Title: TTITRE PLATE AND MEASURING DEVICE FOR CHEMICAL AND/OR BIOCHEMICAL ANALYSIS

Abstract: The invention relates to a titre plate for chemical and/or biochemical analysis based on an illumination measurement comprising a transparent substrate, applied to the substrate there is a metallic layer, which is provided with an array of reaction chambers, wherein the reaction chambers extend from the top side of the metallic layer to, or into, the substrate thereunder, wherein at the side of the metallic layer facing away from the substrate, the same is provided with a transparent cover plate and wherein, viewed in the plane of the metallic layer, the cross-sectional dimension of the reaction chambers is smaller than the illumination wavelength.
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
The invention relates to a titre plate for chemical and/or biochemical analysis.

Such a titre plate is known from practice, for example, in the form of a so-called nanolitre titre plate, which provides a possibility of carrying out analyses using approximately ten reaction chambers per mm² of plate surface.

One object of the invention is to realise a further improvement of the efficiency by significantly raising the number of reaction chambers per unit area and concurrently, to facilitate a simultaneous readout of these reaction chambers.

Another object is to reduce the consumption of reagents and the like that are required in the reaction chambers, so as to limit the costs of using the titre plate.

Still another object is to, based on costs per reaction chamber, reduce the manufacturing costs of the titre plate by increasing the number of reaction chambers per unit area of titre plate.

EP-A-1 353 179 relates to a titre plate for chemical and/or biochemical analysis based on an illumination measurement, comprising a transparent substrate, applied to the substrate there is a metallic layer, which is provided with an array of reaction chambers wherein the reaction chambers extend from the top side of the metallic layer to, or into, the substrate thereunder. The reaction chambers of the titre plate known from this publication, are applied in an irregular pattern and their diameter is at least equal to the wavelength of the applied illumination. Moreover, the illumination measurement is based on a reflection measurement.

In a first aspect, the titre plate according to the invention is characterized by a transparent cover plate at the side of the metallic layer facing away from the substrate, and by reaction chambers whose cross-sectional dimension, viewed in the plane of the metallic layer, is smaller than the illumination wavelength.
Due to both the substrate and the cover plate being transparent so as to allow readout of the reaction chambers to take place by simultaneous illumination through the reaction chambers, the titre plate according to the invention makes it simply and effectively possible to read out all the reaction chambers simultaneously.

It is important for the reaction chambers in the metallic layer to be applied in a regular, periodical pattern and in a density of at least approximately one million per $\text{mm}^2$. The volume of the reaction chambers to be applied is in the order of one or several attolitres.

The titre plate according to the invention is further preferably characterised in that the reaction chambers in the plane of the metallic layer have a cross-sectional dimension in the range from 50 to 150 nm.

On thing and another facilitates the occurrence of the so-called photon-plasmon effect when light is transmitted through the titre plate, resulting in a photon-plasmon coupling among the reaction chambers.

A consequence of the effect just referred to is that
a) the light intensity after passage of the reaction chambers is higher than that predicted on the basis of classic optical calculations;
b) the transmission through the reaction chambers has a spectrum that may be influenced; and
c) the light passing the reaction chambers has a limited diffraction in the order of $3^\circ$.

Thus the titre plate according to the invention makes extremely intensive testing possible, at reduced reagent consumption.

In accordance with the foregoing, the invention is also embodied in a measuring device for chemical and/or biochemical analysis, comprising a titre plate according to the invention, a light source for illuminating said titre plate and a light measuring device.

Another aspect of the invention when applying the above-mentioned effect is that the light sensor is a lens,
positioned closely to the titre plate but outside the near field, and cooperating with an optical sensor.

The preferred optical sensor to use is a CCD camera, which makes it possible to simultaneously read out the light output per reaction chamber.

The distance at which the lens is positioned from the titre plate is preferably in the range from 1 to 1000 μm.

To effectively use the titre plate according to the invention it is further desirable for the cover plate and the metallic layer to be located on either side of a channel that conducts a sample liquid or washing water.

The metallic layer is preferably provided with a hydrophobic coating bordering on the channel. A suitable material for this purpose is, for example, silicon. Such a hydrophobic coating makes a selective supply of reagent and/or sample liquid to the reaction chambers possible. To this end the coating may be applied in a predetermined pattern.

In order to be able to perform a simultaneous multiple assay of a sample of test liquid, it is desirable for at least one of the reaction chambers to be provided with a predetermined selection from the group comprising antibodies, reagents, DNA.

Hereinafter the invention will be further elucidated by way of a non-limiting exemplary embodiment and with reference to the drawing, by way of which various aspects of the invention are explained.

In a single figure, the drawing schematically shows a measuring device provided with a titre plate according to the invention.

The schematically illustrated measuring device 1 serves for chemical and/or biochemical analysis and comprises a titre plate 2, a light source 3 for illuminating the titre plate 2 and a lens 4 positioned outside the near field of the titre plate, which cooperates with an optical sensor 5 in the form of, for example, a CCD camera.

