiding van speciaal van belang zijnde tekstgedeelten of figuren.	Van belang voor conclusie(s) nr:	Classificatie (IPC)
X Y	US 2004/106201 A1 (BLUM HELMUT [DE] ET AL) 3 juni 2004 (2004-06-03) * alineas [0003], [0013], [0018] * * alinea [0023] * * alineas [0036] - [0042], [0044] - [0046] * * alineas [0048], [0051], [0060] * * conclusies 21,25 * -& DE 100 52 511 A1 (HENKEL KGAA [DE]) 2 mei 2002 (2002-05-02) * figuur 1 *	1,7-11, 14 2-6,12, 13	INV. G01N21/25 B01F11/00 B01F15/00
Y	US 2002/168784 A1 (SUNDREHAGEN ERLING [NO] ET AL) 14 november 2002 (2002-11-14) * alinea [0029] *	2	
D,Y	US 2007/256510 A1 (BUCHS JOCHEN [DE] ET AL) 8 november 2007 (2007-11-08) * alineas [0002], [0005], [0006] * & EP 1 730 494 A (RWTH AACHEN [DE]) 13 december 2006 (2006-12-13)	3-6,12, 13	
A	US 6 673 532 B2 (RAO GOVIND [US]) 6 januari 2004 (2004-01-06) * alinea [0065] *	3-6,12, 13	

Onderzochte gebieden van de techniek

G01N
B01F

Indien gewijzigde conclusies zijn ingediend, heeft dit rapport betrekking op de conclusies ingediend op:

Plaats van onderzoek:	Datum waarop het onderzoek werd voltooid:	Bevoegd ambtenaar:
's-Gravenhage	7 Mei 2009	Verdoodt, Erik

[1] CATEGORIE VAN DE VERMELDE LITERATUUR

X: de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur
Y: de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht
A: niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft
O: niet-schriftelijke stand van de techniek
P: tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur
T: na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding
E: eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven
D: in de octrooiaanvrage vermeld
L: om andere redenen vermelde literatuur
&: lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

EOB FORM 02.83 (P0414B)

AANHANGSEL BEHORENDE BIJ HET RAPPORT BETREFFENDE HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK, UITGEVOERD IN DE OCTROOIAANVRAGE NR.

NO 136362
NL 2002055

Het aanhangsel bevat een opgave van elders gepubliceerde octrooiaanvragen of octrooien (zogenaamde leden van dezelfde octrooifamilie), die overeenkomen met octrooischriften genoemd in het rapport.
De opgave is samengesteld aan de hand van gegevens uit het computerbestand van het Europees Octrooibureau per
De juistheid en volledigheid van deze opgave wordt noch door het Europees Octrooibureau, noch door het Bureau voor de Industriële eigendom gegarandeerd; de gegevens worden verstrekt voor informatiedoeleinden.

07-05-2009

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
US 2004106201 A1	03-06-2004	AU 2167202 A	06-05-2002
		DE 10052511 A1	02-05-2002
		WO 0235218 A2	02-05-2002
		EP 1332345 A2	06-08-2003
DE 10052511 A1	02-05-2002	AU 2167202 A	06-05-2002
		WO 0235218 A2	02-05-2002
		EP 1332345 A2	06-08-2003
		US 2004106201 A1	03-06-2004
US 2002168784 A1	14-11-2002	AU 758339 B2	20-03-2003
		AU 5056599 A	14-02-2000
		CA 2337415 A1	03-02-2000
		EP 1099108 A1	16-05-2001
		WO 0005571 A1	03-02-2000
		JP 2002521660 T	16-07-2002
		NO 20010382 A	23-01-2001
US 2007256510 A1	08-11-2007	CA 2563001 A1	20-10-2005
		CN 1938576 A	28-03-2007
		DE 102004017039 A1	03-11-2005
		EP 1730494 A1	13-12-2006
		WO 2005098397 A1	20-10-2005
		JP 2007530270 T	01-11-2007
		KR 20060135858 A	29-12-2006
EP 1730494 A	13-12-2006	CA 2563001 A1	20-10-2005
		CN 1938576 A	28-03-2007
		DE 102004017039 A1	03-11-2005
		WO 2005098397 A1	20-10-2005
		JP 2007530270 T	01-11-2007
		KR 20060135858 A	29-12-2006
		US 2007256510 A1	08-11-2007
US 6673532 B2	06-01-2004	US 2004121453 A1	24-06-2004
		US 2002025547 A1	28-02-2002

Algemene informatie over dit aanhangsel is gepubliceerd in de 'Official Journal' van het Europees Octrooibureau nr 12/82 blz 448 ev

OCTROOICENTRUM NEDERLAND

SCHRIFTELIJKE OPINIE

DOSSIER NUMMER	INDIENINGSDATUM	VOORRANGSDATUM	AANVRAAGNUMMER
NO136362	03.10.2008		NL2002055

CLASSIFICATIE
INV. G01N21/25 B01F11/00 B01F15/00

AANVRAGER
Enzyscreen B.V. te Amsterdam

Deze schriftelijke opinie bevat een toelichting op de volgende onderdelen:

- ☒ Onderdeel I Basis van de schriftelijke opinie
- ☐ Onderdeel II Voorrang
- ☐ Onderdeel III Vaststelling nieuwheid, inventiviteit en industriële toepasbaarheid niet mogelijk
- ☐ Onderdeel IV De aanvraag heeft betrekking op meer dan één uitvinding
- ☒ Onderdeel V Gemotiveerde verklaring ten aanzien van nieuwheid, inventiviteit en industriële toepasbaarheid
- ☐ Onderdeel VI Andere geciteerde documenten
- ☐ Onderdeel VII Overige gebreken
- ☐ Onderdeel VIII Overige opmerkingen

DE BEVOEGDE AMBTENAAR
Verdoodt, Erik

Aanvraag nr.:

SCHRIFTELIJKE OPINIE

NL2002055

Onderdeel I Basis van de Schriftelijke Opinie

1. Deze schriftelijke opinie is opgesteld op basis van de meest recente conclusies ingediend voor aanvang van het onderzoek.