The lens 4 is positioned close to the titre plate 2, however, not in the near field where the distance would be
less than 300 nanometre, but at a distance of 1 to 1000 μm. In accordance with typical practice, the optical sensor cooperating with the lens 4 may be positioned at a greater distance from said lens 4, for example, in the range from 0.1 to 100 cm.

The titre plate 2 is characterized by a transparent substrate 6, applied to the substrate 6 there is a metallic layer 7, which is provided with an array of reaction chambers 8, and a transparent cover plate 9 provided at the side of the metallic layer 7 that faces away from the substrate 6. The metallic layer is preferably a gold coating.

A further characteristic of the titre plate 2 is that the cover plate 9 and the metallic layer 7 are provided at both sides of a channel 10 for conducting a test liquid or washing water.

The metallic layer 7 is preferably also provided with a hydrophobic layer 11 bordering on the channel 10. A suitable material that may be used for this purpose is silicon.

The material to be used for the transparent substrate 6 may be, for example, glass. Glass may also be used for the cover plate 9.

Prior to bringing the titre plate 2 into the ready form shown in the figure, it is preferred for the reaction chambers 8 to be loaded with a substance previously selected from the group comprising antibodies, DNA, reagent. Furthermore, the figure clearly shows that the reaction chambers 8 extend through the entire thickness of the metallic layer 7. It should be mentioned that occasionally a portion of one or several of the reaction chambers 8 may extend into the material of the substrate 6. Some of the material in its surface facing the metallic thin layer 7 may then become etched away.

It is further worth mentioning that the thin metallic layer 7 has a thickness of approximately 100 to 200 nanometres, that the reaction chambers 8 in the plane of the metallic layer 7 have an mean size in the range from 50 to 150 nanometres and that the reaction chambers 8 are provided at a
density of at least approximately one million per mm$^2$ of titre plate area.
CLAIMS

1. A titre plate (2) for chemical and/or biochemical analysis based on an illumination measurement comprising a transparent substrate (6), applied to the substrate (6) there is a metallic layer (7), which is provided with an array of reaction chambers (8), wherein the reaction chambers (8) extend from the top side of the metallic layer (7) to, or into, the substrate (6) thereunder, characterised in that at the side of the metallic layer (7) facing away from the substrate (6), the same is provided with a transparent cover plate (9) and that, viewed in the plane of the metallic layer (7), the cross-sectional dimension of the reaction chambers (8) is smaller than the illumination wavelength.

2. A titre plate (2) according to claim 1, characterised in that the reaction chambers (8) in the metallic layer (7) are applied in a regular, periodical pattern and in a density of at least approximately one million per mm².

3. A titre plate (2) according to claim 1 or 2, characterised in that the reaction chambers (8) in the plane of the metallic layer (7) have a cross-sectional dimension in the range from 50 to 150 nm.

4. A titre plate (2) according to one of the claims 1-3, characterised in that the cover plate (9) and the metallic layer (7) are provided at both sides of a channel (10) for conducting a test liquid or washing water.

5. A titre plate (2) according to one of the claims 1-4, characterised in that the metallic layer (7) is provided with a hydrophobic coating (11) bordering on the channel (10).

6. A titre plate (2) according to one of the claims 1-5, characterised in that at least one of the reaction chambers (8) is provided with a predetermined selection from the group comprising antibodies, reagents, DNA.

7. A measuring device (1) for chemical and/or biochemical analysis, comprising a titre plate (2), a light source (3) for illuminating the titre plate (2) and a light
measuring device (4, 5), characterised in that a titre plate (2) according to one of the claims 1-6 is used and that the light measuring device (4, 5) comprises a lens (4), positioned closely to the titre plate (2) but outside the near field, that cooperates with an optical sensor (5).

8. A measuring device (1) according to claim 7, characterised in that that the distance at which the lens (4) is positioned from the titre plate (2) is in the range from 1 to 1000 μm.

9. A measuring device (1) according to claim 7 or 8, characterised in that the optical sensor (5) is a CCD camera.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

B01L3/00  G01N21/55  G01N33/553  B01L3/00  G01N21/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

B01L  G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No.
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X | WO 03/102559 A (GYROS AB; OESTLIN, HENRIK; ERIKSSON, LARS; LJUNGSTROEM, MAGNUS; AAGREN) 11 December 2003 (2003-12-11) page 4, line 16 - page 5, line 25 page 7, lines 11-15 page 12, lines 24-32 page 13, line 17 - page 14, line 5 page 14, lines 24-27 figures 2-4 | 1-9
X | EP 1 353 179 A (JAPAN SCIENCE AND TECHNOLOGY CORPORATION) 15 October 2003 (2003-10-15) paragraphs ‘0012!’ - ‘0018!’ figures 1,2,4,6,7 | 1-9

* Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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Name and mailing address of the ISA

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