2. Met betrekking tot **nucleotide en/of aminozuur sequenties** die genoemd worden in de aanvraag en relevant zijn voor de uitvinding zoals beschreven in de conclusies, is dit onderzoek gedaan op basis van:

   a. type materiaal:

   ☐ sequentie opsomming

   ☐ tabel met betrekking tot de sequentie lijst

   b. vorm van het materiaal:

   ☐ op papier

   ☐ in elektronische vorm

   c. moment van indiening/aanlevering:

   ☐ opgenomen in de aanvraag zoals ingediend

   ☐ samen met de aanvraag elektronisch ingediend

   ☐ later aangeleverd voor het onderzoek

3. ☐ In geval er meer dan één versie of kopie van een sequentie opsomming of tabel met betrekking op een sequentie is ingediend of aangeleverd, zijn de benodigde verklaringen ingediend dat de informatie in de latere of additionele kopieën identiek is aan de aanvraag zoals ingediend of niet meer informatie bevatten dan de aanvraag zoals oorspronkelijk werd ingediend.

4. Overige opmerkingen:

Aanvraag nr.:

SCHRIFTELIJKE OPINIE

NL2002055

Onderdeel V Gemotiveerde verklaring ten aanzien van nieuwheid, inventiviteit en industriële toepasbaarheid

1. Verklaring

Nieuwheid	Ja:	Conclusies 2-6,12,13
	Nee:	Conclusies 1,7-11,14
Inventiviteit	Ja:	Conclusies
	Nee:	Conclusies 1-14
Industriële toepasbaarheid	Ja:	Conclusies 1-14
	Nee:	Conclusies

2. Citaties en toelichting:

Zie aparte bladzijde

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US 2004/106201 A1 (BLUM HELMUT [DE] ET AL) 3 juni 2004 (2004-06-03)

D2: US 2002/168784 A1 (SUNDREHAGEN ERLING [NO] ET AL) 14 november 2002 (2002-11-14)

D3: US 2007/256510 A1 (BUCHS JOCHEN [DE] ET AL) 8 november 2007 (2007-11-08)

D4: US-B2-6 673 532 (RAO GOVIND [US]) 6 januari 2004 (2004-01-06)

Claim 1

1.1 The claim 1 is not clear for the following reason:

1.2 The feature "afbeeldingen doen genereren tijdens de genoemde onderbreking" (see lines 10-11 of claim 1) in the apparatus claim 1 relates to a method of using the apparatus rather than clearly defining the apparatus in terms of its technical features. The intended limitations are therefore not clear from this claim.

1.3 Furthermore, the above-mentioned lack of clarity notwithstanding, the subject-matter of claim 1 is not new, and therefore the criteria of patentability are not met.

1.4 The document D1 discloses *(the references in parentheses applying to this document):*

Inrichting (see fig .1 in family document DE10052511 A1) geschikt voor het onderzoeken van eukaryote en microbiële celcultures die orbitaal geschud zijn in micotiter platen (§23), omvattende:

- een schudder (17) ingericht om een meervoudig aantal van in de microtiter platen (2) (see § 46) aangebrachte putten orbitaal te schudden *(see claim 21; § 51);* waarbij
- de inrichting verder omvat een optische scaninrichting *(see §36, 39; §18)* ingericht om één of meer tweedimensionale afbeeldingen *(see § 18: "a camera is suitable for spatial distinction of various regions")* van het genoemde meervoudig aantal van putten te genereren en een besturingseenheid (21) ingericht om de schudder te onderbreken *(see §51: control unit (21) controls the mixing unit (17) and sensor unit (8) or camera (10))* en om de scaninrichting de genoemde één of meer afbeeldingen te doen genereren tijdens de genoemde onderbreking.

Claim 11

2.1 The same reasoning applies, mutatis mutandis, to the subject-matter of the corresponding independent method claim 11, which therefore is also considered not new.

2.2 One of the ways of shaking the microtiter plates by the mixing unit (17) is specified in claim 21 of D1 to be with rotating movements, which is similar to the orbital shaking described in claim 11 of the application.
Further, in order for the sensor unit to be moved in relation to the microtiter plate (2) in such a way that the wells (3) can be monitored successively (see §36), it is necessary that the mixing unit (17) holds the shaking temporarily. The interruption and resuming of the shaking to allow the scanning of the microtiter plate, is therefore considered to be implicitly disclosed in D1.

Claim 14

3.1 The subject-matter of the independent claim 14 is also not new, as D1 discloses the use of the control unit (21) to control the separate units. This control unit will have a processor to run a computer program to perform the requires steps.

Dependent claims 2-10, 11-13

4.1 Dependent claims 2-10, 11-13 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of novelty and/or inventive step, see documents D1-D3 and the corresponding passages cited in the search report.